Achieving Equilibrium in Active Staining for MRI of Fixed Mouse Brain

M. D. Wong[1], S. Portnoy[1], C. Laliberté[1], and R. M. Henkelman[1]
[1]Mouse Imaging Centre, Hospital for Sick Children, Toronto, Ontario, Canada

Introduction – The use of a contrast agent for MRI of fixed mice brains is essential for the acquisition of high quality images. Therefore, the optimization of a sample preparation protocol, especially the method of introducing the contrast agent into the sample, is of great importance. One of the parameters on which a protocol should be judged is the length of time required, from the time of perfusion to the time of image acquisition, to obtain this optimal image. Several studies execute active staining protocols, which include the perfusion of the animal with a mixture 4% paraformaldehyde (PFA) and some gadolinium-based MR contrast agent concentration and then placing the extracted brain tissue into a bath solution (either 4% PFA or phosphate buffered saline, PBS) with the same concentration of the contrast agent as the perfusion solution. The hope is for the contrast agent to equilibrate with the brain and bath solution, allowing for the acquisition of equivalent images at any time following equilibrium. This study proposes to dramatically decrease the preparation time by demonstrating a protocol that benefits from the fact that brain vasculature is approximately 5% of the whole brain volume [1,2]. It can be postulated that diffusion of the contrast agent within the brain volume is faster than it is across the skull. Therefore, if one perfuses the mouse with 20 times the concentration of the MR contrast agent than described previously, one would expect that equilibrium can be reached faster than waiting for the contrast agent to diffuse from the bath solution and into the brain. Analysis and comparison of the aforementioned active staining protocols is described.

Sample Preparation - Four mice were perfused with a mixture of 30 mL PBS and then 30 mL of 4% PFA, both with three different concentrations of gadoteridol [ProHance, Bracco Diagnostics, Princeton, NJ], (2mM, 28 mM, 40 mM, and 56 mM). The skulls were excised and allowed to post-fix for 12 hours in 4% PFA with 2mM of ProHance at 4°C. The brains were then in an equilbriated bath in a solution of PBS with 2 mM of ProHance. Between MR image acquisitions the same bath solution was used.

MR Imaging - MR images of three of the brains (28 mM, 40 mM, 56 mM) were acquired at 10 different time points within 2 weeks following perfusion to evaluate the influence of the new proposed protocol on the quality of images. For comparison, MR images of the brain perfused with 2 mM of ProHance were obtained in which 18 scans were performed over 30 days following perfusion. A multi-channel 7.0-T MRI scanner (Varian Inc., Palo Alto, CA) with a 6cm diameter insert gradient set was used. The four brains were placed in 13-mm-diameter plastic tubes filled with fluorocarbon fluid (Flourinert FC-77, 3M Corp., St. Paul, MN) and 3 at a time were imaged in parallel with a custom built array of three 14 mm solenoid coils. A T2-weighted, 3D fast spin-echo sequence (TR/TE = 325/10 ms) was used with six echos (with the center of k-space acquired on the fourth echo, TE_{eff} = 40 ms), FOV = 14 mm × 14 mm × 25 mm, acquisition matrix of 144 × 144 × 258, and two averages. The total imaging time was 37 minutes and images with 97 µm isotropic voxels were obtained.

Data Analysis and Processing – The 3D data sets of each brain were automatically registered using linear transformations (i.e. translations, rotations, scaling and shearing) in order to have all the images for the same brain in the same space. A 3D region of interest in the cerebral cortex was chosen and analyzed for every data set. For each perfused brain (2 mM, 28 mM, 40 mM, and 56 mM) the mean signal was calculated in the specified region of interest for each of the data sets and was plotted against time. This data was fit to the curve $S = S_0 + (S_\infty + S_0)(1 - \exp(-t/C))$, where S is the mean signal intensity, S_0 is the mean signal immediately after perfusion and S_∞ is the mean signal at equilibrium, and C is a time constant.

Results – Table 1 displays the time constant C and the steady state mean signal S_∞ for each of the concentrations of ProHance used during perfusion of the 4 mice. The theoretical optimal contrast agent concentration (20 x 2 mM = 40 mM) is approximately 2 times faster to reach equilibrium within the brain than the more accepted protocol of 2mM. In addition, the time constants for the brains perfused with 28 mM and 40 mM have a percent difference of 4%, while the time constants for the 56 mM and 40 mM brains are different by 1%. Figure 1 is a graph of fitted data for brains perfused with 2 mM and 40 mM ProHance. Figure 2 displays registered images at different time points throughout this study's timeline.

Contrast Agent Concentration (mM)	Time Constant C (days)	R^2 value
28	2.3915	0.9231
40	2.2965	0.8490
56	2.2679	0.9668
2	4.5504	0.9325

Table 1: Time constants and R^2 values for mouse brains perfused with 2 mM, 28 mM, 40 mM, and 56 mM.
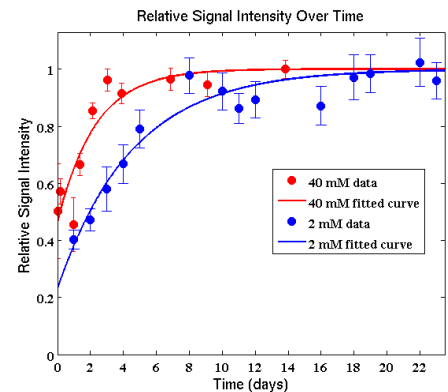

Figure 1: Relative MR signal for both 2 mM and 40 mM concentrations over time.

Figure 2: 2D images of the registered 3D data set of the mice brain perfused with 40 mM of ProHance at different time points after perfusion. a) immediately after perfusion, b) 1 day after perfusion, c) 2 days after perfusion, d) 4 days after perfusion, e) 14 days after perfusion.

Conclusions – From this study it is evident that by perfusing the mouse based on the ratio of brain blood volume to brain volume, one can substantially decrease the time between the perfusion of the animal and time of image acquisition. In this study, the actual MR images of the 40 mM ProHance perfused brains appeared to equilibrate after 4 days following perfusion. In comparison the 2 mM ProHance perfused brains reached an equilibrium after roughly a week. Furthermore, ProHance does reach equilibrium across the brain volume as shown by the goodness of the fit to our data. Lastly, the time constant changes by less than 5% when deviating by √2 from the optimal perfusion concentration of ProHance, therefore, one does not need to be exact in the degree of overdosing to obtain acceptable results.

References- 1. N. Kovacević. Cereb Cortex. 2005 May;15 (5):639-45. Epub 2004 Sep 1.   2. J. Richter. Proc Natl Acad Sci U S A. 1970 Jul; 66 (3):944-51.