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Original Article

Unraveling Cochlear Dynamics: The Effect of Clicks, Tone Burst Frequencies, Polarity, and Stimulus Rates on Cochlear Microphonics in Individuals with Normal Hearing

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BACKGROUND: Despite cochlear microphonic's potential clinical application, especially in ANSD diagnosis, the optimal parameters to record cochlear microphonics and the effect of various stimulus parameters are not well understood yet, which makes its recording a difficult procedure. The present study was undertaken to determine the effect of stimulus polarity, rate, stimulus type, and stimulus frequency on different aspects of cochlear microphonics, which could help to decide an optimal stimulus parameter that can be used to record CM.

METHODS: The study involved 32 normal-hearing adults. CM was recorded from these individuals using extratympanic CM measurement from the ear canal independently for tone burst frequencies (500 Hz, 1 kHz, 4 kHz & 8 kHz) and click stimuli having rarefaction and condensation polarity at 30.1/sec and 59.1/sec repetition rates. Amplitude and latency were measured from the recorded waveforms and compared across and between stimulus conditions.

RESULTS: Results reveal that stimulus frequency and stimulus type have a significant effect on different parameters of CM. However, there was no significant effect of stimulus polarity and rate of stimulus on the amplitude and latency of cochlear microphonics. The amplitude and latency of the cochlear microphonics are inversely proportional to the stimulus frequency.

CONCLUSION: Hence, the study suggests the use of low-frequency tone burst (500 Hz/1 kHz) to elicit robust CM, which has greater application in the assessment of cochlear functioning over OAE as the latter gets affected by environmental and physiological noise and also due to middle ear pathology.

KEYWORDS: Cochlear microphonics, electrococleography, stimulus polarity, extra-tympanic recording, optimization

INTRODUCTION

Cochlear microphonics (CM), as a cochlear potential, can be a potential tool in diagnosing various conditions such as Meniere's disease, auditory neuropathy spectrum disorder, acoustic neuroma, and auditory maturation delay.[1-3] Studies indicate that individuals with cochlear pathology often exhibit diminished amplitude and distorted waveforms in their CM recordings.[4,5] In cases of Meniere's disease, CM recordings have shown enlarged potentials.[6]

Cochlear microphonics is a pre-neural potential unaffected by neural synchrony, meaning it remains present even in cases of ANSD where neural potentials are absent.[7,8] Similar findings can be observed in auditory maturation delay, where CM is present despite delayed or abnormal neural activity. A review article by Soares et al.[9] concludes that the presence of CM alongside the absence of ABR suggests a potential neural lesion.

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The presence of OAEs can also indicate intact OHC functioning. However, OAEs cannot be recorded in cases with middle ear disorders, making them ineffective in such scenarios.[10] Additionally, when background or physiological noise is high, OAEs are not suitable for assessing OHC function, especially for lower frequencies. In these situations, measuring cochlear microphonics may serve as a supplementary approach to OAEs for assessing cochlear functioning in clinical settings. This is supported by studies by Zhang,[11] which demonstrated that low-frequency CM can be measured in individuals with high-frequency hearing loss. Furthermore, Kwak et al[12] concluded that CM might provide more stable information about cochlear hair cell function than the OAE test, which can be easily influenced by middle or external ear conditions, and suggested CM as a useful supplementary tool for OAE testing. Additionally, it can evaluate OHC function in specific regions and help localize peripheral hearing loss, as it reflects the activity of OHCs near the cochlear location tuned to the stimulus frequency.[13]

Studies have documented evidence that in some individuals with ANSD, cochlear microphonics were present while OAEs were absent, highlighting the role of CM in diagnosing ANSD.[14-17] Guidelines recommend assessing cochlear function in newborn hearing screenings to facilitate ANSD diagnosis,[18] with CM preferred over OAE due to the high likelihood of middle ear fluid and background noise in newborns, which can result in absent OAEs. This underscores the need for more studies on CM recording and developing optimal stimulus selection or protocols to better record and identify CM.

Cochlear microphonics, thus, have the potential to be a significant clinical tool. However, the optimal stimulus parameters for efficiently recording cochlear microphonics still need to be fully understood, and there are limited studies exploring how these parameters affect CM. Therefore, this study aims to determine the effect of stimulus polarity, rate, type, and frequency on various aspects of cochlear microphonics to establish optimal stimulus parameters for recording CM. Specifically, the study aims to investigate the effect of stimulus type, frequency, and polarity on cochlear microphonics in individuals with normal hearing.

The objectives of the study were to:

- Explore the effect of stimulus polarity (rarefaction and condensation) on the latency and amplitude of CM,
- Investigate the effect of the rate of stimulation (30.1 and 59.1) on the latency and amplitude of CM,
- Investigate the effect of stimulus type (Clicks vs. Tone bursts) and stimulus frequency (500 Hz, 1 kHz, 4 kHz, 8 kHz) on CM for each polarity and stimulus rate.

MAIN POINTS

- This study delves into the often-overlooked area of cochlear mechanics, specifically examining the effect of stimulus-related parameters on cochlear microphonics, despite their significant applications.
- The findings of the study suggest optimal stimulus parameters for easily recording identifiable cochlear microphonics.
- Arrive at an optimum stimulus parameter to record CM based on the outcome of the findings.

METHODS

The study included 32 normal hearing adult volunteers aged 19-24 years (mean age of 22.5), comprising 18 males and 14 females. Convenience sampling was employed to recruit the participants for the study, and a written informed consent was obtained from all the participants. The study was carried out in compliance with ethical guidelines and adheres to the ethical standards according to the Declaration of Helsinki. Ethical approval was obtained from an institutional ethical committee (SH/AIISH/ERB/Diss/27 /21-22). Participants recruited for the study had transient evoked otoacoustic emissions, indicating normal outer hair cell and middle ear function, and no history of noise exposure or prolonged exposure to music. CM was recorded using a calibrated Bio-logic Navigator Pro Auditory Evoked Potential System Software. A tiptrode was used to present the stimulus and record cochlear microphonics. All audiological tests were conducted in an acoustically treated room with permissible noise levels as specified by ANSI S 3.1.[19]

To record CM, subjects were seated in a reclining chair and instructed to relax and refrain from extraneous body movements to avoid muscle artifacts during recording. The skin surface at the electrode placement sites (forehead and mastoid) was cleaned using NuPrep skin preparation gel. Cup electrodes were placed using conduction paste and surgical plaster. A Tiptrode was placed in the ear canal, which was cleaned with earbuds. The ear canal was gently cleaned using sterile earbuds by inserting them just inside the ear canal and gently rotating to remove any visible earwax or debris. Special care was taken to avoid pushing the earbud too deeply into the ear canal to prevent discomfort or risk of injury. Recordings were obtained using a single-channel horizontal electrode placement. The non-inverting electrode (Tiptrode, +ve) was placed as deep as possible in the ear canal, the inverting electrode (−ve) was placed on the contralateral mastoid (M1), and the ground electrode was placed on the upper forehead (Fz). Absolute electrode impedance was maintained below 5 kOhms, with inter-electrode impedance within 2 kOhms. The Tiptrode was utilized as it is designed to provide a stable and close proximity to the ear canal, enhancing the recording of cochlear potentials while minimizing the influence of external noise and artifacts.

In accordance with the study's objectives, tone bursts of 500 Hz, 1000 Hz, 4000 Hz, and 8000 Hz, as well as clicks, were used to investigate the effect of stimulus type and frequency. The tone burst envelope was set to 2-2-2 to reduce spectral splatter, helping to ensure frequency-specific responses and additionally, this envelope duration is commonly used in auditory research to elicit robust cochlear responses while minimizing non-linear distortions. Since CM is a sustained response that persists as long as the stimulus is present, the recording time window for CM varied with different tone burst frequencies and click stimuli. The test protocol used to record CM is detailed in Table 1. For each stimulus type, cochlear microphonics were recorded separately at repetition rates of 30.1/sec and 59.1/sec. Similarly, CM was recorded separately for condensation and rarefaction polarities for all stimuli. At each combination of stimulus type, rate, and polarity, CM was recorded twice to verify waveform

Table 1. Stimulus and Acquisition Parameters to Record CM

Parameter	Specification
Transducer type	Insert with Tiptrode
Type of stimulus	Tone burst of 500 Hz, 1 kHz, 4 kHz, 8 kHz and Clicks
Stimulus duration	Click-100microseconds Tone burst:2-2-2 cycle
Intensity	100 dBSPL
Stimulus polarity	Rarefaction and Condensation
Stimulus rate	30.1/sec and 59.1/sec
Number of sweeps	2000
Filter setting	300-10 000 Hz
Inter electrode impedance	<2k ohms
Intra electrode impedance	<5k ohms
Gain	100 000
Time window	500 Hz and 1 kHz-15 ms 4 kHz,8 kHz and click-5 ms
Notch filter	Off

replicability. All the stimuli were presented in dB SPL as it provides a direct and objective measure of the sound intensity being delivered to the ear, allowing for precise control across different frequency tone bursts which will have different RETSPLs.

Initially, the response waveforms were recorded with a broader filter setting of 300 Hz to 10 000 Hz. Later, the responses were analyzed using digital offline filtering, applying suitable filters for responses elicited by different tone burst frequencies. Specifically, the response waveform was offline filtered with 300 Hz to 700 Hz, 300 Hz to 1500 Hz, for 500 Hz and 1000 Hz TB, respectively. The waveform of 4 kHz and click was filtered with 300 Hz to 5 kHz, whereas 8 kHz waveform was filtered with 300 to 10 kHz. This helped in possibly removing of unwanted ABR peaks and allowed better visualization of cochlear microphonic responses. The high pass filter was kept at 300 Hz, as the instrument used to record Cochlear Microphonics did not have the capability to increase the high pass filter beyond 300 Hz.

Waveform analysis was carried out after digital offline filtering to note the onset latency and amplitude of CM in each waveform. The latency here is defined as the point where a significant polarity reversal was observed at the beginning of a cycle, as illustrated in Figure 1. A criterion amplitude point was previously established by averaging the initial reversals of CM amplitudes across ten individuals, as detailed in Table 2. On the time scale, the onset latency of cochlear microphonics was identified as the point where the peak amplitude first crossed or equaled the criterion point (Figure 1). The criterion points for different stimuli are listed in Table 2.

For amplitude measurement, the peak-to-peak amplitude was determined for click stimuli. For tone burst (TB) stimuli, the amplitude was measured by averaging the amplitudes of three consecutive highest and stable peaks (indicated with arrow marks) as depicted in Figure 2. Figures 3 and 4 depict the sample recordings of cochlear microphonics elicited by different stimuli under both polarities.

Statistical Analysis

The data were analyzed using SPSS v20 (IBM Statistical Package for Social Sciences, New York, USA).[20] Descriptive statistics were computed to determine the mean and standard deviations of onset latency and amplitude of cochlear microphonics. The Shapiro-Wilk's test of normality indicated that the data significantly deviated from a normal distribution. Therefore, non-parametric tests were selected for inferential statistics. Friedman's test was employed to examine whether there were significant differences in amplitude and latency across the three different tone burst frequencies (excluding 8 kHz) and click stimuli. Wilcoxon signed ranks tests were used to assess significant differences between specific stimuli pairs (e.g., 500 Hz vs. 1000 Hz, 500 Hz vs. 4000 Hz). Additionally, Wilcoxon signed ranks tests were applied to evaluate differences in amplitude and latency between polarity (condensation vs. rarefaction) and between repetition rates (30.1/sec vs. 59.1/sec).

RESULTS

Cochlear microphonics was recorded from thirty-two healthy normal ears, and its occurrence under different stimulus conditions was assessed. All stimulus conditions, except for the 8 kHz tone burst, resulted in a 100% occurrence of CM. Cochlear microphonics recorded using tone burst stimuli had multiple cycles, whereas those recorded from click stimuli resulted in a single cycle CM. Among tone burst stimuli with the same 2-2-2 envelope, lower frequency tone bursts exhibited longer duration CM compared to higher frequencies. Cochlear microphonics also showed higher amplitude and longer duration for lower frequency tone bursts, while it was shorter in duration for higher frequency tone bursts. The amplitude of cochlear microphonics was notably higher during the plateau phase.

Effect of Stimulus Type and Frequency

The results of descriptive statistics show that the mean amplitude of CM is highest and the mean latency is longest when it was elicited with the 500 Hz tone burst stimulus, and lowest when elicited with the 8 kHz tone burst stimulus, regardless of polarity and stimulus rate. This trend in mean amplitude and mean latency across different stimulus types and frequencies is depicted in Figures 5 and 6,

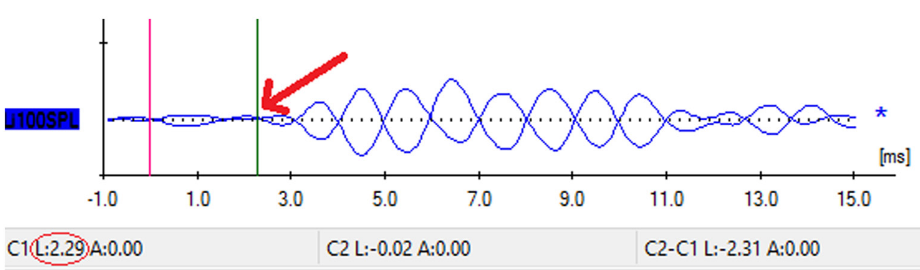

Figure 1. Depicts 500 Hz tone burst-evoked CM waveform and the onset latency. CM, cochlear microphonics.

Table 2. Amplitude Criterion Points

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

respectively. The Friedman test results indicate a statistically significant difference in both amplitude and latency across stimuli (500 Hz, 1 kHz, 4 kHz, and click), with a *P*-value < .05 for all four conditions (rarefaction_30.1/sec, condensation_30.1/sec, rarefaction_59.1/sec, condensation_59.1/sec).

According to the Wilcoxon Signed Ranks test, there is no significant difference in amplitude between 1 kHz versus 500 Hz across all four aforementioned conditions, and for click versus 4 kHz for rarefaction at 30.1 and condensation at 30.1 rates. Table 3 highlights the significant differences observed between specific stimulus pairs. However,

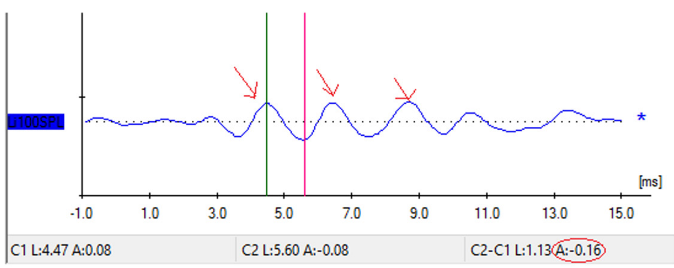

Figure 2. Depicts 500 Hz tone burst-evoked CM waveform and also the amplitude measurement. CM, cochlear microphonics.

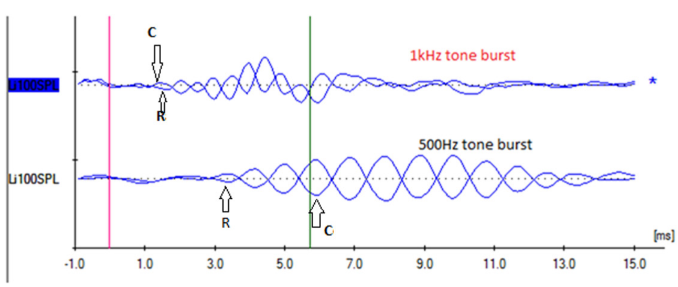

Figure 3. CM recorded using 1 kHz and 500 Hz TB for condensation and rarefaction polarity stimuli. CM, cochlear microphonics; TB, tone burst.

the results of the Wilcoxon Signed Ranks test for latency reveal a significant difference in latency between all pairs compared, regardless of the stimulus condition.

Effect of Stimulus Polarity

The findings of descriptive statistics indicate no discernible trend or polarity effect on both the amplitude and latency of CM, as illustrated in Figures 7 and 8, respectively. According to the Wilcoxon test results, there is no significant difference in amplitude between both polarities across all stimulus conditions, with *P*-values > .05, except for the 4 kHz_59.1/sec condition (*P* = .019). For latency, the Wilcoxon test shows no significant difference between polarities across all stimulus conditions, except for 500 Hz_30.1/sec, 500 Hz_59.1/sec, 1 kHz_30.1/sec, and click_59.1/sec, where significant differences were observed (*P* < .05).

Effect of Stimulus Rate

The descriptive statistics reveal minimal or negligible differences in both mean amplitude and latency between CM elicited by 30.1/sec and 59.1/sec, regardless of stimulus type, frequency, and polarity. Figures 9 and 10 clearly illustrate that there is no discernible trend or rate effect on either the amplitude or latency of CM, respectively. According to the Wilcoxon Signed Ranks test results, there is generally no significant difference in amplitude between stimuli elicited at 30.1/sec and 59.1/sec, with *P*-values > .05, except for the conditions involving 500 Hz, 1 kHz, and click stimuli with condensation polarity. However, for latency measures, the Wilcoxon Signed Ranks test shows no significant difference between stimuli elicited at 30.1/sec and 59.1/sec, with *P*-values > .05 across all stimulus conditions.

DISCUSSION

Effect of Stimulus Frequency and Type on CM

The findings of the study indicate that the amplitude of the CM elicited with tone burst stimuli is greater than that elicited by click stimuli. Across 4 tone burst stimuli, the amplitude of CM decreased as the stimulus frequency increased. The results are in agreement with previous studies[1,21-24] which states that although clicks can be used to record CM, CM is more robust in response to tonal stimulation, and the largest CM responses are produced by 500 Hz and 1 kHz tone bursts. Contrary findings were reported in a study by Heidari et al,[1] where they found that CM amplitude with click stimuli (broadband) is generally larger than with tonal stimuli. However, their comparison involved tonal frequencies of 2, 4, 8, and 16 kHz. Nevertheless, their findings regarding amplitude across tonal frequencies were

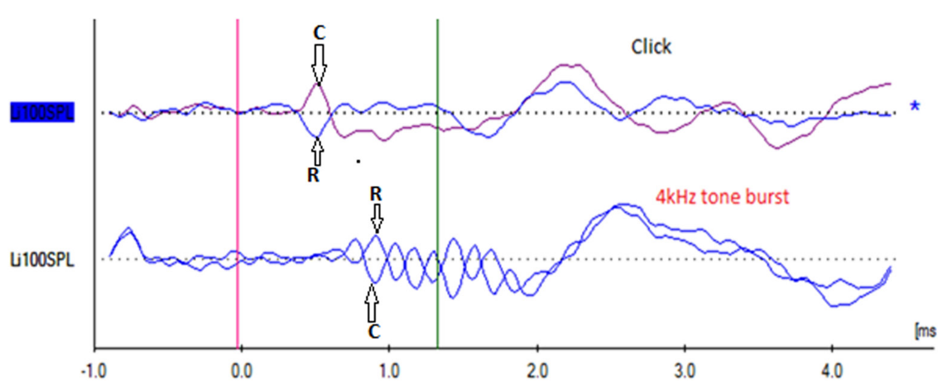

Figure 4. CM recorded using click and 4 kHz TB for condensation and rarefaction polarity stimuli. CM, cochlear microphonics; TB, tone burst.

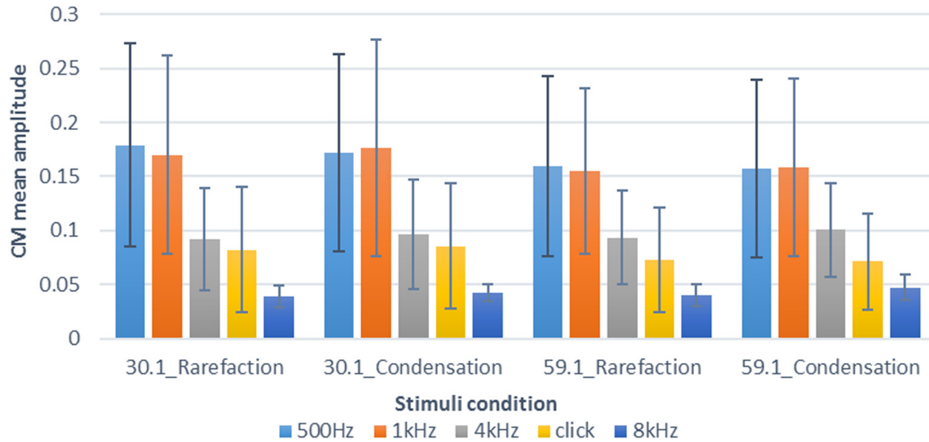

Figure 5. Mean amplitude along with SD across stimulus frequency and type at different polarity and rates.

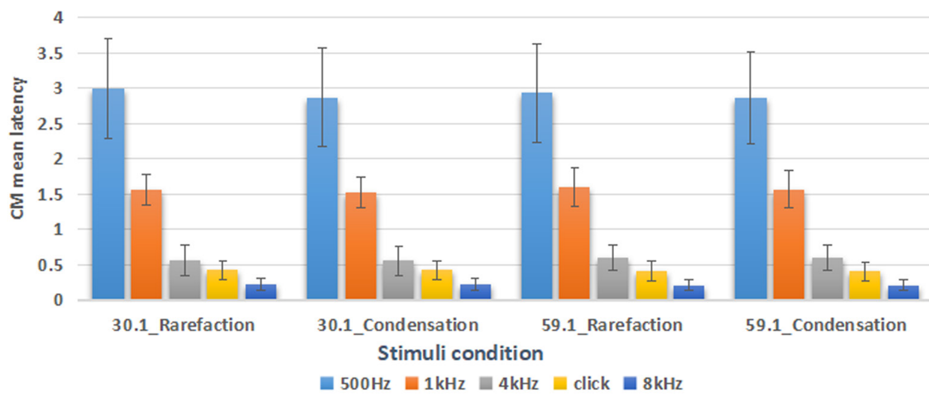

Figure 6. Mean latency along with SD across stimulus frequency and type at different polarity and rates.

consistent with the present study, attributing these results to the influence of stimulus bandwidth. Lower frequency stimuli generate a traveling wave that must travel from the base to the apex, stimulating a larger number of hair cells located predominantly at the apical end. Since CM reflects the spatial summation of hair cell receptor currents,[24,25] its amplitude tends to be larger for lower frequencies. Coraci[26] similarly reported greater CM amplitudes with clicks compared to a 2 kHz tonal stimulus.

Regarding the onset latency of CM, the study found that onset latency is inversely proportional to stimulus frequency, with CM elicited by clicks exhibiting the shortest latency. While there is limited literature analyzing latency across stimulus frequencies, Zhang[27] and Zhang[28] examined the effect of stimulus intensity on CM latency and found it to be intensity-independent, attributing this to physiological features of hair cells, i.e., the hair cells generate CMs almost instantaneously once stimulation reaches the hair cells without any delay, irrespective of any change in stimulus intensities. Although hair cells generate the CM instantaneously, the latency results of the present study can be attributed to travel time for the stimulus to reach the particular set of hair cells on basilar membrane. Hence, the CM elicited by low-frequency tone burst has got a longer latency. The reason for clicks eliciting shorter latency CM could be attributed to the fact that click stimuli mainly stimulate the basal part of the basilar membrane, leading to shorter latency.

One possible limitation of the study could be the exclusion of the 2000 Hz frequency in the evaluation of cochlear microphonics.

Table 3. Z Value and Significance Level for Amplitude Obtained Between the Type of Stimulus at Each Rate and Polarity

	Rarefaction_30.1		Condensation_30.1		Rarefaction_59.1		Condensation_59.1	
	z	*P*	Z	*P*	z	*P*	Z	*P*
1 kHz v/s 500 Hz	−.767	.443	−.088	.930	−.265	.791	−.094	.925
4 kHz v/s 500 Hz	−4.245	<.001	−4.227	<.001	−4.018	<.001	−3.488	<.001
Click v/s 500 Hz	−4.638	<.001	−4.414	<.001	−4.685	<.001	−4.744	<.001
4 kHz v/s 1 kHz	−4.105	<.001	−4.009	<.001	−4.228	<.001	−3.443	<.001
click v/s 1 kHz	−4.517	<.001	−4.422	<.001	−4.783	<.001	−4.682	<.001
click v/s 4 kHz	−1.593	.111	−1.498	.134	−3.410	<.001	−4.167	<.001

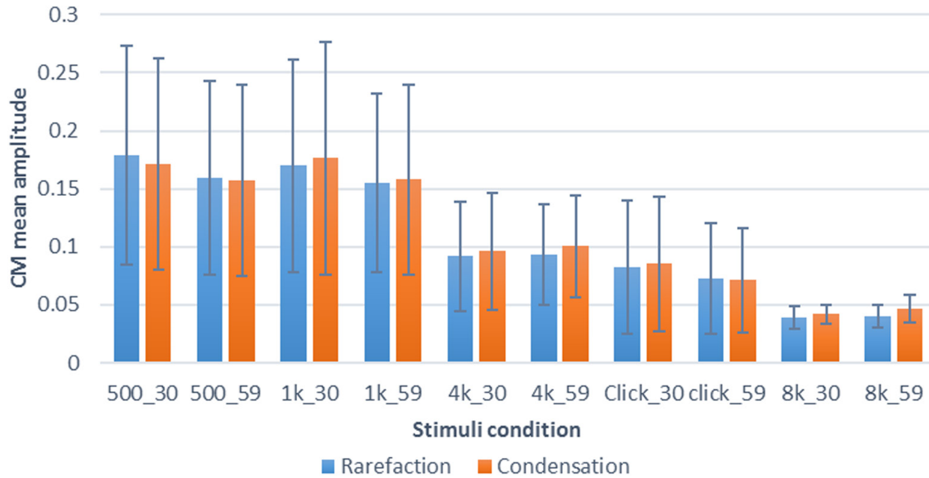

Figure 7. Mean amplitude along with SD depicting the effect of polarity across the type of stimulus and rate.

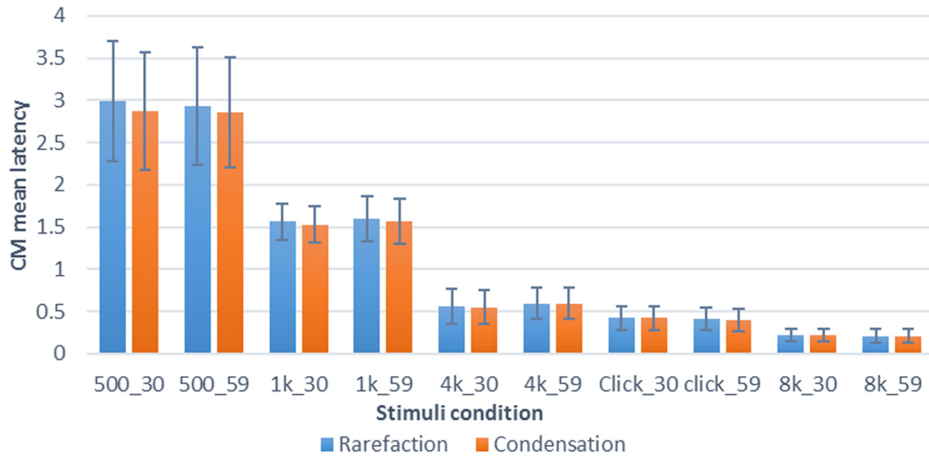

Figure 8. Mean latency along with SD depicting the effect of polarity across the type of stimulus and rate.

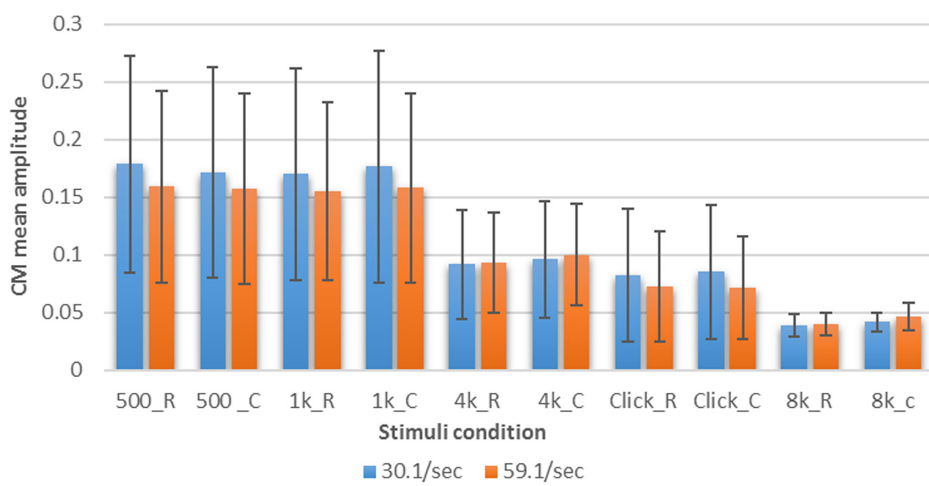

Figure 9. Mean amplitude along with SD depicting the effect of stimulus rate across type of stimulus and polarity.

While the selected frequencies (500 Hz, 1000 Hz, 4000 Hz, and 8000 Hz) and click stimuli provide a comprehensive overview of cochlear responses across low and high frequencies, the absence of 2000 Hz may have resulted in a lack of data from the mid-frequency range. This could limit the granularity of the findings regarding cochlear function at intermediate frequencies, which may be considered in future research.

Effect of Stimulus Polarity

Results suggest that there is no effect of stimulus polarity on the amplitude of the CM regardless of stimulus frequencies and stimulus rates, except a stimulus condition where CM is elicited by 4 kHz at 59.1/sec. This slight variation could potentially be due to chance or the very low amplitude observed at this particular frequency. Similarly, the onset latency of CM was found to have no effect of

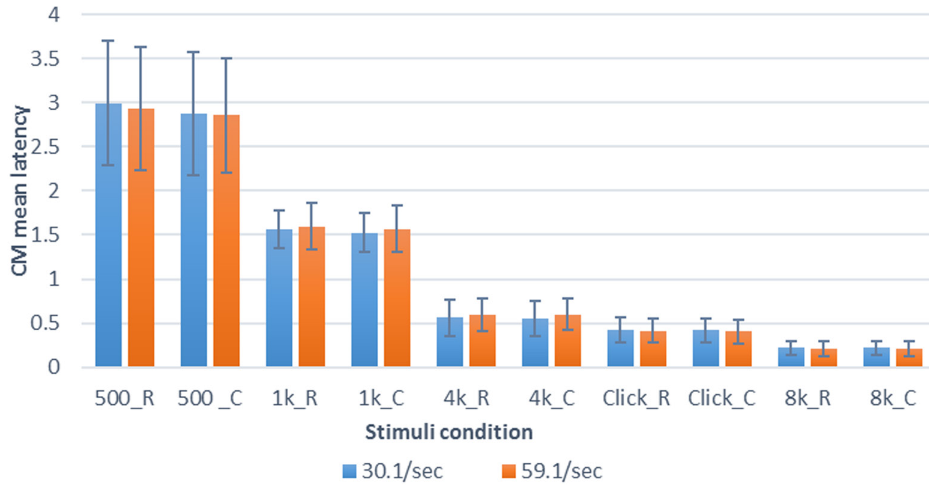

Figure 10. Mean latency along with SD depicting the effect of stimulus rate across type of stimulus and polarity.

stimulus polarity in all stimulus conditions, except at 500 Hz, which may also be due to a chance factor during analysis. The results of the present study can be attributed to full wave rectification, which happens in receptor potential (cochlear microphonics), leading to no observed difference in amplitude and latency elicited by both polarities.

Effect of Stimulus Rate

No stimulus rate effect on CM for click and 4 kHz tone burst, which is in agreement with the guidelines for CM recording in newborn hearing screening given by Lightfoot.[29] They stated that, unlike neural potential, CM may not be subjected to neural fatigue at higher stimulation rates and hence was recommended the use higher rate, which will increase recording speed. This is also supported by a study by Coats[30] where it was found that the CM and AP components of the ECochG remain stable or unaffected by the stimulus rate. This suggested that irrespective of the stimulus type, a stimulus rate below 60/sec may not have significant effect on different parameters of CM.

Stimulus Optimization

The study results indicate that stimulus frequency and type significantly affect CM amplitude and latency. The most robust and longest-duration CM responses were consistently recorded in 100% of participants using low-frequency stimuli (500 Hz and 1 kHz), compared to click and high-frequency tone bursts. This suggests that using low-tone bursts to elicit CM may be particularly valuable for assessing low-frequency cochlear function when OAE measurements are not reliable. Furthermore, the study revealed that stimulus polarity does not significantly affect CM amplitude and latency. However, it is essential to record CM using both polarities to confirm its presence or absence by checking for polarity reversal. Additionally, stimulus rate was found to have no significant effect on CM amplitude and latency. Therefore, the use of a higher stimulation rate is recommended, as it can reduce recording time without compromising the accuracy of CM assessment.

CONCLUSION

The present study underscores the impact of various stimulus parameters on cochlear microphonics, aiding in determining optimal parameters for CM recording. The study highlighted the influence of stimulus type and frequency on CM, demonstrating robust CM responses with low-frequency tone bursts. This suggests that low-frequency tone bursts are effective in eliciting CM for assessing cochlear function. Furthermore, the study generally found no significant effects of stimulus polarity and rate on CM. It is recommended to use both polarities when recording CM to detect any reversal in CM maxima, thereby confirming its presence. Additionally, using a higher repetition rate is advised to streamline recording time without compromising CM assessment accuracy.

Ethics Committee Approval: This study was approved by Institutional Ethical Committee of All India Institute of Speech and Hearing (Approval No.: SH/AI ISH/ERB/Diss/27/21-22, Date: 02-3-2022).

Informed Consent: Written informed consent was obtained from the participants who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – P.K.E., A.B.; Design – P.K.E., A.B.; Supervision – A.B.; Resource – P.K.E., A.B; Materials – P.K.E., G.W. ; Data Collection and/or Processing – P.K.E., G.W.; Analysis and/or Interpretation – P.K.E., G.W.; Literature Search – P.K.E.; Writing – P.K.E.; Critical Reviews – P.K.E., G.W., A.B.

Declaration of Interests: The authors have no conflict of interest to declare.

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