Functional MRI in a zebra finch model of dysfluency shows altered BOLD responses to familiar birdsong stimuli

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Introduction

Stuttering, a speech motor control disorder afflicting about one percent of the world population, can severely alter the quality of life of affected persons. Recognizing the fact that birdsong is analogous to speech in terms of sensorimotor learning and development, we have developed an animal model to study the neuronal basis of a repetitive vocal output characteristic of this disorder in the songbird zebra finch.[1] The utility of songbird models of speech motor control disorders lies in the fact that they might offer insights into the cellular and molecular mechanisms underlying aberrations of vocal learning, perception and vocal motor output. Previously we have described a stuttering-like dysfluency in zebra finches which is characterized by repetitions of birdsong syllables that resemble part-word repetitions of stuttered speech. In trying to investigate brain activation patterns induced by auditory stimulation in zebra finches using functional MRI, we have characterized a distinctive topography of BOLD responses to bird's own song **(BOS)**, tutor song **(TUT)**, conspecific song **(CON)**, and 2 kHz pure tone stimuli **(TONE)**.[2] In the present study we ask if birds producing variant songs containing abnormal syllable repetitions yield different neuronal activations with fMRI compared to normal control birds. Our results show characteristic differences in the BOLD response to familiar song stimuli, namely TUT and BOS, as opposed to an unfamiliar song stimulus (CON) or a non-song stimulus (TONE).

Methods

Experimental set up: Sixteen male zebra finches were tutored by birds that produced songs with repetitions. As a result, half of them became repeaters (repeater birds), and half did not (control birds). The birds were mildly sedated with Diazepam and immobilized in a restraining device made of two soft plastic tubes. The head tube was fixed in a custom made radiofrequency coil made of five tunable inductively coupled elements. The restrained birds were placed in a foam/rubber compound sound isolation box. Auditory paradigms were delivered using a flash memory music player and a pair of stereo headphones with the magnets removed. The sound pressure level of the auditory stimuli at the head position was about 100 dB, the background noise during the EPI sequence about 83 dB. The experiment was approved by the Institutional Animal Use and Care Committees of Cornell University and The Methodist Hospital Research Institute/Texas A&M Institute of Biotechnology.
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 = 25/1000 ms, yielding an effective repeat time of 4 s. Eight sagittal slices of 1 mm thickness, 4 cm FOV, and a matrix size of 128 x 128 were acquired. Slices were prescribed in right-left direction, covering the forebrain. Additionally, in-plane anatomical images and field correction maps[3] were acquired. *Paradigm:* The auditory stimuli, as described in the introduction, were delivered in 16 blocks each consisting of a 32 s "on" and a 32 s "off"' part. The overall scan time per experiment was 1024 s (256 repeats). *Postprocessing:* BOLD sensitive EPI images were corrected for EPI distortions caused by magnetic susceptibility artifacts using field correction maps. The images were then despiked and motion corrected using AFNI[4] and further processed using in-house software written in MATLAB: Data were smoothed slice-wise with a 2D Gaussian filter, detrended, and temporally smoothed. Statistical significance was defined voxel-wise by correlating the signal intensity with the "on-off" block stimulus indicator function. The so obtained statistical parametric maps (SPMs) were registered[5] to a brain template which consisted of the EPI scan that was the least distorted and most symmetric with respect to the sagittal midline. Activations in areas with an EPI intensity baseline less than 20% of the maximum slice intensity and in the eyes were discarded. It was not necessary to discard scans due to bulk motion which was always small (unlike in our first experiment using mild anesthesia[2]). In Fig. 1 a sufficient condition for activation was that at least four of all 16 birds or two of the eight birds in each group had a voxelwise correlation coefficient $R > 0.16$ ($p < 0.005$). In Fig. 2 the strength of BOLD effect was measured in the significantly activated areas by the average percentage of BOLD signal variability against overall signal intensity.

Results

All 16 birds showed significant and reproducible stimulus-evoked BOLD activations of widespread areas within the forebrain. The maximum positive correlation coefficient between the stimulus indicator function and the measured EPI time traces was R=0.78. Activations were bilateral with respect to the sagittal midline of the brain. Averaged BOLD responses in the forebrain auditory areas for the two groups, repeaters and controls, showed greater amount of BOLD activation for CON in repeater birds compared to non-repeater controls (Fig. 1). In contrast, in the repeater birds there was less activation in response to TUT than in the control birds.

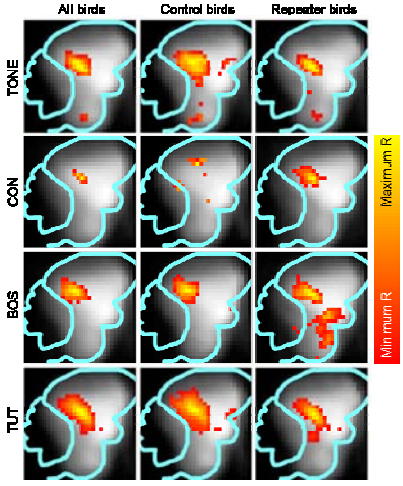

Figure 1 (left): *Mean activation for the four different stimuli TONE, CON, BOS, and TUT in the two medial sagittal slices, for all 16 birds (left), the controls birds only (middle), and the repeater birds only (right). Colors denote correlation coefficients R, individually scaled in each plot, overlaid to averaged EPI images (grey) and a brain template outline (blue).*

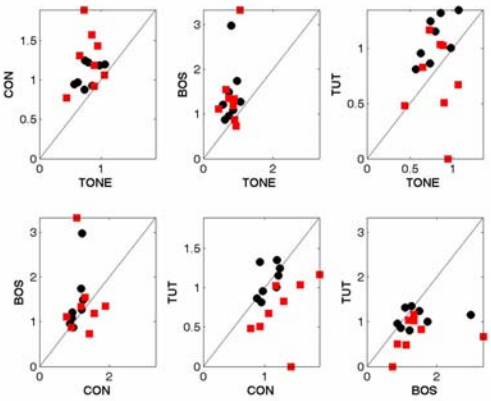

Figure 2 (right): *Quantification of neuronal response given as percentage of BOLD amplitude for control birds (black discs) and repeaters (red squares). The diagonal lines denote where both responses are equal.*

With respect to the percentage of bold response amplitude, in repeater birds TUT stimulation elicited a weaker response as compared to BOS or CON, whereas in control birds it was comparable (Fig. 2). In control birds, TUT response was stronger than TONE, and BOS was stronger that CON, whereas in repeaters they were comparable. (All comparisons significant with $p < 0.05$, Wilcoxon signed rank test for zero median of the differences).

Discussion and Conclusion

Using functional MRI in a songbird model we found significant differences between birds with a stuttering-like variant of song production and normal control birds. These results provide the first evidence for a neural correlate of song representation related to variant song production. It opens new possibilities for the utilization of the songbird model to achieve an understanding of the neural basis of dysfluencies and vocal abnormalities. Our finding that familiar birdsong stimuli (particularly, the tutor song) yield weaker BOLD responses relative to unfamiliar conspecific song stimuli in repeaters compared to non-repeaters suggests a possible deficiency in sensory learning of tutor song or in forming a long-lasting sensory memory of tutor song. A future fMRI study designed to track the development of reduced BOLD sensitivity to tutor song would test which of these alternatives, if any, are tenable.

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