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**Kertas Asli/*Original Article***

# Effects of Plateau Time on Cervical Vestibular Evoked Myogenic Potential (cVEMP) elicited by 500-Hz Tone Burst

(Kesan Masa Penara ke atas Keupayaan Miogen Terbangkit Vestibul Serviks (cVEMP) yang Diperlihatkan oleh Letusan Nada 500 Hz)

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ABSTRACT

*Cervical Vestibular Evoked Myogenic Potential (cVEMP) is a routine vestibular test which checks the integrity of vestibulocollic reflex (VCR) pathway. Clinically, 500-Hz tone burst is widely used stimulus to evoke a cVEMP. Although several studies have suggested different plateau times (PT) for eliciting cVEMPs, but not many have reported the optimal PT for evoking cVEMP using 500 Hz tone burst stimuli. Therefore, the present study aimed to investigate the effect of PT on cVEMPs elicited by 500 Hz tone burst at 95 dBnHL using four different PT (0, 2, 4 and 10 ms). Thirty healthy adults with normal hearing and vestibular systems participated in this study. Results revealed that the P1 latency was significantly longer for PT 10 ms compared to other PTs. N1 latency was significantly prolonged for long PT of 10 ms compared to PT 2 ms. P2 latency showed no significant differences among PTs. The P1-N1 inter-amplitude values however revealed no significant difference across all PTs. It was found that the P1-N1 inter-amplitude was severely affected after 4 ms of PT. This study concluded that the PT of either 0 or 2 ms yielded the most robust cVEMP.*

*Keywords: cVEMP; tone burst; plateau time; P1 latency; N1 latency*

ABSTRAK

*Keupayaan Miogen Terbangkit Vestibul Serviks (cVEMP) adalah ujian vestibul rutin untuk memeriksa integriti laluan refleks vestibulokolik (VCR).Secara klinikal, letusan nada 500 Hz adalah stimulus yang digunakan secara meluas untuk membangkitkan cVEMP.Walaupun beberapa kajian telah mencadangkan masa penara (PT) yang berbeza untuk memperlihatkan cVEMP, tidak banyak yang melaporkan PT optimum untuk membangkitkan cVEMP menggunakan letusan nada 500 Hz. Justeru, kajian ini bertujuan untuk mengkaji kesan PT ke atas cVEMP yang dibangkitkan oleh letusan nada 500 Hz pada 95dBnHL menggunakan empat PT berbeza (0, 2, 4 dan 10 ms). Tiga puluh dewasa sihat dengan sistem pendengaran vestibul normal mengambil bahagian dalam kajian ini. Hasil kajian mendapati kependaman P1 adalah lebih lama secara bererti untuk PT 10 ms berbanding nilai PT lain. Kependaman N1 lebih lama secara bererti berbanding PT 2 ms. Kependaman P2 menunjukkan perbezaan tak bererti di kalangan nilai PT. Nilai antara amplitud P1-N1 bagaimanapun menunjukkan perbezaan tak bererti merentasi semua nilai PT. Didapati bahawa nilai antara amplitud P1-N1 berubah dengan ketara selepas PT 4 ms. Kajian ini menyimpulkan bahawa PT sama ada 0 atau 2 ms menghasilkan cVEMP paling teguh.*

*Kata kunci: cVEMP; tempoh naik/turun; masa penara; kependaman P1; kependaman N1*

## INTRODUCTION

Vestibulocollic reflex (VCR) is a reflex that aids humans to maintain their head position especially while running or walking. VCR is regulated by the force from neck muscle contraction by receiving instruction from the balance organs (Wilson et al. 1995). During vertical linear acceleration, the VCR is controlled by the saccule. Cervical vestibular evoked myogenic response (cVEMP) is the only available balance test for diagnosing the functions of saccule. cVEMP is an electromyogenic response that is recorded by placing four electrodes on upper part of torso; two positive electrodes over one-third of sternocleidomastoid (SCM) muscles, one reference electrode over sternum and one ground electrode over the forehead (Burkard et al. 2007; Cal & Bahmad 2009). An acoustic stimulus such as 500 Hz tone burst with high intensity is commonly used to excite the vestibular hair cells inside saccule.

The response obtained is a biphasic wave potentials consisting of one positive (P1) and one negative peak (N1) with two different absolute latencies (Akin & Murnane 2001). The two later potentials P2 and N2 have limited clinical significance as they are reported to be generated from cochlea (Akin & Murnane 2001). The cVEMP assessment has been clinically accepted in

diagnosing diverse vestibular disorders. Some pathologic factors that could affect cVEMP responses include superior canal dehiscence (SCD), vestibular schwannoma, vestibular neuritis and Meniere's disease (Burkard et al. 2007). To evoke a stable cVEMP response, patients are required to constantly contract their SCM muscle (Barker 2009). This is because the cVEMP pathway includes the contraction of SCM muscles from the inhibitory action potential generated from vestibular nerve (Akin et al. 2004). Contracting SCM muscle could lead to strain and discomfort for the patients. Thus shorter testing time is important to avoid any further neck discomfort and fatigue especially among elderly patients.

The cVEMP response, to an extent, is frequency specific and low frequency tones such as 500 Hz tone burst elicit a robust cVEMP compared to mid frequency tones such as 1000 Hz or 2000 Hz tone bursts. Therefore, low frequency tones are commonly preferred for eliciting cVEMP response. It is also reported that the cVEMPS are highly affected by the 500 Hz tone burst stimulus duration (Singh et al. 2014; Meyer et al. 2015).

Singh et al. (2014) suggested that 2 ms Rise/Fall time (RFT) time and 1 ms of plateau time (PT) yielded robust cVEMP. But a recent systematic review by Meyer at al. (2015) concluded 1 ms of RFT and 2 ms of PT were the most effective RFT and PT for evoking cVEMP. Furthermore, there are many protocols suggesting different RFT and PT for 500 Hz tone burst. For instance, some protocols use various RFT and PT ( 2 ms RFT and 0 ms PT, 1 ms RFT and 0 PT, 1 ms RFT and 2 ms PT, 4 ms RFT and 2 ms PT) for eliciting cVEMP response(Isaradisaikul et al. 2012). These discrepancy in protocols show that the optimum RFT and PT is yet to be obtained and this clearly demonstrate that there is a gap in knowledge regarding the effect of stimulus duration of 500 Hz tone burst on cVEMP.

Majority of the studies have focused only on RFT for eliciting robust cVEMP and only a few have reported the most optimum PT especially using 500 Hz tone burst (Cheng & Murofushi 2001; Singh & Apeksha 2014; Singh et al. 2014; Meyer et al. 2015). Cheng & Murofushi (2001) reported that a shorter PT of 1 ms leads to smaller cVEMP. According to Burkard et al. (2007), 5 ms of PT was the best to elicit robust cVEMP, which demonstrated that the duration of PT had an influence on cVEMP response. Recently, Hain (2014) reported an overall duration of 7 ms, i.e., the combined duration of RFT and PT was accepted in clinical assessment of cVEMPs. These studies reveal that medium-long PT was suitable to evoke a robust cVEMP waveform. However, the discrepancies in PT shows that there is a scant evidence related to the most suitable PT for eliciting robust cVEMP. Therefore, the aim of this study was to investigate different PTs and report the most optimal PT for eliciting robust cVEMPs using 500 Hz tone burst.

## MATERIALS AND METHODS

### PARTICIPANTS

In this study, a total of 30 normal hearing subjects (22 males and 8 females) were recruited from the International Islamic University Malaysia, Kuantan Campus. The mean age of the subjects were 23.7 years (SD = 0.03). Their ages ranged from 23 to 24 years. All study participants met the following criteria (1) Clear or minimal earwax in ear canal with intact tympanic membrane; (2) Normal Middle ear functions: Type A tympanogram and normal middle ear pressure (– 50 to + 50mm dapa); (3) Normal stapedial reflex thresholds at 500, 1000 and 2000 Hz (70-100 dBHL); (4) Audiometry thresholds should be equal to 20 dBHL or lower at all frequencies; (5) No history of vestibular disorder and no history of head and neck weakness or injury. This study was approved by the International Islamic University Malaysia Research Ethics committee (IREC).

### PROCEDURE

Cervical vestibular evoked myogenic potentials All participants were seated on a chair and were instructed to sit upright and turn their head to the contralateral side of the stimulation ear. This position would contract the ipsilateral sternocleidomastoid muscle which is significant for evoking cVEMPs. The Bio-logic Navigator Pro (Natus Medical Incorporated, CA, USA) was used to record cVEMP. The stimulus used in Bio-logic Navigator Pro instrument was calibrated in accordance with ANSI standard. Additionally, a visual feedback was provided for appropriate neck positioning and also to maintain the tonicity of SCM muscle. Three electrode montage was used for recording cVEMP, non-inverting electrode (+ve) was placed in the midpoint of SCM muscle of the contralateral side of stimulation, inverting (-ve) was placed in the sternoclavicular junction and the ground was placed on the lower forehead. The subjects were reminded not to frown or move their faces during the test. Before placing the electrodes, the skin of the three sites were scrubbed with conductive electrode gel (NuPrep) to ensure that the sites had low impedance for optimal recording. The permissible impedance range was below 3-5 kΩ. If the impedance was high ( > 5 kΩ), the subjects' skin was scrubbed again. This would ensure that the signal recorded was devoid of noise and other muscle related artifacts which would suppress the quality of cVEMP recording. Therefore, efforts were made to ensure that the electrode impedance was less than 5 kΩ.

The test stimulus was a 500 Hz tone burst with varying duration was presented at a rate of 5.1/s using rarefaction polarity. Rarefaction polarity of 500 Hz tone burst was used because this would lead to upward shift of basilar membrane which causes endolymphatic fluid displacement in the cochlea which eventually increases the

pressure of endolymph in saccule (Wang et al., 2013). This is due to the unity of saccule and cochlea in membranous labyrinth. The significance of using slower (5.1 /sec) stimulus rate was to prevent adaptation of responses and to ensure clinical relevance (Sheykholesami et al., 2001). Slower stimulus rates of 1 and 5 Hz are optimum due to lesser variance and bigger cVEMP waveform as compared to 20 Hz and higher (Wu & Murofushi 1999). The stimuli were presented using an ER – 3A insert earphones at a constant level of 95 dBnHL.

Four different PT were used in this study (0, 2, 4, and 10 ms) to elicit cVEMP responses. All the stimuli had 0 ms RFT except 0 ms PT because it is not possible to elicit a cVEMP with 0 RFT and PT. Therefore, 0 ms PT had a 2 ms RFT. Although this study had two different RFT (0 ms and 2 ms), a recently study by Singh & Apeksha (2014) found that there was no significant difference between 0 ms and 2ms RFT to evoke a cVEMP response. These stimuli were presented to participants in a randomized order. Each stimulus was presented twice to check the repeatability of the waveforms. After each stimulus presentation, participants were provided with adequate resting time to avoid muscle fatigue. The recording parameters that were used include an amplifier gain of 1000 times to prevent clipping of waveform if the gain was too much since cVEMP is a big response (Akin & Murnane, 2001). Filter setting was set to 10 to 1500 Hz because the dominant energy of EMG signal is between 40 to 150 Hz (Meyer et al., 2015). The cVEMP responses were recorded for 60 ms post-stimulus period and 10 ms pre-stimulus period. The number of sweeps were fixed to 200 sweeps since most protocols apply 64 to 256 number of sweeps (Meyer et al., 2015).

For each participants, there were 16 cVEMP recordings (2 waveforms x 2 ears x 4 PT). The labelling of the peaks were with the consensus of 3 researchers. For P1, the peak was determined from the onset of the stimulus to the first observable positive peak, while N1 was determined from the onset of stimulus to the first observable negative peak. The P1 and N1 were recorded in ms. The P1-N1 inter-amplitude was calculated from P1 to N1 in terms of µV. Each waveform the P1, N1, P2 absolute latencies, and P1-N1 inter-amplitude for both ears were calculated and analysed as a function of different stimuli.

The statistical tests were carried out using one way analysis of variance (ANOVA) at 95% confidence interval [where p-value of less than 0.05 ($p < 0.05$) was considered to be significant]. The parametric test was used because all data were normally distributed based on Shapiro-Wilk test of normality. The comparison between each stimuli duration in terms of P1 latency (ms), N1 latency (ms), P2 latency (ms) and P1-N1 inter-amplitude (µV) were conducted using post-hoc test with Bonferroni-corrected multiple comparison if there was any inequality among the means from each stimulus durations.

## RESULTS AND DISCUSSION

The cVEMP recordings were analysed as a function of stimulus duration among normal adults. The first and the second recording of cVEMP were not statistically significant among the two ears. ($p > 0.05$) , therefore, the cVEMP recordings from both ears were pooled for better statistical analysis.

### COMPARISONS BETWEEN STIMULUS DURATION

The P1 and N1 Latency The mean latencies of P1 and N1 as a function of PT are shown in Figure 1 and Figure 2 respectively. A one-way ANOVA was computed between the P1 latency of four PT and the results showed a significant effect of PT [$F(3,236) = 9.761$, $p < 0.01$]. The Bonferroni multiple comparison revealed that P1 latency of 10 ms PT (19.20 ms ± 5.36 ms) was significantly longer than P1 latency of 0 ms PT (16.60 ms ± 3.14 ms, $p < 0.01$); 2 ms PT (15.33 ms ± 2.96 ms, $p < 0.01$); and 4 ms (16.90 ms ± 4.06 ms, $p = 0.01$). For N1 latency, one-way ANOVA revealed significant difference among the four PT [$F(3,236) = 7.134$, $p < 0.01$]. The Bonferroni multiple comparison revealed a significantly shorter duration for 0 ms (23.75 ± 3.99 ms, $p < 0.01$) and 2 ms (23.00 ± 3.70 ms, $p < 0.01$) compared to 10 ms (26.50 ± 5.32 ms).

This study found that there was a significant delay in P1 and N1 latency when the PT increased from 2 ms to 10 ms ($p < 0.05$) and also from 4 ms to 10 ms ($p < 0.05$) and there was no significant difference among other PTs (0 ms, 2 ms and 4 ms). Based on the results obtained from the present study, it was evident that 0 ms PT yielded earlier P1 and N1 latency compared to 10 ms. These findings suggest that PT does influence P1 and N1 latency in a significant manner.

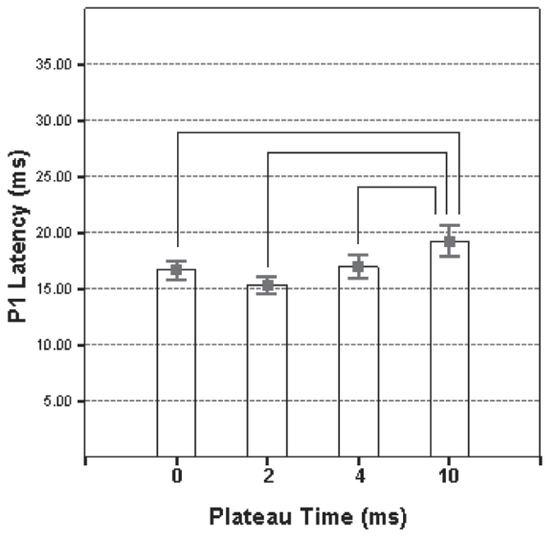


FIGURE 1. The mean of P1 latency as a function of PT. The error bars represent 95% confidence intervals

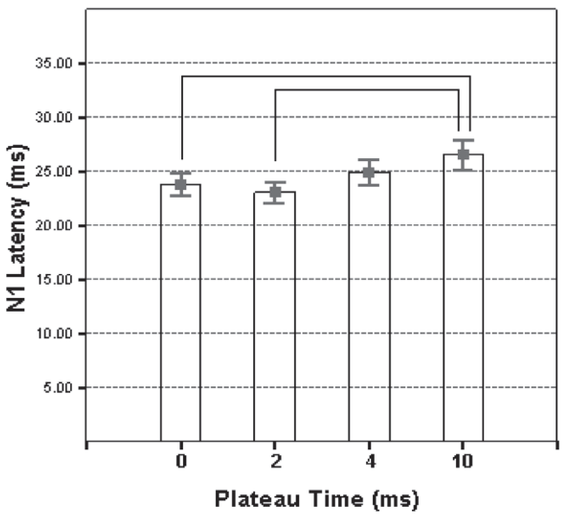


FIGURE 2. The mean of N1 latency as a function of PT. The error bars represent 95% confidence intervals

One possible explanation for this result is that the vestibular neurons needed shorter time to generate synchronous neural stimulation as reported by British Society of Audiology (BSA 2012). Although vestibular nerve is frequency-specific (McCue & Guinan 1994), the nerve still requires neural synchrony to produce stronger action potential (Janky & Shepard 2009). The delayed P1 and N1 latency in the present study could possibly be due to lack of neural synchrony at the vestibular nerve as the PT increased (Singh et al. 2014). Other plausible explanation was that the synaptic potentials last less than 10 ms (Victor & Robert 1999). This could explain the delayed P1 and N1 as the synapse would occur much later for PT of 10 ms or more. Moreover, increase in PT has an effect on the refractory period of vestibular nerve (Avissar et al. 2013). During refractory period, the firing rate would become slower due to shift in phase between each stimulus cycles as the nerve recovers from the continuous firing (Avissar et al. 2013). Besides, the spike trains is enhanced when refractory period is shorter than stimulus period. Thus, when the PT is longer than certain refractory period of the vestibular nerve, the firing rate becomes poorer and results in delayed P1 and N1 latency.

The P2 Latency One way ANOVA between P2 latency of four PTs (0 ms, 2 ms, 4 ms, and 10 ms) revealed no significant difference among the PT ($F(3,236) = 3.285$, $p > 0.05$). The mean of P2 latency as a function of PT is shown in Figure 3. These findings showed that all PTs had no significant difference in P2 latency, which was contrary to P1 and N1 latency. These pattern of results may be attributed to different generator sites for different peaks (Wu et al. 2007). The P1 and N1 latency were derived from the first biphasic waveform occurring at around 10 ms to 25 ms, while P2 was derived from the second biphasic waveform, which occurs later at approximately 34 ms. Watson et al. (1998) observed P2 latency in patients who underwent vestibular neurectomy, but their P1 and N1 latencies were absent. Colebatch et al. (1994) also reported that the P2 latency arises from cochlear pathway rather than the vestibular pathway. However, the exact physiology of cochlear pathway that gives rise to P2 is still undetermined. Therefore, based on the previous studies, it is suggested that P2 response was generated from pathways other than vestibular pathways while P1 and N1 are indeed originated from vestibular organs and its pathways.

The P1-N1 Inter-Amplitude For P1-N1 inter-amplitude, one way ANOVA revealed no significant difference ($F(3,236) = 1.148$, $p > 0.05$) between the four PT. The mean of P1-N1 inter-amplitude are illustrated in Figure 4. The minimum P1-N1 inter-amplitude is 27.97 μV and the maximum is 198.08 μV(Mean = 53.92; SD = 24.43), suggesting large high inter-subject variability among participants. Similar findings were reported by Isaradisaikul et al. (2012) for normal hearing subjects. It was reported that people with normal vestibular function could also have a large asymmetry between ears (Lee et al. 2008).

The P1-N1 inter-amplitudes were similar for 0 ms, 2 ms, and 4 ms and there was a marked decrease in P1-N1 inter-amplitude at 10 ms . Overall, 0 ms produced the largest P1-N1 inter-amplitude, followed by 4 ms, 2 ms, and lastly 10 ms.

This study reported that as the PT increased from 4 ms to 10 ms (fixed rise/fall time of 0 ms) the P1-N1 inter-amplitude deteriorated. Although there was no significant difference between the four stimuli, the morphology of the waveform was severely affected when the PT was 10 ms. It is important to highlight the reduction of P1-N1 inter-amplitude because a successful cVEMP clinical analysis depends on the clarity and robustness of P1-N1 inter-amplitude.

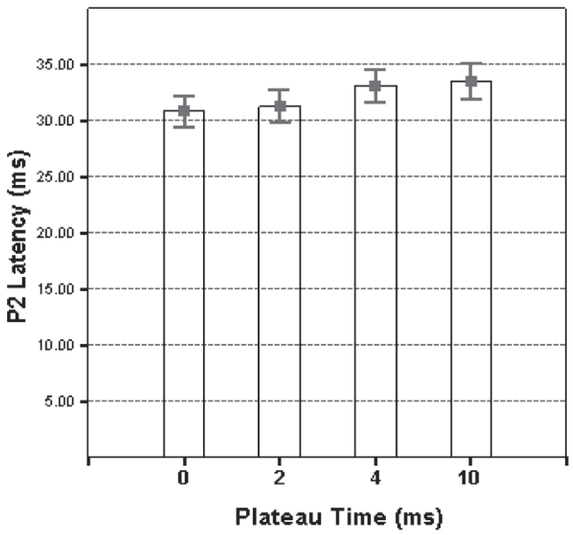


FIGURE 3. The mean of P2 latency as a function of PT. The error bars represent 95% confidence intervals

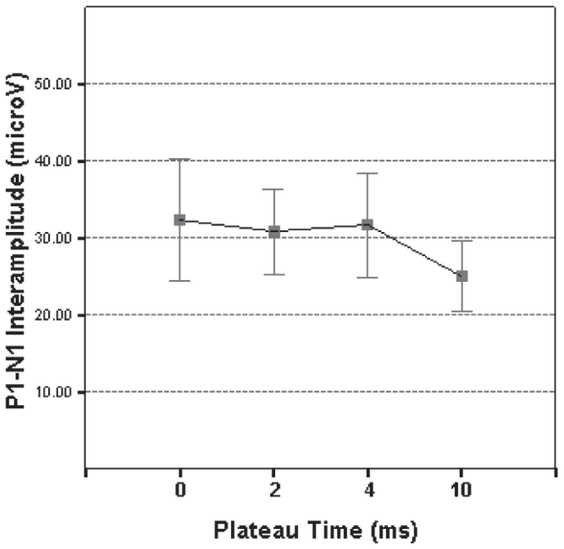


FIGURE 4. The mean of P1-N1 inter-amplitude as a function of PT. The error bars represent 95% confidence intervals

In addition, the increment of PT would contribute to longer time for the tone burst to constantly be at its maximum amplitude (Hall 2007). This means that the longer the PT, the longer the time for the energy being produced by the tone burst. Since all stimuli tested were presented at 95 dBnHL, tone burst with 10 ms PT had the highest energy than the others because the 95dBnHL intensity was maintained for 10 ms. Human ears have special reflex to inhibit loud stimulus from hurting the auditory organ which is the stapedial reflex that stiffens the middle ear system to prevent loud stimulus from injuring the inner ear (Emanuel 2009). In this study, the reduction in energy because of stapedial reflex might cause the P1-N1 inter-amplitude to deteriorate at 10 ms. Due to higher energy distribution at 10 ms, the stapedial reflex becomes stronger and reduces the acoustic energy reaching the saccule (Ochi et al. 2002). The stapedial reflex mainly affects the P1-N1 inter-amplitude since its generation and strength depend on the stimulus intensity. All the participants in this study produced stapedial reflex of below 95 dBHL. Since 95 dBnHL is supra-threshold to their stapedial reflex thresholds, the onset of their stapedial reflex is shorter, which resulted in faster occurrence of stapedial reflex, especially at lower frequencies (Beck & Speidel 2013).

The physiology of vestibular nerve and VCR pathway are yet to be determined specifically due to their complexity. There are still many uncertainties and limited knowledge on the physiology and types of inferior vestibular nerve and vestibular nuclei responding to different stimuli (Victor & Robert 1999). VCR also could be present even when the direct connections between inferior vestibular neurons and SCM motor neurons are interrupted because of lots of disynaptic pathways and undetermined types of neurons contributing to VCR (Wilson 1991; Victor & Robert 1999).

One plausible caveat from this study is that the electromyogram (EMG) activity was not monitored for each subject. Although the EMG was not monitored, it was made sure that the cVEMP waveforms were visually monitored for any insufficient strength of neck contraction. Interestingly, Isaradisaikul et al. (2008) reported that there was no significant difference between the cVEMP waveforms with and without EMG monitoring.

## CONCLUSION

Four different PTs were investigated in normal hearing adults and it was observed that a PT of 0 ms yielded robust cVEMPs. The P1 and N1 latency and P1-N1 inter amplitude are affected when the PT exceeds more than 4 ms. Therefore, the results from this study suggests that a PT of 0 ms or 2 ms should be implemented in cVEMP protocol for optimal cVEMPs recordings.


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