

## RESEARCH ARTICLE

# Morphology variations of click-evoked auditory brainstem response with low and high rate stimuli in rat

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### Abstract

**Background and Aim:** The auditory brainstem response (ABR) is one of the most common objective hearing tests conducting in animal and human. The purpose of this study was evaluating the morphology variations of ABR waveforms in rats with low and high rate click stimuli.

**Methods:** First, rats with ABR thresholds higher than 55 dB SPL were excluded and total 81 ears remained in study. Absolute and interpeak latencies of wave I, II, IV were evaluated at low (17.7 Hz) and high rate (88.7 Hz) for click stimuli at 120 dB SPL.

**Results:** At low rate stimuli, ABR waveforms showed different morphologies. The most common complex for waves II to IV was wave III placed on downward slope of wave II (71% of cases). Almost the same morphologies were seen at higher rate; but in some waves, it rounded and decreased amplitude. For waves IV-V, the most common morphology was equal amplitude of wave IV and V in low and high rates (35% vs 56%, respectively). Generally, the high rate stimuli didn't severely change morphology patterns except for later waves.

**Conclusion:** Normal click-evoked ABR could result in different waveforms. Using click stimuli at low and high rate result in different morphology patterns. Recognizing morphology variations of ABR waveforms are essential for detecting any pathological conditions. The high rate stimuli increased latencies, especially for later waves.

**Keywords:** Auditory brainstem response; rat; latency; morphology; rate

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### Introduction

The auditory brainstem response (ABR) is one of the most common tests for objective hearing assessment in human and animals. It is a clinical and noninvasive test. It usually use for auditory threshold estimation or neurologic evaluation. This response originates from auditory nerve and brainstem structure [1,2]. ABR waveforms were assessed in many species including gerbil, mice [3], dog [4] and rat [5,6]. Some of animals such as guinea pig may have more similar auditory system to human, but rat are used as animal model in many studies, for example, ABR responses in rat frequently used for evaluation of different issue such as tinnitus [7], aging

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[8,9] and ototoxicity [10].

The ABR of rat has five major waves. The wave I to V mainly originated from auditory nerve, cochlear nucleus, superior olivary complex, lateral lemniscus, inferior colliculus and medial geniculate body [5]. First time in 2012, the morphologies of ABR waveforms was evaluated for different tone burst stimuli in rats [5]. But as far as we know the morphologies of click-evoked ABR never evaluated in rat in low and high rate stimuli. The tone burst-evoked ABR showed different morphologies for ABR waveforms. Identifying morphological variations in click-evoked ABR is important to distinguish between normal and abnormal waveforms. It was reported that wave II in rat is the most dominant and reliable wave across different frequencies [5,11,12]. It is present even in low stimulus intensities. The wave II is useful for threshold assessment. Wave III and V have small amplitude and usually found in combination with other waves. Therefore they may show different morphologies. The interpeak latencies in rat also could be calculated for I-II, II-IV and I-IV waves.

The latency of ABR waveforms will change in higher rate stimuli. Increasing stimulus rate will increase latencies of ABR waveforms. But the amount of this increase is not clear for click-evoked ABR in rat. Also, latency increase in different waves may change interpeak latencies of ABR waveforms.

The main purpose of this study was evaluation of morphology variations of click-evoked ABR in normal hearing rats with low and high rate stimuli. We also evaluated the latency variations as additional finding.

## Methods

### *Animals*

Animals were male Wistar rats (250 to 300 grams) were kept under standard condition (Temperature: 22-24 °C, humidity: 40-45%, 12 hour lighting cycle). They were purchased and kept in animal lab of Department of Audiology, Iran University of Medical Sciences. They had complete and free access to water and food. We preferred male rats because they are more

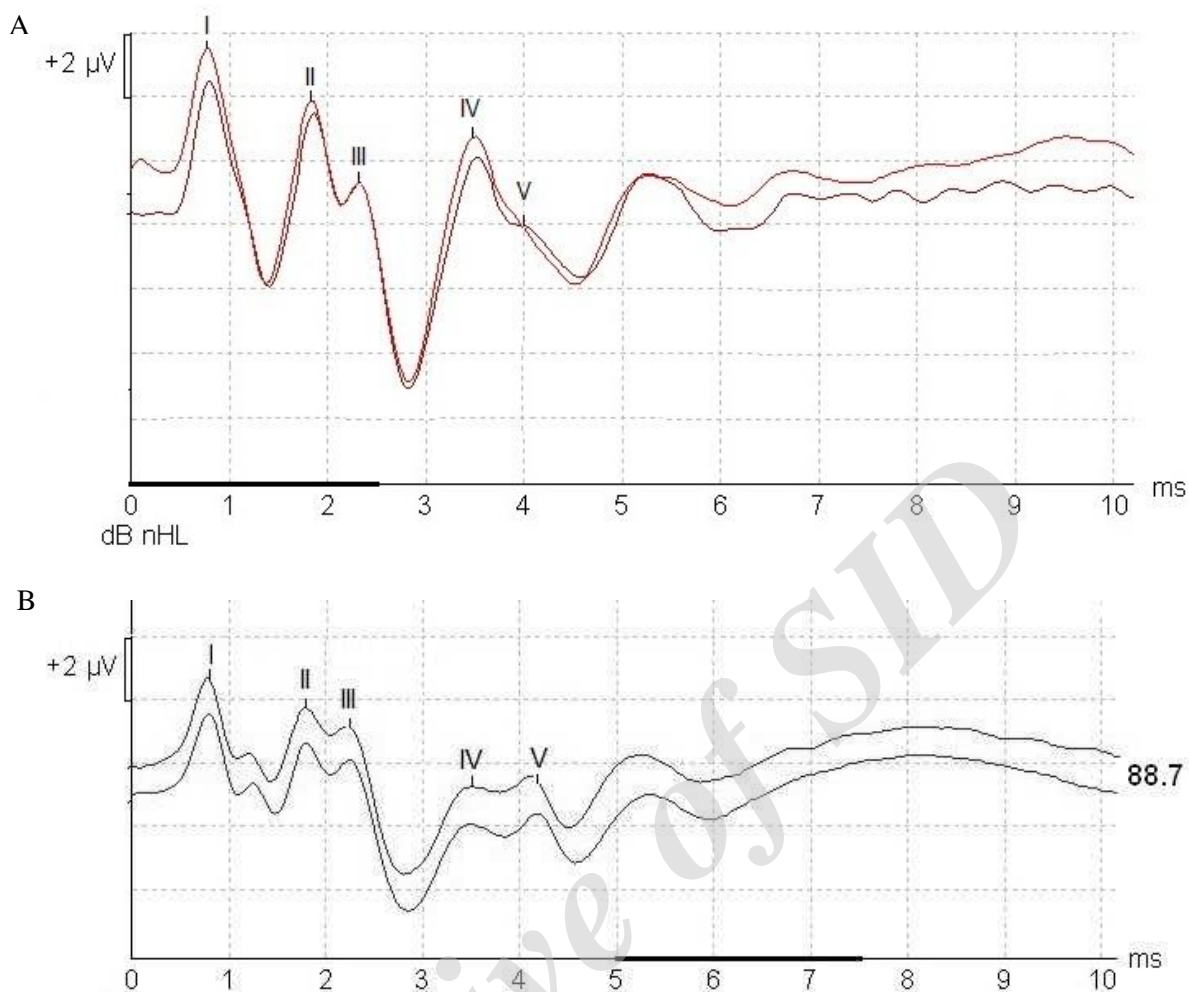
resistant than females. Also, researchers must be cautious about hormonal changes during periods in evaluation of female rats. Study was accepted by Ethics Committee of Iran University of Medical Sciences (Code NO: 930212524757).

### *Auditory brainstem response*

Rats were anesthetized by ketamine (80mg/kg) and xylazine (10 mg/kg) [8]. The body temperature was stable using blanket. Each ear was tested separately by needle electrodes located at the forehead, mastoids, and tail as non-inverting, inverting and ground, respectively. First, the ABR thresholds (Eclipse, EP25, Intracoustic, Denmark) were assessed with click stimuli at rate of 37.7 Hz. The stimulus was sent via insert phone (ER-3A) at 130 dB SPL and decreased to 30 dB SPL in 10 dB steps and 5 dB steps near threshold. Threshold was defined as minimum intensity that could produce repeatable wave II. Up to 2000 stimuli at 100000 x amplification was used for detecting thresholds. Alternate polarity (for optimizing the recording and avoiding any electrical artifact) and 100-3000 Hz filtering were used. The impedance was kept under 5 kΩ and under 2 kΩ between electrodes. The duration of stimulus was the default setting of instrument (0.2 ms). Animals with higher thresholds than normal lab's excluded from study, then with same setting, absolute latencies of wave I, II, IV and interpeak latencies were tested at low (17.7 Hz) and high rate (88.7 Hz) for click stimuli at 120 dB SPL. The intensity was calibrated based on ANSI standards. The 17.7 Hz is low enough for deleting any rate effect on waves. Also, 88.7 Hz was the highest possible rate in our instrument. ABR performed in quiet room with minimum electrical interference.

### *Data analysis*

The analysis performed by SPSS 19. Descriptive analysis included mean and standard deviation for ABR thresholds and absolute and interpeak latencies in low and high rate stimuli. Also, each high rate compared with their low



**Fig. 1. Click-evoked ABR waveforms in rat for A) low and B) high rate stimuli.**

rate associate using pair t-test at p-value level of 0.01.

**Results**

45 rats (90 ears) entered to study and 81 ears had click-evoked ABR thresholds better than the lab’s normal. Fig. 1 shows click-evoked ABR waveforms for low and high rate stimuli. The mean and standard deviation of ABR thresholds for click stimuli were  $46.17 \pm 5.20$  dB SPL. The 74 of 81 ears had thresholds up to 50 dB SPL.

*Morphological variations*

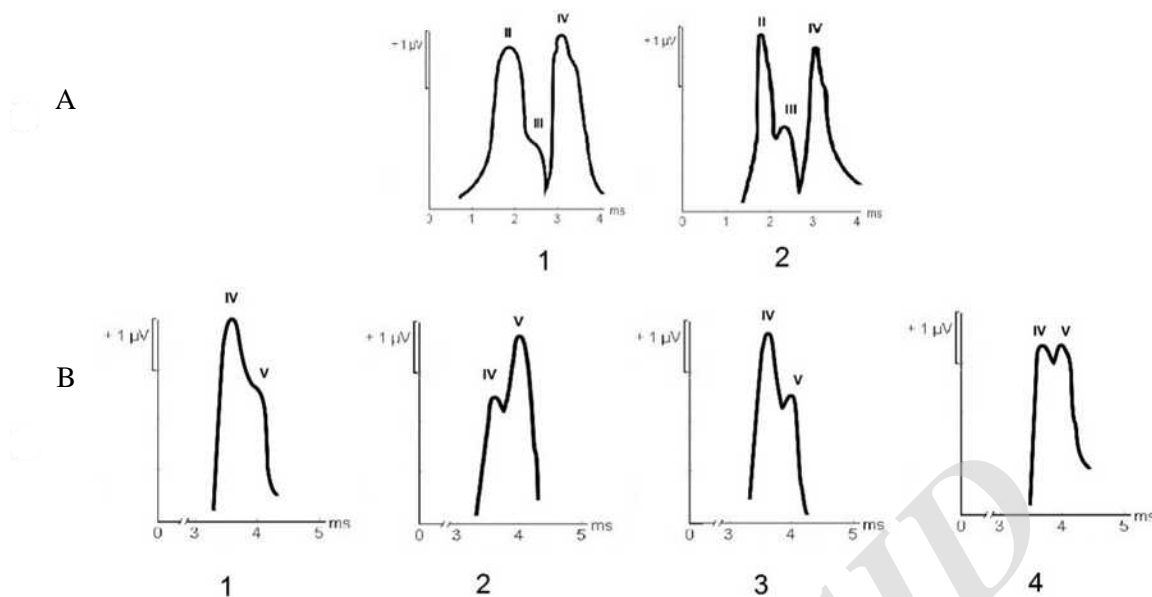
Different morphologies observed for waves II to V, as showed in Fig. 2. In low rate stimuli, about 29 and 71% showed

type 1 and 2, respectively for morphologies of waves II to IV. Generally, the high rate stimuli (88.7 Hz) didn’t severely change morphology of many waveforms (about 28 and 72% showed type 1 and 2 respectively), but it rounded and decreased amplitude of some of waves. (Fig. 2A).

In low rate stimuli, for waves IV-V, the most common morphology was type 4 (35%) followed by 3 (30%) and 2 (30%). type 1 observed in about 5% of cases. In high rate stimuli, 3, 26, 15 and 56% of cases showed type 1, 2, 3 and 4 respectively (Fig. 2B).

*Latency variations*

Table 1 shows the results of absolute latencies and interpeak latencies of waves I, II, IV in rats.



**Fig. 2. Different morphologies for click-evoked ABR for A) waves II to IV and B) waves IV-V in low and high rate stimuli.**

There was no significant difference between latencies of right and left ears ( $p > 0.05$ ). Therefore results of two ears were combined. High rate stimuli changed latencies of all waves but mostly affected later waves.

**Discussion**

In this study, the main purpose was evaluation of morphology variations of click-evoked ABR in normal hearing rats. Numerous types were

observed for wave II to V. wave III to IV usually observed in a complex with other waves. The high rate stimuli did not alter morphology of many waveforms except waves IV-V. Higher number of cases showed type 4 in higher rate stimuli. In Alvarado et al. 'study, as the only published study about morphology in rats [5], the different morphologies for ABR were introduced. However, we did not observe some of previous reported morphologies including

**Table 1. Mean and standard deviation of the absolute and interpeak latencies of ABR wave forms in rats with normal click thresholds at rate of 17.7 and 88.7 Hz (n = 81)**

Rate (Hz)	Absolute latencies (ms)			Interpeak latencies (ms)		
	I	II	IV	I-II	II-IV	I-IV
<b>Right ear</b>						
17.7	0.64 ± 0.08	1.55 ± 0.15	3.50 ± 0.19	0.90 ± 0.09	1.94 ± 0.10	2.85 ± 0.15
88.7	0.74 ± 0.12*	1.77 ± 0.15*	3.81 ± 0.23*	1.02 ± 0.10*	2.04 ± 0.16*	3.06 ± 0.21*
<b>Left ear</b>						
17.7	0.64 ± 0.10	1.55 ± 0.15	3.50 ± 0.18	0.90 ± 0.08	1.94 ± 0.11	2.85 ± 0.14
88.7	0.74 ± 0.13*	1.74 ± 0.13*	3.79 ± 0.27*	1.00 ± 0.10*	2.04 ± 0.18*	3.04 ± 0.24*
<b>Both ears</b>						
17.7	0.64 ± 0.09	1.55 ± 0.15	3.50 ± 0.19	0.90 ± 0.08	1.94 ± 0.11	2.85 ± 0.14
88.7	0.74 ± 0.12*	1.75 ± 0.14*	3.80 ± 0.25*	1.01 ± 0.10*	2.04 ± 0.17*	3.05 ± 0.22*

\*=  $p < 0.01$  when compared to low rate results

types that wave III placed on upward slope of wave IV. This difference may be related to different stimuli used.

In our study, wave II was the most robust waves in ABR as observed in previous studies [5,11,12]. The amplitude of waveforms depends on many factors such as synchronous firing and parallel arrangements of neurons. If more neurons fire synchronously and are parallel and aligned together, the waveforms will be more robust. In rats, the cochlear nucleus generate robust wave II [13] and small wave III mostly originate from superior olivary complex [5]. The amplitude of these wave could be related to structural characteristics of their neural origins. Wave II has high amplitude that could be used for identification of other waves [11,12]. Wave III also was the smallest waves. It was hard to detect it in some cases. For these reasons, interpeak latencies should be determined by using wave II instead of III [5].

As expected, the latency of ABR waveforms increased by higher rate stimuli, especially for later waves. Increased absolute latencies and interpeak latencies of ABR waveforms with higher rate stimuli is similar to human studies [14]. In rat, only increasing rate higher than 40 Hz will cause latency change for later waves and this was similar for all frequencies [15]. For this reason, any rates lower than 40 Hz could be considered low rate stimuli in rats. Higher rate stimuli affect the neural generator of ABR and cause waves with longer latencies. Similar phenomenon is observed in human.

In human, the click-evoked ABR, mainly represent the activity of 1 to 4 kHz region [16], but click stimuli in rat mostly correspond with 8 to 10 kHz frequency in Sprague-Dawley rats by using ASSR [17]. This shows the difference between origins of ABR in rat and human. Amplitude and thresholds of ABR waveforms will change in different frequencies. It was reported that stimulus with lower frequencies has longer latencies [5,6] and even in normal hearing rat, higher frequencies have lower thresholds [5].

The click stimulus is one of the most used stimuli in hearing assessments. Therefore knowing

different morphologies of click-evoked ABR is important. Our limitation in this study was inaccessibility to instrument that could test tone burst stimuli up to 20 kHz. Evaluating different type of stimuli such as click, tone bursts and chirp also could result in more comprehensive view of different morphology and latency variations of ABR waveforms in rat.

## Conclusion

In rat, click-evoked ABR waveforms could have different morphologies. Higher rate stimuli didn't severely altered morphology of waves except for later ones. Knowing these variations could help to better understanding the ABR waveforms in rats. The high rate stimuli increased latencies, especially for later waves.

## Conflict of interest

There was no conflict of interest.

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