^{1}H MRS at 7T demonstrates a strong correlation between stimulus-induced γ-frequency in the visual cortex and the glutamine/GABA ratio.

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Introduction: The peak frequency of stimulus-induced visual γ-oscillations (30-100Hz) measured using magnetoencephalograpy (MEG), has been shown to be predicted by the resting concentration of γ-aminobutyric acid (GABA), measured using Magnetic Resonance Spectroscopy[1] (MRS). Modelling of a cortical network containing GABAergic inhibitory interneurons and excitatory pyramidal cells[2] indicates that the frequency of oscillation depends on the balance between excitatory and inhibitory synaptic currents, suggesting that, whilst GABA may predict the peak γ-frequency, it may be better to consider the ratio of excitatory to inhibitory neurotransmitter levels (e.g. Glu/GABA). In this study we utilize the increased spectral resolution and signal available at 7T to quantify Glu, Gln and GABA, in order to determine whether the peak frequency in stimulus-induced visual γ-oscillations is better predicted by a ratio of excitatory/inhibitory neurotransmitter levels than by GABA levels alone.

Method: 11 healthy subjects (mean age = 35±15 years, mean±standard deviation (SD)) participated in the study. MRS and MEG data were acquired from each subject on the same day.

Visual Paradigm: Subjects viewed a visual stimulus (drifting sinusoidal grating; 3 cycles per degree; 8Hz drift rate; Michelson contrast of 1) presented centrally in a circular window with a 6 degree visual angle. For BOLD, a trial comprised 24s of stimulation with 33s rest with 5 trials presented. For MEG, a single trial comprised 10s of stimulation followed by 10s rest with 40 trials recorded. This paradigm has been shown to induce strong γ-band oscillations in the primary visual cortex[3,4].

MR Measurements: MR measurements were acquired using a Philips Achieva 7T system with a 16 element SENSE head receive coil and surrounding volume transmit coil. EPI BOLD data were acquired to localise a region of interest in visual cortex and allow positioning of the MRS voxel. MR spectra were acquired with a short TE STEAM sequence (TE/TM/TR=16/17/2000ms, bandwidth (BW)=4000Hz, No. points=4096, Volume of Interest (VOI)=20x20x20mm^3). 288 water-suppressed (ws) and 2 non-ws (nws) spectra were collected to allow absolute quantitation of metabolite concentrations. Spectra were phase-corrected before realigning and averaging the ws and nws spectra separately. Metabolite concentrations were estimated, in arbitrary units using LCModel, and were removed from the analysis if SDs from LCModel exceeded 25%.

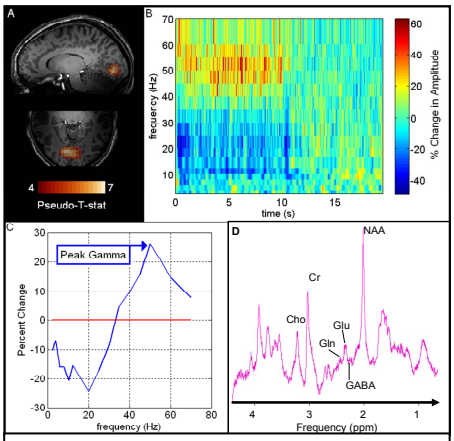

Figure 1: a) MEG pseudo-T stat image b) Time frequency spectrogram c) Difference spectrum (stimulation-rest) d) Example MR spectrum

MEG Measurements: MEG data were acquired using a 275 channel CTF system (sample rate 600Hz). Co-registration of MEG to anatomical MRI was achieved using head digitisation and surface matching. MEG data were band pass filtered into overlapping frequency bands spaced between 0 and 70Hz. Frequency filtered data were processed using synthetic aperture magnetometry[5]. Pseudo-T statistical functional images were constructed by comparison of neural oscillatory power in an active time window (0.5s-2s) to that in a control window (10.5s – 12.5s). Resulting images showed the spatial signature of changes in oscillatory power between the two windows. These images were used to derive locations of interest (LOI) in the visual cortex. Frequency filtered MEG data were projected to each LOI using a beamformer. A Hilbert transform was applied to projected signals in each frequency band yielding the analytic signal; the absolute value of this gave a timecourse showing variation in oscillatory amplitude for each frequency band. Timecourses were concatenated in frequency space yielding a time frequency spectrogram. Peak γ-frequency was measured for each subject and plotted against basal metabolite levels on a subject by subject basis. Correlation between the two measurements was assessed using Pearson's correlation coefficients.

Results and Discussion: Levels of Glu and Gln were measured in 10/11 subjects; GABA was measured in 7/11 subjects. One subject's data were not included due to excessive motion. Fig. 1 shows the peak location of stimulus induced γ-oscillations (a) and the associated time-frequency spectrogram (b). Increased γ and concomitant decreased α/β oscillations are apparent, in agreement with previous studies[3,4]. Fig. 1(c) shows a difference spectrum with peak γ increase at ~50Hz. Fig. 1(d) shows an example spectrum from a single subject with clearly resolved peaks from Glu, Gln and GABA from 2.25-2.45ppm. Fig. 2 shows correlation of peak γ-frequency with measured metabolite levels. In agreement with previous results[1], higher GABA levels tend to be related to higher peak frequencies (p = 0.2, Fig. 2a). Measurements of metabolites that may co-edit with GABA when using MEGA-PRESS[6], as used in previous studies at lower field[1], do not show significant correlation with γ-frequency, which eliminates the possibility that the correlation was due to co-edited metabolites. Correlation of γ-frequency with Glu concentrations (Fig. 2b) indicates that glutamate levels have no effect on γ-frequency. However, it is suggested that a large proportion of the Glu pool is metabolic and is not involved in neurotransmission. The Gln pool size is thought to be closely related to the neurotransmitter Glu pool and interestingly, Gln was found to be negatively correlated (p=0.06, Fig. 2c) with peak γ-frequency. Finally, to test the hypothesis, that γ-frequency is dependent on the excitatory/inhibitory ratio, both Glu/GABA and Gln/GABA ratios were plotted against γ-frequency. Correlation with Glu/GABA (R^2=0.35) is stronger than for GABA alone (R^2=0.26), despite the large metabolic pool. γ-frequency versus Gln/GABA shows the strongest correlation (R^2=0.65, p<0.05) in agreement with our primary hypothesis.

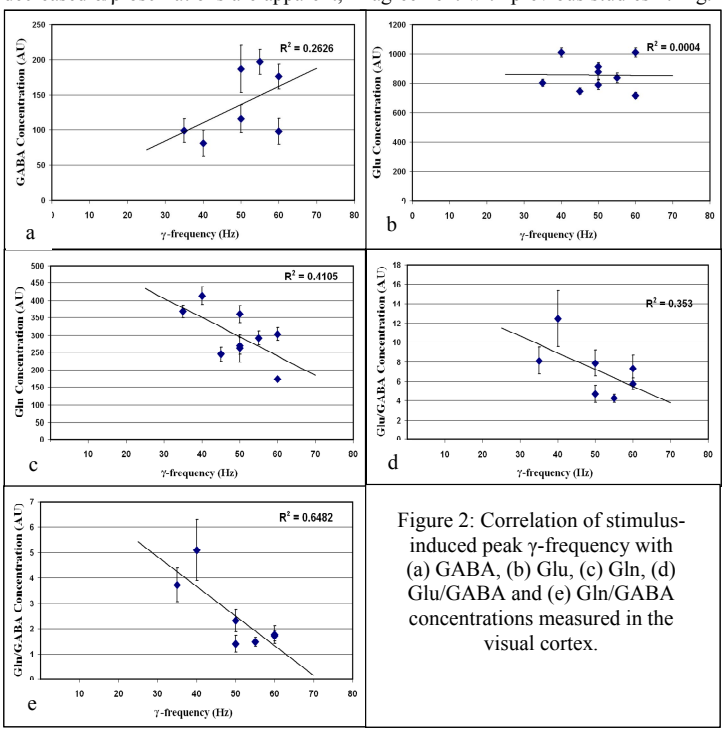

Figure 2: Correlation of stimulus-induced peak γ-frequency with (a) GABA, (b) Glu, (c) Gln, (d) Glu/GABA and (e) Gln/GABA concentrations measured in the visual cortex.

Conclusions: The present study has confirmed recent reports of a link between neurotransmitter levels and the frequency of visually induced gamma oscillations. Our measurements are in general agreement with previous results, however we show that a ratio of excitatory to inhibitory neurotransmitters yields a stronger correlation than just GABA, a finding that agrees with cortical network models.

References: **1**. Muthukumaraswamy et al, PNAS **106** (2009) **2**. Brunel and Wang, *J Neurophysiol.* **90** (2003) **3**. Stevenson *HBM* 2010 in press; **4**. Zumer et al. *NeuroImage*, **49**(2) (2010) **5**. Robinson and Vrba, *Recent advances in Biomagnetism,* (1998). **6.** Mescher et al. *NMR Biomed.* (1998)

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