Research Article

Effects of Repetition Rate on Tone Burst Auditory Brainstem Responses in Normal Young Adult Wistar Rats

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Running title: Rate and Tonal Auditory Brainstem Responses in Rat

Abstract

Background and aim: Repetition rate of stimulus as an audiological assessment criterion plays important role in differential diagnosis in some special populations. Therefore, the goal of this study was to evaluate normal variation of the auditory brainstem response (ABR) parameters including latency, amplitude, morphology and component's duration (width) by using two different rates with tonal stimuli at different frequencies in Wistar rats.

Methods: In this experimental study, forty-five young adult male Wistar rats were subjected to ABR measurements with tone burst stimuli in octave frequencies from 2 to 16 kHz with two rates of 11.1 and 57.1/sec, following the relevant protocols. The stimuli were delivered at an intensity of 80 dB SPL and through a speaker.

Results: At high rate, latency changes in later waves were greater than earlier components whereas amplitude changes in later waves were smaller than earlier ones. Rate-dependent changes as a function of frequency were uniform for latency versus amplitude. Morphologically, ABR components were broadened in a frequency-dependent way. Duration of wave I was shorter than

wave IV as well as wave duration changes influenced by frequency. These findings were statistically significant ($p<0.05$).
Conclusion: The results can be likely due to differences in adaptation mechanisms in auditory system, additive synapse theory and, desynchronization by increasing stimulation rate. Knowledge of the various effects of rate as a function of frequency on ABR parameters in normal rats is basic to understanding how different changes of these parameters at each wave could lead to more precise diagnosis in neuro-pathologic conditions.
Keywords: Auditory brainstem response; Repetition rate; Wistar rat; Morphology; Wave duration; Latency

Introduction

Auditory Brainstem Responses (ABRs) are gross evoked potentials which recorded non-invasively from scalp. These early responses elicit within 10-ms after stimulus onset and have several wave components that provide information about synchronous neural activity in auditory nerve, brainstem and midbrain according to their origin (1). Since it's introducing by Jewett and Williston (1971), as objective and easy technique widespread in both clinical and experimental studies, it has been widely using in threshold estimation and oto-neurologic purposes (2, 3).

ABR for given species, depends on a variety of factors including stimulus parameters such as intensity level, duration, polarity, transducer, etc. One of those effective parameters is stimulus repetition rate (4). In previous human studies, stimulus rates up to approximately 20/sec had little effect on ABR in subjects with normal hearing. However, when stimulation rates reached above 40/sec, various effects on ABR parameters has been reported (3). There is general agreement that ABR latency increases as rate increases (3-10), while in few studies, ABR amplitude was resistant to increasing rate (4, 10-12) and there are some reports that as rate increases, amplitude decreases (12, 13). Changes were not same for different wave components (4, 13), as a result, complex shifts occur on wave morphology and make interpretation of these changes and decision about their sources challenging in various populations. Besides, stimulation type that used in those researches was broad band click that could not show the effects of interaction rate and frequency of narrow band tonal stimuli.

Another key point that can be explained is high rate in some pathologic conditions including multiple sclerosis, auditory neuropathy spectrum disorder and diabetic mellitus (8, 14, 15) provide diagnostic efficiency that is not available with more conventional stimulus low rate. Rate-related effects in non-pathologic conditions such as newborns and infants and aged people (15-17) would also be described.

Some properties of ABR are similar among different mammalian species, while other aspects are more species-specific (18). For instance, in human, wave V is dominant and waves I, III and V are the most common components utilized for analysis. Whereas in rat, the largest wave component is wave II known as a reference for identifying other waves, the smallest is wave III; and wave V is not used for diagnostic purposes, generally (2, 19). Furthermore, the generators of ABR components among mammalian species are not similar. In term of the sources of dominant waves in human and rat, the origin of wave V in human is more central and wave II in rat is relatively peripheral (2).

Rats are one of the animal models which are used commonly in auditory function research. Basic research on ABR features in rats is essential to establish precise perception and appropriate methodological framework investigations on several stimulus variables and pathologic conditions affecting ABR characteristics (19). There is the only one comparable testing standardization for

ABR parameters at octave frequencies in different rates with tonal stimuli in rats (13). In our knowledge, the effect of repetition rate on ABR wave's duration has been measured in current study for the first time. Considering wave's duration of ABR in addition to latency, amplitude and morphology parameters provide more complete information about interaction of repetition rate and frequency for accurate interpretation. Therefore, the aim of this study was to evaluate normal variation of the ABR parameters including latency, amplitude, morphology and wave's duration by using two different rates with tonal stimuli at octave frequencies of 2, 4, 8 and 16 kHz in young adult Wistar rats.

Materials & methods

Animals

Forty-five healthy male young adult Wistar rats weighting 200-250 g were purchased from the center of experimental and comparative studies of Iran University of Medical Sciences (Tehran, Iran) and housed with free access to water and food. The animals were maintained at a temperature 22-24°C, 50% humidity and on a 12/12h light/dark cycle. All procedures were approved by the ethic committee of Iran University of Medical Sciences [No. 93-12-20-6113] before experiment and performed its regulations for the use and care of animals in research.

ABRs recording

ABR were recorded for all subjects using Biologic Navigator pro system (Natus, USA). External custom stimuli were used for the auditory presentation. The stimulus consisted of 5-ms tone bursts at frequencies of 2, 4, 8 and 16 kHz in the WAV format. ABR recordings were performed in a sound-attenuating, electrically shielded booth. Before experiments, anesthesia was induced with a combination of ketamine (80 mg/kg) and xylazine (5 mg/kg) intraperitoneally. Normal body temperature was maintained with a non-electrical heating pad during electrophysiological measurement. Three subcutaneous needle electrodes were placed at vertex (non-inverting), under the right (inverting) and the left (ground) ears (2, 20, 21). Stimuli delivered by loudspeaker located 5 centimeters from the right ear. Prior to experiments, the output of transducer was measured in SPL at all frequencies with sound level meter (Bruel & Kjaer 2250, Denmark). To insure normal hearing sensitivity, threshold estimates were performed at four test frequencies using wave II tracking of the ABR to minimum intensity within the normal range at each frequency where a repeatable response was recorded. During diagnostic ABR recordings, calibrated stimuli were presented by using two different repetition rates at 11.1 and 57.1/sec at 80 dB SPL. The evoked potentials were sampled at 256 points in a 10.66 ms epoch time, amplified by a factor of 100000, band pass filter 100 to 3000 Hz, alternating polarity and averaged 1000 waveforms (13). At each stimulus setting, two recordings were obtained and stored for offline analysis. Latency of the ABR was measured in ms as the time between the stimulus onset and positive peak and the amplitude in µV was defined as the peak to following trough (22-24). Absolute latency and amplitude of five early components (wave I through wave V) acquired. We observed wave morphology visually as well as measured component duration in ms based on distance between initial and end points of waves I, II and IV (4).

Statistical analysis

Mean and standard deviation (SD) were obtained for all data. An analysis of variance (one way ANOVA) test was performed to compare latency, amplitude and duration of wave components at

four tested frequencies on each wave at two different rates and final post hoc analysis employed Scheffe test. $P<0.05$ was statistically considered significant.

Results

Morphology of ABR's waves

In young adult Wistar rats, the ABR consists of four to five successive waves typically (2, 19). At low rate in the current study, those waves peaked distinctly except later ones which formed IV-V complex especially at higher frequencies. Wave III in some cases lied on down-slope shoulder of wave II. Its occurrence depended on frequency and was more common at lower frequency. Extra peak between waves I and II and bifid wave II found in a few subjects regardless of rate.

By increasing rate, beside latencies prolongation and reduction in amplitudes, all waves broadened and peaks and troughs sharpness of ABR components reduced. The strongest wave was wave II and the weakest component was wave III against fast rate. Wave identification became more difficult especially from wave III afterwards, because morphologic changes in later components were more prominent. When wave III at low rate was not a distinct peak, disappeared at high rate. In complex IV-V, detecting each of waves was difficult, due to broadness of components, added shoulder on up-slope of wave IV and multi peaked or rounded without clear peak at wave V.

Interaction of rate and peak identification among later waves were challenging morphologically at 16, 8 and 4 kHz, respectively. This difficulty reduced at 2 kHz, because peaks of later components were clearer, as a result waves IV and V were labeled easier. In contrast, low frequency affected earlier waves. In general, the effects of rate were frequency-dependent. Fast rate influenced morphology of wave III, V, IV and I, respectively. Extra peak between waves I and II and bifid wave II were also revealed like low rate. Fig. 1 presents waveforms recorded for one subject at two rates.

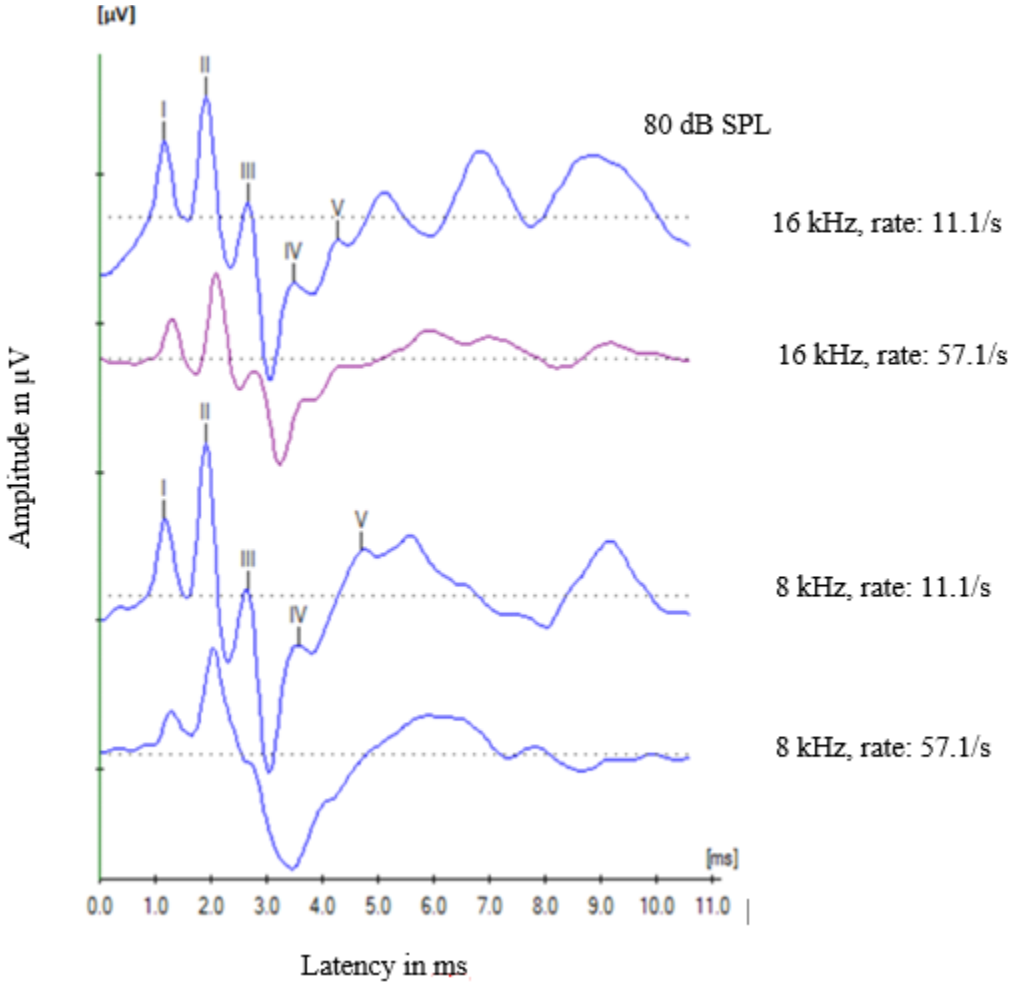


Fig. 1. Representative of waveforms recorded for one Wistar rat.

Latencies of ABR's waves

Latency data at constant supra-threshold intensity level 80 dB SPL. in healthy male young adult Wistar rats showed in Table 1. separated by test frequency, two repetition rates (11.1/sec as low rate and 57.1/sec as high rate) and number of analyzable samples. These findings indicate that as stimulation rate was increased, wave absolute latency became prolonged. Fig. 2 suggests that this occurred for each ABR wave. However, the rate-related effect on wave I through wave V was statistically significant ($P<0.05$). This finding was clearer in later waves; latency shift for each wave was progressively larger in comparison to preceding wave. Fig. 2 also, presents that there was inverse relationship between latency and frequency at two rates. When frequency decreased, absolute latency increased. Analysis of variance showed mean differences of latency at four tested frequencies were statistically significant at two rates ($P<0.001$). Scheffe post hoc test revealed that absolute latencies at 8 and 16 kHz were significantly shorter than absolute latencies at 2 and 4kHz for all waves at two repetition rates ($P<0.001$). Another finding was that rate effect on ABR latency across four tested frequencies was uniform at each wave (Fig. 2).

Table 1. Mean and standard deviation (SD) for absolute latencies

Frequency	Rate	Latency of ABR waves in ms (Mean±SD)				
Wave		**I(N)**	**II(N)**	**III(N)**	**IV(N)**	**V(N)**
2kHz	11.1	1.34±0.11(40)	2.32±0.18(40)	3.04±0.14(40)	3.96±0.12(40)	4.98±0.22(38)
	57.1	1.56±0.12(40)	2.44±0.20(40)	3.25±0.12(37)	4.12±0.18(40)	5.32±0.26(40)
4kHz	11.1	1.22±0.59(42)	2.02±0.10(45)	2.93±0.11(45)	3.85±0.11(45)	4.87±0.16(45)
	57.1	1.31±0.58(42)	2.15±0.09(45)	3.04±0.13(45)	3.98±0.16(45)	5.20±0.18(43)
8kHz	11.1	1.18±0.06(43)	1.98±0.12(45)	2.87±0.09(45)	3.78±0.12(45)	4.81±0.13(45)
	57.1	1.26±0.07(43)	2.07±0.13(44)	2.98±0.10(45)	3.90±0.10(45)	5.11±0.13(44)
16kHz	11.1	1.14±0.05(42)	1.91±0.13(45)	2.78±0.08(45)	3.69±0.08(45)	4.791±0.12(45)
	57.1	1.22±0.06(42)	2.05±0.14(45)	2.90±0.11(38)	3.87±0.11(45)	5.08±0.11(44)

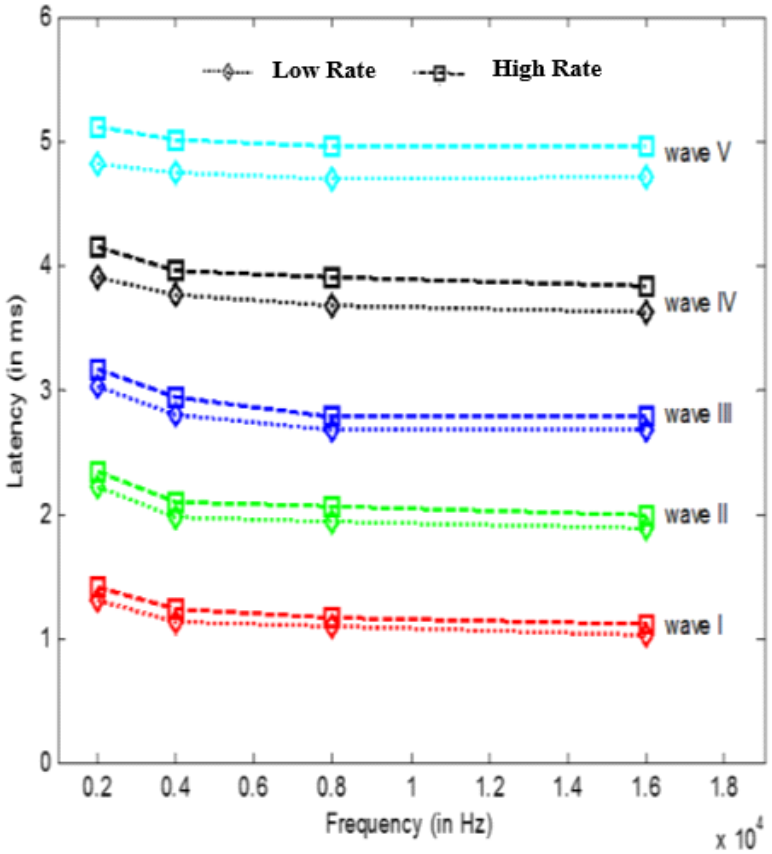


Fig. 2. Line graphs of mean five wave latencies plotted as a function of test frequencies.

Amplitudes of ABR's waves

Table 1 presents the mean amplitudes and standard deviations of all ABR components at four frequencies of low and high rate separately. When stimulation rate increased, amplitude decreased for all waves, magnitude reduction for all components was statistically significant ($P<0.05$). However, the more changes were observed in earlier waves (waves I and II) compared to later

waves. Table 2 suggests that there is direct relation between amplitude and frequency at two rates. As frequency increased, amplitude value grew. Analysis of variance showed that the mean differences of amplitudes across four frequencies were statistically significant at two rates for all components ($P<0.001$). Scheffe post hoc test confirmed that the wave amplitudes at 2 kHz were significantly smaller than 8 and 16 kHz ($P<0.001$). In spite of uniformity in latency changes as function of rate and frequency for all successive waves, variability in amplitude findings was observed. The other data was that, Wave II had the largest amplitude, followed by wave I at two rates (Table 2).

Table 2. Mean and SD values for amplitudes.

Frequency	Rate	Amplitude of ABR waves in μV (Mean±SD)				
Wave		**I**	**II**	**III**	**IV**	**V**
2kHz	11.1	1.86±0.85	2.87±1.18	1.62±0.80	0.75±0.44	0.83±0.70
	57.1	1.15±0.62	2.51±0.96	0.92±0.82	0.32±0.26	0.67±0.42
4kHz	11.1	2.72±1.37	5.31±1.40	2.07±0.74	1.42±1.01	1.41±0.62
	57.1	1.12±0.49	3.37±1.03	1.62±0.72	0.39±0.38	0.79±0.43
8kHz	11.1	3.12±1.48	5.37±1.36	2.90±0.93	1.04±0.72	2.23±0.73
	57.1	1.77±0.91	2.95±0.98	2.43±0.68	0.39±0.25	1.33±0.70
16kHz	11.1	2.97±1.14	5.51±1.22	3.07±0.85	0.86±0.49	2.02±0.70
	57.1	1.94±0.98	3.29±1.07	2.32±0.81	0.44±0.35	1.06±0.50

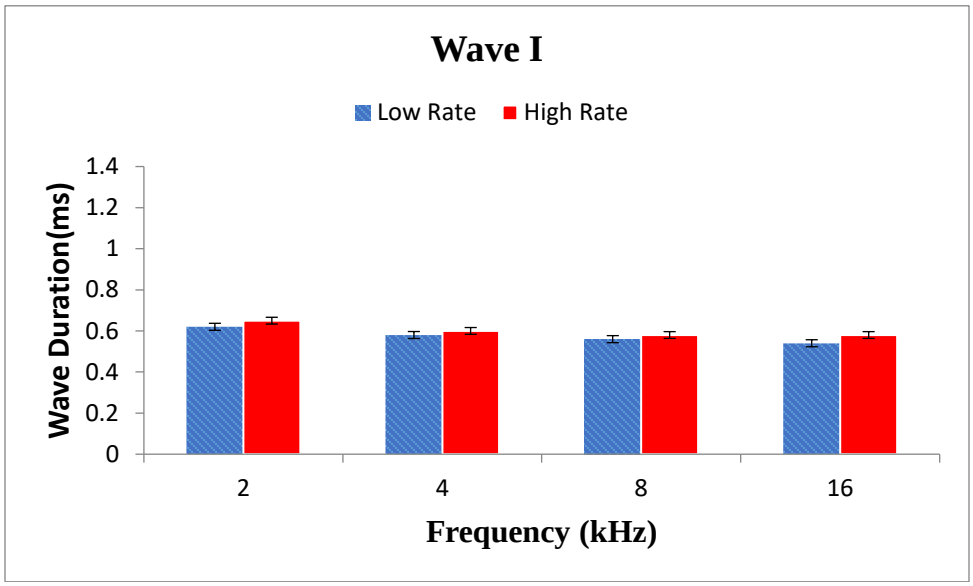
Duration of ABR's waves

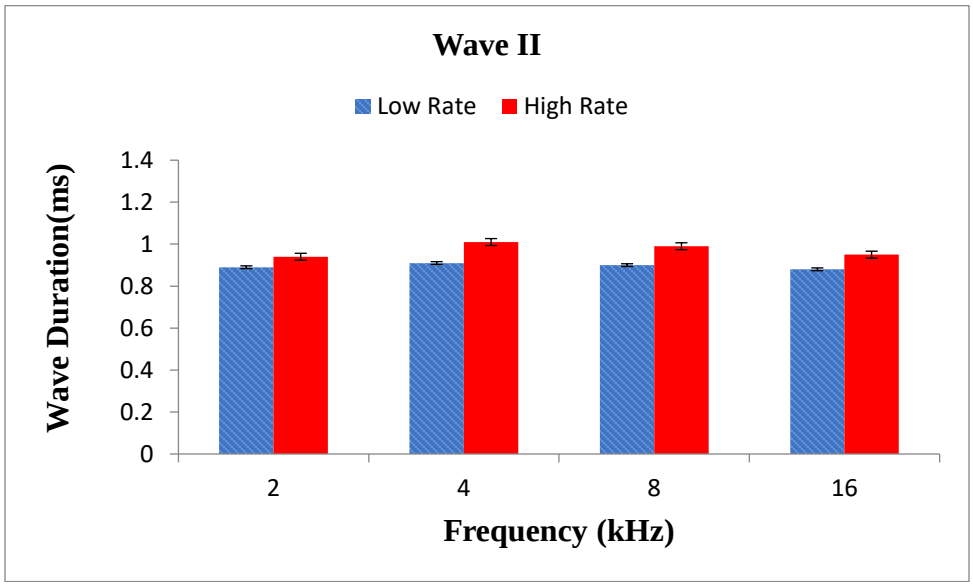
The results obtained from three components (including waves I, II and IV) of ABR wave's duration indicated that, as stimulation rate increased, wave duration of those waves increased. This effect was statistically significant ($P<0.05$).

Fig. 3 displays that interaction value between rate and wave duration was depended on component and frequency. At low and high rates, mean discrepancy of wave I's duration was smaller than wave II and IV, respectively. It means that from wave I to wave IV, waves prolonged progressively in both rates and the effect of increasing rate on wave IV was more than preceding waves.

When comparing the effect of rate on wave I's duration as a function of frequency (Fig. 3), we observed its duration at lower frequencies were longer than higher frequencies, progression of that finding across four frequencies was uniform. In case of wave II, duration of this component at frequencies of 8 and 4 kHz was greater than the other frequencies. Finally, wave IV's duration of higher frequencies were longer than lower frequencies. Those results were unlike wave I.

Analysis variance showed that mean differences of wave I's duration in both rates for all frequencies was statistically significant ($P<0.001$). Scheffe post hoc analysis revealed that wave's duration at 16 kHz was smaller than 2 kHz. However, significant differences obtained at high rate ($P<0.05$) for waves II and IV. Scheffe post hoc analysis confirmed that duration at 16 kHz was different from 4 and 2 kHz for wave II. That discrepancy at wave IV was between 16 and 4 kHz.


Wave I
Low Rate
High Rate
Wave Duration(ms)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
8
16
Frequency (kHz)


Wave II
Low Rate
High Rate
Wave Duration(ms)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
8
16
Frequency (kHz)

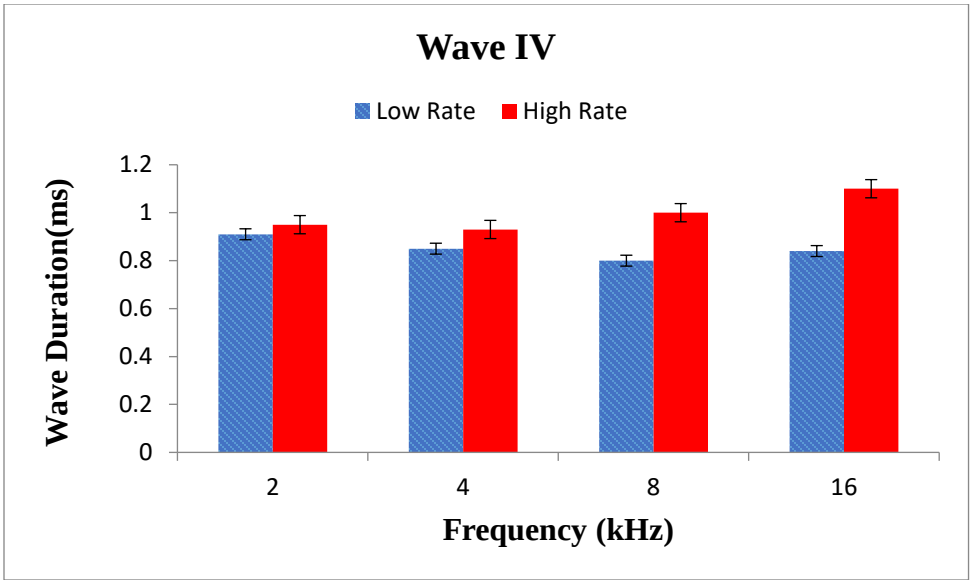


Fig. 3. Mean and standard errors of ABR' wave durations.

Discussion

The purpose of this study was to compare the repetition rate effect on ABR parameters including, latency, amplitude, wave morphology and component's duration in healthy male young adult Wistar rats. Repetition rate is as an important audiological assessment criterion for differential diagnosis in some pathologic conditions (4).

Our study at two rates demonstrated that as rate increased, components latency prolonged progressively through wave I to wave V. The magnitude of those changes was approximately 0.1 ms at wave I and 0.25 ms at wave IV. Also, absolute latency at high frequency was shorter than low frequency. The effect of high rate in interaction with different tested frequencies was uniform. At high rate, ABR amplitude decreased for all waves. In contrast to latency, by increasing rate, amplitude changes on later waves were smaller than earlier components and there was variability in results. Rate-dependent changes as a function of frequency were not same for amplitude versus latency.

Morphologically, by rate enhancement, ABR components were broadened. More changes observed in later waves causing identification those waves difficult. These findings were frequency-dependent. At lower frequency the effect of high rate on earlier waves (waves I and II) was more observed but, higher frequency involved later waves more than earlier waves. Multiphasic earlier waves were seen in both rates.

The final result that has not been reported in previous studies, was wave's duration. By increasing rate, duration of wave I was shorter than wave IV. Wave duration changes influenced by frequency. Wave's duration at high frequency was shorter than low frequency and that effect from high to low frequency was progressive and regular. Whereas at wave IV, between wave duration and frequency in compared to wave I was opposite.

Neurophysiologic mechanisms underlying the effects of rate on ABR latency can be explained based on cumulative sensory and neural adaptation, fatigue and incomplete recovery, involving hair cell-nerve junctions and subsequent synaptic transmission (4). Adaptation defined as a change in responsiveness of sensory and neural systems over-time facing sufficient stimuli (25). When stimulation number per time unit (repetition rate) increases, auditory system stress enhances (4).

Because Hair cells are overstimulated, due to more deflection of the stereocilia and in ascending auditory pathways, responses during time decline (12, 25). Neural responses decrease because of refractory period or reduction of neurotransmitter storage. It means that in a particular nucleous, smaller number of ascending fibers activates, therefore, excitatory post synaptic potential time for reaching to firing threshold become longer (7). Moreover, time course of neural fraction is very short and it is probable that high rate stimulus falls in refractory period of neural populations and leads to desynchronization (26). These changes appear to be more pronounced at the origins of the ABR's later waves.

By increasing rate, latency of each wave progressively delayed compared to preceding wave (accumulative effect) (7). To explain this finding, different adaptation mechanisms in auditory system and additive synapses theory can be stated (4, 7). More peripheral responses of brainstem show less adaptation rather than more central. The other assumption is based on the origin of ABR different components and the number of synapses. In rats, because of shorter length of auditory nerve (AN), it is the only origin of wave I and anatomical source of wave II is cochlear nucleus (CN). Wave III is generated by superior olivary complex (SOC). The sources of wave IV are lateral lemniscus (LL) and inferior colliculus (IC), and wave V is generated by medial geniculate body (MGB) and/or thalamo-cortical radiations (27). Although, there is no definitive data about the sources of ABR components in precise detail, considering multiple sources of the ABR in rats, additive synapses theory seems logical for the results of this study especially in later ABR peaks which are generated by more rostral regions of the brain, where number of synapses traversed increases with increasing ABR peak (4).

In this study, high rate produced uniformed latency increasing for all tested frequencies. Rate-latency functions showed for four frequencies at each wave in Fig. 2. This result indicated that rate effects on latency were not discriminated by spectral content of stimulus. That suggests at least some neural features in a relay station of ascending auditory pathway show similar conduction characteristics regardless of anatomical position (13). All of results that were stated about the effects of rate and frequency were consistent with the only related research in a given species by Newton et al. (13), in relation between rate and latency, significant difference was reported for waves IV and V. This variability can be due to different strain (Sprague-Dowley) (28) or a cutoff for upper limit of normal values that may produce shift in latency. Beside intensity level was lower than our study and different frequencies and repetition rates were used. Interaction these stimulus parameters with rate may affect the results.

We showed smaller amplitude reduction of later waves compared to earlier ones by increasing rate. It can be explained with small dynamic range of brainstem components. When stimulus intensity level increases, the amplitude of later waves reaches to plateau (saturates sooner) (7). In the current study, we utilized high intensity level (80 dB SPL). It is known that in each successive station of ascending auditory pathway, the number of neurons increases. Therefore, small number of lower order firing fibers activates greater number of higher order neurons and each higher order neuron is activated by terminals of many lower order neurons (divergence versus convergence). Hence, higher order neurons in intermediate stations are activated maximally. When stimulation rate increases, the amplitude of wave I decreases due to refractoriness, but fiber number reaching to next station may be still adequate for producing excitation. This can explain smaller reduction of response amplitude in higher order brainstem nuclei. In the other word, lower order neural fibers require sufficient excitation for clear response, whereas lesser stimulation can be adequate for higher order neuron firing (4, 7).

Amplitude and synchrony of neural discharge change in interaction with frequency and predominancy of neuron type, easily. ABR amplitude is susceptible to the fluctuation in background EEG activity. Remaining noise during recording results its variability versus latency (4). To account the inherent amplitude variability, many clinicians use amplitude ratio instead of absolute amplitude. But, the purpose of this study was to report normative data in detail as a reference for future studies. This study was in agreement with previous study (13). They reported only the amplitude of waves I and IV.

Morphologically, at high rate, ABR waves were broadened and the sharpness of peaks and troughs decreased. Possible explanation for this finding may be represented synchronization reduction. Synchronized depolarization reduces from CN toward higher nuclei. Also, peaks and troughs in the ABR are reflections of compound afferent (and presumably efferent) activity from axonal pathways and somatodentric potentials in major neuron groups respectively. Our results showed that rate enhancement involved both potentials in the auditory brainstem (4). Morphologic discrepancy of ABR components in interaction with different rate and frequency suggested that there are various neuron types and discharge patterns along tonotopic portions of bottom-up auditory pathways that have been confirmed in neurophysiologic research (13).

Extra peak between waves I and II and bifid wave II regardless of rate were observed. Extra peak may be related to electrode array (vertex to ipsilateral mastoid), especially in the early portion of ABR waveforms (4), in other words vertical electrode array can effect on the morphology of the earlier waves. Another reason may be auditory neural fibers with broad (tail) and sharp (tip) tuning curves. Earlier peak is derived by activation of broad band portion and longer peak representative of tip tuning curve activation (28). There are some explanations for bifid wave II. Bifid wave II defined as two closely spaced peaks with latency differences less than 0.5 ms. Factors contribute to record this wave include high stimulus intensity level, mastoid or earlobe electrode and possibly stimulus polarity. In this study, we utilized alternating polarity to remove stimulus artifact and cochlear microphonic (CM), in which this polarity helps to the averaged response. A shorter peak may be produced by one polarity (mostly rarefaction portion) while the second peak is generated by the opposite polarity (condensation) (4). In Newton's study (13), the broadening of all components and multiphasic wave I in relation to frequency were reported. Bifid wave II was not pointed out. The possible reason for this difference can be lower intensity level, different polarity or electrode array (vertex (noninverting), chin (inverting) and base of the tail (ground).

Our results showed, by increasing rate, duration of wave I was shorter than wave IV as well as wave duration changes influenced by frequency. In response to acoustic stimulus, travelling wave forms along cochlear partition. Its velocity is different corresponding to frequency. In the basal turn of cochlea, the velocity of travelling wave is considerably faster than in the apical turn (25). In the apical region, travelling wave velocity is not adequate to produce synchronous firing of AN fibers (4). It seems that there is inverse relationship between wave duration (width) and synchronization value at least at wave I that is generated by homogenous discharge patterns of afferent fiber (4). High frequencies are analyzed in basal region of cochlear duct and low frequencies in apical region. Therefore, the velocity of high frequency travelling wave is faster than low frequency. According to tested frequencies in the current study, the location of 16 kHz in cochlear duct is closer to basal turn rather than the rest of frequencies, 8, 4 and 2 kHz, respectively. Hence, the velocity of travelling wave from 16 to 2 kHz reduces progressively. As a result, synchronization value toward low frequencies decreases leading to prolongation in wave width from high to low (Fig. 3). We observed that low frequencies had shorter wave IV duration. At the CN that is the origin of wave II in rat and above, the responses of each cell are determined

not only by the number and patterns of its projections, but also by its intrinsic properties within and between nuclei (13). Beside existence of tonotopic mapping in main neural generators in ascending auditory system, it seems that frequency tuning from cochlea to cortex shifts from high to low frequencies (25). Therefore, spectral energy in higher order auditory system changes toward low frequency. These properties could explain direct relationship between frequency and wave IV' width. The reason of selection three waves was that those components are the most common components in rats which have diagnostic utility and the other important explanation was that waves III and V affected at high rate more and duration calculation of two waves was difficult.
In general, important point based on previous studies is that, two major spectral components form the ABR (29, 30). Spectral energy of slow component is frequencies of 100 Hz and below. Fast component include definitive waves with energy at frequencies in the regions of 500 and 900 Hz. On the other hand, later components trend to low frequency and earlier waves lie upon high frequency (4). There is a physiologically based distinction in the effects of stimulus rate, intensity and frequency on these fast versus slow ABR components. It appears among ABR parameters that investigated in this study, wave's latency and possibly duration provides perception of these basic properties of ABR at least about the effects of rate as a function of frequency.

Limitations

One of the limitations of this study was not possible to record higher frequencies including 32 kHz according to the hearing frequency range of rat (0.25-80 kHz) (28). Also, due to the increased speaker artifact at higher repetition rates, it was not possible to use higher rates and in order to the effect of gender, it was only performed on young male Wistar rats. Not generalizing to human samples is appropriate for the purpose of this study.

Conclusions

In this study, different behaviors of ABR parameters were observed by enhancing rate. Overall divergence of rate effects on latency and amplitude lead to complex variation in component morphology. Considering the interaction of rate and frequency, uniform changes were observed in latency-frequency function in both rates at each ABR components and amplitude values showed variability. Therefore, wave morphology and particularly wave's duration (width) appear valuable criteria for associated assessment the effects of rate and frequency. It seems that frequency-specific stimuli as a function of stimulus rate can provide more sensitive approach of latency, amplitude, wave morphology and especially wave duration of ABR components. These diagnostic potential can be used for more precise determination of dysfunction source.

Conflict of Interest Statement

No conflict of interest was present.

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Authors' Contributions

FH: Study design, Acquisition of data, Statistical analysis, Interpretation of the results and drafting the manuscript; **AP**: Study design and editing of the manuscript.

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