Seasonal plasticity of the olfactory circuit in songbirds assessed with *in-vivo* Manganese Enhanced (MEMRI)

G. De Groof[1], S. Steiger[2], H. Gwinner[2], and A. Van der Linden[1]

[1]Bio-Imaging Lab, University of Antwerp, Antwerp, Antwerp, Belgium, [2]Max Planck Institute for Ornithology, Seewiesen, Germany

INTRODUCTION: Songbirds are known for the pronounced seasonal neuroplasticity of their song control circuit [1]. Size, connectivity and activity of this circuit increases in the breeding season (spring) when birds sing and decreases in the non-breeding season (summer) when the birds no longer sing. It is questioned whether other sensory circuits (e.g. the chemosensory system) display similar plastic seasonal changes. Possibly due to the small size of the olfactory bulb (OB) as compared to rodents [2], it is generally believed that songbirds lack a well-developed sense of smell. Yet there is sporadic evidence that some songbirds are able to detect odors [3,4]. During courtship and nestbuilding, male European starlings (*Sturnus vulgaris*) incorporate aromatic herbs that are rich in volatile compounds (e.g milfoil, *Achillea millefolium*) into the nests [5] and it is likely that they use olfactory cues to identify these plants. Interestingly, European starlings show seasonal differences in their ability to respond to odor cues: odor sensitivity peaks during nest-building in the spring, but is almost nonexistent during the non-breeding season [3], suggesting that the sense of smell might be particularly important during the reproductive season. This study uses repeated *in vivo* Manganese-Enhanced MRI (MEMRI) to quantify possible seasonal changes in the anatomy and activity of the OB in starling brains.

MATERIALS & METHODS: Male starlings (Sturnus vulgaris; N=16) were repeatedly measured in spring (April) and in summer (August). After being anesthetised by an IM injection of xylazine and ketamine [6], droplets of 10µl of 40mM of Manganese Chloride ($MnCl_2$) were administered into each nostril and then the birds were exposed to either a Milfoil oil odor (for 20 minutes) or no odor. This was repeated after two weeks (in the same season) but then each bird was submitted to the opposite experimental condition. In vivo MEMRI on starling was performed on a 9.4T MR Biospec system (Bruker, Germany). A 3D RARE T_1-weighted image (TR/TE = 386/7.5 ms) covering the whole brain was acquired approximately 1 hour after $MnCl_2$ administration. A sagittal slice (thickness 0.5mm) covering the tiny olfactory bulb was obtained using an inversion recovery RARE sequence (TR/TE = 12000/7.5 ms) approximately 1,5 hours after $MnCl_2$ administration and a corresponding T_1-map was calculated. Mean T_1-values (±SD) were calculated for the olfactory bulb (OB) delineated on T_1-maps. Paired T-tests were performed on the T_1-values of the OB obtained at different seasons. The OB was also delineated on the 3D datasets and signal intensity was normalised to the eye of the bird.

RESULTS: Figure 1 shows a MEMRI slice through the OB of a starling in the breeding season. Volumetric analysis of the data showed no seasonal difference in the volume of the olfactory bulb (April: 1,08 ± 0,18 mm³ vs August: 1,12 ± 0,16 mm³, p=0.11). Figure 2 shows the mean (± SD) T_1-values of the OB under different experimental conditions and between two seasons. In the breeding season (April) there is a significant difference between the two stimulations (NoMilfoil: 1412 ± 59 ms vs Milfoil: 1458 ± 66 ms, p=0.028), which disappears in the nonbreeding season (August) (NoMilfoil: 1554 ± 82 ms vs Milfoil: 1576 ± 78 ms, p=0.385). Other significant differences were seen as shown in figure 2 (all $p<0.0001$). The relative signal intensity in the OB (Figure 3) also shows a significant difference between the Milfoil and NoMilfoil condition (NoMilfoil: 178 ± 10 vs Milfoil: 166 ± 13, $p<0.0001$) during the breeding season which disappears in the nonbreeding season (NoMilfoil: 167 ± 10 vs Milfoil: 173 ± 12, p=0.15). A significant difference was seen between the No Milfoil stimulation in April and No Milfoil stimulation in August ($p < 0.001$).

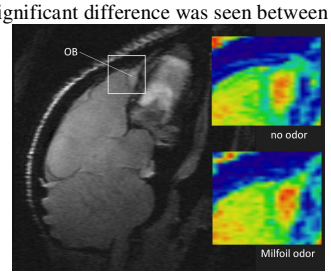

Fig. 1. MEMRI T_1-weighted image of the OB. Inserts show the manganese uptake of OB during different stimuli in April (T_1-maps).

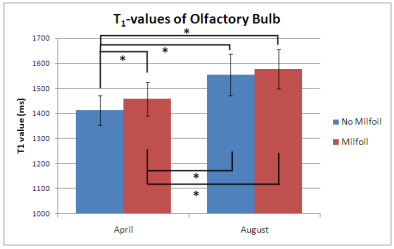

Fig. 2. T_1-values of the OB between experimental conditions and between seasons.

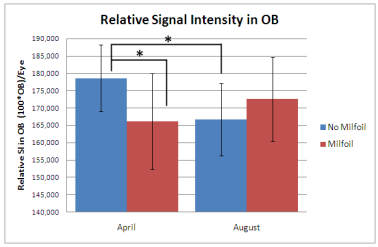

Fig. 3. Relative Signal Intensity of the OB between experimental conditions and between seasons.

DISCUSSION & CONCLUSION: Our data confirm that European starlings can discriminate Milfoil 'better' in the breeding season then outside the breeding season (significant differences in April not in August). The results of this MEMRI study suggest that (i) either the sensory neurons expressing olfactory receptors (OR) that are activated by milfoil have disappeared in summer or that (ii) the expression of ORs that are activated by milfoil is lower or silenced in summer. Both may result in a decreased activity of neurons in the OB and thus in the specific recognition of milfoil only in spring. It has been shown that the incorporation of herbs into the nest benefits the offspring [5] and therefore a seasonal pattern in the ability to respond to milfoil may be evolutionary adaptive [2,4]. However one fact remains puzzling: why is there less $MnCl_2$ in the OB with the Milfoil condition compared to no odor stimulation in the breeding season? A possible explanation could be because Milfoil is a relevant smell in the breeding season the neurons from the OB already transported the manganese to a higher order olfactory region for further processing (e.g. piriform cortex) in the time period measured. As the seasonal changes in birds are driven by changes in steroid plasma levels, future MEMRI studies might provide new insight on the impact of hormones on the olfactory system.

References:

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