# Progression of Whole Mouse Brain Formaldehyde Fixation by T2 and ADC Maps

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**Purpose:** MRI of fixed brains allows to accurately map a number of biophysical parameters (PD, T1, T2, ADC) and to relate these to the microscopic structure of the tissues in normal and pathological conditions [1-2]. MRI has been used to study the formaldehyde fixation of whole human brains both theoretically and experimentally [3-4]. A study on the effect of post-mortem interval (PMI) of the MR properties of rats cortical and spinal cord slices was reported [5]. However, to the best of our knowledge, no data have been reported about the fixation process of whole mouse brain. In this MRI study we have investigated the time course over 18hours from sacrifice of T2 and ADC maps of whole mouse brain during fixation with formaldehyde, comparing the immersion and perfusion methods. A non-fixed whole brain was studied for comparison.

**Methods**: Animal experiments were performed in compliance with the national law 116/95. Three adult Balb/C nude mice (7 weeks, 19g) were sacrificed under deep anesthesia and perfused through the heart with saline at 4 °C containing heparin. Then the brain of two mice were carefully removed from the skull and immersed in 15ml of saline solution (control) or 4% formaldehyde in 0.1M PB at pH 7.4 (infusion) before the MRI scan.

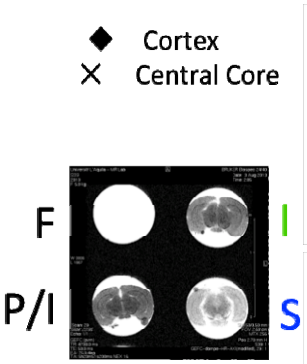
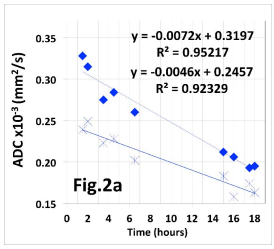
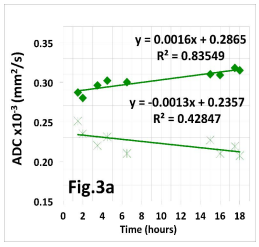
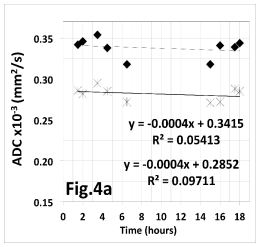
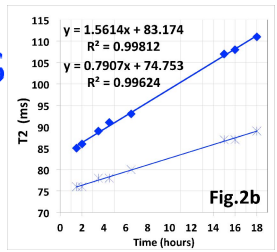


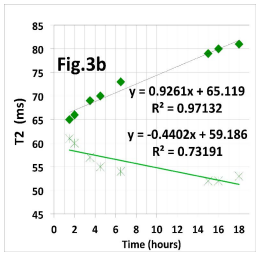

Fig.1

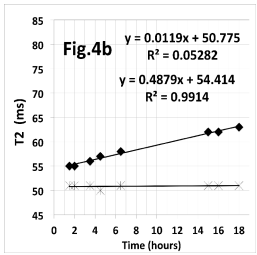



Following the standard procedure with saline, the third mouse was perfused with 4% formaldehyde in PB (perfusion), the brain carefully removed from the skull. The three brains were then positioned each in a 2ml tube containing the respective perfusion solutions (saline or formaldehyde) and inserted in the 2.35T MRI Bruker Biospec equipped with a TX/RX birdcage volume coil (8 rungs, diameter 65mm, length 10cm) tuned at the proton frequency of 100.3MHz. A tube containing the 4% formaldehyde solution was inserted close to the brains as a standard during the measurements (Fig. 1). The time course of brain fixation was monitored during a time window of 18hours by means of: coronal GE images(TR=3600ms; TE=30ms; FOV=2.8cm2; 256*256pixels; FA=75°; BW=8kHz; slice thickness=1mm; NEX=1; TACQ=15min) covering the whole brain; T2 and ADC maps acquired from a single axial slice 2.5mm in thickness positioned in the mid brain. The T2 and ADC mapping sequences were, respectively: MSME (TR=4800ms; 10 echos with TE=16-160ms; FOV=2.5cm2; 256*256pixels; 400µm resolution; BW=67kHz; NEX=1; TACQ=15min) and SE DtiStandard (TR=600ms; TEeff=56ms; diffusion gradients values B=300,600,900,1200s/mm2; diffusion gradient duration and separation 22/26ms, respectively; 256*256 pixels; 510µm resolution; FA=90°; BW=50kHz; NEX=1; TACQ=10min). During the study, an high-resolution axial GE image (TR=4700ms; TE=50ms; FOV=2.5 cm2; 256*256 pixels; 98µm resolution; slice thickness=0.5mm; slice number=25; NEX=16; TACQ=5h20min) was acquired to assess anatomical details and contrast between the three brains (Fig.1). The MRI images were analyzed with Paravision 4.0 to extract T2/ADC maps from specific ROIs.

**Results**: Figure 1 shows the high-resolution axial GE image at 8 hours. It can be seen a different contrast between the infused (I), saline unfixed (S) and perfused/infused (P/I) brains. Figures 2-4 show the ADC and T2 values measured in the cortex (CX) and central core (CC) of the mouse three brains over time. The unfixed brain showed a progressive increase of the T2 values, in accordance with a previous rat model [5]. The superficial layers of the P/I brains showed an increasing trend of T2, with a similar variation of about 20%. However, in the deeper ROI of the brain the P/I condition showed a constant lower T2 value (50ms), while the infused brain showed a steady decrease within the first 10hours, reaching a similar T2 value as the P/I brain over 18 hours. The ADC of unfixed brain presents a large decrease for both superficial and deep ROIs over time. The ADC values of the infused and P/I brains show very little variation over time. Overall, the infused brain shows lower ADC values with respect to the P/I brain and a wider gap between the cortex and central tissue values is evident.

**Conclusions:** In this MRI study we have investigated, for the first time, the progression of whole mouse brain fixation comparing two standard methods (immersion/perfusion). The data about the non-fixed whole brain should be useful to estimate the effect of post-mortem intervals on the MR contrast [5]. In conclusion, the perfused cerebral tissue shows lower T2 and constant ADC values, indicating that the perfusion-fixation process is set with a preserved microstructure over time. A maturation process characterizes the infused-fixed tissue within the first hours of observation, and it should be taken into account for subsequent MRI and histology.

**References:**

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