Investigating the metabolic changes due to visual stimulation using functional proton magnetic resonance spectroscopy at 7T

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Introduction Detection of functional changes in ^{1}H metabolites may enable a greater understanding of neurotransmitter activity and metabolic pathways used for energy synthesis during activation of brain tissue. Previous MRS studies of the activated human brain mainly focused on observing Lactate (Lac) changes [1-3]. More recent studies by Mangia et al [4], taking advantage of the increased signal and spectral resolution at 7T, have investigated the change in the level of Lac, glutamate (Glu), Aspartate (Asp) and Glucose (Glc) during activation. However, Mangia did not measure significant change in the level of gamma aminobutyric acid (GABA) and Glutamine (Gln), which might be expected to change due to increased neurotransmitter cycling rates during activation[5]. Moreover, brain imaging techniques have demonstrated that functionally active neurons show increased metabolic activity and oxygen consumption [6], resulting in increased generation of reactive oxygen species (ROS), which have been shown to cause cell damage [7]. The tripeptide Glutathione (GSH), made up of the amino acids Glu, cysteine (Cys) and glycine(Gly), has been thoroughly investigated as an important intracellular antioxidant/free radical scavenger[7]. No prior studies in ^{1}H-MRS have reported the effect of visual stimulation on brain activity-simulated GSH responses. The purpose of this study was to confirm and quantify the changes in the levels of Glu, GABA, and Gln, and to further investigate GSH, Lac, Asp and Glc in response to functional visual stimulation. In doing so, we hope to establish a clear picture of brain metabolism and neurotransmitter activity during activation.

Methods Nine healthy subjects participated in this study. All MR measurements were acquired using a Philips Achieva 7T MR system and a 16-channel SENSE head coil. An initial fMRI scan (EPI, TE=26ms, TR=2200ms) was performed prior to spectroscopic acquisition in order to determine the activated region in the visual cortex The visual stimulus was projected onto a screen using a LED projector and viewed with prism spectacles. A cubic VOI of 2cm^3 was positioned inside the activated region for functional MRS acquisition. The functional paradigm comprised two 10 minute stimulation periods interleaved with two 10 minute rest periods. A STEAM sequence (TE/TM/TR=15/17/3000ms, spectral width 4000Hz, 4096 time points) was used for acquiring the MRS data. Spectra were collected in blocks of 32 averages, with 6 blocks per 10 minute period. Water suppression was performed using MOIST (Multiply Optimized Insensitive Suppression Train) sequence. 1 acquisition without water suppression was acquired for eddy current correction and for metabolite quantification. Spectral post-processing including frequency alignment and phase corrections were carried out using jMRUI and MATLAB. Summed spectra for visual stimulus off and visual stimulus on (BOLD "free": LB added to compensate for BOLD changes in linewidth) were analyzed separately using LCModel (see Fig.1). The LCModel basis dataset includes the simulated spectra of 20 metabolites [8, 9] and macromolecule baseline experimentally measured from the human visual cortex using an inversion-recovery experiment (TR=2000ms, IR=0.675secs) (see Fig.3(f)). A Wilcoxon signed rank test was conducted to compare the metabolite changes.

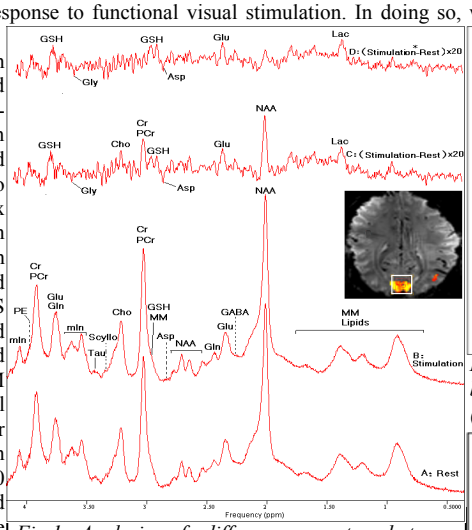

Fig.1 Analysis of difference spectra between stimulation and rest periods, using data from 9 subjects (group analysis): (A) averaged spectrum acquired at rest; (B) averaged spectrum acquired during stimulation; (C) difference between (A) and (B). The peaks seen at1.33, 2.01, 2.35, 3.03 3.18, and 3.78ppm in the difference spectrum are attributed to linewidth changes due to the BOLD effect as well as concentration changes of metabolites. (D) as (C), but the spectrum acquired during stimulation was linebroadened by 0.5Hz to match the linewidth of the spectrum acquired at rest, in order to eliminate the linewidth changes. Lac (1.33ppm), Glu (2.35ppm) and GSH (2.92/2.97/3.78ppm) survive this procedure. Inset: Example acquired BOLD response. The voxel used for MRS is indicated by a white square.

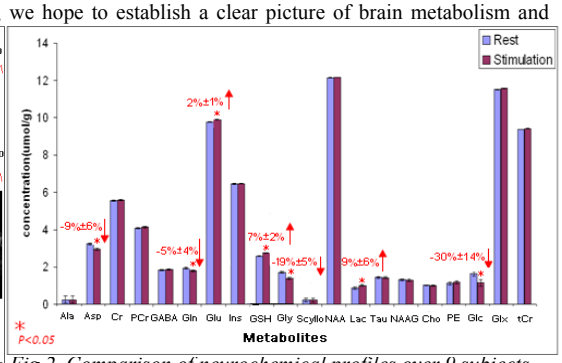

Fig.2. Comparison of neurochemical profiles over 9 subjects between rest and stimulation periods determined using LCModel (error bars= SD).

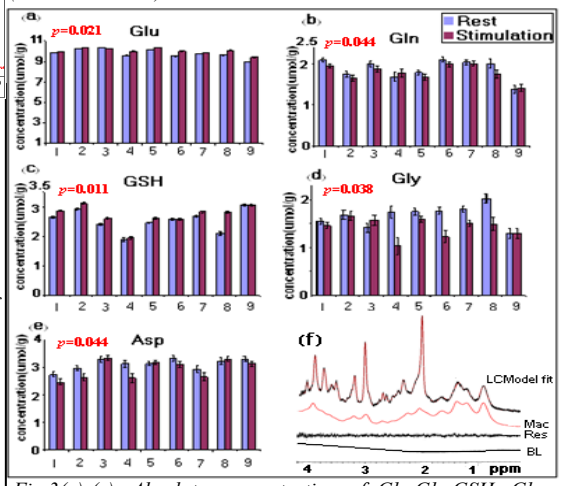

Fig.3(a)-(e). Absolute concentration of Glu,Gln,GSH, Gly and Asp over 9 subjects during rest and visual stimulation (Mean ± SD). (f) Example of LCModel fit; from top to bottom: raw spectrum (black curve) and LCModel fit (red curve), macromolecules, residue and baseline.

Results: Fig.2 shows the comparison of neurochemical profiles over 9 subjects for the measured 20 metabolites between rest and stimulation periods. In good agreement with previously published results [4], during stimulation periods, Glu was found to be significantly increased by 2%±1% (p=0.021, see Fig.3(a)), Asp decreased by 9%±6%(p=0.044, see Fig.3(e)), Lac increased by 9%±6% and Glc decreased by 30%±14%. Interestingly, the brain GSH concentration was found to be elevated by 7%±2% (p=0.011, see Fig.3(c)) during neuronal activation, in parallel with decreased brain Gln and Gly, by 5%±3% (p=0.044, see Fig,3(b)) and 19%±5% (p=0.038, see Fig.3(d)), respectively. No statistically significant difference was observed for GABA and other metabolites.

Discussions and Conclusions: The observed changes of Asp, Glc and Lac concentrations in response to visual stimulation support the suggestion by Mangia [4] that sustained neuronal activation raises oxidative metabolism to a new steady state. Our observations of increased Glu with decreased Gln in the visual cortex during stimulation are consistent with our previous pilot results [10], and indicate a stimulus driven increase in excitatory neurotransmitter cycling. Glu plus Gln did not change, further implying a shift in the Glu-Gln interconversion. No significant change was measured in GABA. The elevated GSH in the visual cortex in response to visual stimulation is a new observation. Possible reasons for it include detoxification of ROS species or clearance of the increased Glu (Glu is a metabolic precursor of GSH), generated during the intense neuronal activation. Gly is also a precursor of GHS and a decrease on activation is consistent with increased GSH synthesis. On the basis of these results acquired from the visual cortex, we propose an increase in oxidative metabolism, excitatory neurotransmitter cycling and GSH synthesis, possible related to Glu clearance and ROS detoxification.

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