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Functional magnetic resonance imaging of auditory cortical fields in awake marmosets

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A B S T R A C T

The primate auditory cortex is organized into a network of anatomically and functionally distinct processing fields. Because of its tonotopic properties, the auditory core has been the main target of neurophysiological studies ranging from sensory encoding to perceptual decision-making. By comparison, the auditory belt has been less extensively studied, in part due to the fact that neurons in the belt areas prefer more complex stimuli and integrate over a wider frequency range than neurons in the core, which prefer pure tones of a single frequency. Complementary approaches, such as functional magnetic resonance imaging (fMRI), allow the anatomical identification of both the auditory core and belt and facilitate their functional characterization by rapidly testing a range of stimuli across multiple brain areas simultaneously that can be used to guide subsequent neural recordings. Bridging these technologies in primates will serve to further expand our understanding of primate audition. Here, we developed a novel preparation to test whether different areas of the auditory cortex could be identified using fMRI in common marmosets (*Callithrix jacchus*), a powerful model of the primate auditory system. We used two types of stimulation, band pass noise and pure tones, to parse apart the auditory core from surrounding secondary belt fields. In contrast to most auditory fMRI experiments in primates, we employed a continuous sampling paradigm to rapidly collect data with little deleterious effects. Here we found robust bilateral auditory cortex activation in two marmosets and unilateral activation in a third utilizing this preparation. Furthermore, we confirmed results previously reported in electrophysiology experiments, such as the tonotopic organization of the auditory core and regions activating preferentially to complex over simple stimuli. Overall, these data establish a key preparation for future research to investigate various functional properties of marmoset auditory cortex.

1. Introduction

The primate auditory cortex comprises anatomically and functionally distinct areas that form the foundation of audition (Morel et al., 1993; Rauschecker et al., 1995; Hackett et al., 1998a, 1998b; Romanski et al., 1999a; 1999b; Tian et al., 2001; for reviews see, Kaas and Hackett, 2000; Rauschecker and Tian, 2000). While neurophysiological studies show evidence for three adjacent tonotopically organized fields, A1, R, and RT (Morel et al., 1993; Aitkin et al., 1986) known as the auditory core, determining the functional contributions of secondary (belt) and tertiary (parabelt) processing fields have proven more challenging (Rauschecker et al., 1995; Rauschecker and Tian, 2004; Bendor and Wang, 2005; Tian and Rauschecker, 2004). Functional magnetic resonance imaging (fMRI) offers a complementary technique that can be used to facilitate neurophysiological research by rapidly characterizing multiple areas of the brain simultaneously and identifying patterns of responses that might not be readily identifiable with single-unit recordings (*e.g.*, Tsao et al., 2006), including the auditory system (*e.g.*, Perrodin et al., 2011). While this approach has been successfully employed in the rhesus monkey (Joly et al., 2012; Ortiz-Rios et al., 2015, 2017; Perrodin et al., 2011), its application to marmosets, a rapidly emerging model system in neuroscience (Miller et al., 2015, 2016; Miller, 2017; Bendor and Wang, 2008; Eliades and Miller, 2017), is likely to yield similarly important insights (*e.g.*, Hung et al., 2015a; 2015b). Because of the small size of the marmoset brain and acoustic interference prevalent in fMRI environments, however, it remains unclear whether distinct fields of the species

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auditory cortex could be distinguished with this method. Here we developed a novel preparation to test the suitability of fMRI for identifying small functional divisions across the marmoset auditory cortical fields.

The marmoset auditory core has been extensively explored using neurophysiological techniques (*e.g.*, Bendor and Wang, 2005; Bendor and Wang, 2008; Sadagopan and Wang, 2009; Zhou and Wang, 2012). Similarly to other primates, the species' auditory core comprises a series of three tonotopically organized fields whose borders can be identified by characteristic frequency reversals (Bendor and Wang, 2008). While these neurophysiological approaches have identified some functionally distinct areas of the marmoset auditory cortex, such as for pitch processing (Bendor and Wang, 2005; Bendor et al., 2012), delineation of the surrounding belt from the auditory core has been limited with these methods. The only previous auditory fMRI experiment in marmosets reported evidence of a vocalization selective response area (Sadagopan et al., 2015), however this study was performed in anesthetized animals, which could significantly affect the response characteristics of different auditory regions (*e.g.*, somatosensory system: Silva et al., 2011; Liu et al., 2013). It is not clear whether a study of awake marmosets would offer the level of precision evident in rhesus monkeys for identifying the auditory cortical fields (Petkov et al., 2006; Tanji et al., 2010), or if the confluence of the acoustic distortions intrinsic to the scanner environment and small brain size would severely limit the suitability of fMRI for marmoset auditory research.

In the current study, we sought to develop a preparation for imaging auditory cortex in the awake marmoset. Our goal was to replicate key findings from neurophysiological studies as a proof of principle that our preparation is effective for future fMRI research. Specifically, we aimed to reproduce frequency reversals in the auditory core (Bendor and Wang, 2008) and demonstrate selectivity for complex stimuli in the belt (Bendor and Wang, 2005; Bendor et al., 2012). We utilized a myelin atlas to illuminate the anatomical delineation of core and belt fields. Previous studies had shown that heavy myelination exists in the auditory core relative to the belt (*e.g.*, Kaas and Hackett, 2000). By registering our functional data to a myelin scan, we were able to visualize this boundary and make a coarse determination of what areas of auditory cortex were activated with specific types of stimuli. Our results reflect principles established with neurophysiological and anatomical techniques. We found frequency selective areas alternating along a caudal-rostral gradient in auditory cortex. Additionally, our results suggest that belt areas outside of the auditory core were activated to complex stimuli with our preparation. These findings establish that fMRI can be used as a complementary technique to neurophysiology to expand our understanding of the functional properties of marmoset auditory cortex.

2. Materials and methods

Magnetic resonance imaging methods. All fMRI experiments were performed in a 7T/30 cm magnet interfaced to an AVANCE AVIII MRI spectrometer (Bruker, Billerica, MA) equipped with a self-shielded 150 mm ID gradient set capable of generating 450 mT/m within 120 μs (Resonance Research Inc., Billerica, MA). An actively decoupled birdcage coil with an inner diameter of 110 mm was used as transmit coil, and the MR signal was acquired from two surface coils placed outside the helmets directly above auditory cortex. BOLD fMRI data were acquired continuously using a gradient-echo echo-planar imaging sequence (EPI). Eight slices were acquired and were oriented parallel to the lateral sulcus, as shown in Fig. 1A. Acquisition parameters for this experiment were: FOV: 2.88 × 2.88 cm^2, matrix: 96 × 96, slice thickness: 0.5 mm, resolution: 300 × 300 μm^2, TE: 26 ms, and TR: 3.6 s (Fig. 1B). All eight slices were acquired within the acquisition time TA = 462 ms so that, within each TR, a silent period of TR-TA = 3168 ms was observed during which auditory stimuli were presented. A 3.6 s TR was chosen based on peak of the marmoset hemodynamic response which is about 4 s (Liu et al., 2013).

Animal preparation. Three adult male common marmosets, weighing between 400 g and 550 g each, were used as subjects in these experiments. The subjects were adapted to the MRI scanner over a period of 30 days with a mock scanning environment described previously by Silva et al., (2011). Individualized, custom-made helmets were built (Papoti et al., 2013) to aid with head immobilization and headphone positioning. After the acclimatization period, the marmosets were scanned in fully awake conditions during all scanning sessions (Fig. 1C). Auditory stimulation was delivered bilaterally and directly into the ear canals through the use of MRI compatible headphones (STAX SR-003, Stax Ltd., Japan). Each headphone was covered with sound attenuating putty (Insta Putty Silicone Earplugs, Insta-Mold Products, Oaks, PA) in order to reduce the loudness of the scanner noise. The sound intensity level of the scanner was measured to be approximately 100 dB, with a center frequency around 2140 Hz. The putty attenuated the scanner noise by approximately 24 dB SPL. Each subject's physiological state was monitored during each scanning session by continuously acquiring its respiration rate (Biopac MP150, Biopac Systems, Inc., Goleta, CA) as well as by visual inspection of the animal via an MR compatible camera (MRC Systems, Heidelberg, Germany) placed in front of the animal's face. Experiments were in full compliance with the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke. Complete care was taken to ensure the wellbeing of the animals involved in these experiments. Two of three monkeys performed well during scanning and exhibited minimal movement. The third monkey moved excessively, and as a result contributed only a small amount of data for this study.

Stimulus presentation. Two types of stimuli were presented in this experiment: 1) A range of pure tones (PTs) and 2) band pass noise (BPN) (Fig. 1D). PTs were varied within three different frequency bands to constitute three different types of PT stimuli (high = 4–16 kHz; medium = 1–4 kHz; and low = 0.25–1 kHz). BPN was generated by band pass filtering random noise. The center frequency of each BPN stimulus was varied within the same frequency bands to control for spectral content. The bandwidth of each type of stimulus was two octaves. A 50 ms PT was randomly generated within each frequency band followed by 50 ms of silence, such that every 100 ms a new pure tone was played within that frequency band (Fig. 1D). BPN was also modulated in this manner (Fig. 1D). All stimuli were synthesized in MATLAB (Mathworks, Inc., Natick, MA). Stimuli were presented at sound intensity levels of 75–80 dB. All stimuli were presented according to a square off-on-off block design in which stimulation periods of 36s were alternated with silence periods of 36s while BOLD fMRI data were acquired continuously (TR = 3.6s) throughout each run (see Fig. 1B). The types of stimuli chosen were based on similarity to those used in other studies across species that successfully examined tonotopy and core/belt delineations (*e.g.*, Humphries et al., 2010; Bendor and Wang, 2008; Petkov et al., 2006; Rauschecker and Tian, 2004) and were well within the hearing range of the common marmoset (125 Hz–36 kHz: Osmanski and Wang, 2011).

Data analysis. Data were preprocessed and analyzed in AFNI (Cox and Hyde, 1997). Acquired volumes were motion corrected using AFNI's function 3dvolreg. Time points with outliers were found visually and with the function 3dToutcount and removed from the analysis. Data were detrended using the function 3dBandPass. Data were registered across sessions (14 runs for Champ, 10 runs for Eli, 4 runs for Scooby) using the function 2dimreg. Runs were concatenated and underwent a multiple linear regression using the function 3dDeconvolve. Six motion regressors were added to the analysis as regressors of no interest. Data were smoothed with the function 3dBlurToFWHM at 0.5 mm. Statistical maps were thresholded at $p < 0.05$ and then cluster thresholded at a size of 10 voxels to correct for multiple comparisons using AlphaSim at an alpha value of 0.05. Voxels outside of the brain were manually segmented and masked out using ITK-SNAP (Yushkevich et al., 2006). To determine whether high and low frequency-selective regions were present in our data, we compared the activation patterns that arose from the presentation of high (4–16 kHz) PT to that of low (0.25–1 kHz) PT stimuli. To