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TOPOGRAPHY OF PROJECTIONS FROM THE PRIMARY AND NON-PRIMARY AUDITORY CORTICAL AREAS TO THE MEDIAL GENICULATE BODY AND THALAMIC RETICULAR NUCLEUS IN THE RAT

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Abstract—The functional significance of parallel and redundant information processing by multiple cortical auditory fields remains elusive. A possible function is that they may exert distinct corticofugal modulations on thalamic information processing through their parallel connections with the medial geniculate body and thalamic reticular nucleus. To reveal the anatomical framework for this function, we examined corticothalamic projections of tonotopically comparable subfields in the primary and non-primary areas in the rat auditory cortex. Biocytin was injected in and around cortical area Te1 after determining best frequency at the injection site on the basis of epicortical field potentials evoked by pure tones. The rostral part of area Te1 (primary auditory area) and area temporal cortex, area 2, dorsal (Te2D) (posterodorsal auditory area) dorsal to the caudal end of area Te1, which both exhibited high best frequencies, projected to the ventral zone of the ventral division of the medial geniculate body. The caudal end of area Te1 (auditory area) and the rostroventral part of area Te1 (a part of anterior auditory field), which both exhibited low best frequencies, projected to the dorsal zone of the ventral division of the medial geniculate body. In contrast to the similar topography in the projections to the ventral division of the medial geniculate body, collateral projections to the thalamic reticular nucleus terminated in the opposite dorsal and ventral zones of the lateral and middle tiers of the nucleus in each pair of the tonotopically comparable cortical subfields. In addition, the projections of the non-primary cortical subfields further arborized in the medial tier of the thalamic reticular nucleus. The results suggest that tonotopically comparable primary and non-primary subfields in the auditory cortex provide corticofugal excitatory effects to the same part of the ventral division of the medial geniculate body. On the other hand, corticofugal inhibition via the thalamic reticular nucleus may operate in different parts of the ventral division of the medial geniculate body or different thalamic nuclei. The primary and non-primary cortical auditory areas are presumed to subserve distinct gating functions for auditory attention. © 2005 Published by Elsevier Ltd on behalf of IBRO.

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Abbreviations: AAF, anterior auditory field; AI, primary auditory area; BF, best frequency; MG, medial geniculate body; MGD, dorsal division of the medial geniculate body; MGV, ventral division of the medial geniculate body; P, posterior field; PB, sodium phosphate buffer; PD, posterodorsal auditory area; Po, posterior thalamic nuclear group; R, rostral auditory field; SG, suprageniculate nucleus; SI, primary somatosensory area; SII, secondary somatosensory area; TB, Tris buffer; Te2D, temporal cortex, area 2, dorsal; TRN, thalamic reticular nucleus; VB, ventrobasal complex.

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Cortical auditory areas appear specialized for processing of different attributes of sound (Rauschecker, 1997) or for cross-modal information processing associated with other sensory systems (Meredith and Clemo, 1989; Kimura and Tamai, 1992; Barth et al., 1995; Brett-Green et al., 2003). Repeated tonotopic representations are then considered a plausible reflection of cortical information processing engaged in diverse functions. The functional significance of parallel and redundant information processing by multiple tonotopic fields in the cortex yet remains elusive. The function of cortical auditory fields has been deduced, so far, mostly in connection with the cascade of information processing linked to higher sensory (Romanski and Ledoux, 1993a,b; Campeau and Davis, 1995; Shi and Cassell, 1997; Bushara et al., 1999; Nakamura, 1999; Romanski et al., 1999; Rauschecker and Tian, 2000; Lewald et al., 2002; Zatorre et al., 2002, 2004; Kimura et al., 2004) or motor function (Azizi et al., 1985; Wilkinson et al., 1996). On the other hand, relatively little is known about the functional significance of multiple tonotopic fields in feedback circuitry. It remains undetermined, for instance, what influences multiple tonotopic fields in the auditory cortex exert on thalamic information processing. In the present study, we attempted to reveal the anatomical framework underlying corticothalamic modulations subserved by multiple tonotopic fields in the rat cortex.

Our previous study (Hazama et al., 2004) suggested that the subfields for high and low frequencies in the primary auditory area in the rat cortex project to the ventral and dorsal zones of the ventral division (MGV) of the medial geniculate body (MG), respectively. It was also suggested that a similar rule of topography could be applied to corticothalamic projections of non-primary auditory areas. Moreover, collateral projections to the thalamic reticular nucleus (TRN) appeared topographic in relation to cortical tonotopy. Since our previous study lacked physiological determination of cortical tonotopy, the rule of topography has yet to be verified. The present study was aimed to reinvestigate corticothalamic projections of physiologically identified multiple tonotopic fields, and consequently to delineate similarities and differences in the pattern of corticothalamic projections between tonotopically compa-

rable subfields in the primary and non-primary auditory areas.

To highlight topography and comparative features of corticothalamic projections of multiple tonotopic fields, we focused on examining cortical subfields that represent similar high or low best frequency (BF) in the primary (AI) and non-primary auditory areas. The rostral part of area Te1 (AI) and posterodorsal auditory area (PD) (Horikawa et al., 1988) were selected as a pair of tonotopically comparable cortical subfields representing high BF in the primary and non-primary areas. PD has been delineated in the dorsal fringe (area Te2D) of area Te1 as a cortical region that receives thalamic afferents specifically from the suprageniculate nucleus (SG) and the dorsal division (MGD) of the MG (Kimura et al., 2003). We have further suggested that PD is uniquely involved in auditory spatial processing through its efferent connections (Kimura et al., 2004). The caudal end of area Te1 (AI) and the ventral margin of the rostral part of area Te1 were selected as a pair representing similar low BF. Based on previous mappings of cortical tonotopy (Horikawa et al., 1988; Rutkowski et al., 2003), the ventral margin of the rostral part of area Te1 is considered a part of anterior auditory field (AAF). Like PD, the cortical region appears to receive thalamic afferents specifically from the MGD (Kimura et al., 2003, 2004). In order to minimize tissue damage during exploratory mapping of cortical tonotopy, we applied recording of epicortical field potentials evoked by pure tones for measurement of BF (Kimura et al., 2004). After determining BF, biocytin was iontophoretically injected into the cortex to produce anterograde labeling in the MG and TRN. The results would provide insight into the corticothalamic connectivity of multiple tonotopic fields.

EXPERIMENTAL PROCEDURES

Surgical preparation

All studies were carried out in accordance with the approved institutional animal care and use protocol of Wakayama Medical University. All experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23; revised 1996) and the guidelines on the ethical use of animals of Japanese Government Notification, and all efforts were made to minimize the number of animals used for experiments and their suffering. Experiments were performed on Wistar rats weighing 268–388 g (mean=305 g). After an initial bolus injection of Nembutal (5–8 mg/100 g body weight, i.p.), chloral hydrate (4–6 mg/100 g body weight/h, i.p.) and Nembutal (0.5–0.8 mg/100 g body weight/h, i.p.) were injected continuously through a cannula placed in the abdomen, using a microinjection pump to avoid supplemental bolus injections and minimize fluctuations of anesthetic level. The animals were maintained in an areflexic state throughout the experiment. The animals were mounted on a stereotaxic apparatus. To avoid damage of tympanic membrane, ear bars with hollow and blunted center were used. The cisterna magna was drained to reduce edema and pulsations of the brain. To record epicortical field potentials evoked by pure tones and inject biocytin into the cortex, a large opening was made in the parietotemporal bone, from 3.0–7.5 mm posterior to bregma, and the dura mater was removed. A local anesthetic (2% Xylocaine) was infiltrated in all wounds. After surgery, the head of animal was suspended in a way that allowed the ear canal contralateral to the recording side to be open to sound stimuli.

Stimulation

Pure tones (frequency, 2, 4, 10, 15, 20, 30, 40 and 50 kHz; duration, 100 ms including 5 ms rise and fall time) were generated by a sound stimulator (DPS 725, Dia Medical System, Tokyo, Japan) and delivered from a free-field speaker (PT-R9, Pioneer, Yamagata, Japan). Intensity was calibrated by free field measurements, using a 1/4 inch condenser microphone (Brüel and Kjaer type 4939). The sound system could effectively deliver sounds from 2 to 50 kHz. The efficacy was flat within $\pm$5 dB between 2 and 50 kHz. The speaker was placed lateral to the ear contralateral to the recording side.

Recording and tracer injection

A tungsten-in-glass electrode (impedance, 0.8–2 MΩ) was used for recording epicortical field potentials evoked by pure tones, and, for tracer injection, it was replaced with a glass capillary (tip diameter, 20–30 μm) filled with 4% biocytin (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 0.9% saline. To estimate BF (de Ribaupierre, 1997), a response profile was made by measuring averaged amplitudes of field potentials evoked by pure tones with various intensities (Galván et al., 2002; Noreña and Eggermont, 2002). A series of 10 stimuli (interstimulus interval, 3–5 s) was delivered to obtain an averaged field potential (Figs. 4 and 7) for a given combination of frequency and intensity. After recording field potentials, the capillary was inserted into the cortex perpendicularly to the cortical surface to inject biocytin. To indicate the other recording site that exhibited a response profile distinguished from that obtained at the injection site, an electrolytic lesion was made in a cortical depth close to the injection site by delivering 10 μA cathodal current for 15 s through the tungsten-in-glass electrode.

Recordings were amplified ($\times$10,000), filtered (10–100 Hz), digitized (20 kHz) through an A-D converter (PCI-MIO-16XE-10, National Instruments, Austin, TX, USA) and stored on a computer for on- and off-line analysis using custom-made programs (LabVIEW, National Instruments) that allowed for measurement of averaged field potentials (Kimura et al., 2004).

Biocytin was injected at two cortical depths (0.6 and 1.2 mm) by delivering 10–20 μA anodal current (7 s on and 7 s off) for 15–20 min. Following completion of injection, the capillary was removed and the wound was closed. The animals were administered antibiotics (gentamicin sulfate, 20 mg/kg body weight, s.c.) and given post-operative care.

Histology

After a survival period of 24 h, the animals were deeply anesthetized with an overdose of Nembutal (150 mg/kg body weight, i.p.) and perfused transcardially with 300 ml of 0.9% saline containing heparin (300 IU), followed by 200 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4). The brains were removed from the skull and postfixed overnight in the same fixative. After storage at 4 °C in 30% sucrose, 0.1 M PB for 3–4 days, the frozen brains were cut at a thickness of 50 μm in the coronal plane with a freezing microtome. The sections were incubated in 1% H_2O_2 in 10 mM PB and saline (PBS) for 1 h to suppress endogenous peroxidase activity. All sections were then incubated in 10 mM PBS containing avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA) and 1% Triton X-100 at room temperature for 2 h (Horikawa and Armstrong, 1988). After bathing in 0.1 M Tris buffer (TB, pH 7.4) containing 0.5% cobalt acetate for 10 min, the sections were processed with 0.02% diaminobenzidine tetrahydrochloride (Sigma), 0.1% H_2O_2 and ammonium nickel sulfate in 0.1 M TB at room temperature for 20–30 min, to visualize labeling.