Auditory frequency-following responses in rat ipsilateral inferior colliculus

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Auditory frequency-following responses (FFRs) are sustained potentials based on phase-locked neural activity preserving low-frequency information. Some neurons in rat inferior colliculus are excited by stimuli at either ear. This study shows that FFRs in inferior colliculus can be elicited by presenting pure tone bursts with frequencies from 225 to 4025 Hz at the ipsilateral ear in anesthetized rats. Moreover, chemical block of glutamate transmissions in the contralateral inferior colliculus markedly reduced the ipsilaterally driven FFRs, which, however, were significantly enhanced by blocking the contralateral dorsal nucleus of the lateral lemniscus. Thus, FFRs in inferior colliculus to ipsilateral stimulation were facilitated by excitatory projections from the contralateral inferior colliculus but suppressed by inhibitory projections from the contralateral dorsal nucleus of the lateral lemniscus. *NeuroReport* 19:1377–1380 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The frequency-following response (FFR) is a short latency response that mimics many acoustic waveform properties [1,2]. Human studies show that the FFR can be affected by top-down influences because of selective attention [3–5], language experience [6], and clinical syndromes such as Rett's syndrome [7] and learning problems [8]. However, in humans it is very difficult to investigate the neural mechanisms underlying the mediation or modulation of FFRs at the circuitry level. Thus, establishing animal models is critical in this line of investigation.

Among the important auditory nuclei, the inferior colliculus is the last nucleus in the primary auditory pathway where FFRs can reliably be recorded, because precise neural phase-locking is increasingly lost at successively higher levels of the brainstem-to-cortex pathway [9]. Moreover, comparisons of recordings in auditory nuclei and far-field placements implicate inferior colliculus as a primary source of scalp-recorded FFRs [10,11].

However, specific coding mechanisms in inferior colliculus underlying FFRs remain elusive, especially the dynamic interaction of excitatory and inhibitory influences converging on the central nucleus of the inferior colliculus. The present research directly evaluates the major excitatory projection from the contralateral central nucleus of the inferior colliculus through the commissure of inferior colliculus, as well as the contralateral dorsal nucleus of lateral lemniscus that has been shown to be an important GABAergic nucleus in the auditory brainstem of the rat and other mammals [12–15]. Effects of these projection pathways were studied by microinjecting the broad-spectrum glutamate receptor blocker kynurenic acid (KYNA) into the contralateral central nucleus of the inferior colliculus and the contralateral dorsal nucleus of the lateral lemniscus.

Methods

Animal preparation

Experiments were conducted in 24 male adult Sprague–Dawley albino rats (300–400 g) obtained from the Beijing Vital River Experimental Animals Technology Ltd. (Beijing, China). They were assigned randomly to two parts of the research: experiment 1 (n=12) and experiment 2 (n=12). Animals were housed individually in a 12-h light–dark cycle (lights on at 7:00 h) with ad-libitum food and water and were allowed 1 week to adapt to the laboratory environment before surgery. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The anesthetic and experimental protocol met all requirements regarding the care and use of small animal subjects in accordance with guidelines of the Beijing Laboratory Animal Center, guidelines of the Canadian Council of Animal Care, and the Policies on the Use of Animals and Humans in Neuroscience Research revised and approved by the Society for Neuroscience (1995).

Stimulus conditions

Pure tones (duration: 40 ms; ramps: 5 ms; intensity: 70 dB SPL) were generated by a TDT system II (Tucker-Davis

Technologies, Alachua, Florida, USA), presented at a rate of 10/s, and delivered through a calibrated earphone (ED1). One end of the 12-cm TDT sound-delivery soft tube was connected to the ED1 earphone, and the other end was inserted into the ear canal on the side ipsilateral to the recording electrode. TDT software (SigCal, SigGen and BioSig, Tucker-Davis Technology) was used to calibrate the earphone, generate acoustic stimuli, monitor neural response properties online, and store data for offline analysis.

Drug administration and electrophysiological recording

Animals were anesthetized with 10% chloral hydrate (400 mg/kg, intraperitoneally) for the initial surgical procedure and a state of areflexia was maintained throughout the experiment by supplemental injections (0.1 ml per hour, intraperitoneally). The animals were fixed in a Kopf stereotactic head holder. A midline incision was made in the scalp, the skin and muscles retracted laterally, and small craniotomies made on the dorsal surface of the skull. Steel electrodes (10–30 kΩ), insulated except at the 0.25-mm diameter tip, and cannula guides were lowered to target nuclei and the assembly fastened to the skull with dental acrylic.

The following two brain structures were approached vertically based on coordinates referring to bregma: (i) central nucleus of the inferior colliculus (electrode ipsilateral to ear stimulated, cannula contralateral to ear stimulated): anteroposterior=−8.80 mm, mediolateral=±1.50 mm, dorsoventral=−4.50 mm and (ii) dorsal nucleus of the lateral lemniscus (cannula contralateral to ear stimulated): anteroposterior=−8.72 mm, mediolateral=±3.00 mm, dorsoventral=−6.80 mm [16].

Drug administration was via the guide cannula connected to a 5-μl microsyringe through polyethylene tubing (inner diameter: 0.38 mm, outer diameter: 1.09 mm; Clay Adams Division, Becton-Dickinson and Co., Parsippany, New Jersey, USA). A total of 1–2 μl KYNA (1 mM) or Locke's solution was injected slowly over a period of approximately 1 min.

FFR recordings were carried out in a sound-attenuating chamber 30 min after surgery during the light phase (8:00–18:00 h). Brain potentials were digitized (20 kHz), amplified (1000×), filtered (0.1–5 kHz bandpass), and averaged (N=500 stimulus repetitions).

Experiment 1: effects of blocking the contralateral inferior colliculus

Twelve rats were randomly divided into two groups that received (i) microinjection of KYNA into the contralateral central nucleus of the inferior colliculus (n=6) and (ii) microinjection of Locke's solution into the contralateral central nucleus (n=6). FFRs were recorded from the ipsilateral central nucleus. Based on the results of our other experiments (not shown here), FFRs were assessed by presenting each of the seven effective pure tone frequencies to the ipsilateral ear: 225, 425, 625, 825, 1025, 2025, and 4025 Hz.

Experiment 2: effect of blocking the contralateral dorsal nucleus of the lateral lemniscus

Twelve rats were randomly divided into two groups that received (i) microinjection of KYNA into the contralateral dorsal nucleus of the lateral lemniscus (n=6) and (ii) microinjection of Locke's solution into the contralateral dorsal nucleus (n=6). All other experiment details were identical to experiment 1.

Data analysis

All neural responses were transformed from time domain into the frequency domain by fast Fourier transformation and the peak spectral value at the center frequency of each stimulus was taken as the measure of FFR amplitude.

A 2×7 (drug by frequency) within-subject analysis of variance was carried out to test for main effect of drug administration and possible interactions.

If the interaction was significant, an additional simple effect analysis was carried out to test for differences at each frequency.

Histology

At the end of testing, each animal was sacrificed with an overdose of chloral hydrate. Lesions were made at the recording electrode tip by an anodal DC current (500 μA for 10 s). The brains were removed, stored in 10% formalin with 30% sucrose until they sank, and then sectioned at 50-μm intervals in the frontal plane in a cryostat (−20°C) and the sections examined to assess electrode placements in inferior colliculus, as well as injection canal placements in inferior colliculus and dorsal nucleus of the lateral lemniscus.

Results

Histology

Histological results confirmed that all recording electrodes and injection cannuli reached the targeted nuclei.

Frequency-following responses

The results of experiments 1 and 2 show that marked FFRs were recorded in the central nucleus of the inferior colliculus between 0.2–4 kHz, which is clearly evident in both the time and frequency domains (representative sample at 425 Hz shown in Fig. 1).

Effects of blocking the contralateral inferior colliculus

In experiment 1, injection of KYNA in the contralateral central nucleus of the inferior colliculus resulted in a significant overall decrease of FFR amplitudes in the ipsilateral central nucleus, $F(1,5)=23.70$, $P<0.05$. The interaction between drug and frequency was also significant, $F(6,30)=3.70$, $P<0.05$. The results show that decreases occurred at all frequencies (Fig. 2), but post-hoc analyses show that the difference was significant only at 425 and 625 Hz. In contrast, after injection of Locke's solution, no significant change in FFR amplitude, recorded in the ipsilateral central nucleus of the inferior colliculus, occurred at any frequency.

Effects of blocking the contralateral dorsal nucleus of the lateral lemniscus

In experiment 2, injection of KYNA in contralateral dorsal nucleus of the lateral lemniscus resulted in a significant increase in FFR amplitudes (Fig. 3), $F(1,5)=21.81$, $P<0.05$. The interaction between drug and frequency was also significant, $F(6,30)=10.07$, $P<0.05$. Post-hoc analysis showed the difference was significant at 425, 625, 825, and

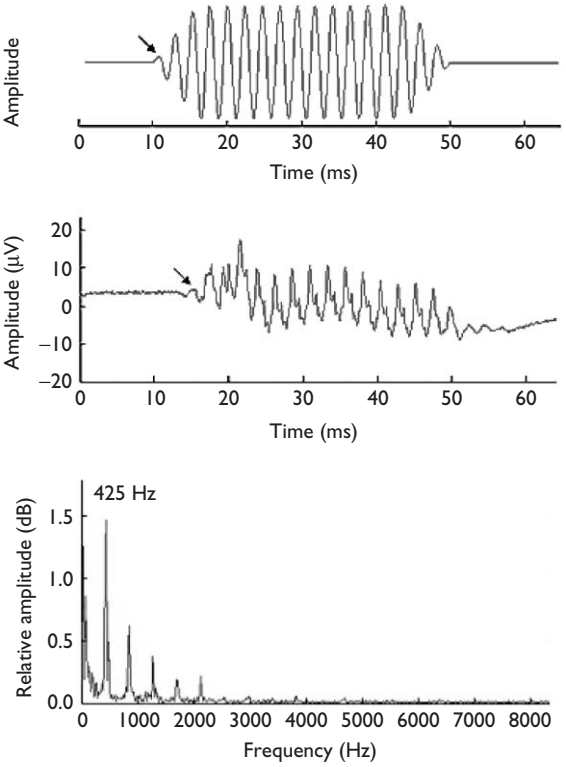

Fig. 1 Example of frequency-following response (FFR) recorded in the central nucleus of the inferior colliculus to a 425-Hz pure tone. Top panel: stimulus waveform (the arrow indicates the onset point of the sound); middle panel: response waveform (the arrow indicates the starting point of FFRs); bottom panel: spectrum of FFRs (displayed in the frequency domain). The results of spectral analyses at each peak stimulus frequency were used to quantify the FFR frequency response range as well as the effects of drug administration.

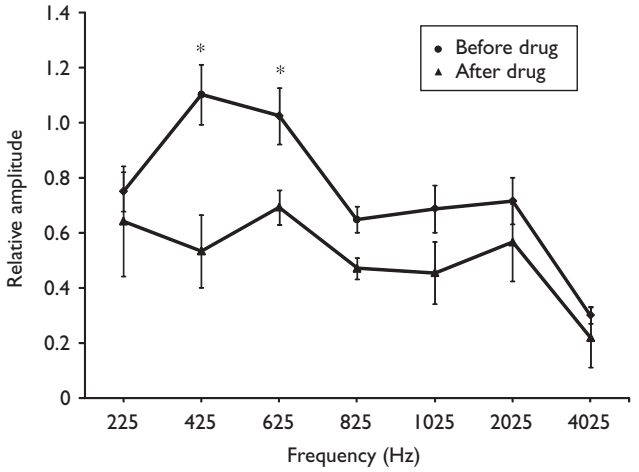

Fig. 2 Frequency-following response (FFR) amplitudes in ipsilateral central nucleus of the inferior colliculus before (filled circles) and after (filled triangles) administration of kynurenic acid (KYNA) into contralateral inferior colliculus. Results are presented for the frequency range (225–4025 Hz) with detectable FFR waveforms. *$P < 0.05$.

2025 Hz. In contrast, after the injection of Locke's solution no significant change in FFR amplitudes occurred at any frequency.

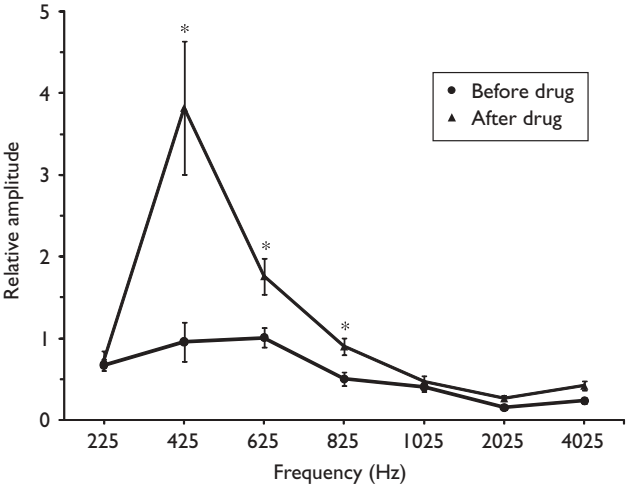

Fig. 3 Frequency-following response amplitudes in the ipsilateral central nucleus of the inferior colliculus before (filled circles) and after (filled triangles) administration of kynurenic acid into contralateral dorsal nucleus of the lateral lemniscus. *$P < 0.05$.

Discussion

FFRs reflect activity of phase-locked neurons in the auditory system. Single-unit recordings show that at each stage along the auditory pathway there is a reduction in the upper frequency limit of the phase-locking. Most phase-locked neurons in inferior colliculus are mainly located in the central nucleus, with a reported response upper limit of 1000 Hz [17]. However, the upper limit in the inferior colliculus has not been studied systematically and previous results seem inconsistent. Indeed, in this study the upper frequency range was 4 kHz, which is higher than earlier findings [2,18,19].

The principal findings of our study strongly indicate that the contralateral dorsal nucleus of the lateral lemniscus and the contralateral inferior colliculus are important nuclei in the formation and modulation of FFRs recorded in the ipsilateral central nucleus of the inferior colliculus. The two paired structures of the inferior colliculus are interconnected by fibers in the commissure of the inferior colliculus. Previous studies have suggested that the commissure of the inferior colliculus interconnects corresponding frequency-band laminae in the two central nuclei and blockade of the commissure of the inferior colliculus influences frequency response areas, spike rate, and monotonicity of neurons in the central nuclei [20,21]. Stimulating fibers of the commissure of the inferior colliculus elicits both inhibitory and excitatory postsynaptic potentials [22,23]. However, there is evidence that this commissural projection is mainly glutamatergic [15,24].

In experiment 1, microinjection of KYNA into contralateral inferior colliculus blocked the neurotransmission to ipsilateral inferior colliculus via the commissure of the inferior colliculus. Consequently, FFR amplitudes in the ipsilateral inferior colliculus decreased significantly. In contrast, after microinjection of Locke's solution into the contralateral inferior colliculus, there was no discernible effect on the FFR amplitudes. These results confirm that the contralateral inferior colliculus is a primary excitatory source affecting ipsilateral inferior colliculus.

In contrast, neurons in the dorsal nucleus of the lemniscus are largely, if not exclusively, GABAergic, making the dorsal

nucleus a major source of inhibitory projection to contralateral as well as ipsilateral inferior colliculus [15].

In experiment 2, microinjection of KYNA into contralateral dorsal nucleus blocked the inhibitory effect of neurotransmission to ipsilateral inferior colliculus. Consequently, FFR amplitudes in the ipsilateral central nucleus increased significantly. In contrast, microinjection of Locke's solution into contralateral dorsal nucleus had no discernible effect. These results confirm that inhibitory projections from the contralateral dorsal nucleus of the lateral lemniscus normally act to suppress the FFRs in ipsilateral inferior colliculus.

Conclusion

The results of this study show that the upper limit of stimulus frequency for inducing FFRs in the inferior colliculus is up to 4 kHz, which is apparently higher than that reported by previous studies. The FFRs are modulated by excitatory projections from the contralateral inferior colliculus and inhibitory projections from the contralateral dorsal nucleus of the lateral lemniscus. These results may increase our understanding of the production of FFRs along the ipsilateral auditory pathway. In future, potential contributions of other auditory brainstem nuclei to the formation of FFRs, such as the ipsilateral medial superior olivary complex [25] and the ipsilateral lateral superior olivary complex [14], need further investigation.

Acknowledgements

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