# Physiological mapping of the songbird brain in relation to their seasonal plasticity: Repeated diffusion-, T2- and brain size measurements

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#### **Introduction**

For a long time, dramatic changes in adult brain size and physiology were exclusively associated with neuropathology. Indeed, Alzheimer and Parkinson Disease are known to imply neuro degeneration and a subsequent degradation of brain structures. Apart from neuropathological and aging related processes, major healthy physiological changes in the brain were only considered to occur at the synaptic level. Recently however, more dramatic neuroplastic changes during different stages in life have become more and more acknowledged and imply changes in neurocellular properties including apoptosis and integration of new neurons within certain brain areas. Songbirds represent an excellent model to study adult brain plasticity under natural conditions in relation to endocrinological changes and learning processes. In this study we made physiological maps of the songbird brain to evaluate neuroplastic features occurring within different brain areas, including the song control system, in which physiological changes regarding seasonal plasticity are very well documented.

## **Methods**

We performed in-vivo MRI of the brain of 18 adult female starlings during and after the breeding season in March and July 2003 respectively. This was done on a 7T (MRRS) MRI system using multislice (FOV=25; image matrix=256x256; slice thickness=1 mm; n° of slices= 16) diffusion-weighted (B-values=0, 286 and 672 s/mm<sup>2</sup>; TE/TR=36/2000 ms) and T2-weighted sequences (TE= 18, 36 and 50ms; TR=2000) in order to make physiological maps as frequently done for the evaluation of brain damage. In this case we aimed at evaluating **seasonal changes** in Apparent Diffusion Coefficient (ADC) and T2-values, which were interpreted as an indication for respectively **changes** in the ratio extra- versus intracellular water and **changes** in the amount of free water (provided no haemodynamic changes had occurred). A T1-weighted 3D sequence (TE/TR=18/300 ms; FOV=25<sup>3</sup> mm; image matrix=256x256x256) with subsequent brain segmentation and volume determinations (Surfdriver 2.5.5) enabled us to relate the physiological changes to changes in brain size. We examined changes within the telencephalon by studying two ROI within and two outside the basal ganglia. In each case we included an ROI from within the song control system and a control region adjacent to it. Seasonal differences in physiological parameters for the different regions were evaluated using a paired T-test.

## **Results**

On the anatomical level, ADC-maps allowed us to discern more detailed structures than T2-maps. Whereas T2-maps only gave differences in contrast between the major subregions of the brain, the ADC maps even revealed brain nuclei from the song control system, such as area X in the basal ganglia and nucleus Robustus Archistriatalis (RA) in the Archistriatum, which had never been discerned on plane MR images (figure 1). Comparison of ADC- and T2-values of these structures and of the adjacent areas obtained during different seasons revealed that ADC values decreased significantly in all areas (table 1; all p<0.001) whereas T2-values did only change (increase) in the area adjacent to RA (p<0.01). According to the overall decrease in ADC, a significant 18 % volume decrease was observed in the telencephalon size (March: 880  $\pm$  56 mm<sup>3</sup>; July: 719  $\pm$  114 mm<sup>3</sup>; p<0.001).

T2 Archistriatum		March July	RA 36.3 ± 3.2 37.2 ± 2.5	Archistriatum 36.4 ± 2.9 38.8 ± 3.0	Area X 34.0 ± 1.5 33.3 ± 1.9	Basal Ganglia 34.9 ± 2.0 34.3 ± 2.5
ADC RA	Area X Basal Ganglia	March July	$\begin{array}{c} 709\pm 64\\ 590\pm 70\end{array}$	$\begin{array}{c} 640\pm84\\ 483\pm82 \end{array}$	$\begin{array}{c} 630\pm65\\ 548\pm82 \end{array}$	$\begin{array}{c} 650\pm81\\ 428\pm62 \end{array}$

**Figure 1**: Illustration of a T2 (top) and an ADC (bottom) map through the archistriatum (left) and the basal ganglia (right).

**Table 1**: Columns: Repeated measures in four brain areas of the songbirds left side telencephalon. Top rows: T2 values (ms); bottom rows: ADC values ( $\mu$ m<sup>2</sup>/sec). Mean values for each time point (March and July) are presented with their standard deviation. Note that the most dramatic changes actually occur outside the song control system.

#### Discussion

We found a dramatic decrease in telencephalon size and ADC values of all tested areas within the telencephalon. These changes occurred in a time span of 3 months under perfectly natural, healthy conditions, i.e. upon transitions from breeding to post breeding season. The decrease in ADC values probably reflects a decrease in extracellular space, which could represent an increased neuronal density. If an increase in cell density would be the only effect one might expect the T2-values, reflecting the tissue's free water content, to decrease as the ADC values decrease. However, within the regions of the song control system the decrease in extracellular space is known to occur in parallel with cell shrinkage. T2-values seem to be either stable or increase during a transfer to the after breeding season. This might be due to the effect of cell shrinkage, leading together with a decreased extracellular space to the decreased telencephalon size. The endocrine changes in female songbirds that take place after the breeding season, including a decrease in estrogen level, could be one of the major driving forces behind this effect. Comparison of our current findings with changes in other brain areas within the telencephalon or the midbrain and the thalamus could point towards new mechanisms of brain plasticity affecting the entire brain apart from the well know plasticity that has been considered as mainly restricted to the song control system and the hippocampus.