Resting State Functional Connectivity in Tuberous Sclerosis Complex and Refractory Epilepsy

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Object: Functional connectivity (FC) MRI has been used to identify seizure onset regions and 'epileptogenic' networks (Stufflebeam et al 2011, Bettus et al 2011). This study aimed to test if FC can be used to differentiate epileptogenic from non-epileptogenic cortical tubers in tuberous sclerosis complex (TSC), by studying the coupling of spontaneous brain activity from one tuber to another, between tubers and thalami, and between tubers and the default mode network (DMN).

Patients and methods: We acquired resting-state fMRI from 10 children with TSC and refractory seizures. The patients were scanned under general anaesthesia, immediately prior to intracranial-EEG seizure localization and tuberectomy. For each patient, 10 minutes of BOLD-weighted functional MRI was acquired (TR 2400 ms, TE 40 ms, 3.2 x3.2 x 3 mm3 voxels). The fMRI data were pre-processed using slice-timing correction, motion realignment and spatial smoothing (FWHM = 6 mm). Before performing seeded FC analysis, each subject's data were first screened using independent component analysis (ICA) to assess the level of FC, which is known to be affected by general anaesthesia (Peltier et al, 2005;Boveroux et al, 2010). Patients were only included in the study if ICA detected recognisable bilateral components in sensorimotor, visual and DMN networks (figure 1). FC was assessed using partial correlation between band-pass filtered (0.01-0.1 Hz) fMRI signal time-courses averaged within regions-of-interest (ROIs) placed in tubers, thalami and posterior DMN regions (posterior cingulate and lateral inferior parietal cortex). The tubers were identified on T2-weighted imaging, and all tubers larger than 3mm were manually outlined to create ROI masks. The thalamic and DMN ROIs were created using 10mm spherical masks. Motion realignment parameters and fMRI time-courses averaged within white-matter and CSF were used as confounds in the partial correlation analysis (figure 2). Tubers were defined as 'epileptogenic' if localized ictal onset patterns were recorded by intracranial-EEG electrodes within or at the surface of tubers. Differences in FC between epileptogenic and non-epileptogenic tubers were tested using two-sample t-tests comparing: tuber to tuber, tuber to thalami, and tuber to DMN.

Results: Four children (3 boys, median 2.8 years) met the inclusion criteria (table 1). All had focal seizures and three had epileptic spasms recorded during intracranial-EEG monitoring. For each subject, total tubers ranged from 5-26 (group total 64), tubers covered by intracranial-EEG electrodes ranged from 3-10 (total 29) and 'epileptogenic' tubers ranged from 1-5 (total 17). For tuber-tuber FC, epileptogenic tubers showed increased connectivity in two subjects and decreased connectivity in two. For tuber-thalami FC, epileptogenic tubers showed increased FC in three subjects and decreased connectivity in one. For tuber-DMN FC, epileptogenic tubers showed increased connectivity in two subjects and decreased connectivity in two. None of these individual analyses, or group level analyses, was statistically significant (table 2).

Discussion: With this approach to FC analysis, we failed to show difference in FC that reliably distinguished epileptogenic from non-epileptogenic tubers. Several factors could account for this negative result. Both propofol (Boveroux et al, 2010) and servofluorane (Peltier et al, 2005) influence cortico-cortical and thalamo-cortical connectivity in a dose-dependent manner. Although robust resting state networks were demonstrated in the four subjects analysed, it is unclear how anaesthesia might modulate 'epileptogenic networks' and tuber epileptogenicity. Also, there is potentially varying epileptogenicity in different parts of a single tuber (i.e. cortical surface, depth of sulci, sub-cortical components), such that using masks of whole tubers may dilute signals of interest. Finally, it is unclear if fluctuation in resting BOLD activity provides a true reflection of epileptogenicity, in contrast to EEG-related BOLD activity (i.e. spike-related EEG-fMRI studies). More subjects, a longer fMRI acquisition and simultaneous EEG monitoring of epileptiform activity and depth of anaesthesia might improve this approach. This study highlights the limitations in translating FC analysis into clinical practice.

References:

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Table 1: clinical details

	sex	sz onset	sz types	tubers	surgery (yr)	anaesthesia
subject 1	M	day 1	FS, ES	15	2	servofluorane
subject 2	F	9 months	FS, ES	18	2.5	servofluorane
subject 3	M	3 months	FS	26	3.2	propofol, servofluorane
subject 4	M	24 months	FS, ES	5	7.3	propofol, servofluorane

FS=focal seizures, ES=epileptic spasms

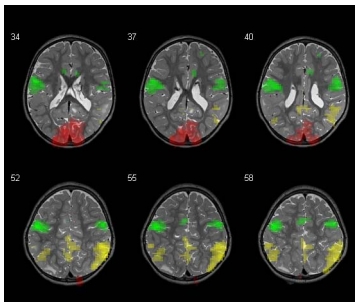

Figure 1: ICA derived networks for subject 2: motor (green), visual (red) and REST (yellow) networks, overlaid on T2-axial MRI

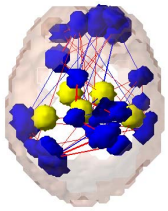

Figure 2: visual summary of correlation analysis for subject 2. The ROIs are shown in a glass-brain, with tubers coloured blue, and the thalami and posterior DMN coloured yellow. The lines signify positive (red) and negative (blue) partial correlations between ROIs, and the line thickness signifies the strength of partial correlation.

Table 2: summary of partial correlation analysis

	Epileptogenic		Non-epileptogenic		
	Mean ϱ	obs	Mean ϱ	obs	p-value
Tuber-tuber connectivity					
subject 1	0.061	5	0.065	4	0.844
subject 2	0.059	4	0.036	3	0.079
subject 3	0.035	2	0.038	8	0.932
subject 4	0.100	1	0.094	2	NA
group	0.059	12	0.050	17	0.507
Tuber-thalami connectivity					
subject 1	-0.014	5	-0.005	4	0.910
subject 2	0.032	4	0.029	3	0.962
subject 3	-0.005	2	0.027	8	0.762
subject 4	-0.072	1	0.042	2	NA
group	-0.002	12	0.022	17	0.556
Tuber-DMN connectivity					
subject 1	0.048	5	0.018	4	0.838
subject 2	-0.005	4	0.067	3	0.391
subject 3	-0.041	2	0.125	8	0.352
subject 4	0.272	1	-0.179	2	NA
group	0.031	12	0.067	17	0.613

ϱ *=partial correlation coefficient, obs=number of tubers*