Combination of super-resolution reconstruction diffusion tensor imaging and track density imaging reveals song control system connectivity in zebra finches

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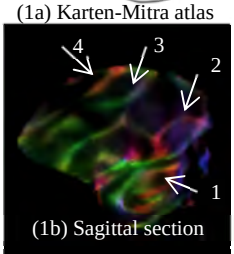
TARGET AUDIENCE: Scientists and clinicians interested in high resolution diffusion imaging of small animals.

PURPOSE: So far, structural investigation of the zebra finch (*Taeniopygia guttata*) brain was mainly performed by invasive methods such as histology [1]. This methodology, however, does not allow quantitative investigation of whole-brain structural connectivity. A recent proof-of-principle study using *in vivo* diffusion tensor imaging (DTI) in adult zebra finches revealed a novel sexual dimorphism in the song control system [2]. However, the diffusion weighted (DW) data acquired in that study, had a low spatial resolution of (0.19x0.19x0.24) mm^3. In order to better understand of the anatomical substrate underlying the observed differences, DTI data with a higher and preferably isotropic spatial resolution is required. Acquiring DW data at such a high isotropic spatial resolution covering the entire brain is, however, not feasible in a reasonable acquisition time using a conventional spin echo DW (SE-DW) or SE-EPI sequence. Therefore, a super-resolution (SR) *ex vivo* DTI protocol and reconstruction (SR-DTI), that improves the trade-off between acquisition time, spatial resolution and SNR of DTI parameters [3], was implemented. The SR-DTI method was combined with track density imaging (TDI) [4], as it has been shown in other small animal studies that TDI facilitates delineation of a large number of brain regions and small white matter bundels [5-6]. Here, the data set of an adult male zebra finch, obtained by the combination of SR-DTI and TDI is presented.

METHODS: *Sample preparation*: One adult male zebra finch kept in normal, non-breeding housing conditions was euthanized by an intramuscular injection of pentobarbital (60 mg/kg) and transcardially perfused first with ice-cold saline and second with an ice-cold 4% paraformaldehyde (PFA) in 0.1 M Phosphate Buffered Saline (PBS; pH 7.4) solution supplemented with gadolinium (1% Dotarem, 0.05 mmol/ml gadoteric acid). Next, the brains were post-fixed overnight with 4% PFA in 0.1 M PBS enriched with 1% Dotarem after which the tissue was transferred to 0.1 M PBS with 1% Dotarem and kept at 4°C. *Acquisition:* Eight hours prior to *ex vivo* imaging the brains were removed from the refrigerator in order to acclimatize to the ambient bore temperature. The zebra finch head was imaged with a spin echo sequence on a 9.4 Tesla MR with a circular polarized transmit resonator, quadrature receive surface coil and a 600 mT/m gradient insert. Fifteen sets of low resolution (LR) images were acquired, each with a different slice orientation, which was rotated around the phase encoding axis at incremental steps of 12°. Each of these sets consisted of 1 non DW image (b=0 s/mm²) and 6 DW images (b=2500 s/mm²) with following acquisition parameters: FOV (15x15) mm2, TE 26 ms, TR 10000 ms, acquisition matrix (192x137) zero-filled to (192x192), in-plane resolution of (0.078x0.078) mm², 37 slices, slice thickness 0.32 mm, b-value 2500 s/mm², δ 6 ms, Δ 14 ms, 1 repetition. Per slice orientation, the scanning time was 2h 40min. As the diffusion gradient directions were different for each slice orientations, a total of 90 unique diffusion gradient directions was acquired. The brains were kept in the skull during the entire procedure as to prevent mechanical damage throughout the different tissue processing and imaging steps. All experimental procedures were approved by the local Ethics Committee for Animal Experiments. *SR reconstruction*: The SR-DTI reconstruction combines the DTI model with an acquisition model, which allows the direct reconstruction of high resolution (HR) DTI parameters from the LR DW images with different slice orientations and diffusion gradient directions. The HR DTI parameters benefit from the high SNR of the LR DW images and the large set of diffusion gradient directions. A set of HR DTI parameters with isotropic voxel size (0.078x0.078x0.078) mm^3 was reconstructed from the acquired data set with this SR-DTI technique. *TDI*: From these HR DTI parameters, the fiber orientation density functions (ODF) with lmax=6, were estimated. Next, 10^8 streamlines, with a threshold of 0.1 on the ODF amplitude, a maximum angle of 9° and a step size of 0.00781 mm, were launched throughout the brain using probabilistic streamlines tractography by second order integration over fiber orientation distributions [7] in MRtrix3 [8]. This tractography result was then used to calculate a track density image with voxel size (0.04x0.04x0.04) mm^3.

(1a) Karten-Mitra atlas



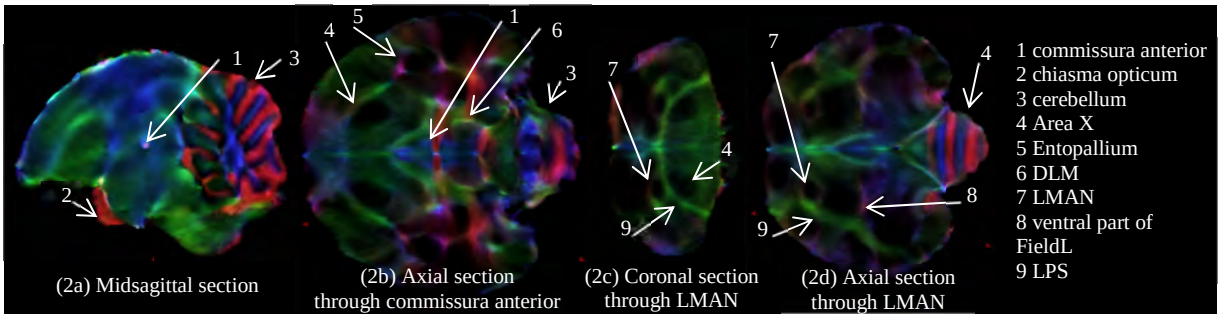
(1b) Sagittal section

1 MLd
2 arcopallium
3 Lamina mesopallialis
4 Lamina frontalis superior

RESULTS: The directionally encoded color SR-TDI maps provide clear anatomical contrast of several components of the song control (e.g. Area X, LMAN, RA; Fig 2b-c), auditory (e.g. FieldL, MLd; Fig 1b-2d) and visual system (e.g. Entopallium; Fig 2b). Structural connectivity such as tracts connecting distinct brain areas (e.g. tractus OM) and laminae subdividing the zebra finch brain in separate parts (e.g. LFS, LaM etc. Fig 1b), are most clearly visualized on the individual and color-coded SR-TDI maps (Fig 2). The obtained TDI maps (Fig 1b) show great similarities with online available myelin stained histological slices (Karten-Mitra atlas, Fig 1a) and with previously published high-resolution starling DTI data [9].

DISCUSSION: The current data set enables 3D whole-brain qualitative assessment of structural connectivity of several areas of the zebra finch brain. The obtained resolution of the acquired dataset and clear anatomical contrast allows delineation of brain regions of interest. This *ex vivo* experiment illustrates that the combination of SR-DTI and TDI can provide clear delineation of the anatomy of the song control system, without proportionally extending the acquisition time. The possibility to perform targeted or even whole-brain fiber tractography on the obtained high-resolution dataset might lead to a further insight into zebra finch brain connectivity both in health, e.g. throughout the critical period of vocal learning, and along the course of pathology.

CONCLUSION: In conclusion, the combination of SR-DTI and TDI has been successfully applied in preclinical small animal research, paving the way for exciting future studies aimed at the establishment of structural connectivity in early development or assessing (early) defects in structural connectivity attributed to neurodegenerative disorders.



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