Normalization of fMRI signal with basal physiologic state improves sensitivity in differentiating subject groups

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INTRODUCTION: Recently, we have developed a T2-Relaxation-Under-Spin-Tagging (TRUST) technique to quantitatively estimate cerebral venous oxygenation (Yv) in units of percentage (1), and have shown that stimulation-evoked fMRI signal is inversely correlated with Yv(baseline) across subjects (2). Specifically, individuals with higher baseline oxygenation tend to have a smaller fMRI signal and vice versa, when using identical stimuli. This provides an opportunity to use TRUST MRI as a normalization factor to reduce inter-subject variations, and to improve sensitivity of fMRI in detecting group differences. This improvement will be beneficial for the study of psychiatric disorders and comparison of pre/post drug treatment, thereby substantially extending the potential clinical utility of fMRI. Here we aim to test the utility of TRUST MRI in distinguishing subject groups in a “model” situation. Checkerboards flashing at 8Hz and 4Hz are presented to two healthy control groups, respectively. Comparison between the groups with and without using TRUST MRI is conducted to assess whether or not TRUST MRI can improve the statistical significance of the results.

METHODS: MR experiments (3T Achieva, Philips) were performed on a total of 13 healthy subjects (7 for 8Hz and 6 for 4Hz, the groups were randomly assigned) with informed consent. It is well established that the BOLD fMRI signal is dependent on the flashing frequency, greatest when using an 8Hz stimulus and lower when using a higher or lower frequencies (3). The vision of the subjects was corrected using MR-compatible corrective lenses, whenever necessary. Vital physiologic signs, including blood pressure, heart rate, arterial oxygenation, and end-tidal CO2, were measured before they entered the magnet room. Stimulus paradigm included 10s flashing and 50s fixation and repeated 4 times. An extra 50s of fixation was used at the beginning of the experiment. Activation detection was based on cross-correlation with a standard BOLD response determined in one of our previous studies (4). The largest activation cluster was determined and this always corresponds to the occipital lobe regions (visually verified). To account for the effect of ROI size on the signal amplitude, only the 500 voxels that have the highest cross-correlation were included in signal average. TRUST MRI (~4 min) was also performed at resting condition to estimate Yv(baseline) (1). The BOLD fMRI parameters were: TR=500ms, flip angle 37 degrees (to maximize CNR and to reduce inflow effect), TE=30ms, SENSE factor 2, matrix 64x64, voxel size 3.44x3.44x5 mm3, 1mm gap.

RESULTS and DISCUSSION: Robust activations were detected in all subjects, including both the 8Hz and the 4Hz groups. An example of the voxels that were used for signal average is shown in Figs. 1a and b. Fig. 1c shows the averaged signal time-courses for both groups. The physiologic parameters, blood pressure, heart rate, arterial oxygenation, end-tidal CO2, and breathing rate, showed no significant differences between the groups. TRUST MRI yielded baseline venous oxygenation of 62.1±2.8% and 51.0±15.1% in the 8Hz group and 4Hz group, respectively. While not planned, the 4Hz group was found to have a wider spread of Yv, and a lower mean value, although the group values are not significantly different (p=0.082, Student t test). The BOLD signal amplitude before TRUST normalization was 2.71±0.20% and 2.44±0.32% for the 8Hz and 4Hz group, respectively. The Student t test yielded p=0.1007, not yet reaching statistical significance. The Yv values from TRUST MRI were then used to normalize the BOLD fMRI signal amplitude by using a linear regression: S=a1•Yv+a0, where S is the BOLD signal amplitude, Yv is the flashing frequency, and a0 is the baseline Yv. The normalized BOLD signal is then calculated as S normalized=S•a1•Yv. It was found that the results after normalization showed significant difference between the 8Hz and 4Hz groups (Fig. 2 middle) and the p value showed an ~100 fold reduction. In TRUST MRI, Yv is estimated by comparing experimentally determined venous blood T2 to a calibration plot specifying the relationship between the baseline T2 and Yv. We also tested to see whether blood T2 can be used as a normalization factor, thereby eliminating the need to assume/measure the calibration plot. Fig. 2 (right) plots the results, showing that venous blood T2 can yield similar normalization effect. One can also use previously established relationship between IMRI signal and Yv to do normalization: i.e. Normalized Signal = Observed Signal + Yv•0.0374, where 0.0374 is the slope obtained from our previous study (2). The results were similar to the direct regression (without using previous data) findings (p=0.0007) and the results were comparable between Yv or blood T2 normalization. Note, however, that similar stimulus types and brain regions should be used when comparing data in different subject data or literature. Regression analysis was also performed using each of the physiologic parameters (i.e. blood pressure, heart rate, arterial oxygenation, end-tidal CO2, and breathing rate) as the regressor, and no significant difference between 8Hz and 4Hz was found using any of these parameters at a p value threshold of 0.05 (corrected for multiple comparisons). Another way to understand the data is to look at them in a scatter plot (Fig. 3). If we consider that the parameter of-interest (in this case the stimulus frequency, but could also be patients vs. controls, or pre vs. post-drug treatment) is the only parameter affecting the IMRI signal amplitude, we are only looking at the data in one dimension (Fig. 3, left), which may show a considerable overlap. If we can identify a physiologic modulator affecting the IMRI signal and measure this modulator, we can then look at the data in two-dimensions (Fig. 3, right), in which the effect of parameter-of-interest is more obvious and detectable. In fact, in this example, the Yv-normalized fMRI signals were completely separated (e.g. by using 2.533% as a threshold) in the right panel, compared to the left panel where the data can only be investigated in one dimension. If we can identify a physiologic modulator affecting the fMRI signal and measure this modulator, we can then look at the data in two-dimensions (Fig. 3, right), in which the effect of parameter-of-interest is more obvious and detectable. In fact, in this example, the Yv-normalized fMRI signals were completely separated (e.g. by using 2.533% as a threshold) in the right panel, compared to the left panel where the data can only be investigated in one dimension.

In summary, TRUST MRI can provide a means to normalize fMRI signals across subjects and to improve the sensitivity of detecting fMRI signal differences between two groups of subjects. This may benefit IMRI studies of psychiatric and neurological disorders.