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# The effects of nembutal anesthesia on the auditory steady-state response (ASSR) from the inferior colliculus and auditory cortex of the chinchilla

Kathleen Szalda [a,*], Robert Burkard [a,b]

[a] *Center for Hearing and Deafness, Department of Communicative Disorders and Sciences, State University of New York at Buffalo, 137 Cary Hall, Buffalo, NY 14214, USA*

[b] *Department of Otolaryngology, State University of New York at Buffalo, Buffalo, NY 14214, USA*

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**Abstract**

We examined the effects of nembutal anesthesia on the amplitude of the auditory steady-state response (ASSR) in the inferior colliculus (IC) and auditory cortex (AC) of the chinchilla. Tungsten electrodes were chronically implanted following anesthesia with ketamine/acepromazine. After a recovery period, the chinchillas were placed in a passive restraining device and put in a sound-attenuating booth. Recordings were made from the right IC and AC simultaneously, while a two-tone stimulus was presented to the left ear. The stimuli consisted of two equal-level tones (F1 and F2) that were mixed acoustically; F1 remained constant at 2000 Hz, while F2 varied between 2029 and 2249 Hz, in steps of ~20 Hz. The stimuli decreased in 10 dB steps from 80 to 30 dB pSPL. Animals were evaluated when unanesthetized, as well as when anesthetized with nembutal (on separate days).

In the IC, the administration of nembutal resulted in either no change in ASSR amplitude or an amplitude increase for difference tone (DT) frequencies below 90 Hz, while an amplitude decrease was typically seen for DT frequencies at or above 90 Hz. In the AC, a decrease in amplitude was seen across DT frequencies and stimulus levels after the administration of nembutal anesthesia. Our results suggest that both the AC and IC may contribute to the scalp-recorded ASSR in the awake state. However, in the nembutal-anesthetized state, it seems unlikely that the AC contributes substantially to the surface-recorded ASSR, as the AC response was greatly attenuated under nembutal anesthesia. In contrast, the IC ASSR responses remained robust, which makes it a likely contributor to the surface-recorded responses under nembutal anesthesia.


*Keywords:* Auditory steady-state response; Difference tone; Auditory evoked potential; Anesthesia; Chinchilla; Nembutal

## 1. Introduction

The auditory steady-state response (ASSR) is a physiological response that follows the envelope of the stimulus (Kuwada et al., 1986, 2002; Arnold and Burkard, 2002). It can be obtained by presenting the ear with periodic stimuli such as clicks or tonebursts, with continuous stimuli that are amplitude-modulated (AM), or with two-tone stimuli (F1, F2) (Dolphin et al., 1994; Galambos and Makeig, 1992). While the

*Abbreviations:* AC, auditory cortex; ASSR, auditory steady-state response; DAC, digital-to-analog converter; DT, difference tone; $F_L$, lower cutoff frequency; $F_U$, upper cutoff frequency; IC, inferior colliculus; IM, intramuscularly; IP, intraperitoneally; ISI, interstimulus interval; MRTF, modulation rate transfer function; PET, positron emission tomography; pSPL, peak sound pressure level; SAM, sinusoidally amplitude-modulated

* Corresponding author. Tel.: +1 716 829 2001x27; fax: +1 716 829 2980.

*E-mail address:* kmszalda@acsu.buffalo.edu (K. Szalda).

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DT or MF is not seen in the acoustic signal, they can be observed in the evoked response due to nonlinearities of the auditory system (Arnold and Burkard, 2002).

The ASSR has been studied in humans (Dimitrijevic et al., 2002; Reyes et al., 2003; Pantev et al., 1996; Dobie and Wilson, 1998) as well as a variety of non-human animals (Kiren et al., 1994; Kuwada et al., 2002; Makela et al., 1990). One of the advantages of using an animal model is in the ability to use near-field electrodes, which allows recordings to be dominated by a known brain region. In chinchillas, using DTs of 20, 40, 80, 160 and 320 Hz, Arnold and Burkard (2002) found that the dominant frequency in the inferior colliculus (IC) was either 80 or 160 Hz, depending on stimulus level, while the dominant frequency from the auditory cortex (AC) was 80 Hz. Arnold and Burkard (2002) used broad frequency steps, and hence details about the chinchilla modulation rate transfer function (MRTF) from the IC and AC are not known.

The ASSR can be used clinically for the estimation of threshold in infants and young children (Cone-Wesson et al., 2002; Rance et al., 1995; Aoyagi et al., 1994a). There are effects of state of arousal/anesthesia on these responses (Gilron et al., 1998; Tiitinen et al., 1993; Jerger et al., 1986; Linden et al., 1985; Plourde and Picton, 1990), and these effects are dependent on the modulation frequency that is used. Studies have shown that the ASSR to lower modulation frequencies (~40 Hz) are more affected by level of arousal or anesthesia than higher modulation frequencies (>~70 Hz) (Cohen et al., 1991; Aoyagi et al., 1993; Levi et al., 1993). Although this technique shows promise for hearing screening and threshold estimation, one of the limitations in the clinical application of the ASSR is that its generators are not known.

The purpose of the present experiment is to define with greater resolution the MRTFs for both the IC and AC of the chinchilla. Defining these functions will aid in determining the contributions of the IC and AC to scalp-recorded responses, as well as optimal stimulation parameters. In addition, we would like to determine the effects of anesthesia on the ASSR, which will also provide insight into where the response is being generated. One theory is that high-frequency modulators arise primarily from the brainstem, while low-frequency modulators come primarily from cortex (Kuwada et al., 1986). As nembutal anesthesia depresses cortical function (i.e., the animal is rendered unconscious), we hypothesize that the ASSR to low frequency DTs from the cortex will be affected by the anesthesia, while responses from the IC will be relatively unaffected, regardless of DT.

## 2. Methods

### 2.1. Surgical procedures

Eleven adult chinchillas were used to examine the effects of nembutal on the ASSR. Two of the 11 chinchillas lost their electrode cap before data collection could be completed, and as a result were excluded from the study. One chinchilla, which received the anesthesia intraperitoneally, died during data collection and was also excluded from the study. Data was collected and analyzed on a total of eight chinchillas, 7 female and 1 male, with weight ranging from 476 to 578 g. All eight of the chinchillas underwent identical surgical procedures. Following injection of ketamine/acepromazine (45 mg/kg ketamine and .6 mg/kg acepromazine), the chinchillas were surgically implanted with tungsten electrodes in the right and left IC, in the right AC, and in the anterior cranium, which served as the common electrode. A stereotaxic device was used to immobilize the animal's head during surgery. A click stimulus was presented to the contralateral ear of the chinchilla while the electrodes were being advanced into the IC and AC. When an adequate response was obtained, the electrode was secured with dental acrylic. All procedures were approved by the Institutional Animal Care and Use Committee at the University at Buffalo.

### 2.2. Data collection

Following a recovery period of at least one week, thresholds were obtained by recording from the right IC, left IC, and right AC, while stimulating the contralateral ear. During data collection, animals were placed in a passive restraining device and put in a sound-attenuating chamber. Thresholds were measured at .5, 2, and 8 kHz, using a 2-1-2 ms (cosine-squared) alternating-phase toneburst. Stimuli decreased in level from 80 to −10 dB peak sound pressure level (pSPL) in 10 dB steps. All sound levels reported in this study were measured in a 0.2cc volume with an Etymotic ER7C probe microphone. Table 1 shows the average thresholds for the 8 chinchillas.

Following threshold estimation, chinchillas were tested both with and without anesthesia. The animals were run on two separate days, with one day in between to minimize any carryover effects of the anesthesia. Half of the animals began the experiment with the anesthe-

Table 1
Average thresholds

| | Left ear/right IC (dB pSPL) | Left ear/right AC (dB pSPL) |
|---|---|---|
| 0.5 kHz | 46.3 | 49.4 |
| 2 kHz | 31.3 | 40 |
| 8 kHz | 17.5 | 23.1 |