Functional connectivity of auditory and visual areas during rest and stimulus presentation.

K. J. Mullinger[1], P. S. Morgan[2], D. P. Auer[2], R. W. Bowtell[1]

[1]Sir Peter Mansfield Magnetic Resonance Centre, School Of Physics and Astronomy, University of Nottingham, Nottingham, United Kingdom, [2]Academic Radiology, University of Nottingham, Nottingham, United Kingdom

Introduction: Inter-regional correlation of low frequency signal fluctuations in resting-state fMRI data has been shown to reveal functional connectivity [1-5], with evidence that the pattern of correlated areas is modulated by sensory stimulation [3] or task execution. Here, the correlation of signal fluctuations in visual and auditory cortices has been studied using whole-brain fMRI and changes in the pattern of connectivity occurring due to presentation of visual and auditory stimuli evaluated. Concurrent EEG recording was used to monitor changes in brain state, and to allow comparison of EEG power fluctuation with BOLD signal variation.

Methods*:* *Data acquisition:* Experiments were performed on 5 healthy volunteers with fMRI and EEG data acquired simultaneously *(i) fMRI:* A Philips Intera Achieva 3.0T MR scanner was used with a whole body rf transmit coil and an 8 channel SENSE head coil. A standard EPI sequence was implemented with *TR*=3 s and *TE*= 35ms (64×64 matrix, 3.25×3.25 mm^2 in-plane resolution and 3 mm slice thickness). 40 transverse slices were acquired, using a SENSE factor of 2. Cardiac and respiratory cycles were simultaneously recorded using the scanner's physiological monitoring system (vector cardiogram (VCG), pulse oximeter and respiratory belt) whose output is sampled at 500 Hz. *(ii) EEG* data was recorded using a BrainAmp MR EEG amplifier, Brain Vision Recorder software (Brainproducts, Munich) and the BrainCap MR electrode cap with 32 electrodes (following the 10/20 system, sampled at 5 kHz) The EEG sampling and scanner waveforms were synchronised by driving the BrainAmp clock cycle using a 5 kHz signal derived from the 10 MHz MR scanner clock.

Paradigm: Five experiments were performed in which: (i) (resting-state experiment) the subject was asked to lie with their eyes closed, without falling asleep; (ii) (acoustic stimulus) the subject was presented with relaxing music (without words); (iii) (visual stimulus) the subject watched a moving, computer-generated image.

Each of these experiments lasted 15 minutes during which 300 image volumes were acquired. The order of implementation of experiments (i-iii) was randomised across subjects and the subjects were not given any prior indication of subsequent tasks to be performed. Subsequently, in order to localise visual and auditory areas, two simple experiments were also undertaken: (iv) visual stimulation using a flashing (8 Hz) checkerboard (v) auditory stimulation using a tone of varying pitch. Experiments (iv) and (v) each employed a block paradigm, involving 5 cycles of 30 s with the stimulus on and 30 s with no stimulation. All the experiments were performed in darkness.

Analysis: *fMRI:* Image processing (realignment, normalization to standard brain and spatial smoothing with a 5 mm FWHM Gaussian kernel) was carried out using SPM99. fMRI models were set up for (iv) and (v) with correction for global effects, high-pass temporal filtering with a cut-off frequency of 0.008 Hz and low-pass filtering using the canonical hrf. The results of this analysis thresholded at $p<0.001$ (uncorrected) were used to identify regions of interest (ROI) in both the auditory and visual cortex of the right hemisphere for each subject.

The time-courses of average signal variation in these ROI's during experiments (i-iii) were then used in the analysis of functional connectivity. Each time-course was temporally smoothed with a high frequency cut-off Gaussian function (< 0.08Hz [1]) so that only low frequency fluctuations were considered. Filtering with a low-frequency cut-off of 0.002 Hz was used to eliminate the effects of slow signal drifts. The resulting time-course was employed as a regressor in statistical analysis of the full-image data sets (temporally smoothed using the same parameters). Levels of connectivity and their variation due to presentation of auditory or visual stimuli, were evaluated by counting the number of voxels showing significant correlation with the ROI's time course within the right and left auditory and visual areas using a threshold of $p<0.05$ (corrected).

EEG: Off-line EEG signal correction was based on averaging and then subtracting gradient and pulse artifacts, as implemented in Brain Vision Analyzer (Brainproducts, Munich) [6]. R-peak markers for pulse artifact correction were generated using the VCG trace from the scanner. The temporal variation of the power in the alpha (8-14 Hz) and beta (17-23Hz) frequency ranges was evaluated using data from electrodes O1 and O2, and the resulting time-courses were exported into Matlab for further analysis. This involved calculating the average alpha power during experiments (i-iii) and evaluating the correlation coefficient of each of the ROI's time courses with the corresponding, temporally smoothed, alpha and beta power time courses.

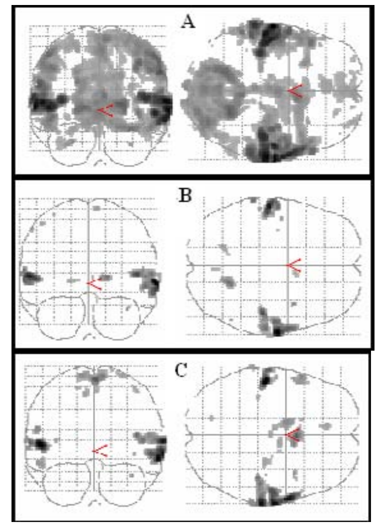

Figure 1: Connectivity at p=0.05 (corrected) with auditory ROI for A: resting state, B: auditory stimulus, C: visual stimulus for subject 1.

Results*:* *fMRI:* Data sets from two subjects could not be used in the analysis: one due to the identification of significant movement (> 5 mm) during image alignment; the second subject fell asleep during the resting state study. Data from the remaining subjects show strong functional connectivity between left and right visual areas and left and right auditory areas in the resting state as previously reported [2]. There was trend for reduction of number of correlated pixels in the presence of visual or auditory stimuli, but this did not reach statistical significance (Fig. 2A). More interestingly, significant numbers of voxels in visual cortex showed fluctuations in intensity that were correlated with the time course of the ROI in right auditory cortex during the resting state study. This finding was confirmed when looking for voxels showing signal fluctuations that were correlated with those measured from the ROI in right visual cortex. This correlation of signal fluctuations in visual and auditory was found to be significantly reduced (P=0.0045 for a one tail paired t-test considering auditory to visual and visual to auditory correlation) when an auditory or visual stimulus was presented (example data shown in Figs. 1 & 2B). *EEG:* Initial analysis of the EEG data showed that the average alpha power decreased when either a visual or auditory stimulus was present compared with the resting state. No significant correlation was found between the ROI time-courses from the image data and the EEG power fluctuations for any combination of ROI and frequency band.

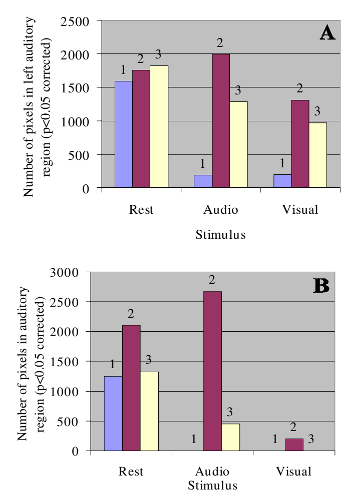

Figure 2 Number of pixels in: (A) left auditory cortex correlated with the right auditory ROI & (B) auditory cortex that correlate with right visual ROI.

Discussion and Conclusions: The data from this study show that in the resting state there are significantly sized regions of visual and auditory cortex that exhibit correlated signal fluctuations. The reduction in this inter-regional correlation when auditory or visual stimuli are presented argues against this finding being due simply to correlated physiological noise or image artefacts. Many previous studies of connectivity have employed small numbers of slices that may have precluded identification of the pattern of correlated fluctuation described here. The variation of alpha power indicates a change in electrical activity during stimulus presentation. The fMRI data imply that a more specific network of brain areas is engaged by attention to sensory stimuli, in agreement with previous findings of a reduction in the extent of areas displaying correlated signal fluctuation in visual cortex during natural viewing compared with the resting state [3] and on moving from sleep to a wakeful state [4].

References: **[1]** Biswal *et al.* MRM **34**(4): 537-541, 1995. **[2]** Cordes *et al.* AJNR **22**: 1326-1333, 2001 **[3]** Bartels and Zeki Neuroimage **24**(2): 339-349, 2005. **[4]** Kaufmann, *et al.* Brain, accepted for publication . **[5]** Laufs *et al.* PNAS **100**(19): 11053-11058, 2003. **[6]** Allen *et al.* Neuroimage **8**: 229-239, 1998