Contrast Enhancement in Preserved Zebra Finch Brains Utilizing Low Temperatures at High Magnetic Fields

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Introduction – The application of MR imaging in neurological studies has increased remarkably during the last decade. Factors such as intrinsic contrast, high spatial resolution and non-invasive volumetric analysis contribute to its usefulness, especially in medical settings. The contrast in an anatomical MR image depends primarily on water relaxation. T_1 (longitudinal), T_2 (transverse) and T_2* relaxation dominate the contrast generated in high-resolution volumetric datasets. These parameters are dependent on temperature, which normally is not a true variable for *in vivo* imaging but can be utilized with preserved (i.e. formaldehyde fixed) specimen to enhance contrast. Lower experimental temperatures decrease molecular motion, thus lowering relaxation times [1-3]. Temperature dependent alterations in T_1 time are usually the most evident; however, contrast enhancement in high-resolution gradient-recalled echo images is largely due to T_2* mechanisms. In this study, the effects of acquisition temperature on contrast in fixed brain tissue from adult male zebra finches were investigated and quantified. In particular, the study was focused on several neuron-dense structures (*i.e.* nuclei) in the brains of these animals. The objective of this study was to determine optimal imaging parameters to highlight nuclei involved in the song acquisition and production pathways as well as the connections between these regions without utilizing exogenous contrast agents [4]. For example, the HVC, which is involved in both song production and acquisition, is connected to the nucleus *robustus arcopallium* (RA) located in the posterior pathway (song production). The nucleus *mesencephalicus lateralis, pars dorsalis* (MLd) is the auditory midbrain region in which multiple parallel inputs from the lower brain stem converge and through which most auditory information passes to reach the forebrain. The lateral magnocellular nucleus of the nidopallium (LMAN) is the output of anterior forebrain pathway essential for learning and maintenance of song.

Imaging Protocol – Excised brains from adult male zebra finches were immersion fixed using 4% paraformaldehyde. All MR data were acquired using an 11.75-T vertical magnet equipped with a Bruker Avance console and Micro2.5 gradients. The brain tissue was washed in phosphate buffered saline (1xPBS) prior to imaging and immersed in Fluorinert (3M, Corp). Using a 10-mm birdcage coil, scans were acquired at the following temperatures: 5, 10, 15, 20 and 25 ˚C. Three-dimensional gradient-recalled echo (GRE) scans (TE/TR=15/200 ms) were acquired at an isotropic resolution of 40 µm. Acquisition time per scan ranged between 3-4 hours depending on the size of the excised tissue. For all temperatures, T_1 relaxation was quantified using multi-slice spin-echo sequences (MSSE) acquired with TE=8 ms and TR =15-0.5 s. T_2 relaxation was measured using MSSE scans acquired with TE=8-96 ms and TR=2.5 s. In addition, to determine T_2* relaxation, multiple gradient echo (MGE) sequences were acquired with the following parameters: TE=3-31 ms and TR=0.75 s. For all relaxation measurements, spatial resolution was 80x80x400µm. Relaxation analysis was performed by assigning regions of interest (ROIs).

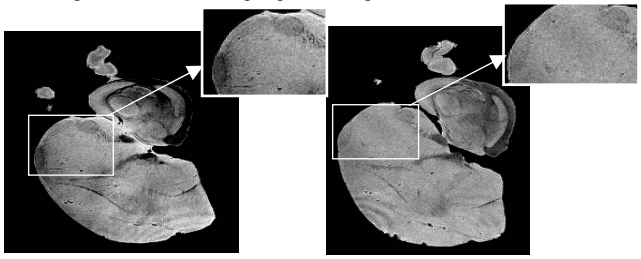

Fig 1. 3D GRE images (40-µm iso. res.) demonstrating the loss of contrast in the HVC and RA with increased acquisition temperatures. L=5˚C; R= 14˚C.

Results & Discussion – Lower experimental temperatures resulted in a significant contrast enhancement. This increase in signal intensity was particularly noticeable for the different neuron-dense nuclei in the zebra finch brain (Fig 1). Significant contrast enhancement could be detected in the different telencephalic layers of the brain as well as in the fiber tracts linking different structures. T_1 and T_2 analysis (Fig 2A and B) demonstrated an expected reduction in values with decreasing temperature, but no significant contrast enhancement due to these mechanisms. However, T_2* measurements displayed decreases in T_2* with decreasing temperature that did enhance contrast (Fig 2C). These contrast enhancements are best illustrated for three structures in the finch brain as shown in Fig 3. In conclusion, reduced acquisition temperatures not only increase the signal-to-noise ratio but can increase contrast enhancement in preserved tissue. Furthermore, the lower acquisition temperatures may protect tissue for further biochemical analysis, such as immunohistochemistry. These techniques could be applied to a range of preserved specimen to develop contrast.

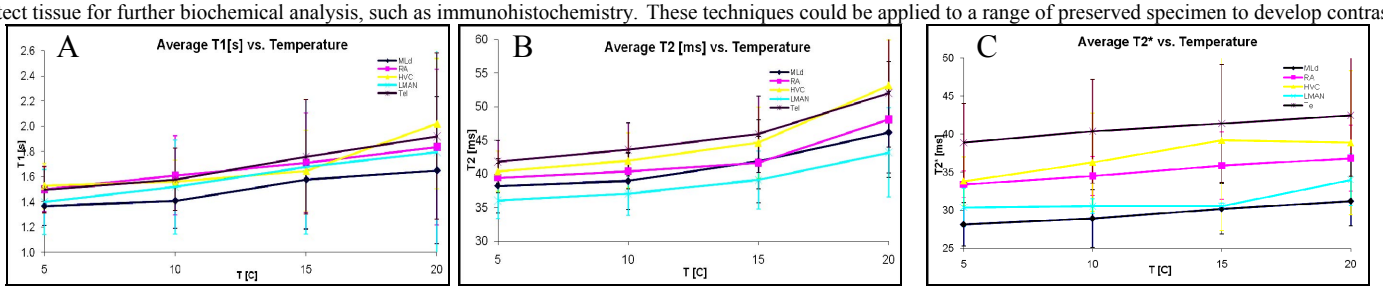

Fig 2. T_1 (A), T_2 (B) and T_2* (C) measurements versus temperature for a representative, fixed zebra finch brain

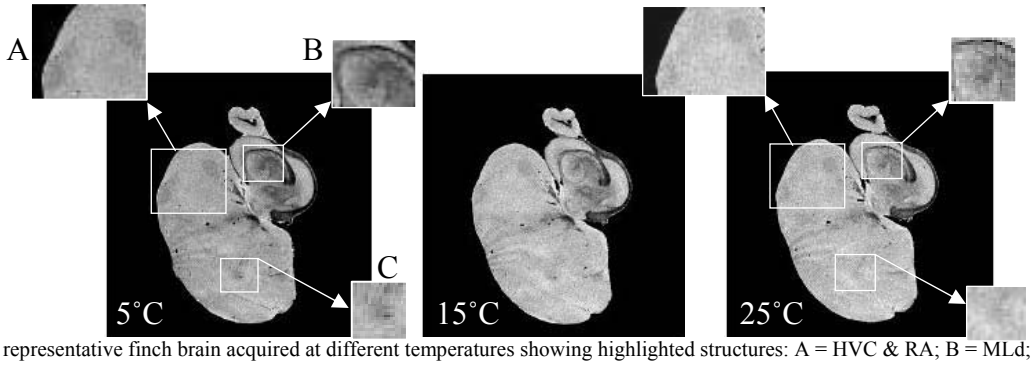

Fig 3. A representative finch brain acquired at different temperatures showing highlighted structures: A = HVC & RA; B = MLd; and C = LMAN

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