Effect of click, tone burst frequency, polarity and rate of stimulus on cochlear microphonics in individuals with normal hearing sensitivity.

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This dissertation is submitted as a part of fulfilment

for the Degree in master of science in Audiology

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July 2020

CERTIFICATE

This is to certify that this dissertation entitled '**Effect of click, tone burst frequency, polarity and rate of stimulus on cochlear microphonics in individuals with normal hearing sensitivity'** is the bonafide work submitted in part of fulfilment for the Degree in master of science in Audiology of the student with registration No. **18AUD027**. This has been carried out under the guidance of a 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 '**Effect of click, tone burst frequency, polarity and rate of stimulus on cochlear microphonics in individuals with normal hearing sensitivity'** has been prepared under my supervision and guidance. It is also certified that it 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 master's dissertation entitled **'Effect of click, tone burst frequency, polarity and rate of stimulus on cochlear microphonics in individuals with normal hearing sensitivity'** is 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.

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Abstract

Cochlear microphonics is a pre neural potential which is generated by healthy outer hair cell as an alternating current response which mirrors the stimulus waveform and sustains as long as stimulus is present. CM being a cochlear potential it has potential in assessing hair cell functioning and there by identification of many cochlear and retro cochlear conditions. Despite CM having potential clinical application the optimal parameters to record cochlear microphonics and effect of various stimulus parameters are not well understood yet. The present study was taken up with an aim of determining the effect of stimulus polarity, rate, stimulus type and stimulus frequency on different aspects of cochlear microphonics which could help us to decide an optimal stimulus parameter that can be used to record CM. The study involved 32 normal hearing individuals in the age range of 18-25 years. CM was recorded from these individuals using extratympanic CM measurement from ear canal. CM was recorded independently for tone burst frequencies (500Hz, 1kHz, 4kHz & 8kHz) and click stimulus having rarefaction and condensation polarity at 30.1/sec and 59.1/sec repetition rate. Amplitude and latency were measured from recorded waveform and compared across and between stimulus conditions. Results reveal that there is effect of stimulus frequency and type on different parameters of CM. Whereas, there was no or negligible effect of stimulus polarity and rate of stimulus on amplitude and latency of cochlear microphonics. The amplitude and latency of the cochlear microphonics are inversely proportional to the stimulus frequency. Hence the study suggests the use of low frequency tone burst (500Hz/ 1kHz) to elicit robust CM which has greater application in the assessment of cochlear functioning over OAE as later get affected by environmental and physiological noise and also due to middle ear pathology. Thus, this study helped in better understanding of effect of few stimulus parameters and gives an optimized stimulus parameter which can be used in clinics to record cochlear microphonics to assess cochlear conditions.

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Chapter-1

INTRODUCTION

Auditory evoke potentials are the electrophysiological responses or change in the neural activity in response to the auditory stimulation which asses the auditory pathway and neural integrity. While most of the AEP evaluate the auditory pathway such as brainstem and auditory cortex, ECochG is a technique which yields information about peripheral portions of the auditory system. ECochG, generally involves measurement of the stimulus-related cochlear potentials as opposed to the resting potentials, and whole nerve or compound action potential (AP) of the auditory nerve. Among the cochlear potentials, cochlear microphonic (CM) is an alternating current (AC) response that mirror the waveform of low to moderately intense sound stimuli and is thought to reflect the displacement-time pattern of the cochlear partition (Dallos, 2012). The CM component may partially obscure later components in the ECochG waveform because, it continues as long as the stimulus is present.

Cochlear Microphonic is phase dependent which changes direction with changing polarity, use of an alternating polarity stimulus effectively abolishes the CM component in an averaged auditory evoked potentials (AEP) recording. Hence, the use of alternating polarity to record CM is not a good option as it cancel the CM obtained from two consecutive different polarity stimulus (Coats, 1981; Hall,2007). With a single polarity stimulus, either rarefaction or condensation, the CM appears as a waveform with a series of repeated upward and then downward peaks which mimics the waveform of the stimulus which can be clearly understood from the

Figure 1.1. Thus, single polarity rarefaction or condensation stimuli are most effective for eliciting CM (Hall,2007).

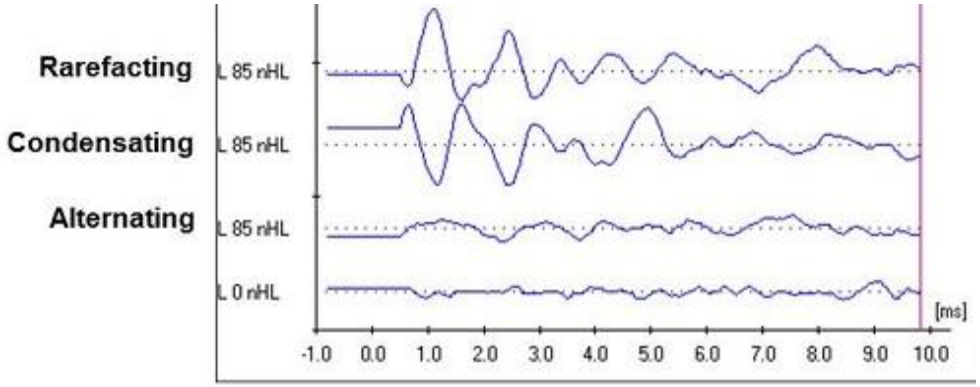

Fig 1.1: Waveforms of Cochlear Microphonics recorded using different polarity stimulus.

Hair cell present in the organ of corti is known to generate CM. Generation of CM depends on movement of hair cells which is depended on basilar membrane happen due to presence of external acoustic stimulus. Alternating potentials generated due to bending of stereocilia present on top of the OHCs (Hall,2007). Modulation of transducer currents is mainly caused by the mechanical stimulation of cochlear hair cells to the incoming acoustic stimulus (Davis et al. 1958; Hudspeth and Corey 1977;Cheatham, Naik & Dallos 2011). Since the cells are embedded in the electrical network of the organ of Corti and surrounding fluid spaces, alterations of transducer currents produce changes in extracellular current flow. They are detectable as a remote reflection of voltage changes measured across any available electrical impedance (Cheatham, Naik & Dallos 2011). Since for any acoustic

stimulation group of hair cells gets stimulated, the recording electrode integrates receptor currents produced by large numbers of individual generators.

The CM can be recorded from fluids and tissues within, around and remote from the cochlea. However, it is best recorded when recording is done near its source as it gives better signal-to-noise ratio. However, the remote recording of CM has technical challenges and difficulties with interpretation (Cheatham, Naik & Dallos 2011).Activity of outer hair cells in the basal portion of the basilar membrane is reflected in the CM if recorded outside the cochlea as recorded clinically with an electrode on promontory or from an electrode in the external ear canal (Aran & Charlet, 1976; Hoke, 1976; Sohmer, Kinarti & Gafni,1981; Euler & Kiessling, 1983).Hence recording from promontory suggest the use of high frequency tone burst could elicit better CM. However, the amplitude of CM is a matter of concern if it is elicited by high frequency tone burst.

Several factors that can influence the recording of CM and also the parameters of CM.CM recorded using click stimulation offers to asses OHC function only in the region of 1-2 kHz, wherein Discovery of a place-specific CM using high pass masking technique offers the possibility of assessing (outer) hair cell function in the apical part of the human cochlea (Ponton, Don & Eggermont, 1992).

However most often clicks are used to record ABR and also CM, the CM can be differentiated from ABR waves with two simple manipulations of the test parameters. Firstly, separate waveforms are evoked with rarefaction and then condensation polarity click stimulus. The CM peak is perfectly inverted with the change in stimulus polarity as they are phase dependent. Peaks for one polarity are valleys for the other polarity, and vice versa. Importantly, an ABR waveform remains essentially identical for both rarefaction and condensation polarity click

stimuli. Polarity of the ABR peaks does not invert when polarity of the stimulus is changed from rarefaction to condensation as it is a neural response. However, there may exists a very slight latency shifts with polarity changes (Hall,2015). And also CM are usually observed with an onset latency of 0.5 msec and shows only one positive or negative hump depending upon the stimulus polarity in normal and then continue to generate action potentials. The second clue is, if waveforms remain with digital addition, then the waveform is likely to be an ABR rather than the CM. If it is CM, the digital addition leads to a flat line result is simple background electrical activity with no detectable CM (Hall,2015).

As we know the waveform of cochlear microphonics mirrors the stimulus waveform, there is a high need to apply proper method to separate the CM from stimulus artifacts. The difficulty in separation of stimulus artifact from the actual CM had limited the clinical usefulness of CM. But it has been observed that the use of ear canal electrode and tone burst stimuli makes this separation task easier (Riazi & Ferraro, 2008). Recording a tube clamped response does confirm whether the response recorded is stimulus artifact or cochlear microphonics. If the waveform continues to be same as unclamped recording when the tube is clamped, it confirms that it is not actual CM response because the CM can't be generated when stimulus is not reaching cochlea in clamped condition.

The amplitude of CM is likely to increase with the stimulus intensity and nonlinear growth function reflecting a nature of cochlear nonlinearity (Zhang, 2013). Hence, CM reflect the physiological integrity of the cochlear partition structures and measurement of CM can be used as non-invasive technique to ascertain outer hair cell functioning (Simmons & Beatty, 1962) though OAEs also reflect the same.

1.1 Need for the study

CM being a cochlear potential can serve as a potential tool in the identification of various conditions such as Meniere's disease, auditory neuropathy spectrum disorder, acoustic neuroma and auditory maturation delay. Studies have shown a diminished amplitude and distorted waveforms when cochlear microphonics are recorded on individuals with cochlear pathology (Kumagami, Nishida &Baba,1982; Morrison, Moffat & O'connor, 1980). CM recorded in Meniere's disease showed enlarged CM (Ge, Shea & Orchik, 1997). A study by Vikas (2013) checked the effect of contralateral noise on cochlear microphonics showed that contralateral noise did have an effect on CM response in normal individuals but didn't show any effect in individuals with Meniere's disease.

Since cochlear microphonic is generated from the outer hair cell, its absence will indicate OHC dysfunction. CM is a pre-neural potential which is not affected by neural synchrony i.e. it is present even in cases of ANSD where the neural potentials are absent. Similar results can also be expected in cases with auditory maturation delay where presence of CM and delayed /abnormal neural activity might be seen. A review article by Soares, Menezes, Carnaúba, de Andrade and Lins (2016) concludes that presence of CM and absence of ABR indicates a possible neural lesion.

The information regarding the intact OHC functioning can also be known by the presence of OAE, but in cases with presence of middle ear disorders, OAEs cannot be recorded (Kreitmayer, Marcrum, Picou, Steffens & Kummer, 2019) rendering it ineffectiveness in such cases. Also, in situations where the background noise, physiological noise is more, OAE is not an option to asses OHC functioning especially for lower frequencies. In such situations, to assess cochlear functioning in the clinics, cochlear microphonic measurement may serve as a supplementary

approach to OAEs. This is supported by a study by Zhang (2012a) who showed that low frequency CM can be measured in individuals with high frequency hearing loss. Kwak et al., 2014 also concluded from their study that CM might provide more stable information about the cochlear hair cell than the OAE test which could be easily influenced by the condition of the middle ear or external ear and suggested CM as a useful supplementary tool for OAE test.

Deltenre et al., (1999) observed that in a few individuals with ANSD cochlear microphonics present, whereas OAEs were absent, which shows the role of cochlear microphonics in diagnosis of ANSD. Rance, et al., (1999) suggests that assessing cochlear functioning as a mandatory protocol in the new-born hearing screening, particularly the CM, when there is absence of ABR, which will facilitate diagnosis of ANSD in new-borns. CM is recommended over the OAE because of high chances of middle ear fluid present in new-born and background noise might lead to the absence of OAEs. Thus it emphasizes the need for more studies on recording of CM and also emphasise on selection of stimulus or protocol which would help to record and identify CM better.

In cases of sloping hearing losses, the cochlear microphonic may/may not be present with click stimulation. However, CM can be recorded with the low frequency tone burst stimulation. This might end up misdiagnosing a sloping hearing loss patient as ANSD as observed by Prabhu,Narne,and Barman (2014).

Cochlear microphonics thus has the potential to be an important clinical tool. However, we are yet to understand the optimal stimulus parameters to record the cochlear microphonics efficiently. There are ample number of studies carried out using CM in clinical population like Meniere's disease (Ge, Shea&Orchik, 1997; Vikas,2013) and ANSD (Soares, et al., 2016). However, there are limited number of

studies which explore how the stimulus parameters affect the CM. Hence this study aims at determining the effect of stimulus polarity, rate, stimulus type and stimulus frequency on different aspects of cochlear microphonics which will help us to decide an optimal stimulus parameter that can be used to record CM. Thus the aim of the study was to investigate the effect of stimulus type, frequency and polarity on cochlear microphonic in normal hearing individuals.

1.2 **Objectives**:

The objectives of the study were to:

- Compare the effect of stimulus polarity (rarefaction and condensation) on latency and amplitude of CM,
- Compare the effect of rate of stimulation (30.1 and 59.1) on latency and amplitude of CM,
- Compare the effect of stimulus type (Clicks Vs Tone bursts) and Stimulus frequency (500Hz,1kHz,4kHz,8kHz) on CM for each polarity and stimulus rate,
- Suggest optimum stimulus parameter to record CM based on the outcome of the findings.

Chapter-2

REVIEW OF LITERATURE

The auditory system conveys the auditory information to the brain via the auditory pathway. It comprises ears and their connections to and within the central nervous system. The auditory system may be divided into the outer, middle, inner ears, the auditory nerve and the central auditory pathways (Gelfand, 2010). The auditory signal is transmitted from the outer ear and through the middle ear before entering cochlea via the conductive mechanism. The cochlea plays the role of a transducer in converting the transmitted vibratory stimulus into a form usable by the nervous system. Organ of Corti in the cochlea is the site of transduction. The potential generated by this sensory transduction is carried further towards the central nervous system via auditory nerves. Pathology can be seen at any above-mentioned levels. Any pathology in the outer and middle ear only interrupts the conductive mechanism of sound transmission leading to conductive hearing loss. If the pathology is in the inner ear it will affect the sensory mechanism of the hair cell and transduction process leading to the sensory hearing loss or cochlear hearing loss. Any pathology beyond cochlea i.e., at the level of the auditory nerve leads to retro cochlear pathology. The battery of audiological tests are useful in assessing the structural deficit or pathology specifically in different parts of the ear. Pure-tone audiometry is the gold standard for diagnostic audiological testing wherein pure tone threshold tells information about the whole auditory system. From pure-tone air conduction and bone conduction thresholds, the degree and type of hearing loss are determined. Speech audiometry assesses the patient's ability to understand speech. The middle ear can be specifically assessed with the help of Immittance measurement. Tympanogram type and acoustic reflexes from Immittance

measurement play a major role in diagnosing or identifying different middle ear pathologies for example eardrum perforation, otitis media, ossicular chain discontinuity, etc. Otoacoustic emissions are a sound generated within the inner ear and recording OAE is a specific test for assessing inner ear pathology basically integrity of outer hair cell(kemp,2002). Recording Auditory evoked potential gives information about the auditory nerve functioning and integrity. Outer hair cell functioning can be assessed with the help of OAE and also cochlear microphonics as it is also generated by OHCs. The assessment of outer hair cell functioning is very important as it helps in the differential diagnosis of cochlear and retro cochlear pathology. And also it is an important aspect of new-born hearing screening.

2.1 Use of OAE

OAEs have a strong tonotopic relationship with the cochlear function. When the middle ear is normal, an OAE outcome reflects contributions that derive directly from cochlear sources. OAEs are unique in this regard. No other clinically available test allows determination of cochlear function in isolation from the rest of the auditory pathway. Therefore, OAEs have powerful utility for clinical applications (Kemp's 1978; Brownell,1983). OAE is found to have a sensitivity of 66.7% and specificity of 98.8 % in neonatal hearing screening (Yousefi, Ajalloueyan, Amirsalari & Fard,2013). OAE data evoked by either transient or pure-tone stimuli provides an objective measure of hearing sensitivity with sufficient accuracy to predict pure-tone audiometric results. DPOAE frequency-grams and TEOAE spectragrams are often displayed in a graphic form similar to audiograms. As evoked OAEs provide objective measures of preneural cochlear function, they assist in the differentiation between "sensory" and "neural" components of the peripheral auditory structures. Most congenital and acquired sensory pathologies result in OHC

dysfunction, which will affect EOAE test results. However, sensory pathologies caused by IHC dysfunction may not be reflected in EOAE measures. For patients with moderate to profound hearing loss, the presence of evoked OAEs supports the diagnosis of retro cochlear lesion, once pseudohypacusis has been ruled out. OAEs can also be used in the assessment of idiopathic sudden hearing loss(ISHL). In these cases, presence of OAE despite of SNHL of 40dB or more suggests the inner ear injury is not to the OHCs but to the other structures (Robinette and Facer, 1991; Sakashita et al, 1991; Schweinfurth et al, 1997; Truy et al, 1993). Schweinfurth et al (1997) support that absent EOAEs in sudden hearing loss are related to an ischemic injury and presence of EOAE in sudden hearing loss is associated with cochlear neuritis, which is often responsive to steroid therapy. The presence of EOAEs in patients with ISHL has been suggested as having prognostic value in predicting hearing recovery (Hoth, 2005; Lalaki et al, 2001). OAEs can also be used for assessing hearing sensitivity during the diagnosis of endolymphatic hydrops using glycerol test. EOAEs have also been found to be more sensitive (Cianfrone et al, 2000; Sakashita et al, 2001) or equally sensitive to pure-tone threshold changes. Studies also indicate DPOAE growth using the I/O function is more sensitive to changes in cochlear function in patients with Endolymphatic hydrops than single stimulus level DPOAEs (Sakashita et al, 1998). EOAEs are a quick and reliable screening test of both unilateral and bilateral pseudohypacusis. Otoacoustic emissions (OAEs) potentially provide ways of detecting subtle inner-ear changes in normal-hearing ears before hearing loss occurs, and ways of detecting who is susceptible to noise-induced hearing loss (NIHL). Rask-Andersen et al, 2000 have shown that the outer hair cells (OHCs) in the inner ear are vulnerable to noise damage, and the damage can be extensive with no concomitant change in hearing

thresholds (Hamernik et al, 1989, 1996). EOAEs could be useful in the evaluation of patients with eighth cranial-nerve tumors for differential diagnosis (Robinette & Durrant, 1997) monitoring cochlear function before, during, and after surgery for removal of the tumor (Telischi et al, 1995); and the prediction of residual hearing following eighth-nerve surgery. Wiederhold (1990) observed a large variability in DPOAE levels and threshold in populations of humans with normal or near-normal hearing. He proposed that a component in DPOAE variability was related to TM or middle-ear pathology. Hence OAE can also be used as an indirect measure of middle ear functioning. Although OAE responses in normal-hearing ears can be used to indirectly assess middle-ear transmission, such an assessment is impossible if the OAE response is absent. A significant concern in NHS programs is the problem of false-positives, that is, those children who initially fail the screen but who are later identified as having normal hearing. A major contributor to this false positive rate is transient ear-canal and middle-ear dysfunction, a leading cause of an absent OAE response. Hence despite having high clinical importance in new-born hearing screening programs to identify sensorineural hearing loss, there exists a significant challenge in such programs in differentiating between an ear with a permanent hearing loss and an ear with transient middle-ear dysfunction. Also, in situations where the background noise, physiological noise is more, OAE is not an option to asses OHC functioning especially for lower frequencies.

2.2 Cochlear microphonics

Cochlear microphonics is an alternating current(AC) voltage generated by the outer hair cells tend to mirror the stimulus waveform. The cochlear microphonics is phase dependent which changes its phase as polarity of the stimulus changes. Several literature supports that different stimulus and recording parameters has an effect on the amplitude and latency of the cochlear microphonic waveform. And also few inner ear pathologies had shown to have an effect on the morphology of cochlear microphonic waveform.

2.2.1 Effect of stimulus intensity

Zhang (2013) investigated the effect of stimulus intensity on ear canal recorded cochlear microphonics. The study was conducted on ten normal hearing individuals of 20-30 years of age. cochlear microphonics were recorded in response relatively long duration 500Hz tone burst stimuli of 14ms duration, consisting of a 2 ms rise-time, a 10 ms plateau-time, and 2 ms fall-time. Stimulus intensity was varied from 80dBnHL to10dBnHL in 10 dB steps. Changes in amplitude and latency of CM were noted from the ear canal recorded waveform. They found that the amplitude of the CM was intensity-dependent, whereas in contrast the latency of the CM was intensity –independent. The results are in agreement with a study by Liu, Chen, & Xu, 1992 where they also found large CM amplitude for higher intensity and lower CM amplitude of low intensity for tone burst stimulation. These findings may be useful for the development of the application of CM measurement as a supplementary approach to Otoacoustic emission (OAE) measurement in the clinic which is severely affected by background acoustic noise, physiological noise and middle ear pathologies etc.

A study by Noguchi, Nishida, & Komatsuzaki, 1999 investigated detection thresholds, amplitudes and input-output curves of cochlear microphonics and the comparison was made between the responses recorded in extratympanic and transtympanic mode. They found that low frequency tone burst of 5ms duration had a detection threshold of 20dBnHL and the CM and AP input-output curves obtained from mean amplitudes at each intensity in normally-hearing ears had similar slopes with the two approaches.

In a study by Riazi & Ferraro, 2008 recorded cochlear microphonics on 11 normal hearing individuals. CM was recorded using both click and tone burst (500Hz,1kHz,2kHz) stimuli presented at 70 dBnHL and 95 dBnHL. They found that the CM is more likely to be recorded when the stimulus is presented at a higher intensity (95 dBnHL) leading to a robust amplitude. The result is in agreement with the previous study by Zhang et al.,2003 where they had used low frequency long duration (14ms) tone burst stimuli and the CM was recorded using ear canal electrode. They attributed this finding to the fact that the CM amplitude is strongly influenced by current flow through OHCs Thus, higher stimulus intensities result in greater current flow through the HCs.

A study by Poch-Broto et al., 2009 carried out on randomly selected 20 adult individuals (40 ears) in hospital setup intending to investigate whether the cochlear microphonics audiometry provides an objective audiometric profile.CM was recorded non-invasively and PTA was carried out to verify and correlate for each patient. Continuous pure tones of 500Hz,1kHz,2kHz,4kHz frequencies were used as stimuli for both cochlear microphonics and PTA. They found that audiometric profiles obtained from Cochlear microphonics audiometry are highly correlated, without statistical differences, to those obtained with PTA. More than 81% of

patients exhibited differences below 10 dB(HL), while a low number of cases showed differences over 20 dB(HL). These findings are in agreement with the study by Sanjuan Juaristi, 2007; Liu, Chen, & Xu, 1992 where they have also concluded that there is a high correlation between subjective audiometry and cochlear microphone audiometry.

2.2.2 Effect of stimulus frequency

A study by Zhang, 2012 was carried out on 10 normal hearing volunteers of age range 18 -35 years. The study was done to demonstrate the response pattern of the cochlear microphonic response across different tone burst frequencies. Tone bursts of 500Hz,1kHz,2kHz &6kHz were considered as stimuli for the study by keeping the duration of the stimuli same across all the frequencies i.e., 14ms.The stimuli were presented at 75 dB nHL at a rate of 22.7/sec and recording was done from ear canal using Tiptrode for a 20ms time window. Peak to peak amplitude of cochlear microphonics was considered for comparison across different frequencies. They found that the amplitude of cochlear microphonics decreased with an increase of stimulus frequency of the tone bursts; and such a decrease occurred at a faster rate at lower frequencies than at higher frequencies. Hence they concluded that low frequency CM is more robust compare to high frequency and thus can be used as an alternative to OAE in assessing cochlear function at lower frequencies. The findings are in agreement with the study by Liu, Chen, & Xu, 1992 where they also found a large amplitude for low frequency and smaller amplitude for high frequency tone burst.

Heidari, Pourbakht, Kamrava, Kamali and Yousefi, 2018 conducted a study on 25 healthy, male, young adult Wistar rats intending to compare cochlear

microphonic responses between broad band click stimuli and narrow band tonal stimuli.CM was recorded with the help of an extratympanic technique in ECochG. Click stimulus of 0.1-µsec duration and 5-ms tone burst at frequencies of 2, 4, 8 and 16 kHz were presented at 80 dB SPL through loudspeakers. They found that the click stimulation had a larger CM amplitude than tonal stimuli showing that the magnitude of CM increases as the bandwidth of the stimuli increases. Across tonal stimuli, results showed that the amplitude decreases as the frequency was increased.

Ponton, Don and Eggermont, 1992 conducted a study on normal hearing individuals of age range 15-30 years of age. The objective of the study was to obtain the derived frequency specific Cochlear microphonics with the use of high pass masking technique. The place specificity of the obtained response was confirmed by verifying whether the derived CM retains the same frequency of the stimulating tone burst. Click and tone bursts (500Hz,1kHz,2kHz) were presented with a high-pass cut-off of the pink noise which was reduced in octave steps from 8 kHz to 0. 5kHz. Derived response was generated by subtracting one high pass masking condition from the other high pass masking condition with cut off of one octave higher. This resulted in 5 sets of narrow band responses and one unmasked response. 0.7 kHz, 1.4 kHz, 2.8 kHz, 5.7 kHz and11.3 kHz were the center frequency(CF) of the derived band responses. They found that as the CF of derived band decreased, the latency difference between this CM peak and Wave **I** (NI) increased. They also observed that the Phase reversal of the microphonic response is most evident and amplitude was relatively more in the lowest derived band conditions. Similarly, with the use of tone bursts, the CM was largest in the two derived bands with CFs above the frequency of the tone burst. They concluded that although clicks can be used to produce the response, the derived CM was more robust in response to tonal

stimulation and the largest CM responses recorded were produced by 500 Hz and 1kHz tone bursts.

2.2.3 Effect of electrode placement

Placement of electrode is one of the important factor that impact the recording of CM. In general, more stable recording can be obtained when the electrode is placed closer to the generator site. CM can be recorded with the help of needle electrode placed on promontory invasively called transtympanic recording whereas, it can also be recorded using Tiptrode in the ear canal non-invasively called extratympanic recording.

Zhang, 2010 conducted a study on 10 individuals of age range 20-30 years having normal hearing sensitivity. The study was carried out to investigate the best electrode placement among mastoid, ear canal and concha for recording a cochlear microphonics. A 14 ms long tone burst stimuli with 4ms rise-fall time was presented at 80dBnHL and CM was recorded from all the 3 above mentioned electrode sites. The amplitude of the cochlear microphonics recorded from each electrode site were compared. They found that CM recorded from the mastoid was smaller than that recorded from either the canal or the concha. However, amplitudes recorded with the concha electrode differ only slightly from those recorded in the canal and that this difference is not statistically significant. Hence they concluded that the concha electrode can be used as an alternative to the ear canal electrode. This study by Noguchi Nishida, & Komatsuzaki, 1999 where they have found similar kinds of values for CM detection thresholds when extratympanic and transtympanic procedures were compared.

Riazi and Ferraro, 2008 carried out a study on 11 normal hearing individuals to compare the cochlear microphonics recorded by placing the negative electrode in the ear canal with those recorded by placing in the mastoid. 2 channel simultaneous recording from both mastoid and ear canal placements were done by presenting click and tone burst (500Hz,1kHz,2kHz) stimuli at 70 dBnHL and 95 dBnHL. The results showed that CM was more likely to be recorded with an EC electrode compared to a surface electrode on the test ear mastoid process. Hence they concluded that it is easy to separate cochlear microphonics from stimulus artifact using an EC electrode and tone burst stimuli. And the results were attributed to the closer generator to the primary electrode, stronger is the response. The results are also in agreement with Ferraro and Ruth, 1994; Ferraro and Durrant, 2002 wherein they stated that it is often difficult to differentiate from stimulus artifact especially in far-field recordings.

2.2.4 Effect of stimulus duration

Zhang, 2013 reported in his study that 3 typical types of cochlear microphonics can be recorded. They are, CM evoked by clicks, CM evoked by short-duration tone burst (<5ms) and CM evoked by relatively long-duration tone bursts(>14ms) which were also mentioned in the study by Zhang, 2012 where he describes cochlear microphonics evoked by clicks appears as only one or two periods of cycles and is represented as ringing in the basilar membrane and it contains many frequencies and less frequency specific(Dauman, Aran, Charlet de Sauvage, & Portmann,& 1988; Arakawa,1998). Whereas the CM evoked by short tone burst appears as a more period of cycles than click-evoked but not sufficient enough to establish a plateau representing fewer frequency, hence relatively more frequency specific compared to click-evoked CM. However, the CM evoked by long tone burst

comprises many more cycles than click and short tone burst and also it possesses the highest frequency specificity among all three as the splatter is less.

2.2.5 Effect of stimulus rate

Guidelines for the CM recording given by Stevens, Sutton, Brockbank, & Mason, 2011 states that since CM is pre-neural potential, it may not be subjected to the neural fatigue, which allows the CM not be affected at higher stimulation rate which is also supported by a study by Coats, 1981. They found that CM and AP component of the EcochG remains stable or unaffected by stimulus rate. But there are not many studies that empirically checked the effect of stimulus rate on CM alone.

2.2.6 Effect of filter setting

Lightfoot, 2011 in their guidelines for the CM also states that the use of high pass filter of 300 Hz if available or the highest value available between 100-300Hz and low pass filter up to 3KHZ/5KHz assist in minimizing the background mayogenic and EEG activity which can be favorable for recording CM.

2.2.7 CM in different pathologies

2.2.7.1 CM in clients with recruitment:

Liu, Chen, and Xu, 1992 conducted a study on 68 cases with unilateral sensory hearing loss, and 5 normal hearing individuals in the age range of 20 -49 years. The study was conducted with an objective of demonstrating recruitment objectively using cochlear microphonics. All the affected ears had a recruitment which was confirmed by the ABLB test. Simultaneous bilateral CM was recorded in the ear canal . Tone burst stimuli of 0.5. 1, 2, 4 and 8 kHz were presented through speakers at different intensity levels. They found that in normal ears cochlear

microphonics had large amplitude for higher intensity and lower amplitude of low intensity and large amplitude for low frequency and smaller amplitude for high frequency tone burst. They also found a positive correlation between PTA and CM detection threshold in normal and affected ears without recruitment. Sixty cases with unilateral hearing loss out of 68 cases had enlarged and prolonged CM in those frequencies where they had recruitment as compared with the opposite normal ears. These included 30 cases of Meniere's disease, 23 cases of sudden hearing loss, and 7 cases of low-tone sensory hearing loss without vertigo. They attributed the results to increased abnormal excitability of the hair cells caused by some pathological stimulations.

2.2.7.2 CM in Meniere's disease

A study by Noguchi, Nishida, Tokano, Kawashima and Kitamura, 2004 was conducted to compare the acute low frequency hearing loss(ALHL) versus Meniere's disease with the help of cochlear microphonics by electrocochleography. It was a retrospective study where they compared electrocochleographic findings from 20 patients with ALHL with those from 58 patients with Meniere's disease (MD) classified into 4 groups (MD1 through MD 4) according to their pure-tone average. They observed that the mean detection threshold of the cochlear microphonics in the ALHL group was 32.0 ± 9.4 dB nHL, which was again similar to that seen in the MD1 group(PTA<25dB). They also observed normal input-output curves of cochlear microphonics in more than 50% of the ALHL. Hence they concluded that pathogenesis of ALHL arises from an endolymphatic hydrops with little or no impairment of hair cells that resembles early-stage of MD.

Vikas, 2013 investigated the effect of contralateral noise on Cochlear microphonics in normal hearing and individuals with Meniere's disease. The study included 12 individuals with Meniere's disease and 10 normal hearing individuals of mean age of 35 years. CM was recorded using Tiptrode from ear canal using click stimulus presented at 80dBnHL. CM amplitude and latency were recorded in 2 conditions, one without contralateral noise and one with contralateral broad band noise at 50dBSL.They found that CM was present in all 10 individuals whereas in Meniere's group it was present in 8/12 individuals. They found a significant difference in amplitude between with noise and without noise condition in the control group but not in the experimental group. The control group showed an enhancement in the cochlear microphonic amplitude on the presence of contralateral noise. They attributed the absence of contralateral noise effect on CM in Meniere's group to the disruption of the efferent pathway.

2.2.7.3 CM in auditory neuropathy(AN)

Shi et al., 2012 conducted a study on 36 infants and children who are divided into 2 groups. Group 1 included 15children with absent ABR and present OAE, group 2 included 21children had both ABR and OAE absent. The study also included 15 normal hearing individuals. CM were recorded for a click stimuli presented at 100, 90, 80 & 70 dB nHL intensities. Latency and amplitude of the recorded cochlear microphonics were noted for both AN group and normal hearing individuals. The input-output curve was also plotted for each group. Results showed no significant difference in latency measure between both AN group and control group. They also found amplitude measure showed no significant difference between group 1 and control group whereas, group 2 had significantly lower amplitude compare to controls and group 1. I/O function showed that there was a nonlinear

trend in decrease in amplitude upon decrease in stimulus intensity in group1 and controls whereas, group2 showed a linear trend. Hence they concluded that CM can be a sensitive indicator of the OHCs functioning that can play an important role in the diagnosis of AN. The amplitude and latency results were consistent with those of Starr et al.,2001 who recorded and analysed CMs in children with AN using a similar method.

Rance et al., 1999 did a study to demonstrate the clinical findings of children with auditory neuropathy spectrum disorder. It was a retrospective study that included 20 infants and young children who had the presence of click-evoked CM with absent ABR. The results suggest that auditory neuropathy is more common in the infant population and all the participants showed clear CM potentials at levels between 60 and 70 dB nHL. Hence they concluded that the presence or absence of CM along with ABR results have a significant clinical application in diagnosing and describing audiological features in ANSD. Similar findings are also reported in a study by Starr et al., 2001 and they also reported that the robust or stronger CM can be recorded by click stimulation in AN group than in normal individuals.

A study by Deltenre et al., 1999 conducted on 2 prelingual children with ANSD with an objective of demonstrating the significance of cochlear microphonics in diagnosing ANSD. They found that there was a selective loss of Otoacoustic emissions whereas cochlear microphonics were preserved. Hence they concluded that one should also record cochlear microphonics when OAEs are absent for a diagnosis of ANSD.

Smith, 2018 in his Thesis studied the cochlear microphonics from ABR of infants with ANSD. It was a retrospective study where click-evoked ABR from 16

infants with ANSD were analysed to compare them with published normative. The ANSD participants were selected based on the presence of OAE, absent ABR and presence of CM, absent ABR. Results showed that CM was significantly longer in duration in ABR waveforms of infants with ANSD than normal healthy infants. They also observed CM amplitude was significantly larger in ABR waveforms of infants with ANSD than normal healthy infants. Among ANSD infants, the infants who had the presence of OAE and those with absent OAE didn't reveal any significant difference in both duration and amplitude but did differ significantly in mean CM amplitude/V peak amplitude ratios. Hence they conclude that duration measure can be used for diagnosis as there was a significant difference between the groups.

The above literature states that a clinical diagnostic feature for ANSD is the presence of cochlear microphonics and absence of ABR. But this notion should not lead to misdiagnosis, which has been studied by Prabhu, P., Narne, V.,and Barman, 2014 where they discussed the case reports of 3 children with sloping hearing loss who were misdiagnosed as auditory neuropathy spectrum disorder based on absence of auditory brainstem response and presence of long ringing cochlear microphonics. They also report 3 children with neurological abnormalities having abnormal cochlear microphonics. They found that 3 children who had been misdiagnosed as ANSD showed the presence of neural response(ABR) upon tone burst stimulation. The study showed the significance of tone burst ABR in the test battery of diagnosis of ANSD and this is in agreement with Ahmmed, Brockbank, and Adshead, 2008. Hence they concluded that abnormal CM detection with absent click ABR is not a distinctive feature of ANSD. The results also showed that the long ringing cochlear

microphonics can also be seen in high frequency hearing loss and neurological abnormalities.

2.2.7.4 CM in ASD, ANSD, SNHL

Dabbous, 2016 conducted a study to investigate the characteristics of CM in different hearing profiles and reflect the usefulness of recording CM simultaneously during Auditory Brainstem Response (ABR) threshold testing in children. This was a retrospective study that included 33 children with autism spectral disorders (ASD), children with SNHL, children with ANSD and 41 normal hearing children as controls. Both CM and ABR were simultaneously recorded. They found that children with ASD had similar CM amplitude and detection threshold as a control group. They also observed in children with ANSD that CM was preserved despite the absence of DPOAE. They concluded that OHC function has remained intact as normal individuals in children with ASD reflecting the absence of any peripheral hyperacusis due to loudness recruitment. It also stated that CM should always be searched for when testing young children when there is an absence of ABR response with absence or presence of Otoacoustic emissions, to avoid any false-negative results for ANSD. CM can be preserved in children with SNHL with loudness recruitment. This finding could be confused with ANSD, so CM should be traced down to its threshold for an appropriate diagnosis.

2.2.7.5 CM in High frequency hearing loss

Zhang, 2012a Conducted a study to investigate the cochlear microphonics in individuals with high frequency hearing loss. Participants of the study were divided into 2 groups, one group included 10 normal hearing and another group included 5 individuals with high frequency hearing loss in the age range of 20-30 years. 14 ms

long tone burst of 500,1000,2000 and 4000Hz frequencies with 4ms rise-fall envelope were presented at 75dBnHL through insert earphones. Ear canal electrode was used to record the cochlear microphonics. They found that CM evoked by 500Hz had almost similar amplitude in both normal hearing and individual with high frequency hearing loss whereas CM evoked by 4kHz was evident in the normal hearing group but was barely recognizable in high frequency hearing loss group. They also stated that CM evoked by 500Hz was robust among 4 frequencies .This was in agreement with the results of Zhang, 2010. They concluded that CM can be recorded using low frequency tone burst in high frequency hearing loss which helps in assessing the cochlear functioning when OAE measurement is difficult in low frequency.

2.2.7.6 CM in New-born Hearing screening

Kwak et al., 2014 in his retrospective study demonstrated the role of cochlear microphonics in newborn hearing screening. The study included reports of 51 new-born infants. TEOAE, ABR and CM data were analysed. They found that Auditory neuropathy was suspected in 2 ears that had the presence of CM and OAE with abnormal ABR waveform. They also observed the correlation between the CM amplitude and the reproducibility of OAE. The subjects with the presence of OAE showed higher amplitude of CM than those with absent OAE. Mean CM amplitude of subjects with normal ABR threshold was higher than those with abnormal ABR. Hence they concluded that CM might provide more stable information about the cochlear hair cell than the OAE test which could be easily influenced by the condition of the middle ear or external ear and suggested CM as a useful supplementary tool for OAE test.

A thorough systematic review of the literature suggests that most of the congenital hearing loss has its pathophysiology in the inner ear basically outer hair cells. Assessment of outer hair cell functioning is very important as it helps in the differential diagnosis, gives information about the degree of hearing sensitivity, new-born hearing screening, etc. As OAE is generated primarily by OHCs, its measurement helps in assessing OHC dysfunction. But because of few limitations of OAE like in conditions of middle ear pathology, noisy situation, low frequency assessment, OAE can't be an option of assessment of OHCs. As cochlear microphonics are also generated by OHCs, it can be used as an option for assessment of OHCs in these conditions.

Because of the stimulus mimicking nature of CM, contamination of CM with stimulus artifacts, lack of understanding about the characteristics of and effect of parameters the clinical applications of CM are limited. The literature discussed above also suggests that different stimulus parameters such as intensity, frequency, duration, rate electrode placement, has a varying effect on the cochlear microphonics characteristics. And also the above studies have shown that characteristics of cochlear microphonics are varied in different pathologies and this helps in diagnostic evaluations of those pathologies like High frequency hearing loss, ANSD, Meniere's disease &to assess recruitment, etc. Cochlear microphonics thus have the potential to be a regular clinical tool. Previous studies have used different stimuli, recording parameters and investigated the effect of them in CM responses. However, despite the information gained by above reviewed literature, there is still not enough data on optimized stimulus for recording the robust cochlear microphonics such that it can be used as a supplementary or an alternative way of assessing OHC dysfunction and few of the studies done were on limited population which is insufficient to conclude

clinical application. Hence there is a need for well controlled study on stimulus optimization for recording cochlear microphonics so that the CM can be a clinically applicable tool in assessing the cochlear function. Hence our study is aimed at investigating the effect of stimulus type, frequency and polarity on cochlear microphonic in normal hearing individuals.

Chapter-3

METHODS

The study was done with the objectives of determining the effect of stimulus polarity, stimulus rate, stimulus type and stimulus frequencies on cochlear microphonics. To fulfil the objectives amplitude and onset latency parameters of cochlear microphonics were considered and compared between 2 polarities, 2 different stimulation rate and across different types of stimulus and also tone burst frequencies.

3.1 Participants

A total of 32 normal hearing adult volunteers with age range of 19-24 years with a mean age of 21.28 years were taken for the study which included 18 males and 14 females. Only left ears were considered to record cochlear microphonics to avoid ear effect if any. Participants in the study had normal hearing sensitivity in both ears. Hearing sensitivity of the participants were confirmed by administering the standard diagnostic audiological test battery, which included, pure tone audiometry, tympanometry and Oto-acoustic emissions. The demographic details and audiological findings of the participants are given below in the table 3.1.

Table 3.1

Demographic details and audiological findings of the participants

Sub- ID	AGE	GENDER	EAR	PTA	TYMP	REFLEXES	TEOAE
CM5	**24**	Male	Left	<15dBHL	"A"Type	Present	Present
CM6	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM7	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM9	**20**	Male	Left	<15dBHL	"A"Type	Present	Present
CM10	**20**	Male	Left	<15dBHL	"A"Type	Present	Present
CM11	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM13	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM14	**23**	Male	Left	<15dBHL	"A"Type	Present	Present
CM15	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM16	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM17	**22**	Female	Left	<15dBHL	"A"Type	Present	Present
CM18	**22**	Male	Left	<15dBHL	"A"Type	Present	Present
CM19	**22**	Female	Left	<15dBHL	"A"Type	Present	Present
CM20	**22**	Male	Left	<15dBHL	"A"Type	Present	Present
CM21	**22**	Male	Left	<15dBHL	"A"Type	Present	Present
CM22	**21**	Male	Left	<15dBHL	"A"Type	Present	Present
CM23	**24**	Male	Left	<15dBHL	"A"Type	Present	Present
CM24	**24**	Male	Left	<15dBHL	"A"Type	Present	Present
CM25	**24**	Male	Left	<15dBHL	"A"Type	Present	Present
CM26	**23**	Male	Left	<15dBHL	"A"Type	Present	Present
CM27	**21**	Male	Left	<15dBHL	"A"Type	Present	Present
CM28	**20**	Male	Left	<15dBHL	"A"Type	Present	Present
CM30	**22**	Female	Left	<15dBHL	"A"Type	Present	Present
CM31	**22**	Female	Left	<15dBHL	"A"Type	Present	Present
CM32	**22**	Male	Left	<15dBHL	"A"Type	Present	Present
CM33	**19**	Male	Left	<15dBHL	"A"Type	Present	Present
CM234	**20**	Male	Left	<15dBHL	"A"Type	Present	Present
CM35	**21**	Male	Left	<15dBHL	"A"Type	Present	Present
CM37	**21**	Male	Left	<15dBHL	"A"Type	Present	Present
CM38	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM39	**20**	Female	Left	<15dBHL	"A"Type	Present	Present
CM40	**20**	Female	Left	<15dBHL	"A"Type	Present	Present

Participants were selected based on the following selection criteria.

3.1.1 Participant selection criteria

- ✓ All the participants had audiometric thresholds within 15dB HL.
- ✓ No history or presence of middle ear pathology, as ascertained while taking case history.
- ✓ All participants had "A" type of tympanogram with both ipsilateral and contralateral acoustic reflexes present indicating normal middle ear functioning.
- ✓ All participants had normal outer hair cell functioning as transient evoked Otoacoustic emissions were present. This also ascertain the absence of middle ear pathology.
- ✓ None of the participants had history of noise exposure or long duration exposure to music.

3.2 Instrumentation

For the routine audiological evaluations the following equipments (calibrated as per standards mentioned by manufacturers) were used:

- ➢ A two channel diagnostic audiometer Inventus Piano coupled with impedance matched TDH-39 earphones and radio ear B-71 bone vibrator was used to obtain air conduction and bone conduction pure tone thresholds.
- ➢ An Immittance meter Grason Stadler Inc., Tympstar was used for Immittance testing to obtain type of tympanogram and measure acoustic reflexes to assess middle ear functioning.
- ➢ Transient Evoked Otoacoustic Emissions were measured with a calibrated OAE analyser- Otodynamics ILO v6 DP Echoport to assess outer hair cell functioning.

- The cochlear microphonic was recorded using Bio-logic Navigator Pro Auditory Evoked Potential System Software. The stimulus intensity and system was calibrated in accordance with ANSI S3.6-2004 standards as specified by the manufacturer. Tiptrode was used to present stimulus and record cochlear microphonics.

3.3 Test Environment

All the audiological tests were conducted in a well illuminated and acoustically treated room with permissible noise level as specified by ANSI S 3.1(1999).

3.4 Procedure

A detailed case history was taken, it included demographic details, information related to ear infection, ear pain, hearing sensitivity etc. Otoscope was done to check wax free ear canal, history of exposure to noise or long duration exposure to music.

Pure tone audiometry was carried out by modified Hughson and Westlake procedure (Carhart & Jerger,1959) to select participants for the study using a two channel diagnostic audiometer to obtain air conduction (250 Hz to 8 kHz) and bone conduction (250 Hz to 4 kHz) thresholds in octave frequencies. Pure tone average was derived by calculating the average of pure tone thresholds obtained at 500, 1000, 2000 and 4000 Hz frequencies.

Immittance measurement was done with a calibrated middle ear analyser using 226Hz probe tone. Type of tympanogram was obtained along with both ipsilateral and contralateral acoustic reflexes at 500 Hz, 1 kHz, 2 kHz frequencies and checked for the fulfilment of participant criteria.

As a last test for the selection of participants Transient Evoked Otoacoustic Emissions were measured using a non-linear method with 80 µsec click stimulation and the stimulus level in the external ear was maintained at around 80 dBpSPL throughout the test. It was made sure that the selected subjects possessed TEOAE. SNR of 6 dB SPL at consecutive 3 frequencies and a reproducibility of >90% was considered as presence of TEOAE.

3.4.1 Recording of Cochlear Microphonics

Subjects were seated on a reclining chair and instructed to relax and refrain from extraneous body movements to avoid muscle artifacts during recording. Skin surface of the electrodes placement (forehead and mastoid placements) were cleaned using Nuprep skin preparations gel. Cup electrodes were placed on mastoid and forehead of the participants with the help of skin conduction paste and surgical plaster was used to hold the electrodes tightly in its respective placement. Tiptrode was placed in the ear canal which was cleaned with the help of earbuds as CM recorded from mastoid was smaller than that recorded from either the canal or the concha (Zhang, 2010).

Recordings were obtained on a single channel Horizontal electrode placement. Non inverting electrode –Tiptrode(+ve) was placed in the ear canal as deep as possible, the inverting electrode (-ve) was placed on the contralateral mastoid and the ground electrode was placed on the upper forehead. Absolute electrode impedance was maintained below 5kohms with interelectrode impedance within 2kohms.

As per the objectives of the study 500 Hz, 1000 Hz, 4000 Hz and 8000 Hz tone burst were used. All these frequencies tone burst were considered as they cover

low, mid and high frequency region. In addition to that click stimulus was also used to record cochlear microphonic.

Tone burst having 2-2-2 cycle envelop was used to record Cochlear Microphonics. Long duration TB was preferred as CM is likely to be present as long as the stimulus is present and hence help in better visualization of CM. Also the CM evoked by long tone burst comprises many more cycles than click and short duration tone burst and also it possesses the highest frequency specificity among all three as the splatter is less (Zhang, 2013; Zhang, 2012a).

As we know the cochlear microphonics is a sustained response and stays as long as the stimulus is present, the time window for recording cochlear microphonics was varied for different tone burst frequencies and click stimuli. Test protocol which was used to record cochlear microphonics, has been mentioned in the table 3.2.

Table 3.2

Stimulus and Acquisition parameters to record CM

Parameter	Specification
Transducer type	Insert with Tiptrode
Type of stimulus	Tone burst of 500Hz, 1kHz, 4khz, 8kHz and Clicks
Stimulus duration	Click-100microseconds
	Toneburst:2-2-2 cycle
Intensity	100dBSPL
Stimulus polarity	Rarefaction and Condensation
Stimulus rate	30.1/sec and 59.1/sec
Number of sweeps	2000
Filter setting	300-10000Hz
Interelectrode impedance	<2k ohms
Intraelectrode impedance	<5k ohms
Gain	100000
Time window	500Hz and 1kHz-15ms
	4kHz,8kHz and click-5ms
Notch filter	Off

For all the different stimuli considered, the recording of cochlear microphonics were done separately for 30.1/sec and 59.1/sec repetition rates. Similarly, cochlear microphonics were also recorded separately for condensation and rarefaction polarity for all the stimuli. At each stimulus type, rate and polarity Cochlear Microphonics was recorded twice to check for replicability of the waveform. Hence a total of 40 [5(type of stimulus) x 2(rate) x 2(polarity) x 2 (replicability] waveforms were recorded in each participant.

The response waveforms were recorded with a broad filter setting of 300Hz to 10000Hz. It was done as we know that cochlear microphonic response mimics the frequency of the stimulus and the selected filter setting can accommodate the CM responses obtained for all stimulus used (tone burst of 500Hz, 2kHz, 4khz, 8kHz & click).

Responses were analysed using digital offline filtering and suitable filters for different tone burst responses were used i.e., response waveform of 500Hz was offline filtered with 300Hz to 700Hz, waveform of 1kHz was filtered with 300 to 1500Hz, waveform of 4kHz and click was filtered with 300 to 5kHz, whereas 8kHz waveform was filtered with 300 to 10kHz.This helped in removing unwanted ABR peaks and allowed better visualization of cochlear microphonic response. High pass filter was kept at 300 Hz as the instrument used to record Cochlear Microphonics did not have the facility to increase the high pass filter beyond 300 Hz.

Waveform analysis was carried out after digital offline filtering to note down latency of onset of the cochlear microphonic and amplitude of cochlear microphonics in each waveform. Thus, 20 (5 stimuli X 2 polarity X 2 rate) latency values and 20 amplitude values were obtained for every subject taken for the study.

The latency here is defined as the beginning of cycle where there is a significant polarity reversal is seen and is considered as the onset of cochlear microphonics as shown in figure 2. Hence, the latency was measured in the waveform by visually inspecting the time at which significant polarity reversal has taken place. A criterion point in amplitude was priorly set to make a decision of significant polarity reversal in the waveform. In a time scale the time at which the peak amplitude crossed or were equal to the criterion point was considered as a significant polarity reversal. Beginning of that peak which can be seen in the Figure 2 was considered as onset latency of cochlear microphonics.

The criterion point was set after taking a grand average of amplitude in 10 subjects for all the stimuli separately which lead to decide different criterion point for different tone burst frequencies. The average waveforms were also given to 3 audiologists to ascertain the onset of the CM. The point at which 2 out of 3 audiologist agreed upon were considered and peak amplitude of that point was considered as criteria point. The criterion points for different stimuli are listed in the table 3.3.

Table 3.3

Amplitude criterion points

Stimulus	Criterion point in micro volts
500Hz Tone burst	0.07
1kHz Tone burst	0.05
4kHzTone burst	0.03
8kHz Tone burst	0.02
click	0.03

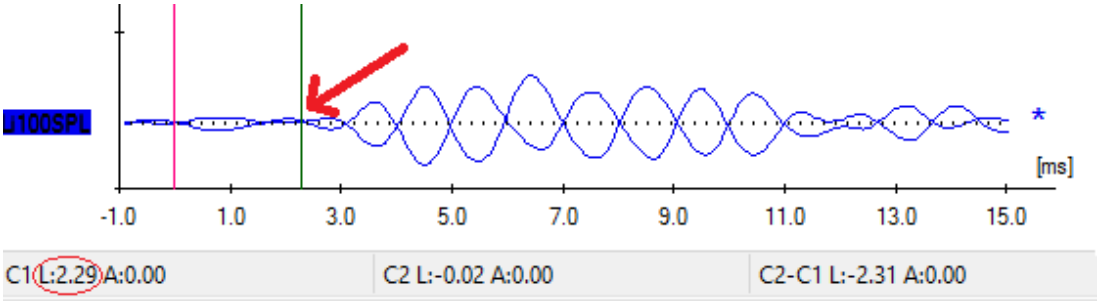

Fig 3.1: Depicts 500Hz tone burst evoked CM waveform and also the onset latency.

For example, in the above figure the first polarity reversal was considered at a latency where the cursor is placed which is shown by an arrow i.e., at 2.29ms. Here at 2.29 ms the amplitude of following peak is more than the criterion point, hence the starting point of that peak was considered as onset latency.

The amplitude parameter was measured as peak to peak amplitude by taking average of three consecutive highest and stable peaks as can be seen in the figure 3.2.

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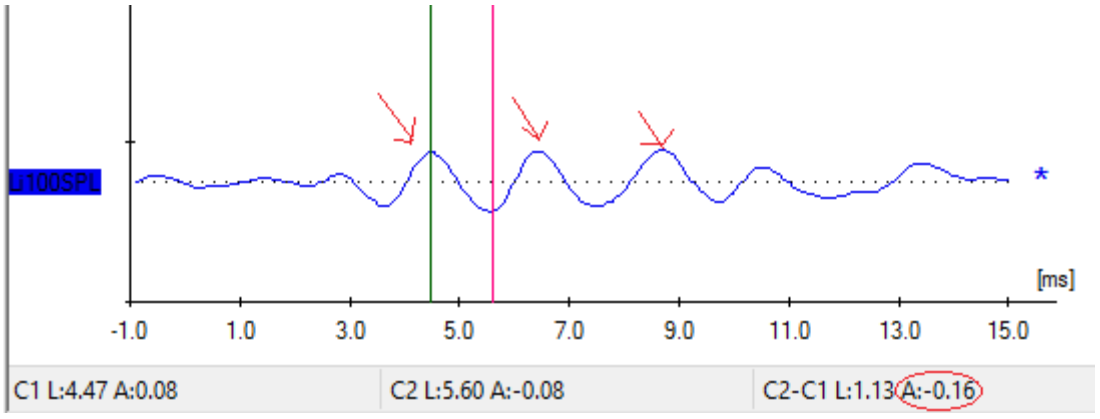

Fig 3.2: Depicts 500Hz tone burst evoked CM waveform and also the amplitude measurement.

For example, in the above waveform those peaks which are marked by arrows were considered as 3 highest or stable peaks. Amplitude analysis was done for each stable peaks by placing one cursor on the peak and other cursor on trough

and the amplitude difference between two cursors was considered as peak to peak amplitude. Here in the above waveform the peak to peak amplitude was 0.16 which has been rounded. Similarly peak to peak amplitude was calculated for all 3 stable peaks and the average of them was taken.

3.5 Statistical analysis

The data were subjected to statistical analysis using SPSS version 20 software. Amplitude and onset latency parameters were taken for the statistical analysis. Amplitude and latency values in different tone burst frequencies and clicks, different stimulation rate and different polarity were noted after the waveform analysis and entered in SPSS software. Descriptive statistics were done to find out the mean and standard deviations of onset latency and amplitude of cochlear microphonic. Distributions of the parameters were examined to decide on the kind of inferential statistics necessary.

Comparisons of the above mentioned parameters were done for the following stimuli conditions

- Parameters were compared between rarefaction and condensation polarities for each tone burst and click stimuli presented at 30.1/sec and 65.1/sec repetition rates separately to determine the effect of stimulus polarity on latency and amplitude of cochlear microphonics.
- Parameters were also compared between stimulus presented at 30.1/sec and 59.1/sec to determine the effect of stimulus rate on latency and amplitude of cochlear microphonics for each stimulus and polarity.
- Parameters were also compared across different tone burst frequencies (500Hz,1kHz,4kHz,8kHz) to determine the effect of stimulus frequency on latency and amplitude of cochlear microphonics.

- Parameters were also compared between tone burst and click stimuli to investigate the effect of stimulus type on latency and amplitude of cochlear microphonics.

Based on the outcome of the results, recommendation to use suitable stimulus parameter to record CM is made.

Chapter-4

RESULTS

The study was conducted on 32 individuals with normal hearing sensitivity. The objective of the study was to compare the parameters of cochlear microphonics across different tone burst frequencies and click. The study was also attempted to examine the effect of stimulus polarity, stimulus rate on parameters of cochlear microphonics. To investigate the above objective, amplitude and onset latency of the cochlear microphonics were considered for the comparison across different stimuli conditions.

Extratympanic mode was utilized to record the cochlear microphonics from the ear canal using tiptrode. Four different tone burst frequencies (500Hz,1kHz,4kHz & 8kHz) and click were presented as stimuli for recording of CM through insert ear phones. The recording of CM was done in rarefaction and condensation polarity separately to investigate the polarity effect. To examine the rate effect, the recording was also done independently for 30.1 and 59.1/sec stimuli conditions.

Amplitude and the latency of the cochlear microphonics were measured from the recorded waveform after the offline filtering which is described in the method part separately for all the variables considered for the study. Hence each subject had 20 amplitude values and 20 latency values.

Shapiro wilks and kolmogrov smirov tests were done to check the normality of the data. The results revealed that the data is significantly different from the normal distribution. The non- parametric tests were considered for the inferential statistics to check the significant difference between the variables of the study

because the data didn’t full fill the assumptions of parametric test such as, the data didn’t follow the normal distribution, data had more standard deviation etc.

Friedman test and Wilcoxon signed ranks tests were the 2 non-parametric tests that were considered for inferential statistics. Friedman test is a non-parametric equivalent of repeated measures of ANOVA used to detect difference across multiple variable. In this study, it was considered to check whether there is any significant difference in amplitude, latency across 3 different tone burst frequencies (8kHz was not considered) and click stimuli. Wilcoxon signed ranks test was considered to check the significant difference between stimuli for example 500Hz v/s 1kHz, 500Hz v/s 4kHz etc. Wilcoxon signed ranks test was also considered to examine the significant difference in amplitude and latency between polarity and also between rate(30.1&59.1/sec). Hence these 2 non parametric tests could help to investigate effect of stimulus type, frequency, stimulus polarity and stimulus rate on amplitude and latency of cochlear microphonics.

Thirty-two healthy normal ears were recruited for the recording of cochlear microphonics. Among 32 normal individuals most of the individuals had a presence of cochlear microphonics according to the set criterion in all the stimuli conditions. Whereas,14 individuals didn’t possess CM when they were stimulated with 8kHz tone burst at 30.1/sec and 10 individuals had absent CM when stimulated with 8kHz tone burst at 59.1/sec. In other words, all the stimuli condition except 8kHz tone burst had 100% occurrence rate of CM. Hence both amplitude and latency values of CM elicited by 8kHz tonebursst stimuli was not considered for the statistical analysis as it had less occurance of CM and could interfere with the statistical results. The details about the stimulus conditions and occurance of cochlear microphonics are shown in the table 4.1.

Table 4.1

Occurance of CM for different stimulus, rate and polarity

Stimulus condition	**No. individuals CM present/No. individuals participated**	**Occurance in %**
500HzTB_rarefraction_30.1/sec	32/32	100
500HzTB_condensation_30.1/sec	32/32	100
500HzTB_rarefraction_59.1/sec	32/32	100
500HzTB_condensation_59.1/sec	32/32	100
1kHzTB_rarefraction_30.1/sec	32/32	100
1kHzTB_condensation_30.1/sec	32/32	100
1kHzTB_rarefraction_59.1/sec	32/32	100
1kHzTB_condensation_59.1/sec	32/32	100
4kHzTB_rarefraction_30.1/sec	32/32	100
4kHzTB_condensation_30.1/sec	32/32	100
4kHzTB_rarefraction_59.1/sec	32/32	100
4kHzTB_condensation_59.1/sec	32/32	100
8kHzTB_rarefraction_30.1/sec	18/32	56.25
8kHzTB_condensation_30.1/sec	18/32	56.25
8kHzTB_rarefraction_59.1/sec	22/32	68.75
8kHzTB_condensation_59.1/sec	22/32	68.75
click_rarefraction_30.1/sec	32/32	100
click _condensation_30.1/sec	32/32	100
click _rarefraction_59.1/sec	32/32	100
click _condensation_59.1/sec	32/32	100

4.1 Properties of the cochlear microphonic waveform

The properties of the CM changes as the stimulus type and frequency varied. Cochlear microphonics recorded using tone burst stimuli found to have many cycles whereas, those recorded from click stimuli restricted to a single cycle. Among tone burst stimuli even though the same envelope of 2 -2-2 were used, the low frequency tone burst had longer duration cochlear microphonics when compared to higher frequency. The cochlear microphonics tend to be present as long as the stimulus is present and CM can be seen having more amplitude and present for a longer duration for low frequency and lesser duration for high frequency tone burst. The amplitude of the cochlear microphonics observed to be more in the plateau duration whereas, the amplitude dampens in raising and falling duration of the stimulus. Figure 4.1,4.2, 4.3 are the sample CM waveforms recorded for different stimuli considered for the study where they depict above described characteristics.

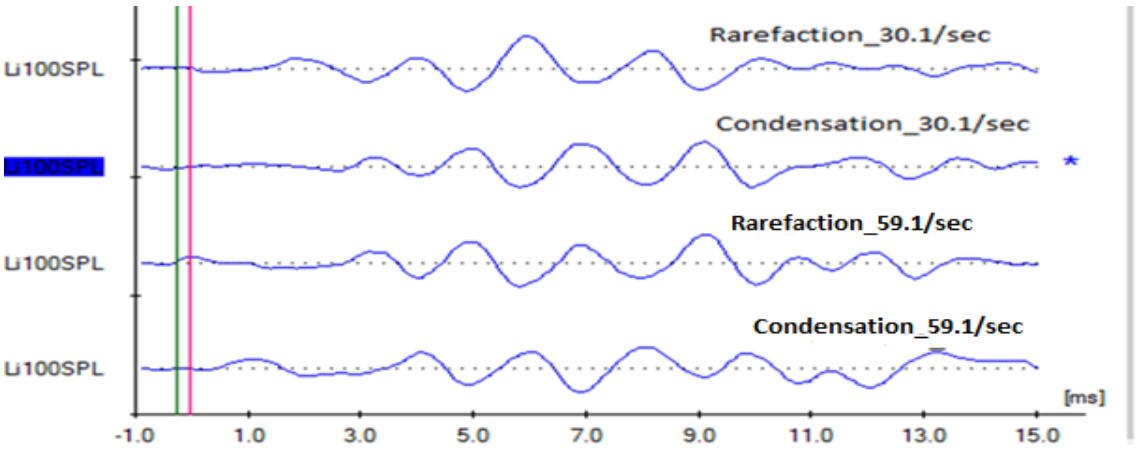

*Fig 4.1:*CM waveforms recorded using 500Hz tone burst for both polarity and rate

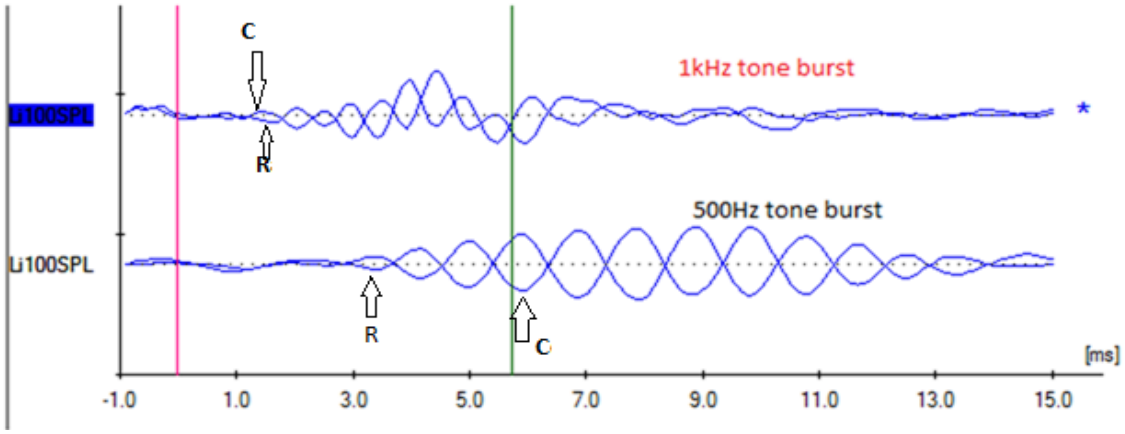

*Fig 4.2:*CM recorded using 1kHz and 500Hz TB for condensation and rarefaction polarity stimuli

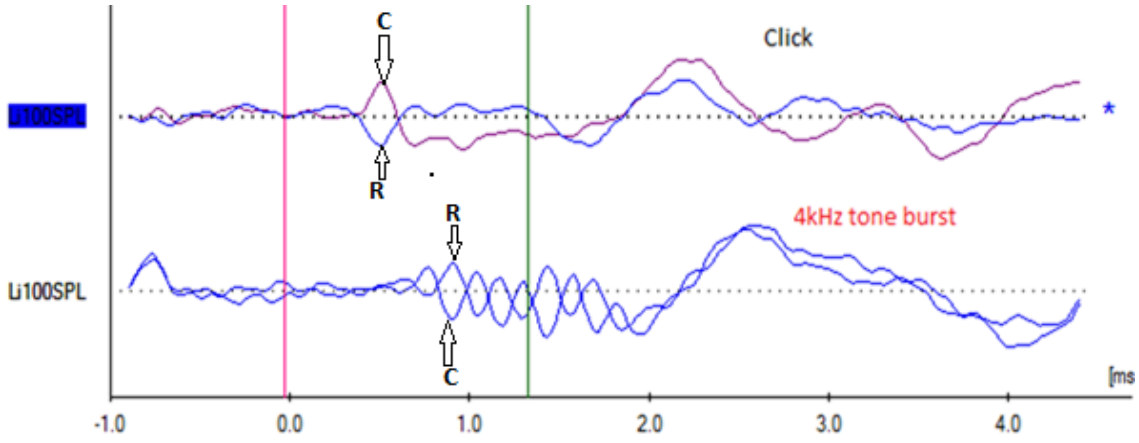

*Fig 4.3:*CM recorded using Click and 4kHz TB for condensation and rarefaction polarity stimuli

4.2 Amplitude of cochlear microphonics

Peak to peak amplitude was considered for the measurement of amplitude of CM. It was measured after the offline filtering of raw waveform as described in methods section and average peak to peak amplitude of 3 consecutive stable peaks were taken as a measure of amplitude. The amplitude measurement was taken separately for all the stimuli condition and was entered in SPSS software for further statistical analysis. Descriptive statistics was done to find out mean, median and standard deviation of CM amplitude. The details are shown in the table 4.2.

Table 4.2

Mean, median and SD of amplitude of cochlear microphonics across stimulus, rate and polarity.

Stimulus condition	N	Mean	Median	SD
500HzTB_rarefraction_30.1/sec	32	0.179	0.148	0.094
500HzTB_condensation_30.1/sec	32	0.171	0.146	0.091
500HzTB_rarefraction_59.1/sec	32	0.159	0.131	0.083
500HzTB_condensation_59.1/sec	32	0.157	0.133	0.082
1kHzTB_rarefraction_30.1/sec	32	0.170	0.156	0.091
1kHzTB_condensation_30.1/sec	32	0.176	0.168	0.100
1kHzTB_rarefraction_59.1/sec	32	0.155	0.141	0.076
1kHzTB_condensation_59.1/sec	32	0.158	0.148	0.081
4kHzTB_rarefraction_30.1/sec	32	0.092	0.080	0.047
4kHzTB_condensation_30.1/sec	32	0.096	0.090	0.050
4kHzTB_rarefraction_59.1/sec	32	0.093	0.080	0.044
4kHzTB_condensation_59.1/sec	32	0.100	0.095	0.044
click_rarefraction_30.1/sec	32	0.082	0.050	0.058
click _condensation_30.1/sec	32	0.085	0.070	0.058
click _rarefraction_59.1/sec	32	0.073	0.060	0.048
click _condensation_59.1/sec	32	0.071	0.050	0.045
8kHzTB_rarefaction_30.1/sec	18	0.039	0.040	0.010
8kHzTB_condensation_30.1/sec	18	0.042	0.040	0.008
8kHzTB_rarefaction_59.1/sec	22	0.040	0.040	0.010
8kHzTB_condensation_59.1/sec	22	0.047	0.050	0.012

Results of the descriptive statistics in the above table reveals that mean amplitude of CM is highest when it was elicited with 500Hz tone burst and it is least when it was elicited with 8kHz tone burst stimulus irrespective of the stimuli polarity and rate of stimulus. The descending order of mean amplitude of CM is 500Hz>1kHz>4kHz>click>8kHz. Though mean amplitude is highest for 500Hz tone burst, there is a negligible or very little difference in amplitude between 500Hz and 1kHz. Similarly mean amplitude of 4kHz tone burst and click stimuli is almost similar. This trend of mean amplitude across different stimulus type and frequencies are clearly depicted in the figure 4.4.

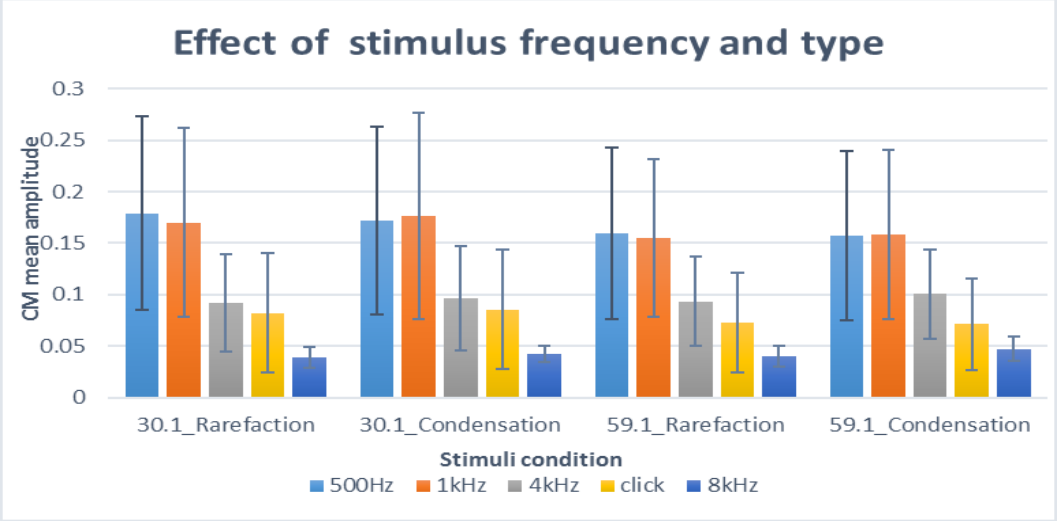

Fig4.4: Mean amplitude along with SD depicting effect of stimulus frequency and type at different polarity and rates.

In terms of comparison of amplitude between polarities, the mean amplitude shows almost negligible difference between CM elicited by rarefaction and condensation irrespective of stimulus frequency, type and rate of stimulus. Hence descriptive statistics shows no trend or no polarity effect on the amplitude of cochlear microphonics which is clearly depicted in a figure 4.5.

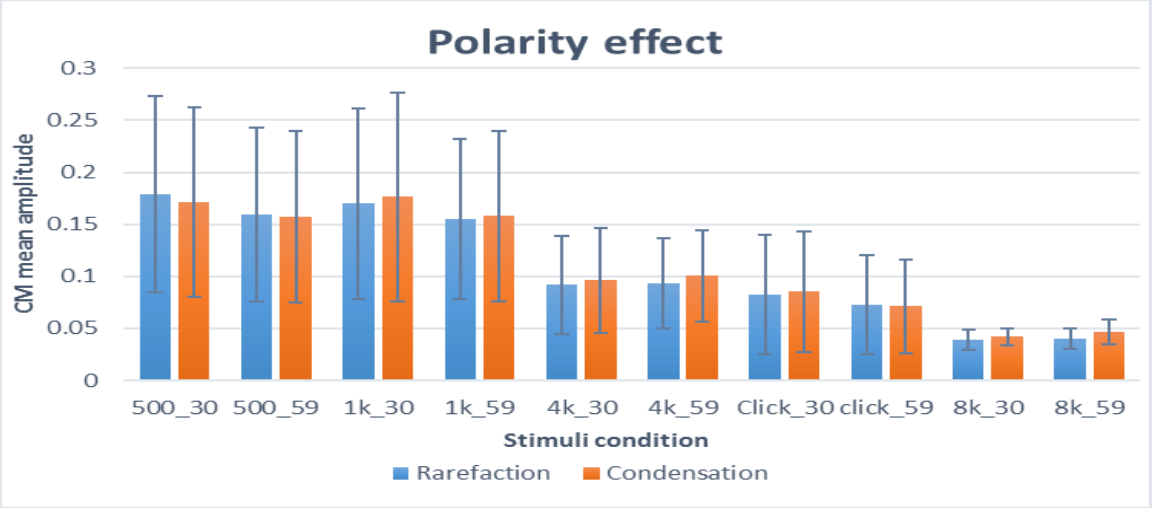

Fig4.5: Mean amplitude along with SD depicting effect of polarity across type of stimulus and rate

With respect to the effect of stimulus rate the results of descriptive statistics shows minimal or negligible difference in the mean amplitude between CM elicited by 30.1/sec and 59.1/sec irrespective of stimulus type, frequency and stimulus polarity. However, the CM elicited by 30.1 /sec shown to have a little higher mean amplitude when compare to 59.1/sec in most of the stimulus condition especially at 500 and 1000 Hz which is clearly shown in the figure 4.6.

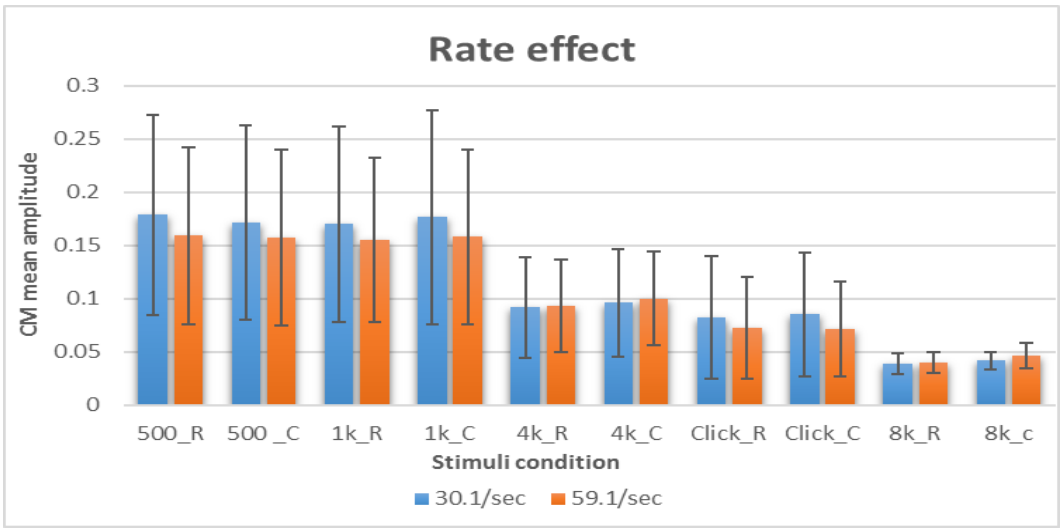

Fig4.6: Mean amplitude along with SD depicting effect of stimulus rate across type of stimulus and polarity.

Descriptive statistics of the amplitude of CM elicited by 8kHz tone burst was not considered for the comparison and also for further inferential statistics as it was difficult to identify the presence of CM in most of the ear and there was no consistency in continuity in CM with the presence of stimulus. Due to variability in CM waveform criterion value established to identify latency was not very accurate. Presence of CM at 8 KHz was more subjective and was considered by visual inspection. Also in many ears CM could not be recorded for 8 KHz tone burst. Hence it was considered for descriptive analysis and was not considered for inferential statistics. However, the mean value shows (table 4.2) that the amplitude of CM obtained at 8KHz is least among all the stimulus and there is no effect of stimulus polarity and rate.

4.2.1 Comparison of amplitude across stimulus

Friedman test was carried out in order to check the significant difference in amplitude across stimulus (500Hz,1kHz,4kHz and click). The test was carried out separately for amplitude recorded in rarefaction at 30.1/sec, condensation at 30.1/sec, rarefaction at 59.1/sec and condensation at 59.1/sec stimulus condition. Results of the Friedman test reveals that there is a statistically significant difference across stimulus in all the above 4 mentioned conditions. The details of statistical results are mentioned in the table 4.3.

Table4.3

Friedman's test results for amplitude comparison obtained across stimulus at each rate and polarity

	Chi-Square	Degrees of freedom(df)	P value
Rarefaction_30.1/sec	46.60	3	0.00
Condensation_30.1/sec	49.13	3	0.00
Rarefaction_59.1/sec	56.15	3	0.00
Condensation_59.1/sec	46.96	3	0.00

Friedman test showed that there is a significant difference in amplitude across the stimulus. In order to check between which two variables has significant difference, pairwise comparison was done using Wilcoxon Signed Ranks Test. As similar to Friedman test, Wilcoxon Signed Ranks Test was also carried out separately for all 4 condition. The results of Wilcoxon Signed Ranks Test reveals that there is no significant difference in amplitude between 1kHz v/s 500Hz in all the 4 above mentioned condition and even click v/s 4kHz didn't show any significant difference in rarefaction for 30.1 and condensation for 30.1 condition. As it can be seen in the table 4.4. Highlighted values obtained between the stimulus paired showed significant difference. Details of test statistic are mentioned in the table 4.4.

Table4.4

Wilcoxon test results for Comparison of amplitude obtained between the type of stimulus at each rate and polarity

	Rare_30.1		**Cond_30.1**		**Rare_59.1**		**Cond_59.1**	
	z	**p**	**Z**	**p**	**z**	**p**	**z**	**p**
1kHz v/s 500Hz	-.767	0.443	-.088	0.930	-.265	0.791	-.094	0.925
4kHz v/s 500Hz	-4.245	0.000	-4.227	0.000	-4.018	0.000	-3.488	0.000
Click v/s 500Hz	-4.638	0.000	-4.414	0.000	-4.685	0.000	-4.744	0.000
4kHz v/s 1kHz	-4.105	0.000	-4.009	0.000	-4.228	0.000	-3.443	0.000
click v/s 1kHz	-4.517	0.000	-4.422	0.000	-4.783	0.000	-4.682	0.000
click v/s 4kHz	-1.593	0.111	-1.498	0.134	-3.410	0.001	-4.167	0.000

4.2.2 Comparison of amplitude between polarity:

Wilcoxon Signed Ranks test was carried out to detect significant difference in amplitude of CM between rarefaction and condensation polarity. The comparison between polarity was made separately for 500Hz_30.1/sec, 500Hz_59.1/sec, 1kHz_30.1/sec, 1kHz_59.1/sec, 4kHz_30.1/sec, 4kHz_59.1/sec, click_30.1/sec and click_59.1/sec stimulus conditions. Test statistics of Wilcoxon Signed Ranks Test reveals no significant polarity effect or no significant difference in amplitude between rarefaction and condensation polarity irrespective of the stimulus conditions mentioned above except for 4kHz_59.1/sec condition where there is a significant difference in amplitude between polarity. Details of test statistic are mentioned in the table 4.5.

Table4.5

Z value and significance level obtained for comparison of amplitude obtained between polarity at each stimulus type/frequency and rate

	Z value	Asymptomatic significance(p)
500Hz_30.1/sec	-1.177	0.239
500Hz_59.1/sec	-.171	0.864
1kHz_30.1/sec	-1.716	0.086
1kHz_59.1/sec	-.140	0.888
4kHz_30.1/sec	-1.108	0.268
4kHz_59.1/sec	-2.354	0.019
click_30.1/sec	-.521	0.602
click_59.1/sec	-.512	0.608

4.2.3 Comparison of amplitude between rate of stimulus

Wilcoxon Signed Ranks Test was carried out to detect significant difference in amplitude of CM between 30.1/sec and 59.1/sec. The comparison of amplitude between stimulus rate were made separately within 500Hz for rarefaction, 500Hz for condensation, 1kHz for rarefaction, 1kHz for condensation, 4kHz for rarefaction, 4kHz for condensation, click for rarefaction and click for condensation conditions. Test statistics of Wilcoxon Signed Ranks Test reveals no significant difference in amplitude between 30.1/sec and 59.1/sec among 4kHz for rarefaction, 4kHz for condensation and click for rarefaction conditions whereas, rest other conditions did show a significant difference which are highlighted in table 4.6. Details of test statistic are mentioned in the table 4.6.

Table 4.6

Z value and significance level obtained for comparison of amplitude between stimulus rate within stimulus type and polarity

	Z value	Asymptomatic significance(p)
500Hz_Rarefaction	-2.891	0.004
500Hz_Condensation	-2.654	0.008
1kHz_ Rarefaction	-2.937	0.003
1kHz_ Condensation	-3.157	0.002
4kHz_ Rarefaction	-.572	0.567
4kHz_ Condensation	-1.215	0.225
click_ Rarefaction	-1.584	0.113
click_ Condensation	-2.145	0.032

4.2.4 Comparison of amplitude difference due to polarity change across stimulus

The amplitude of rarefaction was subtracted from amplitude of condensation for all the stimulus (500Hz_30.1/sec, 500Hz_59.1/sec, 1kHz_30.1/sec, 1kHz_59.1/sec, 4kHz_30.1/sec, 4kHz_59.1/sec, click_30.1/sec and click_59.1/sec) conditions and is termed as amplitude difference. This resultant difference in amplitude was compared across 500Hz, 1kHz,4kHz and click stimuli separately for 30.1/sec and 59.1/sec. Descriptive statistics was carried out to find out the mean amplitude difference due to polarity change along with SD and median. Results of descriptive statistics shows that mean amplitude difference is minimal and seems almost similar across all the stimulus condition which is depicted in figure 4.7 and the details of which is given in the table 4.7.

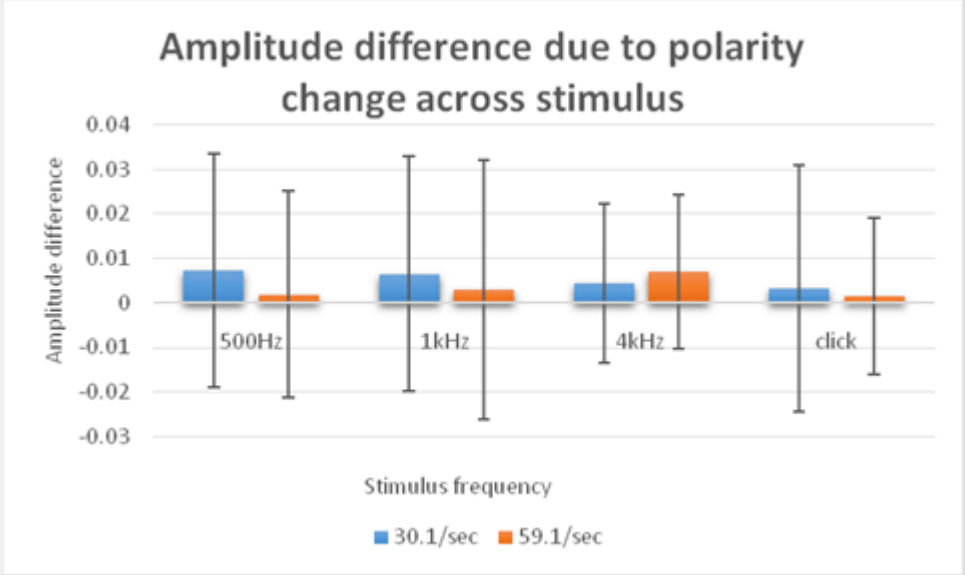

Figure 4.7: Mean amplitude difference along with SD due to polarity change across stimulus and rate

Table:4.7

Mean and median amplitude difference along with SD due to polarity change across stimulus and rate

	Mean	**Median**	**SD**
500Hz_30.1/sec	0.007	0.003	0.026
500Hz_59.1/sec	0.002	0.000	0.023
1kHz_30.1/sec	0.006	0.007	0.026
1kHz_59.1/sec	0.003	0.003	0.029
4kHz_30.1/sec	0.004	0.005	0.018
4kHz_59.1/sec	0.007	0.010	0.017
Click_30.1/sec	0.003	0.000	0.027
Click_59.1/sec	0.001	0.000	0.017

Friedman test was done to find out the significant difference in amplitude difference due to polarity change across stimulus and rate. The test results reveals

that there was no significant difference in amplitude difference across stimulus in both repetition rates (30.1/sec&59.1/sec). Details of the test statistic are mentioned in the table 4.8.

Table 4.8:
Friedman tests results for *effect of polarity across stimulus within stimulus rates*

	30.1/sec	**59.1/sec**
Chi-Square	3.059	2.840
Degrees of freedom	3	3
Asymptomatic significance(p)	0.383	0.417

4.2.5 Comparison of amplitude difference due to rate change across stimulus

Amplitude of 30.1/sec was subtracted from amplitude obtained at 59.1/sec for all the stimulus conditions (500Hz_rarefaction, 500Hz_condensation, 1kHz_rarefaction, 1kHz_condensation, 4kHz_rarefaction, 4kHz_condensation, click_rarefaction and click _condensation) and is termed as amplitude difference due to rate. This resultant difference in amplitude was compared across 500Hz, 1kHz,4kHz and click stimuli separately for rarefaction and condensation polarity. Results of descriptive statistics reveals that the mean amplitude difference due to rate change is minimal. The difference is relatively more at low frequency compare to high frequency and click stimulus which is depicted in the figure 4.8 and details of which is given in the table 4.9.

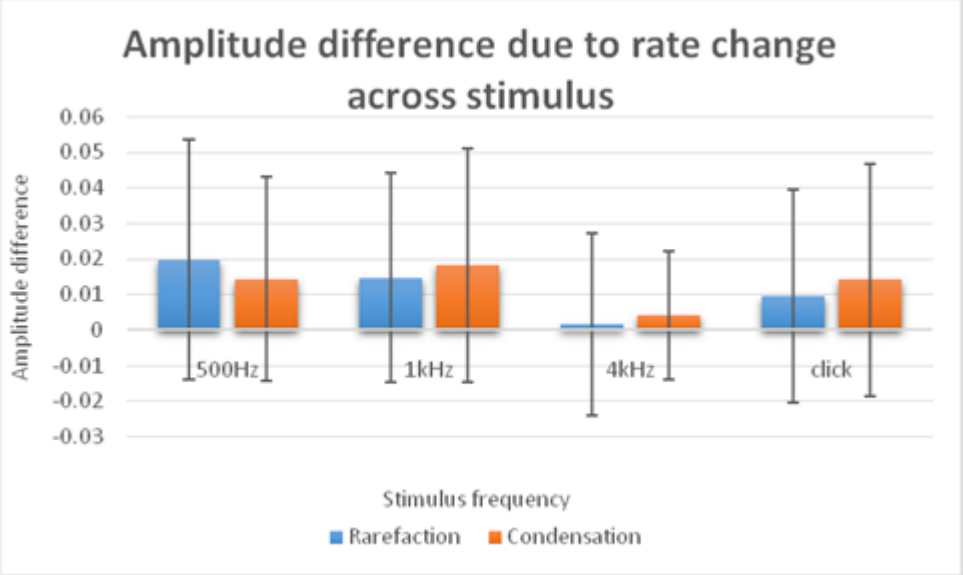

Figure 4.8: Mean amplitude difference along with SD due to rate change across stimulus type and polarity

Table 4.9

Mean and median of amplitude difference along with SD due to rate change across stimulus type and polarity

	Mean	**Median**	**SD**
500Hz_rarefaction	0.020	0.013	0.034
500Hz_condensation	0.014	0.013	0.029
1kHz_rarefaction	0.015	0.011	0.029
1kHz_condensation	0.018	0.017	0.033
4kHz_rarefaction	0.001	0.000	0.026
4kHz_condensation	0.004	0.005	0.018
Click_rarefaction	0.009	0.000	0.030
Click _condensation	0.014	0.005	0.032

Friedman test was done to find out the significant difference in amplitude difference due to rate change across stimulus. Friedman test reveals that there was no significant difference in amplitude difference across stimulus in rarefaction polarity

whereas, there was a significant difference in amplitude difference across stimulus in condensation polarity. Details of the test statistic are mentioned in the table 4.10.

Table 4.10

Effect of stimulus rate across stimulus within polarity

	Rarefaction	**Condensation**
Chi-Square	4.931	14.329
Degrees of freedom	3	3
Asymptomatic significance(p)	0.177	0.002

Friedman test results revealed a significant difference across stimuli in only condensation condition. So in order to check among which 2 stimuli in condensation condition there is a significant difference, pairwise comparison was done using Wilcoxon sign rank test. The results revealed that there was a significant difference between 4kHz v/s 500Hz, 4kHz v/s 1kHz and click v/s 4kHz stimulus which are highlighted. Whereas, rest of the conditions didn't show any significant difference. The details of the tests results are given in the table 4.11.

Table 4.11

Z value and significance level obtained for the comparison of effect of stimulus rate on amplitude difference between stimulus within condensation polarity

	Z value	**Asymptomatic significance(p)**
1kHz v/s 500Hz	-.588	0.557
4kHz v/s 500Hz	-3.251	0.001
Click v/s 500Hz	-.324	0.746
4kHz v/s 1kHz	-3.105	0.002
click v/s 1kHz	-1.039	0.299
click v/s 4kHz	-2.695	0.007

4.3 Onset latency of cochlear microphonics

Onset latency of the CM is the second parameter which was considered in the study to examine the effects of stimulus type, frequency, rate and polarity. The latency here is defined as the beginning of cycle where there is a significant polarity reversal is seen. Hence, the latency was measured in the waveform by visually inspecting the beginning of the first wave reversal which had significant amplitude at its maxima. The latency was measured separately for all the stimuli condition and was entered in SPSS software for further statistical analysis. Descriptive statistics was done to find out mean, median and standard deviation of CM onset latency. The details are shown in the table 4.12.

Results of the descriptive statistics in the above table reveals that mean latency of CM is longest when it is elicited with 500Hz tone burst and it is shortest when it is elicited with 8kHz stimulus irrespective of the stimuli polarity and rate of stimulus. The descending order of mean latency of CM is 500Hz>1kHz>4kHz>click>8kHz. Though the mean latency is longer for 4kHz tone burst when compare to click, there is very little difference in latency between CM elicited by 4kHz and click. This trend of mean latency across different stimulus type and frequencies are clearly depicted in the figure 4.9.

Table 4.12

Mean, median and SD of latency of cochlear microphonics across stimulus, rate and polarity

Stimulus condition	N	Mean	Median	SD
500HzTB_rarefraction_30.1/sec	32	2.99	2.85	0.71
500HzTB_condensation_30.1/sec	32	2.87	2.66	0.70
500HzTB_rarefraction_59.1/sec	32	2.93	2.72	0.69
500HzTB_condensation_59.1/sec	32	2.85	2.72	0.65
1kHzTB_rarefraction_30.1/sec	32	1.56	1.57	0.21
1kHzTB_condensation_30.1/sec	32	1.53	1.54	0.22
1kHzTB_rarefraction_59.1/sec	32	1.60	1.63	0.27
1kHzTB_condensation_59.1/sec	32	1.57	1.54	0.27
4kHzTB_rarefraction_30.1/sec	32	0.56	0.570	0.21
4kHzTB_condensation_30.1/sec	32	0.55	0.570	0.20
4kHzTB_rarefraction_59.1/sec	32	0.59	0.580	0.18
4kHzTB_condensation_59.1/sec	32	0.60	0.58	0.18
click_rarefraction_30.1/sec	32	0.42	0.42	0.14
click _condensation_30.1/sec	32	0.42	0.42	0.13
click _rarefraction_59.1/sec	32	0.41	0.40	0.13
click _condensation_59.1/sec	32	0.40	0.40	0.13
8kHzTB_rarefraction_30.1/sec	18	0.22	0.22	0.08
8kHzTB_condensation_30.1/sec	18	0.22	0.22	0.08
8kHzTB_rarefraction_59.1/sec	22	0.21	0.21	0.08
8kHzTB_condensation_59.1/sec	22	0.21	0.21	0.08

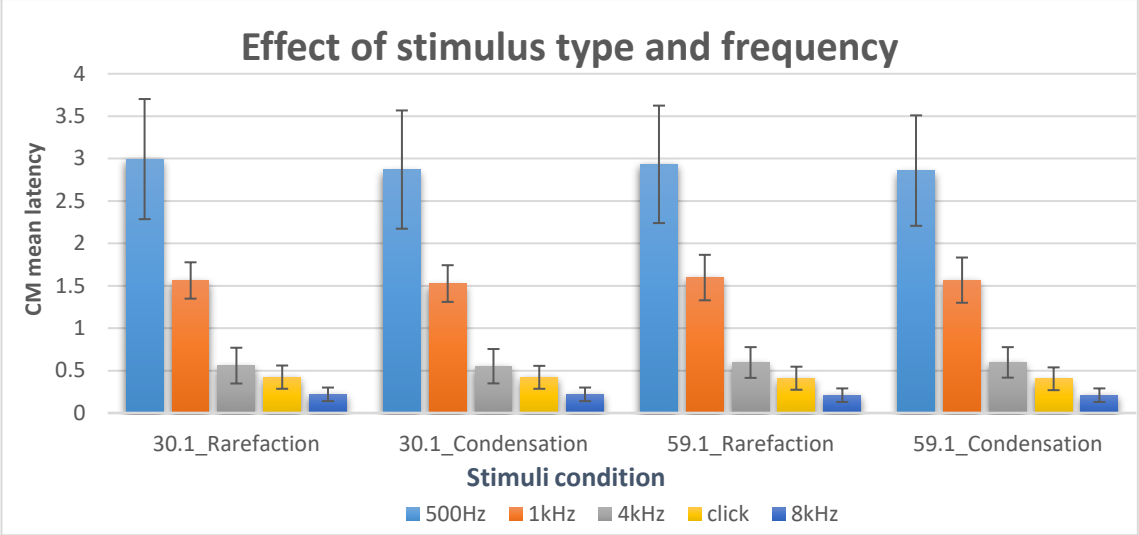

Fig 4.9: Mean latency along with SD across different stimulus conditions

In terms of comparison of latency between polarities, the mean latency shows almost negligible difference between CM elicited by rarefaction and condensation polarity irrespective of stimulus frequency, type and rate of stimulus. However, the CM elicited by low frequency especially 500Hz shows a little difference in latency between polarity having relatively longer latency for rarefaction and shorter for condensation. Hence descriptive statistics shows no trend or no polarity effect on the latency of cochlear microphonics which is clearly depicted in the figure 4.10.

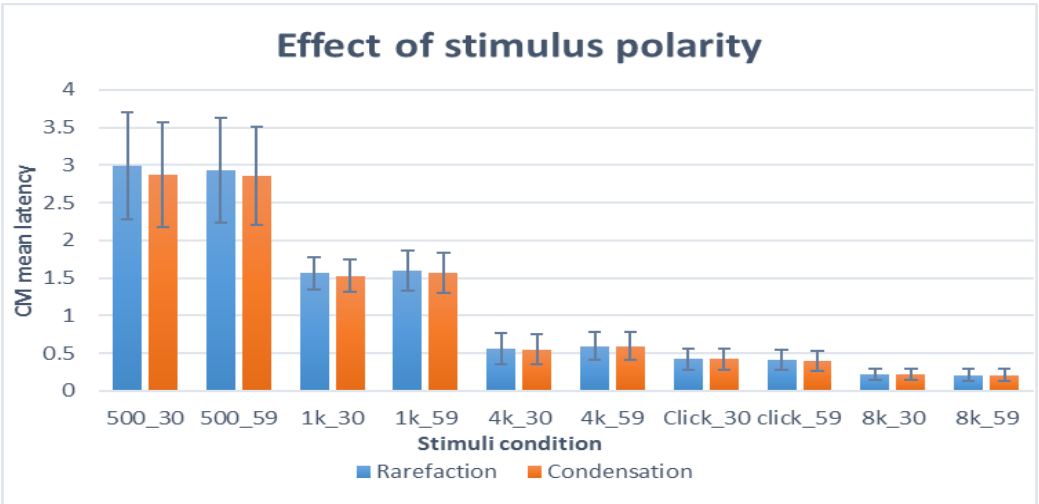

Fig 4.10: Mean latency along with SD depicting effect of polarity across type of stimulus and rate.

With respect to the effect of stimulus rate, the results of descriptive statistics show minimal or negligible difference in the mean latency between CM elicited by 30.1/sec and 59.1/sec stimulus rate irrespective of stimulus type, frequency and stimulus polarity. Hence descriptive statistics shows no trend or no rate effect on the latency of cochlear microphonics which is clearly depicted in the figure 4.11.

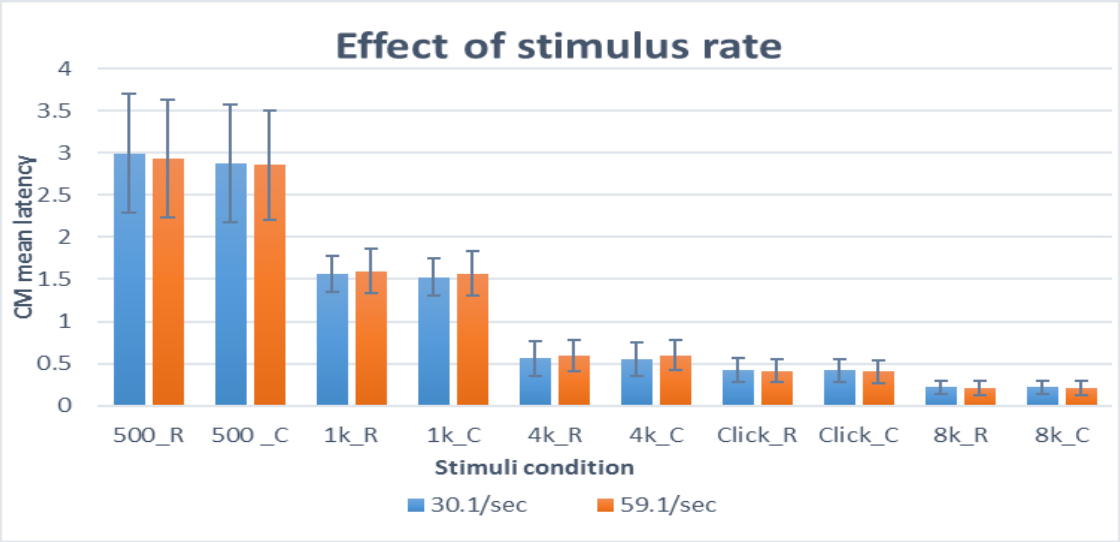

Fig4.11: Mean latency along with SD depicting effect of stimulus rate across type of stimulus and polarity

Similar to amplitude section, onset latency of CM elicited by 8kHz tone burst stimuli considered for descriptive statistics not for inferential statistics because of the same reasons mentioned earlier in amplitude section.

4.3.1 Comparison of latency across stimulus

Friedman test was carried out in order to check the significant difference in latency across stimulus (500Hz,1kHz,4kHz and click). The test was carried out separately for latency recorded using rarefaction at 30.1/sec, condensation at 30.1/sec, rarefaction at 59.1/sec and condensation at 59.1/sec stimulus conditions. Results of Friedman test reveals that there is a statistically significant difference in

the latency across stimulus in all the above 4 mentioned conditions. The details of test statistic are mentioned in the table 4.13.

Table 4.13

Friedman's test results for comparison of latency obtained across stimulus at different rate and polarity

	Chi-Square	Degrees of freedom(df)	P value
Rarefaction_30.1/sec	86.143	3	0.000
Condensation_30.1/sec	86.805	3	0.000
Rarefaction_59.1/sec	92.459	3	0.000
Condensation_59.1/sec	91.382	3	0.000

In order to check among which variables has significant difference, pairwise comparison was done using Wilcoxon Signed Ranks Test. As similar to Friedman test, Wilcoxon Signed Ranks Test was also carried out separately for all 4 conditions. Results of Wilcoxon Signed Ranks Test reveal that there is a presence of significant difference in latency between all the pairs compared irrespective of any stimuli condition. Details of test statistic results are mentioned in the table 4.14.

Table 4.14

Z value and significance level obtained for comparison of latency between the type of stimulus at each rate and polarity

	Rare_30.1		Cond_30.1		Rare_59.1		Cond_59.1	
	z	**p**	**z**	**p**	**z**	**p**	**z**	**p**
1kHz v/s 500Hz	-4.861	0.000	-4.861	0.000	-4.937	0.000	4.339	0.000
4kHz v/s 500Hz	-4.937	0.000	-4.937	0.000	-4.937	0.000	-4.937	0.000
Click v/s 500Hz	-4.860	0.000	-4.860	0.000	-4.937	0.000	-4.937	0.000
4kHz v/s 1kHz	-4.937	0.000	-4.937	0.000	-4.937	0.000	-4.937	0.000
click v/s 1kHz	-4.861	0.000	-4.861	0.000	-4.937	0.000	-4.937	0.000
click v/s 4kHz	-4.938	0.000	-2.736	0.006	-3.793	0.000	-4.019	0.000

4.3.2 Comparison of latency between polarity

Wilcoxon Signed Ranks test was carried out to detect significant difference in latency of CM between rarefaction and condensation polarity. Test statistics of Wilcoxon Signed Ranks test reveals no significant polarity effect or no significant difference in latency between rarefaction and condensation polarity in 4kHz at 30.1/sec, click at 30.1/sec, 4kHz at 59.1/sec and 1kHz at 59.1/sec stimulus conditions. Whereas, conditions such as 500Hz at 30.1/sec,500Hz at 59.1/sec, 1kHz at 30.1/sec and click at 59.1/sec had shown a significant polarity effect. Details of test statistic are mentioned in the table 4.15.

Table 4.15

Z value and significance level obtained for Comparison of latency between polarity at each stimulus frequency and rate

	Z value	**Asymptomatic significance(p)**
500Hz_30.1/sec	-3.306	0.001
500Hz_59.1/sec	-2.549	0.011
1kHz_30.1/sec	-3.201	0.001
1kHz_59.1/sec	-1.602	0.109
4kHz_30.1/sec	-0.730	0.465
4kHz_59.1/sec	-0.447	0.655
click_30.1/sec	-1.414	0.157
click_59.1/sec	-2.032	0.042

4.3.3 Comparison of latency between rate of stimulus:

Wilcoxon Signed Ranks Test was carried out to detect significant difference in latency of CM between 30.1/sec and 59.1/sec. The comparison of latency between

stimulus rate were made separately within 500Hz for rarefaction, 500Hz for condensation, 1kHz for rarefaction, 1kHz for condensation, 4kHz for rarefaction, 4kHz for condensation, click for rarefaction and click for condensation stimulus conditions. The test results reveal no significant rate effect or no significant difference in latency between 30.1/sec and 59.1/sec in all the stimuli conditions. Details of test statistic are mentioned in the table 4.16.

Table 4.16

Z value and significance level obtained for Comparison of latency between rate of stimulus at each stimulus frequency and polarity

	Z value	**Asymptomatic significance(p)**
500Hz_Rarefaction	-1.235	0.217
500Hz_Condensation	-0.292	0.770
1kHz_ Rarefaction	-1.039	0.299
1kHz_ Condensation	-1.180	0.238
4kHz_ Rarefaction	-0.905	0.365
4kHz_ Condensation	-1.008	0.313
click_ Rarefaction	-0.137	0.891
click_ Condensation	-0.065	0.948

4.3.4 Comparison of latency difference due to polarity change across stimulus

The latency of rarefaction was subtracted from latency obtained at condensation for all the stimulus conditions and is termed as latency difference. This resultant difference in latency was compared across 500Hz, 1kHz,4kHz and click stimuli separately for 30.1/sec and 59.1/sec. Descriptive statistics was done to find out the mean, median and SD of the latency difference due to polarity change and reveal that the latency difference is seen to be relatively more in low frequency

compare to higher frequency which is depicted in the figure 4.12 and details of which can be seen in the table 4.17.

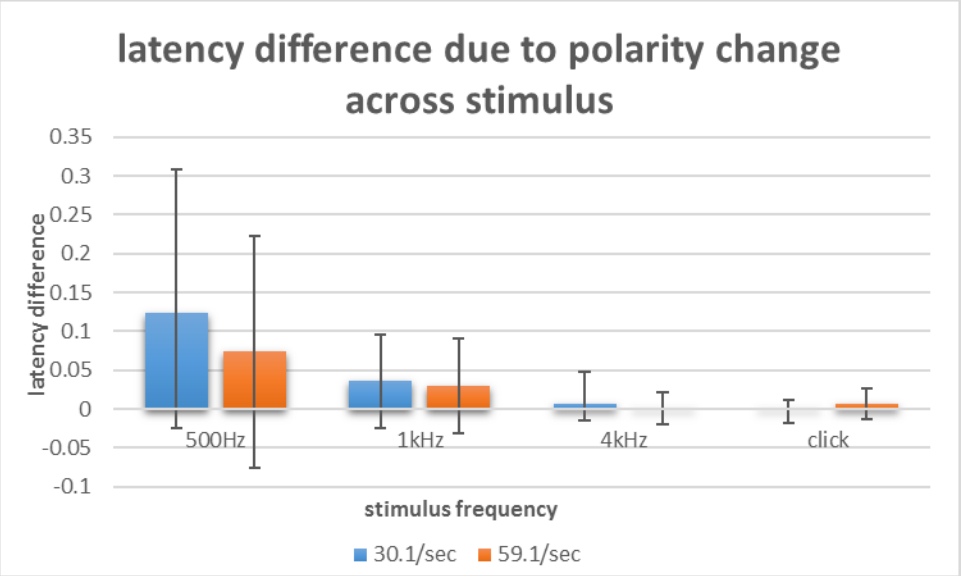

Figure 4.12: Mean latency difference along with SD due to polarity change at each stimulus type and rate

Table 4.17

Mean and median latency difference along with SD due to polarity change at each Stimulus type and rate

	Mean	**Median**	**SD**
500Hz_30.1/sec	0.124	0.000	0.185
500Hz_59.1/sec	0.074	0.000	0.148
1kHz_30.1/sec	0.036	0.000	0.059
1kHz_59.1/sec	0.030	0.000	0.060
4kHz_30.1/sec	0.007	0.000	0.040
4kHz_59.1/sec	0.001	0.000	0.021
Click_30.1/sec	0.002	0.000	0.010
Click_59.1/sec	0.007	0.000	0.020

Friedman test was done to find out the significant difference in latency difference due to polarity change across stimulus. The test results reveals that there is a significant difference in latency difference across stimulus in both repetition rates (30.1/sec&59.1/sec). Details of the test statistic are mentioned in the table 4.18.

Table 4.18

Friedman test results for comparison of latency difference due to polarity change across stimulus at each rate

	30.1/sec	**59.1/sec**
Chi-Square	28.832	8.394
Degrees of freedom	3	3
Asymptomatic significance(p)	0.000	0.039

Friedman test results revealed a significant difference in latency difference across stimuli in both 30.1 and 59.1/sec condition. So in order to check among which pair of stimuli there is a significance difference, pairwise comparison was done using Wilcoxon signed rank test. In 30.1/sec condition results of Wilcoxon signed rank test reveal that there is a significant difference in latency difference between all the pairs except click v/s 4kHz. In 59.1/sec there is no significant difference in latency difference between all the pairs except 4kHz v/s 500Hz and click v/s 500Hz. Details of the test statistic are mentioned in the table 4.19.

Table 4.19

Z value and significance level obtained for Comparison of latency difference due to polarity change between stimulus at each rate

	30.1/sec		**59.1/sec**	
	Z	**p**	**Z**	**P**
1kHz v/s 500Hz	-2.615	0.009	-1.918	0.055
4kHz v/s 500Hz	-3.098	0.002	-2.549	0.011
Click v/s 500Hz	-3.331	0.001	-2.401	0.016
4kHz v/s 1kHz	-2.619	0.009	1.402	0.161
click v/s 1kHz	-3.313	0.001	-1.423	0.155
click v/s 4kHz	-0.948	0.343	-1.214	0.225

4.3.5 Comparison of latency difference due to rate change across stimulus

Latency of 30.1/sec was subtracted from latency obtained at 59.1/sec for all the stimulus conditions and is termed as latency difference. This resultant difference in latency was compared across 500Hz, 1kHz,4kHz and click stimuli separately for rarefaction and condensation polarity. The results of descriptive statistics show the mean latency difference due to rate change is minimal across the stimulus. The details of mean latency difference along with SD is given in the table 4.20 and also depicted in the figure 4.13.

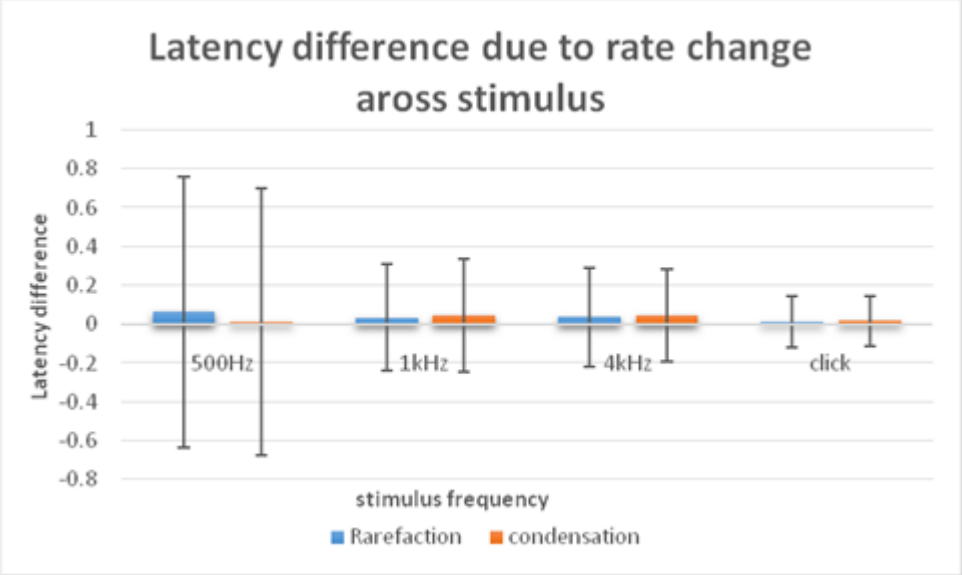

Figure 4.13: Mean latency difference due to rate change along with SD across stimulus type at each polarity.

Table 4.20

Mean and median latency difference along with SD due to rate change at each stimulus type and polarity

	Mean	**Median**	**SD**
500Hz_rarefaction	0.062	0.090	0.700
500Hz_condensation	0.012	0.030	0.687
1kHz_rarefaction	0.034	0.045	0.273
1kHz_condensation	0.044	0.025	0.291
4kHz_rarefaction	0.037	0.080	0.255
4kHz_condensation	0.045	0.080	0.237
Click_rarefaction	0.013	0.000	0.131
Click _condensation	0.017	0.005	0.128

Friedman test was done to find out the significant difference in latency difference due to rate change across stimulus. The test statistic of the Friedman test reveals that there was no significant difference in latency difference across stimulus

in both rarefaction and condensation polarity. Details of the test statistic are mentioned in the table4.21. As there was no significant effect pairwise comparison was not done.

Table 4.21

Friedman test results obtained for comparison of latency difference due to rate change across stimulus at each polarity

	Rarefaction	**Condensation**
Chi-Square	7.657	3.238
Degrees of freedom	3	3
Asymptomatic significance(p)	0.054	0.356

Chapter-5

DISCUSSION

5.1 Effect of stimulus frequency and type on CM

The first objective of the study was to compare the amplitude and latency of the CM with different stimuli such as click and tone burst stimuli with different frequencies. The findings of the study showed that the amplitude of the CM elicited with tone burst stimuli is more than click stimuli. Across 4 tone burst stimuli the amplitude of CM is inversely proportional to the stimulus frequency i.e., the amplitude decreased as the stimulus frequency increased. The above effect had statistically significant difference except between 500Hz v/s 1kHz and 4kHz v/s click however, 8KHz data was not analysed for the comparison. In other words, results conclude that the largest CM amplitude is found for lower frequency (500Hz and 1kHz) tone burst compare to higher frequency(4kHz&8kHz) and amplitude is more for tonal stimuli compared to click stimuli. The results are in agreement with a previous studies by Ponton, Don, and Eggermont, 1992; Liu, Chen, and Xu, 1992; and Zhang, 2012. They have also found large amplitude for low frequency stimulus and smaller amplitude for high frequency tone burst and concluded that although clicks can be used to produce the response, the CM was more robust in response to tonal stimulation and the largest CM responses were produced by 500 Hz and 1kHz tone bursts.

Contradictory results were found in a study by Heidari, Pourbakht, Kamrava, Kamali and Yousefi, 2018 where they stated that the CM amplitude with click (broad band stimuli) is generally larger than tonal stimuli. However, the tonal frequencies taken for the comparison in this study were 2, 4, 8 and 16 kHz which is relatively high frequency which may be the reason for the contradictory results. However, their

findings of amplitude across tonal frequencies were in agreement with the present study i.e., the larger amplitude obtained with lower frequency than higher frequency and they attributed this results to the fact that amplitude is influenced by the bandwidth of stimulation. The generated traveling waves of the basilar membrane displacement on acoustic stimulation travels from base to apex and forms a peak or maximal displacement in the respective position on basilar membrane. Hence the pattern of traveling waves and peak position on basilar membrane is dependent on the stimulus frequency(Gelfand, 2010; Pickles, 2012). Since the low frequency stimulus generates a traveling wave which has to travel from base to apex, region of tail portion is more extended than high frequency. In other words, traveling wave of lower frequencies travel longer distance along basilar membrane and excites more number of hair cells. Additionally, the volume of hair cells is more in apical end than basal which leads to more number of hair cell involvement for lower frequencies. Since CM is a reflection of the spatial summation of hair cell receptor currents(Cheatham et al., 2005; Dallos & Cheatham, 1976) the CM amplitude is found to be larger for lower frequencies.

Findings of onset latency of CM showed that the onset latency of CM is also inversely proportional to the stimulus frequency. In other words, the lower frequencies had a longer latency whereas, higher frequency and click had a shorter latency. The effect of stimulus frequency and type on CM onset latency was statistically significant. CM onset latency measurement is a relatively new and best of our knowledge there are no previous literature where they have analysed latency across stimulus frequency especially for extratympanic CM recording in ear canal. However Zhang (2013) had examined the effect of stimulus intensity on CM latency and found that it is intensity independent and attributed the results to physiological

features of hair cells where he explains that, the hair cells generate CMs almost instantaneously once stimulation reaches the hair cells without any delay, irrespective of any change in stimulus intensities. It has been also stated that, as a physiological response, the CM waveforms occur/appear after a latency but does not occur instantaneously at the same time as the onset of stimulus(Zhang, Paschall, Chandler, Reel& Foster,2003; Zhang, 2010).

Although hair cells generate the CM instantaneously once stimulus reaches, the latency results of the present study can be attributed for travel time for stimulus to reach the particular set of hair cell on basilar membrane. Hence the CM elicited by low frequency tone burst has got a longer latency since it has to travel to the apical end of the basilar membrane compare to higher frequencies.

It was also observed in the study that the CM recorded from tone burst had many cycles whereas, those recorded from click stimuli restricted to single cycle. Among tone burst stimuli those recorded with lower frequencies were longer when compare to higher frequencies and findings are in consistent with the studies by Zhang, 2013 and Zhang, 2012. Since the stimulus envelope was kept constant (2-2-2) for all 3 tone burst frequencies, the low frequency stimulus which had longer duration per cycle had a longer duration of CM when compare to higher frequencies. Also, filter effect or critical bandwidth of different frequencies lead to more ringing in lower frequencies(Moore, 1997). It was also observed that the amplitude of the cochlear microphonics observed to be more in the plateau duration whereas, the amplitude dampens in raising and falling duration of the stimulus and this can be attributed to Maximum SPL at plateau of the stimulus leading to more number of hair cell stimulated.

5.2 Effect of stimulus polarity

Second objective of the study was to compare the amplitude and latency of cochlear microphonics elicited by rarefaction and condensation polarity in order to examine the effect of stimulus polarity. Results suggest that there is no significant difference in amplitude of the CM in both the polarities at all stimulus frequencies and stimulus rate except a stimulus condition where CM elicited by 4kHz at 59.1/sec. This could be due to the chance factor or due to very low amplitude observed at this frequency and slight variation in amplitude might have resulted in significant different. Similarly, onset latency of CM found to have no effect of stimulus polarity as there is no statistically significant difference in the latency elicited by both polarities in all stimuli conditions except at 500Hz which may also be due to a chance factor during analysis. However, the mean value showed very minimal difference. Condensation polarity of stimulus produce an initial inward movement of stapes footplate which leads to downward displacement of basilar membrane creates a hyperpolarization of hair cell which doesn't generate action potential whereas, rarefaction part of the stimulus does upward displacement leading to depolarization generating action potential(Peake & Kiang, 1962). Since only rarefaction part of the stimulus generates a potential, if stimulus starts with a condensation polarity there will be delay in the generation of a potential by half a period of particular stimulus duration. Hence there will be latency and amplitude difference can be seen evidently in lower frequencies as the duration of one cycle is more. The above mentioned phenomenon takes place only in case of neural potential whereas, cochlear microphonics is a receptor potential which generates alternating current in both the polarities. Hence amplitude and latency differences are not seen

in the present study. To the best of our knowledge there are no previous studies in literature where polarity effect was examined.

Amplitude difference due to polarity change was also compared across tone burst frequencies and click which showed that there is no significant difference in amplitude difference across all the stimuli. This reveal that whatever polarity effect is present on amplitude is similar in all the stimuli. Whereas, latency difference due to polarity change had significant difference across stimuli. This could be due to significant polarity effect on latency at lower frequency.

5.3 Effect of stimulus rate

The third objective of the study was to compare the effect of stimulus rate on amplitude and latency of the cochlear microphonics. Findings of the study shows that there is a difference in amplitude of the CM elicited by 30.1/sec and 59.1/sec stimulus rate for 500Hz and 1kHz stimulus conditions and is statistically significant. Whereas, higher frequency and click stimulus didn't show any significant rate effect on amplitude of CM. The onset latency of the CM elicited by 30.1/sec and 59.1/sec showed no significant difference in all the stimulus conditions. The amplitude results of click and 4kHz tone burst are in agreement with the guidelines for the CM recording given by Stevens, Sutton, Brockbank and Mason, 2011 where it was given for CM measurement in new born hearing screening and click was used for the measurement. They stated that unlike neural potential CM may not be subjected to the neural fatigue at higher stimulation rate, which allows the CM not be affected at higher stimulation rate and hence was recommended to use higher rate for screening as it also increase the recording speed. This is also supported by a study by Coats, 1981 where it was found that CM and AP component of the ECochG remains stable

or unaffected by stimulus rate. However, click stimulus was used to record in their study. In the current study different frequency tone burst along with click was used. This suggested that irrespective of stimulus type stimulus rate blow 60/see will not have significant on different parameters of CM. However, there probably a few or not many studies that empirically examined the effect of stimulus rate on CM alone especially across different frequencies tone burst.

Amplitude difference due to stimulus rate change was also compared across stimuli and showed that there is a significant difference across stimuli in condensation condition. This could be due to the significant rate effect in lower frequency which is discussed in the above section. Whereas, latency difference due to rate change had no significant difference across stimuli.

5.4 Stimulus optimization

The importance of cochlear microphonics in assessing cochlear function especially in lower frequency as a supplementary information to Otoacoustic emissions in cases with different auditory neural pathologies and also its role in assessing cochlear functioning in middle ear dysfunction etc are discussed in the earlier section with literature support. But because of few limitations of cochlear microphonics its clinical utility is very limited despite of its great clinical applications. The optimized stimulus parameter or condition for recording of cochlear microphonics is not very well discovered. The present study has made an attempt to examine the effect of few stimulus factors on the properties of cochlear microphonics (amplitude & onset latency) and recommending an optimized stimulus parameter for the recording of cochlear microphonics overcoming the limitations in recording which are discussed earlier. The results of study reveal that there is an

effect of stimulus frequency and type on CM amplitude and latency. The most robust and long duration cochlear microphonics were recorded from the low frequency stimulus (500Hz &1kHz) when compared to click and high frequency tone burst. This suggests that use of low tone burst to elicit will be most useful in assessing low frequency cochlear functioning where OAE measurement is not reliable. Results also revealed that there is no polarity effect on amplitude of the CM and polarity effect had little effect on latency of CM elicited by low frequency tone burst. However, CM has to be recorded using both polarities to check for reversal and to confirm whether CM is present or absent. Onset latency of CM had no stimulus rate effect whereas, lower frequencies had rate effect on amplitude of the CM but the effect is minimal in terms of amplitude which do not affect the identification and recording of CM as, CM elicited by low frequencies tone burst were robust. Hence, use of higher rate of stimulation is recommended which reduces the recording time. Based on the obtained results of the present study it can be concluded that to elicit robust CM and have better identification of CM one must use low frequency tone burst with a repetition rate approximately 60/see. However, to record CM one must use both the polarity of the stimulus separately. Longer plateau duration tone bust is recommended as CM likely to be present as long as the stimulus is present and would assist in better identification. Also low frequencies which are longer in stimulus duration will have less spectral splatter and will give highly frequency specific information.

Chapter-5

SUMMARY AND CONCLUSION

Cochlear microphonics is a pre neural potential which is generated by healthy outer hair cell as an alternating current response which mirrors the stimulus waveform and sustains as long as stimulus is present. As it mimics the stimulus waveform there exists a confusion between CM and stimulus artifacts which could be one of the reason for its limited usefulness. CM can be best recorded trans tympanically using a needle electrode from promontory and it can be non-invasively recorded from extratympanic method using tiptrodes from ear canal. CM being a cochlear potential it has potential in assessing hair cell functioning and there by identification of many cochlear (Meniere's) and retro cochlear conditions(ANSD). It also has literature support for its application of identification of cochlear pathology by its diminished amplitude(Kumagami, Nishida &Baba,1982; Morrison, Moffat & O'connor, 1980). It also has great application in assessing cochlear functioning in individuals with abnormal middle ear functioning where OAE results doesn't give reliable information (Kreitmayer, Marcrum, Picou, Steffens & Kummer, 2019). Thus CM has lot more clinical application than OAEs. Despite CM having potential clinical application the optimal parameters to record cochlear microphonics and effect of various stimulus parameters are not well understood yet. Keeping this limitation in mind this study was carried out with an aim of determining the effect of stimulus polarity, rate, stimulus type and stimulus frequency on different aspects of cochlear microphonics which could help us to decide an optimal stimulus parameter that can be used to record CM.

In order to examine these effects CM amplitude and CM onset latency were considered for the comparison. A total of 32 adult volunteers with normal hearing sensitivity were selected for the study. Normal hearing sensitivity was confirmed with the help of standard audiological test battery. CM was recorded using Bio-logic Navigator Pro Auditory Evoked Potential System Software by placing tiptrode electrode. Horizontal montage was used for recording CM where Non inverting electrode tiptrode(+ve) was placed in the ear canal as deep as possible, the inverting electrode (-ve) was placed on the contralateral mastoid and the ground electrode was placed on the upper forehead. Ears were stimulated with 500 Hz, 1000 Hz, 4000 Hz, 8000 Hz tone burst and click stimulus at100 dB SPL. A relatively long duration tone burst was considered with envelope having 2 cycle rise and fall time and 2 cycles of plateau duration (2-2-2). In order to check stimulus polarity effect CM were recorded separately for rarefaction and condensation polarity in all the stimulus frequency and type. Also CM was recorded independently with 30.1/sec and 59.1/sec stimulation rate in order to examine stimulus rate effect in all the stimulus frequency and type. Initially 300Hz to 10000Hz band pass filter setting was used to record CM under all stimulus condition and it was offline filtered later with the help of suitable filters. Peak to peak amplitude was considered for amplitude measurement by taking average of three consecutive highest and stable peaks. Onset latency was considered for measurement of latency which is measured by visually inspecting the beginning of the first wave reversal which had significant amplitude at its maxima.

The latency and amplitude values were measured separately for all the stimulus conditions and data were analysed using SPSS version 20 software. Descriptive statistics were administered to find out central tendency measures. Since the data didn’t fall under normal distribution non parametric tests such as Friedman

test and Wilcoxon signed ranks test were considered for comparison of amplitude, latency values across and between stimulus conditions respectively.

CM could be obtained and amplitude, latency values could be measured from all 32 individuals in all the stimulus condition except at 8kHz tone burst, where it could be measured from only 18 and 22 individuals in 8kHz at 30.1/sec and 8kHz at 59.1/sec respectively. Therefore, 8kHz data was considered only for descriptive statistics but not for inferential statistics.

Results of descriptive statistics revealed there was an effect of stimulus frequency and type on both amplitude and latency of CM having descending order of mean amplitude of CM 500Hz>1kHz>4kHz>click>8kHz and having descending order of mean latency 500Hz>1kHz>4kHz>click>8kHz. The descriptive data showed no effect of stimulus polarity and stimulus rate on cochlear microphonics however, there is very minimal difference or effect in lower frequency stimulus.

Results of inferential statistics revealed that

- ✓ The overall effect of stimulus type and stimulus frequency on amplitude and latency was statistically evident. However, the amplitude difference between 500Hz v/s 1kHz and 4kHz v/s click stimulus is almost minimal and not statistically significant.
- ✓ Amplitude and latency of cochlear microphonics were inversely proportional to the tone burst frequency.
- ✓ There was no statistically significant polarity effect on amplitude in all stimulus condition except for 4kHz at 59.1/sec.

- ✓ There was no statistically significant polarity effect on latency of CM in all stimulus condition except at lower frequency(500Hz).
- ✓ There was no statistically significant rate effect on amplitude of CM at 4kHz TB and click stimulus whereas, 500Hz,1kHz TB showed a significant rate effect on CM amplitude.
- ✓ Onset latency of CM showed no statistically significant rate effect in all the stimulus conditions.
- ✓ Amplitude difference due to polarity change showed no statistically significant difference across stimulus type and frequency whereas, latency difference due to polarity change had significant difference across stimuli.
- ✓ Amplitude difference due to stimulus rate change showed that there was a significant difference across stimuli in condensation condition but not in rarefaction polarity whereas, latency difference due to stimulus rate change showed no significant difference across stimuli.

6.1 Conclusion

Thus the present study emphasizes the effect of few stimulus parameters on cochlear microphonics which helped us to decide on optimal stimulus parameter that can be used to record CM. The study showed the effect of stimulus type and frequency on CM where robust CM could be obtained using low frequency. Such a result suggest that low frequency CM can be used to assess cochlear functioning. Study also showed majorly no stimulus polarity and rate effect on CM. Thus it can be suggested that both polarity should be used to record CM to identify reversal in CM maxima to confirm presence of CM and also higher repetition rate to save recording time.

6.2 Implications

- ✓ The study gives a better understanding of effect of stimulus parameters on cochlear microphonics. It suggests that low frequency elicits a robust cochlear microphonics and hence CM can be used to assess cochlear function more precisely at lower frequency. Thus it compensates one of the limitation of OAE where it doesn't give reliable information at low frequencies because of background and physiological noise.
- ✓ The study gives an optimized stimulus parameter which can be used in clinics to record cochlear microphonics to assess cochlear conditions.
- ✓ Robust OAE amplitude obtained for low frequency stimulus suggests that it can be used to assess cochlear function in sloping hearing loss where apical hair cells are intact and can be interpreted as OHC dysfunction if assessed by OAE.
- ✓ In cases with middle ear disorder CM can be recorded wherein OAE is not an option. It can also be used to asses cochlear functioning in ANSD assessment where sometimes OAEs are absent.
- ✓ The outcome of the study can add on to the existing literature.

6.3 Limitations

- ✓ Instead of keeping constant stimulus envelope, the total duration of different tone burst frequencies should have been varied. It would have helped us to identify presence or absence ringing in the cochlea.
- ✓ Longer duration of 8 KHz tone burst could have helped to record CM better at 8kHz.

- ✓ The study could have been done on a larger population.
- ✓ Amplitude of CM could have been measured using FFT more precisely.

6.4 Future directions

- ✓ Similar study can be done in a clinical population and compare the data with normals.
- ✓ Effect of stimulus frequency can be checked by keeping total stimulus duration constant by varying plateau duration or envelope.
- ✓ Correlation of CM and OAE in assessment of cochlear functioning can be checked in both normals and clinical population
- ✓ Threshold estimation can be done for CM and correlate with behavioural thresholds.

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