In vivo detection of sexual dimorphisms in the brain of a Passerine songbird, a proof-of-principle study

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Target audience Songbird research, Diffusion Tensor Imaging

Introduction

Vocal learning, the ability to modify the acoustic and syntactic structure of vocalizations forms the basis for spoken human language, a complex socially-learned tool of communication. Because of the convincing similarities between human speech and birdsong learning, zebra finches (ZFs; *Taeniopygia guttata*) are often used as a model to study the neur(ologic)al substrate underlying speech and language.[1] Interestingly, the ZF displays an extreme sexual dimorphism in song behavior: only males produce songs. This dimorphism is reflected anatomically in certain parts of the song control circuitry within the brain e.g. Area X, nucleus HVC and RA are larger in males compared to females.[2] To our knowledge, these sex-related differences have never been studied *in vivo* using a whole-brain approach. The aim of this proof-of-principle study was to implement an *in vivo* non-invasive diffusion tensor imaging (DTI) protocol in the ZF. If this technique proofs itself sensitive enough to detect known sexual dimorphisms in the adult ZF brain, we will use this tool to follow up structural brain development during early life. This will provide us with more insight into neuroplastic processes underlying speech and language acquisition.

Materials and Methods

Male (n=11) and female (n=11) adult ZFs were imaged on a 7 Tesla MR system (PharmaScan, Bruker BioSpin, Germany) equipped with a 400mT/m gradient insert (Bruker BioSpin, Germany). The birds were anaesthetized with isoflurane (induction 3%; maintenance 1.5%) after which coronal diffusion weighted SE-EPI images were acquired with 60 diffusion gradients. The image parameters were: FOV (20x15) mm^2, TE 22 ms, TR 7000 ms, acquisition matrix (105x79), in-plane resolution of (0.19x0.19) mm^2, 28 slices, slice thickness 0.24mm, b-value 670 s/mm^2, δ 2ms, Δ 12ms, 2 repetitions. During experiments respiration rate and temperature were continuously monitored and kept constant. The slice package was positioned along the zebra finch atlas [3] so that the images could be normalized to the atlas space. All data was processed (realignment, coregistration, normalisation, diffusion tensor estimation, smoothing of FA maps) in SPM8 supplemented with the diffusion toolbox (Diffusion II; http://www.fil.ion.ucl.ac.uk/spm/ext/). Voxel-based analysis was performed on the smoothed FA-maps in SPM8 (two sample t-test; FWE correction unless otherwise stated; $p<0.05$ is considered significant). The results are displayed uncorrected ($p<0.001$) and are overlaid to the MRI-based ZF atlas [3].

Results and Discussion

Males display significantly higher FA-values at Area X in both hemispheres compared to female ZFs (left X: p=0.006; right X p=0.019; FWE corrected; Fig.1A). Besides this contrast we tested where female FA values significantly exceed male FA values: three clusters came out. The first is found unilaterally between left HVC and NCM (p=0.004; FWE corrected; Fig.1B), the second appears bilaterally in a rostral region near LMAN (Fig.1C). The shape of the latter cluster resembles an oval structure, which could be co-localized with the lamina frontalis superior (and/or suprema). This lamina contains parts of the anterior forebrain pathway implicated in song learning. Thirdly, females show higher FA values near certain components of the song control and auditory system (Fig.1D). However, the latter clusters only reach statistical significance on cluster level instead of at individual peak level ($p<0.001$ left and right; FWE corrected at cluster level).
In conclusion, the results clearly show that we find the known sexual dimorphisms i.e. Area X, HVC- and RA-related regions using DTI, in addition to our knowledge we reveal one novel bilateral and one novel unilateral dimorphisms between the adult male and female ZF brain. These findings need to be further investigated by histology.

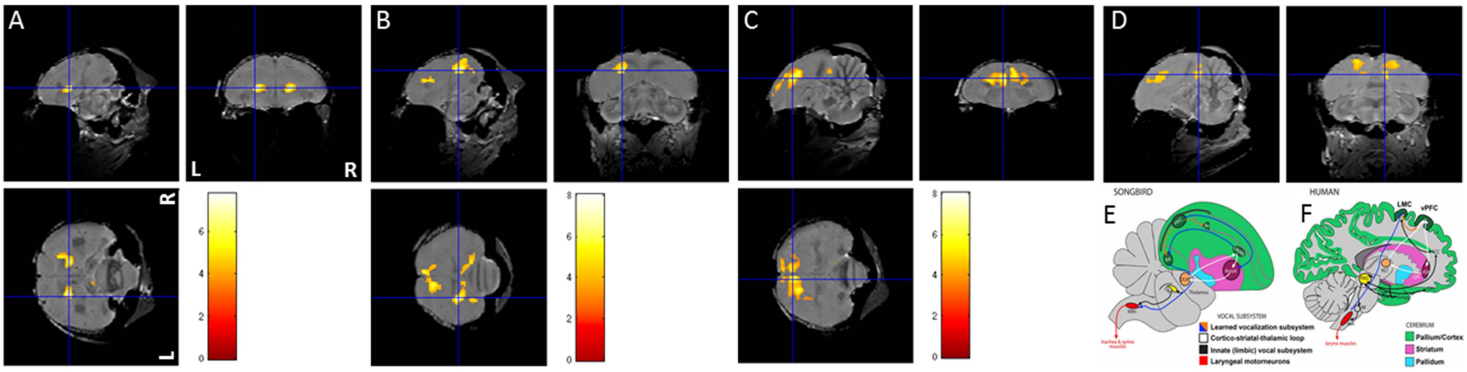

Figure 1: **A**: Statistical map (FA males > FA females): bilaterally appearing clusters co-localized with Area X; **B-C-D**: Statistical map (FA males < FA females): cluster surrounding nucleus HVC and NCM (B); cluster midsaggittal and dorsal to LMAN and Area X, possibly co-localized with a tract (C); cluster either next to brain region between HVC-RA extending to NCM (D) (not significant with FWE-correction). Cross-hairs indicate local maximum in cluster (A-B-C: p-values FWE corrected; all results are displayed as non-corrected). **E-F**: schematic overview of the songbird and human brain with emphasis on the vocal system (modified from [1]).

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