

# Diffusion in the extra-cellular space of the rat cerebral cortex probed by MRI and direct infusion of contrast agents

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## Introduction:

Measurements of the diffusion properties of substances in the cerebral extra-cellular space (ECS) aims to investigate drug delivery/clearance and brain tissue structure [1,2]. Currently used methods have high sensitivity, but are limited to single spatial points (real-time iontophoresis/pressure ejection methods), or are performed post mortem (radiotracer based autoradiograms), or has a limited depth penetration (techniques based on laser-induced fluorescence). Here we explore the use of MRI during direct infusion of T1 relaxating agents and mathematical modelling for investigating ECS diffusion.

## Theory:

The concentration of the contrast agent  $Gd_{conc}$  can be determined from measurements of the relaxation rate measured initially ( $R_1^0$ ) and after ( $R_1$ ) infusion of the contrast agent, provided that the relaxivity,  $r$ , of the agent is known. [Eq. 1]. It can also be determined from strongly T1 weighted images via [Eq. 2], where TR is the repetition time.  $E$  is the tissue enhancement, rescaled to correct for the initial tissue signal, defined in [Eq. 3], where  $S_0$  is the baseline scan acquired prior to infusion, and  $S_{[Gd]}$  is the T1w image acquired after infusion of the contrast agent. This approach is similar to the one presented in [3].

$$[Eq. 1]: Gd_{conc} = \frac{R_1 - R_1^0}{r}; \quad [Eq. 2]: Gd_{conc} = \frac{1}{r \cdot TR} \ln(1 - E); \quad [Eq. 3]: E = \left( \frac{S_{[Gd]}}{S_0} - 1 \right) \cdot \frac{(1 - e^{-TR \cdot R_1^0})}{(e^{-TR \cdot R_1})}.$$

## Methods:

Sprague-Dawley rats (N=11, 220-300g) were included in this study, approved by the local authorities in compliance with guidelines EUVD 86/609/EEC. Physiological signs (heart rate, respiration rate, body temperature, and pO<sub>2</sub>) were monitored throughout the experiment and remained within physiological limits. Surgery was performed after anesthesia with 2.0%isoflurane (Forene, Abbott, Wiesbaden, Germany), urethane (1.5 g/kg, i.p.) and xylocain (locally). A burr hole was made above the primary sensory cortex (S1) and a guiding cannula was implanted and fixed prior to positioning in a stereotaxic holder and insertion of a glass capillary (tip: OD: 21-30μm, ID: 6-10μm) in the center of S1. The capillary was connected to an injection pump (Vici, Valco Instruments) via 6.5m long fused silica tubes and the rat positioned in the center of a 7T Bruker Biospec 70/30 scanner (BGA-9S, Helmholtz RF volume transmission coil, and a 2cm single loop receiving surface coil). After acquisition of anatomical FLASH images, a pH-buffered solution of 20mM Gadoteric Acid, Gd-DOTA (Dotarem, Guerbet) in artificial cerebrospinal fluid with relaxivity in vitro at 7T determined to 3.6mM<sup>-1</sup>s<sup>-1</sup>, was infused as a 1.1±0.3μl slow bolus at a rate of 32±9nl/min. In one control experiment, only ACSF was injected to verify the absence of any medium induced signal alteration in the MR images. For the phantom studies, agar (2%) dissolved in a 0.9% saline (NaCl) solution, doped with 1mM Cu2SO4 was used with the same set-up. Biodistribution and diffusion of the contrast agent was imaged by a T1-weighted RARE sequence (TE=9ms, TR=290ms, voxel-size: 0.15x0.15x0.7mm<sup>3</sup>). Quantitative maps of R1 rates were obtained by a Look-Locker inversion recovery sequence with a single-shot EPI read out (TE=12ms; TR=8s; voxel-size: 0.28x0.28 x0.7mm, 250 kHz read-out bandwidth). Data analysis consisted of calculation of  $Gd_{conc}$  from [Eq. 2] and non-linear fitting of [Eq.4] to determine the wash out time. Next the injection point was identified, and non-linear least squares fitting of the numerical solution to the 3D diffusion equation for a point source and a bolus infusion, [Eq. 5] was performed, with N being the infusion rate in mM/s,  $\theta$  the Heaviside step function,  $t_{stop}$  the duration of the infusion,  $r^2$  the square of the distance to the injection point, and  $D^*$  the apparent diffusion coefficient of the contrast agent.

$$[Eq. 4]: Gd_{conc} = Gd_{max} e^{-t/W_0 t}; \quad [Eq. 5]: Gd_{conc} = N \cdot \int_0^t \frac{1}{(4\pi D^* (t - t_0))^{3/2}} \cdot e^{-\frac{r^2}{4D^* t}} \cdot [\theta(t_0) - \theta(t_0 - t_{stop})] dt_0$$

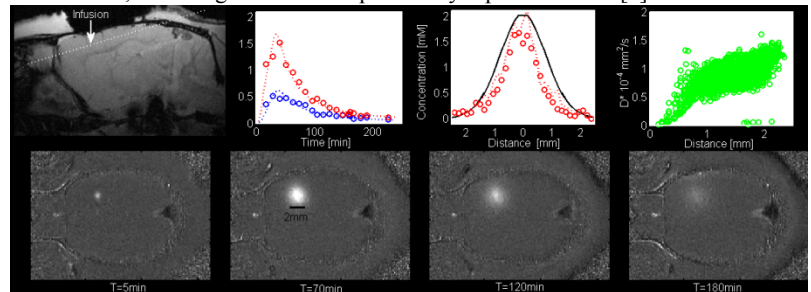
## Results and discussion:

The wash-out time was around 140 minutes and the apparent diffusion coefficient increased linearly close to the injection site (<0.6mm), causing a non-Gaussian shape of the spatial distribution curves (Fig), probably due to an infusion-induced increase of the interstitial volume [4]. Beyond this point, the increase in  $D^*$  with capillary distance leveled-off and reached values expected for molecules of this size [1]. The results obtained will depend on the accuracy of the value used for the relaxivity of Gd-DOTA, which may be influenced by the presence of macromolecules similar to Gd-DTPA [5]. The apparent diffusion coefficient determined in agar, 5·10<sup>-4</sup> mm<sup>2</sup>/s, was in agreement with previously reported values [6].

## Conclusions:

Measurement and modeling of diffusion of MR contrast agents in the extracellular space is feasible, and can be used to study contrast agent delivery/clearance, although infusion-related effects closed to the injection tip must be considered. The proposed methodology applied with contrast agents of variable size may provide an MR tool to study the ECS and brain tissue structure.

**References:** [1]Sykova, Nicholson *Physiol Rev* 88: 1277–1340, 2008; [2]Rusakov et al., *TINS* 34: 359-369, 2011; [3]Tofts, *Magn Reson Imag* 14: 373-80, 1996; [4]Basser *Microvasc Res* 44:143, 1992; [5]Stanisz, Henkelman *Magn Reson Med* 44:665-7, 2000; [6]Fonchy et al., *J Magn Reson Imaging*. 14(2):97-105, 2001.



**Fig.** Spatial and temporal biodistribution of Gd-DOTA determined after a bolus infusion in the primary sensory cortex. The position of the coronal planes is shown in a sagittal plane. Time-course data and spatial distribution are measured (circles) and fitted (dashed lines) values, the Gaussian spatial distribution for a fixed  $D^*$  is shown for comparison (black). The pixel wise  $D^*$  are also shown.