Effect of Age on Spectral Distribution of Click and Toneburst Evoked Otoacoustic Emissions in Infants

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Abstract

Otoacoustic emission (OAE) is a sensitive tool used to assess the functional status of the outer hair cells (i.e., the cochlear component most vulnerable to many of the diseases and disorders that damage hearing) in infants with normal hearing and in different clinical population. The present study aimed to investigate the influence of age on click and toneburst evoked OAEs, to investigate the pattern of frequency shift with age, and to monitor the frequency specific maturational changes in the cochlea. The study was conducted on 40 infants (80 ears) with normal hearing sensitivity in the age range of 0 to 12 months, with subgroups as group I, II, III, IV. The results of the present study revealed that, the mean absolute amplitude of click and toneburst evoked OAEs across age groups increased as the age increased. Similarly, the mean SNR values of click evoked OAEs across age groups increased as the age increases. Whereas, the mean SNR values of toneburst evoked OAEs across age groups increased as the age increases, except for group I. Also, statistically significant difference for mean absolute amplitude was observed between CEOAEs and TBOAEs across all the frequencies. These findings can be used as a clinical tool for screening and diagnostic purposes in the pediatric population.

Keywords: *Otoacoustic emissions, click evoked otoacoustic emissions, toneburst evoked otoacoustic emissions.*

Introduction

Otoacoustic emissions (OAEs) are sounds that originate in the cochlea and propagate through the middle ear into the ear canal, where they can be measured using a sensitive microphone. Because of its relative simplicity, better sensitivity and objectivity of the technique, the OAEs are a promising means for monitoring cochlear function. OAEs are the property of healthy normal functioning cochlea, generated by active frequency selective, non-linear elements within the partition, the critical components being the outer hair cells (Kemp, 1988). The primary purpose of otoacoustic emission (OAE) tests is, to determine cochlear status, specifically the outer hair cell function. This information can be used to screen for hearing, particularly in neonates, infants, or individuals with developmental disabilities, partially estimate hearing sensitivity within a limited range, differentiate between the sensory and neural components of sensorineural hearing loss, and test for functional (feigned) hearing loss. The development of evoked OAEs has gained much interest because they are used as a valid and relatively quick test to assess cochlear integrity in the very youngest subjects.

Transient evoked OAEs (TEOAEs) are frequency dispersive responses following a brief acoustic stimulus such as click or tone burst (Kemp, 1978; Norton & Neely, 1987). The click stimulus has a broad spectrum, and hence consequently it stimulates broad frequency region of the cochlea in a single measurement. Lutman, Mason, Sheppard and Gibbin (1989) opined that, the presence of click evoked OAEs (CEOAEs) are a powerful indicator of normal hearing. Therefore it has been applied as a general tool in universal neonatal hearing screening (UNHS) programs. Toneburst evoked OAEs (TBOAEs) include narrow bandwidth tone stimuli, which has stimulus energy concentrated on a particular area of the basilar membrane and elicits a more frequency-specific cochlear response. An advantage of toneburst stimulus is that more energy can be introduced in a specific frequency range compared to click, which is a more frequency dispersive stimuli. Although tone burst stimuli have greater frequency specificity compared to click stimuli, TBOAEs have not been routinely used in pediatric populations.

Most research works on TEOAEs have been performed using click stimulus. There have been many studies showing that the CEOAEs can be recorded in most normally hearing adults and in normal newborns and in babies admitted to a special care unit (Stevens, Webb, Hutchinson, Connel, Smith & Buffin, 1989). Uziel and Piron (1991) recorded CEOAEs in neonates ranging in age from a few days to 2 months after birth. The results showed that, in neonates, the emissions were stronger and covered a wider frequency range than those from the adults. Stevens, Webb, Hutchinson, Connel, Smith and Buffin (1990) recorded CEOAEs in 30 normal newborns and showed that 96% produced an emission at the highest level tested of 41 dB nHL. These results prove high degree of confidence that can be placed in the test when used to detect hearing impairments.

Probst, Coats, Martin and Lonsbury-Martin (1986) carried out a study on normal hearing young adults using spontaneous, click and toneburst evoked otoacoustic emissions. It was found that toneburst-evoked emissions were often more prominent than click evoked emissions and no spontaneous emissions were detected.

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Highly similar peaks were present in the spectra of toneburst-evoked emissions within the range of toneburst spectra. Wit and Ritsma (1979) documented that the higher the frequency of the stimulus the smaller the response. TBOAEs at 1 kHz were more robust than CEOAEs in terms of emission response level and signal-to-noise ratio (SNR) at both 1 and 1.5 kHz frequency bands. The prevalence rate for CEOAE and TBOAE responses in these two frequency bands was significantly different (Zhang, McPherson, Shi, Tango & Wong, 2007).

Most of the research has been carried out using click stimuli, and also the studies have been concentrated on adult population. Very few studies have been carried out using toneburst stimuli for hearing screening and hearing assessment. As to the research and clinical application of TBOAEs, studies have been mainly focused on adult population. Whereas,there are very limited number of studies which have been done on infants. Hence there is a need to study the utility and efficacy of TBOAEs among infants. Also very limited studies have been done on the spectral information of TEOAEs as the age advances. The information regarding the comparison between CEOAEs and TBOAEs in infants is limited. Hence the present study was undertaken. The aims of the present study were, to investigate the influence of age on click and toneburst evoked OAEs; to investigate the pattern of frequency shifts with age; to study the sensitivity of click and toneburst evoked OAEs; and, to monitor the frequency specific maturational changes in the cochlea.

Method

Participants

The study was conducted on forty infants (80 ears) in the age range of 0 to 12 months. The forty infants were further categorized into four subgroups based on their age as, Group I: 0 to 4 months; Group II: 4 to 6 months; Group III: 6 to 8 months, and; Group IV: 8 to 12 months. Each age group had 10 infants each. All the infants had normal otoscopic examination indicating absence of external and middle ear pathology. They were healthy with no symptoms of cold or ear discharge at the time of assessment. They had no complaints and prior histories of any high risk factors and neurological symptoms. All the infants had age appropriate minimum response levels in behavioral observation audiometry, normal outer hair cell functioning ensured by recording TEOAEs and normal hearing sensitivity ensured by recording auditory brainstem responses (ABR).

Instrumentation

An otoscope was used to observe the status of external auditory canal and tympanic membrane. To present the stimuli for behavioral observation audiometry or visual reinforcement audiometry, a calibrated two-channel diagnostic audiometer Madsen Orbiter-922 (version 2) with impedance matched loudspeakers were used. A calibrated Grason Stadler Inc. Tympstar middle ear analyzer (GSI Tympstar, version 2) was used to carry out tympanometry and acoustic reflexometry. A personal computer based Intelligent Hearing Systems Smart EP version 3.94 evoked potential system to record ABR, and a calibrated otoacoustic emission system ILO version 6 software (Otodynamics Ltd., UK) was used to record TEOAEs for both clicks and tone burst stimuli.

Test Environment

All the tests were carried out in a sound treated room with noise levels within permissible limits as per ANSI S3.1 (1991). The test room was comfortable enough for the infants in terms of light and temperature.

Test Procedure

Case history: Detailed information regarding the history of prenatal, natal and postnatal medical conditions were collected for all the infants. A detailed report regarding the auditory behavior of infants at home for various environmental sounds like calling bell, voices from TV or radio, pressure cooker whistle etc was obtained from the parents or caregivers.

High Risk Register: Medical reports were reviewed to make sure that all the infants were devoid of various risk factors and other medical conditions. This was done by administering the modified high risk register developed by Anitha & Yathiraj (2001) to rule the high risk factors in infants.

Otoscopic Examination: The visual examination of the ear canal and the tympanic membrane of infant's ear were done using a hand held otoscope. This was done to rule out the presence of wax, foreign bodies in the ear canal and/or tympanic membrane pathologies.

Table 1: Parameters used to record click and toneburst evoked TEOAEs

Parameters	
Stimulus type	Click and Toneburst
Stimulus intensity	80 dBpk SPL
Stimulus rate	50 stimulus/sec
No. of stimulus	260
Stimulus polarity	Non-linear
Amplification	100-10,000 times
Filter settings	High pass filtered at 300 or 400 Hz

Tympanometry and Acoustic Reflex Measurements: Tympanograms were obtained using 1000 Hz probe tone frequency for infants till the age of 6 months and 226 Hz probe tone frequency for infants above 6 months of age. The pressure was swept from positive to negative (+200 to -400 daPa) with the pump speed of 200 daPa/sec and the probe intensity was 85 dB SPL for 226 Hz and 75dB SPL for 1000 Hz probe tone frequency. Ipsilateral acoustic reflex thresholds (ARTs) were measured for pure tone stimuli of 500 Hz, 2 kHz and 4 kHz using 226 Hz and 1 kHz probe tone frequencies. ART for 1 kHz was not recorded when 1 kHz probe tone was used as it might interact with the reflex activator signal frequency causing artifacts (Wilson & Margolis, 2001). ARTs were determined using an ascending technique by increasing the intensity of the activating stimuli in 5 dB steps from 60 dBHL as the starting intensity until reflex was obtained or equipment limit was reached. The minimum intensity at which the repeatable change in the admittance value is observed by taking the criterion as 0.03 mmhos was considered as an acoustic reflex threshold.

Behavioral Observation Audiometry: The behavioral responses of the infants were observed in the free field condition using warble tones from 500 Hz to 4000 Hz separated in octaves and for speech stimuli. The lowest levels of presentation of each of the stimuli, at which the subject exhibited some sort of auditory behavior was noted down.

Visual Reinforcement Audiometry: Thresholds for the infant's natural response (head turn) to the warble tones at octave frequencies from 250 Hz to 8 kHz and also for speech stimuli were obtained. Head turn towards the stimuli or the LCD TV monitor, was considered as a response only when it occurred within 3 seconds of the stimulus presentation.

Auditory brainstem response: ABR was recorded using IHS Smart EP system, version 3.94 with insert earphone (ER- 3A) using repetition rate of 11.1/s and filter setting of 30 to 3000 Hz with an analysis time of 15 ms. Initially, the electrode sites were cleaned with the help of skin preparing gel. Vertical electrode montage was used, where the non inverting electrode was placed on high forehead, inverting on the test ear mastoid and common on the non test ear mastoid. Electrodes were placed on the recording sites with the conduction paste and then were fixed with the help of surgical tape. It was ensured that the independent electrode impedance was less than 5 kΩ and inter electrode impedance was within 2 kΩ. The subjects were considered to have normal hearing sensitivity if the ABR wave V was clearly seen at 30 dB nHL

Transient evoked otoacoustic emissions (TEOAEs): Infants were tested during natural or sedated sleep or in quiet condition while recording TEOAEs. The probe was inserted gently into the ear canal with an appropriate sized probe tip so as to give a flat stimulus spectrum across the frequency range. Both click and tone burst-evoked OAEs were recorded. The evoked response for click stimulus included the frequency range from 500 Hz to 6 kHz, and the evoked response for toneburst was recorded individually at 500 Hz, 1 kHz, 2 kHz and 4 kHz. The stimulus was clear, with a positive and negative deflection followed by a flat line. The stimulus spectrum was smooth, with a rounded curve. The protocol used for recording click and tone burst evoked TEOAEs are mentioned in the Table 1.

The stimuli for clicks are trains of 4 biphasic clicks of 80 μs in the non-linear position. The non-linear protocol removes the stimulus artifacts of linear nature which can be misinterpreted as TEOAE response. In order to ensure a significant artifact rejection, the first 2.5 ms of the recording was eliminated. On termination of the test, the OAE response data that is obtained is averaged and an OAE waveform is displayed in the time domain. A Fast Fourier Transform (FFT) is performed on the OAE response spectrum and the OAE and noise energy are displayed in a frequency spectrum. The responses were considered as emissions based on the signal-to-noise ratio (SNR) and reproducibility. The overall SNR of greater than or equal to +3 dB and the reproducibility of greater than 50% were considered for the presence of otoacoustic emissions to determine normal outer hair cell functioning.

Results and Discussion

Absolute amplitude and SNR for different frequencies of CEOAEs and TBOAEs across age groups

The mean, SD and range values for absolute amplitude and SNR for both CEOAEs and TBOAEs at 1 kHz, 2 kHz and 4 kHz, across different age groups are depicted in the Figure 1 and 2. The results revealed that, the mean absolute amplitudes increase with increase in age for 1 kHz click frequency. But such a trend was not observed for 2 kHz and 4 kHz click frequencies. The mean absolute amplitude was more at 2 kHz and 4 kHz click frequencies compared to 1 kHz across all the age groups. Also, as it is evident from Figure 1, no definite trend was followed for the mean absolute amplitude across the age groups for different toneburst frequencies. However, the absolute amplitude was found to be higher at 2 kHz and 4 kHz compared to 1 kHz toneburst frequency (Figure 1). The mean SNR values also increased with increasing age only for 1 kHz click frequency. The mean SNR was more at 2 kHz and 4 kHz compared to 1 kHz across all the age groups (Figure 2). When SNR values for different toneburst stimulus frequencies were considered, no definite trend was observed. However, the mean SNR was found to be higher at 2 kHz and 4 kHz compared to 1 kHz toneburst frequency.

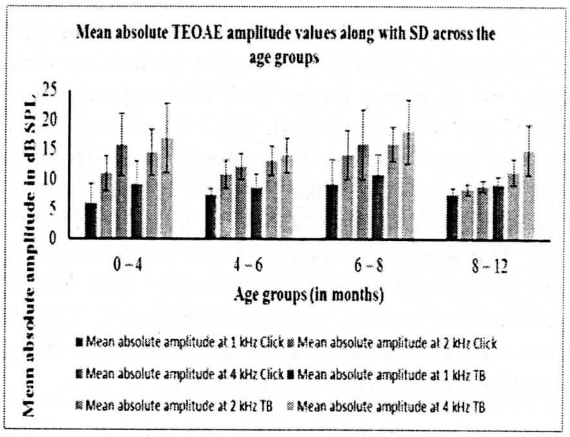

Figure 1: Mean, SD and range for absolute amplitude at 1 kHz, 2 kHz, and 4 kHz for click and tone burst stimuli across age groups.

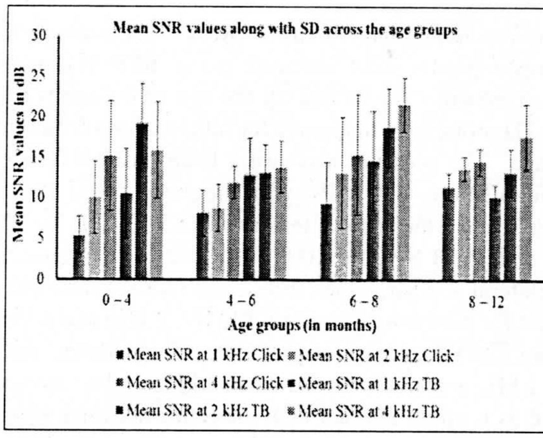

Figure 2: Mean, SD and range for SNR at 1 kHz, 2 kHz, and 4 kHz for click and tone burst stimuli across age groups.

A possible explanation for higher absolute amplitude at 2 kHz and 4 kHz is that, the resonance frequency of the infants ear canal is of high frequency. Thus at higher frequencies the absolute amplitude is more. As the age increases, the resonant frequency of the ear canal shifts towards the mid frequencies. This would have resulted in an increase in the absolute amplitude at 1 kHz with increase in the age. The reduction in the absolute amplitude at other frequencies with increasing age could be attributed to the fact that, with the increase in age, the ear canal volume increases and because the SPL measured inside the cavity is inversely proportional to the volume, this could have led to the decrease in absolute amplitude with increase in age.

The findings of the present study are in accordance with the findings stated by Smurzynski (1994). It was found that the click evoked TEOAE levels increases with the post conceptional age. The TEOAE levels were found to decrease with increasing age (Engdahl, Arnesen & Mair, 1994; Glattke, Pafitis, Cummiskey & Herer, 1995; Norton & Widen, 1990; Nozza & Sabo, 1992; Prieve, Fitzgenald, & Schuttle, 1997; Speak, Leonard, Kim, Jung & Smurzynski, 1991), which is also evident from the present study, where, TEOAE amplitudes were reduced for 8 to 12 months age group infants at all the frequencies. Kapoor and Panda (2006) analyzed SNR values for TEOAEs in neonates (0 to1 month) and infants (1 to 12 months). The results showed that the neonates had the lowest SNR ranging between 3.47 to 9.62 dB whereas the infants showed the highest SNR values ranging between 6.13 to 13.11 dB. These findings support the present study where similar results were found. The results of the present study can also be supported by results of the study done by Shi, Wang, Yuan and Zhang (2010), where they reported that lowest SNRs were obtained at low frequency (0.8 kHz) for neonates and at higher frequency (4 kHz) for younger adults.

Zhang et al. (2008) investigated the characteristics (amplitude and SNR) of the 1 kHz TBOAE response in neonates. The results showed that, the mean TBOAE response at 1 kHz was significantly higher than that of 2 kHz ($p < 0.05$), whereas, the SNR at 1 kHz was significantly lower than that at 1.5 kHz and 2 kHz frequency bands. The main reason for these findings relates to the greater noise in the 1 kHz frequency region, which may mask out the weaker TBOAE response. And also, a default low frequency filter setting in the ILO system may also reduce the noise levels at lower frequencies, and also reduces the OAE response. The mean TBOAE responses obtained at 1 kHz in the present study did not correlate with the results obtained from Zhang and McPherson (2008) study. Whereas, in the present study, the SNR values at 1 kHz were in accordance with Zhang and McPherson (2008) study.

Comparison of Absolute Amplitude and SNR across Age groups for Different Frequencies of CEOAEs and TBOAEs

Mixed ANOVA was carried out to see the significant interaction across age groups, frequencies and stimuli for amplitude and SNR measures separately. The results of mixed ANOVA for absolute amplitude revealed, a high statistically significant difference between the age groups [$F (3, 76) = 13.24$, $p < 0.05$]; between different frequencies [$F (2, 152) = 88.89$, $p < 0.05$]; between the two different stimuli [$F (1, 76) = 105.7$, $p < 0.05$] for all the four age groups; between frequencies and age groups [$F (6, 152) = 9.09$, $p < 0.05$]; between age groups and stimuli [$F (3, 76) = 1.53$, $p < 0.05$]; between stimuli and frequencies [$F (2, 152) = 8.37$, $p < 0.05$]; also between the stimuli, frequencies and age groups [$F (6, 152) = 3.52$, $p < 0.05$]. The results of mixed ANOVA for SNR revealed, a high statistically significant difference between the age groups [$F (3, 76) = 5.70$, $p < 0.05$]; between different frequencies [$F (2, 152) = 55.04$, $p < 0.05$]; between the two different stimuli [F

(1, 76) = 95.8, $p < 0.05$] for all the four age groups; between frequencies and age groups [F (6, 152) = 4.44, $p < 0.05$]; between age groups and stimuli [F (3, 76) = 4.35, $p < 0.05$]; between stimuli and frequencies [F (2, 152) = 10.04, $p < 0.05$]; and also between the stimuli, frequencies and age groups [F (6, 152) = 8.41, $p < 0.05$].

Effect of Age on Absolute Amplitude and SNR at Each Click Frequency

As mixed ANOVA showed significant interaction across click frequencies on amplitude and SNR measures, to see the effect of age on amplitude and SNR, multivariate analysis of variance (MANOVA) was done at each click frequency. The results for amplitude measures showed that there was significant difference in amplitude across age groups for 1000 Hz [F (3, 76) = 4.40, $p < 0.05$]; 2000 Hz [F (3, 76) = 13.61, $p < 0.05$]; and for 4000 Hz [F (3,76) = 13.39, $p <0.05$] click frequencies. The results for SNR measures showed that there was significant difference in SNR across age groups for 1000 Hz click frequency [F (3, 76) = 12.56, $p < 0.05$]; and for 2000 Hz click frequency [F (3, 76) = 5.89, $p < 0.05$]. There was no significant difference in SNR for 4000 Hz click frequency [F (3,76) = 1.86, $p > 0.05$] across age groups.

As MANOVA showed significant difference between age groups for 1000 Hz, 2000 Hz and 4000 Hz click frequency for amplitude measures, and significant difference between age groups for 1000 Hz and 2000 Hz click frequency for SNR measures, further analysis using the Duncan post hoc analysis test was done to see between which two age groups, the amplitude and SNR differ significantly. The results of Duncan post hoc test for absolute amplitude for each click frequency revealed that, for 1 kHz click frequency, group I was statistically significant from group III and vice versa. For 2 kHz click frequency, group III and IV significantly differed from all the age groups. However, group I and II significantly differed from both the groups III and IV. And for 4 kHz click frequency, group I was statistically significantly different from all the other age groups except group III. Similarly group II showed significant difference from all the age groups except from group IV. These results were similar to the results obtained by Norton et al. (2000) where, there was no much difference in the TEOAE levels for infants born between 28 and 40 weeks of GA and those between birth and 28 days after birth. And a significant group effect was observed at 2 kHz click frequency.

The results of Duncan post hoc test for SNR for each click frequency showed that, for 1 kHz click frequency, only group I was found to be significantly different from the other age groups. And group II was significantly different from group I and IV. Whereas, group III was significantly different from only group I. For 2 kHz click frequency, group I showed no statistically significant difference between the age groups. Whereas, group II was statistically significant from group III and IV. For 4 kHz click frequency, the SNR values did not show any significant difference across the age groups.

Effect of Age on Absolute Amplitude and SNR at each Toneburst Frequency

To see the effect of age on absolute amplitude and SNR measures, MANOVA was done at each tone burst frequencies. The results showed that, there was no significant difference in absolute amplitude [F (3, 76) = 2.41, $p > 0.05$] across age groups for 1000 Hz tone burst frequency. However significant difference in absolute amplitude across age groups for 2000 Hz and 4000 Hz tone burst frequency [F (3, 76) = 9.03, $p < 0.05$] and [F (3,76) = 2.94, $p < 0.05$] respectively was observed. A significant difference in SNR across age groups for 1000 Hz, 2000 Hz, and 4000 Hz tone burst frequency [F (3, 76) = 3.86, $p < 0.05$], [F (3, 76) = 12.86, $p < 0.05$], and [F (3,76) = 11.75, $p < 0.05$] respectively was also observed.

As MANOVA showed significant difference between age groups for 1000 Hz, 2000 Hz and 4000 Hz toneburst frequency on absolute amplitude and SNR, further analysis using the Duncan post hoc analysis test was done to see between which two age groups, the absolute amplitude and SNR differ significantly. The results of Duncan post hoc test for absolute amplitude for each toneburst frequency revealed that, 1 kHz toneburst frequency showed statistically no significant difference across the different age groups. For 2 kHz toneburst frequency, group I and II were statistically significant from group III only; whereas, group III was statistically significant from all the age groups, and group IV was significantly different from the other age groups except from group II. For 4 kHz toneburst frequency, group I and IV showed no statistically significant difference across the age groups. Whereas, group II was statistically significant from group III and vice versa.

The results of Duncan post hoc test for SNR for each toneburst frequency showed that, for 1 kHz toneburst frequency, group II showed no significant difference with the other age groups. When 2 kHz toneburst frequency was considered, the SNR values for group I was statistically significant from group II and group IV. And the group II showed statistically significant difference from all the other age groups except from group IV. Similarly, group III was statistically significant from all the other age groups except from group I. Group IV was statistically significant from all the other age groups except from group II. For 4 kHz frequency, group III was found to be significantly different from all the age groups. Group II and IV showed statistically significant difference from all the other age groups except from Group I. The findings of Zhang and McPherson (2008) were in support with the present study where, the age of the neonate (mean test age: 2.54 days) had no effect on the mean response and SNR at each frequency band (1 kHz, 1.5 kHz and 2 kHz). But this was not evident for all the frequencies in the present study.

Effect of click frequency on absolute amplitude and SNR at each age group

As mixed ANOVA showed significant interaction across click frequencies, repeated measure ANOVA was done at each age group to see the effect of age on amplitude and SNR. The results showed that there was significant difference in absolute amplitude across click frequencies in group I, II, III, IV [F (2, 38) = 29.14, p < 0.05], [F (2, 38) = 46.49, p < 0.05], [F (2, 38) = 24.29, p < 0.05], and [F (2, 38) = 37.23, p < 0.05] respectively. A significant difference in SNR values across click frequencies in group I, II, III, IV [F (2, 38) = 26.61, p < 0.05], [F (2, 38) = 22.10, p < 0.05], [F (2, 38) = 8.17, p < 0.05], and [F (2, 38) = 20.02, p < 0.05] respectively were also observed.

As repeated measure ANOVA showed significant difference between click frequencies across age groups on absolute amplitude and SNR, further analysis using the Bonferroni's multiple pair wise comparison was done to see between which two click frequencies, the absolute amplitude and SNR differed significantly. The results of Bonferroni's multiple pairwise comparison test for absolute amplitude, for each age group revealed that, for group I and IV, the absolute amplitude for the click stimulus of 1 kHz, 2 kHz and 4 kHz was significantly different from the other frequencies (p < 0.05). Whereas, group II showed statistically significant difference for 1 kHz and 2 kHz, but 4 kHz was significantly different only from 1 kHz, and for group III, only 1 kHz was significantly different from the other frequencies.The results of Bonferroni's multiple pair wise comparison test for SNR for each age group revealed that, group I was significantly different across all the frequencies. For group II, 1 kHz and 2 kHz showed significant difference only from 4 kHz click frequency. For group III and IV, the SNR values at 1 kHz were found to be statistically significantly from the other frequencies. Whereas, 2 kHz and 4 kHz was significantly different only from 1 kHz click frequency.

Effect of tone burst frequency on absolute amplitude and SNR at each age group

Repeated measure ANOVA was done at each age group to see the effect of age on absolute amplitude and SNR values. The results showed that there was significant difference in absolute amplitude across tone burst frequencies in group I, II, III, IV [F (2, 38) = 21.15, p < 0.05], [F (2, 38) = 64.73, p < 0.05], [F (2, 38) = 24.61, p < 0.05], and [F (2, 38) = 52.04, p < 0.05] respectively. The results of repeated measure ANOVA for SNR revealed, significant difference across click frequencies in group I, III, and IV [F (2, 38) = 24.34, p < 0.05], [F (2, 38) = 14.61, p < 0.05], and [F (2, 38) = 58.33, p < 0.05] were observed respectively. Whereas, group II showed no significant difference in SNR [F (2, 38) = 1.39, p > 0.05] across click frequencies.

As repeated measure ANOVA showed significant difference between tone burst frequencies across age groups on absolute amplitude and SNR, further analysis using the Bonferroni's multiple pair wise comparison was done to see between which two tone burst frequencies, the absolute amplitude and SNR differ significantly. The results of Bonferroni's multiple pair wise comparison test for absolute amplitude, for each age group revealed that, for group I, 1 kHz toneburst frequency was statistically significant from all the other frequencies and, 2 kHz toneburst frequency was not significantly different from 4 kHz frequency and vice versa. Whereas, statistically significant difference was observed between 2 kHz and 1 kHz, and 1 kHz and 4 kHz. For group II, the absolute amplitude at all the tone burst frequencies was statistically significant, except for the 4 kHz which did not have a significant difference with 2 kHz. Similarly, the 4 kHz tone burst frequency showed statistically no significant difference with 2 kHz frequency and vice versa for group III. There was statistically significant difference between 2 kHz and 1 kHz, and 4 kHz and 1 kHz (p < 0.05). Whereas, group IV showed statistically significant difference across all the tone burst frequencies. The results of Bonferroni's multiple pair wise comparison test for SNR, for each age group revealed that, all the age groups, except group II, showed a statistically significant difference (p < 0.05) across all the toneburst frequencies tested.

Comparison of absolute amplitude and SNR across click and tone burst stimuli at each age group for each frequency

As mixed ANOVA showed significant interaction across stimuli, paired sample t-test was done, in order to compare the absolute amplitude of click frequencies (1 kHz, 2 kHz & 4 kHz) and tone burst frequencies (1 kHz, 2 kHz & 4 kHz) at each age group for each of the frequencies. The findings for the comparison of absolute amplitude revealed that, for group I, there was statistically significant difference at 1 kHz [t (19) = 2.69, p < 0.05] and 2 kHz [t (19) = 4.67, p < 0.05] click and toneburst stimuli. Whereas, no significant difference was observed for the comparison of 4 kHz [t (19) = 0.93, p > 0.05] click and toneburst stimulus. For group III, statistically significant difference was observed only for 1 kHz click and toneburst frequencies [t (19) = 2.43, p < 0.05]. Statistically no significant difference was obtained for 2 kHz [t (19) = 1.69, p > 0.05] and 4 kHz [t (19) = 1.49, p > 0.05] click and toneburst stimuli. Whereas, for group II, statistically significant difference was observed at all the frequencies that is, at 1 kHz [t (19) = 3.54, $p < 0.05$], at 2 kHz [t (19) = 2.99, $p < 0.05$], and at 4 kHz [t (19) = 2.87, $p < 0.05$] click and toneburst stimuli. Similarly, for group IV, statistically significant difference was observed at all the frequencies, that is, at 1 kHz [t (19) = 4.46, $p < 0.05$], at 2 kHz [t (19) = 6.46, $p < 0.05$], and at 4 kHz [t (19) = 7.04, $p < 0.05$] click and toneburst stimuli.

The findings for the comparison of SNR revealed that, for group I, there was significant difference for the comparison of SNR values between click and toneburst stimuli at 1 kHz [t (19) = 3.96, $p < 0.05$] and 2 kHz [t (19) = 5.95, $p < 0.05$]. Whereas, 4 kHz click and toneburst stimulus showed no significant difference [t (19) = 0.34, $p > 0.05$]. Similarly, for group II, there was significant difference for the comparison of SNR values between click and toneburst stimuli at 1 kHz [t (19) = 3.94, $p < 0.05$] and 2 kHz [t (19) = 5.02, $p < 0.05$]. Whereas, 4 kHz click and toneburst stimulus showed no significant difference [t (19) = 1.98, $p > 0.05$]. For group III, the comparison of SNR values between click and toneburst stimuli was found to be significantly different at all the frequencies, that is, at 1 kHz [t (19) = 3.14, $p < 0.05$], at 2 kHz [t (19) = 4.87, $p < 0.05$], and at 4 kHz [t (19) = 3.20, $p < 0.05$] click and toneburst stimuli. Whereas, for group IV, except at 2 kHz [t (19) = 0.61, $p > 0.05$] click and toneburst stimulus, all the other frequencies, that is, 1 kHz [t (19) = 2.40, $p < 0.05$] and 4 kHz [t (19) = 3.69, $p < 0.05$] click and toneburst stimuli showed a statistically significant difference.

Conclusions

From the present study it can be concluded that, TBOAEs showed a stronger response level than CEOAEs. Hence, to obtain a more frequency specific response, a toneburst stimulus can be used instead of click stimulus. A significant difference across the age groups was found for both CEOAEs and TBOAEs. The response in infants is more robust and it contains more high frequency energy. As the mean SNR value is more at higher frequencies for both click and toneburst stimulus, while evaluating the response in infants not only the amplitude of the response should be considered but also the SNR values.The values obtained from the present study can be used as a clinical tool for screening and diagnostic purposes in the pediatric population. Also, toneburst stimuli can be used instead of click stimuli to obtain a more frequency specific response in both pediatric and adult population. As the prevalence of TBOAEs are higher compared to CEOAEs in infants, it can be included in the screening and diagnostic test battery.

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