Seasonal changes in the auditory network of female starling assessed with rsfMRI

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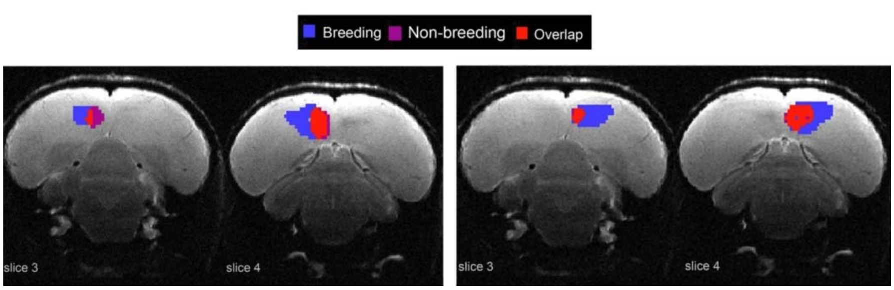
Introduction: European starlings sing both during and outside of the breeding season but the primary function of male song shifts depending upon the season[1]. During the breeding season (BS) one of the functions of male starling song is that of immediate mate attraction. Mate attraction is characterized by particular displays (wing waving) and a clear increase in the production of particular song elements (high-pitched trills). The fact that female starlings select mates based on song characteristics such as bout length[2] could possibly indicate an increased relevance for auditory discrimination in breeding season compared to non-breeding season (NBS). In this study we investigated if this increased discrimination is reflected in an increased local functional connectivity (FC) within the major auditory areas, NCM and Field L that could reflect improved information transfer within this region.

Materials and methods: In this study, 11 female European starlings (*Sturnus vulgaris*) were used. Both in BS (March-April) as the NBS (October) resting state fMRI scans were acquired for all birds. During scanning the birds were anesthetized with an intramuscular bolus injection containing a mixture of 10ml medetomidine (Domitor, Pfizer, 1mg/ml) and 2,5ml ketamine (Anesketine, Eurovet, 100mg/ml) of which 0,3ml was administered. Subsequently, an intramuscular catheter was inserted that administered the same mixture at a speed of 0,12ml /hr during the entire acquisition time. The resting state fMRI data was recorded using a 9.4T Biospec scanner (Bruker, Ettlingen, Germany). The resting state fMRI data was acquired using single shot GE-EPI using a repetition time of 2000ms, and an echo time of 16ms. 13 axial slices with a slice thickness of 0.8mm were recorded. 150 repetitions of each image were acquired, resulting in a measuring time of approximately 5 minutes per sequence. Pre-processing was carried out using a standard procedure using the Statistical Parametric Mapping 8 program (SPM 8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). To estimate FC, Independent component Analysis was performed using a pre-set of 25 components, applied in GIFT (Group ICA of fMRI toolbox: http://icatb.sourceforge.net/).

Resulting components were matched to anatomical meaningful regions using a homemade MRI starling atlas. Those components coinciding with the auditory system (namely Field L-NCM complex) were selected for further analysis.

Results: For both measurements two components could be identified co-localized with the NCM-Field L complex one in the left hemisphere, one in the right hemisphere (figure). Overall, the central part of the component is present in both measurements. Both the left as the right component is more spread out laterally in BS compared to NBS. Paired t-tests showed a difference in cluster size between both seasons ($p=0.002$ for the left component, $P<0.0001$ for the right component). Moreover cross-correlation values indicating the inter-hemispheric FC between left and right NCM-Field L complex test showed a significant lower FC in NBS (0.19+/-0.02) compared BS (0.46+/-0.02) ($p=0.003$).



Mean components co-localized with the left NCM-Field L complex and right NCM Field-L complex overlaid on an anatomical MRI image using a colour code indicating the similarity between both seasons.

Discussion and conclusion: Increased spontaneous and induced activity of Field L in BS has been reported before using invasive electrophysiological methods. These could be explained by an improved inter-communication between neurons and seems to be reflected as an increased local FC. Nevertheless by using resting state fMRI this seasonal variation in neuronal communication could be studied for the first time in a non-invasive set-up measuring the same animals repetitive over time.

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