Optimizing stimulus repetition rate for recording ocular vestibular evoked myogenic potential elicited by air-conduction tone bursts of 500 Hz

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Abstract

Amidst several publications reporting the effects of stimulus-related parameters on ocular vestibular evoked myogenic potential (oVEMP), the effect of the repetition rate on oVEMP responses has largely gone unexplored. Studies have used a repetition rate of ~5.1 Hz mainly due to a presumption that oVEMP, like cervical VEMP, should produce best responses for ~5 Hz, although there is paucity of experimental evidence to support this hypothesis. S2 healthy individuals in the age range of 17-35 years underwent air-conduction oVEMP elicited by 500 Hz tone-bursts using seven different repetition rates (3.1, 5.1, 10.1, 15.1, 20.1, 25.1 and 30.1 Hz). The results revealed a tendency for prolongation of latencies and reduction in amplitude with increasing repetition rate. However, significantly longer latencies were observed only for 20.1 Hz and larger amplitudes for 3.1 and 5.1 Hz (P<0.05). There was no significant difference between the rates of 3.1 Hz and 5.1 Hz. However 3.1 Hz produced poorer signal-to-noise ratio and required considerably longer time and thereby had lesser efficiency than 5.1 Hz (P<0.05). This would also result in higher fatigue and irritation levels considering the physical act of maintaining a supero-medial gaze. Thus the use of 5.1 Hz is recommended for clinical recording of oVEMP.

Introduction

Vestibular evoked myogenic potentials (VEMPs) are short latency electromyograms elicited by presentation of loud acoustic, vibratory or galvanic stimuli. These biphasic responses can be elicited from several muscles of the body including the sternocleidomastoid muscle,1 the trapezius muscle,2 the triceps muscle,3 and the inferior oblique muscle of the eye.4 When elicited from the inferior oblique muscle of the eye, it is referred as an ocular vestibular evoked myogenic potential.4,6 The ocular vestibular evoked myogenic potential (oVEMP) is believed to be an excitatory biphasic potential with an initial negative peak around 10 ms (referred as N10 or n1) and a subsequent positive peak around 15 ms (referred as P17 or p1) in response to loud acoustic stimulation.4,5 These peaks have also been referred to as n1 and p1 respectively.7 Clinical studies on individuals with a normal audio-vestibular system and those with different cochlear and vestibular pathologies have confirmed the otolithic and speculated utricular relevance of this potential, although not everyone agrees regarding the utricular origin alone.8-13 Subsequently the pathway for oVEMP has been reported to be similar to that of the transverse vestibulo-ocular reflex pathway, originating from the otolith organs and ending on the contralateral inferior oblique muscle of the eye, on the way crossing via the superior vestibular nerve, vestibular nuclei, and oculomotor nuclei.6,14,15 During and even before the complete exploration of its pathway, oVEMP was being explored through clinical and basic research. The basic research, which later contributed to its clinical application in a big way, was mainly concentrated around its stimulus parameters. Since one of its earliest reports by Todd et al.,16 the effect of several stimulus parameters like stimulus intensity, stimulus frequency, and stimulus type on oVEMP responses have been explored.4,17,18 The rate at which the stimulus is delivered has been shown to affect all the acoustically evoked potentials and oVEMP should be no different. However this aspect of oVEMP has largely gone unexplored. Studies using oVEMP have by and large used a repetition rate of 5.1 Hz which is mainly due to a presumption that oVEMP, like cervical VEMPs (cVEMP), are myogenic potentials and hence should produce best responses for the repetition rate found optimum for acquisition of cVEMP.19 There is lack of experimental evidence though to support this
Materials and Methods

Participants

The study adhered to the general ethical standards for protection of human subjects during experiments and included 52 healthy volunteers in the age range of 17-35 years (mean age=24, standard deviation=±4.6 years) after obtaining informed written consent and following the institutional ethical guidelines strictly. Their otological and neurological well-being was ensured through detailed structured case history, pure-tone audiometry, speech audiometry, immittance evaluation and auditory brainstem response assessments. The vestibular well being was ensured through a screening by an experienced otolaryngologist. Individuals with a history related to vestibular, otological or neural pathologies were not considered for the study.

All the participants had pure-tone thresholds within 20 dB HL. They further demonstrated at least a fair agreement (±±±12 dB HL) between pure-tone average and speech recognition threshold, in addition to the speech identification scores in excess of 90%. In addition to the above, their uncomfortable levels were in excess of 100 dB HL. Normal middle ear status was ascertained by presence of ‘A’ type tympanograms and acoustic reflex thresholds (ipsilateral and contralateral) within 100 dB HL. The existence of retro-cochlear pathology was ruled out by normal clicks-evoked auditory brainstem responses (absolute latencies of waves I, III, and V of less than 2, 4 and 6 ms respectively, inter-peak latency difference ≤2.0 ms, inter-aural latency difference ≤0.2 ms, wave VI amplitude ratio ≤0.5).

The otolaryngologist screened for balance related dysfunction through the use of Fukuda stepping test, Romberg test, Tandem gait test and Past pointing test. The Fukuda stepping test was performed in the centre of two concentric circles in the standard format described by Fukuda. The examiner stood directly behind the participant during the test and the participant was instructed to keep his/her eyes closed during the standard stepping test. He/she was instructed to stand with his/her arms outstretched and extended at an angle of 90° in front of the body and to march at a place for 50 steps at the rate of about 1step/s. An angle of deviation ≤45° was considered for abnormality (our clinical norm, established on 100 healthy participants). The Romberg test was performed in a way similar to the Fukuda stepping test but without marching. Sway towards sides, forward or behind was considered an abnormal result. Tandem gait test was administered by asking the participant to walk heel-to-toe on an imaginary straight line and sway towards either side was taken for abnormality. Finally, the Past pointing test (popularly known as finger-to-nose test) was performed by asking the participant to touch his/her nose tip and clinician’s index finger tip, the position of which kept varying in terms of distance and angle, alternately. Inability to perform the task, evident tremors and overshooting or undershooting of the target was considered as abnormal response. Abnormal response on any of these tests served as exclusion criteria.

Procedure

Following the fulfillment of the subject selection criteria, the participants underwent oVEMP recordings using the Biologic Navigator Pro (Natus Medical Inc., San Carlos, CA, USA) with ER-3A insert earphones. For the acquisition of oVEMP, the participants were seated in an upright position in an acoustically treated room with ambient noise levels within permissible limits. A commercially available abrasive gel was applied and the skin overlying forehead (Fz) and the inferior oblique muscle was scrubbed to achieve absolute and inter-electrode impedance of ≤5 kΩ and ≤2 kΩ respectively. The non-inverting electrode was placed on the cheek approximately 1 cm below the centre of the lower eye lid, directly below the pupil when in forward centre gaze. The inverting electrode was placed 2 cm below the non-inverting and the ground electrode on the forehead. The electrode placement was similar to those used previously for appropriate recording of oVEMP.

The insert earphone was placed in the ear contralateral to eye of electrode placement as oVEMP has been found to be mainly a contralateral response. Seven different repetition rates of 3.1, 5.1, 10.1, 15.1, 20.1, 25.1 and 30.1 Hz were used for the presentation of alternating polarity short tone-bursts of 500 Hz at 95 dB nHL (equivalent to 125 dB pSPL). The stimuli were ramped using a Blackman window with 2 ms rise/fall time and 1 ms plateau time. To avoid the order effect, odd numbered participants of each of the groups were tested by varying the repetition rate in ascending order while the even numbered were presented the stimuli in descending order of the repetition rate. The subjects were instructed to maintain a 30-35° superio-medial gaze position, a gaze position found most appropriate for oVEMP recording. The responses were band-pass filtered between 1 and 1000 Hz and amplified by a factor of 30,000. An epoch of 42 ms was used for all repetition rates but 25.1 and 30.1 Hz. For these two rates, an epoch of 32 ms was used in order to discount for the overlapping responses. A pre-stimulus baseline recording of 7 ms was incorporated within the above-mentioned epochs, irrespective of the repetition rate. The responses for 200 sweeps were averaged per recording. An inter-recording rest period of 1-2 min was granted to ensure fatigue and irritation free recording of oVEMP.

The obtained waveforms were marked for peaks by two independent experienced audiologists. The parameters documented were the individual peak latencies (n1 and p1) and peak-to-peak amplitudes. Inter-observer reliability was assessed using Chronbach's alpha test and Pearson's correlation analysis. There was a significantly high positive correlation between the observers (r>0.9, P<0.001) and excellent inter-tester reliability (α>0.9) for all of the parameters. Therefore due to equivalence between the markings of the two audiologists, marking of only one audiologist was considered for further data analysis. The response rate at each of the repetition rates was calculated in terms of percentage of participants in whom the peaks were identifiable. Further, the peak-to-peak amplitude was used for the calculation of inter-aural asymmetry ratio using the formula used previously. As per this formula, the absolute difference in the peak-to-peak amplitudes between the two side responses is divided by their sum and the thus obtained value is multiplied by 100 to obtain the percentage interaural asymmetry ratio. The signal-to-noise ratio (SNR) was obtained for all waveforms using a MATLAB software. The SNR was calculated as:

\[
\text{SNR} = 20\log(\text{RMS}_1/\text{RMS}_2) 
\]

where:

SNR is signal-to-noise ratio in dB;
RMS$_{oV}$ is the root-mean-square of the oVEMP response in the time range of 7 to 30 ms; RMS$_{b}$ is the root-mean-square of the pre-stimulus baseline.

**Statistical analysis**

The latency, amplitudes, asymmetry ratio and SNR values were tabulated and statistical analysis was performed using a commercially available statistical tool- Statistical package for social science (SPSS, version 17.0). The descriptive analysis was performed for obtaining mean and standard deviation values. This was followed by one-way repeated measures analysis of variance (one-way repeated measures ANOVA). Bonferroni adjusted multiple comparisons were performed for pair-wise comparisons whenever necessary. For evaluating the effect of order of presentation, multivariate analysis of variance (MANOVA) was used.

**Results**

The individual and grand averaged waveforms across the repetition rates have been displayed in Figure 1. The ocular VEMPs could be successfully recorded at all the seven repetition rates of the study; however the number of ears showing presence of oVEMP varied from rate to rate. The response rate tended to drop with increasing repetition rate of the stimuli beyond 5.1 Hz. The response rate was 100% at the repetition rate of 3.1 Hz and 5.1 Hz and reduced thereafter to 80.76%, 67.30%, 53.84%, 32.69% and 17.30% of the ears at repetition rates of 10.1 Hz, 15.1 Hz, 20.1 Hz, 25.1 Hz and 30.1 Hz respectively. Table 1 shows the mean and standard deviation (SD) values of various amplitude and latency related parameters of oVEMP with changes in repetition rate of the stimulus.

The latencies of peaks demonstrated a trend towards prolongation with increase in repetition rate. The statistical significance of this trend was evaluated using one-way repeated measures ANOVA. Due to absence of oVEMP at 25.1 Hz and 30.1 Hz in a large majority of individuals, these rates were not included for one-way repeated measures ANOVA. The results revealed a significant main effect of the repetition rate on n1 latency [F(4,412)= 13.90, P<0.001]. The Bonferroni adjusted multiple comparisons showed significantly longer latencies only for 20.1 Hz compared to all other rates (P<0.01). One-way repeated measures ANOVA for p1 latency also revealed a significant main effect of repetition rate [F(4,412)=7.92, P<0.001]. For comparison of the pairs of repetition rates for p1 latency, the Bonferroni adjusted multiple comparisons was done which revealed no significant difference between any of the pairs of repetition rates until 15.1 Hz. Nonetheless, the laten-

![Figure 1. The individual ocular vestibular evoked myogenic potential waveforms and the resultant grand averaged waveforms.](image)

Table 1. Mean and standard deviation of various parameters of ocular vestibular evoked myogenic potential across repetition rates.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Repetition rate (in Hz)*</th>
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<tbody>
<tr>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td>n1 latency (in ms)</td>
<td>11.01 (0.60)</td>
</tr>
<tr>
<td>p1 latency (in ms)</td>
<td>16.68 (1.20)</td>
</tr>
<tr>
<td>Peak-to-peak amplitude (in µV)</td>
<td>10.26 (7.40)</td>
</tr>
<tr>
<td>IAAR (in %)</td>
<td>27.90 (21.75)</td>
</tr>
<tr>
<td>SNR (in dB)</td>
<td>17.41 (14.71)</td>
</tr>
</tbody>
</table>

*Standard deviations are mentioned within brackets. IAAR, inter-aural asymmetry ratio; SNR, signal-to-noise ratio.
cy for 20.1 Hz was significantly longer than all others (P<0.01) except 15.1 Hz (P>0.05). Figure 2 shows the graph demonstrating the effect of change in repetition rate on the latencies of n1 and p1.

The one-way repeated measures ANOVA was also done to investigate the effect of changing repetition rate on peak-to-peak amplitude. The results revealed a significant main effect of repetition rate on the peak-to-peak amplitude [F(4,412)=37.83, P<0.001]. The pair-wise comparison using the Bonferroni adjusted multiple comparisons revealed no significant difference between 3.1 and 5.1 Hz repetition rates (P>0.05). However, these two rates were significantly different (produced higher amplitudes) from all other rates (P<0.05). Apart from this, the Bonferroni adjusted multiple comparisons also revealed no significant difference between 15.1 Hz and 20.1 Hz (P>0.05). The repetition rate of 25.1 Hz and 30.1 Hz were not considered due to the absence of oVEMP in most of the individuals at these rates. Figure 3 shows the comparison of mean and 95% confidence intervals between repetition rates for peak-to-peak amplitude of oVEMP.

The one-way repeated measures ANOVA was applied to evaluate the effect of repetition rate on the inter-aural asymmetry ratio which revealed no significant main effect of changing the repetition rate [F(4,412)=1.84, P>0.05] on the inter-aural asymmetry ratio. Therefore a post hoc test was not necessitated. Figure 4 shows the effect of varying repetition rate on inter-aural asymmetry ratio by way of depicting mean and 95% confidence intervals.

The effect of presenting the stimuli in a particular order of repetition rate was evaluated using MANOVA. The results revealed no significant main effect of order of presentation on n1 latency, p1 latency, peak-to-peak amplitude and inter-aural asymmetry ratio (P>0.05). The Bonferroni adjusted multiple comparisons were not required due to lack of main effect for any of the parameters.

Signal-to-noise ratio

The signal-to-noise ratio was obtained for all the waveforms using a MATLAB program. The largest SNRs were obtained for 5.1 Hz. There was a general trend of reduction in SNR with increase in repetition rate. However, 30.1 Hz rate produced larger mean SNR than 25.1 Hz whereas 3.1 Hz produced smaller SNR than 5.1 Hz. Table 1 shows the mean and standard deviation of SNR values for each repetition rate. One-way repeated measures ANOVA was done for SNRs across the repetition rates. For this, only the repetition rates up to 20.1 Hz were used owing to absence of responses in a large majority of individuals at 25.1 Hz and 30.1 Hz. The results revealed a significant main effect of repetition rate on SNR [F(4,412)=13.65, P<0.001]. The Bonferroni adjusted multiple comparisons revealed significant differences between only some of the repetition rates. The result of these comparisons is shown in Figure 5.
Relative efficiency

The efficiency, which can be defined as the SNR divided by recording time, was obtained only for 3.1 Hz and 5.1 Hz since only these two rates produced 100% response rates. The efficiency was observed to be higher for 5.1 Hz (mean=0.57, SD=0.39) compared to 3.1 Hz (mean=0.29, SD=0.24). The statistical significance of comparison of efficiency between the two repetition rates was evaluated using one-way repeated measures ANOVA. The results revealed a significant main effect of repetition rate on efficiency [F(1,103)=34.82, P<0.001]. Therefore, the efficiency of 5.1 Hz was higher than that of 3.1 Hz.

Discussion

Several studies have explored oVEMP for either normative or clinical purposes previously and used a stimulus repetition rate in the vicinity of 5 Hz.4-6,15,16,27,29-39 No study has ever explored the effects of different stimulation rates on AC-oVEMP, although its impact on BC-oVEMP has been examined and 20 Hz was reported as best repetition rate for clinical recording of BC-oVEMP.21 However, it is little known through experimental evidence whether 5 Hz stimulation rate (which is best for evoking cVEMP and have been repeatedly used for recording AC-oVEMP) or 20 Hz (which is found optimal for acquiring BC-oVEMP) is best suited to the recording of AC-oVEMP.

The results of the present study found that tone-burst stimuli presented at rates of 3.1 and 5.1 Hz produced 100% response rates and the response rate gradually declined with increasing stimulus repetition rate thereafter. Similar findings have been reported for air-conduction stimuli, though for cVEMP only.19 The results of the study demonstrated evidence against the reason for such a finding being the order effect. There was a lack of statistically significant difference between ascending and descending testing order (MANOVA, P>0.05) for all the parameters of the present study. The reduction in response rate with increasing repetition rate might rather be attributed to the adaptation creeping in to the vestibular afferents.

There was also a trend towards prolongation of the latencies of n1 and p1 peaks of oVEMP with increasing repetition rate. To the best of our knowledge, there are no studies reporting the effect of repetition rate of the stimulus on air-conduction evoked oVEMP. However the studies evaluating the effect of repetition rate of air-conducted clicks on latencies of auditory brainstem response (ABR) and cVEMP, close associates of oVEMP owing to sameness in the nerve for generation (vestibulocochlear nerve), have shown a prolongation of latencies with increased repetition rate.19,40-42 This phenomenon in the ABR literature has been attributed to the refractory period of the nerve fibers and the same concept might be useful in explaining similar findings in oVEMP responses. A rapid stimulation rate results in a change in receptor function, better known as fatigue or adaptation.40,41 Increasing the repetition rate of air-conducted stimuli might cause refractoriness and decreased synaptic efficacy to generate action potentials,43 thereby causing progressive prolongation of the oVEMP latencies, more so for an increase in repetition rate beyond 20.1 Hz.

In terms of amplitude, largest values were attained for lower repetition rates. The rates of 3.1, 5.1 and 10.1 Hz produced significantly larger amplitude compared to the others and the amplitude progressively diminished with increasing repetition rate. There are no reports of the effects of repetition rate of the stimulus presentation on oVEPs evoked by air-conduction stimuli. However, Chang et al., using bone-conducted (BC) tone-bursts of 500 Hz, reported no difference in amplitudes of oVEPs and cVEPs between the repetition rates of 5, 10 and 20 Hz and accordingly recommended the use of 20 Hz for clinical recording of BC evoked cervical and ocular VEMPs.21 The differences in the findings of the present study and those of Chang et al. may be attributed to the use of different parameters for arriving at the conclusion along with the use of different modes of stimulation (AC in present study compared to BC in Chang et al.).21 Chang et al. used only amplitude and variability as the parameters for reaching the conclusion of best repetition rate whereas the present study also incorporated SNR and efficiency calculation in addition to the parameters used by Chang et al. Studies have also been done using air-conducted click stimuli to check for the effect of repetition rate on a close cousin of oVEMP, namely cVEMP.19 Wu and Murofushi reported a similar trend, as observed in the present study, of progressive reduction in amplitude with increasing rate of stimulus delivery.19 They further reported best amplitudes for 1 Hz and 5 Hz rates.

The reason behind largest amplitudes at lowest rates could be hidden in the pathway involved in production of oVEMP and its characteristics. oVEMP is a reflex generated by acoustic stimulation which is conducted via the neuro-receptors in the utricle, vestibular afferent transmission to the vestibular nucleus, central nervous system conduction from the vestibular nucleus along and across the medial longitudinal fasciculus to the inferior oblique muscle. This trend in reduction of amplitude with increasing repetition rate may be attributed to progressive adaptation of the superior vestibular nerve fibers, similar to those reported in auditory nerve fibers.44 However, the refractory period of the mammalian myelinated neural fibers varies from 1 to 10 ms and the central mammalian neurons can fire up to 1000 spikes per second.45 Additionally, the muscle fibers can respond to stimulation frequencies up to around 100 Hz.46 Considering all the above, an inter-stimulus interval of 50 ms produced by stimulation rates below 20.1 Hz appears to be well within the physiological capacity for the central neurons and efferent fibers. Further, the most important frequencies of head perturbations, which naturally initiate the vestibulo-ocular reflex, fall mainly in the region of 0.5 to 5 Hz.47 Therefore, the adaptation phenomenon, along with the potential metabolic exhaustion within the sensory cells to support the sustained high firing rate, might explain amplitude reduction following rate increase.

An additional contribution could come from the impact of stapedial reflex on the energy transfer to the otolith organs which produce cervical and ocular VEMPs. The onset and impact of stapedial reflex cannot
be completely eliminated when recording oVEMP. This is because of the fact that oVEMP thresholds in healthy individuals have been reported in the range of 90 dB nHL (equivalent to 110 dB pSPL) to 105 dB pSPL where as acoustic reflex thresholds have been reported to occur around 100-105 dB pSPL. This shows that oVEMP cannot be elicited below the level of acoustic reflex threshold and therefore the effect of stapedial reflex will be an integral part of any oVEMP recording. However, the rate of stimulus presentation could affect the acoustic reflex amplitudes and thresholds differentially. Higher rate has been shown to be associated with higher acoustic reflex amplitude and lower thresholds than lower rates. Nonetheless this has been reported for click rates from 50 Hz to 300 Hz but not for ±30.1 Hz, the maximum rate used in the present study. None of the other studies have reported the effects in the range of repetition rates used in the present study. Admittedly though, an effect even at these rates is a possibility which was not incorporated in the present study. Future studies in this regard could be conducted by accounting for the effect of stapedial reflex by using a possible reduction in the intensity, especially at higher rates of stimulation.

The asymmetry ratio did not show any consistent pattern with change in repetition rates, although largest values were obtained for higher repetition rates. Asymmetry ratio is used to calculate the side-to-side amplitude difference. Since the comparison is made between the two sides at a given rate, the finding of no significant change in asymmetry ratio with increasing repetition rate is expected. This finding of no significant difference between the rates might be aided by presence of large inter-subject variability which might result in overlap of asymmetry ratios between the repetition rates. However, a close scrutiny of the asymmetry ratios revealed lowest mean values for 5.1 Hz, probably reflecting lower variability produced at this rate.

The SNRs obtained were largest at 5.1 Hz for oVEMP response, which is in accordance with those reported previously for cVEMP. None of the other studies have explored this aspect in oVEMP. There was a trend towards reduction in SNRs with increasing repetition rate which probably reflects reduction in amplitude of response.

The signal-to-noise ratio was lower and overall morphology poorer for a given number of averages for 3.1 Hz compared to 5.1 Hz. Additionally, this rate would require the subject to maintain constant gaze elevation for nearly 20 seconds more than when using 5.1 Hz rate. At the rate of 3.1 Hz the time required for 200 averages would be about 60 s as against 40 s for 5.1 Hz. This might result in considerably higher level of discomfort through fatigue and watering of eyes, especially when doing multiple recordings required during threshold assessments or obtaining frequency tuning. Additionally, the stimulation rate of 5.1 Hz produced significantly higher efficiency than 3.1 Hz. Therefore, with lowest asymmetry ratio and highest efficiency, 5.1 Hz is recommended as the optimal stimulation rate for the clinical use of oVEMP.

Conclusions

The results of the present study revealed maximum response rate and largest amplitudes for repetition rates of 3.1 Hz and 5.1 Hz. However, a rate of 3.1 Hz requires considerably longer time than 5.1 Hz which would result in higher fatigue and irradiation levels considering the physical act of maintaining a supero-medial gaze position for oVEMP recording. The stimulation rate of 5.1 Hz produced higher efficiency than 3.1 Hz. Thus the use of 5.1 Hz repetition rate is recommended for clinical recording of oVEMP.

References