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Research Article

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

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## ABSTRACT

**Introduction:** The repetition rate of a stimulus serves as a crucial criterion in audiological assessments for differential diagnosis in certain special populations. Therefore, this study aimed to evaluate the normal variation of auditory brainstem response (ABR) parameters, including latency, amplitude, morphology, and component duration (width), using two different rates of tonal stimuli with varying frequencies in Wistar rats.

**Materials and Methods:** In this experimental study, 45 young adult male Wistar rats were subjected to ABR measurements using tone burst stimuli at octave frequencies ranging from 2 to 16 kHz, with two rates of 11.1 and 57.1/s, following the relevant protocols. The stimuli were delivered at an intensity of 80 dB SPL and through a speaker.

**Results:** At a high rate, latency changes in later waves were greater than those in earlier components, whereas amplitude changes in later waves were smaller than those in earlier ones. Rate-dependent changes, as a function of frequency, were uniform for both latency and amplitude. Morphologically, ABR components were broadened in a frequency-dependent way. The duration of wave I was shorter than that of wave IV, and the wave duration changes were influenced by frequency. These findings were statistically significant (P<0.05).

**Conclusion:** The results can be attributed to differences in adaptation mechanisms within the auditory system, the additive synapse theory, and desynchronization resulting from increased stimulation rates. Knowledge of the various effects of rate as a function of frequency on ABR parameters in normal rats is essential to understanding how different changes in these parameters at each wave could lead to a more precise diagnosis in neuro-pathological conditions.

**Keywords:**

Auditory brainstem response; Repetition rate; Wistar rat; Morphology; Wave duration; Latency

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## Introduction

Auditory brainstem responses (ABRs) are gross evoked potentials that are recorded non-invasively from the scalp. These early responses, elicited within 10 ms after stimulus onset, have several wave components that provide information about synchronous neural activity in the auditory nerve (AN), brainstem, and midbrain, according to their origin [1]. Since it was introduced by Jewett and Williston (1971) as an objective and easy technique widely used in both clinical and experimental studies, it has been commonly used in threshold estimation and oto-neurological purposes [2, 3].

ABR for a given species depends on various factors, including stimulus parameters such as intensity level, duration, polarity, and transducer type. One of those effective parameters is stimulus repetition rate [4]. In previous human studies, stimulus rates of up to approximately 20 Hz had little effect on ABR in subjects with normal hearing. However, when stimulation rates reached above 40/s, various impacts on ABR parameters have been reported [3]. There is general agreement that ABR latency increases as the rate increases [3-10].

In contrast, a few studies have found that ABR amplitude is resistant to increasing rate [4, 10-12], while some reports indicate that amplitude decreases as the rate increases [12, 13]. Changes were not uniform across different wave components [4, 13]. As a result, complex shifts occur in wave morphology, making it challenging to interpret these changes and decide about their sources in various populations. Besides, the stimulation type used in those studies was a broadband click, which could not show the effects of interaction rate and frequency of narrow-band tonal stimuli.

Another key point that can be explained is the high rate in some pathologic conditions, including multiple sclerosis, auditory neuropathy spectrum disorder, and diabetic mellitus [8, 14, 15], which provides diagnostic efficiency that is unavailable with a more conventional stimulus low rate. Rate-related effects in non-pathological conditions, such as newborns and infants, and in older 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 humans, wave V is the dominant component, and waves I, III, and V are the most commonly used components for analysis. In rats, the largest wave component is wave II, which serves as a reference for identifying other waves, while the smallest is wave III. Wave V is not typically used for diagnostic purposes [2, 19]. Furthermore, the generators of ABR components differ among mammalian species. In terms of the sources of dominant waves in humans and rats, the origin of wave V in humans is more central, and wave II in rats is relatively peripheral [2].

Rats are one of the animal models commonly used in auditory function research. Basic research on ABR features in rats is essential to establish precise perception and an appropriate methodological framework for investigating various stimulus variables and pathological conditions that affect ABR characteristics [19]. There is only one comparable testing standardization for ABR parameters at octave frequencies in different rates with tonal stimuli in rats [13]. To our knowledge, the effect of repetition rate on the ABR wave's duration has been measured for the first time in the current study. Considering the wave's duration, ABR latency, amplitude, and morphology parameters provide more comprehensive information about the interaction between repetition rate and frequency, facilitating accurate interpretation. Therefore, this study aimed to evaluate the normal variation of ABR parameters, including latency, amplitude, morphology, and wave duration, using two different rates with tonal stimuli at octave frequencies of 2, 4, 8, and 16 kHz in young adult Wistar rats.

## Materials and Methods

### Animals

A total of 45 healthy male young adult Wistar rats, weighing 200-250 g, were purchased from the center for experimental and comparative studies at Iran University of Medical Sciences (Tehran, Iran) and housed with free access to water and food. The animals were maintained at a temperature of 22-24 °C, 50% humidity, and on a 12-hour light/12-hour dark cycle.

### ABRs recording

ABRs were recorded for all subjects using the 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 the vertex (noninverting), under the right (inverting), and the left (ground) ears [2, 20, 21]—stimuli delivered by a loudspeaker located 5 cm from the right ear. Before the experiments, the output of the transducer was measured in SPL at all frequencies using a sound level meter (Bruel & Kjaer, 2250, Denmark). To ensure normal hearing sensitivity, threshold estimates were performed at 4 test frequencies using wave II tracking of the ABR to the minimum intensity within the normal range at each frequency, where a repeatable response was recorded. During diagnostic ABR recordings, calibrated stimuli were presented at two different repetition rates: 11.1 and 57.1 Hz, both at an intensity of 80 dB SPL. The evoked potentials were sampled at 256 points within a 10.66-ms epoch, amplified by a factor of 100000, and filtered with a band-pass filter of 100 to 3000 Hz. The alternating polarity was used, and the 1000 waveforms were averaged [13]. At each stimulus setting, two recordings were obtained and stored for offline analysis. The latency of the ABR was measured in ms as the time between stimulus onset and the positive peak, and the amplitude was defined as the peak-to-following trough [22-24]. Absolute latency and amplitude of 5 early components (wave I through wave V) were acquired. We visually observed wave morphology and measured component duration in milliseconds based on the distance between the initial and endpoint of waves I, II, and IV [4].

### Statistical analysis

Mean±SD were obtained for all data. An analysis of variance (one-way ANOVA) test was performed to compare the latency, amplitude, and duration of wave components at four tested frequencies for each wave at two different rates. The final post hoc analysis employed the Scheffé test. $P<0.05$ was statistically considered significant.

## Results

### Morphology of ABR's waves

In young adult Wistar rats, the ABR consists of 4 to 5 successive waves typically [2, 19]. At the low rate in the current study, those waves peaked distinctly, except for the later ones, which formed an IV-V complex, especially at higher frequencies. Wave III, in some cases, lay on the down-slope shoulder of Wave II. Its occurrence depended on frequency and was more common at a lower frequency. An extra peak between waves I and II, and a bifid wave II, were found in a few subjects, regardless of the rate.

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

The interaction of rate and peak identification among later waves was challenging morphologically at 16, 8, and 4 kHz, respectively. This difficulty reduced at 2 kHz because the peaks of later components were clearer; as a result, waves IV and V were labeled more easily. In contrast, low-frequency waves were affected earlier. In general, the effects of the rate were frequency-dependent. The high rate influenced the morphology of waves III, V, IV, and I, respectively. An extra peak between waves I and II, as well as a bifid wave II, was also revealed, indicating a low rate. Figure 1 presents waveforms recorded for one subject at two rates.

### Latencies of ABR's waves

Latency data at a constant supra-threshold intensity level (80 dB SPL) in healthy young adult male Wistar rats are shown in Table 1. They are separated by test frequency, two repetition rates (11.1/s as the low rate and 57.1/s as the high rate), and the number of analyzable samples. These findings indicate that as the stimulation rate increased, the wave absolute latency became prolonged. Figure 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; the latency shift for each wave was progressively larger compared to the preceding wave. Figure 2 also presents an inverse relationship between latency and frequency at the two rates. As the frequency decreased, the absolute latency increased. Analysis of variance revealed statistically significant mean differences in latency at four tested frequencies at two rates ($P<0.001$). Scheffé post hoc tests revealed that absolute latencies at 8 and 16 kHz were significantly shorter than those at 2 and 4 kHz for all

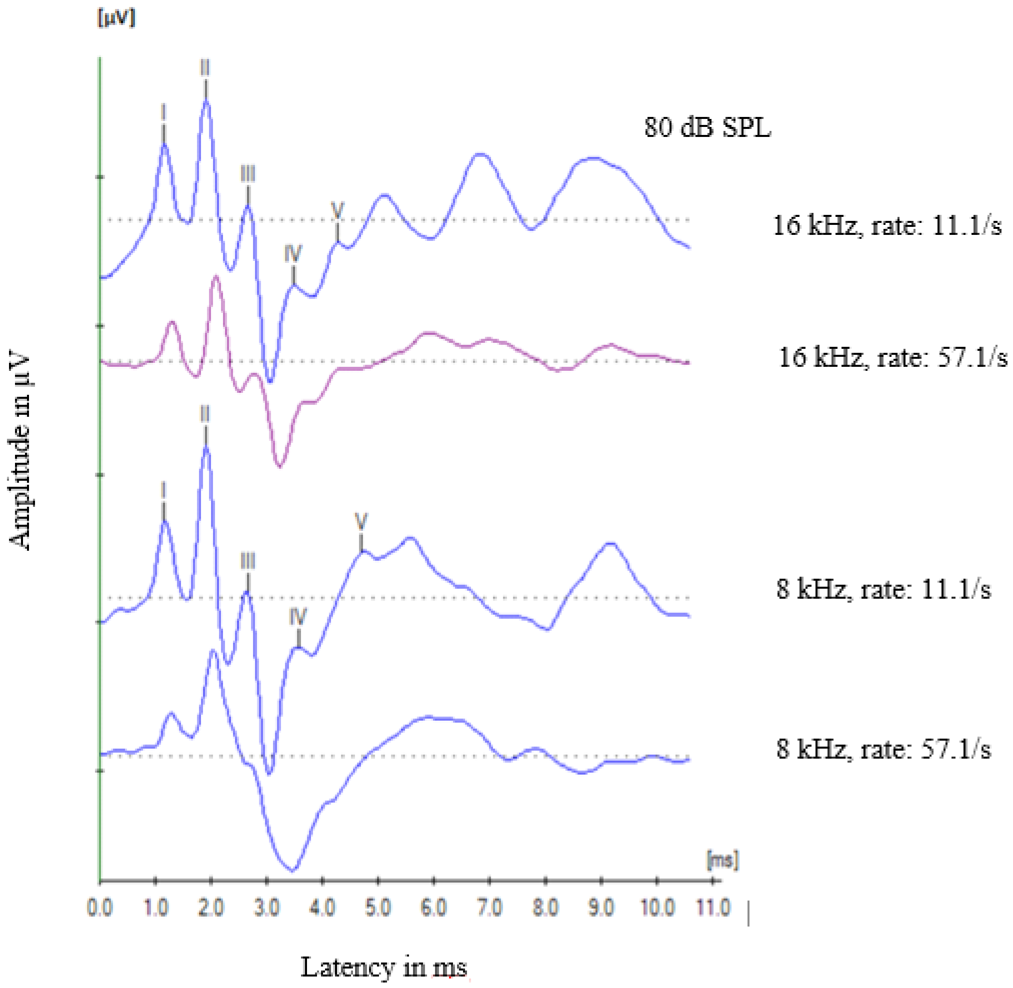


**Figure 1.** Representative of waveforms recorded for one wistar rat

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waves at both repetition rates (P<0.001). Another finding was that the rate effect on ABR latency across four tested frequencies was uniform at each wave (Figure 2).

## Amplitudes of ABR's waves

Table 1 presents the mean amplitudes and standard deviations of all ABR components at four frequencies, both low and high rates, separately. When the stimulation rate increased, the amplitude decreased for all waves, and the magnitude reduction for all components was statistically significant (P<0.05). However, more changes were observed in earlier waves (waves I and II) compared to later waves. Table 2 suggests a direct relationship between amplitude and frequency at two rates. As frequency increased, the amplitude value grew. Analysis of variance revealed that the mean differences in amplitudes across the four frequencies were statistically significant at two rates for all components (P<0.001). The Scheffé post hoc test confirmed that the wave amplitudes at 2 kHz were significantly smaller than those at 8 and 16 kHz (P<0.001). Despite uniformity in latency changes as a function of rate and frequency for all successive waves, variability in amplitude findings was observed. The other data showed that Wave II had the largest amplitude, followed by Wave I at two rates (Table 2).

## Duration of ABR's waves

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

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

**Table 1.** Mean±SD for absolute latencies

| Wave (kHz) | Rate | Mean±SD/No. | | | | |
|---|---|---|---|---|---|---|
| | | Latency of ABR Waves in ms | | | | |
| | | I | II | III | IV | V |
| 2 | 11.1<br>57.1 | 1.34±0.11 (40)<br>1.56±0.12 (40) | 2.32±0.18 (40)<br>2.44±0.20 (40) | 3.04±0.14 (40)<br>3.25±0.12 (37) | 3.96±0.12 (40)<br>4.12±0.18 (40) | 4.98±0.22 (38)<br>5.32±0.26 (40) |
| 4 | 11.1<br>57.1 | 1.22±0.59 (42)<br>1.31±0.58 (42) | 2.02±0.10 (45)<br>2.15±0.09 (45) | 2.93±0.11 (45)<br>3.04±0.13 (45) | 3.85±0.11 (45)<br>3.98±0.16 (45) | 4.87±0.16 (45)<br>5.20±0.18 (43) |
| 8 | 11.1<br>57.1 | 1.18±0.06 (43)<br>1.26±0.07 (43) | 1.98±0.12 (45)<br>2.07±0.13 (44) | 2.87±0.09 (45)<br>2.98±0.10 (45) | 3.78±0.12 (45)<br>3.90±0.10 (45) | 4.81±0.13 (45)<br>5.11±0.13 (44) |
| 16 | 11.1<br>57.1 | 1.14±0.05 (42)<br>1.22±0.06 (42) | 1.91±0.13 (45)<br>2.05±0.14 (45) | 2.78±0.08 (45)<br>2.90±0.11 (38) | 3.69±0.08 (45)<br>3.87±0.11 (45) | 4.791±0.12 (45)<br>5.08±0.11 (44) |

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effect of the increasing rate on wave IV was greater than that of the preceding waves.

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

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

## Discussion

The purpose of this study was to compare the repetition rate effect on ABR parameters, including latency, am-

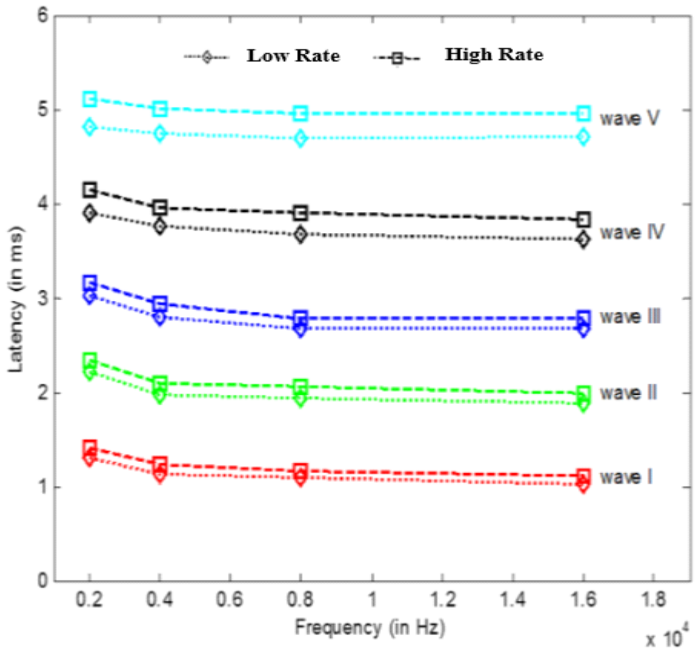


**Figure 2.** Line graphs of mean five wave latencies plotted as a function of test frequencies

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**Table 2.** Mean±SD values for amplitude

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

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plitude, wave morphology, and components' duration in healthy male young adult Wistar rats. Repetition rate is a crucial audiological assessment criterion for differential diagnosis in certain pathological conditions [4].

Our study at two rates demonstrated that as the rate increased, the latency component prolonged progressively from 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. Additionally, the absolute latency at high frequencies was shorter than that at low frequencies. The effect of a high rate in interaction with different tested frequencies was uniform.

At a high rate, ABR amplitude decreased for all waves. In contrast to latency, increasing the rate resulted in smaller amplitude changes on later waves compared to earlier components, and there was variability in the results. Rate-dependent changes as a function of frequency were not the same for amplitude versus latency.

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

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

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 is defined as a change in the responsiveness of sensory and neural systems over time in response to sufficient stimuli [25]. When the stimulation number per time unit (repetition rate) increases, auditory system stress enhances [4]. Because hair cells are overstimulated, due to the increased deflection of the stereocilia and in the ascending auditory pathways, responses decline over time [12, 25]. Neural responses decrease due to the refractory period or a reduction in neurotransmitter storage. It means that in a particular nucleus, a smaller number of ascending fibers activate; therefore, the excitatory postsynaptic potential's time to reach the firing threshold becomes longer [7]. Moreover, the time course of neural fraction is very short, and high-rate stimuli probably fall within the refractory period of neural populations, leading to desynchronization [26]. These changes appear to be more pronounced at the origins of the ABR's later waves.

By increasing the rate, the latency of each wave progressively increases compared to the preceding wave (accumulative effect) [7]. To explain this finding, various adaptation mechanisms in the auditory system and the additive synapses theory can be cited [4, 7]. More peripheral responses of the brainstem show less adaptation rather than more central ones. The other assumption is based on the origin of ABR's different components and the number of synapses. In rats, due to the shorter length of the AN, it is the only origin of wave I, and the ana-

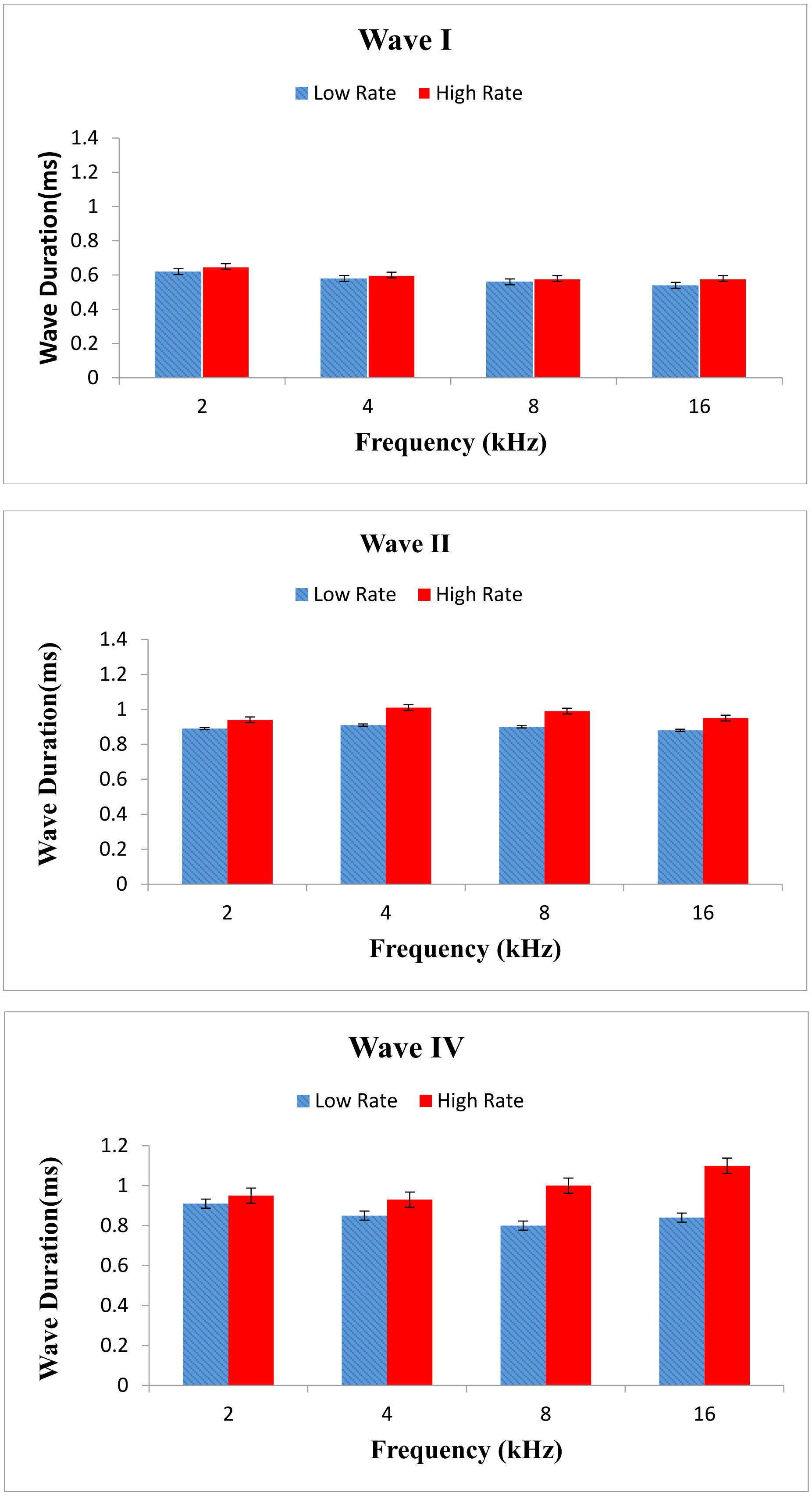


**Figure 3.** Mean±SE of ABR wave durations

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tomical source of wave II is the cochlear nucleus (CN). Wave III is generated by the superior olivary complex (SOC). The sources of wave IV are the lateral lemniscus and inferior colliculus, and the medial geniculate body generates wave V and or thalamocortical 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, the 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 brainstem, where the number of synapses traversed increases with increasing ABR peak [4].

In this study, a high rate produced uniform latency increases for all tested frequencies. Rate-latency functions showed for four frequencies at each wave in Figure 2. This result indicated that the spectral content of the stimulus did not discriminate rate effects on latency. This finding suggests that at least some neural features in a relay station of the ascending auditory pathway exhibit similar conduction characteristics regardless of their 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-Dawley) [28] or a cutoff for upper limit of normal values that may produce shift in latency. Additionally, the intensity level in our study was lower, and different frequencies and repetition rates were employed. Interacting these stimulus parameters with the rate may affect the results.

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

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

Morphologically, at a high rate, ABR waves were broadened, and the sharpness of peaks and troughs decreased. A possible explanation for this finding may be represented by the reduction of synchronization. Synchronized depolarization reduces from CN toward higher nuclei. Additionally, peaks and troughs in the ABR reflect compound afferent (and presumably efferent) activity from axonal pathways and somatodendritic potentials in major neuron groups, respectively. Our results showed that rate enhancement involved both potentials in the auditory brainstem [4]. Morphologic discrepancies in ABR components, in interaction with different rates and frequencies, suggest that various neuron types and discharge patterns exist along tonotopic portions of the bottom-up auditory pathways, as confirmed in neurophysiological research [13].

An extra peak was observed between waves I and II, and bifid wave II occurred regardless of the rate. The extra peak may be related to the electrode array (vertex to ipsilateral mastoid), especially in the early portion of ABR waveforms [4]. In other words, a vertical electrode array can affect the morphology of the earlier waves. Another reason may be auditory neural fibers with broad (tail) and sharp (tip) tuning curves. The earlier peak is derived from the activation of the broad band portion, and the longer peak represents the activation of the tip tuning curve [28]. There are some explanations for bifid wave II. Bifid wave II is defined as two closely spaced peaks with latency differences less than 0.5 ms. Factors contributing to this wave include high stimulus intensity levels, mastoid or earlobe electrodes, and possibly stimulus polarity. In this study, we utilized alternating polarity to remove stimulus artifact and cochlear microphonic (CM), as this polarity helps to enhance 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 was reported. Bifid wave II was not pointed out. The possible reason for this difference may be a lower intensity level, different polarity, or electrode array: Vertex (noninverting), chin (inverting), and base of the tail (ground).

Our results showed that by increasing the rate, the duration of wave I was shorter than that of wave IV, and wave duration changes were influenced by frequency. In response to an acoustic stimulus, a travelling wave forms along the cochlear partition. Its velocity is different, corresponding to frequency. In the basal turn of the cochlea, the velocity of the 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 an inverse relationship between wave duration (width) and synchronization value, at least at wave I, which is generated by homogenous discharge patterns of afferent fiber [4]. High frequencies are analyzed in the basal region of the cochlear duct, and low frequencies in the apical region. Therefore, the velocity of a high-frequency travelling wave is faster than that of a low-frequency wave. According to the tested frequencies in the current study, the location of 16 kHz in the cochlear duct is closer to the basal turn than the rest of the frequencies, 8, 4, and 2 kHz, respectively. Hence, the velocity of the travelling wave from 16 to 2 kHz reduces progressively. As a result, the synchronization value toward low frequencies decreases, leading to a prolongation in wave width from high to low frequencies (Figure 3). We observed that low frequencies had shorter wave IV durations. At the CN, the origin of wave II in rats 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]. Besides the existence of tonotopic mapping in the main neural generators of the ascending auditory system, it appears that frequency tuning shifts from high to low frequencies from the cochlea to the cortex [25]. Therefore, spectral energy in the higher-order auditory system changes toward lower frequencies. These properties could explain the direct relationship between frequency and the width of wave IV. The reason for selecting three waves was that these components are the most common in rats and have diagnostic utility. The other important explanation was that waves III and V affected the high rate more, and the duration calculation of the two waves was difficult.

In general, a key point based on previous studies is that two major spectral components form the ABR. The spectral energy of the slow component is at frequencies of 100 Hz and below. Fast components include definitive waves with energy at frequencies in the regions of 500 and 900 Hz. On the other hand, later components tend to low frequency, and earlier waves lie at 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 that among the ABR parameters investigated in this study, the wave's latency and possibly duration provide a perception of these basic properties of ABR, at least regarding the effects of rate as a function of frequency.

## Conclusions

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

### Study limitations

One of the limitations of this study was that it was not possible to record higher frequencies, including 32 kHz, which falls outside the hearing frequency range of rats (0.25-80 kHz). Additionally, due to the increased speaker artifact at higher repetition rates, it was impossible to use higher rates. Furthermore, considering the effect of gender, the study was only performed on young male Wistar rats. Not generalizing to human samples is appropriate for this study.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran before the experiment and were performed in accordance with the regulations governing the use and care of animals in research.

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## Authors' contributions

Study design, Data collection, Statistical analysis, Interpretation, and drafting the manuscript: Fatemeh Heidari; Study design and editing of the manuscript: Akram Pourbakht.

## Conflict of interest

The authors declared no conflict of interest.

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