Neuroscience Letters 534 (2013) 24–29

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Audiovisual integration in the primary auditory cortex of an awake rodent

Kohta I. Kobayasi [1], Yoichi suwa, Hiroshi Riquimaroux [*,1]

Department of Biomedical Information, Faculty of Life and Medical Sciences, Doshisha University, 1-3Miyakodani, Tatara, Kyotanabe-city, Kyoto, 610-0321, Japan

HIGHLIGHTS

- We investigated the temporal integration of audiovisual stimuli in auditory cortex.
- Mongolian gerbils were used in an awake condition to avoid artifacts of anesthesia.
- Flashing light altered the auditory responses of about one fourth A1 units.
- Auditory cortical responses were affected mostly by the concurrent visual stimulus.
- The species can be a valuable model for studying audio–visual integration.

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form
17 September 2012
Accepted 24 October 2012

Keywords:
Mongolian gerbil
A1
Temporal integration

ABSTRACT

Much is known about the behavioral and physiological aspects of multimodal integration in primates, whereas less is known about the extent of audiovisual integration in other species. This study investigated the temporal integration of audiovisual stimuli in the primary auditory cortex (A1) of a standard animal model of auditory physiology: the Mongolian gerbil (*Meriones unguiculatus*). We recorded single unit responses to auditory and visual stimuli in the A1 of awake gerbils. A tone burst (auditory stimulus) paired with a flashing light (visual stimulus) at differing lag times (from 0 to ±160 ms) was presented contralateral to the recording site. As a result, the auditory response was altered significantly by the visual stimulus in more than 25% of the A1 units. The effect of the visual stimulus on the auditory response decreased as the time lag between the two modalities increased. The influence of the visual stimulus remained relatively greater when it preceded rather than followed the auditory stimulus. These results suggest that the A1 and earlier (subcortical) auditory structures of the rodent are capable of temporally integrating information from auditory and visual modalities.

1. Introduction

Animals have to reconstruct a coherent perceptual world from multisensory information. Understanding how and where information for different sensory modalities is integrated in the brain is a central question of sensory neuroscience. Audiovisual interactions in the auditory cortex have been well described in several mammal species (*e.g.*, nonhuman primates [10,16], cat [15], ferret [2,4]). Those studies showed that the neural responses to a sound stimulus in both the non-primary and primary auditory cortices are modulated by visual input, and several functions of the visual effect on auditory cortices have been proposed. Primate studies have suggested that the visual influence on the auditory response is involved in vocal communication. The neural activity of the auditory cortex is modulated by watching a moving mouth, but not by moving circles, in both monkeys [8] and humans [14]. Bizley et al. investigated the functions of multisensory neurons in sound localization in the ferret and showed that the spatial tuning of auditory responses can be shaped by a visual stimulus when the stimulus is presented from the same location as the sound [2,4]. Those studies demonstrated that the visual stimulus needs specific properties (*i.e.*, mouth shape in primates or the location in space) for the modulation to happen. Nevertheless, the importance of the relative timing of the stimuli has not been studied in depth.

In this study, we used awake animals to avoid possible artifacts due to the influence of anesthesia. Because anesthetic agents influence the discharge characteristics of neurons [7], and more importantly, significantly affects the latency of the visual response [1], and the temporal properties of the auditory response in both the midbrain and cortex [18], it is difficult to quantify detailed temporal interactions in anesthetized animals. This study used Mongolian gerbils and investigated the degree of audiovisual integration in the rodent auditory system by recording the neural activities of A1 in response to auditory and visual stimuli presented with various temporal delays.

* Corresponding author. Tel.: +81 774 65 6956; fax: +81 774 65 6500.
E-mail addresses: hrikimar@mail.doshisha.ac.jp, hrikimar@gmail.com (H. Riquimaroux).
[1] Also at Neurosensing and Bionavigation Research Center, Doshisha Univeristy, Japan.

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.10.056

2. Materials and methods

2.1. Animal preparation

Eight 20- to 72-week-old gerbils (60.0–86.5 g) were studied. For the surgery, anesthesia was induced with an intramuscular injection of a mixture of ketamine hydrochloride (35 mg/kg) and xylazine (14 mg/kg). The temporal muscle and bone over the auditory cortex in the right hemisphere were removed. A metal rod was fixed to the top of the cranium with dental cement. After a recovery period (2–3 days), an awake animal was held on a platform and free to move its limbs while its head was immobilized by the metal rod in a pitch-dark soundproof Faraday cage. All husbandry and experimental procedures were approved by the Animal Experiment Committee of Doshisha University.

2.2. Stimulus

Auditory stimuli were presented using a sound card (UA-101, Roland, Japan) and a loudspeaker of 3.2-cm diameter (AT-SP91, Audio-Technica, Japan). The loudspeaker was placed to the left (contralateral to the recording hemisphere) of the animal, 13 cm from its head. As a visual stimulus, a white light-emitting diode (LED: OSWT3166B, Optosupply, China) was fixed to the top of the loudspeaker and controlled by a different channel in the same sound card used for sound production. The intensity of the light at the animal's eye was 0.41–0.57 cd/m^2 (measured with a GL-03, be-s, Japan). All sound stimuli were generated using custom-made software developed in C++ (192 kHz, 16 bits). By applying a digital filter to the sound stimuli (calibrated by a microphone: type 7016, ACO, Japan), the frequency was flattened (1–45 kHz, ±3 dB) at the position of animal's head.

To identify auditory units, constant frequency (CF) tone bursts (of eight different frequencies logarithmically divided between 1 and 45 kHz, 70 dB SPL), frequency-modulated tone bursts (1–45 kHz, 70 dB SPL), and white noise bursts (1–45 kHz, 70 dB SPL) were delivered as search stimuli at a rate of 1 or 2/s. After finding an auditory unit, 32 CF tone bursts (1–45 kHz, divided logarithmically) were presented in pseudo-random order and the sound pressure level of the tones was varied from 70 dB in 10 dB steps to determine the best frequency (BF) of the unit; *i.e.*, the frequency of the CF tone that elicited observable neural responses at the lowest sound pressure level. The duration of all acoustic stimuli was 150 ms, which included 5 ms linear ramps at onset and offset. After the BF of the neuron had been determined, combined audiovisual stimuli were presented. The auditory stimulus was a 150 ms tone burst at the BF presented at 15 dB above the threshold of the unit [13] and the visual stimulus was a light flash from an LED with the same 150 ms duration. The stimulus types were defined according to the temporal lag between the sound and light. Synchronized presentation is presented as Audio (A) = Visual (V); the stimuli involving presentation of the auditory stimulus 10, 20, 40, 80, and 160 ms before the visual stimulus were called A10 V, A20 V, A40 V, A80 V, and A160 V, respectively; and the stimuli involving presentation of the visual stimulus 10, 20, 40, 80, and 160 ms before the auditory stimulus were expressed as V10A, V20A, V40A, V80A, and V160A, respectively. Presentation of the auditory or visual stimulus alone was defined as A or V, respectively. These 13 stimulus types were presented in 20 trials in pseudo-random order every 1000 ms.

2.3. Electrophysiological recordings

Enamel-coated Elgiloy microelectrodes (1–5 MΩ) were introduced into the auditory cortex with a hydraulic micromanipulator (MW-8, Narishige, Japan). All penetrations were orthogonal to the brain surface. The recordings were obtained at intracortical depths (10–500 μm). The action potentials were amplified and band-pass filtered (0.3–10 kHz) with a differential amplifier (DAM 80, WPI, USA), and was stored (50 kHz, 16 bits) in a personal computer *via* an A/D converter (Micro 1401, CED, UK). In all experiments, the location of the electrode in A1 was determined based on anatomical landmarks (cranial bone sutures and patterns of cortical vasculature [6]) and a tonotopic map of the gerbil auditory cortex (Fig. 1) [19]. In some penetrations, the recording site was marked by an electric lesion with a current injection (50 mA, 2 min) and later determined in a Nissl-stained section.

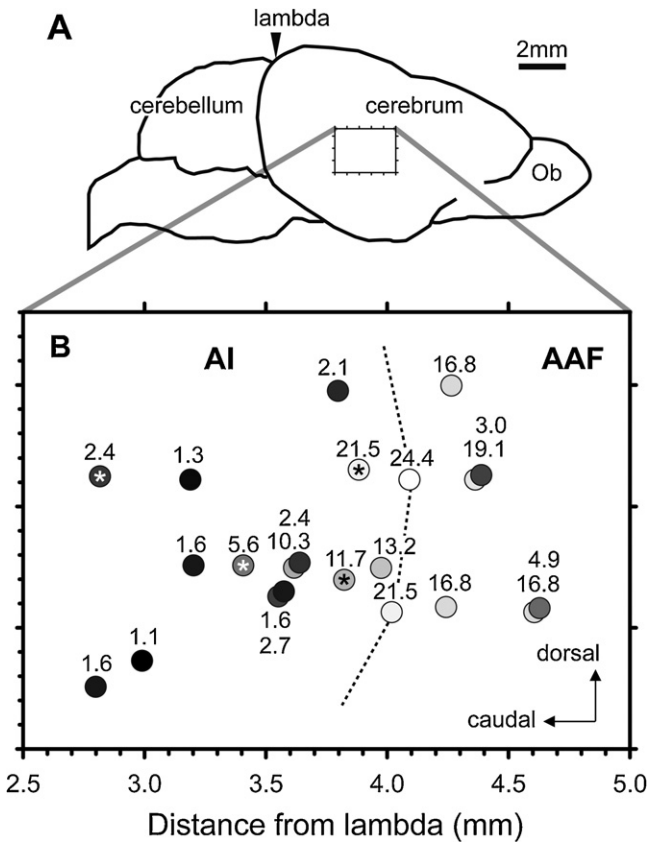

Fig. 1. Distribution of recording sites and estimation of the border between the A1 and anterior auditory field (AAF). (A) Side view of the gerbil brain. (B) Distribution of the best frequency (BF) and response types in the A1 of one example gerbil. The BFs in kHz of each unit are indicated by the number above the recording site. Asterisks indicate sites where the visual stimulus had a significant effect ($P < 0.05$). The borders between A1 and AAF were estimated from the BF of each unit, and are indicated by dashed lines. *Ob*, olfactory bulb.

2.4. Data analysis

Single units were isolated from digitized signals using a spike recognition and analysis program (Spike 2, CED, UK). The spike rates of each unit for each stimulus were calculated to create the peri-stimulus time histogram (bin = 10 ms). Response latency was estimated from the median of first-spike latencies to the BF tone. In each unit, the spontaneous spike rates and the spike rates during auditory and visual stimulation (from the beginning to the end of the stimulus: 150 ms) were calculated. To quantify the visual effect along with the different stimulus delay in each unit, the absolute difference in the spike rates (spike rate difference) between the auditory stimulus alone (A) and each combination (*e.g.*, A160V) was first determined, and then each absolute spike rate difference was divided by the maximum spike rate difference to generate the normalized spike rate difference. This is a normalized measure between 0 and 1, with 1 indicating the stimulus condition affected