Electrocochleography (ECochG) with a non-invasive tympanic membrane (TM) electrode in normally - hearing subjects

Radboudumc
university medical center

Master's thesis
Author: L. M. Coraci (s1005887)
Supervisor: Dr. A. J. Beynon (Radboudumc, department of Otorhinolaryngology)
Second assessor: Dr. E. Janse (Radboud University Nijmegen)
Date: November 4th, 2019

Abstract

Background: Cochlear microphonics (CMs) may play an important role in the diagnosis of auditory neuropathy/dyssynchrony spectrum disorder (ANSD), in the prognosis of cochlear implant (CI) outcome and in the preservation of residual hearing in cochlear implantation. Due to a wide variety of parameter settings, there is a lack of data for non-invasive tympanic membrane (TM) electrocochleography (ECochG) with the current setup and hardware. *Purpose:* The aim of the study was to determine the optimal parameter settings for the recordings of CMs and to obtain reference data for the summating potentials (SPs), action potentials (APs) and CMs recorded by non-invasive ECochG with TM electrodes in normal-hearing adult subjects. *Methods:* A total of 24 normal-hearing subjects (right ear pure tone thresholds ≤ 20 dBnHL) aged between 20 and 32 years (10 males and 14 females) were tested. SPs and APs were elicited by a click at 90 dBnHL. CMs were elicited by a click at 100 dBnHL or by a tone burst (2,000 Hz) or broadband (BB) chirp, both at 80 dBnHL. *Results:* CMs occurred for 100% of clicks, for 79% of tone bursts and for 63% of BB chirps. The click stimulus elicited significantly larger CM amplitudes than the tone burst and BB chirp did. CM amplitudes evoked by tone bursts and BB chirps did not differ from each other. High-pass filter (HPF) settings did not significantly change the CM recordings. There was no relationship between the parameter settings and both, CM latency and duration. *Conclusions:* The present study provides reference SP, AP and CM values for TM ECochG. It is recommended to use a click for elicitation of CMs with an HPF setting of 100 Hz.

Keywords: electrocochleography, cochlear microphonics, tympanic membrane electrode

Content

Abbreviations

ABR	Auditory brainstem responses
AEP	Auditory evoked potential
ANN	Auditory nerve neurophonic
ANSD	Auditory neuropathy/dyssynchrony spectrum disorder
AP	Action potential
BB	Broadband
BERA	Brainstem evoked response audiometry
BM	Basilar membrane
CI	Cochlear implant
CM	Cochlear microphonic
dBnHL	Normal hearing level in decibels
ECochG	Electrocochleography
EEG	Electroencephalogram
ET	Extra-tympanic
Hz	Hertz
IHC	Inner hair cells
IT	Intra-tympanic
MD	Meniere's disease
ms	Milliseconds
NB	Narrowband
OAE	Otoacoustic emissions
OCB	Olivocochlear bundle
OHC	Outer hair cells
RW	Round window
SNR	Signal-to-noise ratio
SP	Summating potential
TM	Tympanic membrane
TT	Trans-tympanic

List of figures

List of tables

1 Introduction

In the seventies, trans-tympanic (TT) electrocochleography (ECochG) was used to examine the function of the inner ear objectively. TT ECochG is an invasive procedure because it requires a physician to insert the needle through the tympanic membrane. In the eighties, this technique was replaced by a non-invasive and more efficient measurement, the Brainstem Evoked Response Audiometry (BERA). Both methods examine the function of the inner ear: where the BERA focuses on the activity of the brainstem, the ECochG focuses on the peripheral cochlea. Later, ECochG appeared to be required for the diagnosis or evaluation of hearing losses in specific patient groups. Extra-tympanic (ET) ECochG proved to be an alternative for TT ECochG, whereby an electrode is inserted in the ear canal near the tympanic membrane. ET ECochG is often performed by two types of electrodes: tiptrodes and TM electrodes. Bonucci and Hyppolito (2009) compared both electrodes within-subject and concluded that TM electrodes give larger amplitude responses than tiptrodes. Non-invasive ECochG enabled researchers to perform recordings without a guiding physician due to the less invasive nature of the electrode position. Nowadays, ET ECochG is most often used as an additional measurement in the diagnosis of patients with Meniere's Disease (MD). It may also be a promising tool for the diagnosis and evaluation of hearing loss in children with an auditory neuropathy/dyssynchrony spectrum disorder (ANSD) and potential candidates for a cochlear implant (CI; McMahon, Patuzzi, Gibson & Sanli, 2009).

An adequate diagnosis requires the collection of normalized data from normal-hearing subjects. ECochG with TM electrodes (also called TM ECochG) have been used in more recent studies, but these were conducted with different stimulus and recording parameter settings (e.g., Grasel et al., 2017; Lake & Stuart, 2019; Redondo-Martínez et al., 2016). This wide variety of implemented parameter settings has led to a lack of normalized data for ET ECochG. The main objective of the present study is to determine which parameter settings give optimal CM responses in normal-hearing subjects using TM ECochG and the hardware available in this clinic. The following parameters will be investigated: stimulus type and filter setting.

1.1 The human ear

The perception of an acoustic signal in the human ear is a complex process. This process involves three major anatomical structures: the outer ear, the middle ear and the inner ear (see Figure 1.1). These structures and functions are briefly described below.

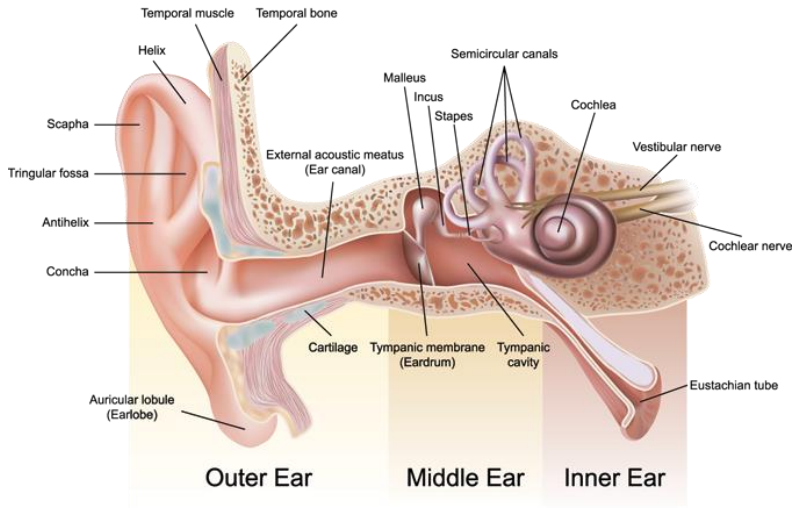

Figure 1.1 The anatomy of the human ear. Retrieved from https://www.listen-2-life.com/how-hearing-works/

1.1.1 The outer ear and middle ear

The outer ear plays a role in both the enhancing strength of specific speech sounds and the localization of a signal. The signal travels from the outer ear through the auditory canal and sets the tympanic membrane in motion. The tympanic membrane functions as a bridge between the outer- and middle ear. Subsequently, the ossicles (malleus, incus and stapes) that are located in the middle ear are set in motion. These bones serve as an impedance adjuster needed to transmit vibrations from air to liquids in the inner ear, starting at the tympanic membrane and ending at the oval window (Emanuel & Letowski, 2009).

1.1.2 The inner ear

The inner most sophisticated 200 mm^3 of our ear structures (Buckingham & Valvassori, 2001), the inner ear, includes organs responsible for our ability to hear (cochlea) and to maintain balance (semi-circular canals and vestibule) (Emanuel & Letowski, 2009). The cochlea, being bilaterally located inside the temporal lobe, is a spiral which forms a coiled tunnel. The beginning of the cochlea is the widest coil of the spiral and is called the base. The end is the narrowest coil and is called the apex. The base is responsible for the higher frequencies, and the lower frequencies are recognised by the apex. The tonotopic organisation of the cochlea was first described by von Békésy (1960).

The cochlea has three separate channels, namely the scala vestibuli, the scala media and the scala tympani and those are filled with fluids (perilymph and endolymph). The basilar membrane (BM) is located in the scala media (see Figure 1.2), which contains the inner hair cells (IHCs) and outer hair cells (OHCs). When the fluids in the scalae are set in motion by a travelling wave entering from the oval window, the hair cells will eventually move accordingly. IHCs are afferent sensory receptors and communicate with neurons from the hearing nerve. OHCs receive efferent input from the olivocochlear bundle (OCB). The stereocilia, on top of the hair cells, will deflect by friction with the tectorial membrane and cause a subsequent flow of transducer currents (Emanuel & Letowski, 2009).

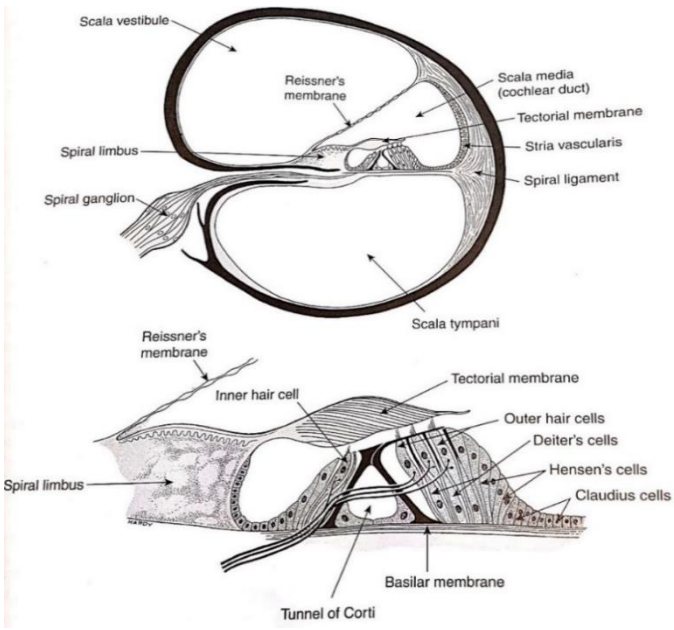

Figure 1.2 A cross section of the cochlea. Retrieved from Emanuel, D. C., & Letowski, T. (2009). Hearing science. Philadelphia, PA: Wolters KluwerHealth/Lippincott Williams and Wilkins.

1.1.3 Auditory Evoked Potentials

The central nervous system of the human body generates electrical potentials that can be recorded in electroencephalogram (EEG; Arns, Gunkelman, Olbrich, Sander & Hegerl, 2010). EEG signals that are derived as such from a single modality, in specific from the hearing system, are called auditory evoked potentials (AEPs). AEPs are gradually generated by the auditory pathway from the most peripheral part, i.e. from the cochlea to the central auditory cortex.

1.2 Electrocochleography

The AEPs from the cochlea and the auditory nerve (i.e., VIIIth n. vestibulocochlearis) are created by concentrations of positively and negatively charged ions in the endolymph and perilymph, the inner hair cells (IHC) and the outer hair cells (OHC) activities. The cochlear responses are objectively assessed by electrocochleography (ECochG) captured by electrodes (Emanuel & Letowski, 2009). The first ECochG measurements in humans during surgery were obtained by Perlman and Case (1941). The development of computer averaging algorithms enabled the first non-surgical ECochG recordings with local anaesthesia (Yoshie, Ohashi & Suzuki, 1967). The four basic components which are discriminated from an ECochG waveform occur within the first 5 ms after stimulus onset (Minaya & Atcherson, 2015). An advantage of ECochG is that the masking of the contralateral ear is not necessary, because the amplitude of the response in the opposite ear is too small for interference (Ferraro & Ruth, 1994; Hall, 2015). There are many parameters involved in the ECochG setup, such as materials (electrode brand and hardware), electrode positioning, stimulus (type and repetition rate) and filters.

1.2.1 Potentials in ECochG waveforms

There are four basic components that can be distinguished in ECochG recordings. First, the (compound) action potential (C/AP), which can be described as a reflection of the combined

firing of cochlear nerve fibres. The AP is a short alternating current potential (i.e., its signal reverses its direction periodically) which occurs only at the onset of an acoustic stimulus. This amplitude is the actual auditory response or 'hearing potential' (Ferraro & Ruth, 1994). The AP amplitude increases with increasing stimulus intensity, while its latency negatively correlates at the same time (Eggermont, 1974). The potential corresponds with wave I in auditory brainstem responses (ABR) but has a larger amplitude and needs less averages. Accordingly, the sensitivity is enhanced in determining peripheral cochlear nerve functions using ECochG (Kileny, 2019).

Second, the summating potential (SP), which reflects the non-linear distortion from the OHC. The typical SP and AP waveform is shown in Figure 1.3. The SP does not indicate an actual auditory response. It is a direct current potential (i.e., its signal flows in a constant direction) and lasts the duration of the auditory stimulus. Its positive of negative orientation is inconsistent and will depend on the position of the electrode and the stimulus (Ruth, Lambert & Ferraro, 1988).

Third, the cochlear microphonic (CM), which is a pre-neural reproduction of the acoustic signal that 'mirrors' the movement of the BM. Figure 1.4 depicts an example of this potential. CMs are mainly generated by the OHC (80 – 85%) and possibly by some IHC (15 – 20%). It is an alternating current potential (i.e., its signal reverses its direction periodically) that occurs immediately at stimulus onset and possibly lasts up to 5 ms (British Society of Audiology, 2019). The potential is the spatial summation of transducer currents produced by a large number of OHC (Cheatham, Naik & Dallos, 2011). CMs are difficult to distinguish from artefacts what had led to the thought that CMs had little clinical utility (von Békésy, 1960). Recently, the clinical application of the CM has been revised. It may be a promising tool for the diagnosis and evaluation of hearing loss in children with ANSD and potential candidates for a CI (Pienkowski, Adunka & Lichtenhan, 2018). It is this promising potential that will be investigated in the present study.

In contrast to the previously described potentials, the fourth, named the auditory nerve neurophonic (ANN), has gotten relatively less attention. This potential has been found in human TT ECochG recorded from the round window by Choudhury et al. in 2012. It occurs as a sinusoidal waveform of twice the frequency of the presented sound. An ANN reflects the auditory nerve firing and is most likely to occur in low frequency tones. It may reveal information about the capabilities of temporal processing (e.g., sound localization and pitch perception). The CMs and ANNs are hard to distinguish because of their sinusoidal nature (Choudhury et al., 2012). Figure 1.5 shows the ANN for both polarities and merged, resulting in an alternating polarity.

1.2.2 Subject factors in ECochG

Although muscle movements minimally effect the ECochG recordings, relaxation does facilitate the measurement. A comfortable and still lying subject requires less averaging and a lower rejection level (ideally ±40µV or less) compared to a tensed one. The state of arousal and specific disabilities (e.g., autism and development delay) have no effect on the ECochG waveforms (Hall, 2015).

A young age seems to have little effect on the ECochG waveforms because human cochleae are fully developed at birth. Aging, on the other hand, affects the waveforms due to increased high-frequency threshold (presbycusis), resulting in a progressive delay and decrease of the AP amplitude compared to younger subjects (Oku & Hasewega, 1997). Likewise, CM amplitudes decrease with age (Starr et al., 2001).

There are no significant differences between the right and left ear (Grasel et al., 2017; Wilson & Bowker, 2002; Zakaria, Othman & Musa, 2017), suggesting that ear selection does not bias ECochG recordings.

Opinions among authors differ on whether gender influences the ECochG waveforms. Some found small differences (Chatrian et al., 1985; Coats, 1986) and others none (Franco & Chiong, 2002; Zakaria et al., 2017). However, these possible small differences are negligible.

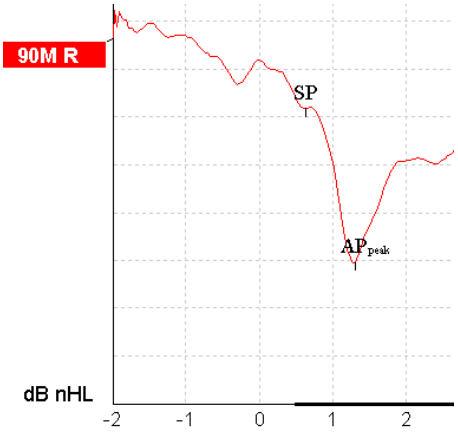

Figure 1.3 A typical example of the SP and AP captured with TM electrodes. Elicited by a click on 90 dBnHL with alternating polarity.

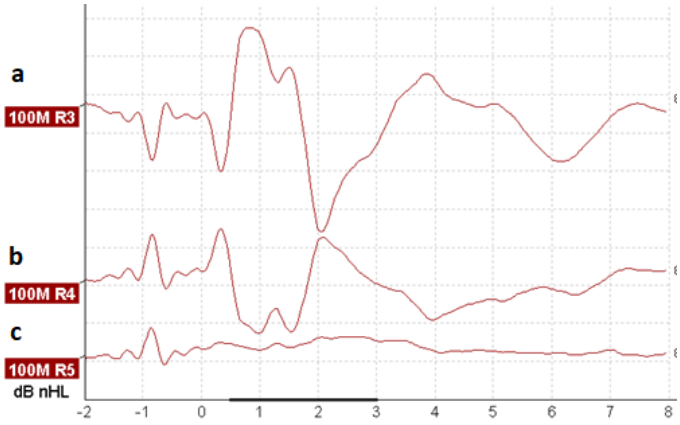

Figure 1.4 A typical example of the CM captured with TM electrodes. Elicited by a click at 100 dBnHL. Condensation (a) and rarefaction (b) are phase reversible and there is no sinusoidal waveform in the clamped condition recorded with rarefaction (c).

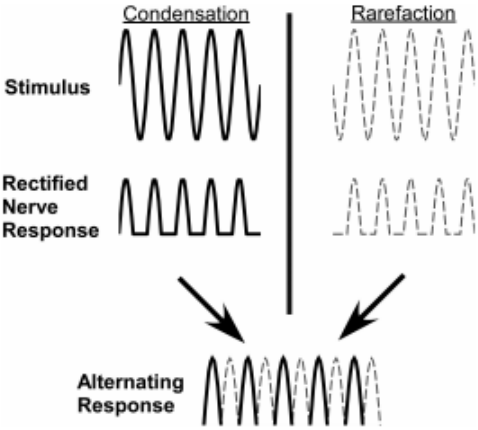

Figure 1.5 Schematic showing the origin of the ANN in the alternating response waveform. The first row (labelled as 'stimulus') shows a tone burst for both polarities (condensation and rarefaction). The second row (labelled as 'rectified nerve responses') shows the ANN of the response to each phase of the sinusoidal tone burst. The third row (labelled as 'alternating response') shows the combination of the ANN response to each phase. An alternating polarity isolates these neural responses. Retrieved from Choudhury, B., Fitzpatrick, D. C., Buchman, C. A., Wei, B. P., Dillon, M. T., He, S., & Adunka, O. F. (2012). Intraoperative round window recordings to acoustic stimuli from cochlear implant patients. Otology and Neurotology, 33(9), 1507-1515. doi:10.1097/MAO.0b013e31826dbc80

1.3 Parameters in ECochG

Little is known about normalized values for ECochG recordings because these are dependent on many parameters that may change the amplitude or latency of the potentials, leading to a varying cut-off score for pathological hearing. These parameters are subdivided in recording parameters (i.e., electrode position, filter settings and hardware) and stimulus parameters (i.e., stimulus type, stimulus repetition rate and polarity). Redondo-Martínez et al. (2016) mentioned each clinical centre should create their own normalized ECochG values based on the specific recording conditions.

1.3.1 Active electrodes

Active ECochG electrodes could be placed intra-cochlear, trans-tympanic (TT) or extra-tympanic (ET). In intra-cochlear recording the CI electrode is used as a recording electrode inside the cochlea during surgery. In TT ECochG the electrode is placed with a needle

through the tympanic membrane on the promontory or round window. In an ET configuration the electrode is placed in the ear canal near the tympanic membrane. Both IT and TT are invasive for the patient and need a physician because the patient needs to be anesthetized and the tympanic membrane must be perforated. In ET it is possible to place a flexible electrode near or against the tympanic membrane in the ear canal without making an incision (Pienkowski et al., 2018). Although the amplitudes in ET ECochG are four times smaller (expressed in µV) than in TT ECochG, as a result of the less proximity to the cochlea (Bonucci & Hyppolito, 2009), ET is still preferred due to the less invasive nature. Another disadvantage of ET ECochG, however, is the lower signal-to-noise ratio (SNR), which is resolvable by more averages (i.e., more stimulus repetitions in one measurement to filter out the noise; Bonucci & Hyppolito, 2009).

Nowadays, ET ECochG recordings are most regularly made with tiptrodes or TM electrodes. Tiptrodes are foam plugs wrapped in gold foil, whereby it functions as a recording site, around insert phones (Ruth et al., 1988). TM electrodes are placed directly near the tympanic membrane (Stypulkowski & Staller, 1987). Bonucci and Hyppolito (2009) compared both electrodes within-subject. No significant differences were found between the two electrode positions, but ECochG with a TM electrode (also called TM ECochG) revealed a greater amplitude and reproducibility by reason of its closer proximity to the cochlea. A slight discomfort (i.e., a sensation of pressure) and the importance of a good placement can be considered as an obstacle in the use of TM ECochG.

1.3.2 Filters

Filters are tools to reduce the noise leading to an increased SNR. The high-pass filter (HPF) is designed to attenuate the low-frequency signals and the low-pass filter (LPF) is designed to attenuate the high-frequency signals. The lowest and highest frequencies that are expected to be recorded, should fall within this range (Ferraro & Ruth, 1994). Wuyts, Van de Heyning, Van Spaendonck and Molenberghs (1997) concluded that the HPF is often set to 3 or 5 Hz (12 dB/octave) in the determination of SP/AP ratios. They also mentioned an LPF of 3,000 Hz as widely accepted with a large deviation between 1,500 and 30,000 Hz.

The British Society of Audiology (2019) recommends an HPF between 100 and 300 Hz with an LPF between 3,000 and 5,000 Hz for CM recordings. An HPF of 100 Hz is used repeatedly (Heidari, Pourbakht, Kamrava, Kamali and Yousefi, 2018; Shi et al., 2012; Zhang, 2012a), but filters of 5 Hz (Zhang, 2012b), 10 Hz (McMahon et al., 2009) and 30 Hz (Anastasio, Alvarenga & Costa Filho, 2008) have also been used. It is the hardware that finally determines which exact filter cut-offs are available to apply.

The options ‘Bayesian weighting’ and ‘minimize interference’ are rarely used in ECochG recordings because these are typically developed in order to reduce noise in ABR recordings.

1.3.3 Auditory stimulus types

Several types of auditory stimuli have been used to evoke the cochlear potentials such as broadband (BB) clicks, BB chirps, narrowband (NB) chirps and tone bursts. BB clicks are the most traditional stimuli used to evoke all cochlear potentials. BB clicks are characteristically brief with an abrupt onset and a broad frequency spectrum; thus, they are not frequency specific (Chertoff, Lichtenhan & Willis, 2010). Due to the tonotopic character of the BM, lower frequencies will be more temporal delayed than higher frequencies when using a click stimulus (von Békésy, 1960; Kiang, 1965). This leads to a spread of neural activation over time, which in turn causes a smaller AP amplitude. There is no research that examines whether the click is the best stimulus to evoke CMs. However, the click stimulus is still being used for evoking the CM in the majority of studies, because of its abruptness.

BB chirps are created to compensate for the time-lag of low frequencies. The low-frequency sounds are presented first and subsequently followed by high-frequency sounds. This stimulus has the same frequency spectrum as the standard BB click. The aim is synchronous displacement and neural discharges from all frequencies, leading to a larger AP amplitude (Elberling, Don, Cebulla & Stürzebecher, 2007). The difference between BB chirps and clicks is visually depicted in Figure 1.6. The NB chirp, on the other hand, has a smaller frequency spectrum and is more useful for frequency specific evaluation (Bell, Allen & Lutman, 2002). These stimuli are used in ABR and SP/AP recordings. The studies of Elberling et al. (2007) and Bell et al. (2002) can be consulted for a more extensive description of both chirp stimuli. To date, no study has used BB chirps to elicit CMs.

A tone burst is presented on one specific frequency (e.g., 2,000 or 4,000 Hz) and has a longer duration than the ones described above (range: 1.5 – 15 ms). A tone burst is more frequency specific than the previous specified stimuli because of its limited bandwidth. This stimulus is defined by their rise, plateau and fall cycles. Most commonly used, is a rise and fall time of two cycles and a plateau of one cycle (2-1-2; see Figure 1.7). The first CM recordings elicited by tone bursts recorded from the ear canal were ranging from 500 to 1,000 Hz (Elberling & Salomon, 1973; Yoshie & Yamaura, 1969). Nowadays, tone bursts are still used to assess the function of specific frequencies on the BM (e.g., McMahon et al., 2009; Zhang, 2012a).

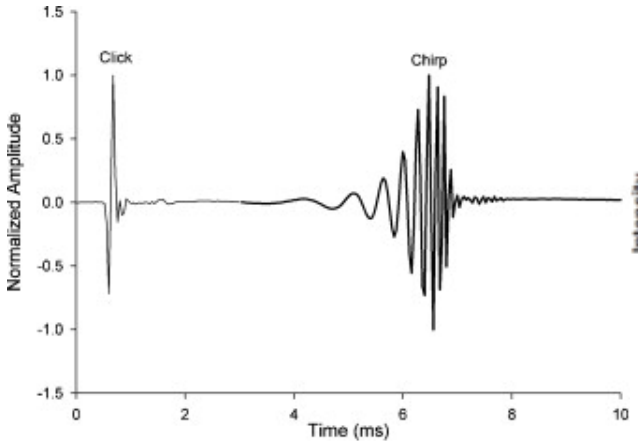

Figure 1.6 The acoustic waveforms of the standard click and the BB chirp stimuli. Retrieved from Chertoff, M., Lichtenhan, J., & Willis, M. (2010). Click-and BB chirp-evoked human compound action potentials. The Journal of the Acoustical Society of America, 127(5), 2992-299

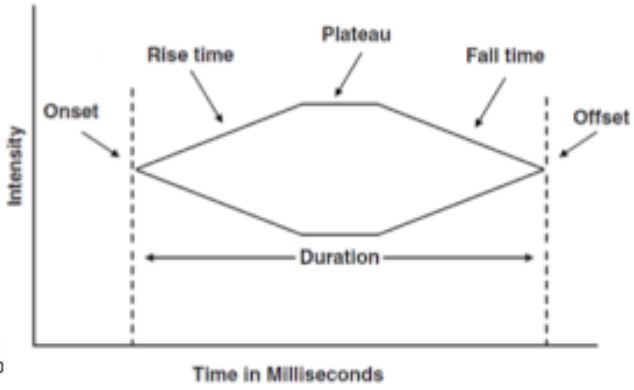

Figure 1.7 A schematic example of a tone burst. Retrieved from van Bommel, E. (2014). Narrow band and level specific CE-BB chirps in Auditory Brainstem Responses and the relationship between objective and subjective hearing thresholds. Unpublished Master thesis Radboud University Medical Centre Nijmegen,

1.3.4 Stimulus repetition rate

The amount of repetitions or sweeps needed for an appropriate averaged AEP is proportional to the SNR and the amplitude of the potential of interest. When the SNR improves and the amplitude increases, the amount of repetitions decreases. Ferraro and Ruth (1994) mentioned that the repetition rate should ideally be between 5 and 11 repetitions per second to record the SP and AP properly. A rate of 30 repetitions per second will probably cause a transformation of the AP. Two studies reported that a high stimulus repetition rate enhances the SP amplitude, leading to an increased SP/AP amplitude ratio (Lake & Stuart, 2019; Wilson & Bowker, 2002) and SP/AP area ratio (Lake & Stuart, 2019). This greater stability of the SP suggests that the pre-neural nature causes less sensitivity for fatigue (Jiang, 1996) and changes in the amount of activated afferent fibres (Moore, 1997). Added to the findings of

high repetition rate studies, Wilson and Bowker (2002) reported a latency delay in both potentials and Luke and Stuart (2019) found a loss of all potentials in some patients.

The CM remains unaffected under a high stimulus repetition rate. According to the British Society of Audiology (2019), this is explained by the resistance to neural fatigue (Kiang & Peake, 1960), because it is a pre-neural occurrence. They recommended 87.1 repetitions per second for CM recordings to preserve valuable time in clinical practice and scientific research. In summary, researchers agree that a low stimulus repetition rate is required for a proper recording of SPs and APs, whereas CMs allow a high repetition rate.

1.3.5 Polarity

The term polarity refers to the initial direction of pressure of the stimulus waveform as measured at the front of the transducer. Three polarity types in AEP recordings have been described by Hall (2015). Condensation starts with the movement to the positive direction (i.e., the movement of the transducer diaphragm towards the tympanic membrane), rarefactions starts with the movement to the negative direction (i.e., the movement of the transducer diaphragm away from the tympanic membrane) and alternating polarity is the switching between the two directions. The first two polarities are visually depicted in Figure 1.8. The upward movement of the BM in rarefaction causes excitation of sensory hair cells and may lead to earlier peak responses than in condensation. However, the cochlear mechanisms are too complex for such a simple statement (Hall, 2015).

The polarities could be summed or subtracted to amplify a specific ECochG potential. The SP and AP require a summation of both condensation and rarefaction (i.e., alternating polarity), while the CM appears when the polarities are subtracted. Thus, CM requires measurements in both polarities, condensation and rarefaction, separately.

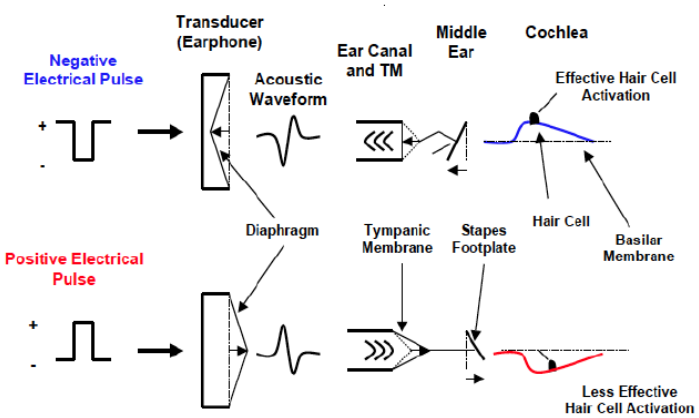

Figure 1.8 A schematic representation of both polarities: rarefaction (top in blue) and condensation (bottom in red). Retrieved from Hall, J.W. (2015). eHandbook of auditory evoked responses: principles, procedures & protocols. Pretoria: Pearson.

1.4 Clinical applications

As mentioned earlier, ECochG recordings reveal the electrical potentials derived from the cochlea and the auditory nerve. These potentials can be useful in the diagnosis, evaluation and the prognostic value of patients with specific deficits.

1.4.1 Auditory neuropathy/dyssynchrony spectrum disorder

A hearing loss that is characterized by normal OHC function and a lack of neural synchrony at a higher level, is indicative for an auditory neuropathy/dyssynchrony spectrum disorder (ANSD) which may occur in all age groups (Cardon & Sharma, 2013). ANSD can be provoked by infectious, metabolic, hereditary, developmental problems and oncological drug side effects (Starr et al., 2004). ANSD can typically be diagnosed by the presence of intact CMs (obtained by ECochG recordings) normal otoacoustic emissions (OAEs), while ABR and stapedial reflexes are abnormal or absent. Thus, hearing thresholds may be relatively normal in comparison with an ABR, where a big number of active neurons are required (Harrison, Gordon, Papsin, Neghandi & James, 2015). Although both OAEs and CMs can assess OHC activity, the lower frequencies (e.g., 500 Hz) are more difficult to obtain with OAEs when compared to CMs. Therefore, CMs may have a higher diagnostic value (Zhang, 2012b). To date, more studies aiming to measure CMs in the diagnosis, used invasive TT ECochG than non-invasive ET ECochG (Soares, Menezes, Carnaúba, de Andrade & Lins, 2016). Anastasio et al. (2008) declared ET ECochG with tiptrodes as more detailed and thus as having a higher clinical applicability for diagnosing ANSD than ABRs. One caveat is that this conclusion was based on one single case study.

Neural dyssynchrony causes particularly problems in the temporal processing, leading to difficulties in speech perception (Cardon & Sharma, 2013). Therefore, an early detection of ANSD is important to reduce or prevent a delay in the speech- and language development of young children. Several studies explored the specific CM waveforms of patients with ANSD. One study determined the differences between 33 patients with ANSD and 4 normal-hearing subjects recorded with scalp electrodes. They found abnormal increased CM amplitudes for children with ANSD less than 10 years old. Three hypothetical mechanisms may explain this increase in amplitude. First, contractions of the middle ear muscles could enhance specific tonal frequencies and cause increased amplitudes, even when the middle ear reflexes are typically missing. Second, the efferent OCB function could be altered in this disorder. If the OCB is overactive in this patient group, it would lead to hyperpolarization of the OHC accompanied by an increase of receptor potentials. Finally, some subjects may have a metabolic hair cell comorbidity causing increased amplitudes and altered functioning of the OCB (Starr et al., 2001). However, due to the small control group, it is unclear whether this was an abnormal process or whether this result was influenced by a sampling bias. Santarelli et al. (2008) found normal and elevated CMs recorded with TT ECochG in eight subjects (5 – 48 years old) with ANSD in comparison to 16 normal-hearing controls. An enhancement of the CM, according to them, is specific for several patients with ANSD. Also, some experimental models of ANSD found elevated CM amplitudes in patients with ANSD (e.g., El-Badry, Ding, McFadden & Eddins, 2007). Overall, consensus have been reached about elevated CMs in patients with ANSD.

1.4.2 Cochlear implants

The function of damaged auditory neurons or hair cells, being caused by either congenital or neurosensory hearing loss, can be adopted by a biomedical device, called a CI. Although there exist few differences between manufacturers, the basic components are equal. The external part consists of a microphone, a speech processor and a radio transmitter. These elements are responsible for the transmission of sounds to the internal part through the skin. The conversion into a series of bipolar square-wave signals is enabled by the array electrodes located in the scala tympani so as to stimulate the (remaining) fibres of the auditory nerve. The aim is to restore some degree of auditory perception (Peterson, Pisoni & Miyamoto, 2010).

Options for CI use are unilateral CI fitting, bilateral CI fitting and bimodal fitting (i.e., unilateral CI fitting and contralateral HA use). The second option seems to improve speech perception more than the other (Blamey et al., 2015), suggesting that CI research is essential. Not all CI recipients experience equal benefit from an CI, possibly due to cochlear trauma. Intra-cochlear recording of the CM through the CI electrode is an upcoming research area, given that it is a promising tool in detecting trauma to cochlear tissue. The CM appeared to be more sensitive to detect damage than the AP (Choudhury et al., 2011).

1.4.3 Meniere's disease

Meniere's disease (MD) is an inner ear chronic disorder that is clinically characterized by episodic attacks of vertigo (dizziness), nausea, tinnitus, fluctuating deafness and aural fullness (i.e., ear pressure). A pathophysiologic feature of the disease is endolymphatic hydrops (ELH; Goebel, 2015). They explained ELH as an increased pressure in the scala media, filled with endolymph, which causes a break in the membrane that separates this fluid from the perilymph. The resulting change in chemical proportions (i.e., potassium and sodium concentrations) leads to the disturbance in question.

ECochG is an important tool for the diagnosis and evolution of ELH, with an existing preference for the ET electrode positioning (Lamounier, Gobbo, De Souza, De Oliveira, & Bahmad, 2014). Increased SPs were found in the ET ECochG study of Kumar and Peepal (2012), which involved patients with MD. This enlarged SP amplitude causes a deviant summating potential-to-action potential (SP/AP) amplitude ratio for MD patients in comparison to normal hearing. No consensus cut-off score is reached for the SP/AP amplitude and area ratios. In an TM ECochG study, Ferraro and Tibbils (1999) labelled a SP/AP area ratio greater than 1.37 and a SP/AP amplitude ratio greater than 0.41 as abnormal. Based on a small group of MD patients, 0.53 is reported as the upper limit of normal for the SP/AP amplitude ratio and 1.94 for the SP/AP area ratio (Devaiah, Dawson, Ferraro & Ator, 2003). Pappas Jr., Pappas Sr., Carmicheael, Hyatt and Toohey (2000) preferred a SP/AP amplitude ratio cut-off of 0.50 rather than 0.40 because of a decreased chance of false positives. It should be stated these two lasts were based on ET ECochG recordings with tiptrodes.

1.5 The present study

The first aim of the present study is to determine the optimal parameter settings of the non-invasive TM ECochG for the CMs with the current setup in the Radboud University Medical Centre Nijmegen. The parameters to be determined are the filter setting and stimulus type. The second aim is to gather normalized data for the SP, AP and CM for normal-hearing subjects.

For an adequate diagnosis, a sizable set of normalized values is required. Due to the big variety of possible parameters in ET ECochG recordings, it is difficult to mutually compare the previous ET ECochG studies. The AP latency (i.e., wave I in ABR) has been normalized for normal-hearing subjects using the current hardware, albeit with an ABR setup (van Bommel, 2014). Other studies aiming to normalize the AP latency (and amplitude) employed other recording and stimulus parameters (Lake & Stuart, 2019; Redondo-Martínez et al., 2016; Wilson & Bowker, 2002; Zakaria et al., 2017). To date, only one study has used both the exact same TM electrodes and hardware to gather normative data in 100 normal-hearing subjects (Grasel et al., 2017), as in the current study which does indicate the current setup must be feasible to perform. Distinct from the current study, Grasel and colleagues' (2017) main goal was to gather normalized data for the SP and AP, for the diagnosis of MD. Accordingly, they did not employ the CM. In other studies where the CM was captured non-invasively, other electrodes than the TM electrode were used (e.g., tiptrodes by Zhang, 2012a; 2012b) and other aims were pursued (e.g., capturing characteristics of ANSD in infants by Shi

et al., 2012). In contrast to the previous studies, this study aims to contribute to the field of CM research, in particular recorded with TM electrodes, by creating a protocol and gathering reference data for normal-hearing subjects.
The following research questions belong to these aims:

1. What is the prevalence of the SP, AP and CM recorded by non-invasive TM ECochG in normal-hearing subjects?
2. What are the normalized amplitudes and latencies for the SP, AP and CM?
3. What are the normalized response durations for the CM?
4. Which parameter settings (stimulus type, filter settings) give the largest CM amplitudes recorded by non-invasive TM ECochG and the setup used in the current clinical setting?
5. What is the relationship between parameter settings (stimulus type, filter setting) and CM latency and CM duration?

The CM is the main dependent variable of the present study. Therefore, the research questions about CM amplitudes are most intriguing to answer. The subsequent important variable is the CM latency, which is often reported briefly in ANSD research (e.g., Shi et al., 2012; Starr et al., 2001). The least is known for the CM duration. Even though this variable may not seem important, the present study involved it to broaden the reference data for future research.

Considering the TM ECochG study of Grasel and colleagues (2017) with the exact same electrodes and hardware, it has been hypothesized that CM recordings are feasible when the TM electrode is properly placed near the TM and the impedance is low according to the recommendations.

Concerning the stimulus types, BB chirps are exclusively designed for ABRs and not used in CM research for this particular reason. Therefore, the following hypothesis about the BB chirps are based on literature and common sense. The higher frequencies are delayed in BB chirps what leads to a simultaneous arrival at the BM. Since the CM rests on the spatial summation of OHC currents, it is expected for the BB chirps to evoke larger amplitudes than both clicks and frequency-specific tone bursts (Cheatham et al., 2011).

Heidari et al. (2018) compared CM amplitudes of clicks with tone bursts (2,000, 4,000, 8,000 and 16,000 Hz) captured by ET ECochG with ET electrodes (not further specified) in 25 healthy rats. Larger amplitudes were found for clicks when compared to all tone burst frequencies, which is probably explained by the greater amount of involved OHC on the BM. Accordingly, clicks are expected to give larger CM amplitudes than these frequency-specific tone bursts.

As last, the tone burst is expected to evoke the smallest CM amplitudes due to its narrow activation of OHCs. In addition to this argument, the 2,000 Hz tone burst in the current study, has relatively low frequencies leading to a greater travel distance from the apex to the TM electrode than the other stimuli. A greater distance to the active electrode, creates a smaller amplitude (Bonucci & Hyppolito, 2009).

Because of the variety of HPFs (3 – 300 Hz) that are used in CM recordings, it is unclear which filter fits in the optimal parameter setting. Little is known about the relationship between stimuli and CM latency or CM duration. If there are any differences between males and females, these are expected to be negligible.

2 Methods

2.1 Participants

This prospective pilot study engaged 28 normal-hearing subjects from May 2019 to July 2019. In total, four subjects were excluded from analysis. Two of those subjects were excluded due to practice errors in the pilot phase. The third was not feasible for ECochG recordings because of a troubled ear. The fourth was excluded post hoc due to deviant outcomes and age. Twenty-seven out of twenty-eight subjects met the inclusion criteria (normal otoscopy and a pure tone thresholds ≤ 20 dBnHL from 250 to 8,000 Hz for both ears) and exclusion criteria (i.e., not being familiar with hearing and otoneurologic problems). A hearing loss of 25 dBnHL was established for two frequencies of one subject's left (non-test) ear, but since only the right ear was tested, this was considered as irrelevant. After all, ECochG values of 24 subjects (10 males and 14 females) aged between 20 and 32 years (M = 24.6, SD = 2.6) were analysed. Due to some unusual findings, it was decided to retest two subjects. All subjects read the information brochure and had the opportunity to ask questions about the study. They all signed the informed consent before participation. Participation was completely voluntarily. The total measurement lasted up to 70 minutes.

2.2 Materials

A clinical otoscope was used by a specialised physician to inspect and cleanse the subject's ear. The pure tone audiometer Interacoustics AD629 diagnostic audiometer was used to test hearing acuity. The air conduction thresholds were obtained using the Interacoustics TDH-39 supra-aural headphones.

All ECochG recordings were performed with the Otoaccess software version 1.2.1 running on the InterAcoustics Eclipse II ® with standard clinical EP25 software (Assens, Denmark). Electrodes were inserted in the EPA4 cable collector. Foam E-A-RLINK 3A tips rolled into the E-A-RTONE^{TM} 3M insert phones were used to reduce electrical stimulus artefacts on the ECochG signal, to stabilize the TM electrode (Sanibel, Denmark) and to decrease the SNR (Ferraro, 2010). A shortcut (green and red TM cable) and a jumper were used to create one single recording channel.

2.3 ECochG parameter settings

In general

A one channel recording system was created by short cutting the TM electrode with the contralateral (here: left) reference. Only the right ear was stimulated. TM ECochG responses were recorded at least twice in each polarity to confirm good reproducibility. Subsequently, these were averaged resulting in one waveform for each polarity. The impedances of the surface electrodes were ≤ 5 kΩ and the inter-electrode impedance was ≤ 10 kΩ. A time window length of 10 ms was used. Considering the travel time through the 26.6 cm silicon tube, the stimuli travelled with a speed of -0.9 ms and resulting in a sound arrival at the tympanic membrane at 0 ms. The responses were amplified 100,000 times. No additional filters were used.

Different protocols were created to obtain SPs, APs and CMs. Those protocols varied with respect to the stimulus and recording parameters. Considering the main goal of the current study, the protocols are focused on determining the optimal parameter settings for the CM. The order of data acquisition was: SP/AP, CM click, CM tone burst and finally CM BB chirp.

SP/AP protocol

In the SP/AP protocol, stimuli were offered with alternating polarity at 90 dBnHL with a slow stimulus repetition rate of 11.3 per second. The filter setting was set on 3.3 – 3,000 Hz. To ensure the quality of the response, the stimuli were presented with 1,000 sweeps in each condition. Stimulus parameters are depicted in Table 2.1 and recordings parameters in Table 2.3.

Table 2.1 Stimulus parameters for the SP/AP protocol.

SP/AP protocol	
Stimulus parameters	**Protocol 1**
Stimulus type	Click
Stimulus repetition (rate/s)	11.3
Polarity	Alternating
Duration (ms)	1
Envelope (ms)	-
Intensity (dBnHL)	90

Table 2.2 Stimulus parameters for the CM protocols (click, tone burst 2,000 Hz and BB chirp).

CM protocols			
Stimulus parameters	**Protocol 1**	**Protocol 2**	**Protocol 3**
Stimulus type	Click	Tone burst 2,000 Hz	BB chirp
Stimulus repetition (rate/s)	87.1	87.1	87.1
Polarity	Rare & cond	Rare & cond	Rare & cond
Duration (ms)	0.1	1.5	5
Envelope (ms)	-	0.5 rise/fall time, 0.5 plateau	-
Intensity (dBnHL)	100	80	80

Note: BB = broadband, rare = rarefaction, cond = condensation.

CM protocols

For CM recordings, each stimulus type (i.e., click, tone burst and BB chirp) had its own protocol. The stimuli were presented at a stimulus intensity of 100 dBnHL (click) and 80 dBnHL (tone burst and BB chirp) respectively. The measurements were repeated with a fast stimulus repetition rate (87.1/s) with a narrow- or broadband filter settings (i.e., 3.3 – 3,000 Hz or 100 – 3,000 Hz). For each condition, ECochG responses to condensation and rarefaction stimuli were obtained. According to protocol of the British Society of Audiology (2019), CMs were confirmed by an additional recording with a clamped tube. The stimuli were presented with 1,500 sweeps in each condition. Stimulus parameters are depicted in Table 2.2 and recordings parameters in Table 2.3.

Table 2.3 Recording parameters for the SP/AP and CM protocols.

Recording parameter	**CM protocols (1 – 3)**	**SP/AP protocol**
Stimulation	Monoaural	Monoaural
Headset	Insert phone	Insert phone
Type electrode	TM electrode	TM electrode
Electrode positioning	Vertical montage	Vertical montage
Averaging (total)	2x1,500 (3,000)	2x1,000 (2,000)
HPF – LPF (Hz)	3.3 – 3,000 or 100 – 3,000	3.3 – 3,000
Extra filters	Off	Off
Amplification (times)	100,000x	100,000x

2.4 Procedure

Prior to the experimental phase of the study, a small pilot (N = 2) was carried out. It enabled the executive investigator to practise without affecting the results and to establish the optimal order of protocols.

Before the measurement, the right ear canal was properly cleaned by an experienced specialist, followed by conventional tonal audiometry, which was obtained by the executive investigator in order to confirm the normal air-conduction thresholds of both ears. Furthermore, the recommended procedure for pure-tone air-conduction threshold audiometry without masking was used (British Society of Audiology, 2018). When normal hearing was confirmed, the skin of the high-forehead and middle-forehead was prepared with chlorhexidine 0.5% in ethanol 70%, followed by Nuprep, a mild abrasive gel. Its aim was to obtain low electrical skin impedance. The surface electrodes were subsequently covered with Ten20 conductive electrode paste and established on the skin as shown in Figure 2.1: vertex Fz and ground Fpz; 'vertical montage'. Due to a dipole shift, this montage enhances the AP and CM (Interacoustics, 2019). The electrodes were connected to the EPA4 as followed: right (-) connected with TM electrode; vertex (+) green cable connected with Fz; ground connected with Fpz; left (+) connected with right through by a cable jumper (see Figure 2.2). This electrode montage was set up for all subjects in the experimental phase, except for the first three. Their configuration (N = 3) is known as the 'horizontal montage', where the reference electrodes were attached to both mastoids (M1/2). This difference in electrode montage was attributed to the fact that the correct electrode montage was not found yet.

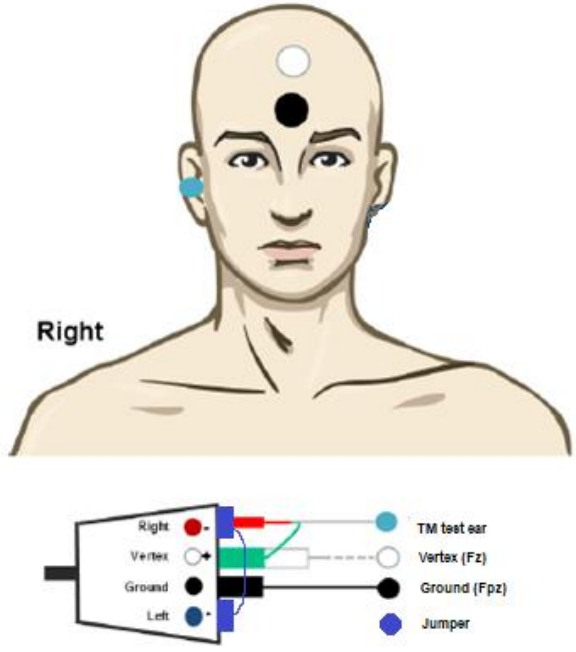

Figure 2.1 The electrode positioning using the TM electrode and EPA4. Retrieved and adjusted from Interacoustics (2019).

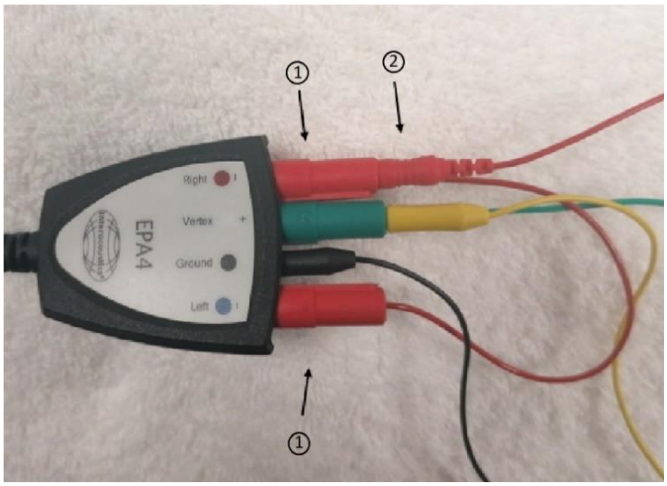

Figure 2.2 The EPA4 with inserted electrodes. From the top to the bottom: Right, jumper (1) connected with TM electrode (2); Vertex (+) green cable connected with Fz; Ground connected with Fpz; Left connected with right by the jumper (1).

The patient was instructed to lie down comfortably on a bed on their left side, and this was followed by a puff of 10% xylocaine spray in the right ear canal to avoid any discomfort. At the same time, the TM electrode was placed in a bath of saline and Lectron II conductivity gel (1:1 ratio) at room temperature. After a ten-minute break, the ear canal was dried with a cotton bud. The TM electrode was subsequently placed in the right ear canal on the superior quarter of the tympanic membrane such that there was resistance or the subject appointed sensation (see Figure 2.3). Lastly, the insert phone connected to a fitting earplug was tucked

in while the electrode was still being held to prevent misplacement. The earplug was cut at top, so it did not obstruct the soundwave of the stimuli. The subjects were instructed to lie down still and to listen passively. The importance of little movement was emphasized. When ECochG recordings were completed, a final ear canal inspection and cleansing were carried out. The whole experiment was executed in a soundproof and light-dimmed research lab.

The retest's procedure was exact the same as in the first test round, except for the ear preparation. After the effect of the anesthetic, the ear was dried out more attentively than in the first round. Finally, the ear was examined with an otoscope to verify this issue.

Figure 2.3 The top view of the ear with the subject lying horizontally. The TM electrode (TM) with a relatively superior (s) position compared to the insert phone (IP) which is relatively anterior.

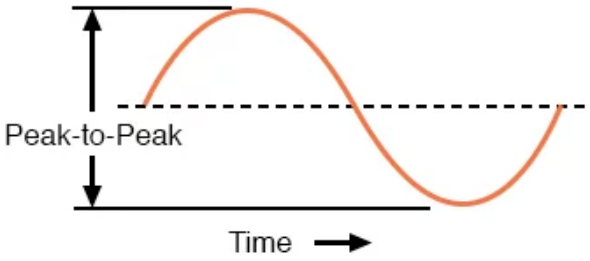

Figure 2.4 An example of the peak-to-peak CM amplitude calculations. Retrieved from allaboutcircuits.com/textbook/alternating-current/chpt-1/measurements-ac-magnitude/

2.5 Analysis

First, the overall prevalence of the SP, AP and CM waveforms were analysed and categorized as follows: the neural response is present 'yes' or 'no'. In the presence of an evident SP or AP peak within the first 2.5 ms post stimulus onset, they were noted as 'yes' with the corresponding peak-to-peak amplitudes and latencies. 'No' was assigned if the waveform was flattened. A 'yes' was ascribed to CMs when both polarities were phase reversible with an absence sinusoidal waveform in the clamped condition (British Society of Audiology, 2019). 'No' was ascribed when both polarities were not reversible or when the signal was not flattened in the clamped condition. If there was a definable neural response for only one HPF setting (i.e., for 3.3 Hz or 100 Hz), the stimulus type of this specific subject was still labelled as 'yes'.

The current study had three independent variables stimulus type (click, tone burst and BB chirp), polarity (condensation and rarefaction), HPF (3.3 Hz and 100 Hz) and three dependent variables (CM amplitude, latency and response duration). These variables were combined into all possible conditions, resulting in six ECochG waveforms for each CM protocol. The absolute wave latency and amplitude of the CM were identified separately from the raw data for each stimulus type and polarity. CM amplitudes were measured peak-to-peak, biggest peak minus the smallest peak of the same sinus depicted in Figure 2.4. CM latencies were defined as the time window from the onset of the stimulus to the point where the maximal peak amplitude was reached. CM duration was calculated from the onset till the end of the largest CM sinus.

Statistical analysis was performed with IBM SPSS Statistics, version 21. Firstly, the prevalence of the potentials (SPs, APs and CMs) were determined for each protocol. Secondly, means and standard deviations of SPs and APs were obtained and descriptively compared to the previous reference values. No statistical tests were used for this comparison. Likewise, means and standard deviations were obtained for each CM protocol. The dependent variables (CM amplitude, latency and duration) were independently explored by tables and

bars to evaluate normality and outliers for each stimulus type. Thirdly, the ANOVA assumptions were controlled. Independent variables were stimulus type, polarity, HPF and sex. Differences between males and females were analysed using a long data format in a multifactorial ANOVA design. A repeated measures ANOVA design with repeated and simple contrasts was used to compare the dependent variables for each stimulus type (click, tone burst and BB chirp) and polarity (condensation and rarefaction). Notably, polarities were not pooled. The effects of the HPFs (3.3 Hz and 100 Hz) were compared for each stimulus type while taking polarity into account. Paired sample t-tests were used to compare condensation with rarefaction for each stimulus type. Effect sizes were given for the ANOVA (partial eta squared) and t-test (Cohen's d).

3 Results

3.1 Prevalence of evoked potentials

TM ECochG recordings of responses to acoustic stimuli were obtained in 24 normal-hearing adults. The prevalence of neural responses are presented in Table 3.1. SPs were present in 33% of the subjects and APs in 79%. The recorded ECochG signals were most often characterized as CMs in response to a click stimulus (100%), followed by the tone burst presented at 2,000 Hz (79%) and the BB chirp (63%). A few examples of ECochG responses to each stimulus are given in Appendix I.

Table 3.1 Prevalence of definable SPs, APs and CMs in ECochG recordings among normal-hearing subjects (N = 24).

Response	SP	AP	CM click	CM burst	CM BB chirp
Yes	8/24 (33%)	19/24 (79%)	24 / 24 (100%)	19/24 (79%)	15/24 (63%)
No	16/24	6/24	0/24	5/24	9/24

Note: N = number of observations.

3.2 SPs and APs

Descriptive statistics (i.e., standard deviation, range, minimum and maximum) are given in Table 3.2 for both the SP and AP amplitudes (μV) and latencies (ms). SP latencies ranged from 0.37 to 1.90 ms after stimulus onset (M = 1.12, SD = 0.43), and AP latencies ranged from 0.97 to 2.43 ms after stimulus onset (M = 1.92, SD = 0.37). SP amplitudes ranged from 0.04 to 0.32 μV (M = 0.14, SD = 0.09) and AP amplitudes from 0.11 to 1.87 μV (M = 0.70, SD = 0.47).

Table 3.2 Descriptive statistics for amplitudes and latencies of SPs and APs.

	N	Mean	Standard Deviation	Range	Minimum	Maximum
SP amplitude (μV)	8	0.14	0.09	0.28	0.04	0.32
SP latency (ms)	8	1.12	0.43	1.53	0.37	1.90
AP amplitude (μV)	19	0.70	0.47	1.76	0.11	1.87
AP latency (ms)	19	1.92	0.37	1.46	0.97	2.43

Note: N = number of observations.

3.1 Analysis of CMs

A 3 x 2 x 2 repeated measures ANOVA design was executed with independent factors stimulus type (click, tone burst and BB chirp), polarity (condensation and rarefaction), HPF (3.3 Hz and 100 Hz) and three dependent variables (CM amplitude, latency and response duration). The normality of the sample mean distributions was violated (Shapiro-Wilk $p <$ .05) for all dependent variables (amplitude, latency and response duration) indicated by stimulus type, except for the BB chirp latency (Shapiro-Wilk $p = .241$). Violation of normality would indicate a non-parametric test at which the normality of sample mean distribution does not apply. However, the dataset of the current study is not dissectible to a simple dataset required for separate non-parametric tests. For this reason, the a priori plan was retained. A view of the CM amplitude's abnormality is shown in Appendix II. Data values from the three subjects with different electrode positioning were pooled with data from the others because these values did not differ considerably from each other. Mauchly's test indicated that the assumption of sphericity had been violated for the majority of the sample mean distributions. Therefore, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon < .75$). It cannot be stated that all observations were

independent, because the same subjects were used, which may lead to correlation of some condition results.

3.1.1 CM amplitude

Sexes

A multifactorial ANOVA design was used to detect the between-subject differences by sex. Means and standard deviations of males and females are given for each dependent variable in Appendix III. No significant differences between males and females were found for the click stimulus, although differences were found for the tone burst and BB chirp stimuli. The following conditions were significantly different between males and females:

- CM amplitude:
    - Condensation, HPF 100 Hz, tone burst
    - Rarefaction, HPF 3.3 Hz, tone burst
    - Rarefaction, HPF 100 Hz, tone burst
- CM latency:
    - Condensation, HPF 100 Hz, BB chirp
- CM response duration:
    - Condensation, HPF 3.3 Hz, BB chirp
    - Condensation, HPF 100 Hz, tone burst
    - Rarefaction, HPF 3.3 Hz, BB chirp

Stimulus type

The following appeared from a 3 x 2 x 2 repeated measures ANOVA design. A significant main effect for stimulus ($F(1.01, 9.12) = 28.71$, $p < .001$, $\eta^2_p = .76$) and a significant interaction effect for stimulus*polarity ($F(1.04, 9.36) = 25.87$, $p < .001$, $\eta^2_p = .74$), both corrected with Greenhouse-Geisser, were found. Planned contrasts revealed that the click stimulus evoked a significantly larger CM amplitude (M = 1.36, SD = 0.94) than the tone burst stimulus ($F(1, 9) = 28.49$, $p < .001$, $\eta^2_p = .76$) and the BB chirp stimulus ($F(1, 9) = 24.41$, $p < .001$, $\eta^2_p = .73$). CM amplitudes evoked by the tone burst stimulus (M = 0.13, SD = 0.05) and the BB chirp stimulus (M = 0.18, SD = 0.10) did not differ significantly ($F(1, 9) = .001$, $p = .98$, $\eta^2_p = .00$). These differences are shown in Figure 3.1. Table 3.3 presents the means and standard deviations of those stimulus types. Complete descriptive statistics are given in the last column of Appendix III.

Polarity

The following results were from a paired-sample t-test design. To continue as before, the violation of normality was neglected. Condensation (M = 1.60, SD = 1.05) and rarefaction (M = 1.12, SD = 0.75) deviated significantly when the CM amplitude was evoked by a click stimulus ($t(45) = 6.78$, $p < .001$, $d = 0.998$). The polarities did not deviate significantly for CM amplitudes evoked by a tone burst stimulus ($t(35) = -.69$, $p = .496$, $d = -0.015$) or a BB chirp ($t(27) = -1.41$, $p = .172$, $d = -0.265$).

Table 3.3 Means and standard deviations of CM amplitudes (in µV) evoked by a click, a 2,000 Hz tone burst and a BB chirps.

Stimulus type	Polarity	CM amplitude Mean (SD)
Click	**Cond**	1.60 (1.05)
	Rare	1.12 (0.75)
	Total	1.36 (0.94)
Tone burst	**Cond**	0.13 (0.45)
	Rare	0.13 (0.05)
	Total	0.13 (0.05)
BB chirp	**Cond**	0.17 (0.10)
	Rare	0.19 (1.0)
	Total	0.18 (0.10)

Note: Cond = condensation, Rare = rarefaction.

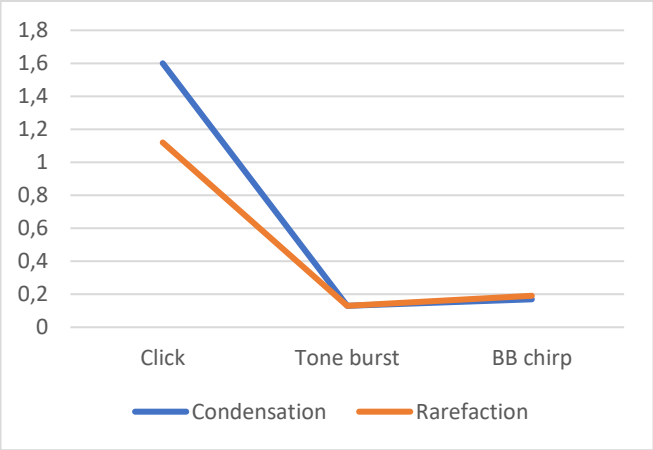

Figure 3.1 Mean CM amplitudes (Y axis) for each stimulus type (X axis) and each polarity (blue = condensation, orange = rarefaction).

HPF setting

The corrected main effect of the HPF ($F(1, 9) = 7.87$, $p < .05$, $\eta^2_p = .47$) and the interaction effect of HPF*stimulus ($F(1.01, 9.11) = 5.15$, $p = 0.49$, $\eta^2_p = .36$) were significant for the CM amplitude, while the corrected interaction effect of HPF*polarity*stimulus ($F(1.01, 9.17) = 2.79$, $p = 0.128$, $\eta^2_p = .24$) was not significant for the CM amplitude. Unmerged totals (C/R) are given in Appendix IV.

3.1.2 CM latency

The largest CM amplitude occurred within 1.24 ms (SD = 0.62) after click onset, within 1.28 ms (SD = 0.51) after tone burst onset and within 0.72 ms (SD = 0.53) after BB chirp onset. All stimulus types had considerable variation among subjects. Unmerged totals (C/R) are given in Appendix III. The main effect of stimulus ($F(2, 18) = 6.88$, $p < .05$, $\eta^2_p = .43$) was significant for CM latency, while the main effects of polarity ($F(1, 9) = 0.66$, $p = .437$, $\eta^2_p = .07$) and HPF ($F(1, 9) = 0.59$ $p = .462$, $\eta^2_p = .07$) were not significant. The interaction effect stimulus*polarity was not significant ($F(2, 18) = 0.39$, $p = .68$, $\eta^2_p = .04$) for CM latency. Thus, no relationship between CM latency and stimulus type was found when considering either the polarity only or the polarity and HPF together.

3.1.3 CM response duration

The duration of the largest CM amplitude was 1.18 ms (SD = 0.59) for the click, 0.55 ms (SD = 0.13) for the tone burst and 0.62 ms (SD = 0.19) for the BB chirp. Duration of the largest CM amplitudes evoked by a click had the greatest variation among subjects. Unmerged totals (C/R) are again given in Appendix III. For the CM response duration, the corrected main effect of stimulus was significant ($F(1.53, 13.78) = 13.82$, $p < .01$, $\eta^2_p = .60$), while the corrected main effect of polarity ($F(1, 9) = 4.23$, $p = .07$, $\eta^2_p = .32$) and HPF ($F(1, 9) = 0.09$, $p = .768$, $\eta^2_p = .77$) were not significant. The corrected interaction effect stimulus*polarity was also not significant ($F(1.12, 10.11) = 4.13$, $p = .066$, $\eta^2_p = .31$) for the CM response duration.

4 Discussion

4.1 Interpretation of the results

To the best of our knowledge, no studies have reported results of recording the CMs with TM electrodes and the current hardware in normal-hearing subjects. Only one group of researchers has used the exact same electrode and hardware setup, but they recorded SPs and APs (Grasel et al., 2017). Therefore, the optimal parameter settings for the specific TM ECochG used in the current study were not yet known. This pilot study identified the best recording and stimulus conditions for CMs captured with TM electrodes in normal-hearing adults aged between 20 and 32 years. It also gathered reference data for the three main ECochG potentials.

4.1.1 Prevalence of evoked potentials

APs (79%) were recorded more often than SPs (33%). The low prevalence of the SP could be explained by the 'vertical montage', which was intended to enhance APs and CMs. Grasel et al. (2017) found the AP in 100% and the SP in 65% of their normal-hearing subjects with a 'horizontal montage' (non-inverting electrode on non-test mastoid). Thus, that contradicts the previous statement that a 'vertical montage' is better than a 'horizontal montage' for enhancement of the AP. An explanation for the relatively low prevalence of APs in the current study could be an inadequate position of the TM electrode – for example, it may have been in contact with the external ear canal instead of the tympanic membrane.

The highest prevalence of CMs was evoked by a click (100%), followed by a tone burst at 2,000 Hz (79%) and then by a BB chirp (63%). According to these outcomes, it could be stated that CMs are recordable with TM electrodes accompanied by a 'vertical montage' and the current hardware (Eclipse). Differences in prevalence are possibly due to the features of the stimulus which do or do not foster the OHC transducer currents.

4.1.2 Comparing SP and AP amplitudes and latencies

The current mean and standard deviation of SP (M = 0.14, SD = 0.09) and AP (M = 0.70, SD = 0.47) amplitudes were approximately equal to those seen in previous studies (see Appendix II). The mean SP amplitudes of those studies were between 0.08 and 0.28 μV, with standard deviations between 0.08 and 0.17 μV. The mean AP amplitudes were between 0.45 and 0.87 μV, with standard deviations between 0.18 and 0.37 μV (Lake & Stuart, 2019; Redondo-Martínez et al., 2016; Wilson & Bowker, 2002; Zakaria et al., 2017). The AP amplitudes of the current study had a larger standard deviation, thus greater variation among subjects compared to previous studies.

The mean and standard deviation of the SP (M = 1.12, SD = 0.43) and AP (M = 1.92, SD = 0.37) latencies, on the other hand, were delayed and had much more variation among subjects than those found in previous studies (see Appendix V). The mean SP latencies in those studies were between 0.82 and 0.88 ms, with standard deviations between 0.12 and 0.13 ms (Redondo-Martínez et al., 2016; Wilson & Bowker, 2002). Their mean AP latencies were between 1.32 and 1.71 ms, with standard deviations between 0.08 and 0.20 ms (van Bommel, 2014; Grasel et al., 2017; Lake & Stuart, 2019; Redondo-Martínez et al., 2016; Wilson & Bowker, 2002; Zakaria et al., 2017). Lake and Stuart's (2019) AP peak had the greatest delay but was recorded with another brand of TM electrode and other stimulus repetition rates (7.7/s versus 11.3/s in the current study). Even in comparison to those results, the current AP peak is delayed. Van Bommel (2014) used the current Eclipse hardware to record wave I with an ABR setup. The electrodes were placed further from the cochlear nerve fibres where the signal was generated. One would expect this setup to lead to a delay of wave I in comparison to a setup with TM electrodes in closer proximity to the cochlea. The opposite was observed:

van Bommel (2014) had earlier AP peaks. Even the most comparable study (Grasel et al., 2017), with identical parameter and stimulus settings (TM electrode brand, Eclipse hardware, 11.3/s), differed in AP latency (right M = 1.45, SD = 0.16; left M = 1.47, SD = 0.20) from the current study.

A possible explanation for this study's unusual result may be an inaccuracy in the preparation of the subjects (e.g., too much fluids remaining in the ear canal). Other explanations might be technical ones, such as calibration or tube length. The first issue was explored post hoc by conducting a second ECochG recording round involving two of the subjects. The first subject's ear was directly dried and controlled by a specialist with an otoscope after anaesthetizing, before the second measurement was performed. The second subject first underwent the measurement again as previously performed, after which the ear was dried, and another measurement was performed. In total, the first subject had two measurements and the second subject had three. The retests had shorter SP ($\Delta = 0.44$ ms; $\Delta = 0.06$ ms) and AP ($\Delta = 0.53$ ms; $\Delta = 0.37$ ms) latencies than the original tests. This finding suggests that the ear canals were not sufficiently dry during the original round of tests. The remaining fluids caused a conduction hearing loss, with an overall delay induced by the additional barrier the soundwaves had to cross. The stimulus types with a lower CM prevalence may have been more strongly affected by this barrier. Tables of absolute SP and AP values of both subjects are presented in Appendix VI. CM values are only presented for the first retested subject, because the second had too much noise due to the subject's agitation. In addition, Appendix VI shows the actual SP and AP waveforms for both subjects.

4.1.3 Relation between parameter settings and CM amplitude

The implication that there might have been an overall potential delay caused by fluid, is not an issue for the comparison of stimulus types. All evoked potentials were measured under the same conditions and compared within subjects.

Clicks evoked larger CM amplitudes than tone bursts and BB chirps. CM amplitudes were the same for both tone bursts and BB chirps. The a priori expectation was that the BB chirp would evoke the greatest CM amplitude, followed by the click and then the tone burst. In contrary to these expectations, however, the click evoked the greatest CM amplitude, equally followed by both the BB chirp and tone burst. A contributing characteristic of the click was its abruptness. The fast activation of the BM enhances the CM amplitude, because this potential is a summation of the spatially activated OHC currents (Cheatham et al., 2011). A second characteristic of the click is the relatively high-frequency spectrum. High frequencies are processed in the base of the cochlea, which is relatively close to the recording TM electrode. Proximity of the active electrode causes a larger amplitude (Bonucci & Hyppolito, 2009). Another factor that clearly had an impact on the amplitude was the intensity. Clicks were presented at a level 20 dBnHL louder than the other stimuli.

According to the BB chirp, one would expect that the synchronized stimulation of the whole BM would activate a large number of OHCs, causing a larger amplitude than the click and tone burst. This hypothesis was contradicted in the present study. Instead, the BB chirp evoked smaller amplitudes than the click, and approximately equal CM amplitude as the tone burst. The principal of simultaneous masking could explain this finding. Simultaneous masking occurs when, for example, multiple BB chirps are presented to the human ear such that some sounds simply dissolve in the presence of other sounds with specific characteristics. Low-frequency tones are more likely to mask high-frequency tones because of the remaining swell pattern on the BM (Rietveld & Van Heuven, 2009). Since the BB chirp has a broad frequency spectrum and more low-frequency tones than the click, the impact is presumably larger than for the other stimuli. To date, no literature has proved this suggestion. However, the evoked amplitudes of the tone burst and the BB chirp did not differ, yet the BB chirp

generated a lower response rate than the tone burst. This might be caused by an order effect, because the BB chirp was always presented last. However, fatigue is the only plausible order effect, and this possibility can be dismissed due to the pre-neural nature of the CM.

The last stimulus was the tone burst (2,000 Hz), which is not abrupt, because of its rise and fall time. Additionally, the relatively low-frequency tones of the tone burst are processed in an area of the cochlea less proximate to the TM electrode, what undoubtedly played its part in the results (Bonucci & Hyppolito, 2009).

Differences between males and females emerged only for the tone bursts and BB chirps. There were, however, fewer observations of these than of clicks. Moreover, the male and female groups differed in size. Given these facts, the observed differences seem not very reliable and thus received no further attention.

One atypical finding was a significantly larger amplitude for condensation than for rarefaction evoked by clicks. This was not common in previous research, and no explanation was found for this result. Finally, no differences were found for the HPF settings.

4.1.4 Relation between parameter settings and CM latency and duration

The current study showed no significant difference in CM latency or CM duration when the parameter settings were changed. No latency shift (i.e., the potential's peak appearing earlier in rarefaction than in condensation) was found in the present study.

Although there was no relationship between the parameters and the CM latency or duration, this does not mean these variables should be neglected in CM research involving patients. Based on previous literature about CMs in ANSD and CI, it is evident that CM amplitudes are most indicative for diagnosis and/or prognosis. Starr and colleagues (2001) compared CM peak latencies in ANSD patients to those in control subjects. No significant differences were reported. One figure showed a difference in CM duration between one child with ANSD and its matched control; the CMs of the child with ANSD continued in comparison to those observed in the control.

4.2 Limitations

This study is limited by the subjectivity involved in determining CM variables such as amplitudes. This is a limitation which applies to all ECochG studies examining CM variables. Although ECochG is an objective tool, it still has its subjective hindrances. This is mainly the result of a lack of standardization due to divergent parameter settings in ECochG measurements.

This study is also limited by the fact that its results are based on a small group of subjects (N = 24). Furthermore, not every subject had the same number of observations per stimulus type, resulting in missing values in the data set. This contributed to even smaller groups of data, which may affect the significance of the statistical tests.

Finally, the retest would be more useful for further interpretation if more recordings were established under the same conditions. In this case, only two subjects were measured for a second time.

4.3 Future clinical applications

Now that the optimal parameter settings for CM recordings in the current clinical setting have been established, these settings can be applied in further scientific research into issues where CM recording is potentially a valuable tool. As discussed, patients with ANSD and CI recipients could benefit from progress in CM research.

4.3.1 Auditory neuropathy/dyssynchrony spectrum disorder

As discussed, the CM recording is one of the requisite tools for the diagnosis of ANSD. In practice, not all ANSD patients with normal OHC functioning, experience the same benefit of HAs. Sound amplification appears to offer louder and more distorted signals only (Berlin, 1999). A diversity in physiological mechanism underlying ANSD may be the cause of this deviation among patients. Investigation of these mechanisms could guide a more effective clinical decision making for interventions (e.g., HA and CI fitting). McMahon and colleagues in their studies (2008; 2009) found two physiological mechanisms underlying ANSD. A presynaptic underlying deficit, showing a delayed SP waveform (result of changes in IHC activity) and normal ABR, and a postsynaptic underlying deficit, showing a normal SP, no AP and abnormal ABR (result of disruptions in nerve fiber initiation or brainstem dysfunction). There may be a correlation between ANSD subtype and HA or CI outcome. Based on these results, one might expect that children with normal cochlear nerves are more likely to benefit from CI implantation than those with abnormal cochlear nerves. Walton, Gibson, Sanli and Prelog (2008) confirmed this hypothesis in their study of 54 recipient children up to the age of 15. However, to date, it is still not possible to make definitive statements about the prognosis of hearing with a CI for ANSD patients, because of the heterogenicity. Overall, ECochG recordings seem adequate for the diagnosis of ANSD subtypes but the results do not correlate with the severity of ANSD (Cone, 2008). In conclusion, SPs, APs and CMs are a valuable tool in ANSD research, preferably measured via non-invasive ECochG

4.3.2 Cochlear implantation

Criteria for cochlear implantation are expanded, that younger patients and patients with a growing amount of residual hearing are implanted by reason of advanced surgical and technological developments (Kuang, Haversat & Michaelides, 2015). Due to these adjustment of terms for cochlear implantation, there are more patients with residual hearing, both children and adults, who could be implanted with a CI.

Because of the high risk of intra-cochlear trauma during array insertion, more studies are dedicated to searching for a reliable instrument for monitoring cochlear function. Campbell, Kaicer, Briggs and O'Leary (2014) demonstrated the feasibility of intra-cochlear measurement of all ECochG potentials using CI electrodes in implanted patients postoperatively. Intra-cochlear ECochG was even sensitive in detecting changes during the actual implantation in a study consisting of 31 CI recipients, but it had little prognostic value related to hearing preservation (Adunka et al., 2015). In contrast to Adunka and colleagues (2015), Campbell et al. (2016) indicated that the intra-cochlear monitoring of CMs may help to predict early post-operative hearing loss. It was hypothesized that physical contact with or elevation of the BM is likely to cause hearing loss. CMs can also give real-time feedback during surgery. How the surgeon should interpret and react to such real-time feedback is not yet clear and needs further investigation. In a more recent study, of Giardina and colleagues (2019), the CM recordings were also declared as feasible during cochlear implantation. They stated that changes in the amplitudes only were not accountable for the amount of hearing preservation. Other factors, such as neural contribution, latency and phases could help clarifying the changes, and possibly improve the sensitivity and specificity.

4.4 Future research and recommendations

The present study is a first step towards the use of TM ECochG recordings in both clinical practice and scientific research at the Radboud University Medical Centre Nijmegen. The

findings of this study have a number of important implications for the future use of TM ECochG.

The first recommendations relate to subject preparation. Three subjects were advised by the specialist to drip oil into their ear and come back in a week because of persistent cerumen. Aside from saving time, the dripping makes the cleaning less uncomfortable for the subject. A reasonable approach to tackle this issue is to instruct the subject a priori to use oil. Another hindrance was that one subject appeared not feasible for ECochG because of a troubled ear (i.e., complaints about an obstructed ear). A cold should be an exclusion criterion for further research in normal-hearing subjects. Another essential practical implication is the need to dry out the ear completely after anesthetizing it. This action is required to avoid any chance of self-created conduction hearing losses resulting in a latency delay in ECochG potentials.

In view of the parameter results, a click stimulus can still be recommended for the elicitation of CMs. Although the click gave the largest CM response amplitudes, the tone burst and BB chirp can still be used in further CM research. The prevalence of CMs may be higher and the quality of the waveforms may be better if the ears are dried out. Despite the fact that the HPF settings did not change CMs, use of it at 100 Hz is recommended, as any 50-Hz noises (i.e., electric grumble) will be attenuated using this filter setting. The CM should still be established by recording in both polarities separately with the clamping method as confirmation. CMs seemed to stay constant after 1,000 sweeps, which may allow less response repetitions in future research. Finally, the high stimulus repetition rate (87.3/s) can be maintained in future research to save time.

Now that the optimal parameter settings for recording CMs with TM electrodes are known, future research can include both normal-hearing subjects and subjects with hearing loss (e.g., patients with ANSD or CI recipients). In CM research, amplitudes should be the main variable, while SP and AP research should be focussed on both amplitudes and latencies. The CM duration of the highest peak or of all CMs together could be used for the detection of probable differences between patient groups and controls. First, additional efforts are needed to ensure that the results of the present study's retests were due to fluids that had remained in the ear. For further research involving patients, a controlled pre- and post-test design, coupled with measures of speech perception in quiet and noise, is recommended to guide diagnosis and prognosis (Cone, 2008).

5 Conclusions

The purpose of this study was to determine the best recording and stimulus conditions for capturing CMs with TM electrodes in normal-hearing adults. It also gathered reference data for the three main ECochG potentials.

The study found that APs were evoked in 79% of subjects and SPs in 33%. ECochG results were most often characterized as a CM when evoked by a click stimulus (100% of stimuli), followed by a tone burst at 2,000 Hz (79%) and a BB chirp (63%).

The findings for SP and AP amplitudes (μV) were approximately equal to those of previous studies. The current findings for SP (M = 1.12 ms, SD = 0.43 ms) and AP (M = 1.92 ms, SD = 0.37 ms) latencies, on the other hand, were longer and had much more variation among subjects than what has been seen in previous studies. Two ECochG retests showed an earlier SP and AP when the ear was completely dried out. Hence, a possible explanation for the latency delay is the additional fluid barrier for the soundwaves to cross before reaching the tympanic membrane. With the use of TM electrodes, it is important to dry out the ear completely after anesthetizing the tympanic membrane.

Clicks evoked the largest CM amplitudes (M = 1.60 μV, SD = 1.05 μV) in comparison to those evoked by tone bursts (M = 0.13 μV, SD = 0.05 μV) and BB chirps (M = 0.18 μV, SD = 0.10 μV). Tone bursts and BB chirps did not differ from each other in terms of CM amplitude. The HPF setting made no difference in CM amplitude. There also appeared to be no relationship between parameter settings and CM latency or CM duration.

The results of this study indicate that the TM electrode can be used in ECochG research. Overall, this study strengthens the idea that click stimuli at 80 dBnHL are adequate to evoke large CM amplitudes. An HPF of 100 Hz is recommended, and stimulus repetitions of 1,000-1,500 times should be continued in further CM research.

Future TM ECochG research should involve both normal-hearing and hearing-impaired subjects (i.e., ANSD or CI recipients) to gather normalized data for these groups. This will guide diagnosis and prognosis in clinical and scientific settings.

References

Adunka, O. F., Giardina, C. K., Formeister, E. J., Choudhury, B., Buchman, C. A., & Fitzpatrick, D. C. (2015). Round window electrocochleography before and after cochlear implant electrode insertion. *The Laryngoscope*, *126*(5), 1193-1200. doi:10.1002/lary.25602

Anastasio, A. R. T., Alvarenga, K. D. F., & Costa Filho, O. A. (2008). Extratympanic electrocochleography in the diagnosis of auditory neuropathy/auditory dyssynchrony. *Revista Brasileira de Otorrinolaringologia*, *74*(1), 132-136. http://dx.doi.org/10.1016/j.ijporl.2014.11.025 0165-5876

Arns, M., Gunkelman, J., Olbrich, S., Sander, C., & Hegerl, U. (2010). EEG Phenotypes of common neuropsychiatric syndromes: implications for intervention. In R. Cohen (Reds.), *Neurofeedback and Neuromodulation Techniques and Applications* (1st edition) (pp. 79-435). Cambridge, MA: Academics Press.

Von Békésy, G. (1960). *Experiments in Hearing*. New York, NY: McGraw Hill.

Bell, S. L., Allen, R., & Lutman, M. E. (2002). An investigation of the use of band-limited chirp stimuli to obtain the auditory brainstem response. *International Journal of Audiology*, *41*(5), 271–278. doi:10.3109/14992020209077186

Berlin, C. (1999). Auditory neuropathy: using OAEs and ABRs from screening to management. *Seminars in Hearing*, *20*(04), 307–314. doi:10.1055/s-0028-1082946

Blamey, P. J., Maat, B., Baskent, D., Mawman, D., Burke, E., Dillier, N., ... & Huber, A. M. (2015). A retrospective multicenter study comparing speech perception outcomes for bilateral implantation and bimodal rehabilitation. *Ear and Hearing*, *36*(4), 408-416. doi:10.1097/AUD.0000000000000150

van Bommel, E. (2014). *Narrow band and level specific CE-BB chirps in auditory brainstem responses and the relationship between objective and subjective hearing thresholds.* (Unpublished Master's thesis, Radboud University Medical Centre Nijmegen).

Bonucci, A. S., & Hyppolito, M. A. (2009). Comparison of the use of tympanic and extratympanic electrodes for electrocochleography. *The Laryngoscope*, *119*(3), 563-566. https://doi.org/10.1002/lary.20105

British Society of Audiology, (2018). *Recommended procedure: pure-tone air conduction and bone-conduction threshold audiometry with and without masking* [guideline]. Retrieved from http://www.thebsa.org.uk/wp-content/uploads/2014/04/BSA_RP_PTA_FINAL_24Sept11_MinorAmend06Feb12.pdf

British Society of Audiology, (2019). *Recommended procedure: cochlear microphonic testing* [guideline]. Retrieved from https://www.thebsa.org.uk/wp-content/uploads/2019/01/FINAL-JAN2019-Recommended-Procedure-for-Cochlear-Microphonic-Testing-GL21-01-19.pdf

Buckingham, R. A., & Valvassori, G. E. (2001). Inner ear fluid volumes and the resolving power of magnetic resonance imaging: can it differentiate endolymphatic structures? *The Annals of Otology, Rhinology and Laryngology*, *110*(2), 113-117. https://doi.org/10.1177/000348940111000204

Campbell, L., Kaicer, A., Briggs, R., & O'Leary, S. (2014). Cochlear response telemetry: intracochlear electrocochleography via cochlear implant neural response telemetry pilot study results. *Otology and Neurotology*, *36*(3), 399-405. doi:10.1097/MAO.0000000000000678

Campbell, L., Kaicer, A., Sly, D., Iseli, C., Wei, B., Briggs, R., & O'Leary, S. (2016). Intraoperative real-time cochlear response telemetry predicts hearing preservation in cochlear implantation. *Otology and Neurotology*, *37*(4), 332-338. doi:10.1097/MAO.0000000000000972

Cardon, G., & Sharma, A. (2013). Central auditory maturation and behavioural outcome in children with auditory neuropathy spectrum disorder who use cochlear implants. *International Journal of Audiology, 52*(9), 577-586. doi:10.3109/14992027.2013.799786

Chatrian, G. E., Wirch, A. L., Edwards, K. H., Turella, G. S., Kaufman, M. A., & Snyder, J. M. (1985). Cochlear summating potential to broadband clicks detected from the human external auditory meatus. A study of subjects with normal hearing for age. *Ear and Hearing, 6*(3), 130-138. doi:10.1097/00003446-198505000-00002

Cheatham, M. A., Naik, K., & Dallos, P. (2011). Using the cochlear microphonic as a tool to evaluate cochlear function in mouse models of hearing. *Journal of the Association for Research in Otolaryngology, 12*(1), 113-125. https://doi.org/10.1007/s10162-010-0240-5

Chertoff, M., Lichtenhan, J., & Willis, M. (2010). Click-and BB chirp-evoked human compound action potentials. *The Journal of the Acoustical Society of America, 127*(5), 2992-2996. https://doi.org/10.1121/1.3372756

Choudhury, B., Adunka, O. F., DeMason, C. E., Ahmad, F. I., Buchman, C. A., & Fitzpatrick, D. C. (2011). Detection of intracochlear damage with cochlear implantation in a gerbil model of hearing loss. *Otology and Neurotology, 32*(8), 1370. doi:10.1097/MAO.0b013e31822f09f2

Choudhury, B., Fitzpatrick, D. C., Buchman, C. A., Wei, B. P., Dillon, M. T., He, S., & Adunka, O. F. (2012). Intraoperative round window recordings to acoustic stimuli from cochlear implant patients. *Otology and Neurotology, 33*(9), 1507-1515. doi:10.1097/MAO.0b013e31826dbc80

Coats, A. C. (1986). The normal summating potential recorded from external ear canal. *Archives of Otolaryngology- Head and Neck Surgery, 112(7), 759-768.* doi:10.1001/archotol.1986.03780070071016

Cone, B. (2008). The electrophysiology of auditory neuropathy spectrum disorder. In D. Hayes (Red.), *Guidelines for identification and management of infants and young children with auditory neuropathy spectrum disorder* (pp. 20-27). Italy, Como: Guidelines Development Conference at NHS.

Devaiah, A. K., Dawson, K. L., Ferraro, J. A., & Ator, G.A. (2003). Utility of area curve ratio electrocochleography in early meniere's disease. *Archives of Otolaryngology- Head and Neck Surgery, 129*(5), 547-551. doi:10.1001/archotol.129.5.547

Eggermont, J. J. (1974). Basic principles for electrocochleography. *Acta Oto-Laryngologica, 77*(316), 7-16. https://doi.org/10.1080/16512251.1974.11675742

El-Badry, M. M., Ding, D. L., McFadden, S. L., & Eddins, A. C. (2007). Physiological effects of auditory nerve myelinopathy in chinchillas. *European Journal of Neuroscience, 25*, 1437–1446. https://doi.org/10.1111/j.1460-9568.2007.05401.x

Elberling, C., Don, M., Cebulla, M., & Stürzebecher, E. (2007). Auditory steady-state responses to chirp stimuli based on cochlear traveling wave delay. *Journal of the Acoustical Society of America, 122*(5), 2772-2785. https://doi.org/10.1121/1.2783985

Elberling, C., & Salomon, G. (1973). Cochlear microphonics recorded from the ear canal in man. *Acta Oto-Laryngologica, 75*, 489-495. doi:10.3109/00016487309139780

Emanuel, D. C., & Letowski, T. (2009). *Hearing science*. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams and Wilkins.

Ferraro, J. A. (2010). Electrocochleography: A review of recording approaches, clinical applications, and new findings in adults and children. *Journal of the American Academy of Audiology, 21*(3), 145–152. doi:10.3766/jaaa.21.3.2

Ferraro, J. A., & Ruth, J. A. (1994). Electrocochleography. In: J. T. Jacobson (Ed.). (1994). *Principles and applications in auditory evoked potentials*. Upper Saddle River, NJ: Prentice Hall.

Ferraro, J. A., & Tibbils, R. P. (1999). SP/AP Area Ratio in the Diagnosis of Meniere's Disease. *American Journal of Audiology, 8*(1), 1-8. doi:10.1044/1059-0889(1999/001)

Franco, C. M. C., & Chiong, C. M. (2002). Electrocochleography findings in normal and pathologic ears: a local experience. *Otolaryngology- Head and Neck Surgery, 17*(4), 87-93.

Giardina, C. K., Brown, K. D., Adunka, O. F., Buchman, C. A., Hutson, K. A., Pillsbury, H. C., & Fitzpatrick, D. C. (2019). Intracochlear electrocochleography: response patterns during cochlear implantation and hearing preservation. *Ear and hearing*, *40*(4), 833-848. doi:10.1097/AUD.0000000000000659

Goebel, J. A. (2016). Equilibrium Committee Amendment to the 1995 AAO-HNS guidelines for the definition of Meniere's disease. *Otolaryngology- Head and Neck Surgery*, *154*(3), 403-404. doi: 10.1177/0194599816628524

Grasel, S. S., de Oliveira Beck, R. M., Loureiro, R. S. C., Rossi, A. C., de Almeida, E. R., & Ferraro, J. (2017). Normative data for TM electrocochleography measures. *Journal of Otology*, *12*(2), 68-73. https://doi.org/10.1016/j.joto.2017.04.005

Hall, J., W. (2015). *eHandbook of auditory evoked responses: principles, procedures & protocols*. [EPUB], Pretoria, South-Africa*:* Pearson.

Harrison, R. V., Gordon, K. A., Papsin, B. C., Negandhi, J., & James, A. L. (2015). Auditory neuropathy spectrum disorder (ANSD) and cochlear implantation. *International Journal of Pediatric Otorhinolaryngology*, *79*(12), 1980-1987. https://doi.org/10.1016/j.ijporl.2015.10.006

Heidari, F., Pourbakht, A., Kamrava, S. K., Kamali, M., & Yousefi, A. (2018). Comparison of cochlear microphonics magnitude with broad and narrow band stimuli in healthy adult Wistar rats. *Iranian journal of child neurology*, *12*(2), 58-65.

Jiang, Z. D. (1996). Binaural interaction and the effects of stimulus intensity and stimulus rate in human auditory brain-stem. *Electroencephalography and Clinical Neurophysiology, 100*(6), 505-516. https://doi.org/10.1016/S0168-5597(96)96519-3

Kiang, N. Y. S. (1965). *Discharge patterns of single fibres in the cat's auditory nerve.* Cambridge, MA: The Massachusetts Institute of Technology Press.

Kiang, N. Y. S., & Peake, W. T. (1960). Components of electrical responses recorded from the cochlea. *The Annals of Otology, Rhinology and Laryngology, 69*(2), 448-458.

Kileny, P. (2019). *The audiologist's handbook of intraoperative neurophysiological monitoring.* San Diego, CA: Plural Publishing.

Kuang, H., Haversat, H. H. & Michaelides, E. M. (2015). Impairment of caloric function after cochlear implantation. *Journal of Speech, Language, and Hearing Research*, *58*(4), 1387-1395. https://doi.org/10.1044/2015_JSLHR-H-15-0010

Kumar, P., & Peepal, P. (2012). Electrocochleography in individuals with Meniere's disease. *Indian Journal of Otology*, *18*(2), 72-75.

Lake, A. B., & Stuart, A. (2019). The effect of test, electrode, and rate on electrocochleography measures. *Journal of the American Academy of Audiology*, *30*(1), 41–53. doi:10.3766/jaaa.17081

Lamounier, P., Gobbo, D. A., De Souza, T. S. A., de Oliveira, C. A. C. P., & Bahmad, F. (2014).Electrocochleography for ménière's disease: Is it reliable? *Brazilian Journal of Otorhinolaryngology*, *80*(6), 527-532. https://doi.org/10.1016/j.bjorl.2014.08.010

McMahon, C. M., Patuzzi, R. B., Gibson, W. P., & Sanli, H. (2008). Frequency-specific electrocochleography indicates that presynaptic and postsynaptic mechanisms of auditory neuropathy exist. *Ear and Hearing, 19*, 314-325. doi:10.1097/AUD.0b013e3181662c2a

McMahon, C. M., Patuzzi, R. B., Gibson, W. P., & Sanli, H. (2009). *Identification of different subtypes of auditory neuropathy using electrocochleography*. In K. Kaga (Eds.) Neuropathies of the Auditory and Vestibular Eighth Cranial Nerves. Japan, Tokyo: Springer. https://doi.org/10.1007/978-4-431-09433-3_3

Minaya, C., & Atcherson, S. R. (2015). Simultaneous extratympanic electrocochleography and auditory brainstem responses revisited. *Audiology research, 5*(1), 105-110. doi:10.4081/audiores.2015.105

Moore, B. C. J. (1997). *An introduction to the psychology of hearing (4th edition).* England, London: Academic Press.

Oku, T., & Hasegewa, M. (1997). The influence of aging on auditory brainstem response and electrocochleography in the elderly. *Journal of Oto-Rhino-Laryngology*, *59*(3), 141 -146. https://doi.org/10.1159/000276927

Redondo-Martínez, J., Morant-Ventura, A., Robledo-Aguayo, D., Ayas-Montero, A., Mencheta-Benet, E., & Marco-Algarra, J. (2016). Extra-tympanic electrocochleography in a normal population. A descriptive study. *Acta Otorrinolaringológica Española, 67*(5), 254-260. https://doi.org/10.1016/j.otoeng.2015.10.001

Pappas, D. G. Jr., Pappas, D. G. Sr., Carmichael, L., Hyatt, D. P., & Toohey, L. M. (2000). Extratympanic electrocochleography: diagnostic and predictive value. *The American Journal of Otology, 21*(1), 81-87.

Perlman, M. B., & Case, T. J. (1941). Electrical phenomena of the cochlea in man. *Archives of Otolaryngology- Head and Neck Surgery 34*, 710-718. doi:10.1001/archotol.1941.006600407 66003

Peterson, N. R., Pisoni, D. B., & Miyamoto, R. T. (2010). Cochlear implants and spoken language processing abilities: review and assessment of the literature. *Restorative Neurology and Neuroscience, 28*(2), 237-250. doi:10.3233/RNN-2010-0535

Pienkowski, M., Adunka, O. F., & Lichtenhan, J., T. (2018). Editorial: New advances in electrocochleography for clinical and basic investigation. *Frontiers in Neuroscience, 12*(310), 1-5. doi:10.3389/fnins.2018.00310

Rietveld, T., & Van Heuven, V. J. (2009). *Algemene Fonetiek (3rd edition)*. The Netherlands, Bussum: Coutinho.

Ruth, R. A., Lambert, P. R., & Ferraro, J. A. (1988). Electrocochleography: methods and clinical applications. *The American Journal of Otology, 9*, 1-11.

Shi, W., Ji, F., Lan, L., Liang, S. C., Ding, H. N., Wang, H., ... & Wang, Q. J. (2012). Characteristics of cochlear microphonics in infants and young children with auditory neuropathy. *Acta Oto-Laryngologica, 132*, 188-196. doi:10.3109/00016489.2011.630016

Soares, I. D. A., Menezes, P. D. L., Carnaúba, A. T. L., de Andrade, K. C. L., & Lins, O. G. (2016). Study of cochlear microphonic potentials in auditory neuropathy. *Brazilian Journal of Otorhinolaryngology*, *82*(6), 722-736.

Starr, A., Isaacson, B., Michalewski, H. J., Zeng, F. G., Kong, Y. Y., Beale, P., ... & Lesperance, M. M. (2004). A dominantly inherited progressive deafness affecting distal auditory nerve and hair cells. *Journal of the Association for Research in Otolaryngology*, *5*(4), 411-426. https://doi.org/10.1007/s10162-004-5014-5

Starr, A., Sinniger, Y., Nguyen, T., Michalewski, H. J., Oba, S., & Abdala, C. (2001). Cochlear receptor (microphonic and summating potentials, otoacoustic emissions) and

auditory pathway (auditory brain stem potentials) activity in auditory neuropathy. *Ear and Hearing*, *22*(2), 91-99.
Stypulkowski, P. H., & Staller, S. J. (1987). Clinical evaluation of a new ECoG recording electrode. *Ear and hearing*, *8*(5), 304-310. doi:10.1097/00003446-198710000-00010
Walton, J., Gibson, W. P. R., Sanli, H., & Prelog, K. (2008). Predicting cochlear implant outcomes in children with auditory neuropathy. *Otology and Neurotology*, *29*(3), 302 -309. doi:10.1097/MAO.0b013e318164d0f6
Wilson, W. J., & Bowker, C. A. (2002). The effects of high stimulus rate on the electrocochleogram in normal hearing subjects. *International Journal of Audiology, 41*(8), 509-517. https://doi.org/10.3109/14992020209056071
Wuyts, F. L., Van de Heyning, P. H., Van Spaendonck, M. P., & Molenberghs, G. (1997). A review of electrocochleography: instrumentation settings and meta-analysis of criteria for diagnosis of endolymphatic hydrops. *Acta Oto-Laryngologica, 117*(526), 14-20. https://doi.org/10.3109/00016489709124014
Yoshie, N., Ohashi, T., & Suzuki, T. (1967). Non-surgical recording of auditory nerve action potentials in man. *The Laryngoscope*, *77*(1), 76-85. https://doi.org/10.1288/00005537-196701000-00006
Yoshie, N. & Yamaura, K. (1969). Cochlear microphonic responses to pure tones in man recorded by a non-surgical method. *Acta Oto-Laryngologica*, *67*(252), 37-69. doi:10.3109/00016486909120513
Zakaria, M. N., Othman, N. A. N., & Musa, Z. (2017). Electrocochleographic recording in Asian adults: Preliminary normative data and demographic analyses. *Journal of Otology*, *12*(1), 29-33. https://doi.org/10.1016/j.joto.2017.02.002
Zhang, M. (2012a). High-frequency hearing impairment assessed with cochlear microphonics. *Acta Oto-Laryngologica, 132*(9), 967-973. https://doi.org/10.3109/00016489.2012.679688
Zhang, M. (2012b). Response pattern based on the amplitude of ear canal recorded cochlear microphonic waveforms across acoustic frequencies in normal hearing subjects. *Trends in Hearing*, *16*(2), 117-126. https://doi.org/10.1177/1084713812448547

Appendix I: Examples of ECochG potentials

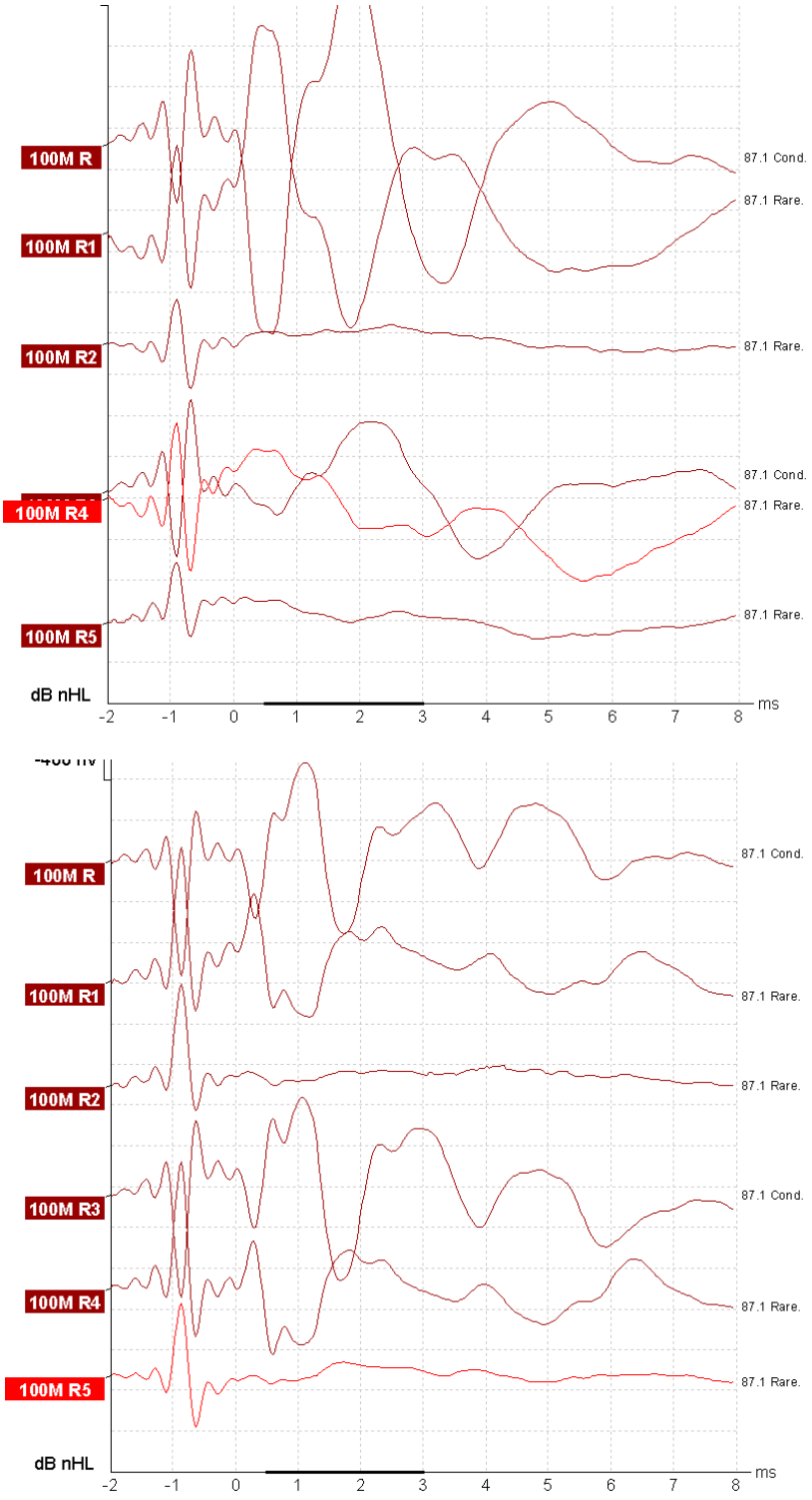

Figure 1a-b. Examples of repeated CM responses in two subjects, obtained with a click stimulus at 100 dBnHL (repetition rate: 87.1/s). Each ECochG acquisition contains six waveforms: the top three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 3.3 Hz and the bottom three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 100 Hz.

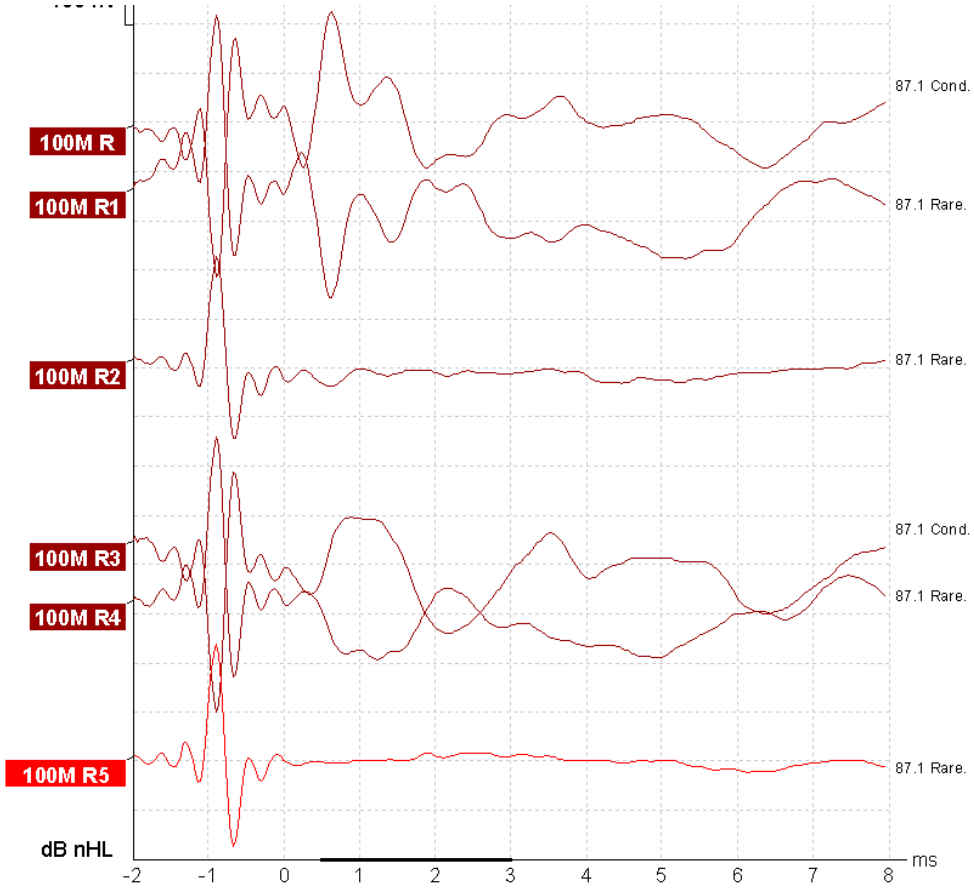

Figure 1c. Examples of repeated CM responses in one subject, obtained with a click stimulus at 100 dBnHL (repetition rate: 87.1/s). Each ECochG acquisition contains six waveforms: the top three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 3.3 Hz and the bottom three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 100 Hz.

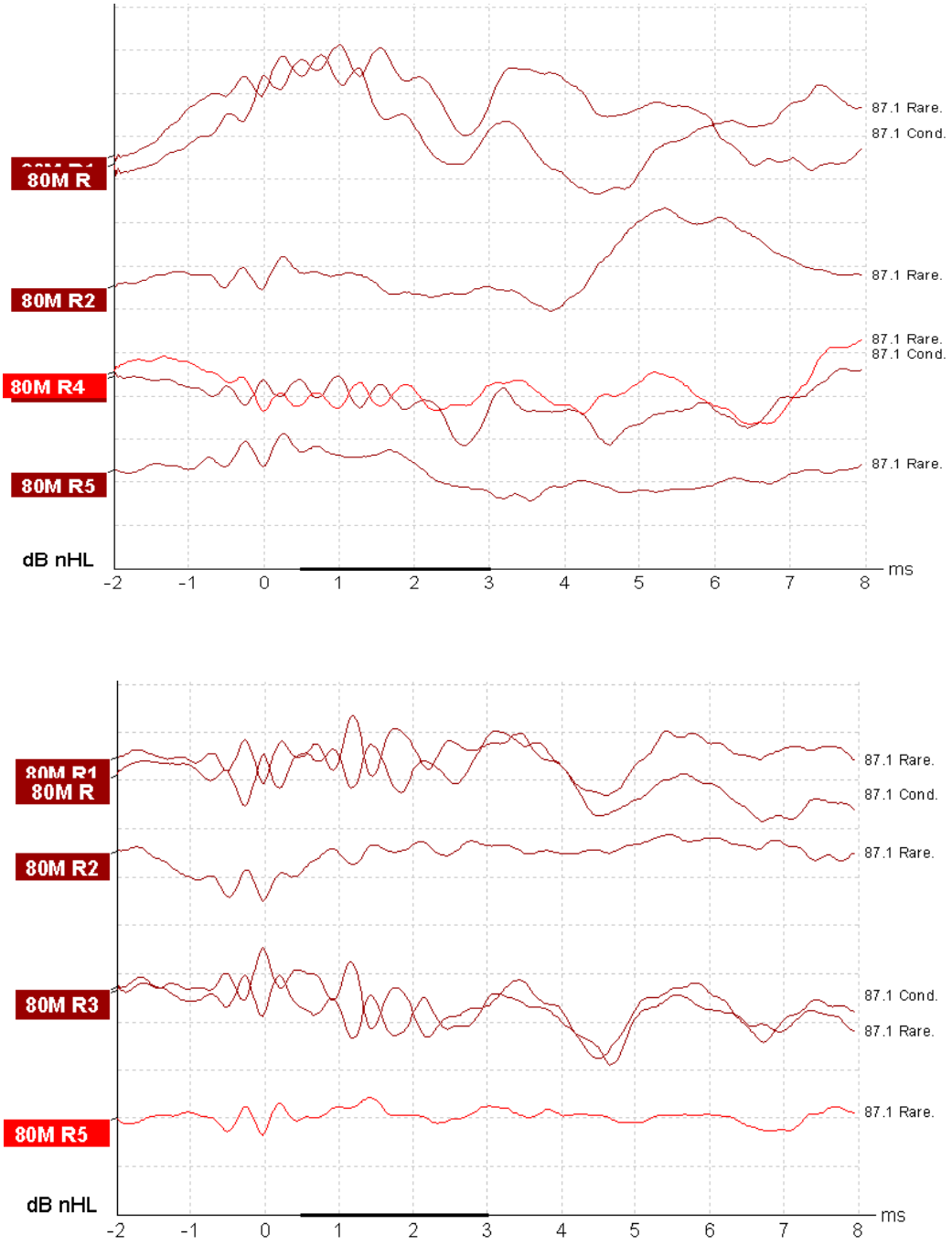

Figure 2a-b. Examples of repeated CM responses in two subjects, obtained with a tone burst stimulus at 80 dBnHL (repetition rate: 87.1/s). Each ECochG acquisition contains six waveforms: the top three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 3.3 Hz and the bottom three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 100 Hz.

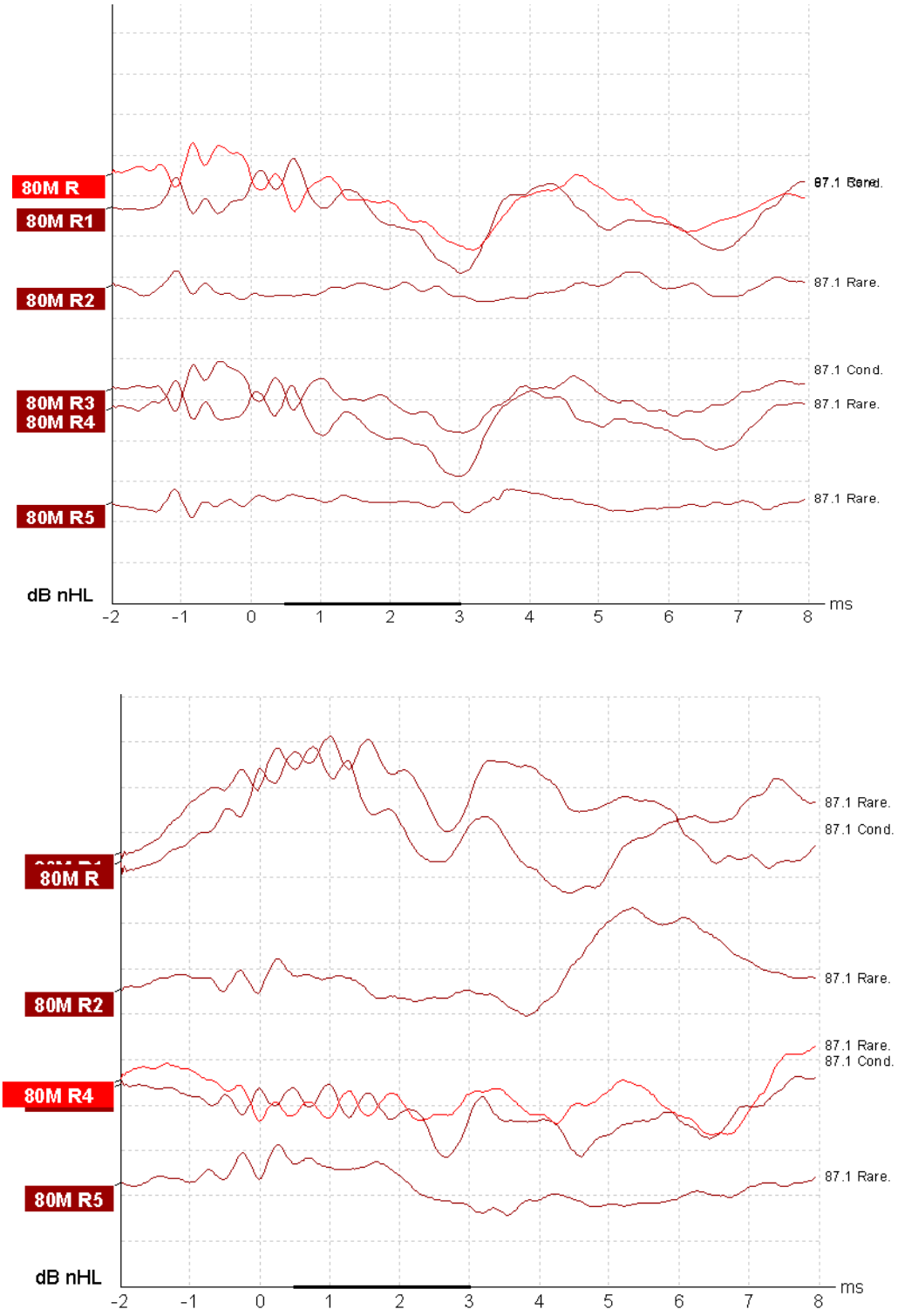

Figure 3 a-b. Examples of repeated CM responses in two subjects, obtained with a BB chirp stimulus at 80 dBnHL (repetition rate: 87.1/s). Each ECochG acquisition contains six waveforms: the top three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an HPF of 3.3 Hz and the bottom three waveforms (condensation, rarefaction and the clamped condition with rarefaction) were recorded with an

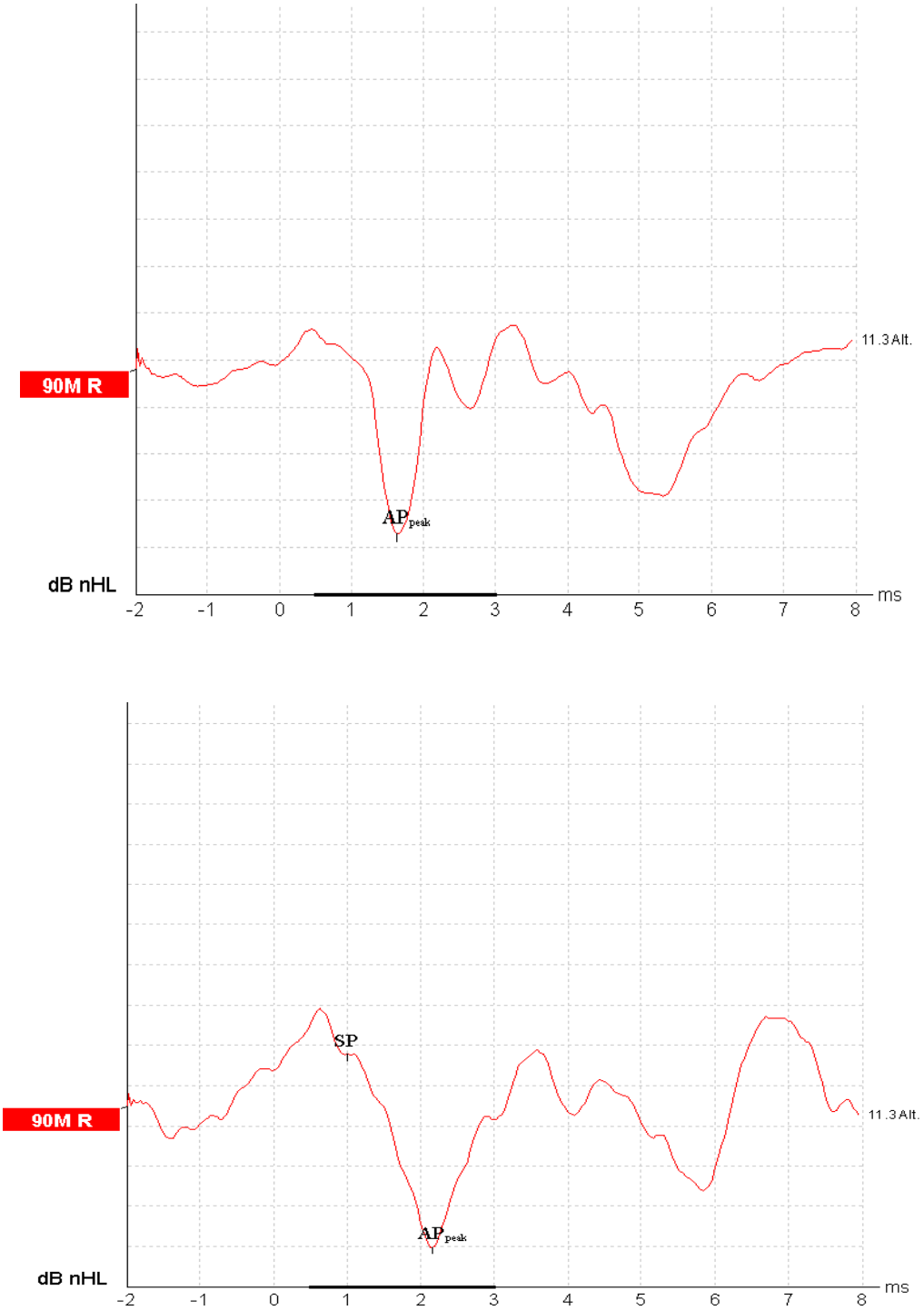

Figure 4 a-b. Example of the SP and AP evoked by a click at 90 dBnHL in two subjects. Polarity: alternating. Repetition rate: 11.3/s. Filter setting: 100 – 3,000 Hz.

Appendix II: Normality histograms for the CM amplitudes and each stimulus type

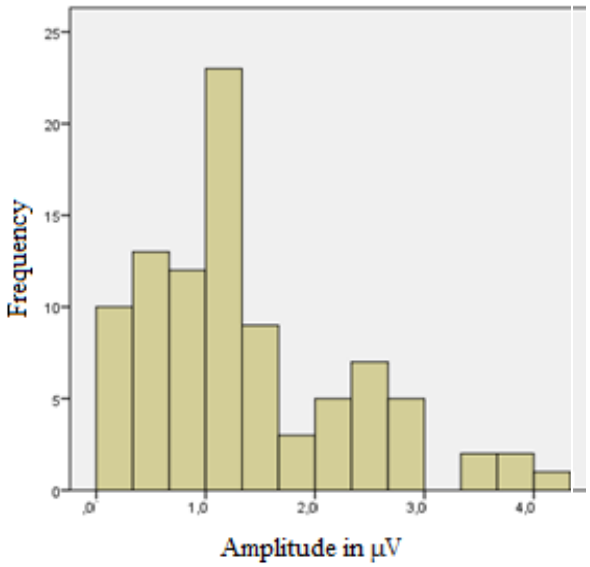

Figure 1. Normality of the click stimulus.

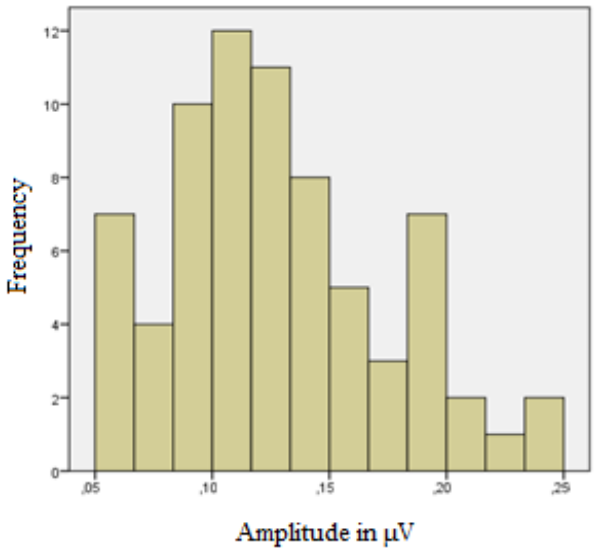

Figure 2. Normality of the tone burst stimulus.

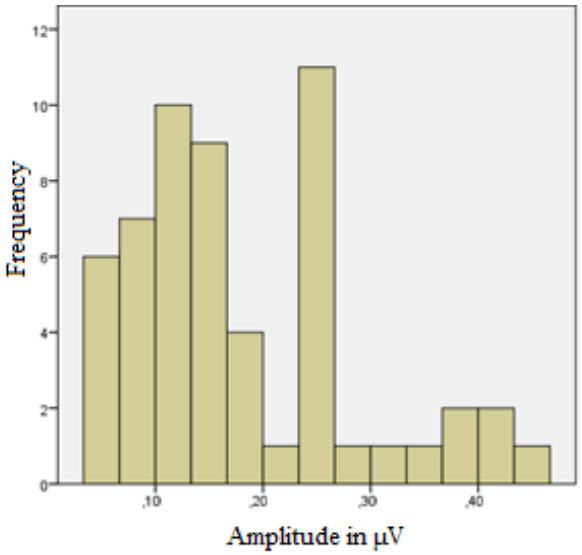

Figure 3. Normality of the BB chirp stimulus.

Appendix III: Means and standard deviations of CM values for males, females and the summation

Dependent variable	Stimulus	Polarity	Female Mean (SD)	Males Mean (SD)	Summed Mean (SD)
Amplitude	**Click**	**Cond**	1.29 (0.86)	2.03 (1.17)	1.60 (1.05)
		Rare	0.93 (0.64)	1.38 (0.84)	1.12 (0.75)
		Total	1.11 (0.77)	1.70 (1.06)	1.36 (0.94)
	Tone burst	**Cond**	0.13 (0.05)	0.12 (0.04)	0.13 (0.45)
		Rare	0.14 (0.57)	0.12 (0.03)	0.13 (0.05)
		Total	0.14 (0.05)	0.12 (0.03)	0.13 (0.05)
	BB chirp	**Cond**	0.19 (0.12)	0.16 (0.89)	0.17 (0.10)
		Rare	0.18 (0.10)	0.20 (0.10)	0.19 (1.0)
		Total	0.18 (0.11)	0.18 (0.09)	0.18 (0.10)
Latency	**Click**	**Cond**	1.28 (0.60)	1.23 (0.64)	1.26 (0.61)
		Rare	1.29 (0.64)	1.12 (0.63)	1.22 (0.64)
		Total	1.28 (0.61)	1.18 (0.63)	1.24 (0.62)
	Tone burst	**Cond**	1.28 (0.52)	1.26 (0.51)	1.28 (0.51)
		Rare	1.38 (0.55)	1.19 (0.56)	1.29 (0.55)
		Total	1.33 (0.53)	1.23 (0.53)	1.28 (0.53)
	BB chirp	**Cond**	0.50 (0.33)	0.93 (0.41)	0.70 (0.42)
		Rare	0.54 (0.33)	0.95 (0.45)	0.73 (0.44)
		Total	0.52 (0.32)	0.94 (0.42)	0.72 (0.42)
Duration	**Click**	**Cond**	1.20 (0.61)	1.32 (0.63)	1.25 (0.62)
		Rare	1.24 (0.65)	0.94 (0.31)	1.12 (0.55)
		Total	1.22 (0.62)	1.13 (0.53)	1.18 (0.59)
	Tone burst	**Cond**	0.59 (0.19)	0.53 (0.09)	0.56 (0.15)
		Rare	0.55 (0.12)	0.53 (0.07)	0.54 (0.10)
		Total	0.57 (0.16)	0.53 (0.08)	0.55 (0.13)
	BB chirp	**Cond**	0.66 (0.23)	0.55 (0.14)	0.61 (0.20)
		Rare	0.65 (0.22)	0.59 (0.15)	0.63 (0.19)
		Total	0.65 (0.22)	0.57 (0.14)	0.62 (0.19)

Note: cond = condensation, rare = rarefaction, total = sum of both polarities, summed mean = sum of males and females, SD = standard deviation.

Appendix IV: Means and standard deviations of CM values for each HPF setting and stimulus type

Dependent variable	Stimulus	Polarity	HPF 3.3 Hz Mean (SD)	HPF 100 Hz Mean (SD)
Amplitude	**Click**	**Cond**	1.86 (1.16)	1.36 (0.89)
		Rare	1.27 (0.79)	0.98 (0.71)
		Total	1.56 (1.03)	1.17 (0.82)
	Tone burst	**Cond**	0.12 (0.05)	0.13 (0.45)
		Rare	0.13 (0.06)	0.13 (0.04)
		Total	0.13 (0.05)	0.13 (0.04)
	BB chirp	**Cond**	0.18 (0.10)	0.17 (0.11)
		Rare	0.17 (0.10)	0.21 (0.11)
		Total	0.17 (0.10)	0.19 (0.11)
Latency	**Click**	**Cond**	1.17 (0.57)	1.35 (0.64)
		Rare	1.06 (0.59)	1.37 (0.65)
		Total	1.11 (0.57)	1.36 (0.64)
	Tone burst	**Cond**	1.24 (0.50)	1.31 (0.59)
		Rare	1.30 (0.50)	1.29 (0.63)
		Total	1.27 (0.46)	1.30 (0.60)
	BB chirp	**Cond**	0.70 (0.42)	0.70 (0.44)
		Rare	0.67 (0.37)	0.79 (0.50)
		Total	0.69 (0.39)	0.75 (0.46)
Duration	**Click**	**Cond**	1.29 (0.64)	1.21 (0.61)
		Rare	1.07 (0.51)	1.16 (0.59)
		Total	1.18 (0.58)	1.18 (0.59)
	Tone burst	**Cond**	0.54 (0.11)	0.58 (0.19)
		Rare	0.52 (0.08)	0.56 (0.12)
		Total	0.53 (0.94)	0.57 (0.16)
	BB chirp	**Cond**	0.61 (0.21)	0.61 (0.18)
		Rare	0.61 (0.20)	0.64 (0.19)
		Total	0.61 (0.20)	0.63 (0.18)

Note: cond = condensation, rare = rarefaction, total = sum of both polarities, SD = standard deviation.

Appendix V: Latencies and amplitudes (mean and standard deviations) of the SP and AP from previous studies

Authors	Specification	Ear	SP amp Mean (SD)	AP amp Mean (SD)	SP lat Mean (SD)	AP lat Mean (SD)
Wilson & Bowker, (2002)	N = 20 18 – 30 years Biologic TM 7.1/s 90 dBnHL	Left Right	0.08 (0.08) 0.08 (0.08)	0.59 (0.18) 0.54 (0.21)	0.87 (0.13) 0.88 (0.12)	1.54 (0.12) 1.56 (0.13)
Redondo-Matínez et al., (2016)	N = 30 15 – 50 years tiptrode Unknown 90 dBnHL	Sum both ears	0.12	0.45	0.82	1.44
van Bommel, (2014)	N = 54 18 – 59 years ABR setup 90 dBnHL	Male Female	*	*	*	1.37 (0.10) 1.32 (0.08)
Lake & Stuart, (2019)	N = 84 20 – 30 years Lily TM 7.7/s 90 dBnHL	One ear	0.28 (0.17)	0.87 (0.37)	*	1.71 (0.13)
Zakaria et al., (2017)	N = 84 20 – 49 years Unknown TM** 7.1/s 95 dBnHL	Sum both ears	0.20 (0.08)	0.65 (0.25)	*	*
Grasel et al., (2017)	N = 200 19 – 71 years Sanibel TM 11.3/s 90 dBnHL	Left Right	*	*	*	1.45 (0.16) 1.47 (0.20)

Note: amp = amplitude, lat = latency, SD = standard deviation.
** = variable has not been investigated.*
*** = brand of the TM electrode was not further specified.*

Appendix VI: Within-subject differences

Table 1. SP and AP latency values of the first and second round ECochG of the first retested subject.

Retest first subject			
Potential	**1st recording**	**2nd recording**	**Difference**
SP	1.07	0.63	0.44
AP	1.83	1.30	0.53

Table 2. SP and AP latency values of the first, second and third round ECochG of the second retested subject.

Retest second subject				
Potential	**1st recording**	**2nd recording**	**3rd recording**	**Difference**
SP	1.80	0.83	0.77	0.06
AP	1.90	1.67	1.30	0.37

Table 3. CM amplitude, latency and duration values of the first retested subject.

Condition	**1st recording CM amp/lat/dur**	**2nd recording CM amp/lat/dur**
CM click Cond 3,3 Hz	**1.45**/1.60/1.20	**0.74**/0.37/0.53
CM click Rare 3,3 Hz	**1.23**/1.67/1.37	**0.75**/0.37/0.57
CM click Cond 100 Hz	**1.23**/1.50/1.37	**0.69**/0.40/0.57
CM click Rare 100 Hz	**1.37**/1.57/1.33	**0.63**/0.85/0.53
CM TB Cond 3,3 Hz	**0.14**/1.37/0.43	**0.53**/1.00/0.47
CM TB Rare 3,3 Hz	**0.17**/1.33/0.43	**0.59**/1.03/0.47
CM TB Cond 100 Hz	-/-/-	**0.54**/1.23/0.50
CM TB Rare 100 Hz	-/-/-	**0.61**/1.27/0.50
CM BB chirp Cond 3,3 Hz	-/-/-	**0.73**/0.50/0.57
CM BB chirp Rare 3,3 Hz	-/-/-	**0.73**/0.87/0.47
CM BB chirp Cond 100 Hz	-/-/-	**0.71**/0.47/0.50
CM BB chirp Rare 100 Hz	-/-/-	**0.73**/0.50/0.57

Note: cond = condensation, rare = rarefaction, amp = amplitude, lat = latency, dur = duration, - = no response.

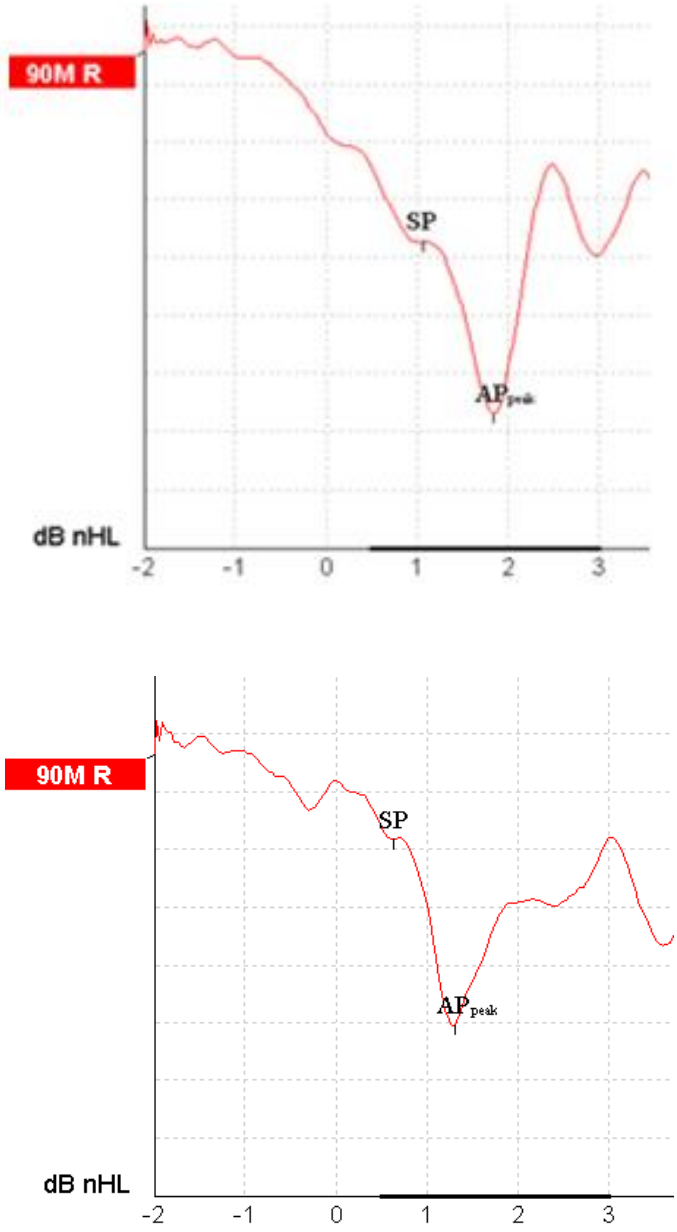

Figure 1a-b. Results of the first retested subject. SP and AP waveforms evoked by a click at 90 dBnHL in alternating polarity. Repetition rate: 11.3/s. Filter setting: 100 – 3,000 Hz. First ECochG acquisition (top): subject's ear canal with residual xylocaine. Second ECochG acquisition (bottom): subject's ear canal without residual xylocaine (after cleaning).

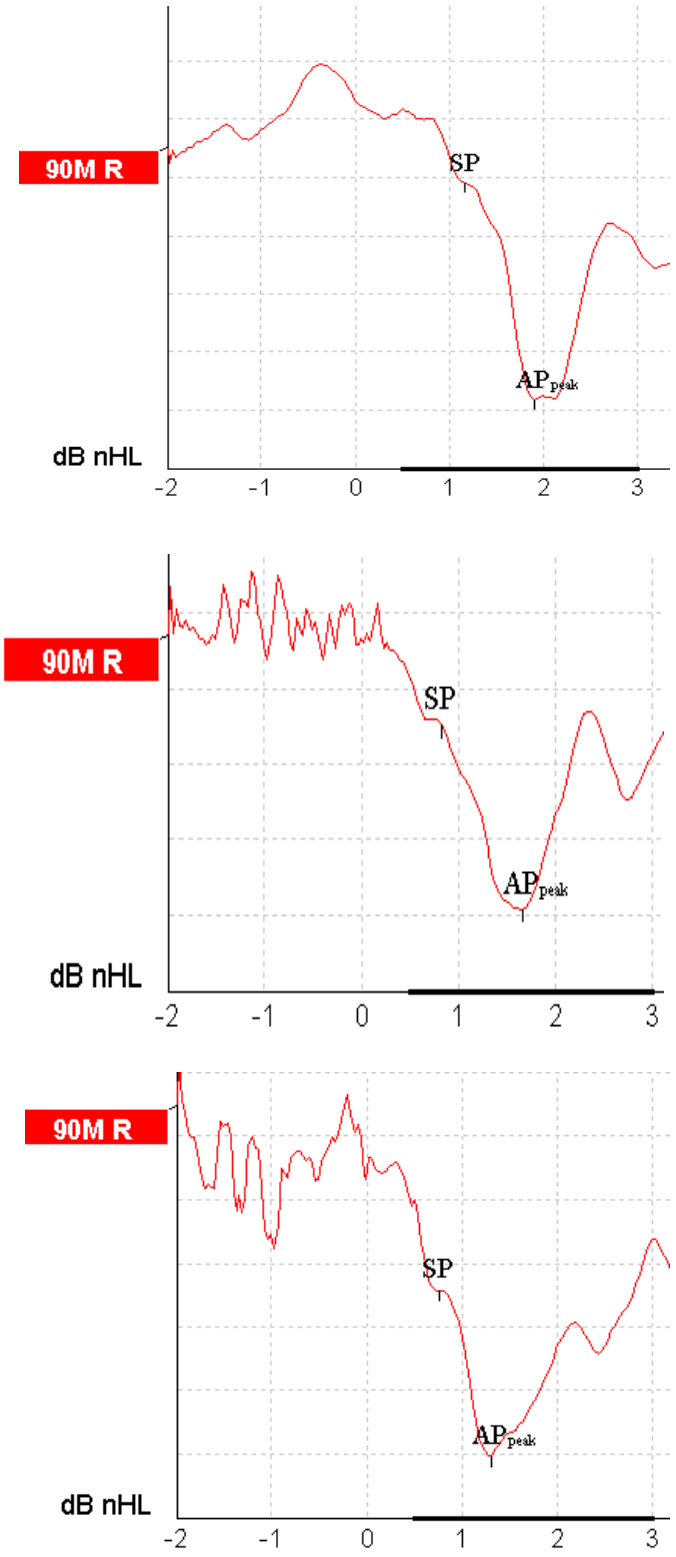

Figure 2a-c. Results of the second retested subject: SP and AP waveforms evoked by a click at 90 dBnHL in alternating polarity. Repetition rate: 11.3/s. Filter setting: 100 – 3,000 Hz. First ECochG acquisition (top): subject's ear canal with residual xylocaine. Second ECochG acquisition: subject's ear canal with residual xylocaine as baseline (middle), followed by an acquisition where the subject's ear had no residual xylocaine (after cleaning: bottom).