RESEARCH ARTICLE

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Evoked Potentials by Tone Burst on the Auditory Cortices in Cats -Comparison of Off Responses in Awake and Anesthetized Conditions

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ABSTRACT

Background: Auditory Brainstem Response (ABR) recording in awake is essential to detect off-responses. This study clarified whether after-termination responses on ABR were offset responses, off-responses or a mixture of the two.

Methods: Evoked potentials in the auditory cortex of cats in response to tone burst stimuli were recorded, and off responses were examined with chronically implanted electrodes.

Results: When the fall time at the end of sound stimuli was 5ms or longer, the amplitude of click responses was extremely small. Under this condition, evoked potentials in response to two types of tone bursts (long and short) were recorded.

By calculating the differences in evoked potentials between the two-tone bursts, off responses were separated. Off responses were generated during wakefulness by auditory cortex stimulation but were not observed under anesthesia. Pronounced off responses, which were middle latency responses exhibiting bis positive waves, were obtained in response to sound stimuli with a frequency of 2 kHz or higher. Vertex stimulation did not induce off responses either during wakefulness or under anesthesia.

Conclusion: Off responses are derived from synchronous responses of neurons in the auditory cortex, which are generated when the neurons detect attenuation in the stimulus strength at the end of tone burst stimuli.

Keywords: Auditory brainstem response, Off response, Offset response, Tone burst stimuli, Auditory cortex.

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INTRODUCTION

In an experimental study of Auditory Brainstem Response (ABR), Hecox et al. reported that an on-response of ABR was evoked by the beginning part of tone bursts because the latency of the on-response was affected by the rise-fall time and duration of sound stimuli[1]. It was also reported that a brainstem response was evoked just after the termination of tone bursts lasting longer than the latency of ABR[2, 3]. As co the cause of the after-termination response, two explanations can be proposed. Firstly, the response can be evoked by a click noise (a noise with a broad frequency spectrum) produced by attenuating vibration of the headphone at the termination of sound stimuli. Secondly, the response can be associated with central recognition of the termination or rapid decay of sound stimuli.

Responses to the click noise can be considered to be evoked by the same mechanism as with on-response and they can be called “offset response.” On the other hand, responses with central recognition of the termination of sound stimuli can be called “off-response” because they are completely different from offset response in respect of the mechanism.

Although the presence of offset response has been demonstrated, off-response after the termination of sound stimuli has not been confirmed in ABR studies. If off-responses were evoked, they should be originated from the auditory cortex.

Most experimental studies on after-termination responses have been performed in cats under anesthetized conditions. However, evoked responses recorded in the auditory field of anesthetized cats consisted of several spikes of the “on-response” type. Therefore, it is considered that anesthetized conditions are not suitable for recording off-responses. ABR recording in awake is essential to detect off-responses. It is also important to examine evoked responses under anesthesia and in an awake state.

Before recognizing offset responses and off-responses separately, acoustic outputs from the headphone should be analyzed to quantitatively evaluate the click noise produced at the termination of sound stimuli.

To clarify whether after-termination responses on ABR were offset responses, off-responses or a mixture of the two, this study was conducted in chronic experimental cats both in waking state and under anesthesia.

On a side note, a part of this study was presented at a meeting of the American Research of Otolaryngology about 20 years ago. However, we had no time to write the manuscript. Now that due to the increasing movement of animal protection, this animal experiment is difficult to carry out. Time passed by since the experiment, however, we believe our research will be useful to this field.

METHODS

Animals: Eight adult cats (between 4 and 7 years old) weighing between 2.4 and 2.8 kg (CAT 1-8) were used.

Implantation of Electrodes: After sedation with intramuscular injection of xylazine (2 mg/kg) and anesthesia with intravenous injection of pentobarbital (25 mg/kg), animals were held in a brain stereotaxic apparatus for cats. The skull was exposed through an incision created on the parietal skin. Stainless-steel screw electrodes were placed as active electrodes over the vertex and bilateral primary auditory cortices (AI). Screw electrodes for reference were placed on the bilateral postauricular skin.

Fixation of the Head: The head of the animal was attached with 2 aluminum pipes embedded in a head holder made of dental cement. Screws were inserted in the pipes to hold the head in the stereotaxic apparatus during the experiment.

Recording of evoked responses was performed 1 week after implantation of electrodes both in awake and under anesthesia with intraperitoneal injection of pentobarbital (35 mg/kg). After adequate adaptation to the surrounding environment, the head holder was fixed with the brain stereotaxic apparatus. The body was gently supported using a soft bag. A needle electrode for grounding was inserted in the posterior neck skin. An evoked potential analyzer, Neuropack 8 (MEE-4108) (Nihon Koden Co., Tokyo, Japan), was used for recording and analyzing evoked responses.

Sound Stimuli: Sound stimuli were given through a headphone (AD-05) (Rion Co.,Ltd, Tokyo, Japan) placed adjacent to the bilateral auricles. Evoked responses to 100 tone bursts at a frequency of 1/sec were recorded and analyzed using a conventional signal averaging process. Unilateral auditory stimulation was performed with a masking noise on the contralateral side.

To confirm the reproducibility of responses, the recording was repeated more than twice and obtained waveforms were traced on the same sheet.

A tone burst consisted of a plateau phase with a constant sound pressure level and rise-fall phases located before and after the plateau, respectively. When the plateau phase has 50 ms duration and rise-fall phases have 5 ms duration, for example, the sound burst has a 60 ms duration in total. In the following text of this paper, the plateau phase had a constant duration of 50 ms, unless defined otherwise, and the duration of rise-fall phases was variable.

As for the standard condition to achieve adequate sedation in cats, tone bursts were given at a frequency of 3 kHz, sound pressure level of 70 dB, duration of rise-fall phases of 5 ms and duration of the plateau phase of 50 ms. After the experimental study, all cats were anesthetized with isoflurane and euthanized by cervical dislocation.

Ethics and Other Information: This experimental study was conducted according to the protocol approved by Teikyo University School of Medicine Animal Ethics Committee. Protocol number was not assigned for this

study. And it was conducted in January 1995 at the laboratory of Teikyo University School of Medicine.

RESULTS

Click Noise Just After the Termination of Sound Stimuli: The amplitude ratio of the click noise to the tone burst in terms of acoustic output was analyzed to evaluate the sound pressure level of the click noise produced by the headphone just after the termination of sound stimuli. The driving voltage and acoustic output from the headphone connected with the evoked potential analyzer (Neuropack 8) which generated tone bursts were recorded using another evoked potential analyzer, Neuropack sigma (MEB-5504) (Nihon Koden Co., Tokyo, Japan).

Figure 1 show waveforms of the input (driving voltage) and output (acoustic output) of the headphone. A residual component of the acoustic output (click noise) is noted just after the termination of the driving voltage.

To quantitatively analyze the click noise, the attenuation rate (dB) was calculated using the following equation; attenuation rate (dB) $=20 \cdot \log (a/b)$, where a = sound pressure of tone burst, b = sound pressure of click noise (Figure 1).

Figure 2 shows changes in the click noise produced just after the termination of tone bursts which had a frequency of 3 kHz, sound pressure of 70 dB and duration of rise-fall phases varying from 0.1 ms to 7 ms. The click noise was clearly detectable with the duration of rise-fall phases of 0.1 ms, very small with the duration of 3 ms and almost negligible with the duration of 5 ms and longer.

Table 1 shows the attenuation rate of the click noise to tone bursts with a sound pressure of 70 dB, varying frequency from 500 Hz to 4,000 Hz and varying duration of rise-fall phases from 0.1 ms to 7 ms. Based on the above-mentioned equation, the smaller value in the table corresponds to the larger sound pressure of the click noise. For example, when the tone burst had a frequency of 3 kHz and a rise-fall phase duration of 0.1 ms, the attenuation rate was 6.6 dB (sound pressure of tone burst=70 dB and sound pressure of click noise=63.4 dB). The sound pressure of the click noise was about 47% of the sound pressure of the tone burst. When the tone burst had a rise-fall phase duration of 5 ms, the attenuation rate was 34.0 dB. In this case, the sound pressure of the click noise was 36 dB and about 2% of the sound pressure of the tone burst.

Separation and Extraction of Off-Responses: Figure 3 shows evoked responses obtained from the left auditory cortex in a cat in awake (CAT 2) undergoing sound stimuli in the right ear. In Figure 3A, a bimodal positive response is detectable following an on-response just after the termination of the tone burst with a frequency of 3 kHz, sound pressure of 70 dB, rise-fall phase duration of 5 ms and total duration of 60 ms. In Figure 3B, however, the response in the analysis period was considered to be an on-response to sound stimuli with an increased total duration of 100 ms. Figure 3 A-B shows the difference in evoked responses between A and B, which was considered to separate and extract the off-response.

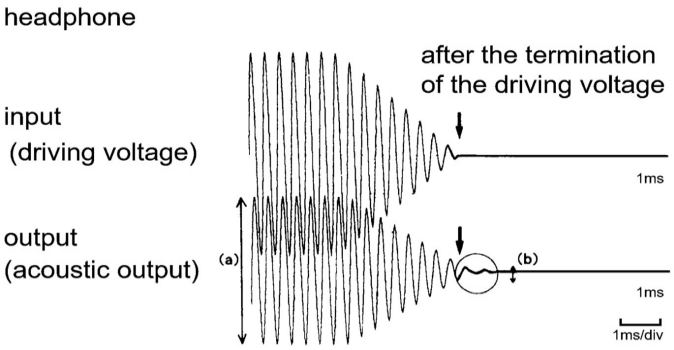

Figure 1: Headphone output and click noise.

The figure shows sound pressure from acoustic output (a) and click noise output (b) after the termination of driving voltage. Tone frequency, 3 kHz; sound pressure, 70 dBSPL, rise-fall time, 3 ms.

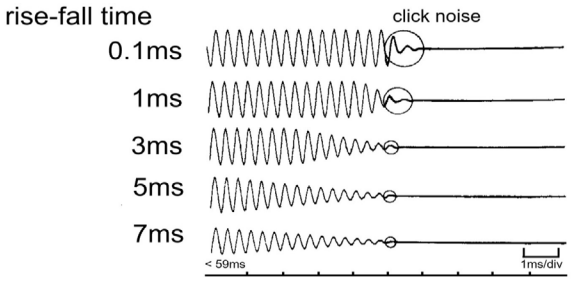

Figure 2: Click noise and rise time.

The rise-fall time varied from 0.1 ms to 7 ms under tone frequency of 3 kHz, sound pressure of tome bursts of 70 dBSPL and plateau phase of 50 ms. Click noise increases along with shorter rise time.

Table 1: Attenuation rate of click noise.

Sound pressure 70 dB	(dB)				
Frequency	Rise-Fall Time				
	0.1ms	1ms	3ms	5ms	7ms
4kHz	4.1	19.1	26.7	27.1	32.8
3kHz	6.6	21.2	30.8	34.0	36.2
2kHz	5.1	24.5	31.4	35.7	38.2
1kHz	-0.5	20.3	30.2	34.1	37.1
500Hz	-0.5	23.5	35.1	>40	>40

Attenuation rate of click noise pressure (b, Figure 2) to a constant sound pressure of tone bursts (a) is shown according to tone frequency and rise-fall time. Small values in the table correspond to large click noise pressure by the definition of attenuation rate. The unit is dB.

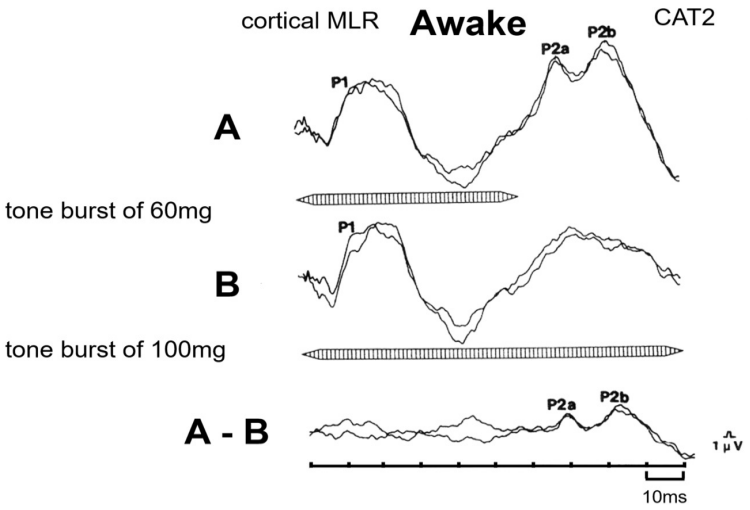

Figure 3: Isolation of off-response in a waking cat.

Tone burst stimuli were applied to the right ear with tone frequency of 3 kHz, sound pressure of 70 dBSPL and rise-fall time of 5 ms. Evoked potential was measured in the left auditory cortex (AI). A: 60 ms in total. On-response (P1) and off-response (P2a, P2b) are observed. B: Total duration as well as analysis time is 100 ms. Only on-response (P1) is thought to occur at least with this duration. A-B: The difference between A and B was calculated and off-responses (P2a, P2b) were isolated.

Off-responses with a similar appearance were detected in 5 (63%) of 8 animals.

Positive peaks of the off-response in Figure 3 were marked with P2a and P2b. The latency from the start of sound stimuli to the peak of responses was 69.97 ms for P2a and 80.39 ms for P2b.

DISCUSSION

Responses Evoked Just after the Termination of Sound Stimuli: Using a signal averaging process, Jewett first recorded ABR in cats undergoing click sound stimuli[4]. Buchwald demonstrated that ABR originate from the brainstem auditory pathway in an animal experiment with a central nerve sectioning technique[5].

Kodera et al. reported that ABR responses were noted just after the termination of tone bursts in cats and humans[6]. They speculated that such after-termination responses also originated from the brainstem because they exhibited a similar behavior to on-responses while changing the sound pressure and rise-fall time of tone bursts under anesthesia with pentobarbital. Clear responses were often detectable after sound stimuli with a very short duration of rise-fall phases. It was considered that after-termination responses were caused by the click noise produced at the termination of tone bursts. We defined this type of after-termination responses as “offset response” in this study.

Van Campen et al. reported that evoked responses were noted just after the termination of sound stimuli with the duration of rise-fall phases varying from 0.5 ms to 5 ms and the frequency varying from 500 Hz to 2,000 Hz in humans in awake[7]. It was suggested that a similar offset response as shown in Kodera’s study was detectable in humans.

Keidel et al. found that an action potential (AP: compound action potential of the cochlear nerve induced from the middle ear or external ear skin surface) was evoked just after the termination of tone bursts with a longer duration than the latency of AP in cats[2, 3]. This evoked potential is also considered to be “offset response” because AP corresponds to wave I in ABR.

As mentioned above, after-termination responses reported in the literature were classified as “offset response.”

Takahashi et al. has densely mapped tone-burst evoked potentials over the auditory cortex in experiments with rats and examined the overall characteristics of offset responses[8]. They reported that offset responses were facilitated when the intensity of test tones was high, the fall time was short, and the duration was long. And they also reported offset responses did not appear tonotopically, but at the fringe of tonotopic onset distributions. They indicated that the rebound after the inhibition in the presence of a stimulus was likely to be a major cause of offset responses in the auditory cortex. Regarding the offset response of awake animals, not only the rat report by Takahashi et al., But also the monkey, cat, and mouse have been reported[9-11]. However, there are no reports of off-response in awake and under anesthesia.

Responses in Awake and Under Anesthesia: The effect of anesthesia on Middle Latency Response (MLR) has not been clarified, although several studies suggested the difference in MLR between subjects in awake and under anesthesia[12, 13].

In a study of the effect of pentobarbital on MLR, Buchward et al. reported that A wave (P1 wave consisting of positive components in the present study) disappeared in “he anesthetized condition[14]. It was also reported that bimodal peaks of PA and PB (P1 wave consisting of positive components in the present study) changed into a monomodal peak with about 5 times greater amplitude than before the induction of anesthesia[15]. In the present study, a monomodal peak (P1) with a slightly greater amplitude was detected in pentobarbital anesthetized cats.

Under the anesthetized condition, off-response was not detected in the present study. This can be caused by several factors including 1) a decrease in the amplitude of off-response, 2) a loss of synchronization of off-response, and 3) suppression of off-response by anesthesia (pentobarbital).

The most reliable explanation for the absence of off-response under the anesthetized condition should be the suppression of ABR responses by pentobarbital because this anesthetic drug more strongly affects ABR responses with longer latencies due to more synaptic connections[16].

Bimodal off-response was detected on ABR recording in awake from the auditory cortex (Figure 4). On the other hand, off-response was not recorded from the vertex either in awake or under anesthesia (Figure 5). It was suggested that off-response was originated from the auditory cortex.

Analysis of Click Noise: When the driving voltage of tone bursts has a very short rise-fall time less than 1 ms, a click noise is produced just after the termination of sound stimuli because of attenuating vibration of the inertial mass (headphone).

When the rise-fall time was 0.1 ms, for example, a noticeable offset response with a duration of about 1 ms was observed just after the termination of tone bursts (Figure 2). This finding was remarkable with lower frequency stimulation, such as 1 kHz and 0.5 kHz. Based on the results of acoustic outputs and hearing impression (Table 1, Figure 2), offset responses were considered to be negligible after tone bursts with the rise-fall time of 5 ms or longer.

Offset and Off-Responses: The present study demonstrated the presence of off-response evoked in the auditory cortex which recognized the termination of sound stimuli. Off-response was detectable exclusively in auditory cortices in awake and considered to be different from offset response. Offset response was caused by the click noise produced just after the termination of sound stimuli with the same mechanism as on-response.

On-responses are detectable under anesthetized conditions just after the beginning of sound stimuli. Offset responses should be also detectable under anesthetized conditions because of the same mechanism of evoked responses.

However offset responses were very small because the click noise produced just after the termination of sound stimuli had a lower sound pressure compared with the beginning part of sound stimuli (Table 1).

As shown in Figure 4, on-response (P1) has a greater amplitude under anesthesia than in awake, although the difference was very small. It was considered that offset response was negligible in the condition shown in Figure 4 because it was undetectable under anesthesia. Therefore, P2a and P2b responses evoked just after the termination of sound stimuli in awake were considered to be “off-response.”

A previous experimental study on ABR in cats under anesthesia showed shortening of the latency of off-responses from the initiation of sound stimulation with prolongation of rise-fall times[16]. Though that study cannot be directly compared with our study because of differences in the tone burst pattern, off-responses in our study were derived from the auditory cortex, the latency from the initiation of the attenuation of sound stimuli became almost constant with a fall time of 3ms or more (Figure 6). The auditory cortex plays an important role in sound localization in these animals[17]. Therefore, off-responses appear to be derived from the detection of attenuation and changes in the intensity of sound stimuli by the auditory cortex.

In summary the present study conducted in chronic experimental cats both in awake and under anesthesia showed that off-response, rather than offset response to the click noise, was evoked in the auditory cortex just after tone bursts with a duration of rise-fall phases of 5 ms or more.

There are several limitations in this study. At first, cerebral blood flow measurement such as functional magnetic resonance imaging, single-photon emission computed

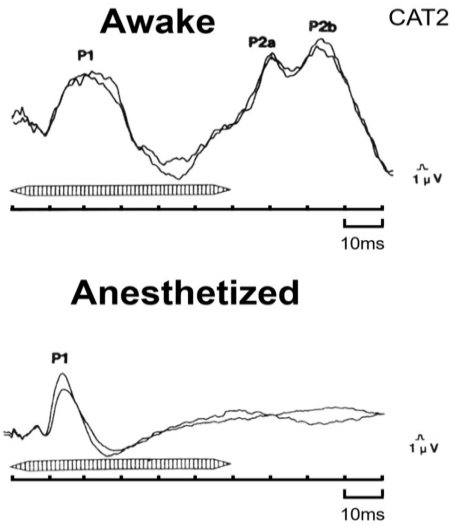

Figure 4: Response in auditory cortex in waking and anesthetized states.

Tone burst stimuli were applied to the right ear with tone frequency of 3 kHz, sound pressure of 70 dB, rise-fall time of 5 ms and total duration of 60 ms. Evoked potential was measured in the left auditory cortex (AI). 2-peak off-response observed in waking state (Awake, P2a, P2b) disappeared in anesthetized state (Anesthetized).

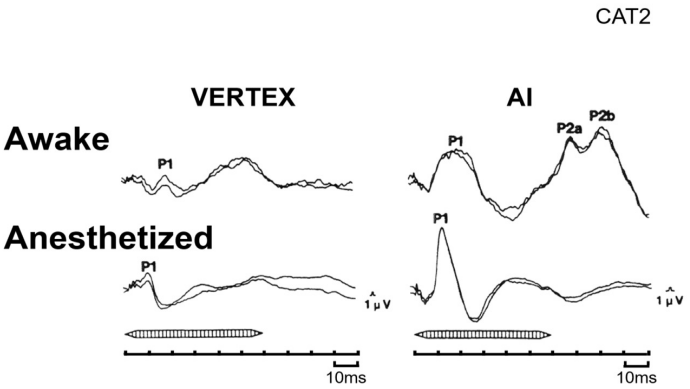

Figure 5: Comparison of responses in vertex and auditory cortex.

The tone burst stimuli were applied to the right ear with tone frequency of 3 kHz, sound pressure of 70 dBSPL, rise-fall time of 5 ms and total duration of 60 ms. Evoked potential was measured in the left auditory cortex (AI). Off-response (P2a, P2b) was observed only in the auditory cortex, and was completely absent in both waking and anesthetized states of the vertex (VERTEX).

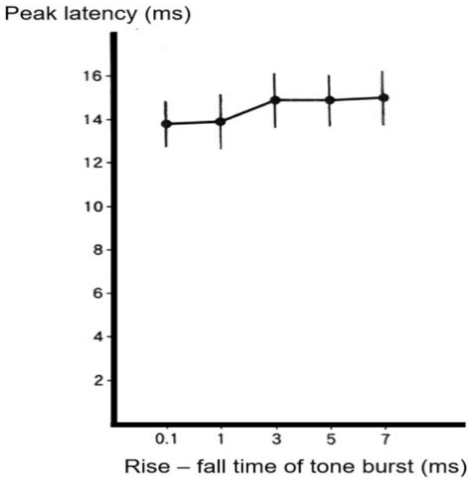

Figure 6. Latency and rise-fall time of off-response.

The latency of off-response is plotted against varying rise-fall time. Tone burst stimuli were applied to the right ear with tone frequency of 3 kHz, sound pressure of 70 dBSPL and constant plateau phase of 50 ms. Evoked potential was measured in the left auditory cortex (AI). Latency is defined as the duration from the attenuation start of the tone stimuli to the peak of P2a, Noticeable is the nearly constant latency for rise-fall time > 3 ms (mean + SD). SD: standard deviation.

tomography, and near-infrared spectroscopy is difficult to carry out in animals, and actual brain activity cannot be verified. In addition, since the experiment was conducted on cats, it cannot be asserted that the results are the same for humans.

CONCLUSION

We clarified the existence of an off-response from this study. This fact suggests the possibility of activation of cerebral blood flow by auditory on-off stimulation, as well as visual light-dark adaptation and olfactory on-off responses. In addition, the confusing responses that were said to be artifacts among the electrophysiological responses such as ABR, MLR, and slow vertex response will be clarified in the future based on the results of this study.

ETHICS COMMITTEE APPROVAL

This experimental study was conducted according to the protocol approved by Teikyo University School of Medicine Animal Ethics Committee. And it was conducted in January 1995 at the laboratory of Teikyo University School of Medicine.

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