In-vivo Longitudinal Relaxation Enhancements (LREs) of Central-Nervous-System Metabolites at 21.1 T upon Stroke

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Target Audience. Researchers and clinicians interested in stroke and MR spectroscopic characterizations.

Introduction. The effective T_1 of macromolecular resonances may be shortened significantly by harnessing spectrally selective excitations[1], which focus on specific (e.g., amide) resonances while leaving a large reservoir of solvent and/or macromolecular magnetization unperturbed. This phenomenon–termed Longitudinal Relaxation Enhancement (LRE)–is driven by exchange or cross-relaxation in slowly tumbling molecules, and can be used to enhance NMR spectral sensitivity[2]. A recent study directly measured the apparent T_1s of non-labile metabolic resonances in intact *ex vivo* mice brains by a similar approach[3], revealing unexpected apparent T_1 reductions even for methyl resonances of endogenous metabolites upon switching from broadband excitation with active water suppression to a spectrally selective sequence targeting only the resonances of interest[3]. Here, we implement an *in vivo* localized version of the ensuing LRE-MRS sequence. The potential of LRE as a biomarker in a rat model of *in vivo* ischemia is evaluated.

Purpose. To employ LRE for detecting superior *in vivo* metabolic spectra and assess the latter's effectiveness as novel stroke biomarkers.

Methods. All experiments were performed in the National High Magnetic Field Laboratory's 21.1 T MRI operating at a ^{1}H frequency of 900 MHz and equipped with a Bruker Avance III console. The middle cerebral artery of juvenile Sprague-Dawley rats (*N=6*) was occluded for 1.5 h, followed by reperfusion. Rats were scanned at 3 h, 24 h and 1 w post ischemia. ^{1}H MRS spectra were acquired using a localized LRE sequence[3], which spectrally targeted only four prominent resonances of interest (Lac, NAA, Cre and Cho) by means of SLR-designed[4] polychromatic excitation and single-band refocusing pulses, 8 and 4 ms long, respectively. Spatial localization was achieved by a 3D LASER module inserted just prior to acquisition (Figure 1). Metabolic apparent T_1s were measured by a progressive saturation (PS) approach[5]. In separate acquisitions on the stroked rats, LRE-MRS data were collected from 5x5x5 $(mm)^3$ voxels positioned in the ipsi- and contralateral hemispheres.

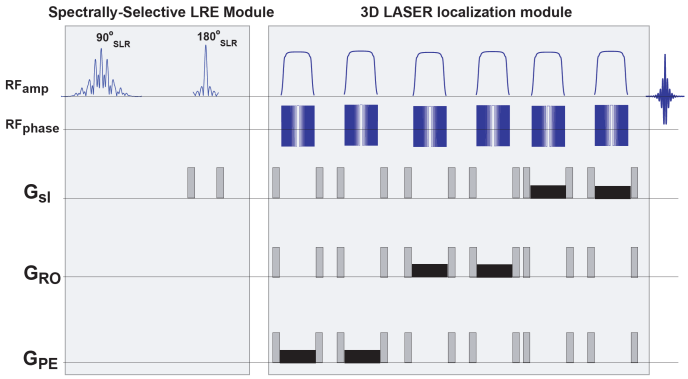

Figure 1. Localized LRE MRS sequence, involving a selective excitation and refocusing of Lac, NAA, Cre and Cho resonances, and 3D LASER spatial localization. Localizing gradients are shown in black and crushers in gray.

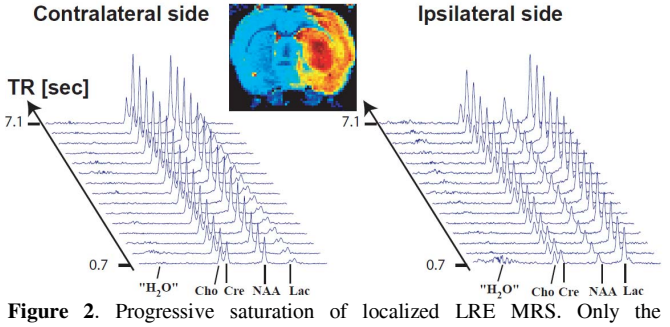

Figure 2. Progressive saturation of localized LRE MRS. Only the metabolites of interest are excited; no active water suppression was used, and the signal was averaged with 48 repetitions (~30 minutes to acquire the entire curve). Spectra allow for robust quantification of apparent T_1s.

Results and Discussion. Localized LRE-MRS acquisitions from the *in vivo* stroked rat brain at 21.1 T show clear resonances from the targeted metabolites with no excitation of water (Figure 2). SNR for the shortest TR was typically very high exceeding 100 for Cre in only 48 repetitions. Marked increases of Lac and decreases of NAA levels in the ipsilateral hemisphere were noted upon stroke (Figure 2). The peaks' TR dependence was used to extract the apparent metabolic T_1s using the LRE-MRS sequence (Figure 3), and these were compared with a water-suppressed CHESS-Spin-Echo experiments where water magnetization is actively excited and crushed prior to the application of the sequence shown in Figure 1. Apparent T_1 reductions in the spectrally selective LRE sequence were evident for Cre at all three times post ischemia ($p<0.05$); by contrast, the NAA and Cho resonances do not exhibit any LRE. Interestingly, Lac appears to show a trend towards LRE only at 24 h post ischemia, although statistical significance was not achieved. An increase is shown in the apparent T_1 of Lac 3 h post stroke, which further increased at 24 h but subsequently decreased after 1 w. These transient apparent T_1 changes of Lac may be indicative of its chemical environment; the initial increase observed 3 h post stroke may reflect a release of Lac to an environment in which it undergoes fewer interactions with macromolecules or membranes, and the subsequent apparent T_1 decrease may reflect the transport of excess Lac. Interestingly, a statistically significant transient increase in Cre apparent T_1 was evident between 3 and 24 h in the ipsilateral hemisphere, which at 1 w decreased back to its initial value. This differential response was only observed in the LRE sequence. The absence of LRE for NAA is also consistent with a recent *ex vivo* study[3]; however, we do not find statistically significant differences for Cho as previously reported *ex vivo* at 9.4 T[3]. This difference may be a result of the different magnetic fields employed, of the profile of the selective pulses, or from inherent differences between *ex* and *in vivo* tissue. Notably, there is a similarity in apparent T_1s of small metabolites for 9.4 and 21.1 T. Unexpected for small molecules in solution, this result underscores the complex and dynamic interactions between small endogenous metabolites and their host tissues.

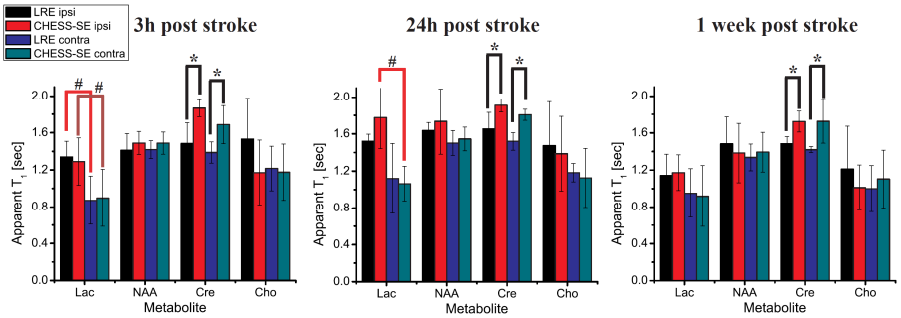

Figure 3. LREs and apparent T_1s in stroked rats for the four resonances of interest. *p<0.05, #p<0.05 (ANOVA, Fisher means). * indicates LRE; # indicates differences between ipsi- and contralateral.

Conclusions. Superior MRS traces and differential LRE effects were detected for the first time *in vivo* via a novel localized MRS sequence. The spectra arising from the spectrally-selective excitation obviate water suppression, significantly enhancing MRS quality while providing a commensurate increase in SNR per unit time. LRE also was found to be time-dependent post ischemia, highlighting the complex nature of interactions between metabolites and their tissue under dynamic conditions in normal and diseased states.

Acknowledgements. This work was supported by an ERC Advanced Grant (#246754), a Helen and Martin Kimmel Award for Innovative Investigation, and the generosity of the Perlman Family Foundation, as well as the American Heart Association (10GRNT3860040) and NHMFL (NSF DMR-0654118), including a visiting scientist grant (#12601).

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