The BOLD FMRI response to spontaneous fluctuations in CO2, evaluated in the human brainstem

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INTRODUCTION: The aim of this study was to map BOLD reactivity to CO_2 in the human brainstem by correlating natural fluctuations in CO_2 with changes in BOLD signal. Areas in the brainstem that are responsible for the control of breathing are known to be exquisitely sensitive to CO_2 (chemoreceptors). Resting fluctuations in CO_2 are correlated with significant low frequency variations in breathing [1] and with the BOLD signal [2]. They therefore provides us with a physiologically realistic model for examining CO_2 sensitivity in the natural state. We compare the results with those from external CO_2 challenges in the same subjects examined during the same session (more detail in another abstract that we have submitted to this conference).

METHODS: 12 healthy volunteers (2 female) took part in the study performed on a Siemens Trio 3T scanner. T2* weighted gradient-echo EPI scanning was performed (TE=30 ms, TR=1000 ms, 1130 volumes, voxel size 2.5x2.5x3mm). The field of view comprised 16 coronal oblique slices of the brainstem. The subject wore a tight fitting face mask attached to a breathing system which delivered air at 30 litres per minute. The subject was asked to perform no particular task and to keep their eyes open throughout. Recordings were made of tidal CO_2 and O_2, respiratory volume, and pulse oximetry. The responsiveness of BOLD signal to CO_2 (defined as the % BOLD signal change per unit change in $PETCO_2$) was compared between different areas. Analysis was carried out using FSL [3], including correction for B_0 distortion with FUGUE [3] and RETROICOR [4] to correct for cardiac cycle and respiratory cycle related variations in BOLD. Voxel-wise statistical analysis was extended to a second (group) level in a mixed effects analysis using FLAME [3]. Registration to standard images was carried out using FLIRT [3], using a three stage registration procedure making use of a single volume whole brain EPI, a T1 high resolution structural and a brainstem weighting mask in standard space [5]. A region of interest analysis (ROI) was performed, that compared the BOLD response to CO_2 in predefined areas (pons, medulla, thalamus, putamen, rostral ventral lateral medulla (RVLM)) and also areas identified by the voxel-wise analysis from the CO_2 challenge study to have the strongest response to CO_2 (areas in the pons and subthalamic nuclei). We estimated the time delay between the CO_2 change and the BOLD response, by calculating the time lag of maximal cross-correlation. To examine the magnitude of the BOLD response to CO_2, we performed a linear regression between CO_2 and BOLD at this optimal delay time. The slope of the BOLD response to CO_2 was compared between areas using repeated measures ANOVA and differences were located using Tukey's Least Significant Difference post-hoc test, P values <0.05 were considered significant. Finally, the impulse response (IR) function between $PETCO_2$ and BOLD signal was calculated by expansion of the former in terms of the Laguerre basis and least-squares estimation of the expansion coefficient [6].

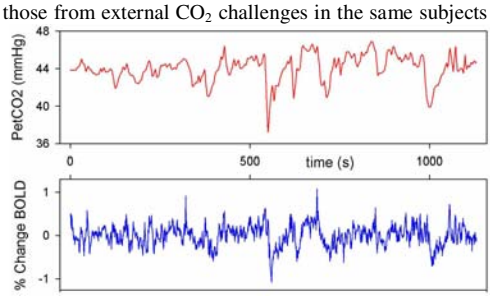

Figure 1. PetCO2 (red)and BOLD signal (blue) in one ROI (subthalamic activation) recorded breath-by-breath from one volunteer during an FMRI session lasting ~19 min.

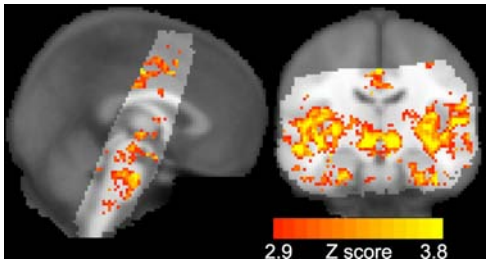

Figure 2. Group map of statistical significance of BOLD signal change per mmHg change in $PETCO_2$. Significant regions are displayed with a threshold of Z>2.9 and with a cluster probability threshold of P<0.05. The area scanned is shown in lighter gray scale, and superimposed on MNI standard brain (darker gray)

RESULTS: The mean $PETCO_2$ (SD) in the 12 subjects was 44.4 (1.1) mmHg. The voxel-wise analysis (figure 2) revealed significant BOLD reactivity to CO_2 bilaterally in an area comprising caudal thalamus and subthalamic nuclei, and in midline in the pons. As the input stimulus is relatively small (CO_2 fluctuations are approximately 1mmHg) the statistical map of BOLD response is noisier than that seen with CO_2 challenges, however the overall pattern of activation appears to be similar. Cross-correlation showed variation in BOLD signal change between the different ROI's (table 1 and figure 3). The RVLM and subthalamic activation exhibited greater BOLD reactivity to CO_2 than either the pons or the medulla ($P<0.01$). These results are comparable with those obtained from the external CO_2 challenge experiment in that the relative BOLD sensitivity between regions of interest remains similar, however the overall BOLD sensitivity to CO_2 was considerably lower when examined using natural fluctuations in this experiment. The IR analysis, revealed a similar pattern between the regions of interest (figure 4), yielding maximum magnitude values and fastest dynamics for the RVLM and subthalamic areas (not in diagram for clarity). Interestingly, the RVLM exhibited larger magnitude values than the subthalamic area, in contrast to external CO_2 challenges, in agreement to figure 3. On the other hand, the smallest maximal IR magnitude values and slowest dynamics were observed in the medulla, with the characteristics of the RLVM IR being considerably different than the rest of the medulla. When compared to the IR's obtained from external CO_2 challenges, the fluctuation IR's exhibited similar magnitude, but also exhibited an undershoot after approximately 30 seconds (figure 4), which explains the smaller CO_2 sensitivity shown in figure 3.

area	r^2
medulla	0.03 (0.01)
pons	0.05 (0.01)
putamen	0.06 (0.01)
thalamus	0.09 (0.03)
pons activation	0.06 (0.01)
RVLM	0.04 (0.01)
subthalamic activation	0.10 (0.03)

Table 1. regression coefficients (s.e.m) in ROI's

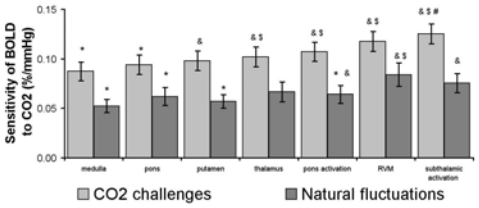

*Figure 3. % BOLD signal change per mmHg CO2 (+/-s.e.m.) from cross-correlation. P<0.05 compared with * RVLM, & medulla, $ pons, # thalamus.*

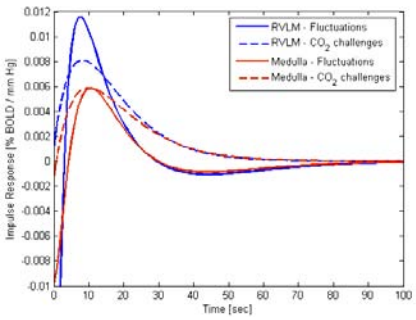

Figure 4. Averaged impulse responses between CO2 and BOLD for two ROI's and comparing data from CO2 challenges with natural fluctuations.

DISCUSSION: We have mapped BOLD reactivity to CO_2 in the human brainstem using two different techniques of CO_2 stimulus. Although the pons and the medulla have lowest overall BOLD response to CO_2 we have found that the RVLM appears to have a greater response than the rest of the medulla. The RVLM is hypothesised to contain the Pre-Botzinger complex, which is considered to be the kernel of respiratory rhythm generation [7], and is highly chemosensitive. By investigating the BOLD response to CO_2 in the natural state, we have shown differing behaviour of the BOLD response to CO_2, specifically the undershoot following the BOLD response is abolished in the unnatural experimental condition of external CO_2 challenges, and the integral of the IR is smaller, however the magnitude of response is similar. Simple cross-correlation analysis gives poorer sensitivity, because of this undershoot. It has been suggested that gain of the central chemoreceptors is maximal at natural CO_2 levels [8], our data support this. Our data suggest differing behaviour of these chemosensitive networks at rest compared to when being stimulated. Examining natural CO_2 fluctuations provides us with a physiologically realistic method to investigate CO_2 sensitivity and has a potential application for investigation of respiratory related paradigms.

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