

FUNCTIONAL MRI OF THE SONGBIRD ZEBRA FINCH AT 3 TESLA

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Introduction

Birdsong in songbirds has emerged as a useful model of human speech in terms of its perception, acquisition and production. For example, simple models of speech motor control disorders are being developed in zebra finches.¹ We studied the feasibility of functional BOLD MRI in small song birds on a 3 Tesla whole body MRI scanner and found significant BOLD signal intensity changes for different kinds of stimuli across different brain regions. By quantifying the activation intensity, it was possible to discriminate between tutored song and other song stimulations. Finally, we discuss how the experienced limits of BOLD imaging of songbirds at 3T could be overcome.

Methods

Experimental set up: Nine male zebra finches were tutored by three different songs of increasing complexity (song "1"- "3"), each bird by one song. The birds were mildly anesthetized and immobilized in an in-house built RF coil. The coil was put into a foam/rubber compound sound isolation box. Auditory paradigms were delivered using a flash memory music player, a headphone volume booster, and a pair of Ti-diaphragm stereo headphones with a frequency response of 15-25000 Hz, with the magnets removed. The sound pressure level of the auditory stimuli was about 100 dB, the background noise during the EPI sequence about 83 dB. Visual paradigms were delivered to four birds using two white LED's and a parabolic reflector. The experiment was approved by the Institutional Animal Use and Care Committees of the three participating universities.

MRI parameters: BOLD sensitive Images were acquired on a GE Excite 3T scanner with 50 mT/m gradients using a four-shot 2D gradient echo EPI sequence with TE/TR = 18.5/1000 ms. (The effective repeat time was thus 4 s). Seven coronal slices of 0.8 mm thickness, 0.2 mm spacing, 4 cm FOV, and a matrix size of 128 x 128 were acquired. Slices were prescribed in rostral-caudal direction, covering the forebrain. The overall scan time per experiment was 512 s (128 repeats). Additionally, in-plane anatomical images (spin-echo sequence, TE/TR=14/367 ms, matrix size 256 x 192, four acquisitions) and phase maps² were acquired.

Paradigm: All stimuli were delivered in eight blocks consisting of a 32 s "on" and a 32 s "off" part, totaling 512 s. The auditory stimuli were the same three songs as used for tutoring the birds and a pure tone of 2 kHz, all played out repeatedly during the "on" part of each block. The visual stimulus was flashing at a rate of about 2 Hz during the "on" part. All birds were tested for all five stimulus types.

Postprocessing: Images were motion corrected using AFNI³ and further processed using in-house software written in MATLAB (The MathWorks): The data was spatially smoothed with a 2D Gaussian filter, temporally smoothed with a binomial filter, thresholded, and detrended. All eight block repeats were averaged. Statistical significance of activation was defined voxel-wise by comparing the mean signal intensity of the "on" state with that of the "off" state using a two-sided t-test. Only positive differences of the mean were considered. Volumes with false activations due to bulk motion and slices with false activations due to eye motion were discarded.

Results

Visual inspection of raw data averaged over all "on-off" blocks revealed frequent BOLD activations in several birds. Counting all voxels with significance $p < 0.0001$, the average BOLD effect was 5.7 % of the mean signal intensity for auditory and 5.3 % for visual stimuli. It was largest for pure tone stimulation (6.4 %) and smallest for the most complex song (Song 3, 5.2 %). For auditory stimuli, activations were mainly observed in the most caudal slices presumably corresponding to the auditory areas, including Field L, caudal medial nidopallium, caudal lateral mesopallium and caudal medial mesopallium, and song control nuclei, high vocal center and robust nucleus of arcopallium. Bilateral clusters of activations were observed in response to most stimuli (Fig. 1). By counting significantly activated voxels over the whole brain volume, we found that for all cases where a comparison between the three songs was possible, stimulation with the song used for tutoring the bird caused the

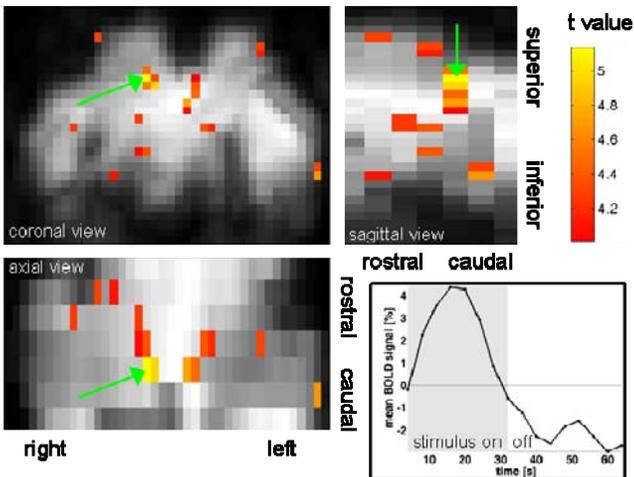


Figure 1 (color): Example for a maximum intensity projection of both the EPI images and color coded t-statistics along three orthogonal views. In all three views, the arrow points to the same voxel, located in a bilaterally activated forebrain area. The averaged time course of all activated voxels in this area is shown as well (average over the eight stimulation blocks and over voxels). All voxels shown are significant with $p < 0.0001$. This bird was both stimulated and tutored with song 2.

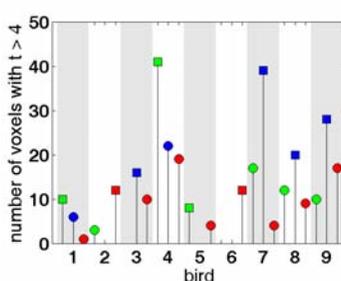


Figure 2 (color): Number of activated voxels in the whole brain for all nine birds and the three stimulation songs (green, song 1; blue, song 2; red, song 3). In all cases, stimulation with the tutored song (shown as a square symbol) caused the strongest activation.

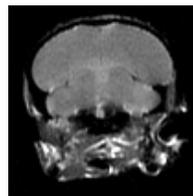


Figure 3: Coronal anatomical image.

high-throughput imaging.

Conclusions

We have demonstrated that it is feasible to obtain localized BOLD activations in mildly anesthetized zebra finches using a 3T whole body MRI scanner, and that the activations reflect which song had been tutored to the birds. Our results provide insights into how to optimize fMRI in songbirds at 3T with respect to a more accurate localization of activations, a better differentiation of response curves with stimulus type, a higher signal-to-noise ratio, and reduced geometric distortions.

¹ Helekar, S. A., Espino, G. G., Botas, A. & Rosenfield, D. B., Development and adult phase plasticity of syllable repetitions in captive zebra finches, *Behav. Neurosci.*, 117, 961-969 (2003).

² P. Jezzard and R. S. Balaban, Correction for geometric distortion echo planar images from B0 field variations. *Magn. Res. Med.* 34, 65-73 (1995).

³ R.W. Cox, AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages, *Computers and Biomed. Res.*, 29, 162 (1996).

⁴ H.U. Voss et al., Correction of spatial and intensity distortions in functional magnetic resonance imaging, *Proc. Intl. Soc. Mag. Reson. Med.* 13, 1544 (2005).

⁵ V. Van Meir et al., Spatiotemporal properties of the BOLD response in the songbirds' auditory circuit during a variety of listening tasks, *Neuroimage* 25, 1242 (2005).