Neuroscience 332 (2016) 38–52

MICROELECTRODE MAPPING OF TONOTOPIC, LAMINAR, AND FIELD-SPECIFIC ORGANIZATION OF THALAMO-CORTICAL PATHWAY IN RAT

TOMOYO ISOGUCHI SHIRAMATSU, [a] **KAZUSA TAKAHASHI,** [a,b†] **TAKAHIRO NODA,** [a‡] **RYOHEI KANZAKI,** [a] **HARUKA NAKAHARA** [c¶] **AND HIROKAZU TAKAHASHI** [a*]

[a] *Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan*

[b] *Graduate School of Information Science and Technology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*

[c] *Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan*

Abstract—The rat has long been considered an important model system for studying neural mechanisms of auditory perception and learning, and particularly mechanisms involving auditory thalamo-cortical processing. However, the functional topography of the auditory thalamus, or medial geniculate body (MGB) has not yet been fully characterized in the rat, and the anatomically-defined features of field-specific, layer-specific and tonotopic thalamo-cortical projections have never been confirmed electrophysiologically. In the present study, we have established a novel technique for recording simultaneously from a surface microelectrode array on the auditory cortex, and a depth electrode array across auditory cortical layers and within the MGB, and characterized the rat MGB and thalamo-cortical projections under isoflurane anesthesia. We revealed that the ventral division of the MGB (MGv) exhibited a low–high–low CF gradient and long-short-long latency gradient along the dorsolateral-to-ventromedial axis, suggesting that the rat MGv is divided into two subdivisions. We also demonstrated that microstimulation in the MGv elicited cortical activation in layer-specific, region-specific and tonotopically organized manners. To our knowledge, the present study has provided the first and most compelling electrophysiological confirmation of the anatomical organization of the primary thalamo-cortical pathway in the rat, setting the groundwork for further investigation.

Key words: auditory cortex, auditory thalamus, microelectrode array, thalamo-cortical system, tonotopic map.

INTRODUCTION

Thalamo-cortical interactions play a crucial role in sensory perception (Singer and Gray, 1995; Usrey and Reid, 1999; Jones, 2001; Spencer et al., 2004; Kondo and Kashino, 2009). In addition, synaptic plasticity in the thalamo-cortical system underlies cortical map reorganization during perceptual learning (Armstrong-James et al., 1994; Buonomano and Merzenich, 1998). This thalamo-cortical communication is mediated by functional maps in several thalamic divisions and cortical fields, and also by the laminar structure of the cortex (Kelly and Wong, 1981; Huffman and Henson, 1990; Buonomano and Merzenich, 1998; Lee and Winer, 2008a,b,c; Lee and Sherman, 2011). Thus, a better understanding of functional maps and projection pattern in the thalamo-cortical system helps to clarify how this system contributes to sensory perception.

The thalamo-cortical auditory system in rats has been recognized as a useful experimental preparation to investigate neural mechanisms of sensory perception and perceptual learning (Butt et al., 2009; Hui et al., 2009; Takahashi et al., 2011; Funamizu et al., 2013; Headley and Weinberger, 2013; Noda et al., 2013) because of its extensive characterization in terms of both anatomical and electrophysiological properties. In the auditory cortex (AC), previous anatomical and electrophysiological studies have confirmed delineation of subfields with distinct frequency and temporal properties, i.e., different tonotopic and latency maps (Doron et al., 2002; Rutkowski et al., 2003; Takahashi et al., 2004; Polley et al., 2007; Noda and Takahashi, 2015). In addition, the laminar structure that underlies the thalamo-cortical projection has also been characterized (Barth and Di, 1990; Huffman and Henson, 1990; Kimura et al., 2003; Szymanski et al., 2009, 2011; Smith et al., 2012). Similarly, the auditory thalamus, or the medial geniculate

*Corresponding author. Fax: +81 3 5452 5196.
E-mail address: takahashi@i.u-tokyo.ac.jp (H. Takahashi).
† K. Takahashi is now with Komatsu Ltd., 2-3-6, Akasaka, Minato-ku, Tokyo 107-8414, Japan.
‡ T. Noda is now with the Institute of Neuroscience, Technical University Muinch, Arcisstraße 21, 80333 Munich, Germany.
¶ H. Nakahara is now with the Edogawa-bashi Clinic, 1-19-6 Sekiguchi, Bunkyo-ku, Tokyo 112-0014, Japan.

Abbreviations: A1, primary auditory cortex; AAF, anterior auditory field; AC, auditory cortex; AEP, auditory-evoked potential; BF, best frequency; CF, characteristic frequency; CSD, current source density; FRA, frequency responsive area; FSL, first spike latency; LFP, local field potential; Lv, pars lateralis; MGB, medial geniculate body; MGm, medial division of the medial geniculate body; MGv, ventral division of the medial geniculate body; MUA, multi-unit activity; Ov, pars ovoidea; P1, middle-latency auditory-evoked potential; PSTH, post-stimulus time histogram; SRAF, suprarhinal auditory field; VAF, ventral auditory field.

http://dx.doi.org/10.1016/j.neuroscience.2016.06.024
0306-4522/

body (MGB), has been anatomically characterized in terms of delineation of subdivisions (Clerici and Coleman, 1990; Winer et al., 1999a,b), and layer-specific, field-specific, and tonotopic projections to the AC (Scheel, 1988; Roger and Arnault, 1989; Arnault and Roger, 1990; Winer et al., 1999b; Kimura et al., 2003; Lee et al., 2004; Polley et al., 2007; Read et al., 2008; Storace et al., 2010). However, the tonotopic and latency maps within each subdivision in the MGB have not yet been fully characterized, and the field-specific, layer-specific and tonotopic thalamo-cortical projections have never been confirmed electrophysiologically, possibly because such characterization is technically demanding.

In the present study, we have established a novel experimental setup to characterize the rat MGB and thalamo-cortical projections by combined use of surface and depth microelectrode arrays. The MGB and AC in rats are located in the same coronal plane, and both nuclei can be accessed with a single penetration of a probe perpendicular to the cortical surface (McCarthy et al., 2011a,b). Therefore, a depth array with recording sites aligned in the depth direction characterizes the MGB and the layer-specific activation in the AC. In addition, a surface array measures electrocorticogram (ECoG) on the cortical surface, and visualizes the tonotopic activation in each subfield of the AC on the basis of the auditory-evoked potentials (AEPs) (Takahashi et al., 2004, 2005a; Shiramatsu et al., 2013). Of our first interest are the tonotopic and latency maps in the MGB. Second, we deliver electrical stimulation to the MGB and map the evoked activities in each cortical layer and on the cortical surface. Such characterization reveals whether and how the thalamo-cortical projections are organized according to the laminar structure, subfield, and tonotopic map.

EXPERIMENTAL PROCEDURES

This study was carried out in strict accordance with "Guiding Principles for the Care and Use of Animals in the Field of Physiological Science" published by the Japanese Physiological Society. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments at the Research Center for Advanced Science and Technology, the University of Tokyo (Permit Number: RAC130107). All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering. After the experiments, animals were euthanized with an overdose of pentobarbital sodium (160 mg/kg, i.p.).

Design of microelectrode arrays

We designed a surface microelectrode array and a depth electrode array (NeuroNexus Technologies, Ann Arbor, MI, USA) to measure neural activity simultaneously in the AC and MGB (Shiramatsu et al., 2015; Takahashi et al., 2015).

The surface microelectrode array was designed to measure local field potential (LFP) from the surface of the AC (Fig. 1A). The array was made on a flexible polyimide substrate to conform to the curvature of the cortical surface. The recording sites formed a 10×7 grid within an area of $4.5 \times 3.0\ mm^2$, out of which 64 sites were available for the recording. Each electrode was made of platinum and coated with platinum black. The diameter of the recording sites was 30 μm, and the center-to-center inter-electrode distance was 500 μm. On the substrate, 54 holes with a diameter of 300 μm were made between the recording sites at an interval of 500 μm to insert a depth array.

The depth microelectrode array was designed to measure LFP and multi-unit activities (MUAs) in the AC and MGB (Fig. 1B–D). The depth array was a silicon probe with three shanks. Each shank was 6 mm long and 50 μm thick, and the inter-shank distance was 500 μm. The shank width was 42 μm at the most distal recording site and 97 μm at the most proximal site. Each shank had 32 recording sites in total; 15 distal sites targeted the MGB while 17 proximal sites targeted the AC (Fig. 1B–D). The diameter of the recording sites was 30 μm, and the center-to-center inter-electrode distance was 120 μm. The most distal site was placed 100 μm from the tip of the shank, and the distance between the most proximal site in the MGB and the most distal site in the AC was 1200 μm. Each electrode was made of iridium oxide and coated with platinum black.

Animal preparation

Eight male Wistar rats, at postnatal week 8–12, with a body weight of 250–300 g, were used. Rats were anesthetized with isoflurane in conjunction with air (3% for induction and 1–2% for maintenance), and were held in place with a custom-made head-holding device. Atropine sulfate (0.1 mg/kg) was administered at the beginning and at the end of the surgery to reduce the viscosity of bronchial secretions. A heating blanket was used to maintain body temperature at approximately 37 °C. A skin incision was made at the beginning of the surgery under local anesthesia using xylocaine (0.3–0.5 ml). A needle electrode was subcutaneously inserted into the right forepaw, and used as a ground. A small craniotomy was performed near the bregma landmark to embed a 0.5-mm-thick integrated circuit socket as a reference electrode, with an electrical contact to the dura mater. The right temporal muscle, cranium, and dura overlying the AC were surgically removed, and the exposed cortical surface was perfused with saline in order to prevent desiccation. Cisternal cerebrospinal fluid drainage was performed in order to minimize cerebral edema. The right eardrum, i.e., ipsilateral to the exposed cortex, was ruptured and waxed to ensure unilateral sound inputs from the ear contralateral to the exposed cortex. Respiratory rate, heart rate, and hind-paw withdrawal reflexes were monitored throughout the experiment in order to maintain an adequate anesthetic level as stably as possible.

Neural recording

LFPs were obtained from both the surface and the depth microelectrode arrays with an amplification gain of 1,000, digital filter bandpass of 0.3–500 Hz, and sampling frequency of 1 kHz (Cyberkinetics Inc., Salt Lake City,