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BOLD fMRI study of ultrahigh frequency encoding in the inferior colliculus

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ABSTRACT

Many vertebrates communicate with ultrahigh frequency (UHF) vocalizations to limit auditory detection by predators. The mechanisms underlying the neural encoding of such UHF sounds may provide important insights for understanding neural processing of other complex sounds (e.g. human speeches). In the auditory system, sound frequency is normally encoded topographically as tonotopy, which, however, contains very limited representation of UHFs in many species. Instead, electrophysiological studies suggested that two neural mechanisms, both exploiting the interactions between frequencies, may contribute to UHF processing. Neurons can exhibit excitatory or inhibitory responses to a tone when another UHF tone is presented simultaneously (combination sensitivity). They can also respond to such stimulation if they are tuned to the frequency of the cochlear-generated distortion products of the two tones, e.g. their difference frequency (cochlear distortion). Both mechanisms are present in an early station of the auditory pathway, the midbrain inferior colliculus (IC). Currently, it is unclear how prevalent the two mechanisms are and how they are functionally integrated in encoding UHFs. This study investigated these issues with large-view BOLD fMRI in rat auditory system, particularly the IC. UHF vocalizations (above 40 kHz), but not pure tones at similar frequencies (45, 55, 65, 75 kHz), evoked robust BOLD responses in multiple auditory nuclei, including the IC, reinforcing the sensitivity of the auditory system to UHFs despite limited representation in tonotopy. Furthermore, BOLD responses were detected in the IC when a pair of UHF pure tones was presented simultaneously (45 & 55 kHz, 55 & 65 kHz, 45 & 65 kHz, 45 & 75 kHz). For all four pairs, a cluster of voxels in the ventromedial side always showed the strongest responses, displaying combination sensitivity. Meanwhile, voxels in the dorsolateral side that showed strongest secondary responses to each pair of UHF pure tones also showed the strongest responses to a pure tone at their difference frequency, suggesting that they are sensitive to cochlear distortion. These BOLD fMRI results indicated that combination sensitivity and cochlear distortion are employed by large but spatially distinctive neuron populations in the IC to represent UHFs. Our imaging findings provided insights for understanding sound feature encoding in the early stage of the auditory pathway.

Introduction

Many vertebrates have evolved the ability to communicate with ultrahigh frequency (UHF) vocalizations to decrease the likelihood of detection by predators (Brudzynski, 2009, 2013; Gadziola et al., 2012; Portfors, 2007; Takahashi et al., 2010). For example, male mice emit vocalizations with frequencies up to 110 kHz when encountering female mice, and female mice produce frequency-modulated upsweeps above 60 kHz when their pups are removed from the nest (Portfors, 2007). Rats emit short chirping 50 kHz vocalizations during juvenile play, male agonistic behaviors and sexual behaviors (Brudzynski, 2009; Portfors, 2007). Recently, there is increasing interest in studying the neural mechanisms underlying the learning, production and representation of such vocalizations (Arriaga and Jarvis, 2013; Woolley and Portfors, 2013) because it may improve our understanding of complex sound (e.g., speeches) processing in humans (Rauschecker and Scott, 2009).

One important question is how the auditory system encodes UHFs, in order to process vocalizations that contain them (Romanski and Averbeck, 2009; Woolley and Portfors, 2013). In the auditory system, the majority of neurons are sensitive to only a narrow spectrum of sounds centered about a characteristic frequency (CF). The topographic alignment of the CFs (i.e., the tonotopy) is a fundamental organizing principle maintained from the cochlea to the auditory cortex (Ehret and Fischer,

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1991; Malmierca, 2003). For example, in the primary auditory nucleus in the midbrain, called the inferior colliculus (IC), which is a compulsory relay for all ascending auditory projections (Malmierca, 2003) and a region that efficiently encodes vocalizations (Holmstrom et al., 2010), the tonotopic organization is a basic feature in its central nucleus (CNIC), where the CFs of neurons run from low to high along the dorsolateral-ventromedial dimension (Cheung et al., 2012a, 2012b; De Martino et al., 2013; Malmierca et al., 2008; Ress and Chandrasekaran, 2013; Schreiner and Langner, 1997; Yu et al., 2005). In the cortical regions of the IC, the frequency organization is usually non-tonotopic, with neurons showing complex frequency tuning properties, such as broadband or multipeak receptive fields (Duque et al., 2012; Hernandez et al., 2005; Malmierca et al., 2011; Stebbings et al., 2014). Given the importance of UHFs in social communications, they are expected to occupy a substantial area in the tonotopic map of the CNIC, or the frequency receptive fields of neurons in non-tonotopic IC regions. But surprisingly, there is limited representation of UHFs in the auditory system of many species. In mouse IC, neurons with CF higher than 60 kHz are rarely found and occupy a very small tonotopic area compared to those with lower CFs (Stiebler and Ehret, 1985; Yu et al., 2005). Similarly, in rat IC, representations of frequencies higher than 40 kHz are seldom delineated (Cheung et al., 2012a, 2012b; Malmierca et al., 2008). This mismatch immediately poses a basic question how UHFs are encoded in the auditory system of these species.

Current evidence of UHF encoding is found in electrophysiological studies that probed neuronal responses to behaviorally relevant sound in the auditory system. In the mustached bat, neurons in the auditory cortex as well as the IC were found to display excitatory or inhibitory responses when two tones with different frequencies were presented simultaneously (two-tone paradigm) with one of them in the frequency range of bat biosonar or vocalizations (Leroy and Wenstrup, 2000; Mittmann and Wenstrup, 1995; Suga et al., 1979). This neuron property, i.e., responding best to the combination of two different frequency components, was called combination sensitivity (Leroy and Wenstrup, 2000; Portfors and Felix, 2005; Portfors and Wenstrup, 2002). Combination sensitivity was believed to allow neural representation of spectral and temporal information conveyed in echolocation signals or social vocalizations, and was suggested to be a common mechanism for processing complex sound (Portfors, 2004; Portfors and Wenstrup, 2002). Combination sensitive neurons were also found in mouse IC (Felix and Portfors, 2007; Portfors and Felix, 2005). In both bat and mouse, the facilitatory or inhibitory frequencies were often found to be in the UHF range (Portfors and Felix, 2005; Portfors and Wenstrup, 2002), indicating that combination sensitivity might be a neural mechanism underlying UHF encoding shared by different mammals.

Recently, it was observed that neurons in the central auditory system could also respond to the two-tone paradigm by exploiting cochlear nonlinearities. When simultaneously stimulated with two different frequencies (f1 and f2, f2 > f1), the cochlear characterized by mechanical nonlinearity will generate distortion tones with frequencies at e.g. f2–f1 (quadratic) (Robles et al., 1991). The distortion products can propagate along the cochlea to their CF site on the basilar membrane and then excite neurons with the same CF at higher levels of the auditory pathway, including the cochlear nucleus (Faulstich and Kossl, 1999), the IC (Abel and Kossl, 2009; McAlpine, 2004) and the auditory cortex (Purcell et al., 2007). It has been shown that at least in some cases, cochlear distortion products were responsible for the neuronal activities that were thought to arise from combination sensitivity (McAlpine, 2004). It was also noticed that some IC neurons could respond to the cochlear distortion of UHF tones (Portfors and Roberts, 2014; Portfors et al., 2009), suggesting that cochlear distortion could also be a mechanism underlying UHF encoding.

By demonstrating the neuronal responses in multiple auditory nuclei that arise from combination sensitivity and cochlear distortion, the above-mentioned studies provided important clues for understanding UHF encoding. However, it remains unclear how prevalent these mechanisms are in an auditory nucleus, since relatively small numbers of neurons were usually recorded in those electrophysiological studies. Furthermore, the previous studies were limited by the large variation of frequency values used in the two-tone paradigm. Since different neurons display different tuning properties, one of the two frequencies was usually fixed at the CF or limited in the frequency range of a vocalization that the neuron was sensitive to (Portfors and Felix, 2005; Portfors et al., 2009; Portfors and Wenstrup, 2002). This strategy could effectively reduce the large number of combinations to test, but also induced large variation in the frequency values tested in a study. With such limitations, it was not addressed whether UHFs are encoded in a generally organized way in the auditory system as like the tonotopic encoding of lower frequencies is, or only particular UHFs can be encoded by some specialized neurons. Moreover, it remains unknown how the two mechanisms are combined by the auditory system in processing UHFs, e.g. whether they are adopted by spatially different neuron populations or the same set of neurons at different temporal stages.

Blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) (Ogawa et al., 1990) with large field of view is a non-invasive technique that can measure the hemodynamic responses as neural correlates (Logothetis et al., 2001) in multiple brain structures with relatively high spatial and temporal resolutions. Recent development in BOLD fMRI has been demonstrated for studying the rodent auditory system (Cheung et al., 2012a, 2012b; Gao et al., 2014; Lau et al., 2013, 2015; Yu et al., 2009; Zhang et al., 2013), along with previous auditory fMRI studies conducted on humans (Barton et al., 2012; De Martino et al., 2013; Sigalovsky and Melcher, 2006), primates (Baumann et al., 2011; Kayser et al., 2007; Tanji et al., 2010) and songbirds (Boumans et al., 2007; Van Meir et al., 2005; Voss et al., 2007).

In this study, BOLD fMRI was applied to investigate the responses to UHF sounds in rat auditory system. Rat UHF vocalizations, single UHF pure tones, and pairs of UHF tones interleaved with pure tones equivalent to their quadratic distortion products were presented to the animals. The UHF values were systematically varied between 45 kHz and 75 kHz with 10 kHz steps, in order to examine the organization of UHF representation that may potentially exist. The findings were mainly focused on the IC and lateral lemniscus (LL), a subcollicular auditory nucleus that projects to the IC (Malmierca, 2003).

Methods

Animal preparation

All animal experiments were approved by the local animal research ethics committee. Animals were prepared for fMRI experiments as described in our previous studies (Chan et al., 2010; Cheung et al., 2012a, 2012b; Gao et al., 2014; Lau et al., 2011, 2013, 2015; Zhang et al., 2013; Zhou et al., 2012, 2014). Briefly, male Sprague-Dawley rats (300–350 g, N = 10) were used in this study. They were initially anesthetized with 3% isoflurane and then mechanically ventilated via oral intubation. They were then placed on a holder in the prone position with a tooth bar to restrict head motion. Throughout the course of MR scanning, anesthesia was maintained with 1.2% isoflurane and warm water was circulated to maintain the animal temperature constant. Animal heart rate, respiration rate, oxygen saturation and rectal temperature were continuously monitored (SA Instruments).

Auditory stimulation

Auditory stimulation was controlled by a computer and produced by a pair of high-frequency magnetic speakers (MF1, TDT) driven by an amplifier (SA1, TDT) (Cheung et al., 2012a, 2012b; Gao et al., 2014; Lau et al., 2013, 2015; Zhang et al., 2013). Each speaker was connected to a 26 cm long rigid tube. The two tubes were merged by a Y-shape connector and catenated to a 144 cm long rigid tube and then a 6.5 cm long flexible tube. Monaural stimulation was delivered through this custom-made tube system (see illustration in Supplementary materials) into