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Interaural phase difference modulates the neural activity in the nucleus angularis and improves the processing of level difference cue in the lateral lemniscal nucleus in the chicken

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ABSTRACT

We investigated the chicken auditory system to understand how an interaural level difference (ILD) is processed. Sound intensity is extracted in the nucleus angularis (NA) and an ILD is processed in the dorsal lateral lemniscal nucleus (LLD). We found that the neural activity in these nuclei is affected by the interaural phase difference (IPD). Activity in the NA was suppressed by strong contralateral sound when binaural stimuli were presented in-phase, but the activity was enhanced by out-of-phase stimuli. These IPD dependent suppression or enhancement probably occurs through acoustic interference across the interaural canal connecting the middle ears of the two sides. The LLD neurons were excited by contralateral sound and inhibited by ipsilateral sound, reflecting excitation by the contralateral NA and inhibition from the ipsilateral NA, probably through the contralateral LLD as in the barn owl. The LLD unit activity encoded an ILD and was strongly modulated by the IPD. We propose a simple model to explain the interaural coupling effects and IPD modulation of LLD activity, and conclude that the modulation of neuronal activity by IPD may improve ILD processing and the direction sensitivity of LLD neurons to the contralateral ear, compensating for the small ILD cues.

1. Introduction

Sound source localization is an important auditory function. The direction from which sound emanates affects the level and arrival time of sound between the ears. These two cues, known as interaural level difference (ILD) and interaural phase difference (IPD), are used by the auditory nervous system for sound source localization (Rayleigh, 1907; Klump, 2000; Yin, 2002; Konishi, 2003). These interaural cues depend on the animal's head size, with small-headed animals having small cues. Some small-headed mammals, such as bats and rodents, hear high-frequency sound; a small diffraction of the sound around the head creates a large level difference between two ears for high frequencies (Ehret and Dreyer, 1984; Heffner and Heffner, 1992; Firzlaff and Schuller, 2004). On the other hand, most birds have small heads but use low-frequency sound, below 5 kHz, for communication (Dooling et al., 2000). The interaural level differences at these frequencies are small, suggesting that the neural coding strategies for this cue must be specialized.

Some enhancement of the binaural cues occurs due to acoustic interactions within the interaural canal, an air-filled connection between the two middle ear cavities in birds (Klump, 2000). The difference between the external and internal pressure at the tympanic membrane depends on the phase difference of sound between two ears, even when the sound pressure level is the same. It has been hypothesized that the direction of sound can be detected, in part, at the tympanic membrane due to the difference in the acoustic path length and the coupling between the two ears by the interaural canal (Coles and Guppy, 1988; Hyson et al., 1994; Larsen et al., 2006; Koppl and Carr, 2008). In the barn owl, however, the effects of acoustic coupling are negligible above 3 kHz because of a sharp attenuation of coupling in the sound frequency ranges important for sound localization in this species (Moiseff and Konishi, 1981), though clear directionality was reported at the level of cochlear microphonics (Coles and Guppy, 1988). Despite the wealth of information about interaural coupling, few studies have focused on the effect of interaural phase difference on the processing of level difference cue in such cases.

Abbreviations: ANF, auditory nerve fibre; BF, best frequency; ILD, interaural level difference; IPD, interaural phase difference; NA, nucleus angularis; NL, nucleus laminaris; LLDa, anterior part of the dorsal lateral lemniscal nucleus; LLDp, posterior part of the dorsal lateral lemniscal nucleus; LLD, dorsal lateral lemniscal nucleus; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; SLu, nucleus semilunaris; SPL, sound pressure level; VLV, ventralis lateral lemniscus.

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In the barn owl, sound level information is extracted in the nucleus angularis (NA) and the level difference between two ears is processed in the posterior portion of the dorsal lateral lemniscal nucleus (LLDp), which receives excitatory input from the contralateral NA and inhibitory input from the ipsilateral NA via the contralateral LLDp (Fig. 1A; Manley et al., 1988; Takahashi and Konishi, 1988; Mogdans and Knudsen, 1994; Konishi, 2003). The anterior portion of the dorsal lateral lemniscal nucleus receives projections from the contralateral nucleus laminaris (NL; Takahashi et al., 1987). However, a clear distinction between the anterior and posterior portions of the lateral lemniscal nucleus has not been made in the chicken (Conlee and Parks, 1986). We followed new nomenclature introduced for the lemniscal nuclei by Arends and Zeigler (1986). With this terminology, the lateral lemniscal nucleus is subdivided into three sub-nuclei: ventral, intermediate, and dorsal nucleus of the lateral lemniscus (LLD). This LLD is the same as the nucleus ventralis lateral lemniscus (VLV) in the previous nomenclature for the chicken and barn owl (Conlee and Parks, 1986; Takahashi and Konishi, 1988; Wild et al., 2001).

We measured the effect of the sound phase difference between two ears on the processing of sound level difference in the chicken.

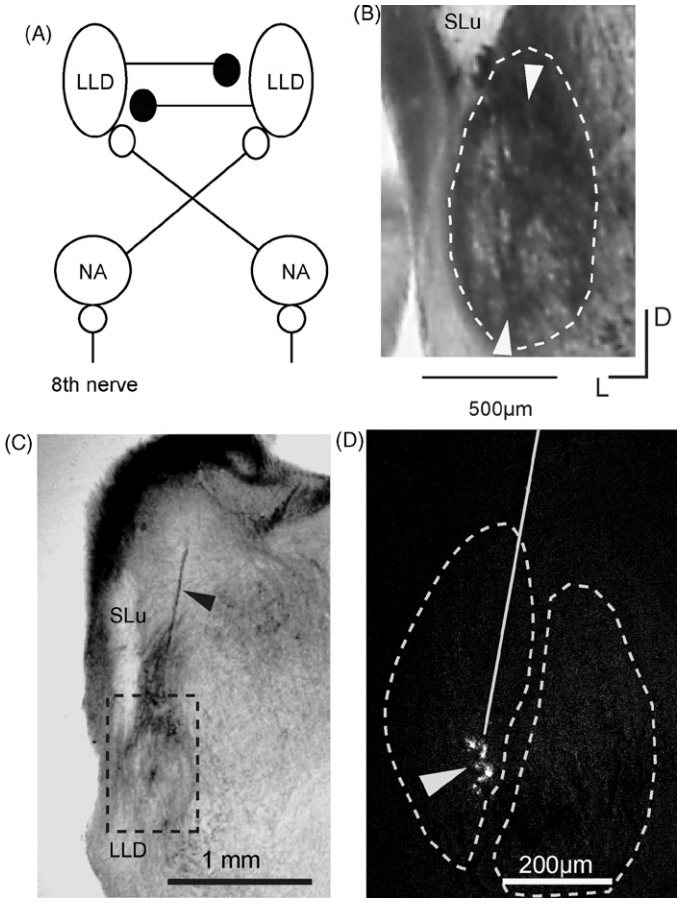

Fig. 1. Diagram of fibre projections and the recording site in the LLD. (A) Schematics to show the excitatory projections from the nucleus angularis (NA) to the dorsal lateral lemniscal nucleus (LLD) and inhibitory projections between the bilateral LLDs. Open circles indicate excitatory projections; filled circles represent inhibitory projections. (B) A bright field image of the LLD. Arrowheads indicate fibre projections separating the LLD into two parts. A region of the LLD is encircled. SLu, nucleus semilunaris. The orientation bars are for (B–D). d, Dorsal; l, lateral. (C) Bright field image showing an electrode track (arrowhead). The square indicates a region of the LLD shown in d. (D) Fluorescence image of the LLD. The arrowhead indicates Alexa488 fluorescence particles. A solid line indicates the electrode track. Background auto-fluorescence was removed by image processing (see Section 2). The nuclei boundaries and fibre projections in-between are drawn by broken lines, in reference to the Nissl staining of adjacent slices and background signal.

Responses in both NA and LLD neurons were sensitive to the sound level difference and were affected by the sound phase difference between two ears. Furthermore, we hypothesized that the sensitivity to the sound phase difference may improve the direction sensitivity of LLD neurons by binaural acoustic interference through the interaural canal in the chicken.

2. Materials and methods

2.1. Animals

Recordings in the NA and LLD neurons were made in 48 juvenile chickens aged 3–5 days post-hatch (P3–5). Chickens were anaesthetized by an intra-muscular injection of chloral hydrate (160 mg/kg); in some cases, an additional dose was injected (50 mg/kg) to maintain the level of anaesthesia. The bird's cloacal temperature was monitored and maintained (40 °C) with a heating pad. The surgical procedures were described previously (Fukui et al., 2006). These procedures conformed to the principles for the care and use of animals in the field of physiological sciences set by the Japanese Physiological Society.

2.2. Unit recordings

The NA was accessed by tilting the beak 30° downward around the axis connecting the two ear openings, and the electrode was inserted 1.0–2.0 mm lateral and 0.5 mm rostral to the inion. The electrode was tilted 5° lateral to the vertical angle. The LLD was accessed by tilting the beak 45° downward. The electrode was tilted 5° laterally and inserted 1.5–2.5 mm lateral and 0.5–1.0 mm rostral to the inion.

The recording electrodes were made from quartz capillaries (No. 100-70-10, Sutter Instrument, California, USA), pulled (P-2000, Sutter Instrument), and filled with 1 M sodium chloride (electrode impedance, 8–20 MΩ) containing dextran Alexa488 (5% w/w, Molecular Probes, Oregon, USA). The recording sites were labelled by ionophoretic dye injection (−200 nA, 1 s duration, 2 s intervals for 20 min) and confirmed by post hoc visualization of the electrode track and bright fluorescent particles (Fig. 1C and D) in brainstem slices. The fluorescence of Alexa488 was extracted from the background by image processing, which un-mixed the Alexa488 image and the auto-fluorescence image in separate colour channels by utilizing the spectral pattern of the dye sampled by a 3CCD camera (C7780-20, U9677, Hamamatsu Photonics, Hamamatsu, Japan). The brainstem slices (100-μm-thick) were made with a cryo-microtome (Leica CM 3050) after perfusion of the animal with 4% formaldehyde in PBS through the heart and cryoprotection (30% w/w sucrose in PBS). The number of electrode insertions was limited to a maximum of three to facilitate the identification of the electrode tracks. Recordings were made from a single nucleus, either NA or LLD, in each animal. Most NA units were recorded from the left NA (47 of 67 units), and most LLD units were recorded from the right LLD (45 of 49 units).

Single unit activity was recorded extracellularly as described previously (Fukui et al., 2006) using an AxoClamp-2B amplifier (Molecular Devices, California, USA). Signals were band-pass filtered (2-pole) between 150 Hz and 5 kHz. Data were collected with 12-bit resolution at a 50 kHz sampling frequency. Sound presentation and data acquisition were controlled by customized software written in MATLAB.

2.3. Sound stimulation

Sound stimuli were presented through a pair of hollow ear bars in a closed acoustic system using a pair of earphones (EF-1935, Knowles Electronics Japan, Tokyo, Japan). We adopted closed field