DTI eigenvalues in grey matter: a songbird's perspective

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INTRODUCTION:

Despite the abundant use of DTI in investigating brain white matter changes in aging and neurodegenerative diseases, the use of DTI parameters in investigating changes in gray matter (GM) is relatively recent. However, theoretically DTI can provide quantitative data about changes in magnitude and orientation of diffusivity resulting from intracellular changes dealing with alterations in protein synthesis, vesicle formation or distribution and concentration of organelles and from extracellular changes such as dendrite branching.

Male starlings sing throughout the year, but in the breeding season (spring), when circulating concentrations of the steroid hormone testosterone (T) are elevated [1], the song is highly sexually motivated [2]. It has been shown that the brain regions of the so-called 'Social Behaviour Network' also change seasonally. These changes are linked to the activity of Testosterone (T) converting enzymes such as aromatase. Apart from this, there is evidence that sex steroids also modulate perception of socio-sexual signals by affecting sensory processing. Therefore instead of focussing only on the seasonal plasticity of the song nuclei, we adopted a whole brain GM approach which allowed us to investigate regions known for motivation and sexual behaviour and regions involved in auditory processing.

MATERIALS & METHODS:

Nine male starlings (Sturnus vulgaris) were measured in spring (March) as well as in summer (July). The starlings were sedated with an IM injection of xylazine and ketamine as described before in De Groof et al. [3]. In vivo Diffusion Tensor Imaging (DTI) on starling was performed on a 7T MR system (MRRS, UK). Sagittal slices (thickness 0.4mm) were obtained covering one hemisphere of the starling brain. DW-SE images were obtained with diffusion applied in 7 directions. The image parameters were: FOV 25 mm, TE 43 ms, TR 2200 ms, acquisition matrix (256x128), δ 12ms, Δ 20ms, 14 averages (zero-filled to obtain an in-plane resolution of 0.1 mm²). Data analysis was done with SPM5. Diffusion-weighted images were first realigned using the procedure of the 'Diffusion II Toolbox' for SPM5. The spring and summer B_0-image of the same starling were then realigned to each other. The mean images of the realigned data were spatially normalized onto one subject's spring B_0-image (= study specific template), and the normalization parameters were reapplied to the individual Diffusion weighted data. The diffusion tensor was estimated from the DW-data, and mean diffusivity (MD), eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and fractional anisotropy (FA) maps were derived. The voxel-based analysis (Paired T-test) of these maps was performed in SPM5. A P value of 0.05 (False Discovery Rate (FDR) of 0.05) was the threshold for statistical significance. Also mean DTI-values ($\pm$ SD) were calculated for relevant regions of interest (ROI) delineated on FA-maps and this for the spring and the summer data. Paired T-tests were performed on the DTI-values of the same ROI obtained at different seasons. Each season, after MRI testing blood samples were taken from each male to assay T concentrations.

RESULTS & DISCUSSION:

Testosterone concentrations were significantly higher in spring compared to summer [$P = 0.017$; mean $\pm$ SEM: spring, 0.946 $\pm$ 0.231 ng/ml; summer, 0.174 $\pm$ 0.156 ng/ml]. It has been shown that in caudal-medial nidopallium (NCM, a secondary auditory region) the concentration of the (testosterone converting) enzyme aromatase is highest in spring, when the birds hear the most song. A decreased FA ($P = 0.017$, see table) and a distinct increased third eigenvalue (λ_3) ($P = 0.012$, see table) were observed in NCM between the seasons. The figure shows the statistically significant difference of λ_3 between the seasons in NCM. Top image is an FA map. The inset shows the map of the paired Student's t statistic for the (summer-spring) difference in the λ_3 parameter overlaid on a high resolution T_2 weighted image. The colour scale has been constrained to the range of $0.05 > P > 0.0005$ (reddish colours for decrease of λ_3 during spring).

Brain regions within the 'social behaviour network' are the medial preoptic nucleus (POM), the bed nucleus of the stria terminalis (BNST) and the nucleus ventromedialis hypothalami (VMN). The POM plays a key role in regulating male reproductive behaviour in birds, the nucleus taeniae of the amygdala (TnA) is known to be involved with social and motivated behaviour, such as aggression and reproduction and the 'nuclei of the social behaviour network' have been directly implicated in the regulation of singing behaviour in songbirds. Studies have indicated that T and aromatase are involved in the regulation of aggressive and reproductive behaviour, particularly in the 'social behaviour network' [4]. While no significant change was detected in the BNST between seasons (see table), the VMN showed a reduced FA due to an increased λ_3 (see table). In POM we identified a seasonal decrease in FA and an increase in MD, λ_3 and T_2 (see table). In TnA several DTI parameters (decrease of FA and increase of MD, λ_1, λ_2 and λ_3) changed between the breeding and the non-breeding season (see table).

CONCLUSION:

It has been shown that aromatase positive neurons have highly complex branching patterns and large nuclei [5] and changes in aromatase expression and activity could modify this cell phenotype. This observation could explain the FA and λ_3 change we observed in NCM between the breeding and non-breeding season. In all except one region expressing aromatase (of which the activity is known to change between breeding and non-breeding season) that were studied here, the DTI parameter λ_3 was altered between seasons and may even be considered as an indirect marker of the metabolic changes that accompany changes in aromatase activity keeping in mind that repeated measures in the same bird can be done. Due to the *in vivo* nature of the applied MRI/DTI tools, the causality of these features could be monitored in the future by using for instance aromatase inhibitors. These results show how the *whole* songbird brain is prepared for the breeding season.

References: [1] Riters LV et al., 2002. J Comp Neurol 444: 63-74. [2] Riters LV et al., 2000. Horm Behav 38: 250-261. [3] De Groof et al., 2008. Eur J of NeuroScience (in press). [4] Thompson RR et al., 1998. Brain Behav Evol 51: 215-229. [5] Saldanha CJ et al., 2000. J Comp Neurol 423: 619-630.
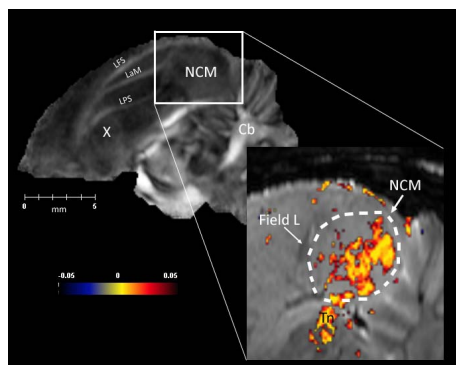
Figure: Statistical comparison of the third eigenvalue λ_3 measure between brains (n=9) from the spring breeding season and those from the summer non-breeding season (repeated measures). Abbreviations: NCM: Caudal-medial Nidopallium; X: Area X; Cb: cerebellum; LFS: lamina frontalis superior; LPS: lamina pallio-subpallialis; LaM: lamina Mesopallialis; TnA: nucleus taeniae of the amygdala.

Table: Results ($\pm$ SD)of DTI and T_2 metrics from breeding (April) and non breeding (July) starlings. MD, λ_1, λ_2 and λ_3 in $\mu m^2/ms$ and T_2 in ms. *P-value < 0.05 (Wilcoxon signed ranks test; April vs July).

ROI	Month	FA	MD	λ_1	λ_2	λ_3	T_2
NCM	April	**0.19 ± 0.02**	0.60 ± 0.02	0.72 ± 0.03	0.60 ± 0.02	**0.49 ± 0.02**	39 ± 1
	July	**0.16 ± 0.02***	0.62 ± 0.03	0.73 ± 0.04	0.60 ± 0.03	**0.52 ± 0.02***	38 ± 2
POM	April	**0.26 ± 0.02**	0.68 ± 0.02	0.87 ± 0.03	0.65 ± 0.02	**0.52 ± 0.02**	37 ± 3
	July	**0.25 ± 0.02***	**0.69 ± 0.02***	0.88 ± 0.03	0.66 ± 0.02	**0.54 ± 0.02***	39 ± 3*
BNST	April	0.24 ± 0.04	0.66 ± 0.04	0.84 ± 0.07	0.61 ± 0.03	0.55 ± 0.03	40 ± 3
	July	0.25 ± 0.03	0.67 ± 0.02	0.86 ± 0.04	0.62 ± 0.01	0.53 ± 0.03	40 ± 2
VMN	April	**0.24 ± 0.02**	0.67 ± 0.03	0.79 ± 0.05	0.65 ± 0.03	**0.48 ± 0.02**	39 ± 3
	July	**0.21 ± 0.02***	0.65 ± 0.03	0.86 ± 0.04	0.66 ± 0.04	**0.51 ± 0.03***	36 ± 2
Tn	April	**0.17 ± 0.04**	**0.79 ± 0.06**	**0.93 ± 0.07**	**0.78 ± 0.06**	**0.67 ± 0.06**	46 ± 3
	July	**0.14 ± 0.02***	**0.93 ± 0.16***	**1.07 ± 0.19***	**0.92 ± 0.16***	**0.80 ± 0.14***	49 ± 7