Normalization of fMRI signal improves group differentiation in Alzheimer's Disease

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INTRODUCTION: Early stage Alzheimer's disease (AD) is characterized by memory deficit, which is thought to be a sign of dysfunction in the medial temporal lobe (MTL) of the brain. Functional MRI provides a great opportunity to study the mechanism of these neural deficits in humans [1]. Results of such studies have, however, been inconsistent, with findings suggesting increased/decreased/unchanged activation in AD patients compared to elderly controls [1]. Moreover, the amplitude of fMRI signal is often uncorrelated with behavioral data, making it even harder to interpret the fMRI findings. In this context, it is important to note that the BOLD contrast is an indirect measure of neural activity and is influenced by various factors including baseline physiology (e.g. venous oxygenation, $Y_{v,baseline}$) [2] and changes in underlying vasculature [3,4]. This is particularly relevant for applications in patient populations such as AD where one or more of these factors may be changed. Therefore, in order to properly quantify neural activity from fMRI signals, these non-neural components need to be factored out. Here we compared BOLD fMRI signal during a visual memory task in AD to normal controls (NC). Importantly, we applied two normalization techniques [2,3] to remove the effects of basal physiology and cerebrovascular reactivity (CVR), respectively. The signals before and after normalization were assessed in terms of group differentiation and the correlation with post-MRI memory scores.

METHODS: A total of 16 subjects (8 AD: age 68.3±10.8 yrs, Mini-mental State Exam (MMSE) scores 23.1±3.8; 8 NC: age 68.4±8.5 yrs, MMSE scores 29.5±0.5) were recruited for study following informed consent. Imaging was performed on a 3T Philips scanner. The visual memory BOLD fMRI scan was conducted with TR/TE=1500/30ms, resolution 3.44x3.44x6 mm^3, duration 11min. During the activation block, subjects were asked to judge if the scenes displayed were outdoor/indoor and then memorize them. During baseline, subjects identified whether arrows pointed to left/right (to control for attention). A post-MRI memory test was used to identify how well they memorized the scenes. For CVR measurement, the subjects breathed room-air and 5% CO2 in an interleaved fashion (switching every minute), while BOLD MRI (resolution 1.8x1.8x6 mm^3, TR/TE=3000/30ms, duration 7min) scans were acquired similar to a typical fMRI scan. End-tidal CO2 which is the vasodilatory input function to brain vasculature was recorded throughout the scan. For basal physiology measurement, a recently developed technique, TRUST MRI (~4 min), was performed at resting state to estimate venous oxygenation [2]. A T1w MPRAGE (1x1x1mm^3) was also acquired for structural assessment. Standard processing was performed on memory BOLD data using SPM. CVR data was processed similarly using a general linear model, except that the regressor was the end-tidal CO2 trace rather than the "fMRI paradigm". The resulting CVR is in units of %BOLD/mmHg CO2. Both BOLD and CVR images were spatially normalized into MNI space using the T1w image. We used HAMMER software [5] to do this spatial normalization since it corrects for brain atrophy. Hence, both CVR and BOLD maps were partial volume corrected for group comparisons. For fMRI signal normalization, the *normalized BOLD signal* was defined as *un-normalized signal / CVR / (1 – $Y_{v,baseline}$)*, following procedures used by Thomason *et al.* [3] and Lu *et al.* [2].

RESULTS AND DISCUSSION: All subjects could perform the CO2 task well with no adverse side effects. No differences were found in the arrow and scene identification tasks (p>0.2), suggesting that both groups paid good attention to the stimuli. However, the AD patients performed significantly worse than NC in the post-MRI memory test (Table 1, p<0.001). The MTL was found to be activated in both AD and NC following group-level analysis (Fig. 1). Next, we assessed the amplitude of un-normalized fMRI signals in the two groups (Table 1). For this, we defined the MTL ROI anatomically (not functionally defined, to avoid circular argument) and the voxels inside the ROI were averaged. The signal amplitude in AD and control groups were similar (Table 1) (S_{AD}/S_{NC}=105%). If anything, AD had a slightly higher value than NC. In addition, the un-normalized BOLD signal showed no correlation with the post-MRI memory scores (p>0.25).

Previous studies (in healthy controls) have suggested that fMRI signal is correlated with baseline oxygenation [2] and CVR [3,4] across subjects. However, such a relationship has not been confirmed in patient populations. Our data extended these findings to AD patients and to MTL regions: we found that subjects with higher baseline oxygenation tend to have lower fMRI signal (Fig. 2) and those having higher CVR tend to have higher fMRI signal (Fig. 3). These correlations were found both for the entire subject group and for the AD group only (p<0.05). Once we establish this relationship, the fMRI signal normalization can be carried out using the equation described above. For the normalized fMRI signals, we found that the AD group had considerably lower signal amplitude compared to controls (S_{AD}/S_{NC}=75%) (Table 1). Furthermore, we found that the normalized BOLD data was now positively correlated to the post-MRI memory scores (both for entire subject group and for AD only, p<0.05, Fig. 4). Thus, overall, the fMRI data was now much more interpretable after normalization: patients with early AD showed deficits during the memory task; the neural substrate for this deficit was that MTL showed less activation as shown by reduced fMRI signal; within AD group, patients with worse symptoms showed even less signals.

PARAMETERS:	AD	NC
Post scan score (%)	63.3±13.6	86.8±6.9
BOLD signal (%)	0.21±0.15	0.20±0.05
Normalized BOLD signal	1.95±1.82	2.59±1.15
Baseline oxygenation (%)	59.4±9.8	57.7±6.3
CVR (%BOLD/mmHg)	0.25±0.11	0.19±0.05

Table 1: Memory test and ROI results for AD and NC. Values are expressed as (Mean±SD).

Comparing the normalized and un-normalized signals, a possible reason for the different finding is that the higher CVR in the AD group (Table 1) within the MTL region could have been responsible for their original high BOLD signal. Once differences in the CVR were accounted for, the normalized BOLD signal now reflects the 'real' trend in neuronal activation across the two groups.

In summary, our results emphasize the importance of normalizing the BOLD signal to remove the influence of physiologic modulators such as baseline oxygenation and CVR. This is especially important for the correct interpretation of hypo- or hyperactivation results in fMRI studies that compare diseased with healthy groups. This has important implications on the potential clinical utility of BOLD fMRI.

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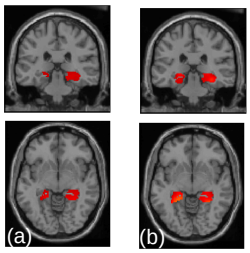

Fig.1. Activation in MTL during visual memory task in (a) AD and (b) NC groups. (1 sample t test, uncorrected p<0.005, k=200). Only activations in MTL are shown.

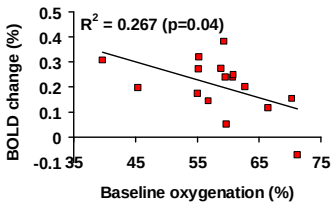

Fig 2. Scatter plot of BOLD against baseline oxygenation showing significant negative correlation (n=16).

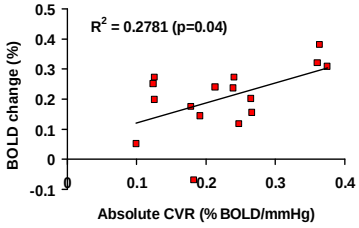

Fig 3. Scatter plot of BOLD against CVR showing significant positive correlation (n=16).

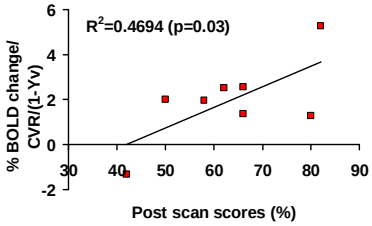

Fig 4. Scatter plot showing positive correlation between normalized BOLD signal and memory scores in the AD group (n=8).