# Observation of Frequency Shifts Induced by Chemical Exchange in Brain Tissue

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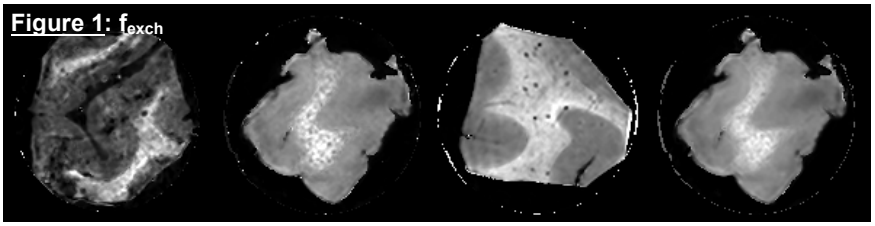
**Introduction:** Substantial resonance frequency shifts among human and animal brain tissues have been reported in high-field MRI studies [**1,2**]. The gradient-echo phase, directly proportional to the frequency, may provide complementary or improved information to conventional (magnitude) contrast mechanisms. Susceptibility differences have been widely thought to give rise to most of the phase contrast but the precise mechanisms underlying the frequency shifts are still poorly understood. Recently, chemical exchange between water and macromolecular protons has been proposed as a source of phase contrast between gray and white matter (GM, WM) [**3**] and exchange-induced frequency shifts have been observed in protein solutions [**3,4**]. Here we extended this work to investigate whether the proposed exchange mechanism contributes to the phase contrast in brain tissue. We carried out MR spectroscopic imaging experiments designed to observe directly any exchange-induced frequency shifts ($f_{exch}$) in fixed human and fresh pig brain tissue.

**Methods:** As in previous experiments [**3,4**], reference chemicals were used whose protons are assumed not to undergo chemical exchange. Because the local susceptibility-induced frequency shifts are identical for both the water and reference protons, $f_{exch}$ can be measured by subtracting the reference frequency from the water frequency in every voxel. Reference chemicals 3-(trimethylsilyl)-propionic acid-$d_4$ sodium salt (TSP) (50% w/v in saline) and 1,4 dioxane (50% v/v in saline) were added separately to two formalin-fixed samples from the human visual cortex and together to a fresh sample of pig cortex. Dioxane was used in addition to TSP as it has been suggested that TSP, unlike dioxane, may interact with proteins [**4,5**]. 3-D chemical shift MR imaging (CSI) was performed in both fixed and fresh brain tissues soaked for at least 5 days in reference chemical solutions. CSI was performed using a 600 MHz vertical bore spectrometer (Bruker) at constant room temperature after the tissue samples had been allowed to equilibrate to this temperature. CSI scans had isotropic spatial resolution = 100 μm, matrix size = 150 x 150 x 32, spectral width = 10 kHz, 1024 time points, TR = 120 ms and a delay of 1.367 ms before acquisition. The data were band-pass filtered (FWHM ~550 Hz) to generate water and reference time-domain signals. These were spatially Fourier-transformed, resulting in both water and reference magnitude and phase images at each time point. $f_{exch}$ was obtained from a linear fit of the phase difference between the reference and water signal in every voxel over time. Only timepoints having a magnitude signal-to-noise ratio greater than 10 were included in the fit. Regions of interest (ROIs) were drawn in the GM and WM to allow calculation of the exchange-induced GM-WM contrast, $\Delta f_{exch}$.

**Results:** Water and reference magnitude images were very similar, showing that the reference chemicals permeated the tissues. Raw phase images showed intracortical contrast (human cortex) and GM-WM contrast. The phase of all the band-pass filtered signals depended linearly on echo time as expected. Maps of $f_{exch}$ observed in each tissue sample are shown in Figure 1 (scaled from 0 to 30 Hz except Fig. 1A which is scaled from 0 to 10 Hz) together with ROI results in Table 1. There is strong GM-WM contrast in all the $f_{exch}$ maps but almost no intracortical contrast in the human visual cortex maps. WM has a higher $f_{exch}$ than GM in all the samples, both fresh and fixed tissues for both TSP and dioxane. The human visual cortex soaked in TSP has smaller $f_{exch}$ and GM-WM $\Delta f_{exch}$. Fresh and fixed tissues have similar $f_{exch}$ and $\Delta f_{exch}$ although using dioxane as a reference leads to higher $f_{exch}$ and GM-WM $\Delta f_{exch}$ than with TSP.




**A: Human, TSP** **B: Pig, TSP** **C: Human, Dioxane** **D: Pig, Dioxane**

| Table 1 Tissue | Reference Chemical | GM-WM $\Delta f_{exch}$ (ppb) Mean | S.E. |
|---|---|---|---|
| Fixed Human Visual Cortex 1 | Dioxane | -13.45 | 0.08 |
| Fixed Human Visual Cortex 2 | TSP | -6.30 | 0.08 |
| Fresh Pig Cortex | Dioxane | -10.30 | 0.13 |
| | TSP | -10.00 | 0.17 |

**Discussion and Conclusions:** Exchange-induced frequency shifts $f_{exch}$ have been observed for the first time in fixed human visual cortex and fresh pig brain cortex using reference chemicals that are not involved in chemical exchange. Substantial exchange-induced frequency shifts and GM-WM contrast were observed in both fresh and fixed brain tissue with both TSP and dioxane reference chemicals. $f_{exch}$ and GM-WM $\Delta f_{exch}$ are smaller when TSP is used as a reference, perhaps because it interacts to some extent with proteins [**4,5**]. Despite the raw water and reference phase images having strong intra-cortical contrast similar to that in vivo for the human visual cortex sample soaked in TSP, there was almost no intra-cortical contrast in $f_{exch}$ (Fig. 1A). This suggests that the primary source of intracortical contrast is increased susceptibility in the stripe of Gennari due to its high iron content [**6,7**]. The sign of $f_{exch}$ observed in these experiments is positive everywhere but the GM-WM contrast $\Delta f_{exch}$ is negative and opposite to the GM-WM frequency contrast observed in vivo [**1**]. This suggests that exchange is an important source of GM-WM frequency contrast but is not sufficient to explain the GM-WM frequency contrast observed in vivo because it has the opposite sign. These results imply that the amplitude of tissue magnetic susceptibility differences may be even greater than is currently thought. These experiments suggest that exchange should be included in future models of frequency contrast although further studies are needed to quantify the contribution of exchange in vivo. The presence of this macromolecular exchange-based contrast mechanism may expand the applicability of frequency and phase imaging for the investigation, diagnosis and staging of diseases involving alterations in tissue macromolecular content.

**References:** **1.** J.H. Duyn et al. *PNAS* **2007**, 104(28), 11796-801 **2.** J.P. Marques et al. *Neuroimage* **2009**, 46(2) 345-352 **3.** K. Zhong et al. *Neuroimage* **2008**, 40(4), 1561-6 **4.** J. Luo et al. *Proc. ISMRM* **2009**, 863 **5.** A. Shimizu et al. *J. Biomolec. NMR.* **1994**, 4(6), 859-862 **6.** Li T-Q et al. *Proc. ISMRM* **2008**, 885 **7.** M. Fukunaga et al. *Proc ISMRM* **2009**, 47