Hearing Research 296 (2013) 51–59

Contents lists available at SciVerse ScienceDirect

Hearing Research

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

Research paper

The response properties of neurons in different fields of the auditory cortex in the rat

Oliver Profant [a,b,*], Jana Burianová [a], Josef Syka [a]

[a] Department of Auditory Neuroscience, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Czech Republic
[b] Department of Otorhinolaryngology and Head and Neck Surgery, 1st Medical Faculty of Charles University, Faculty Hospital Motol, Institute of Postgraduate Medicine, Czech Republic

ARTICLE INFO

Article history:
Received 4 July 2012
Received in revised form
19 October 2012
Accepted 18 November 2012
Available online 6 December 2012

ABSTRACT

The auditory cortex (AC) of the rat has been the subject of many studies, yet the details of its functional organization are still not well understood. We describe here the functional organization of the AC in young rats (strain Long Evans, aged 30–35 days, anesthetized with ketamine/xylazine) on the basis of the neuronal responses to acoustic stimuli. Based on the neuronal responses to broad band noise (BBN) and pure tone bursts, the AC may be divided into the primary auditory cortex (AI) and three other core fields: anterior (AAF), suprarhinal (SRAF) and posterior (PAF) as well as an unspecific region (UR) inserted between the AI and AAF. The core fields are surrounded by a belt area. Neurons in the AI, AAF, SRAF and PAF showed well defined characteristic frequencies (CF) in response to pure tone stimulation; in contrast, UR neurons responded only at high intensities without a clear CF. Neurons responding only to BBN stimulation were found mostly in the belt area. The putative borders between the core fields were determined by changes in their tonotopic gradient; however, no tonotopic organization was found in the PAF. Neurons with the shortest response latencies to BBN stimulation were found in layer 4 (L4) and layer 6 (L6) in the AI, while those with the longest latencies in the superficial layers (L1/2) of the belt area. Similar principles of responsiveness were observed when the spike rate in response to BBN stimulation was evaluated, with the highest rate present in L4 of the AI and the lowest in L1/2 of the belt area. According to the shape of the peristimulus time histograms, the responses of neurons in the AC of the rat may be classified as pure onset, sustained, onset-sustained, double peak or late onset. The most dominant in all fields, as well as in all layers, was the pure onset response. Our findings offer further cues for understanding the functional organization of the AC in the rat.

1. Introduction

The mammalian sensory cortices can be divided into different subdivisions or fields based on their functional organization. Such organization is present in the somatosensory (Petersen, 2007), visual (White and Fitzpatrick, 2007) and auditory cortices (Schreiner and Winer, 2007).

In the auditory cortex (AC), as in the subcortical levels of the auditory pathway, the main criterion for functional parcellation is tonotopy, which is a systematic spatial representation of the sound frequencies driving the neurons. This feature of auditory neurons has been used in the AC for characterizing the core areas in the cat (Heil et al., 1994), monkey (Cheung et al., 2001a), ferret (Bizley et al., 2005), guinea pig (Redies et al., 1989; Wallace et al., 2000), mouse (Stiebler et al., 1997) and gerbil (Thomas et al., 1993). In the original work of Sally and Kelly (1988) in the rat, the primary auditory field (AI) was distinguished on the basis of its tonotopic gradient with high frequencies represented rostrally and low frequencies caudally. In the same paper surrounding fields with no clear tonotopy were described. In subsequent investigations (Doron et al., 2002; Kalatsky et al., 2005; Pandya et al., 2008; Polley et al., 2007; Rutkowski et al., 2003) this finding was confirmed in principle, and in addition to the AI an anterior auditory field (AAF), based on the reversal of the tonotopic gradient in the rostral region adjacent to the AI, was described. The other areas belonging to the

Abbrevations: AI, primary auditory field; AAF, anterior auditory field; AC, auditory cortex; AP, action potential; CF, characteristic frequency; BBN, broad band noise; FRA, frequency response area; MCA, middle cerebral artery; GABA, γ-butyric acid; L, layer; PAF, posterior auditory field; PSTH, peristimulus time histogram; SRAF, suprarhinal auditory field; TC, tuning curve; UR, unspecific region; VAF, ventral auditory field.

* Corresponding author. Department of ENT, Charles University in Prague, 1st Faculty of Medicine, Faculty Hospital Motol, V Úvalu 84, 150 06 Prague 5, Czech Republic. Tel.: +420 224434301, +420 224434358, +420 608610117 (mobile); fax: +420 224434310.
E-mail address: profant@biomed.cas.cz (O. Profant).

0378-5955/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.heares.2012.11.021

core part of the auditory cortex are the posterior auditory field (PAF), with no clear tonotopy, and the suprarhinal auditory field (SRAF) (Pandya et al., 2008; Rutkowski et al., 2003). This distribution is based on the results of electrophysiological experiments; however, there is also available a scheme based on the histological differences within the AC. The core region is Te1, which is surrounded by the secondary areas Te2 and Te3 (Zilles and Wree, 1985, Zilles et al., 1980). Although the cytoarchitectonic distribution can be roughly correlated with the electrophysiology, a fine differentiation into several auditory fields is not possible on the basis of cytoarchitectonic (Kelly, 1990).

Although tonotopy provides a clear basis for the functional parcellation of the AC, neurons in relation to their position in the auditory fields also differ in many other parameters such as their response latencies, thresholds (Rutkowski et al., 2003), spectral tuning bandwidth and the duration of the response (Polley et al., 2007). In addition, other features of the neuronal responses, such as the frequency of the occurrence of different types of peristimulus time histograms (PSTHs), can be used to characterize the fields in the AC of the rat. Characterization of the auditory fields or nuclei on the basis of the occurrence of neuronal PSTH types was previously used in the subcortical (Kvasnák et al., 2000; Nuding and Nadelhaft, 1998; Suta et al., 2003) as well as the cortical parts of the auditory system (Recanzone, 2000; Valentine and Eggermont, 2004).

The AC as well as other parts of the neocortex consists of 6 layers in mammals. The position of a neuron within an individual cortical layer is related to its functional properties. In comparison with other sensory areas the borders between the layers in the AC, especially layer 3 and 4, are not clearly defined. This can be related to the lack of granule cells (Smith and Populin, 2001), which are replaced by small pyramidal cells in the AC. Regardless of this histological ambiguity, the microcolumnar organization in the AC is maintained (Jones, 2000), and neurons in the column share some similar features across all layers: characteristic frequency (Abeles and Goldstein, 1972; Merzenich et al., 1975), intensity threshold (Suga, 1965) and intensity tuning (Clarey et al., 1994). On the other hand, neurons within the columns may differ in their morphology, tuning bandwidth (Wallace and Palmer, 2008) and their neuronal inputs and outputs (Linden and Schreiner, 2003; Sakata and Harris, 2009; Winer and Lee, 2007). A detailed comparison of neuronal properties based on the involvement of individual neurons in the types of auditory processing in different auditory regions as well as in different cortical layers has not yet been performed in the rat.

In the present study our aim is to contribute to the classification of the individual auditory cortical fields in the rat on the basis of a description of the different properties of the neuronal responses to sound stimuli. The response properties are correlated with the position of the analyzed neurons in different regions of the AC as well as their position in different cortical layers.

2. Material and methods

Ten young rats (strain Long Evans; 2 males, 8 females; age 30–35 days; weight 80–105 g) with no pathology were used in this study. Relatively young animals were used because in the accompanying experiments we studied the relationship between the biophysical properties of the neuronal membranes recorded with the patch clamp technique and the position of the neurons within the cortical fields. The age of 30–35 days was found to be the maximum age for optimal use of the patch clamp technique. All animals were obtained from a local supplier. The care and use of animals reported in this study were approved by the Ethics Committee of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, and followed the guidelines of the Declaration of Helsinki.

2.1. Surgical preparation

Each animal was initially anaesthetized with an intramuscular injection of a mixture of 33 mg/kg of ketamine (Narkamon 5%, Spofa) and 6 mg/kg of xylazine (Sedazine, Fort Dodge Inc.). Supplementary injections of one half of the original dose of the ketamine–xylazine mixture were administered approximately every hour to maintain a sufficient level of anesthesia. Respiratory and heart rates, along with corneal and tail reflexes, were monitored. Body temperature was held at 37 °C using an electrically controlled heating pad.

The skin and muscles above the right temporal region of the skull were removed, and the base of a S-shaped holder was fixed by two stainless steel screws to the skull above the frontal part of the left hemisphere. A craniotomy (diameter ~5 mm) was performed above the AC area. The rat was placed in a stereotaxic frame, and the head was fixed to the frame by the S-shaped holder, leaving the pinnae and outer ear canal intact. The dura mater was removed. Electrode probe penetrations were made perpendicular to the surface of the cortex. During the recording of neuronal responses, the cortex was covered by warm agar.

2.2. Stimulation and recording

Acoustical stimulation was performed in free field-conditions in a sound-attenuated and anechoic room from a two frequency band loudspeaker system (Jamo woofer and SEAS T25CF 002 tweeter) placed 60 cm in front of the animal's head. The frequency characteristics of the speaker system were relatively flat over the range 1–40 kHz (±4 dB). Broadband noise bursts (BBN; 100 Hz-40 kHz) and pure tone bursts were used for acoustical stimulation. The acoustic system was calibrated with a Bruel and Kjaer 4939 microphone, a ZC0020 preamplifier and a B&K 2231 Sound Level Meter. A TDT (Tucker Davis Technologies, Florida) System III setup was used for acoustical stimulation and signal acquisition. The extracellular activity of AC neurons was recorded by a 16 channel multielectrode probe from NeuroNexus Technologies (single shank, site spacing 50 μm). A photograph of the vascular pattern of the AC was taken with a digital camera (Olympus C-3000 ZOOM) to mark the individual penetration sites.

Penetration sites were chosen to avoid blood vessels. At the beginning of the electrode penetration, the electrode tip was inserted 100 μm into the cortex with an electrical microdrive (0.1 μm step; MCL, Märzhäuser Wetzlar) under visual inspection through an operating microscope (32× magnification) and then withdrawn to the level of the surface, to prevent denting of the cortex. The electrode probe was then inserted again perpendicularly into the auditory cortex (depth 800–950 μm). Broadband noise bursts (60 ms duration, 5 ms rise/fall times, 80 dB SPL, 30 trial repetitions) were used to evoke neuronal responses with the aim of checking the localization of the electrode in the auditory cortex.

For cortical frequency mapping, pure tones (0.5–36 kHz, 60 ms duration, 5 ms rise/fall times, 1 repetition) were presented in a random order of changing frequency (1/4 octave steps) and intensity (5 dB steps, between 100 and 0 dB SPL).

Neuronal activity was amplified and digitized by a Medusa RA16PA preamplifier (A/D sample rate 25 kHz); the amplified digital signal was sent to a Pentusa RX5-2 base station via optic fibers. Data were further processed by Brainware software and then analyzed by custom-made Matlab routines.

2.3. Position of the recording site according to its depth

To confirm the depth of the electrode, at the end of the first 4 experiments, the position of the deepest recording site was marked