Cochlear microphonics: A comparison between scalp recording vs. intracananicular recording procedure using tone bursts and click.

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CERTIFICATE

This is to certify that this dissertation entitled **"Cochlear microphonics: A comparison between scalp recording vs. intracananicular recording procedure using tone-bursts and click"** is a bonafide work submitted as a part for the fulfilment for the degree of Master of Science (Audiology) of the student with Registration Number: 18AUD012. This has been carried out under the guidance of the faculty of this institute and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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CERTIFICATE

This is to certify that this dissertation entitled **"Cochlear microphonics: A comparison between scalp recording vs. intracananicular recording procedure using tone-bursts and click"** has been prepared under my supervision and guidance. It is also being certified that this dissertation has not been submitted earlier to any other University for the award of any other Diploma or Degree.

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DECLARATION

This is to certify that this dissertation entitled **"Cochlear microphonics: A comparison between scalp recording vs. intracananicular recording procedure using tone-bursts and click"** is the result of my own study under the guidance of Dr. Animesh Barman, Professor in Audiology, Department of Audiology, All India Institute of Speech and Hearing, Mysuru and has not been submitted earlier to any other University for the award of any other Diploma or Degree.

Mysuru

July, 2020

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Dedicated to Appa

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TABLE OF CONTENTS

List of Tables

List of Figures

Chapter 1

Introduction

Electrocochleography (ECochG) is an auditory evoked potential which yields information about synchronous electrical activity exhibited by the cochlea and auditory nerve together i.e., the peripheral part of the auditory system. It generally involves measuring the cochlear potentials, which are cochlear microphonics (CM) and summating potential (SP) and, also compound action potential (CAP) of the auditory nerve.

The cochlear microphonic (CM) is a part of ECochG and is an alternating electrical current measured from hair cells in the cochlea. CM was first recorded by Wever and Bray (1930) and was initially thought to be neural responses. Researches did over the years eventually proved CM to be a response originating from the cochlea. The history of the study of human cochlear responses began with a series of initial works conducted by Fromm, Nylen and Zotterman (1935), Andreev, Aranova and Gerschuni (1939), Perlman and Case (1941) and Lempert, Meltzer, Wever and Lawrence (1950). They could record the CM with a gross electrode placed on the round window niche by a surgical approach during operations on the ears. It was concluded, after several considerable efforts, that the recording of the CM in humans was not a practical clinical procedure due to technical difficulties (Lempert, Meltzer, Wever & Lawrence, 1950). It is not surprising that the matter has not been considered in clinical audiology until these early researches began on the human CM. Since then till date, the recording of CM has undergone tremendous changes. CM is an indicator of the physiological integrity of the hair cells in the cochlea. In other words CM is the summation of receptor potentials of individual sensory hair

cells. Therefore CM can be used as potential gauze of the degree of hair cells impairment (Simmons & Beatty, 1962). CM reflects the oscillation of the basilar membrane induced by stimulus and grossly has the tendency to mimic the various characteristics of the acoustic input. This characteristic of CM has led to difficulty in its recording and has created problem to separate and identify from artifacts.

One of the most important factors that impact the recording of CM is the placement and type of the electrode used. Placing the electrode as close as to the source of the potential generator increases the signal to noise ratio which further results in better amplitude and stable recordings of the CM. Based on the placement of the electrode, recordings can be divided into transtympanic and extratympanic recordings. Transtympanic recording have been used successfully to record CM. As the name implies, transtympanic electrodes are passed through the tympanic membrane and placed on either the round window niche or the promontory of the middle ear cleft. This approach uses a ball-tipped electrode or a needle electrode positioned on the place of interest through an invasive procedure called Myringotomy (Aran & Lebert, 1968). Extratympanic recording is a non-invasive procedure to record CM using electrodes placed on epidermal layer of the tympanic membrane which uses the wick electrode and/or in the ear canal which uses TIPtrode (Cullen, Ellis & Berlin, 1972).

Recording CM in response to an acoustic stimulation can be accomplished by using same instruments that are used to measure ABRs. Though only a few recording parameters are different from those used to measure ABR, the similarities are valid enough to record both ABR and CM together. The active (non-inverting) electrode is typically placed in the ear canal or on the tympanic membrane. The inverting electrode is usually placed on the contralateral ear lobe, contralateral

mastoid, or on the vertex. Ground (common) electrode is placed on the forehead or any other far-field location (Hood, Katz, Chasin, English, & Tillery, 2009)

The factors which affect the recording of CM are many such as type of stimulus, frequency of the stimulus, intensity of stimulus, recording site used etc. For click stimuli, a relatively short window of about 5ms is sufficient. Tone burst stimuli which are longer in duration needs sufficient broader time windows to capture the responses.

Several studies have investigated the CM and have done it across acoustic frequencies and intensities (Zhang, 2012, 2013; Heidari, Pourbakth, Kamrava, Kamalo & Yousefi, 2018; He, Porsov, Kemp, Nuttall & Ren, 2013). The aim of such studies was basically to find the importance of CM and to explore its unique features and usefulness in regards to gain knowledge on the cochlear functions, therefore, aiding in the diagnosis of auditory disorders. Few of these studies are done by Zhang (2012), where a comparison of CM amplitude across different frequency tone bursts was made. Results revealed that as a function of frequency, the amplitude of CM was seen to decrease. However, this was prominent more w.r.t the lower frequencies than higher frequencies. In another study done by Zhang (2013) on the effects of intensity at low frequency tone burst on CM amplitude, and saw that the CM waveforms are intensity-dependent. However, it is contrary, that the delay of CM waveforms is intensity-independent, which is different from neural responses as their delay of latency is intensity-dependent (Zhang, 2013). And they also suggested usage of long duration tone burst for better recordability of CM using ear canal electrodes.

There are studies even in the literature which talks about recording CM using clicks. These studies conclude that recording with clicks yields higher amplitude

than tone burst (Heidari, Pourbakth, Kamrava, Kamalo & Yousefi, 2018; Coraci, 2019). CM recorded with click stimuli, enables to assess outer hair cells in the region of 1 KHz to 2 KHz. Wherein recording place-specific CM using high pass masking technique led to the possibility of measuring (outer) hair cell function in the apical part of the human cochlea (Ponton, Don & Eggermont, 1992).

There is enough evidence therefore, to understand that CM can altogether be a supplementary tool in the cochlear function assessment as both Oto-acoustic emissions and CM are generated from outer hair cells. They also provide knowledge regarding the hair cells integrity (Norton, Ferguson & Mascher, 1989). However, it is no longer an unknown fact that in the clinical setting, measuring OAEs at low frequencies have always been a hindrance and has poor test-retest reliability. These marked difficulties are mainly due to background noise, breathing, and cardiovascular murmurs (Tognola, Ravazzani & Grandori, 1995). Both Transient evoked otoacoustic emissions (TEOAEs) and Distortion product otoacoustic emissions (DPOAEs) have their shortcomings. While TEOAEs typically cannot give information above ~5 KHz, on the other hand, the DPOAEs (2f1-f2) fail to provide frequency specific information. Keeping these limitations of OAEs in mind, it is only logical to think of CM as an answer to the above mentioned flaws, only if CM can be optimized to fetch reliable and instant information regarding the cochlear functions. These are evidenced by the facts in literature that CM is not affected by any environmental related factors as it is an electrical response (Zhang, 2010).

In the advent of renewed interest regarding CM, (Wilson, Sininger & Starr, 2003) have thrown light towards the point that in patients with ANSD, CM can be recorded to get confirmation on the diagnosis. Even with conditions where there is a partial loss of OHCs, resulting in the absence of OAEs, CM has been found to be

preserved (Deltenre et al., 1999). Another study, which took subjects with ANSD also showed that CM was present even in the absence of OAEs (Yathiraj & Mathew, 2012). Therefore, it provides even more confirmation to show the sensitivity of CM towards assessing the functional integrity of cochlea.

Need for the study

CM is known to assess the cochlear function. Recent researches have shown CM to have other diagnostic utility as well. The CM is found to be one of the significant tools in diagnosing Meniere's disease (Ferraro & Durrant, 2006; Ge, Shea & Orchik, 1997; Gibson & Beagley, 1976). The presence of CM has found a very important value in the assessment and diagnosis of auditory neuropathy spectrum disorders (Berlin, Hood, Morlet, Rose & Brashears, 2003; Deltenre et al., 1999). Inclusion of CM in the test battery to diagnose any hearing and balance related disorders have shown to increase the sensitivity and specificity of the diagnosis made. This advantageous application of CM in the clinical scenario, as mentioned above, has paved the way for more research regarding CM over the recent years. CM has been comprehensively studied in animals (Dallos, 2012). However, the amount of studies investigating CM recordings in humans and their utility in the clinical applications is relatively limited, and such a limitation may be due to technical complexity (Ferraro & Durrant, 2006) or may be due to lack of adequate knowledge regarding the recording and interpretation of CM measured from a far-field electrode in the clinical scenario. Therefore there is an increasing need to enrich our understanding of CM measurements in humans.

Also, there has been a limited study showing whether a click or a tone burst of short duration or long duration should be used for the clinical recording of CM as

all of the stimuli hold its benefits and is able to show diagnostic utility (Heidari, Pourbakth, Kamrava, Kamalo & Yousefi, 2018). It is a common practice amongst the Audiologists to record auditory brainstem response (ABR) as it is established that they have a significant role in the accurate assessment of hearing sensitivity. So there is also a need to probe into the presence of CM when recorded with the ABR protocol without changing the efficacy of CM in its various diagnosing capability. This might lead to a cost and time effective procedure wherein the audiologist could be able to record both cochlear potentials and potentials generated by the auditory nervous system simultaneously. The study will use various stimulus and stimulus parameters to find out the optimum stimulus to record the CM using the ABR protocol and ECochG protocol or in other words, study will try to throw an insight into which stimuli can be used for best recording of CM. There have been studies to see the recording of CM simultaneous to ABR in children with different hearing conditions, and it was concluded that even in the absence of ABR, CM could be traced to its threshold level for appropriate diagnosis (Dabbous, 2016). Riazi and Ferraro (2008) observed that on mastoid and ear canal recording of CMs were difficult due to ineffective ways to extract CM, which is contaminated by stimulus artifacts in both click and tone bursts. Therefore, the point that most importantly needs to be noticed is that CM is most likely to get masked while recording ABR in individuals with normal hearing. Thus, this study aimed at comparing the CM recorded with ABR protocol vs. ECochG protocol to check for discrepancies in the CM waveform, if any. This study also tried to explore whether any modification in the filter settings using ABR protocol would help to record CM, thus reducing the time and cleaning procedure.

Aim

The aim of the study was to record CM using ABR protocol and ECochG protocol using various types and duration of the stimulus (clicks-0.1ms, tone burst-10ms and tone burst- 18ms) and compare the response rate and different parameters of CM.

Objectives

Thus, the objectives of the study were to:

- Find the occurrence of CM with ABR protocol using click and different duration of 500Hz tone burst.
- Find the occurrence of CM with ECochG protocol using click and different duration of 500Hz tone burst.
- Compare the presence of CM between ABR protocol and ECochG protocol at each type and duration of the stimulus.
- Compare different parameters of CM recorded using different stimuli and also protocol.
- Determine the optimum stimulus based on CM's frequency of occurrence and the amplitude and onset latency of CM.

Chapter 2

Review of Literature

The Human auditory system consists of the outer ear, middle ear, inner ear, eight cranial nerve and central auditory nervous system, which include the auditory brainstem and auditory cortex. The outer ear and middle ear are collectively called the conductive system since their most apparent function is to bring the sound signal from air to the inner ear. The inner ear includes the vestibular system and the cochlea, structurally both of these organs contain bony and membranous labyrinth, which consists of sensory structures in the form of hair cells responsible for hearing and balance function. The auditory nerve comprises of both the vestibular nerve branch and the cochlear nerve branch. The signal then passes from various stations and reaches auditory cortex, which is the final region where sound, reaches and gets coded.

Evaluation of the complete auditory system requires a combination of psychoacoustic and physiological measures. A variety of tests of auditory function targeting peripheral and central structures of the auditory system have been applied to accomplish the assessment. However, this proves to be highly cost and time consuming. Psychoacoustic tests tend to provide a global measure to assess the function of auditory system as well as target the integration of auditory information across the two ears, such as behavioral hearing thresholds, which include air conduction and bone conduction measurement. These cumulatively make the pure tone audiometry, which is the most fundamental aspect of the audiologic assessment, and it involves the determination of the pure-tone thresholds across audiometric frequencies and tests of speech perception with or without background distracters.

Behavioral tests are till date considered being Gold standard. Physiological tests additionally aid in the determination of the general site of lesion in the peripheral auditory system and offer objective measures of auditory function. Tests such as otoacoustic emissions (OAEs) and auditory brainstem responses (ABRs) are few popular physiological tests which have gained high value in the diagnostic audiology field for its prompt and efficient results in order to give detailed knowledge regarding the integrity of the auditory system. There are few other tests for physiological assessment, such as Cochlear Microphonics (CM), which due to its cumbersome recording procedure and lack of optimization was unable to gain popularity. However, lately, literature is scanning through the uniqueness of CM and is gaining its lost touch. Research investigations pertaining to the optimization of CM is under highlight in many of such investigations.

2.1 Overview of Otoacoustic emissions.

Otoacoustic emissions (OAEs), first described by David Kemp in 1978, are one amongst various fascinating phenomena of the auditory system. OAEs are those preneural sound which originates in the cochlea and propagates through the middle ear and into the ear canal where it can be measured using a sensitive microphone. Extensive research has been done regarding many aspects of OAEs and their relation to auditory functioning.

OAEs have gained immense consideration in the assessment of the auditory system as they can be measured even when the eighth nerve has been compromised in its function (Siegel & Kim, 1982) or even when nerve activity is chemically blocked (Arts, 1990). They are unaffected by stimulus rate or polarity changes. Liberman, Zuo and Guinan Jr, (2004) showed evidence that both Outer Hair Cells

(OHCs) somatic motility and steriocilia may contribute to the production of OAEs in mammals and that their contribution may be stimulus- dependent. There are various kinds of OAEs which are documented in the literature, such as Transient evoked otoacoustic emissions (TEOAEs), Distortion product otoacoustic emissions (DPOAEs), Stimulus frequency otoacoustic emissions (SFOAEs). Amongst them, TEOAEs and DPOAEs have received the most attention in the field.

OAEs found its mammoth importance in universal newborn screening programs as they provide a quick, non-invasive measurement of cochlear function hearing loss (Thompson, McPhillips & Davis, 2001). They also offer the greatest insight into the integrity of the CANS when suppression effects are measured in the presence and absence of noise (Smart, Kuruvilla, Kelly & Purdy 2019). It became a major clinical test battery tool in differentially diagnosing various audiological disorders, to monitor the effects of ototoxic medications, to detect cochlear abnormalities in patients with tinnitus and normal audiograms and help in identifying malingerers. However, this test came with its shortcoming. Few of which are discussed below.

The absence of OAEs can be used as a confirmatory diagnostic tool for sensorineural hearing loss only when middle ear function is relatively normal (Owens, McCoy, Lonsbury-Martin & Martin, 1993). Due to high noise floor OAEs are challenging to measure at low frequencies (Zhang, 2013). The high noise floor either conceals the OAE signal or results in a high detection threshold. Even if a high detection threshold can be utilized in the clinic, its measurement is challenging because the measurement of high detection thresholds requires high stimulus intensities. In addition, the results of low-frequency OAEs can vary greatly. For example, response amplitudes in lower frequency DPOAEs and those in higher

frequency DPOAEs differ significantly as they include individual's own breathing patterns (Zhang, 2012). Despite these obstacles, obtaining a measurement at low frequencies is essential. However, the low frequency of 500Hz is an important audiometric frequency in the clinical assessment as this is included in tuning fork tests, pure tone average tests, acoustic reflex measurement, and tone burst-ABR tests (Wang, Tymczyszyn, Yu, Yin, Bance & Robertson, 2011).

2.2 Cochlear microphonics

CM measurements may be considered as an accompanying approach to OAE measurements in assessing low-frequency cochlear function. Besides OAEs, CMs can also be used for evaluating cochlear conditions as hair cells are involved in the generation of both CMs and OAEs. CM measurements might be an answer to the limitations that OAE measurements have. For example, acoustic noise will not hamper CM recording or occurrence, as CMs are electrical signals (Zhang, 2010, 2012 & 2013).

Although EcochG is available to hearing scientists since 1930, the discovery of other early potentials and late latency potential masked the attention to the peripheral system. Eventually, over the years, investigations are coming up with techniques and approaches for recording CM in a non-invasive manner in order to identify, adapt, and expand its clinical applications. It also broadens its horizon and aims to improve the sensitivity and specificity in the diagnosis, assessment, and management of inner ear and auditory nerve disorders (Burkard, Eggermont & Don, 2007)

Cochlear microphonics reflects the integrity of the peripheral auditory system, particularly outer haircells. CM can be elicited by various kinds of acoustic

stimulus and also has an ability to replicate the various acoustic characteristics like frequency, the polarity of the stimulus (Deltenre et al., 1999). Cochlear microphonics is an important electrophysiological response and is part of the audiological test battery, which allows the assessment of the extent of haircells impairment (Simmons & Beatty, 1962). Despite its massive application in the diagnosis of various auditory disorders, the recording of CM is a concern to the scientific community due to meagre knowledge about it in humans and its characteristics (Zhang, 2013)

2.3 Factors affecting Cochlear microphonics

There are numerous factors that have been seen to significantly alter the characteristics of CM. Few of these factors are type of stimulus, duration of the stimulus, frequency of the stimulus, intensity of the stimulus, electrode placements, recording sites etc.

Cochlear microphonics and type of stimulus

The history of the human cochlear microphonics began with elaborative works carried out by several authors such as Fromm, Nylen and Zotterman (1935), Andreev, Aranova and Gerschuni (1939), Perlman and Case (1941), and Lempert, Meltzer, Wever and Lawrence (1950) and Yoshie & Yamaura (1969). An extensive and series of studies in recent years done by several authors on several parameters affecting CM has helped the professionals and clinicians to understand this particular preneural potential better. Investigations also will help to optimize a certain protocol best suited for recording CM. However, any such study is lacking in literature, which reports of a specific protocol that can be used for recording CM with good morphology. Despite this shortfall, several studies have discussed about the various factors that can affect the recording of CM. Taking these factors into consideration,

it is only reasonable for one to set an optimised protocol which will consider every lacuna and therefore give the best for clinical use.

Zhang (2012) had recorded CM using tone burst frequencies of 500 Hz, 1 KHz, 2 KHz and 6 KHz from ear canals of 10 normal hearing individuals. The stimulus duration used was 14ms.The positive/non inverting disc electrode was placed on upper forehead (Fz), negative/inverting electrode was a TIPtrode placed inside the ipsilateral ear canal of the subject and the ground disc electrode on the contralateral mastoid. It was found that amplitude of CM was reduced as a function of frequency, and this observation was prominent at lower frequencies than higher frequencies. The author had discussed the differences and importance of clicks and long and short duration tone burst in recording CM. Tone bursts are more frequency specific as it contains fewer frequencies than click evoked CM. 14ms tone burst yielded higher CM amplitudes than 6ms tone burst. The author has used frequency fourier transformation (FFT) to discuss the reasons for better frequency specificity provided by the long duration tone burst stimuli(14ms) in relation to shorter duration tone burst stimuli (6ms). The power spectrum revealed that 6ms tone burst had much wider bandwidth and capable of covering a larger frequency range on basilar membrane than that of the 14ms tone burst. The narrower bandwidth indicates better frequency sensitivity and forms sufficient number of sinusoidal cycles to form a plateau with increase in the of the duration stimulus. Such narrow frequency band stimuli could help professionals to assess specific frequency region on the basilar membrane.

Heidari, Pourbakth, Kamrava, Kamalo and Yousefi (2018) experimented using broad and narrower stimulus i.e., click and tone bursts respectively on 25 healthy adult wistar rats. Authors used an extratympanic approach for ECochG

recording, CMs in response to click of duration 0.1-µsec and tonal stimulus of 5ms with different octave frequencies (2 KHz, 4 KHz, 8 KHz and 16 KHz) were recorded at a high intensity level of 80 dB SPL in all the subjects. Three subcutaneous needle electrodes were placed at vertex non-inverting/positive, under the right ear inverting/negative and the left ear as ground as in conventional vertical montage ABR electrode placements. Results indicated that there was a direct relation between bandwidth of the stimulus and magnitude of CM. The amplitude of CM at low frequencies kept increasing with the increase in stimulus intensity. They attributed this to the reason that click stimulation leads to larger spectrum stimulation on the basilar membrane and its traveling wave is spread along the cochlear partition from base to apex. Thus, it involves the stimulation of more hair cells than narrow band stimuli. Therefore, CM most likely is a reflection of spatial summation of voltage drops generated by hair cell groups in response to acoustic stimulation. Tonal stimuli yielded very small magnitudes of CM, and authors recommend clicks for CM potential recording. This study contraindicates results of the study done by Zhang (2012).

Cochlear microphonics and intensity

Further investigations were conducted by Zhang (2013), to evaluate effect of intensity on cochlear microphonics where they recruited 10 individuals with normal hearing sensitivity. Stimulus used was 500Hz tone burst with 14ms duration (1-5-1) cycle with blackmann gated function. Since, author's previous study supported and proved that with the use of long duration tone bursts produced robust CM compared to shorter duration tone bursts and clicks. The possibility of evoking ABR would be less when long duration tone burst are used, and CM wouldn't be contaminated by ABR waveforms, which leads to easier data analysis. CM has been recorded only in

rarefaction polarity and various intensity levels like 80, 60, 40, 30, 20 and 10dBnHL. The Author has incorporated the same electrode placements like his previous studies, negative electrode which is a TIPtrode inside the ipsilateral ear canal of the subject, ground electrode on the contralateral mastoid and positive electrode at the upper forehead (Fz). The results indicated that the ear canal recorded CM was robust. The amplitude of CMs was intensity-dependent i.e., the amplitude of CM decreased as a function of intensity. In contrast, delay or latency of CM is intensity independent i.e., the latency of CM remained unchanged as a function of intensity. The delay in the peak of CM waveforms was not substantially significant. The Author suggests the reason for such finding could be that the temporal locations for the generation of such potentials remain same as a function of intensity. The study concluded that these findings might be useful for developing CM measurement applications as a supplementary approach to oto-acoustic emission (OAE) measurement. The intensity-independent nature of CMs with regards to delay measurements may also become an impacting factor for differential diagnoses and for designing new research studies.

Another study done by He, Porsov, Kemp, Nuttall and Ren (2013) quantified the intra-cochlear group delay by measuring Round Window CM and vibrations at the stapes and basilar membrane. Twenty-eight Mongolian Gerbils were taken for the study. the stimulus used was 20ms single tone burst with 1ms rise and fall time at frequencies from 200 Hz to10 KHz with 200 Hz steps and at sound levels varying from 10 to 90 dB SPL with 10 dB per step. The results demonstrate that the round window CM showed no significant delay at all measured frequencies above 2 KHz. As the sound level increased, the peaks of CM waveform became broader and fused together. The magnitude of CM at frequencies below 2 KHz increased nonlinearly

with the increase in sound level. At frequencies above 2 KHz, high sound levels above 80 dB SPL, the CM magnitude decreased slightly with frequency and showed no obvious peak. The relative contribution of the OHCs from the best frequency location to the RW CM varies with the sound pressure level. This was reasoned to be because at low levels, the low-frequency RW CM comes mainly from the best frequency location while, at high sound levels, it partially comes from the cochlea base hence compromising with the frequency specificity in recording CM.

2.4 Effect of electrode montage and recording sites

In the past 80 years, with the advancement of technology, the electrode location for CM measurement has migrated from inside the cochlea, to outside cochlea, the round window niche or promontory area, outside the middle ear, the tympanic membrane, and eventually the external ear canal (Ferraro & Durrant, 2006).

Riazi and Ferraro (2008) have examined techniques that can be employed to optimize the far-field recording of CM in humans by comparing different electrode sites and stimulus parameters. In this study, authors recorded CM in a simultaneous two channel fashion from ear canal in channel 1 and from mastoid in channel 2 where 7 newborn and 4 adults were part of the study. A surface electrode placed on the high forehead (Fz) served as the noninverting/positive common recording site for both channels. In channel 1, a TIPtrode placed in the ear canal of the stimulated ear served as the inverting/negative electrode site. For channel 2, a surface electrode placed on the test ear mastoid served as the secondary minus electrode site. A surface electrode placed on the non-test ear mastoid served as ground. Stimuli used were clicks (100μs) and tone burst of 500 Hz, 1 KHz and 2 KHz and stimulus level

delivered at 70 dBnHL and 95 dBnHL. Results of the study showed out of 7 newborns, CM was present in only one when recorded using tone burst stimulus. It was difficult to differentiate between CM and stimulus artifact; hence CM was absent in 5/7 individuals.

Authors suggested that electromagnetic shielding and grounding of the electrode cables and the acoustic transducer were effective in reducing and/or eliminating electromagnetic stimulus artifact paved to better recording of CM compared to non-shielded condition. CM was most readily recorded with an ear canal electrode than the surface electrode on the mastoid process. Authors discuss that reasons for failure in far-field recording of CM could be generation of the potential from trailing end of the traveling wave and limited contribution of the characteristic frequency from apical regions, resulting in a low amplitude potential that was difficult to elevate above the electrical noise floor.

Zhang (2010) explored the recording of CM from 3 different electrode sites from 10 human participants. The inverting/negative electrode placed at earcanal/concha/mastoid prominence, non-inverting/positive electrode placed on upper forehead and ground electrode placed on contralateral mastoid prominence. Results found that amplitude of CM recorded at ear canal was higher than concha recordings followed by mastoid recordings. There wasn't statistical significance between the amplitude of CM ear canal vs. concha site recorded. These results are expected because concha anatomically adjoins ear canal. The latency of CM recorded at the concha was longer than at the canal but shorter than at the mastoid.

Clinical applications of cochlear microphonics - Comparison between CM with other tests.

A retrospective study conducted by Dabbous (2016) was aimed at profiling the usefulness of recording CM simultaneously with the ABR threshold estimation testing. Subjects were children with autism spectrum disorder, children with cochlear hearing loss (SNHL), auditory neuropathy spectrum disorder and individuals with normal hearing sensitivity. Both the CM and ABR were recorded simultaneously using insert phones to ensure similarity of stimulus level for both recordings. CM was recorded at 90, 80 and 70 dBnHL and amplitude of CM decreased as a function of intensity. Author speculates the probable reason for such phenomenon could be because of the direct relation between CM amplitude and stimulus level. Higher stimulus intensity levels produce greater basilar membrane displacement and proportionally greater CM responses. The important findings of the study are that even though DPOAEs were not preserved in most of the ANSD population, CM was present with/without the presence of ABR. The amplitude of CM was significantly higher in ANSD individuals with preserved DPOAEs than individuals with no DPOAE responses. CM can be preserved in children with high frequency SNHL with loudness recruitment. This finding could be confused with ANSD, so CM should be traced down to its threshold for an appropriate diagnosis.

Zhang (2012) extended the applications of CM to assess high frequency hearing loss. The study included five individuals with high frequency hearing loss and ten individuals with normal hearing sensitivity. A set of 4 different frequencies such as 500 Hz, 1 KHz, 2 KHz and 4 KHz tone bursts of 14ms duration was used to record CM. In individuals with high frequency impairment, with an increase in the frequency CMs became barely recognizable. The amplitude of CMs in both normal

and clinical population decreased as a function of frequency. When cochlear functions of the individuals were compromised at the higher frequencies hearing threshold was also affected. Therefore, CM may be used to assess and monitor the cochlear function and to estimate hearing thresholds in subjects with high-frequency hearing loss under certain conditions. This trend is mainly consistent and in accordance with the measurements of CM and hearing threshold. In addition, both OAE and CM tests indicated that high-frequency function was impaired in the cochlea. Therefore, the CM results in the high-frequency region were supported by the OAE measurement.

Another retrospective study conducted by Narne, Prabhu & Mahadeva (2014) aimed at exploring the audiological characteristics in individuals with auditory neuropathy spectrum disorder (ANSD). As a part of the study, authors recorded CM was recorded using click stimulus in horizontal montage. The study emphasized on importance of CM in differential diagnosis of ANSD. Authors reported about 75% of the patients with ANSD had OAEs present which helped in differentiating of cochlear and retro-cochlear pathology. But, individuals with severe to profound hearing loss clearly exhibit abnormal or absent ABRs and are likely to be absent of OAEs. Apart from the 25% of the population who didn't exhibit OAEs had preserved CM in them. Hence, concluded that OAE alone cannot be used to diagnose patients with ANSD and CM have to be recorded in patients in whom OAEs are absent to confirm ANSD.

However, from the review of literature on CM, it is clear that there is uncertainty in recording CM. And no uniformity in the conclusion about an optimum stimulus whether a click or tone burst of short duration or long duration or broadband chirp to be used to increase CM's occurrence, better amplitudes and

latency measurements (Zhang, 2012; Heidari, Pourbakth, Kamrava, Kamalo & Yousefi, 2018; Coraci, 2019), There are only limited human researches on the optimization of the stimulus in recording this pre-neural potential (Ferraro & Durrant, 2006). Among professionals, it is a regular practice to record ABR as it established well in gauzing the thresholds of the individual's objectively and also enhancing sensitivity of the test battery in diagnosing cochlear and retro-cochlear pathologies (Ferraro & Ruth, 1994). There is a need for exploring the CM and incorporating it in the test battery without trading its effectiveness and adding up its value in screening, diagnosing and rehabilitating various audio-vestibular disorders (Ferraro & Ruth, 1994). This study will need to compare the CM recorded with ABR protocol vs. EcochG protocol to check for differences in the CM waveform, if any. This study also intent to optimize various acquisition parameter setting mainly where filter setting and electrode placements will be explored and an effective stimulus and stimulus parameter settings to record CM. This approach would help researchers and clinicians in recording CM by reducing the time used and limiting the cleaning procedures.

Chapter 3

Methods

The current study was taken up to compare the Cochlear microphonics (CM) recorded using Auditory brainstem response (ABR) protocol and Electrocochleography (ECochG) protocol. The stimulus used was clicks and tone burst with two different durations. The subjects taken up for the study were individuals with normal hearing sensitivity. To arrive at the aim of the study, the following methods were adopted.

3.1 Participants

The present study included 30 adult volunteers. The participants consisted of 16 males and 14 females. The age of the participants ranged from 15 years to 25 years (mean age – 20 years). The subjects underwent otoscopic evaluation, pure tone audiometry, immittance evaluation, and otoacoustic emission (OAE) testing for both the ears to ensure normal hearing sensitivity. However, for the recording of CM using auditory brainstem response (ABR) protocol and Electrocochleography (ECochG) protocol in each participant was done in a single ear, which was randomly selected.

3.1.1 Subject selection criteria

The subjects taken for the study was assessed using a test battery approach to rule out the presence of peripheral auditory abnormalities. Each participant in this study was selected based on the following criteria.

- Otoscopic evaluation was done to ensure normal tympanic membrane with the cone of light present, along with no visually detectable external and middle ear pathology.
- The participants underwent pure tone audiometry and were selected if their air conduction (AC) thresholds and bone conduction (BC) thresholds were ≤15 dBHL and ≤10 dBHL respectively across all the octave frequencies from 250 Hz to 8 KHz (BC thresholds were measured till 4 KHz).
- Immittance evaluation was also included to ensure normal middle ear status. The selection criteria was A type of tympanogram with presence of ipsilateral and contralateral acoustic reflexes at least in three frequencies among 500 Hz, 1000 Hz, 2000 Hz or 4000 Hz.
- Participants were also assessed using OAEs and at least 3dB SNR in three consecutive frequencies in transient evoked otoacoustic emissions (TEOAE) was mandatory to ascertain a healthy outer hair cells function, in particular.
- A structured interview was carried out with all the participants. Any history of exposure to noise for a longer duration, loud music, or intake of any ototoxic medicines was asked. If the subject reported any such history, they were not taken as a part of the study.
- Presence or history of neurological, otological, or any other associated problems at the time of assessment was ruled out.

3.2 Instrumentations

The instruments used in the present study were:

- A two channel independent diagnostic audiometer, Inventis piano (Inventis Incorporation, Italy) was calibrated with the TDH- 39P headphones and Radio ear

B-71 bone vibrator, according to ANSI S3.6 (2004) was used for pure tone threshold estimation.

- A Calibrated middle ear analyser, GSI-Tympstar (Grason-Stadler Incorporation, USA) was used for tympanometry and to measure the acoustic reflex thresholds.
- An ILO V-6 clinical OAE software (Otodynamics Ltd., UK) was used to measure and analyze TEOAEs.
- The recording of CM was done in the Natus Bio-logic navigator pro (Natus Medical Incorporated, San Carlos, USA) instrument, which is coupled with Etymotic ER-3A insert ear phones as default transducer.

3.3 Test environment

All the required testing procedures were carried out in a sound treated double walled air-conditioned room set-up. The noise level in the testing room was maintained within the permissible limits as per the ANSI S3.1-1999-R2013 (American National Standard Institute, 1999) guidelines.

3.4 Procedure

3.4.1 Basic audiological evaluation

A detailed case history was taken from all the participants before the commencement of the audiological evaluation. Participants were enquired if they had any history or active auditory problems such as otitis externa, occlusion due to ear wax, acute or serous otitis media and chronic suppurative otitis media (CSOM). The participants were also enquired if they are exposed to noise or intake of medicines for long duration that can affect the ear. These participants were investigated for ear pain, itching sensation, presence of tinnitus or any ear related

surgeries. The participants were also enquired for blocked nose and fullness in the ear due to the cold present during the time of testing. Structural abnormalities was checked for to rule out stenosis, atresia etc. Also, the tympanic membrane was viewed under the otoscope to rule out any perforation, scar or infection. If so, the participants were either excluded from the study or asked to report back once the cold resolved.

- To determine the air conduction and bone conduction pure tone thresholds, modified Hughson and Westlake procedure (Carhart & Jerger, 1959) was used. It was assessed at octave frequencies, between 250 Hz to 8 KHz for air conduction and 250 Hz to 4 KHz for bone conduction thresholds.
- Tympanometry was conducted using probe tone frequency of 226 Hz, along with pressure varying from +200 daPa to -400 daPa. The ipsilateral and contralateral acoustic reflexes were obtained for pure tone frequencies of 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz.
- TEOAEs were obtained for nonlinear click stimuli presented at around 75 dBpeSPL. It was considered to be present if the SNR was at least 3 dB SPL or more in at least three consecutive frequencies, with reproducibility greater than 50%.

The above mentioned tests were carried out to select the participants for the study.

3.4.2 The tests administered to achieve the objectives of the study

The participants underwent cochlear microphonics (CM) recording twice using two different protocols. Auditory Brainstem Response (ABR) protocol and the other one was with Electrocochleography (ECochG) protocol. Each recording was done with three different stimuli, which were,

- Clicks of 0.1milliseconds.
- 500Hz tone burst of 10milliseconds.
- 500Hz tone burst of 18milliseconds.

3.4.3 CM recording procedure using ABR protocol (Scalp recording)

The participants were made to sit comfortably on a reclining chair. The recording sites were identified and cleaned with commercially available abrasive gel (nuprep gel). The non-inverting electrode (positive) was placed on the mastoid of the test ear. The inverting electrode (negative) was placed on the mastoid of the non-test ear. The ground electrode was placed on the upper forehead. The electrodes were fixed with the help of surgical tape. The impedance of each electrode was ensured to be below 5 KΩ and the inter electrode impedance was maintained below 2 KΩ. The stimulus and acquisition parameters used to record CM are shown in Table 3.1.

Table 3.1

Stimulus and acquisition parameters used to record CM in ABR protocol

Stimulus parameter	
Stimulus type	Click and Tone burst (TB)
Stimulus frequency	TB of 500 Hz
Window	Blackmann window
Intensity	100 dB SPL
Stimulus duration	2-1-2 cycle (10ms) for TB of 500 Hz, 2-5-2 cycle (18ms) for TB of 500 Hz and for click – 0.1ms
Repetition rate	30.1/sec
No. of sweep	1500
Polarity	Rarefaction and condensation
Acquisition parameter	
Filter	100 Hz to 3000 Hz for clicks and 100 Hz to 800 Hz for tone bursts.
Notch filter	Off
Analysis time	5ms for clicks, 16ms for 2-1-2 tone burst and 25ms for 2-5-2 tone burst.
Amplification	1,00,000
Transducer	Etymotic ER-3A Insert phone

3.4.4 CM recording procedure using ECochG Protocol (Intracanicular recording)

The participants were made to sit comfortably on a reclining chair. The recording sites were identified and cleaned with commercially available abrasive gel (nuprep gel). The recording for CM with ECochG protocol required both TIPtrode

and disc electrodes to be used. TIPtrode placement required the ear canal to be cleaned. The pinna was pulled upward and backward in the process of cleaning the ear canal with cotton swab in a circular motion. The non-inverting (positive) electrode i.e., TIPtrode was placed in the ear canal of the test ear. The inverting (negative) disc electrode was placed on the mastoid of the non-test ear. Ground electrode was placed on the upper forehead (FPz) and within electrode impedance was maintained below 5 KΩ and between electrode impedance was maintained below 2 KΩ. The stimulus and acquisition parameters used to record CM using ECochG protocol was same as the parameters used for ABR protocol (as mentioned in table 3.1) except the type of electrode and placement of the electrode.

CM was recorded twice for each stimulus type and protocol to look for replicability and better identification of CM. The parameters analyzed are latency and amplitude of the CM. In this study, CM was recorded in both rarefaction and condensation polarity to enhance the effectiveness in identifying CM. Another recording was taken by clamping the sound delivering tube which is coupled with insert ear phones. This maneuvre helped in controlling negatives i.e., effective recording and differentiating CM and also to minimize the contamination of stimulus artifacts in the current study.

Recorded CM waveforms were then further subjected to digital offline filtering. Suitable filters for both clicks and tone burst responses were used i.e., response waveform of 500Hz tone burst was offline band pass filtered with the settings of 300Hz to 700Hz and 300Hz to 3000Hz for click stimulus. This helped in minimizing unwanted ABR peaks and other artifacts like posterior auricular muscle responses and also allowed better visualization of CM responses.

After digital offline filtering, the resulted waveforms was analysed in order to document the onset latency and the amplitude of CM. The onset latency for the current study is defined as the beginning of a cycle or a response where significant polarity reversal is identified at its maxima. The criterion point for locating CM's onset was decided after considering the grand average of amplitude for different stimuli from 10 subjects of the study. These waveforms were then given to three Audiologists to establish the onset latency criteria of CM. The point at which 2 out of 3 observers agreed upon was considered as criteria point which was 0.1 mV for 500 Hz TB and 0.03 mV for click evoked CM. Hence, the point where peak to peak amplitude is greater or equal to the criterion point and a significant polarity reversal was easily detectable was considered as an indication for the onset latency of CM for the rest of the waveforms. A depiction for determining the onset latency of CM is shown in Figure 3.1.

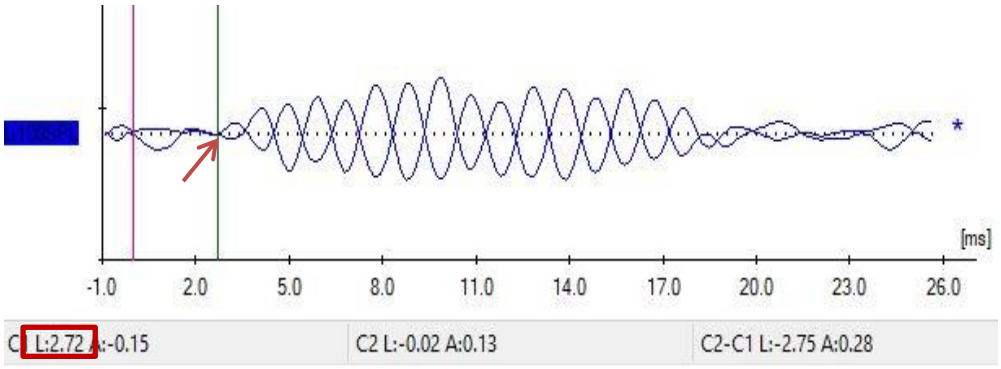

Figure 3.1: Depiction of the waveform of 500 Hz 18ms tone burst evoked CM for identification of onset latency.

For example, as shown in the figure above, the initial significant polarity reversal is seen at 2.72ms as pointed with the arrow. At 2.72ms, the amplitude of the following peak is more than the criterion point. Therefore, the starting point of this peak was considered as the onset latency of CM.

Amplitude analysis was done for each stable peak by calculating peak to peak amplitude i.e., from peak to trough and the amplitude difference between those two points was considered to be the peak to peak amplitude. Average of three consecutive, stable peak to peak amplitude was considered as the amplitude of CM as shown in figure 3.2.

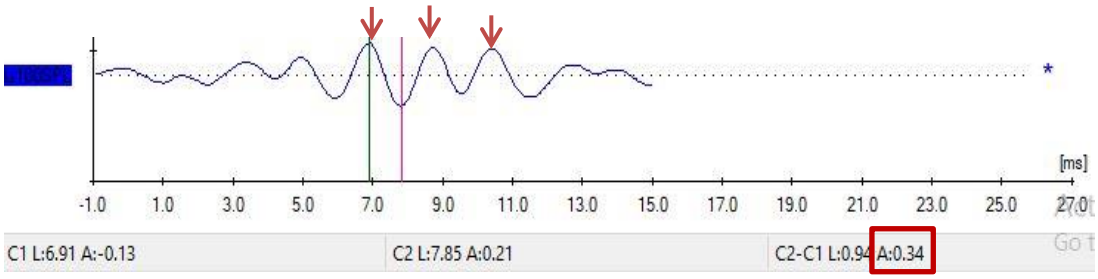

Figure 3.2: Depiction of the waveform of 500 Hz 10ms tone burst evoked CM for identification of amplitude.

The arrow displayed in the figure point towards the CM peaks which were considered as robust and stable. The peak to peak amplitude of the first peak of CM (from left) was 0.34mV (highlighted in the rectangle). Similarly, the peak to peak amplitude was also calculated for the remaining two consecutive stable peaks.The amplitude for each of the three peaks was noted and then averaged. The averaged cumulative value was then considered as the amplitude of CM for each respective stimuli used in the study.

3.5 Statistical Analyses

The percentage occurrence of CM in both ABR and ECochG protocol across all the three stimuli was compared. This was done to check the efficacy of the protocol in recording CM. Onset latency and amplitude of CM for two protocol and three stimuli were tabulated for statistical analysis SPSS software version 20. The mean and standard deviation of onset latency and amplitude of CM for various

stimuli was analyzed. The data analyzed showed normal distribution following which parametric tests were carried out. These tests were done in order to optimize the protocol for the better recordability of the CM.

Chapter 4

Results

The aim of the present study was to record CM using ABR protocol and ECochG protocol using various types and duration of the stimulus (clicks-0.1ms, tone burst- 10ms & tone burst- 18ms). A total of 30 participants were taken as subjects for the study. All the participants underwent a recording of CM using ABR protocol and ECochG protocol. The data collected was then subjected to statistical analyses using Statistical Package for social sciences (SPSS) version 20.

4.1 Occurrence of CM in ABR protocol.

CM was recorded using ABR protocol with tone burst of two different durations and click stimulus in all the subjects. The percentage of occurrence of CM using ABR protocol (scalp recording) with both kinds of stimuli and duration was analyzed. The total number of participants in whom CM was present was arithmetically divided by the total number of participants in the study, which was later converted into a percentage. Results revealed 100% occurrence when CM was recorded using 500 Hz tone burst of both duration (long = 18ms & short = 10ms). The occurrence percentage of CM, however, using click stimulus was only 80%. It was seen that for 20% of the subjects who underwent recording using the click stimulus in ABR protocol, the CM was absent. Figure 4.1 shows the percentage of occurrence of CM recorded using ABR protocol.

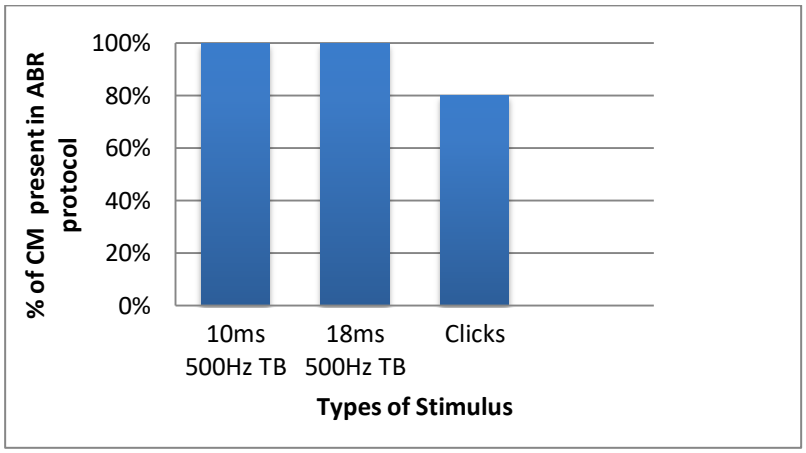

***Figure 4.1*:** Representing the percentage of CM present/occurrence in ABR protocol across stimulus.

4.2. Occurrence of CM in ECochG protocol.

CM was recorded using ECochG protocol (Intracanicular recording) also for tone bursts of two different durations and click stimulus. To find the percentage of occurrence of CM, total number of participants in whom CM was present was arithmetically divided by total number of participants in the study which was later converted into percentage. Results revealed 100% of occurrence of CM when it was recorded using 500 Hz tone burst of both duration (long = 18ms and short = 10ms). The occurrence percentage of CM, however, using click stimulus was only 86.7 %. In 13.3% of the subjects, CM was found absent for click stimulus. Figure 4.2 shows the percentage of occurrence of CM using ECochG protocol.

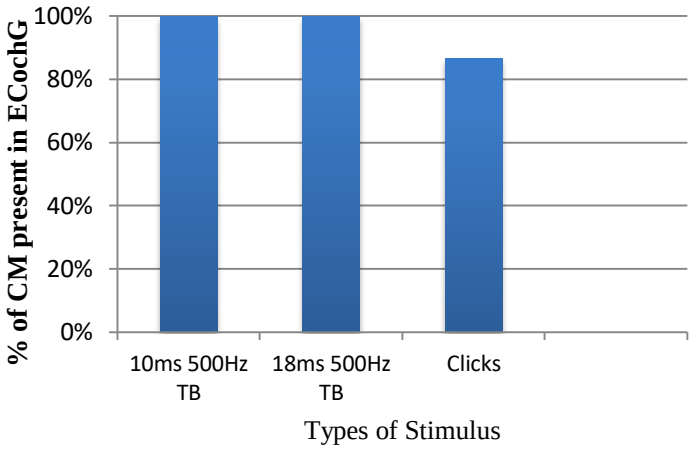

Figure 4.2: Representing the percentage of CM present/occurrence in ECochG protocol across stimulus.

4.3. Comparison of occurrence of CM recorded using ABR and ECochG protocol

The third objective of the current study was a cumulative comparison of the percentage of occurrence of CM in ABR and ECochG protocol. The total percentage of occurrence of CM potential was compared between the protocols across three different stimuli i.e., 500Hz tone burst of 10ms, 500Hz tone burst of 18ms and clicks of 0.1ms duration. Results revealed that robust, replicable and stable CMs were present in 100% of the subjects (n=30) when recorded with 500 Hz tone burst of 10ms in both ABR and ECochG protocol. Similarly CMs were also present in 100% of the subjects (n=30) when recorded with 500Hz tone burst of 18ms duration equally in both ABR and ECochG protocol. CM recorded with click stimulus of 0.1ms duration were present in about 80% of the subjects (n=24) in ABR protocol and was slightly higher in ECochG protocol which is about 86.3% of the subjects (n=26). No statistical tests were used for this comparison. Figure 4.3 depicts the percentage of occurrence of CM elicited using both the protocols.

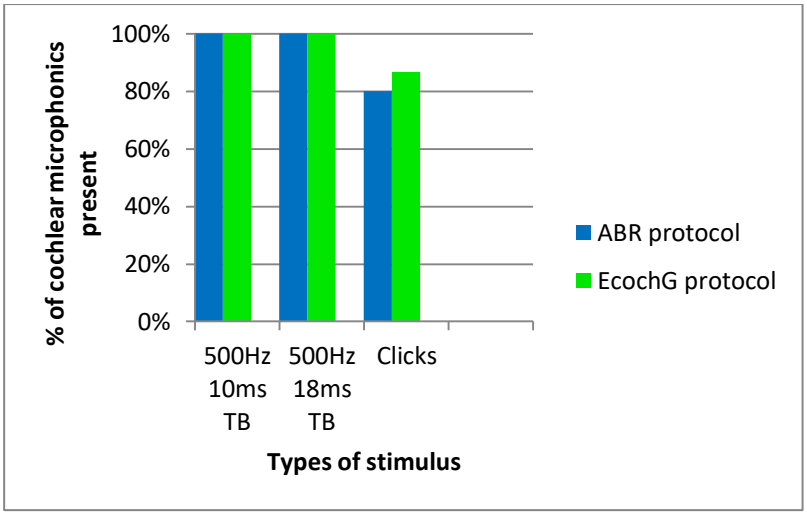

***Figure 4.3*:** Percentage of occurrence of CM in ABR and ECochG protocol across 3 different stimuli.

4.4 Effect of stimulus and recording protocol on parameters of Cochlear Microphonics.

Descriptive statistics was carried out to find the mean and standard deviation of onset latency and amplitude of CM recorded using 500Hz tone burst of 10ms, 500Hz tone burst of 18ms and clicks of 0.1ms. Shapiro Wilk test of normality showed normal distribution of data ($p > 0.05$). Hence, parametric tests were carried to see the significant effect of different variables on different parameters of CM. The CM was compared between different stimuli within the protocol by noting the amplitude and onset latency of CM waveforms (as defined in the method section) and also CM parameters were compared between ABR and ECochG protocol for respective stimulus.

4.4.1 Onset latency of CM recorded across different types of stimuli using ABR and ECochG protocol

In ABR protocol (scalp recording), the onset latency of the CM was earlier when the stimulus used to record was clicks (0.61ms) compared to tone bursts. Amongst two different durations of tone bursts of 500Hz, 18ms long duration tone burst had earlier latencies (2.57ms) compared to 10ms short duration tone burst (2.91ms).

The onset latency of CM recorded using ECochG protocol also showed similar results. The latency of CM was earlier when clicks (0.65ms) was used to record compared to tone bursts. Amongst 500 Hz tone burst of different duration, tone burst with 18ms longer duration had earlier latencies (2.31ms) compared to tone burst of 10ms shorter duration (2.62ms). Mean and standard deviation of onset latency for different stimulus used for recording CM in ABR protocol and ECochG protocol is given in Table 4.1.

Table 4.1

Mean and Standard deviation of onset latency of CM for different stimuli obtained in ABR protocol and ECochG protocol

	ABR protocol		ECochG protocol	
	Mean	Standard Deviation	Mean	Standard deviation
Toneburst 500Hz-(10msec)	2.91	1.16	2.62	1.12
Toneburst 500Hz-(18msec)	2.54	1.32	2.31	1.31
Click-100µsec	0.61	0.61	0.65	0.57

4.5 Amplitude of CM recorded across different types of stimulus using ABR and ECochG protocol

In ABR protocol, the CM recorded with click stimulus had lesser amplitude (0.06µV) compared to CM amplitude recorded using tone burst. Amongst the two different duration of 500 Hz tone burst, 10ms short duration tone burst elicited slightly higher amplitudes (2.62µV) compared to 18ms longer duration tone burst (2.31µV).

In ECochG protocol, CMs recorded with click and tone burst also showed similar results. CMs recorded with click stimulus showed lesser amplitude (0.08µV) when compared to tone bursts. Amongst the two different durations of tone burst of 500 Hz, longer duration (18ms) tone burst showed slightly lesser amplitude CM (0.25 µV) unlike shorter duration (10ms) tone burst of 500Hz with a mean amplitude

of 0.28 µV. Mean and standard deviation of amplitude for different stimulus used for recording CM in ABR protocol and ECochG protocol is given in Table 4.2.

Table 4.2

Mean and standard deviation of amplitude of CM for different stimuli obtained in ABR protocol and ECochG protocol

	ABR protocol		ECochG protocol	
	Mean	Standard Deviation	Mean	Standard deviation
Toneburst 500Hz-(10msec)	0.22	0.07	0.28	0.088
Toneburst 500Hz-(18msec)	0.21	0.06	0.25	0.087
Click-100µsec	0.06	0.04	0.08	0.056

4.6 Comparison of different parameters of CM

In order to optimize the best stimulus to record CM, onset latency and amplitude recorded using various stimuli were compared. The data showed normal distribution ($p > 0.05$) on Shapiro Wilk test of normality. Hence, to see significant effect of stimulus type (click, 500Hz tone burst of 10ms and 500Hz tone burst of 18ms), repeated measures ANOVA (analysis of variance) was administered for amplitude and latency measures of the CM in both ABR protocol and ECochG protocol, separately.

4.7 Comparison of onset latency of CM across different types of stimuli within protocol.

4.7.1 In ABR protocol

The repeated measures of ANOVA for onset latency of CM elicited across stimuli (clicks, 10ms and 18ms 500 Hz tone burst) in ABR protocol showed a significant main effect of the stimulus [$F(2, 58)= 48.090$, $p< 0.05$]. Further post hoc analysis to account for a significant difference in onset latency obtained between the types of stimulus, Bonferroni's test was done. It revealed that CM onset latency was significantly earlier when recorded with clicks ($p<0.05$) than CM recorded using tone bursts. Between 10ms and 18ms tone burst of 500Hz the results showed no significant differences ($p> 0.05$) in the onset latency of CM.

4.7.2 In ECochG protocol

The analysis of the onset latency parameter between clicks and two different duration of 500Hz tone burst in ECochG protocol, showed the significant main effect of the stimulus [$F(2, 58)= 37.133$, $p< 0.05$]. Additional post hoc analysis using Bonferroni's test revealed similar results across three different stimulus regarding CM onset latency as observed for ABR protocol. CM onset latency were significantly earlier when recorded with clicks ($p<0.05$) than CM recorded using tone bursts. The Onset latency of CM was much delayed/longer when recorded with tone burst of 500Hz. Amongst 10ms and 18ms tone burst of 500 Hz the results showed no significant differences ($p> 0.05$) in the onset latency of the CM.

4.7.3 Comparison between onset latency of CM recorded using ABR and ECochG protocol at various stimuli

The paired sample t-test was done to compare the onset latency of CM between ABR and ECochG protocol at each stimulus. The results revealed that CM onset latency recorded using 500Hz tone burst of 10ms in ECochG protocol was significantly earlier, [t(29)= 3.168, $p< 0.05$] than that of ABR protocol. CM onset latency in ECochG protocol recorded using 18ms tone burst of 500 Hz was also significantly earlier [t (29)= 3.282, $p <0.05$] than that of ABR protocol. The onset latency of click evoked CM in ECochG protocol and ABR protocol showed no significant difference [t (29) = -1.045, $p >0.05$]. Figure 4.4 depicts the comparison of mean onset latency of cochlear microphonics using different stimuli in ABR and ECochG protocol.

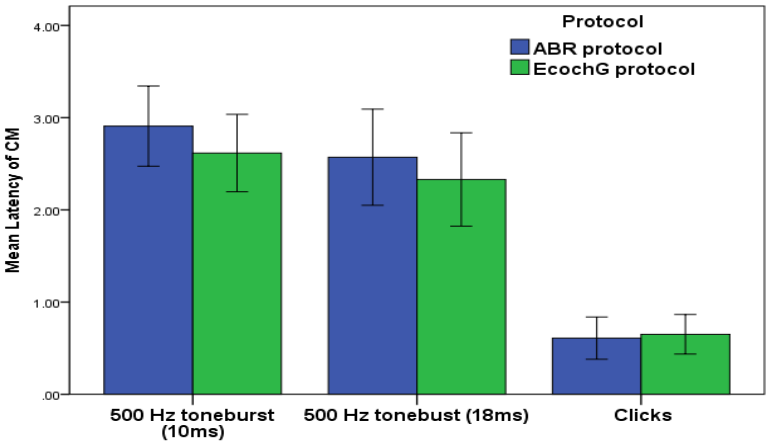

***Figure 4.4*:** Comparison of mean latency of CM in milliseconds with 95% confidence interval between ABR and ECochG protocol across different stimuli.

4.8 Comparison of amplitude of CM across type of stimulus within protocol.

4.8.1 In ABR protocol

Repeated measures of ANOVA was done to check the significant main effect of stimulus type on amplitude of CM recorded in ABR protocol, and results showed a significant main effect of the stimulus [$F(2, 58) = 110.336$, $p < 0.05$]. Since results of repeated measures of ANOVA showed a significant effect across three stimulus conditions, post hoc analysis were carried out using Bonferroni's test.

The paired wise comparisons were made between the amplitude of CM recorded using three different types of stimulus in ABR protocol. It showed the amplitude of CMs was significantly higher ($p < 0.05$) when recorded with 500Hz tone burst stimulus of 10ms duration than with clicks. Also, the amplitude of CM recorded using 500Hz tone burst of 18ms duration was higher and showed a significant difference ($p < 0.05$) when compared with clicks. However, there was no significant difference found between 500Hz short and long duration tone bursts ($p > 0.05$).

4.8.2 In ECochG protocol

Repeated measures of ANOVA results for the amplitude of CM recorded using three different stimuli in ECochG protocol also showed a significance main effect of the stimulus [$F (2, 58) = 128.667$, $p < 0.05$]. Post hoc analysis using Bonferroni test, showed that CM of both 500Hz tone burst of 10ms and 18ms duration had significantly higher amplitudes ($p < 0.05$) compared to the amplitude of click evoked CM. When compared between the 500 Hz tone burst of both durations, the 10ms tone burst had significantly higher amplitudes of CM ($p < 0.05$) than 18ms

duration tone burst in ECochG protocol. Such a significant difference in amplitude of CM between 2 different durations of tone bursts was not found in ABR protocol.

4.8.3 Comparison between the amplitude of CM recorded using ABR and ECochG protocol at various stimuli

The paired sample t-test was done to compare the amplitude of CM between ABR and ECochG protocol at each stimulus. Results showed that CM recorded using 500 Hz tone burst of 10ms in ECochG protocol was significantly higher [t (29) = -5.484, $p< 0.05$] than that of ABR protocol. The amplitude of CM recorded using 500 Hz tone burst of 18ms was significantly higher in ECochG protocol [t (29) = -4.430, $p< 0.05$] than in ABR protocol. Also, the amplitude of CM in ECochG protocol recorded using clicks was significantly higher [t (29) = -3.597, $p< 0.05$] than ABR protocol. Figure 4.5 depicts the mean amplitude of CM across different stimuli used in ABR and EcochG protocol.

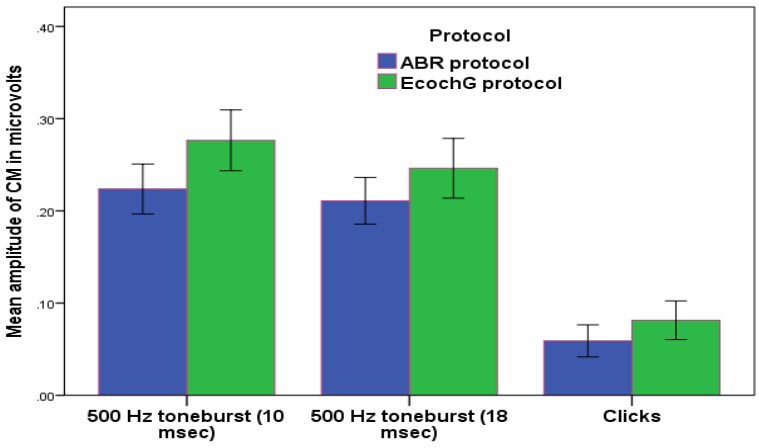

Figure 4.5: Comparison of mean amplitude of CM in microvolts with 95% confidence interval between ABR and ECochG protocol across different stimuli.

The results in the present study, therefore directed towards a focused outlook on optimization of the protocol in order to record CM in the interest of assessing the functional integrity of cochlea.

4.9 Optimizing the protocol for recording CM

The analyses of data, therefore give a vivid idea of what parameters should be focused in order to record and to interpret CM efficiently. It is also well understood that importance should be given to its presence/occurrence, having better morphology, more amplitude, which would help in better identification of CM. However, the latency of CM may not be called as significant parameter as it is a pre-neural potential which is insignificantly affected by any eliciting parameter. In the present study, ECochG protocol was not seen as a standalone protocol to visualize CM. ABR protocol also showed the equal occurrence of CM with appropriate amplitude therefore, indicating that both ECochG and ABR protocol are feasible for recording CM. It is also clear from the results that tone bursts of 500 Hz, evoked the highest amplitudes of CM with good morphology. Clicks however, showed an

entirely different picture wherein CM waveforms were not recorded with good morphology, which is evident in figure 4.6. It is also important to focus on the fact that the amplitude of CM using 10ms 500 Hz tone burst was significantly higher than 18ms 500 Hz tone burst. However, 10ms and 18ms duration of 500Hz tone burst didn't show statistical significance in ABR protocol. Hence, it can be concluded that both the duration of TBs can be used to record CM in ABR protocol. All these points ultimately go to say that the stimulus which can produce the highest amplitude along with the best morphology and suited protocol that yields the highest occurrence of CM should be considered for clinical use.

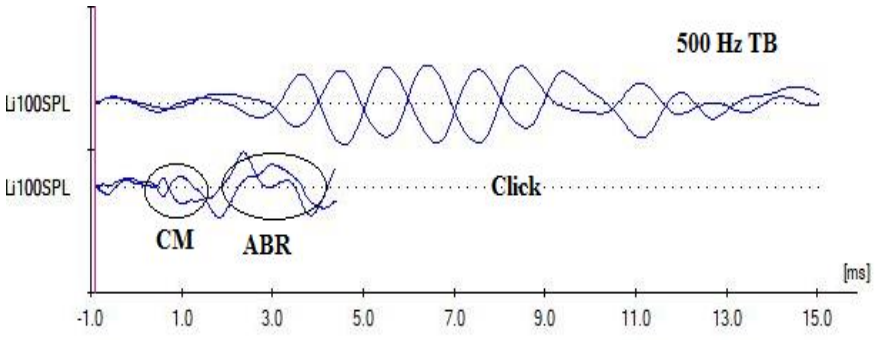

Figure 4.6: CM waveform recorded using 500 Hz TB of 10ms and clicks.

In summary,

- CM was present in 100% of the participants in both ABR and ECochG protocol when 10ms and 18ms duration of 500 Hz tone bursts were used.
- For the click stimuli, the presence of CM in ECochG protocol was slightly higher (86.7%) than in ABR protocol (80%).

- The amplitude of CM recorded using 10ms tone burst of 500 Hz, 18ms tone burst of 500 Hz and clicks of 0.1ms, in ECochG protocol was significantly higher than CM of ABR protocol.
- The amplitude of CM recorded using 10ms tone burst of 500Hz was significantly higher than 18ms tone burst of 500 Hz in ECochG protocol only.
- Click evoked CM had the least amplitude among three different stimuli used in both ECochG and ABR protocol.
- Click evoked CM had significantly earlier onset latency when compared with the onset latency of both the duration of tone bursts in both ABR and ECochG protocol.
- No significant difference found in the onset latency of click evoked CM between ABR and ECochG protocol.
- No significant difference found in the onset latency of CM recorded between 10ms tone burst of 500 Hz and 18ms tone burst of 500 Hz in both ABR and ECochG protocol.

Chapter 5

Discussion

To fulfil the aim of the study, 30 healthy individuals with normal hearing sensitivity were included as participants. All the 30 participants underwent testing using ABR and ECochG protocol to record CM. CM was also recorded using different types of stimuli of two different durations. This data was then analyzed and results revealed significant findings. The possible reasons that can be attributed to such revelation in the results are discussed under each objective of the study.

5.1 Percentage of occurrence of CM in ABR and ECochG protocol

It was seen that the occurrence/presence/prevalence of CM was 100% when CM was recorded using a 500 Hz tone burst of both duration using ABR and ECochG protocols. Meanwhile, when CM was recorded using click stimuli, it was noticed that only 80% of the participants had CM when recorded using ABR protocol and 86.6% of the participants had CM when recorded using ECochG protocol.

Although research investigation has not reported of any such finding which has compared the occurrence of CM between the protocols set in ABR and ECochG, the reason of 100% occurrence in tone burst versus reduced occurrence of CM for clicks can be attributed to the fact that with the increase in frequency the occurrence of CM reduces. This can be supported by investigations done by Zhang (2012) that, amplitude and identification of the CM reduced, as a function of frequency. It can also be said with certitude that clicks evoke response mainly from the higher frequency region in the basilar membrane (Tognola, Grandor & Ravazzani, 1997;

Ohashi, Ochi, Nishino, Kenmochi & Yoshida, 2005) therefore it is only reasonable to suggest that clicks have lesser likelihood for contribution to CM occurrence as high frequency region has lesser number of sensory cells than apical region (Bredberg, 1968). Also shreds of evidence show that identifying CM becomes difficult and shows no obvious peak of CM ≥2 KHz (He, Porsov, Kemp, Nuttall & Ren, 2013).

The current study is in slight disagreement with the study done by Coraci (2019), where he compared the occurrence of ear canal recorded CM between clicks and tone bursts stimulus. The difference in the studies can be because of clear demarcations such as usage of stimulus intensity of 100 dBnHL to record click evoked CM and only 80 dBnHL to record tone burst evoked CM. Usage of high band pass filter of 3.3Hz or 100Hz to record CM for click and tone burst. Majorly present study used 500 Hz tone burst wherein 2000 Hz tone burst in the Coraci (2019) study.

5.2 Amplitude of CM recorded using ABR and ECochG protocol

The amplitude of CM across all three different stimuli (500 Hz TB-10ms, 500 Hz TB- 10ms and clicks) was significantly higher ($p<0.05$) in ECochG protocol when compared to ABR protocol. Similar results have been reported in the investigations of Riazi and Ferraro (2008), where they found amplitude of ear canal recorded was higher than the amplitude of mastoid recorded CM.

By the logical explanation for such a finding can be understood to be as the distance between the active electrode and site of generation of the CM potential. The current study can clearly state that in ECochG protocol, the active electrode was placed inside the ear canal of the participants, thereby reducing the distance of the

electrode to the outer hair cells which is the source of generation of CM, and this might be the reason for resulting higher amplitude. However, the active electrode in the ABR protocol was placed on the mastoid process of the participants resulted in increasing the distance between the sites of generation to the active electrode. This might have resulted in the reduced amplitude of the CM. Other studies that showed a direct relationship between the amplitude of potential and distance between the active electrode and site of generation in their investigations are Ruth, Lambert and Ferraro, 1988; Ruth and Lambert, 1989; Bonucci and Hyppolito, 2009; Zhang, 2010; Coraci, 2019.

5.3 Amplitude of CM recorded across different types of stimulus using ABR and ECochG protocol

The result in the present study revealed that the amplitude of CM recorded using both the protocol (ABR & ECochG) for the stimuli of 500 Hz tone burst of 10ms and 18ms durations were significantly higher ($p<0.05$) than the amplitude of the click evoked CM. The results are in accordance with the investigations carried out by Zhang (2012, 2013), where he found amplitude of CM recorded using 14ms and 6ms 500Hz tone burst was significantly higher than clicks. The results of the current study are in disagreement with the findings of Heidari, Pourbakth, Kamrava, Kamalo and Yousefi (2018) and Coraci (2019). These studies suggest the use of clicks for the better amplitude of CM. Even the British society of Audiology (2019) is in favors of using clicks for the recording of CM. The reason that they provide for using the click as an ideal stimulus is that they argue that click is a broadband stimulus and can provoke a large number of hair cells at the same point of time, resulting in a high amplitude CM. However, the current study strongly supports the evidence of Zhang (2012, 2013) and speaks in favor about the fact of decrement seen

in the amplitude of CM w.r.t to frequency i.e., the higher the frequency lesser the CM amplitude. And as discussed earlier, there is enough evidence in review to strongly stand on the points that click is sensitive to provoke the base of the basilar membrane which in turn responds for high frequency stimuli (Grandori, Ravazzani & Tognola, 1995; Ohashi, Ochi, Nishino, Kenmochi & Yoshida, 2005). It can also be reasoned out that amplitude patterns on basilar membrane are spatially distributed where maximum displacement is dependent on the frequency of excitation. Click stimulus has relatively high frequency spectrum such reasoning's can explain as to why clicks failed to evoke high amplitude CM.

The explanation for yielding robust, replicable and stable amplitudes of CM using low frequency tone burst can be explained through anatomical variations along the basilar membrane and the non-linear property of the cochlea. The displacement amplitude of the basilar membrane at the apical end is much greater than basal end since the apical end is relatively much less stiff (Bekesy, 1960). The displacement of the basilar membrane is in direct relation to the amplitude of CM. This anatomical evidence could possibly explain the enhancing the amplitude of low frequency tone burst evoked CM. Also, the number of hair cells varies along the length of the basilar membrane. More hair cells are found on the apical end of the basilar membrane. About 3 to 5 rows of outer hair cells can be found at the apex (Retzius, 1881). The volume and length of hair cells at the apical end of the basilar membrane which is much greater than at basal end (Bredberg, 1968), could probably the reason for the greater amplitude of CM for a low frequency tone burst stimulus.

Amplitude of 500Hz 10ms vs. 500Hz 18ms tone burst.

Though there was no difference ($p>0.05$) in the amplitude of CM recorded by 10msec and 18msec 500Hz tone burst frequency in ABR protocol, however, there was a significant difference ($p<0.05$) in the amplitude of CM evoked by 10ms 500 Hz TB being greater than 500 Hz 18ms tone burst in ECochG protocol. The results of the current study contradict observations of Zhang (2012). Zhang (2012) used two durations of 14ms and 6ms 500Hz tone burst. The study revealed a robust amplitude of CM when recorded using 14ms 500 Hz tone burst in ear canal. This finding poses disagreement to the present study which can be due to variation in the objective analysis of amplitude parameter where peak to peak amplitude of single highest peak in the waveform of CM was considered to be the amplitude of CM wherein in the present study peak to peak average of 3 consecutive, robust and stable peaks of CM is considered as the amplitude of CM.

The reasons of why the current study found higher amplitude for shorter duration tone burst (10ms) in ECochG protocol can be explained if considered the energy spectrum of the tone burst. Based on the duration of the TB, a certain amount of spectral splatter will always result in the energy that will spread to various neighboring distant frequencies starting from the nominal frequency of tone burst (Davis, Hirsh, Popelka & Formby, 1984). It is also a fact that longer the duration of the tone burst, narrower will be the spectrum hence, lesser the spectral splatter. This logically explains that 10ms tone burst might have resulted in higher splatter, therefore, excited a higher number of hair cells on the basilar membrane and resulted in a higher amplitude of CM.

5.4 Onset latency of CM recorded using ABR and ECochG protocol

From the results of this section, it can be deciphered that the onset latency of 500 Hz 10ms TB and 18ms 500 Hz TB CM recorded in ECochG protocol were significantly earlier than the onset latency of CM recorded in ABR protocol. Also, the onset latency between 500 Hz 10ms and 18ms evoked CM showed no significant difference in both ABR and ECochG protocol independently. Such findings are also reported in a study conducted by Zhang (2013). The current study showed significant earlier onset latency of CM when it was recorded with click stimulus than tone bursts. These findings are in consonance with the results of the study done by Zhang (2013) and Coraci (2019). The onset latency of CM was earlier when click stimulus was used than tone bursts even in their study.

CM doesn't really show a latency effect since they are generated by hair cells and are preneural in nature. It can be understood that it should be present/evoked as soon as the recording stimulus is present and as long as the recording stimulus is present (Zhang, 2013). The rationale behind such a finding can be described through Bekesy's travelling wave theory. The Basilar membrane is narrower at the base, and gets wider towards the apical region. Because of this anatomical variation along the length of the basilar membrane, there is a gradation of stiffness on the basilar membrane where it is stiffest near the base and gets least stiff towards the apex (Bekesy, 1960). As a result of this gradation in stiffness, an acoustical signal which enters the inner ear always travels from the basal region of basilar membrane to apical region as a special kind of wave pattern. High frequencies are represented towards the base of the basilar membrane, and successively lower frequencies are represented towards the apex (Bekesy, 1960). As clicks energy spectrum majorly exhibits in high frequency region (Grandori,

Ravazzani & Tognola, 1995; Ohashi, Ochi, Nishino, Kenmochi & Yoshida, 2005), it will reach the base earlier compared to low frequency stimulus where stimulus translates into a place of stimulation (Tondorf, 1960). Hence, click exhibits earlier onset latency than tone bursts (low frequency) evoked CM.

The possible reason for not finding a significant difference between the onset latency of CM recorded by 500 Hz 10ms and 18ms tone burst can be explained through the best frequency location where signal frequency translated into a place of stimulation. Since the generation of CM potential is the same location on the basilar membrane for both 10msec and 18msec 500 Hz tone burst, hence a significant latency difference could not be observed.

The significant difference in onset latency of CM recorded between the protocol could be attributed to the fact that, the active electrode to record CM in both the protocol was different. In ECochG protocol active electrode placed was much closer to the site of CM generation than the electrode site of ABR protocol. Thus the time taken for the generated potential to reach the electrode was faster in ECochG protocol leading to significantly shorter latency.

Based on the results and looking broadly onto the possible reasons for findings in the current study, it can be recommended that in order to record CM with the best morphology and amplitude, either ABR or ECochG protocol can be used. However, the study would like to highlight the recoding of CM made handier and less time consuming if done alongside an ABR testing, which can now be possible with the optimized protocol advocated in the current research investigation. The choice of stimulus for recording CM should be 500Hz tone burst of either 10ms or 18ms duration as per the current study; meanwhile, if recording using ECochG

protocol, a short duration low frequency tone burst is endorse such as 500Hz 10ms tone burst likely as per the present study. This will ensure the procurement of CM in an utmost manner with the highest amplitude. From the findings of the study it is also suggested that the wave morphology of tone burst of 500Hz frequency was superior to what was recorded by clicks giving another reason to use tone burst to record CM. Another observation noticed was that there was a direct relationship between tone burst duration and number of sinusoidal peaks of CM, adding more evidence to use tone burst instead of clicks. Additionally, a very interesting point to focus on the duration of tone burst and therefore which is better to choose, the longer or shorter duration, (10ms or 18ms) definitely poses a dilemma as in the study it was observed that there was a better identification of CM with 10ms tone burst as they gave higher CM amplitudes while the findings with 500 Hz tone burst of 18ms was that presence of CM was prolonged adding to better and easy identification of CM. Therefore the study would likely recommend the usage of both or either duration in order to record the best CM. Another useful insight aiding in the process of optimization was the recording of CM in both the polarities and clamped response for better identification, differentiation and minimize the contamination from stimulus artifacts. It is indeed very necessary to be mindful regarding the filter settings used for the recording. Usage of 300Hz to 700Hz offline filtering was critical and therefore recommended because it could help in the removal of 60Hz electrical artifacts, posterior auricular muscle (PAM) artifacts and other myogenic potential, which could potentially mask CM and pose problems in analyzing CM waveform.

Chapter 6

Summary and Conclusion

Cochlear Microphonics is a part of Electrocochleography (ECochG) which is usually generated by the outer hair cells. Over the years, CM lost its charm due to tediousness in its recording procedure and failure to observe its occurrence as it's known to mimic the stimulus characteristics. However, given the recent years, CM has managed to regain its popularity amongst the scientific fraternity in the field of audiology as an answer to the shortcomings of recording OAE, especially in the low frequencies under noisy situations. This created a new genre of interest to set an appropriate stimulus and acquisition parameters for the ease of recording and visualizing CM in the best manner possible. The present study was decided to be done in the same line of interest and expand to create an optimized protocol with an accurate approach towards the recording parameters to be used for recording CM. Hence, the aim of the current study was to record CM using ABR protocol and ECochG protocol using various types and duration of the stimulus (clicks-0.1ms, tone burst- 10ms and tone burst- 18ms) and compare the response rate and different parameters of CM, thus, arrive at a suitable stimulus protocol to record CM.

In order to fulfill the aim, 30 healthy adult participants were taken in the age range of 15-25 years (mean age = 20). All the participants underwent recording of CM in ABR protocol and ECochG protocol. The three different stimuli used for recording CM are 500 Hz -10ms tone burst, 500 Hz – 18ms tone burst and clicks of 0.1ms in both ABR (scalp recording) and ECochG protocol (intracananicular recording).

The non-inverting electrode (positive) was placed on the mastoid of the test ear in ABR protocol and inside the ear canal (TIPtrode) in ECochG protocol. The inverting electrode (negative) was placed on the mastoid of the non-test ear and the ground electrode was placed on the upper forehead in both ABR and ECochG protocol. A calibrated constant high level stimulus of 100 dB SPL was delivered through default insert ear phones (Etymotic ER-3A) across various stimuli to evoke the CM. The tone bursts were gated through the Blackmann window such that the stimulus duration for short duration tone burst was 10ms (4ms-rise/fall time and 2ms-plateau time) and for long duration tone burst was 18ms (4ms-rise/fall time and 10ms-plateau time). Filter settings and amplification for CM was set at 100 Hz to 3000 Hz for clicks and 100 Hz to 800 Hz for tone bursts in both ABR and ECochG protocol. Thousand five hundred sweeps of stimuli were delivered at the rate of 30.1/s for each stimulus. The recorded waveforms of CM were offline filtered through band pass filter of 300 Hz to 700 Hz for tone bursts and 300 to 3000Hz for click stimulus. Later, the amplitude of CM in each stimulus was calculated after taking the average peak to peak amplitude of 3 consecutive, robust and stable peaks of CM. The onset latency of CM in each stimulus was decided after setting a criterion point.

The percentage of occurrence of CM in ABR protocol and ECochG protocol across three different stimuli was calculated. Later, the subjective comparison of occurrence of CM across stimulus within the protocol and also at each stimulus recorded between ABR and ECochG protocol was carried out.

A repeated measure of ANOVA followed by Bonferroni test was done to see the effect of stimulus and also between the stimulus effects within the protocol, respectively for onset latency and amplitude and of CM. A paired sample t-test was

done to see the significant effect of protocol at each stimulus condition. All statistical analyses were done using SPSS software version 20.

Results showed that CM was present in 100% of the population in both ABR and ECochG protocol when 10ms and 18ms duration of 500Hz tone bursts were used. With click stimuli, the CM occurrence in ECochG protocol was relatively higher (86.7%) than in ABR protocol (80%). The CM amplitude recorded using ECochG protocol at 10ms tone burst of 500 Hz, 18ms tone burst of 500Hz and clicks of 0.1msec was significantly higher than CM recorded using ABR protocol. Similarly, the amplitude of CM recorded using 10ms tone burst of 500Hz was seen to be significantly higher than 18ms tone burst of 500 Hz in ECochG protocol only. Amongst all the three stimuli used for recording in both ECochG and ABR protocol, click evoked CM had the least amplitude. The onset latency of CM recorded using a 10ms tone burst of 500 Hz, 18ms tone burst of 500 Hz in ECochG protocol was observed to be significantly earlier than CM latency of ABR protocol. However, no significant difference found in the onset latency of click evoked CM between ABR and ECochG protocol.

The possible reasons of these findings in the present study are attributed majorly to the understanding of the stimuli and how it is coded on the basilar membrane with regards to the various parameters of the stimuli. It was already clear from the previous studies that increasing frequency leads to a reduced occurrence of CM (Zhang, 2012). The present study, therefore, tried to relate the finding to a logical anatomical outlook, wherein reasons which were given for the occurrence of CM, in 100% of the participants using tone burst of both the duration in both the protocols was considered due to the usage of low frequency stimuli. However, this was not the case using click stimuli which are known to excite the high frequency

regions on the basilar membrane. Low frequencies exhibiting higher amplitude could be due to the more number of hair cells at the apical part than the basal part.

The reason for higher amplitude of CM when recorded across three stimulus using ECochG protocol than ABR protocol was thought to be due to the active electrode place and its direct relationship to the site of generation of the CM. The latency was concluded as an unimportant factor to note the occurrence of CM. This was in unison with the other studies, which also said that the latency does not hold an essential parameter of CM. The reason, however, for earlier latency when recorded using click was a rationale to the fact that clicks are coded in the basal end which is nearer than the apical end, which is considered to code for the low frequency, therefore delaying the onset latency of 500 Hz tone burst.

Conclusion

It can be thus concluded that the CM recorded using both ABR and ECochG protocol with tone burst stimulus of both durations was present in 100% of the normal hearing population with good morphology and amplitude. However, with clicks, there was less than 100 % occurrence of CM with poor morphology and amplitude. The results of the study were successful in finding an optimum stimulus best suitable for recording the CM and concluded that recording CM with ABR protocol, preferably with tone burst of either duration. This method has its advantages and reliability. The study also concluded that if recording CM using ECochG protocol, its best to use short duration (10ms) tone burst for good morphology and amplitude with a mindful offline filter setting of 300 Hz to 700 Hz for better identification of CM. In comparison, the findings with 500 Hz tone burst of 18ms were that the presence of CM was prolonged, adding to better and easy

identification of CM. Therefore the study would likely recommend the usage of both or either duration of low frequency tone burst to record the best CM.

Clinical Implications of the study

- The results of the study suggest that 500 Hz tone burst as the preferred stimulus to record CM.
- The study also leads to show the advantages and reliability of recording CM with ABR protocol in a manner that the audiologist now will no longer require to change the electrode placement on the subject of interest minimizing the time taken to clean and set the electrode in place, after recording ABR.
- This study also tried to explore the best suitable filter settings for easy recording of the CM, to be used with ABR protocol and ECochG protocol as the identification of CM of 500 Hz was better with a filter setting of 100Hz to 800 Hz and offline filtering to be done with 300 Hz to 700 Hz for better morphology and identification.

Limitations and future directions

The study would have provided better generalization if a larger sample group was taken. The study also did not take tone burst of a high frequency along with low frequency, which could provide elaborate reliability recording CM in ABR protocol. The study also did not correlate behavioral thresholds with the thresholds of CM, which can also be a future goal of investigation. In future, the feasibility of recording CM under the mentioned protocol of the present study in the disordered population can also be explored.

REFERENCES

American national Standard Institute, 1999. Maximum permissible noise levels for audiometric test rooms. New york. ANSI S3.1-1999.

Andreeff, A. M., Aranova, A. A., & Gerschuni, G. V. (1939). *On the electrical potentials of the human cochlea*. 26, 205.

Aran, J. M., & Lebert, G. (1968). Les reponses nerveuses cochléaires chez l'homme, image du fonctionnement de l'oreille et nouveau test d'Audiométrie objective. *Rev Laryngol Otol Rhinol*. 89, 361-378.

Arts, H. A. (1990). Influence of perilymphatic tetrodotoxin and calcium concentration on hair cell function. *In Assoc. Res. Otolaryngol. Abstract*, 13, 194.

Beatty, D. L., & Simmons, F, B. (1962). The Significance of Round Window-Recorded Cochlear Potentials in Hearing: An Autocorrelated Study in the Cat. *Annals of Otology, Rhinology & Laryngology*, 71(3), 767-800.

Beagley, H. A., & Gibson, W. P. R. (1976). Electrocochleography in the diagnosis of acoustic neuroma. *The Journal of Laryngology & Otology*, 90(2), 127-139.

Berlin, C. I., Hood, L., Morlet, T., Rose, K., & Brashears, S. (2003). Auditory neuropathy/dys-synchrony: Diagnosis and management. *Mental retardation and developmental disabilities research reviews*, 9(4), 225-231.

Bredberg, G. (1968). Cellular pattern and nerve supply of the human organ of Corti. *Acta Oto-Laryngol Supply*, *236*, 1–135.

British Society of Audiology, (2019). Recommended procedure: cochlear microphonic testing [guideline]. Retrieved from

https://www.thebsa.org.uk/wpcontent/uploads/2019/01/FINAL-JAN2019-Recommended-Procedure-for-CochlearMicrophonic-Testing-GL21-01-19.pdf

Burkard, R. F., Eggermont, J. J., & Don, M. (2007). *Auditory Evoked Potentials: Basic Principles and Clinical Application.* Philadelphia: Lippincott Williams & Wilkins. 536-544.

Coraci, L. M. (2019). *Electrocochleography (ECochG) with a non-invasive tympanic membrane (TM) electrode in normally-hearing subjects.* Radboud University, Nijmegen , Netherlands.

Cullen, J. K., Ellis, M. S., Berlin, C. I., & Lousteau, R. J. (1972). Human acoustic nerve action potential recordings from the tympanic membrane without anesthesia. *Acta Oto-Laryngologica,* 74(1-6), 15-22.

Davis, H., Hirsh, S. K., Popelka, G. R., & Formby, C. (1984). Frequency selectivity and thresholds of brief stimuli suitable for electric response audiometry. *Audiology*, 23(1), 59-74.

Deltenre, P., Mansbach, A. L., Bozet, C., Christiaens, F., Barthelemy, P., Paulissen, D., & Renglet, T. (1999). Auditory Neuropathy with Preserved Cochlear Microphonics and Secondary Loss of Otoacoustic Emissions. *Audiology, 38*(4), 187–195.

Ferraro, J. A., & Durrant, J. D. (2006). Electrocochleography in the evaluation of patients with Meniere's disease/endolymphatic hydrops. *Journal of the American Academy of Audiology*, 17(1), 45-68.

Ferraro, J. A., & Ruth, J. A. (1994). Electrocochleography. In: J. T. Jacobson (Ed.). (1994). *Principles and applications in auditory evoked potentials*. Upper Saddle River, New Jersey: Prentice Hall. 145-147.

Fromm, B., Nylen, C. O., & Zotterman, Y. (1935). Studies in the mechanism of the Wever and Bray effect. *Acta oto-laryngologica*, 22(3), 477-486.

Ge, N. N., Orchik, D. J., & Shea, J. J. (1997). Cochlear microphonics in Ménière's disease. *The American journal of otology*, 18(1), 58-66.

Grandori, F., Ravazzani, P., & Tognola, G. (1995). An optimal filtering technique to reduce the influence of low-frequency noise on click-evoked otoacoustic emissions. *British journal of audiology*, 29(3), 153-160.

Heidari, F., Pourbakth, A., Kamrava, S. K., Kamalo, 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.

He, W., Porsov, E., Kemp, D., Nuttall, A. L., & Ren, T. (2012). The group delay and suppression pattern of the cochlear microphonic potential recorded at the round window. *Public Library of Science One*, 7(3).

Hood, L. J., Katz, J., Chasin, M., English, K. M., & Tillery, K. L. (2009). *Handbook of clinical audiology*. Philadelphia: Wolters Kluwer Health. 251-252

Jijo, P. M., & Yathiraj, A. (2012). Audiological characteristics and duration of the disorder in individuals with auditory neuropathy spectrum disorder (ANSD) – a retrospective study. *Journal of Indian Speech Hear Association*, 26(1), 17-26.

Kemp, D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *The Journal of the Acoustical Society of America*, 64(5), 1386-1391.

Lempert, J., Meltzer, P. E., Wever, E. G., & Lawrence, M. (1950). The cochleogram and its clinical application: concluding observations. *Archives of Otolaryngology*, 51(3), 307-311.

Liberman, M. C., Zuo, J., & Guinan Jr, J. J. (2004). Otoacoustic emissions without somatic motility: can stereocilia mechanics drive the mammalian cochlea?. *The Journal of the Acoustical Society of America*, 116(3), 1649-1655.

Norton, S. J., Ferguson, R., & Mascher, K. (1989). Evoked otoacoustic emissions and extratympanic cochlear microphonics recorded from human ears. *Abst Assoc Res Otolaryngoly*, 12, 227.

Narne, V. K., Prabhu, P., Chandan, H. S., & Deepthi, M. (2014). Audiological profiling of 198 individuals with auditory neuropathy spectrum disorder. *Hearing, Balance and Communication*, *12*(3), 112-120.

Ohashi, T., Ochi, K., Nishino, H., Kenmochi, M., & Yoshida, K. (2005). Recovery of human compound action potential using a paired-click stimulation paradigm. *Hearing research*, 203(1-2), 192-200.

Osman Dabbous, A. (2016). Cochlear microphonics recording during ABR threshold testing in children. *Hearing, Balance and Communication*, 14(4), 163-182.

Owens, J. J., McCoy, M. J., Lonsbury-Martin, B. L., & Martin, G. K. (1993). Otoacoustic emissions in children with normal ears, middle ear dysfunction, and ventilating tubes. *Otology & Neurotology*, 14(1), 34-40.

Perlman, H. B., & Case, T. J. (1941). Electrical phenomena of the cochlea in man. *Archives of Otolaryngology*, 34(4), 710-718.

Ponton, C. W., Don, M., & Eggermont, J. J. (1992). Place-Specific Derived Cochlear Microphonics from Human Ears. *Scandinavian Audiology*, *21*(3), 131–141.

Retzius, G. (1881). Uber die peripherische Endigungsweise des Gehornerven. *Biologische Untersuchungen*, 3, 1-51.

Riazi, M., & Ferraro, J. A. (2008). Observations on mastoid versus ear canal recorded cochlear microphonic in newborns and adults. *Journal of the American Academy of Audiology*, *19*(1), 46-55.

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

Ruth, R. A., & Lambert, P. R. (1989). Comparison of tympanic membrane to promontory electrode recordings of electrocochleographic responses in patients with Meniere's disease. *Otolaryngology—Head and Neck Surgery*, 100(6), 546-552.

Siegel, J. H., & Kim, D. O. (1982). Efferent neural control of cochlear mechanics? Olivocochlear bundle stimulation affects cochlear biomechanical nonlinearity. *Hearing Research*, *6*(2), 171–182.

Smart, J. L., Kuruvilla-Mathew, A., Kelly, A. S., & Purdy, S. C. (2019). Assessment of the efferent auditory system in children with suspected auditory processing disorder: the Middle ear muscle reflex and contralateral inhibition of OAEs. *International Journal of Audiology*, 58(1), 37-44.

Thompson, D. C., McPhillips, H., Davis, R. L., Lieu, T. A., Homer, C. J., & Helfand, M. (2001). Universal newborn hearing screening: summary of evidence. *Journal of the American medical association*, 286(16), 2000-2010.

Tognola, G., Ravazzani, P., & Grandori, F. (1995). An optimal filtering technique to reduce the influence of low-frequency noise on click-evoked otoacoustic emissions. *British journal of audiology*, 29(3), 153-160.

Tognola, G., Grandori, F., & Ravazzani, P. (1997). Time-frequency distributions of click-evoked otoacoustic emissions. *Hearing research*, *106*(1-2), 112-122.

Tonndorf, J. (1960). Shearing motion in scala media of cochlear models. *The Journal of the Acoustical Society of America*, 32(2), 238-244.

Von Békésy, G., & Wever, E. G. (1960). Experiments in hearing (Vol. 8). New York: McGraw-Hill.28-29.

Wang, J., Tymczyszyn, N., Yu, Z., Yin, S., Bance, M., & Robertson, G. S. (2011). Overexpression of X-linked inhibitor of apoptosis protein protects against noise-induced hearing loss in mice. *Gene Therapy*, 18, 560-568.

Wever, E. G., & Bray, C. W. (1930). AUDITORY NERVE IMPULSES. *Science*, *71*(1834), 215.

Wilson, C. Sininger Y, Starr A. (2003). Auditory Neuropathy. A new perspective on hearing disorders. Canana. Singular Publishing Group. 67-82.

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.

Zhang, M. (2010). Using concha electrodes to measure cochlear microphonic waveforms and auditory brainstem responses. *Trends in amplification*, 14(4), 211-217.

Zhang, M. (2012). Response pattern based on the amplitude of ear canal recorded cochlear microphonic waveforms across acoustic frequencies in normal hearing subjects. *Trends in amplification*, 16(2), 117-126.

Zhang, M. (2013). Effects of stimulus intensity on low-frequency toneburst cochlear microphonic waveforms. *Audiology Research*, 3(1), 3.