Title: Auditory Efferent System Influences the Auditory Overshoot Phenomenon- An Auditory Brainstem Response Study in Guinea Pigs

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Abstract

**Introduction:** Detection of a brief tonal signal at the beginning of a longer masking noise is difficult, but it becomes easier when the onset of signal is delayed. This phenomenon is known as overshoot or temporal effect. Aiming of our study was to investigate the effect of the auditory efferent nerves (AENs) function on the auditory electrophysiological overshoot, further introducing an objective tool examining one of the AENs performances. Therefore, the effect and the trend of changes induced by low and high-frequency stimuli on electrophysiological overshoot in different delay onset time (DOTs) between the signal and the noise before and after dissecting the AENs were studied.

**Methods:** Right internal auditory canals were exposed in 16 young male guinea pigs weighing 250 to 350 g. The inferior and posterior vestibular nerves that are known to carry AENs were transected in half of the subjects. Then, the ABR waveforms were recorded at 16 & 8 kHz tone burst stimuli at 0, 30, 60, 100 ms DOTs relative to wide-band noise. The value of latency of ABR waves I and III were compared among the different DOTs and five signal-to-noise ratios (SNRs) before and after the surgery.

**Results:** By increasing DOTs, the latency of ABR waves I & III decreased in the control group before and after the surgery and the case group before surgery at 16 kHz. However, the observed overshoot-like effect disappeared after the surgery in the case group. The wave's latency I & III increased from 0 to 30 ms, remained approximately constant from 30 to 60 ms, and then began to decrease toward 100 ms DOTs. However, none of the measurements at 8 kHz, before and after surgery at both groups showed an overshoot effect.

**Conclusion(s):** By using the ABR paradigm, the overshoot phenomenon disappeared after the transection of AENs. The results confirmed the role of the efferent system on auditory overshoot. Therefore, an objective tool to measure auditory efferent function is provided.

**Keywords:** Auditory efferent nerve, Auditory brainstem response, Overshoot, Temporal effect, Delay onset time, Latency
Introduction

The caudal auditory efferent system or the olivocochlear nerve bundle is a descending pathway that originates in the brainstem and ends in the organ of Corti of the cochlea. It has been identified more than 70 years ago. (Rasmussen, 1946) A lot of researches conducted on its anatomy and functions thereafter. (Brown, 1987; Liberman, 1980; Warr, Boche, & Neely, 1997; Warr & Guinan, 1979) The anti-masking (Liberman & Guinan, 1998), protection against loud noise (Cody & Johnstone, 1982; Handrock & Zeisberg, 1982; Liberman & Gao, 1995; Rajan, 1990), auditory and visual attention (Guinan, 2018; Igarashi, Alford, Gordon, & Nakai, 1974; Oatman, 1976; Scharf, Magnan, & Chays, 1997), and auditory development are among the roles attributed to this system. (E. J. Walsh, McGee, McFadden, & Liberman, 1998) Despite much research on the characteristics of efferent nerves, many of the functional aspects of this system remain unclear.

Several psychoacoustical studies have been carried out on the effect of perception in noise. In the last theories about the functioning of olivocochlear nerves, a phenomenon called auditory overshoot has been discussed. It used to study the effects of background noise that we encounter in everyday hearing. In fact, the investigation of auditory overshoot may help to understand the ability to hear a brief sound in background noise. It is known as a temporal auditory effect (Bacon, 1990; Hicks & Bacon, 1991; Schmidt & Zwicker, 1991; von Klitzing & Kohlrausch, 1994) that depends on the timing between the onset of the signal and the masker noise, called delay onset time (DOT). When a listener must detect a brief tonal signal presented in a longer masking noise and the onset of the signal is slightly delayed from the onset of the masker, the behavioral detectability of the signal becomes easier. (Chatterjee & Smith, 1993) It was shown that this phenomenon can also be recorded in the auditory brainstem (Chatterjee & Smith, 1993; Haddadzade et al., 2019). We realized overshoot in our previous study that the latencies of ABR's waves, especially the first waves decrease and the amplitude increases when DOT increases. Classic firing rate adaptation and medial olivocochlear feedback (Jennings, Heinz, & Strickland, 2011) are considered proposed physiological mechanisms of overshoot. Some basic characteristics of overshoot are obligatory consequences of cochlear function, as modulated by the olivocochlear efferent system (McFadden, Walsh, Pasanen, & Grenwelge, 2010). For the subjects showing overshoot, detectability remained approximately constant for at least 20-30 ms of signal delay, and then detectability began to improve gradually toward its maximum (McFadden et al., 2010).
It is known that in guinea pigs, the discharge peak of the auditory efferent nerves (AENs) in ipsilateral and contralateral neurons is 20-40 and 25-45 ms, respectively (Brown, 1989). It was compatible with the time course that the auditory overshoot observed. Therefore, we aimed to prove the effect of efferent system on overshoot by extending the previous ABR study on different DOTs within and out of discharge peak of AENs by dissecting the nerves. The cochlear efferent innervation in guinea pigs is carried in the inferior and also superior vestibular nerves, at the point of entry into the medial bulla (Littman, Bobbin, & Cullen, 1991). Therefore, we sectioned both right inferior and superior vestibular nerves at the point of entry into the internal acoustic canal, and then examined ABR by using the low-frequency tone burst in comparison to the high-frequency tone burst at 0, 30, 60, 100 ms DOTs before and after the interruption.

A convenient tool for confirming the section of the AENs is the immunohistochemical staining for acetylcholinesterase (ACHE). Cholinesterase was confined to the spiral ganglion and organ of Corti. The efferent nerves sectioning at the brainstem level in guinea pigs results in significant reduction in cholinesterase-positive structures within the cochlea. Aiming of our study was to investigate the effect of the AENs function on the auditory electrophysiological overshoot, and introducing an objective tool to examine one of the AENs performances.

**Materials and Methods**

**Animals**

Sixteen male albino guinea pigs of two-month-old (250-350 g) were purchased from the Pasteur Institute (Tehran, Iran). The animals were housed in cages with free access to water and food in a temperature-controlled room (20-25°C) with a 12h light/dark cycle. In the present study, a checklist of working with laboratory animals approved by the Ministry of Health was used. Before surgery, the animals were kept for three days for adapting with the new living environment. The guinea pigs were randomly divided into two groups: the first group (cases) with sectioning the AENs and the second group (control) just exposing the nerves (n=8, each). The present study with the ethics code of, IR.IUMS.REC-1396.9311303001, date of, 01/20/2018 was conducted in the Animal Hearing Research Laboratory at the School of Rehabilitation Science of Iran University of Medical Sciences.
Procedure

ABR recording

The animals were anesthetized with a IP injection of the mixture of ketamine (40 mg/kg body weight) and xylazine (4 mg/kg body weight). Ketamin was repeated half dose during surgery, if needed. The ABR was recorded using a Biologic Navigator Pro AEP (USA). The inverting needle electrode was set on the vertex, the non-inverting on the right, and the common on the left mastoid. The impedance difference between electrodes was kept less than 3 kOhm. The animals were placed in a double-walled sound-proofed booth throughout the ABR recording. The body temperature was measured during surgery and testing with a digital thermometer and controlled with a heating pad. The threshold was defined as the minimum level at which the wave III could be repeatedly detected and disappeared at 5 dB less. Subjects with a threshold within ±1 standard deviation of the lab's normal range were included for further data gathering. The threshold was re-measured after the completion of surgical procedures. In case the threshold shifted more than 10 dB, that animal was excluded from the study. To evaluate auditory overshoot, the ABR was recorded using the noise level fixed at 60 dB PeSPL\(^1\) and the signal level modulated for the five signal to noise ratios (SNRs: 0, +5, +10, +15, +20 dB) and the four DOTs (0, 30, 60, 100ms) at 8 & 16 kHz. The stimuli (noise and signal) were combined by Cool Edit Pro (version 2.1) and presented to the right ear at 45° to the head of the animal by the speaker (ONKYO DWASK001, Korea). The other ear was blocked. To control the effect of noise, the waves achieved by noise and signal were subtracted from those with noise alone, then, the absolute latencies of waves I and III for the four DOTs and each SNRs at two different frequencies were determined pre and post-surgery.

Surgery approach

In the case group, AENs were sectioned. But in the control group, the nerves were exposed but kept healthy. A tracheotomy was performed and the animal ventilated with room air. Epinephrine administered to reduce blood pressure at the point of surgery in the skull. A posterior craniotomy was performed. A tinny hole was drilled into the skull at the intraparietal triangle on the right side. Using a small clamp, the bone was removed from the parietal crest to the right temporal line. The dura was opened and the cerebellum retracted medially using small pieces of

\(^1\) dB PeSPL: decibel Peak Equivalent Sound Pressure Level
moistened cotton and para-flocculus was aspirated. Then, the brain stem was retracted medially and the cochlear and vestibular and facial nerves exposed at the medial aspect of the right bulla. By a stapes hook, the inferior and posterior vestibular nerves were transected and the cochlear and facial nerves and artery maintain. Figure (1) schematically illustrates the relative location of the nerves of interest. Animals underwent the surgical procedure up to exposure of the nerves without transection (control). After surgery the dura was closed using small pieces of moistened cotton and the scalp incision sutured. The animal was monitored for three to six hours and allowed to recover. Once awake, pain was controlled with acetaminophen (10mg/kg). Tetracycline was given orally (415mg/l) via drinking water, for 5 to 7 days following surgery.

(Fig 1 here)

**Immunohistochemical Staining**

Twenty days’ post-surgery, the animals were sacrificed. Both bullae removed and fixed with 10% formalin and the cochleae stained for cholinesterase using the envision IHC\(^2\) staining technique. The primary antibody for IHC was Anti-acetylcholinesterase antibody ab31276 (Santa Cruz production). Slides were prepared from the first and second turns of the guinea pig cochlea because of higher density of efferent innervation in this region. (Smith, 1961; Smith & Rasmussen, 1965; Wright & Preston, 1973) The changes in staining after vestibular nerves transection observed via light microscopy.

**Stimuli and recording parameters**

Specific parameters were adopted to record the overshoot electro-physiologically. We applied a 16 and 8 kHz tone burst stimulus and wide-band noise for the signal and noise, respectively. The signals with different DOTs were compared to the noise. The signal (the ABR stimulus) was presented simultaneously with the noise onset and 30 ms and 60 ms after noise onset and also immediately after the completion of the noise. The signal was included of five tone burst complexes with 5 ms duration and 1 ms rise/fall time. For each tone burst at 16 kHz and 8 kHz, 11 ms interstimulus intervals were considered to overcome the minimal nerve response recovery time. The duration of the noise was 100 ms (0.1-8 kHz). The intensity level of the signal and noise was set at 60 dB PeSPL.

\(^2\)IHC: Immuno-Histochemical
The gain to ABR recordings was set as 100000 and a total of 250 stimuli were used for averaging. High-pass and Low-pass filter was 100-3000 Hz and the time window set at 10 ms. The stimuli intensity (signal and noise) was calibrated with a 2250 L sound level meter (B&K) at 5 cm from the speaker. The speaker covered high frequency sound of up to 20 kHz.

**Statistical Analysis**

Statistical analysis was performed in SPSS (version 19.0; USA). All data had a normal distribution \( p > 0.05 \). The statistical significance was tested using global linear models. For each ABR, Tukey one-way ANOVA analysis was conducted to analyze the calculated latency of waves I and III, to the subtracted waves across the factors of time delay (four conditions) and SNR (five conditions) as within subject variables. A pairwise comparison was conducted using Bonferroni analysis of the significant effects. The significance level was 0.05 for all statistical analyses.

**Results**

In our previous study on the electrophysiological overshoot, changes in latency of subtracted ABR waves especially at the first waves showed overshoot phenomenon. Therefore, we measured and compared the latencies of subtracted waves I and III in both groups before and after surgery, for both stimuli separately under the different stimuli conditions.

Since data displayed a normal distribution, we compared the latency waves I & III before and after surgery at both groups and both stimuli by using Tukey one-way ANOVA statistical analysis. There were no significant changes between the control group before and after surgery and the case group before surgery \( p > 0.05 \). There were significant changes after surgery in the case group. Therefore, we reported the variables at the case group for both stimuli, separately before and after surgery.

(Fig 2 here)

**Electrophysiological result (case group)**

**16 kHz stimulus**

Analysis of the data in the case group showed that there were significant changes between the latency waves I and III, separately at 16 kHz before and after surgery \( p < 0.05 \).

Repeated measures analysis for absolute latency of subtracted wave I before and after surgery, separately showed a significant main effect for DOT \[ F (3.0/21.00) = 10.252; p < 0.001; \eta^2 = 0.893 \] and no significant main effect for SNR \[ F (1.889/13.226) = 0.08; p > 0.05; \eta^2 = 0.106 \] before
surgery. There was also a significant interaction effect for DOT and SNR [F (12.00/84.00) = 0.174; p < 0.05; η² = 0.532] before surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave I before surgery showed that a significant changes between the 0 ms and (30, 60) ms DOTs and the (30, 60) ms with 100 ms DOTS respectively for 0, 5 and 10 dB SNRs (p < 0.05). For the other SNRs (15, 20 dB), there were significant changes between the 0 ms and other DOTs (30, 60 and 100 ms) and between the (30, 60) ms and 100 ms respectively (p < 0.05). No significant changes between the 30 ms and 60 ms and also between the 0 ms and 100 ms were observed for all of the SNRs (p > 0.05).

Analysis of data with repeated measures for absolute latency of subtracted wave I before and after surgery, separately showed a significant main effect for DOT [F (3.0/21.00) = 13.491; p < 0.001; η² = 0.927] and no significant main effect for SNR [F (2.353/16.471) = 0.025; p > 0.05; η² = 0.409] after surgery. There was also a significant interaction effect for DOT and SNR [F (12.00/84.00) = 0.302; p < 0.05; η² = 0.477] after surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave I after surgery showed significant changes between the 0 ms and other DOTs for all of the SNRs (p < 0.05) and there were no significant changes between the 30 ms and the (60, 100) ms DOTs separately (p > 0.05)

(Fig 3 here)

Analysis of data with repeated measures for absolute latency of subtracted wave III before and after surgery, separately showed there was a significant main effect for DOT [F (2.186/15.304) = 6.522; p > 0.005; η² = 0.839] and a significant main effect for SNR [F (4.00/28.00) = 0.042; p > 0.05; η² = 0.512] before surgery. There was also a significant interaction effect for DOT and SNR [F (12.00/84.00) = 0.333; p < 0.05; η² = 0.767] before surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave III before surgery showed no significant changes between the 0 ms and three other DOTs and between the 30 ms and 60 ms for the 0, 5, 10 dB SNRs (p > 0.05). There were significant changes between the (30, 60) ms and 100 ms DOTs (p < 0.05). Significant changes between the 0 ms and (30, 60) ms DOT and the (30, 60) ms and 100 ms DOT were noticed (p < 0.05) and also there were no significant changes between the 0 ms and 100 ms DOT for the 15 and 20 dB SNRs (p > 0.05).
Analysis of data with repeated measures for absolute latency of subtracted wave III before and after surgery, separately showed that there was not a significant main effect for DOT [F (2.019/21.00) = 16.484; \( p < 0.001; \eta^2 = 0.918 \)] and not a significant main effect for SNR [F (4.00/24.00) = 0.020; \( p > 0.05; \eta^2 = 0.148 \)] after surgery. There was also a significant interaction effect for DOT and SNR [F (12.00/84.00) = 0.388; \( p < 0.05; \eta^2 = 0.567 \)] after surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave III after surgery showed that for all of the SNRs, there were significant changes between the 0 ms and other DOTs (\( p < 0.05 \)) and no significant changes between the 30 ms and the (60, 100) ms DOTs separately (\( p > 0.05 \))

(Fig 4 here)

8 kHz stimulus

There were significant changes between the latency waves I and III, separately at 8 kHz before and after surgery in the case group (\( p < 0.05 \)). Analysis of data with repeated measures for absolute latency of subtracted wave I before and after surgery, separately showed a significant main effect for DOT [F (3.0/21.00) = 15.895; \( p < 0.001; \eta^2 = 0.827 \)] and a significant main effect for SNR [F (4.00/28.00) = 0.214; \( p < 0.01; \eta^2 = 0.411 \)] before surgery. There was also a significant interaction effect for DOT and SNR [F (12.00/84.00) = 0.764; \( p < 0.05; \eta^2 = 0.419 \)] before surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave I before surgery showed that for all of the SNRs, there were significant changes between the 0 ms and other DOTs (\( p < 0.05 \)). No significant changes between the 30 ms and 60 ms (\( p > 0.05 \)) and also no significant changes between the (30, 60 ms) and 100 ms DOTs respectively (\( p > 0.05 \)) were found.

Analysis of data with repeated measures for absolute latency of subtracted wave I before and after surgery showed a significant main effect for DOT [F (2.255/21.00) = 13.737; \( p < 0.001; \eta^2 = 0.885 \)] and no significant main effect for SNR [F (1.916/28.00) = 0.003; \( p > 0.05; \eta^2 = 0.026 \)] after surgery. There was also a significant interaction effect for DOT and SNR [F (12.00/84.00) = 0.173; \( p < 0.05; \eta^2 = 0.292 \)] after surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave I after surgery showed that for all of the SNRs, there were significant changes between the 0 ms and other DOTs (\( p < 0.05 \)) and no significant changes between the 30 ms and the (60, 100) ms DOTs separately (\( p > 0.05 \)).
Repeated measures analysis for absolute latency of subtracted wave III before and after surgery, separately revealed a significant main effect for DOT \([F(3.00/21.00) = 14.754; p < 0.001; \eta^2 = 0.739]\) and no significant main effect for SNR \([F(2.179/15.250) = 0.011; p > 0.05; \eta^2 = 0.180]\) before surgery. There was also a significant interaction effect for DOT and SNR \([F(12.00/84.00) = 0.229; p < 0.05; \eta^2 = 0.452]\) before surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave III before surgery determined that for the 0 dB SNR, there were no significant changes between the 0 ms and (30, 60 ms) DOTs \((p > 0.05)\) and significant changes between the 0 ms and 100 ms DOTs and between the (30, 60 ms) and 100 ms DOTs \((p < 0.05)\). There were no significant changes between the 0 ms and 30 ms DOTs for 5 dB SNR \((p > 0.05)\). For the other SNRs, there were significant changes between the 0 ms and other DOTs and also between the (30, 60 ms) and 100 ms DOTs \((p < 0.05)\). There were no significant changes between the 30 ms and 60 ms DOT for all of the SNRs \((p > 0.05)\).

Analysis of data with repeated measures for absolute latency of subtracted wave III before and after surgery, separately showed that there was not a significant main effect for DOT \([F(3.00/21.00) = 17.596; p < 0.001; \eta^2 = 0.860]\) and not a significant main effect for SNR \([F(4.00/28.00) = 0.065; p > 0.05; \eta^2 = 0.129]\) after surgery. There was also a significant interaction effect for DOT and SNR \([F(12.00/84.00) = 0.446; p < 0.05; \eta^2 = 0.258]\) after surgery.

Main effect Bonferroni analysis at absolute latency of subtracted wave III after surgery showed that for all of the SNRs, there were significant changes between the 0 ms and other DOTs \((p < 0.05)\) and no significant changes between the 30 ms and the (60, 100) ms DOTs separately \((p > 0.05)\).

**Immunohistochemical staining**

We used immunohistochemical staining for acetylcholinesterase (ACHE) to confirm the interruption of the AENs. Figures 7a and 7b demonstrate the significant decrease in cochlear cholinesterase staining after IVN and SVN transection compared to a normal, control cochlea. Transection of the VN consequently reduced the cholinesterase in the right cochlea of the case.
group. The density of spiral ganglion fibers (arrow) was greatly reduced, although not eliminated. No decrease in cholinesterase staining was detected in the right cochleae of the control group (B). (Fig 7 here)

Discussion

Aiming of our study was to investigate the effect of AENs function on the auditory electrophysiological overshoot, and then to introduce an objective tool for examining one of the AENs performances. Therefore, we studied the effect of low and high-frequency stimuli on electrophysiological overshoot and the trend of ABR changes in different DOTs in a guinea pig model of auditory de-efferentation. The model was developed when the AENs were sectioned at the point of entry into the internal acoustic canal from the cerebellum side. Then the absolute latencies of waves I and III obtained by subtraction of the waveform generated by the noise and signal together from that by noise alone were compared before and after the interruption of the nerves. ABR was measured at two different frequencies (16 kHz and 8 kHz), separately with DOTs of 0, 30, 60, and 100ms between the noise and signal for the five SNRs.

Considering different DOTs at 16 kHz before surgery, the latency of waves increased from 0 to 30 ms, unchanged from 30 to 60 ms, and decreased from 60 to 100 ms, but at 8 kHz increased significantly from 0 to 100 ms. After surgery, the trend of changes at 16 kHz was different and increased from 0 to 100 ms. But, there was no change at the course of changes at 8 kHz. As shown in our previous study, the trend of changes was more evident in the latency of wave I compared to the latency of wave III. Therefore, ABR especially primary waves (wave I) at different DOTs showed the expected overshoot-like effect. It means that the detectability of signal (decrease of wave's latency) was better with increasing the DOT as seen in our previous study.

In psychoacoustic studies, it has been said that with increasing DOT over a certain period onset of noise, changes in detectability of signal are almost constant and then it will be better. McFadden et al (2010) reported, detectability remained approximately constant for at least 20–30 ms of signal delay and then began to improve gradually toward its maximum. It means there was a “hesitation” prior to detectability beginning to improve, and its duration was similar to that seen in physiological measurements of the medial olivocochlear function. In other words, the time histograms of auditory ipsilateral and contralateral efferent nerve function indicated a peak of 20 to 45 ms (Brown, 1989; Robertson & Gummer, 1985). In the present study, the absence of changes
in the latency of waves before surgery and changes after surgery at a time interval between the 30 to 60 ms at 16 kHz stimulus (high-frequency), could be related to the probable effect of the AENs. However, the latencies of waves (I and III) between 30 to 60 ms DOT were changed at 8 kHz. As shown in previous psychoacoustic studies, the magnitude of overshoot is greater at high-frequency and is lesser at low-frequency stimuli.

To investigate the probable effect of the efferent system on auditory overshoot, transection of the entire efferent bundle as it enters the medial aspect of the internal auditory canal was considered. A convenient and valuable tool for confirming the location of the efferent pathway between the brainstem and the cochlea is immunohistochemical staining for acetylcholinesterase (ACHE). ACHE is consistently identified and traced in and around cochlear structures associated with efferent innervation. Cholinesterase staining in a de-efferented cochlea, 20 days after transection was greatly reduced or absent but it was normal in the control guinea pig's cochlea. So, the transection procedure and de-efferented model was confirmed. It can be concluded that the AENs have been affected and destroyed in the case group and those in the control group were intact and healthy.

After surgery, with transection of the AENs, the trend of changes differed: the latency of waves I& III increased from 0 to 100 ms DOTs at 16 kHz stimulus. There was no “hesitation” prior to detectability beginning to improve at the 30 to 60 ms. As already mentioned, the hesitation has been related to the performance of the AENs and thus, the absence of plateau in our post-surgery data between 30 to 60 ms DOT could be explained. This means that the auditory efferent increases SNR when a signal is masked by noise, thereby enhancing the encoding of the signals in the noise (Nieder & Nieder, 1970; Tomchik & Lu, 2006). With a greater DOT, the effect of efferent nerves decreases and the detectability of the ABR waves increases, decreasing the latency and increasing the amplitude, especially for earlier waves such as wave I. It can be concluded that increasing the latency of waves from 0 to (30- 60) ms and the plateau between 30 to 60 ms DOT, which was abolished after AENs transection is explained by auditory efferent nerves function.

At the 8 kHz stimulus, the course of changes was similar before and after surgery: the latency of waves I & III, increased from 0 to 100 ms DOT, and the overshoot phenomenon was not evident. It was also reported in our previous study, that an increase in DOT would decrease the latencies of the waves and increased their amplitudes. In the current study, by comparing the changes in the variables mentioned between the 16 kHz and 8 kHz stimuli, the overshoot
phenomenon could be obviously observed by 16 kHz and to a lesser extent at 8 kHz stimuli. It showed that the auditory overshoot is frequency-dependent. As in another study, the magnitude of the overshoot was influenced by the frequency of the signal; the higher the frequency, the magnitude variation greater (Liberman & Gao, 1995; Liberman & Guinan, 1998; K. P. Walsh, Pasanen, & McFadden, 2010). As for psychoacoustic overshoot, the effect of the noise on the signal decreases and signal detection becomes easier (Fletcher, de Boer, & Krumbholz, 2013), the detectability of the signal in our study reflecting the neural activity became much easier.

As mentioned, the proposed factors or mechanisms that influence overshoot are peripheral and central (Keefe, Schairer, Ellison, Fitzpatrick, & Jesteadt, 2009; Lichtenhan, Wilson, Hancock, & Guinan, 2016; K. P. Walsh et al., 2010). The role of attention and efferent nerve function as central factors has been linked to overshoot (Guinan, 2018). In psychoacoustic overshoot studies, the listener is alert and attends to the task. As a result, attention attenuates irrelevant auditory stimuli through the function of the caudal efferent system (Overson, Bacon, & Webb, 1996; Schmidt & Zwicker, 1991). In our study, the guinea pigs were anesthetized. It is thought that anesthesia diminishes the effect of attention and the medial olivocochlear reflex. To reduce the effect of the noise, we subtracted waves due to noise alone from waves produced by the combination of signal and noise. In fact, subtraction of the waves lessened the effect of noise on the signal and important features were extracted.

Moreover, in the case of anesthesia, the functioning of the AENs is reduced but not completely eliminated. Therefore the comparison of the magnitude of the overshoot phenomenon in psychoacoustic studies (attending subjects) with electrophysiological studies (anesthetized animals) is not easy. Thus, the present study is only to track this phenomenon as an electrophysiologic tool, and the effects of the AENs function on them. Even though the efferent nerve function decreased, but did not disappear completely (Chambers, Hancock, Maison, Liberman, & Polley, 2012), therefore, the magnitude of electrophysiological overshoot was rather lower.

In examining the post-intervention changes due to DOTs, as shown in the figure (3-6), the latency of the waves relative to the pre-intervention period slightly increases despite the lack of change in hearing threshold. It can be attributed to the effect of AENs function on the performance of the afferent system (Zheng, Henderson, McFadden, Ding, & Salvi, 1999). In our study, the noise was broadband while the signal was a tone burst and transient. It is known that the medial
olivocochlear reflex acts to minimize the steady response to the noise by auditory nerve fibers, thereby maximizing the response to a transient signal and making the signal easier to detect. To reach the extent of the changes related to the phenomenon of overshoot compared with the psychoacoustic studies, and also to evaluate the magnitude of the overshoot phenomenon, we used different SNRs and studied the amplitude of the waves by conveying the changes due to the increase in the signal intensity compared with the noise. With regard to the variability of waves amplitude, as well as the effect of the process of subtraction the waves from each other, did not achieve significant results in using this parameter, so our focus was on the changes created at the latency of the waves.

Conclusions

Comparing the trend of changes in latency of waves at different DOTs, especially the lack of changes at 30- 60 ms speculate that the auditory electrophysiological overshoot was influenced by the efferent system. By transection of AENs, the overshoot phenomenon by using the ABR paradigm disappeared and the role of efferent system on the overshoot was confirmed.

In the present study, the overshoot phenomenon was observed the 16 kHz and not at 8 kHz, so we can claim that the detected overshoot is frequency-dependent.

The findings of this study relate only to the study itself and cannot be generalized. Further understanding is required about changes in waves occurring due to changes in the other DOTs and different frequencies in un-anesthetized conditions.

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Authors Contribution

Authors had the main idea of the project, designed the study, cooperated in study implementation and data collection, interpreted the data, critically reviewed the manuscript, and approved the final manuscript as submitted. Dr Haghani helped in analyzed and interpreted the data.
| Abbreviation | Description                  |
|--------------|------------------------------|
| AEN          | Auditory Efferent Nerve      |
| ABR          | Auditory Brainstem Response  |
| DOT          | Delay Onset Time             |
| SNR          | Signal to Noise Ratio        |
| WBN          | Wide Band Noise              |
| SPL          | Sound Pressure Level         |
| ACHE         | Acetyl-Cholinesterase        |
| IHC          | Immuno-Histochemical         |
| dB PeSPL     | decibel Peak Equivalent Sound Pressure Level |
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Fig 1. Internal aspect of temporal bone. (present study) Arrows (FF; Facial Foramen, TCIAC; Transverse Crest of Internal Auditory Canal, CN; Cochlear Nerve opening, SF; Sub-arcuate Fossa, SVN; Superior Vestibular Nerve, IVN; Inferior Vestibular Nerve.)
Fig 2. The grand averages of ABR waveforms in case group at 16 kHz (left column) and 8 kHz (right column). a1, a2: The ABR waveforms in response to signal (16 kHz) and noise alone, respectively pre- surgery. c1, c2: The ABR waveforms in response to signal (16 kHz) and noise alone, respectively post- surgery. e, g: Subtractions of the ABR waveforms in response to signal (16 kHz) and noise together from the waveforms of noise alone in four DOTs at 0 dB SNR pre- and post- surgery, respectively. b1, b2: Pre surgery (case groups) The ABR waveforms in response to signal (8 kHz) and noise alone, respectively pre- surgery. d1, d2: The ABR waveforms in response to signal (8 kHz) and noise alone, respectively post- surgery. f, h: Subtractions of the ABR waveforms in response to signal (8 kHz) and noise together from the waveforms of noise alone in four DOTs at 0 dB SNR pre- and post- surgery, respectively. The noise was a wide band noise (0.1-8.0 kHz, 100ms) at 60 dB PeSPL.
Fig 3. The chart shows that the trend of change in the latency of wave I differed with an increase in DOT at 16 kHz signal. Pre-surgically, the latency increased from 0 to (30, 60) ms DOTs and then decreased from the 60 to 100 ms DOTs (p < 0.05). Post-surgically, the latency increased from the 0 to 100 ms DOTs.

Fig 4. The chart shows that the trend of change in the latency of wave III differed with an increase in DOT at 16 kHz signal. Pre-surgically, the latency increased from the 0 to (30, 60) ms DOTs and then decreased from the 60 to 100 ms DOTs (p < 0.05). Post-surgically, the latency increased from the 0 to 100 ms DOTs.
**Fig 5.** The chart shows that the trend of change in the latency of wave I differed with an increase in DOT at 8 kHz signal. Pre- and post-surgically, the latency increased from 0 to 100 ms DOTs (p < 0.05).

**Fig 6.** The chart shows that the trend of change in the latency of wave III differed with an increase in DOT at 8 kHz signal. Pre- and post-surgically, the latency increased from 0 to 100 ms DOTs (p < 0.05).
Fig 7. (A) Cholinesterase staining in a guinea pig cochlea 20 days after VN transection. (B) Cholinesterase staining in a control guinea pig cochlea. In (B), dark staining is seen in spiral ganglion fibers which appeared normal. Staining is greatly reduced or absent in (A). (SV; Scala Vestibuli, SM; Scala Media, ST; Scala Tympani, RM; Reissner's Membrane, TM; Tectorial Membrane, BM; Basilar Membrane, SG; Spiral Ganglion).