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Hearing Research 206 (2005) 177–184
HEARING RESEARCH
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Responses to species-specific vocalizations in the auditory cortex of awake and anesthetized guinea pigs

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Received 22 November 2004; accepted 14 January 2005
Available online 12 April 2005

Abstract

Species-specific vocalizations represent an important acoustical signal that must be decoded in the auditory system of the listener. We were interested in examining to what extent anesthesia may change the process of signal decoding in neurons of the auditory cortex in the guinea pig. With this aim, the multiple-unit activity, either spontaneous or acoustically evoked, was recorded in the auditory cortex of guinea pigs, at first in the awake state and then after the injection of anesthetics (33 mg/kg ketamine with 6.6 mg/kg xylazine). Acoustical stimuli, presented in free-field conditions, consisted of four typical guinea pig calls (purr, chutter, chirp and whistle), a time-reversed version of the whistle and a broad-band noise burst. The administration of anesthesia typically resulted in a decrease in the level of spontaneous activity and in changes in the strength of the neuronal response to acoustical stimuli. The effect of anesthesia was mostly, but not exclusively, suppressive. Diversity in the effects of anesthesia led in some recordings to an enhanced response to one call accompanied by a suppressed response to another call. The temporal pattern of the response to vocalizations was changed in some cases under anesthesia, which may indicate a change in the synaptic input of the recorded neurons. In summary, our results suggest that anesthesia must be considered as an important factor when investigating the processing of complex sounds such as species-specific vocalizations in the auditory cortex.

Keywords: Anesthesia; Ketamine; Vocalization; Guinea pig; Auditory cortex; Multiple-unit activity

1. Introduction

Most information about the function of the mammalian sensory systems (including the auditory system) has been accumulated in electrophysiological studies performed on anesthetized animals. Anesthesia, similarly as a state of vigilance (Edeline et al., 2001), can affect sensory processing, therefore the investigator must be aware of the influences of anesthetics on neural processing and the relevancy of the obtained data when interpreting results in a non-anesthetized animal. The first studies of unit activity in the auditory cortex already demonstrated strong effects of anesthesia. Anesthesia was found to reduce the number of units encountered by a micro-electrode (Katsuki et al., 1959) and to reduce the capacity of units to respond to auditory stimuli (Thomas, 1952; Erulkar et al., 1956). Several studies reported a mainly suppressive effect of various anesthetics on spontaneous activity in different subcortical nuclei (e.g., pentobarbital, chloralose, and halothane, Evans and Nelson, 1973; pentobarbital, Kuwada et al., 1989; ketamine and pentobarbital, Zurita et al., 1994), but less is known about the impact of anesthetics on sound-evoked activity in the auditory system and signal processing in neuronal

Abbreviations: AC, auditory cortex; DE, drug effect; MU, multiple-unit; PSTH, peri-stimulus time histogram

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doi:10.1016/j.heares.2005.01.013

circuits. Kisley and Gerstein (1999) reported that the variability of stimulus-evoked responses in the cortex is modulated by the depth of ketamine anesthesia. The authors showed that trial-to-trial variability was usually lowest under light anesthesia and highest under moderate anesthesia. Gaese and Ostwald (2001) found a loss of tuning in some neurons and a sharpening of the frequency response areas in other neurons in the auditory cortex (AC) of the rat after pentobarbital/chloral hydrate anesthesia.

The effect of anesthesia on the processing of acoustical information has been studied not only at the cortical level, but also at subcortical levels of the auditory system such as the cochlear nucleus (Anderson and Young, 2004), the inferior colliculus (Astl et al., 1996; Torterolo et al., 2002) and the medial geniculate body (Massaux et al., 2004).

The effects of anesthesia seem to be even more important when we attempt to understand the processing of sounds such as species-specific vocalizations. These calls are typically complex sounds characterized by time-varying amplitudes and spectral features (Syka et al., 1997; Šuta et al., 2003). It is possible that the processing of such sounds in the auditory cortex depends on the level of vigilance of the animal, especially in the case of calls with a high behavioral impact. The aim of this study was therefore to investigate the effects of ketamine–xylazine anesthesia on the responses of neurons in the auditory cortex of the guinea pig to a set of spectrally and temporally different complex sounds – guinea pig calls. The responses of multiple units in the auditory cortex of the guinea pig were recorded first in an awake and weakly restrained animal and then after the injection of the anesthetic.

2. Methods

2.1. Animal preparation

Experiments were performed on 12 adult, healthy, pigmented male guinea pigs, 3–9 months old (mean age 6 ± 1.7 months), weighing 300–500 g. The care and use of animals reported in this study were approved by the Ethics Committee of the Institute of Experimental Medicine and followed the guidelines of the Declaration of Helsinki.

2.2. Recording of neuronal activity in the AC

Neuronal activity in the AC was recorded by either of two procedures. In the first procedure, four platinum–iridium electrodes (Bionic Technologies, impedance 0.5–2 MΩ) were implanted into the AC. Animals were anesthetized with an intramuscular injection of a mixture of 33 mg/kg ketamine (Narkamon 5%, Spofa) and 6.6 mg/kg xylazine (Sedazine 2%, Fort Dodge). The skin and underlying muscles on the skull were retracted to expose the dorsal cranium between points bregma and lambda. A small hole (diameter 5 mm) was made by a trephine in one side of the skull above the AC, and the electrode array was introduced into the AC through the dura mater and fixed to the skull by acrylic resin. A small connector was fixed to the dorsal skull by two screws, electrodes were soldered to the pins and the connector was secured to screws by acrylic resin. The exposed tissue was treated with an antibiotic (Framykoin, Spofa) to prevent inflammation, and the wound was sutured. The recording of neuronal activity was performed at least 10 days after the surgery. In the second procedure, a hole (diameter 5 mm) was made in the skull above the AC by the same procedure as described above. A small plastic tube was fixed by two screws above the hole as a support. The wound was treated with antibiotic, covered by the tissue, and the support filled with isotonic solution and plugged. A few days after the surgery a miniature mechanical electrode driver was fixed on the support to insert the electrode array (four epoxylite insulated tungsten electrodes, impedance 0.4–2 MΩ) into the AC. Both types of recording resulted in the same responses of multiple units, therefore the results of the recordings are presented together. Some animals were used in two or three experimental sessions. In both types of experiments the neuronal activity was recorded in guinea pigs placed in a plastic box, securing their heads by a sliding ring over the nose. This type of fixation enabled the animal's head to be free for electrode penetration and for free-field acoustical stimulation. The neuronal responses were recorded first in an awake and weakly restrained animal and then after the intramuscular injection of the anesthetic. At the beginning of the experiment the syringe needle was prefixed into the leg muscle to minimize manipulations with the animal during the later anesthetic injection. A DC-powered electric heating pad maintained body temperature at 37–38 °C.

The signal from the electrodes was amplified by a custom made four-channel differential amplifier and band-pass filtered in the range of 300 Hz to 10 kHz (filter slopes 12 dB/octave). The signal was transmitted via a Cambridge Electronic Design (CED 1401plus) interface into a PC computer running the Spike2 program, where the activity was saved and later analyzed. The neuronal activity was recorded simultaneously from one or more microelectrodes in the form of multiple-unit (MU) activity.

2.3. Acoustic stimulation

Electrophysiological recordings were made in a soundproof anechoic room. The walls and ceiling inside the room were covered by cones from phono-absorbent material; the attenuation was 55 dB at 250 Hz and 60–70