

Passive staining: a novel procedure for 3D MR histology - Description and application to the characterization of cerebral anomalies in doublecortin knockout mice

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Introduction: Unlike classical histology, magnetic resonance microscopy (μ MRI) allows one to record three dimensional (3D) images from intact samples making it easier to evaluate complex 3D structures, for example, by virtually sectioning the sample in any direction or angle or by measuring tissue volumes [1]. To be useful, μ MR images must have a high resolution and contrast to noise to display small structures. Optimization of high resolution μ MRI may be achieved with non specific contrast agents reducing tissue T1. An elegant method, called "active staining", based on the peri-mortem intracardiac perfusion of animals with gadolinium-based contrast agents has been developed to improve signal and contrast to noise ratio in post-mortem images from excised mouse brains [2]. One aim of our study was to evaluate an easier method to improve μ MRI. Our protocol, called "passive staining", is based on the post-mortem staining of samples by gadoteric acid. The effect of the staining procedure on contrast and relaxation times was first evaluated on control samples. Then the method was applied to characterize the brains of doublecortin (Dcx) knockout (KO) mice, a model of type 1 lissencephaly, a disease responsible for a large proportion of cases of mental retardation and epilepsy in children.

Materials and methods: The passive staining protocol consists of soaking a brain in a 1:40 mixture of 0.5 mmol/ml gadoteric acid (Dotarem®, Guerbet, France) and 10% buffered formalin. Images from the brains embedded in an agar gel were then recorded at 4.7 T. The effect of brain immersion in the contrast agent on relaxation times was assessed by measuring T1, T2 and T2* before (n=2) and 1, 3, 4, 7, 9, 11, and 16 days after passive staining (n=7). To assess the stability of relaxation times after brain inclusion in agar, T1, T2 and T2* were evaluated at least four times during a period of 32 hours after brain inclusion in the agar gel (n=2). Then, T2*-weighted (T2*w) 3D gradient echo (GE) images were recorded for all the brains on which we measured relaxation times (TR/TE=100/10 and 20 ms, $\alpha=90^\circ$, resolution=63x47x59 μ m³, signal averaging (NA)=8 to 10). Passive staining was then used to evaluate brain alterations in Dcx-KO mice, a model of lissencephaly, generated using the Cre-loxP site specific recombination system (Sv129Pas background) [3]. Fixed brains from hemizygotes (DcxY/- (Dcx is localized on the chromosome X); n=2), heterozygotes (X/-; n=1), and wild type animals (male, Y/+ n=2) were passively stained and 3D-GE images were recorded (TR=100 ms, TE=15 ms). The brain and hippocampus were manually delineated (Amira 3.1) and their volumes and profiles were assessed. Corpus callosum length was measured using Display (ftp.bic.mni.mcgill.ca).

Results: T1, T2 and T2* relaxation times decreased rapidly after immersion of the brains in the contrast agent and stabilized at very low values after 3 days of immersion (see for example T1 values in figure 1, values without staining > 700 ms whatever the brain structure). After inclusion of the brains in the agar gel, T1, T2 and T2* relaxation times remained low for at least 30 hours and a strong difference between tissues was maintained for at least 20 hours. The contrast to noise ratio was poor on 3D-GE images from unstained brains and was improved after the passive staining protocol, mainly because of a signal decrease within the white matter (WM) and thalamus (Th) (figure 2). The contrast improvement after passive staining allowed a highlighting of the border of several structures such as the corpus callosum or the hippocampus in DCX and control mice. Corpus callosum anomalies ranging from complete agenesis in DcxY/- mice to partial agenesis in heterozygote mice were detected. In addition, the delineation of the hippocampi revealed a modified shape of the hippocampi in the DcxY/- mice (figure 3), despite similar hippocampus (and brain) volumes. Other alterations such as unsuspected ectopic fiber bundles in rostral regions, as well as abnormally extended cingulate cortex could also be detected.

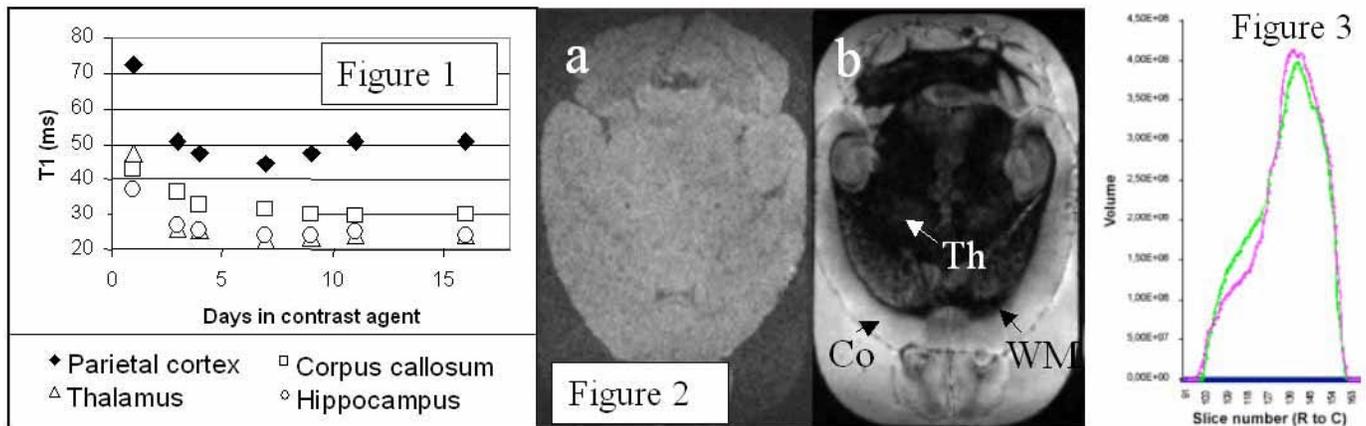


Figure 1. T1 relaxation times as a function of the time of immersion in the passive staining solution. **Figure 2.** Fixed brains imaged without passive staining (a) and after passive staining (b). Co: Cortex. **Figure 3.** Profiles of coronal sections of the hippocampi from a control (green) and DcxKO mouse (pink). The hippocampal slice volumes rose more slowly in the DcxKO mouse compared to the control.

Discussion and conclusion: A μ MRI strategy based on "passive staining" of brain samples by gadoteric acid strongly reduced cerebral relaxation times and permitted the recording of high resolution 3D-GE images with a good contrast to noise ratio between cortex and subcortical structures, at 4.7 Tesla. It allowed us to perform a systematic study of brain morphology in the DCX KO mouse model, providing information such as modified hippocampal shape or ectopic fiber bundles that could hardly be obtained with non-stained post-mortem MRI or classical histological methods.

References 1. Dhenain M et al. Dev. Biol. 2001. 2. Benveniste H et al. NeuroImage 2000. 3. Kappeler C et al. Submitted.

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