Seasonal changes in neuronal connectivity in the songbird brain discerned by repeated in vivo DTI

G. De Groof[1], M. Verhoye[1,2], A. Leemans[2], A. Van der Linden[1]

[1]Bio-Imaging Lab, University of Antwerp, Antwerp, Belgium, [2]Vision Lab, University of Antwerp, Antwerp, Belgium

INTRODUCTION: The neural substrate for song behaviour in songbirds, the so called song control system (SCS), is thus far the best documented brain circuit to study seasonal neuroplasticity. Not only the volume of the key song control nuclei (SCN) HVC, RA and X change in size, but also the density of the connections between them change as a function of seasonal and hormonal influences. Changes in volumes of SCN were determined in previous studies using in-vivo Manganese Enhanced (ME) MRI [1,2]. This study uses repeated *in vivo* Diffusion-Tensor MRI (DTI) and the resulting Fractional Anisotropy (FA) maps (as a measure of axonal outgrow) to quantify seasonal changes in the connections between the SCN in starling brains.

MATERIALS & METHODS: Ten male starlings (Sturnus vulgaris) were measured in spring (March 2004) as well as in summer (July 2004) following exactly the same experimental protocol. The starlings were sedated with an IM injection of xylazine and ketamine as described before in Van Meir et al. [2].

In vivo Diffusion Tensor Imaging (DTI) on starling (N=10) was performed on a 7T MR system (MRRS, UK). Sagittal slices (thickness 0.4mm) were obtained covering one hemisphere of the starling brain. The b-matrices were calculated using the analytical expressions [3] incorporating diffusion gradients (70mT/m, δ 12ms, Δ 20ms) and image gradients. DW-SE images were obtained with diffusion applied in 7 non- collinear directions. The image parameters were: FOV 25 mm, TE 43 ms, TR 2200 ms, acquisition matrix (256x128), 14 averages (to obtain the required image resolution of 0.1 x 0.1 x 0.4 mm^3).

The 6 DW-images were co-registered to the non-DW image by maximization of mutual information (MIRIT). Diffusion tensor images and Fractional Anisotropy (FA)-maps were calculated using Matlab routines. Mean FA-values (± SD) were calculated for relevant regions of interest (ROI) delineated on FA-maps and this for the spring and the summer data (IDL). Paired T-tests were performed on the FA-values of the same ROI obtained at different seasons.

RESULTS & DISCUSSION:

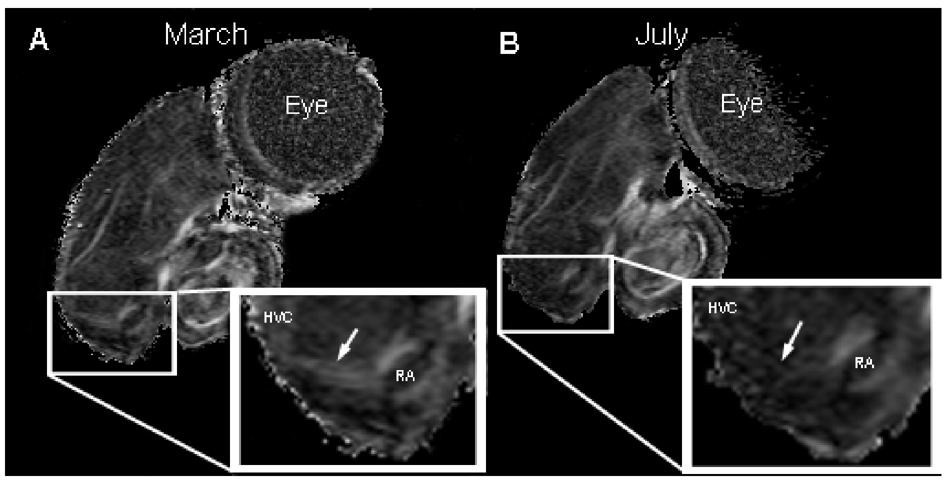

Figure 1 shows two sagittal FA maps of the same starling brain obtained during spring (A) and summer (B). The arcopallium (enlarged in the insert) reveals the Nucleus Robustus arcopallialis (RA) as the dark core surrounded by white fiber layers and the tract between HVC and RA.

The mean (± SD) FA-values of the region encompassing the HVC-to-RA tract (indicated by the arrows) were significantly higher (0.263 ± 0.052) in spring than in summer (0.187 ± 0.044) (29 % decrease, $p < 0.01$). Other ROI's including the lamina which carry connections between HVC and X (Lamina frontalis superior: spring 0.379 ± 0.035, summer 0.385 ± 0.037, p = 0.688) and between other SCN (Lamina pallio-subpallialis (spring 0.339 ± 0.044, summer 0.349 ± 0.047, p = 0.606), Lamina mesopallialis (spring 0.255 ± 0.052, summer 0.237 ± 0.074, p = -0.462), and the SCN themselves, e.g. RA (spring 0.159 ± 0.023, summer 0.145 ± 0.027, p = 0.127) and area X (spring 0.162 ± 0.035, summer 0.176 ± 0.029, p = 0.209) did not display any significant seasonal changes in FA-values. These increased FA values in spring correlate perfectly with literature findings showing that the HVC-RA connection is thus far the only connection which is known [4] to display an increase in axonal projections (from HVC to RA) in spring. Although in this particular study HVC and RA can be used as accurate internal reference points for selecting identical slices comprising the tract connecting them, current efforts to optimize the registration techniques for FA maps might allow more accurate ROI delineation and comparison between different seasons. This could assist us to discern smaller and less obvious seasonal changes in other brain areas, if existing. In a second step and within the same bird these quantitative seasonal neuroanatomical (FA) changes can be compared with the quantitative seasonal behavioural changes related to the amount of singing and/or song complexity (songbirds typically sing in spring and not in summer).

References:

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