Resting-state functional connectivity predicts the amplitude of the BOLD response to thermal pain stimulation in humans

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Introduction: Spontaneous, low-frequency (<0.08Hz) fluctuations in BOLD fMRI signal are highly synchronised between functionally connected regions in the human brain [1]. These intrinsic connectivity networks (ICNs) present a popular and valuable methodology for studying both the functional architecture of the human brain in health and disease; and the functional significance of fluctuations in ongoing brain activity that occur in the absence of external stimuli or task performance. Recent evidence suggests that brain activity immediately preceding a stimulus, as indexed by the amplitude of oscillatory EEG activity or fMRI signals, can affect the magnitude of the brain's response to that stimulus [2,3,4,5]. However, the broader relationship of whether the resting-state signal coherence between nodes of a functional brain network is related to how those nodes respond to stimulation remains unclear. The "*pain matrix*" is a well studied, distributed network of brain regions that generate the perception of pain from nociception [6]. Here we use BOLD fMRI to investigate how the strength of resting-state functional connectivity (FC) between the primary nodes of the pain matrix is correlated with the amplitude of the BOLD response during noxious thermal heat stimulation in those areas.

Methods: *Paradigm:* Thermal pain stimuli were applied to the peroneal area of the right leg at two temperatures (PATHWAY CHEPs, Medoc, Israel). During a preliminary testing session prior to scanning the high temperature condition was selected as that which elicited an average subjective pain report of 7/10 on a numerical rating scale (NRS). High temperatures delivered were: 53°C (2 subjects); 52°C (9 subjects); 51°C (2 subjects). The low condition was always set 2°C below the high temperature. Individual runs consisted of 36 trials of one temperature condition. Two of these runs were acquired for each temperature, in each subject. Run order was counterbalanced across subjects. The inter-stimulus interval (ISI) was 20s. Individual trials of the experiment consisted of a single delivery of the stimulus followed by a 10s fixation period before a visual cue instructed the subject to provide a behavioural pain rating. This cue lasted 6s during which time subjects were instructed to report a subjective pain rating using a 0-4 NRS (0 = no pain and 4 = severe pain). *Imaging:* fMRI was recorded in 16 subjects. Data from three subjects was discarded due to gross movements (>3mm) that resulted in poor quality fMRI data. Thirteen subjects (6 female, 24.9±3.8 years, mean age±SD) remained for further analysis. Four stimulus runs during fMRI were acquired using a 3T Philips Achieva scanner (367 volumes/run, 32 slices oriented parallel to the AC–PC axis, 3x3x4 mm voxels, TR=2000 ms, TE=35 ms, SENSE factor=2, flip angle=80°). In addition a 6-minute (180 volumes) resting-state scan with no task was also acquired, during which subjects were instructed to relax, keep their eyes open and remain awake.

Analysis: fMRI analyses were carried out using FSL 4.1 (www.fmrib.ox.ac.uk/fsl). RETROICOR was used to reduce physiological noise in the BOLD data. All data were routinely preprocessed, spatially smoothed (5mm) and registered to MNI standard space. *Stimulus GLM:* First-level GLM analysis was performed in FEAT using a constant amplitude regressor of stimulus timings, convolved with the canonical HRF, to identify regions of significant positive BOLD response. Results were combined across runs at the second-level using fixed effects, and combined across all subjects at the third-level using FLAME 1+2 mixed effects (cluster corrected p<0.05). To compare the characteristics of resting FC and the BOLD response to thermal pain in pain processing regions, three regions of interest (ROIs) were defined from the most significant regions of the group Z-statistic activation map: anterior insula (AntIns), anterior cingulate (ACC) and secondary somatosensory cortex (SII). To ensure comparison of equal voxel volumes between subjects and regions, all ROIs were formed by centering a 3x3x3 voxel cube on the peak voxel in each area. Individual masks were made by registering ROIs to stimulus runs and the resting scan. For each ROI and each stimulus run, the mean BOLD timecourse across voxels was calculated. Single-trial HRFs were extracted based on stimulus timings, converted to percentage signal change relative to the last 6s and averaged across each run. The peak BOLD amplitude was then calculated. *Resting FC:* BOLD data from the separate resting scan were further low-pass filtered (0.008<f< 0.08Hz). The following trends of no-interest were removed with linear regression: six motion parameters, global signal calculated by averaging across all voxels, and ventricular and white matter signals [7]. FC analysis for each individual was performed to investigate the strength of BOLD signal correlation between ROIs at rest. The stimulus run ROIs were used to extract an average voxel seed timecourse from the resting-state data. This seed timecourse was correlated with the BOLD signal in all other brain voxels. Pearson correlation coefficent (R) values were used to create a map of the voxels significantly correlated with the seed timecourse of each ROI. The average correlation between two ROIs was calculated from these correlation maps to measure resting-state FC between two distinct brain areas. These FC values were correlated with the mean peak amplitude of the BOLD response that was extracted from stimulus run data using the same ROIs.

Results: Mean pain ratings were significantly larger for high temperature than low temperature stimuli (p<0.001 respectively paired t-test, data not shown). Only results from high temperature condition are reported here, though similar results were observed for the low temperature condition. Significant positive BOLD responses (PBR) to thermal pain stimuli were observed in: cerebellum, thalamus, bilateral insula cortex, contralateral SII, bilateral precentral gyrus, prefrontal cortex (PFC), ACC and SMA (Fig 1A). FC analysis showed that resting BOLD signal in SII, ACC and AntIns nodes of the pain matrix were mutually and selectively highly correlated (Fig 1BCD). The spatial pattern of resting FC was qualitatively very similar to that of the BOLD response evoked by thermal pain stimuli in separate runs (Fig 1A vs Fig 1BCD). The strength of the BOLD signal correlation between these ROIs in the *independent resting dataset* was significantly correlated with the mean amplitude of the peak BOLD response to the high temperature stimulus in these same areas (Fig 2). The mean peak BOLD response in AntIns was predicted by the resting FC between this area and both ACC and SII. Interestingly, the ACC and SII peak BOLD response was predicted by resting FC between spatially distinct brain regions: SII-AntIns and ACC-AntIns respectively.

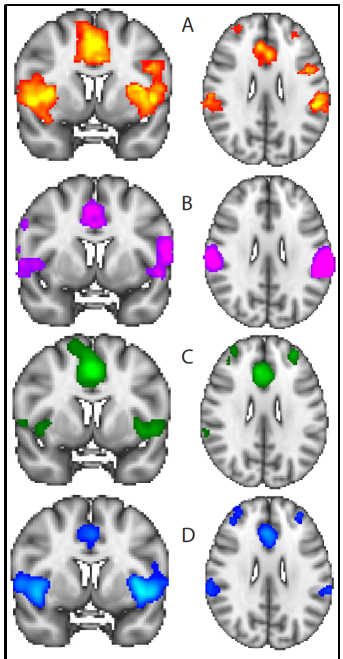

Figure 1: Group BOLD response to CHEPS stimulus (A), group mean resting FC seeded in SII (B), ACC (C) and AntIns (D)

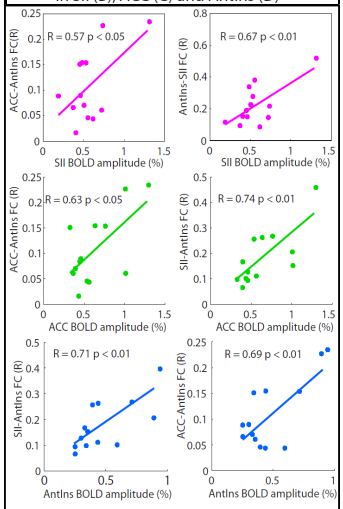

Figure 2: The functional connectivity between pain processing regions during rest is significantly correlated with the mean peak amplitude of BOLD response to CHEPs in SII (purple), ACC (green) and anterior insula (blue) ROIs.

Discussion: Here we demonstrate that the strength of resting FC between spatially distinct areas of the brain network responsible for processing pain perception has a direct functional relevance to the magnitude of the BOLD response to a painful stimulus, and by extension the subject's perception of a painful stimulus. Subjects who had greater resting FC in pain-responsive regions had a larger BOLD response to thermal pain stimulation, demonstrating that part of the inter-individual variability in evoked BOLD responses is explained by the intrinsic properties of the pain network. In a previous study using a cognitive task paradigm, inter-individual differences in regional BOLD signal changes were shown to be positively related to resting-state FC in the task-positive network, or negatively related to FC in the default mode network [8]. However, our work is the first demonstration of a functional relationship between FC and BOLD response in the same pain processing areas. Our findings are important for BOLD fMRI studies of pain as the ability to use measures of resting-state functional connectivity as a representative marker for a subjects' neural and behavioural response to a painful stimulus would require shorter, simpler and less painful scanning sessions and could be of great benefit in patient studies.

References [1] De Luca et al Neuroimage **29**(4):2005. **[2]** Fox et al Neuron **56**:2007. **[3]** Reinacher et al J. Neurosci Meth.183:2009. **[4]** Becker et al J. Neurosci **31**(30): 2011. **[5]** Scheeringa et al J. Neurosci. **31**(10) 2010. **[6]** Peyron et al Neurophysiol. Clin. **30**:2000. **[7]** Fox et al PNAS **102**:2005. **[8]** Mennes et al Neuroimage **50**(4):2010.