Disrupted intrinsic functional connectivity in the vegetative state

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Received 12 December 2007
Revised 2 July 2008
Accepted 10 July 2008

ABSTRACT

It is debatable as to whether the spontaneous blood-oxygen-level dependent fluctuations that are observed in the resting brain in turn reflect consciously directed mental activity or, alternatively, constitute an intrinsic property of functional brain organisation persisting in the absence of consciousness. This report shows for the first time, in three patients, that the persistent vegetative state (PVS) is marked by a dysfunctional default mode network, with decreased connectivity in several brain regions, including the dorsolateral prefrontal cortex and anterior cingulated cortex, especially in the right hemisphere. This finding supports the view that the resting state is involved in self-consciousness, and that the right-hemisphere default state may play a major role in conscious processes. It is speculated that the default state may act as a surrogate marker of PVS with awareness contents and, therefore, could replace a more complex activation paradigm.

In neuroimaging studies, the persistent vegetative state (PVS) is characterised by the hypometabolism of several associated areas (precuneus, posterior cingulate cortex, BA 19, 22, 30 and 39), with impaired thalamocortical and corticocortical effective connectivity.[1 2] A similar pattern is seen during anaesthesia.[3] These areas are believed to be a part of a default mode network (DMN), which may, or may not, support self-awareness.[4] To investigate this issue, we studied the resting state (RS) of patients in PVS for the first time.

We used functional magnetic resonance imaging (fMRI) to measure the resting state (RS) activity of three patients in PVS, two of which followed traumatic brain injury 20 months previously (one female aged 19: Disability Rating Scale (DRS) 25/ Cat. 9, one male aged 21: DRS 23/ Cat. 8) and one of mixed origin (a female aged 78: DRS 28/ Cat. 9; see Supplementary Online Material for full details), and a control group of six healthy subjects. These patients were enrolled in an approved coma-recovery cortical stimulation protocol.

Data acquisition was performed on a 1.5 T scanner (Philips Medical Systems). The resting state functional T_2-weighted images were acquired using EPI sequences. A total of 300 volumes were acquired, each consisting of 25 axial slices, covering the whole brain. In the same session, a set of 3D high-resolution T_1-weighted structural images was acquired for each participant. Functional connectivity was measured via independent component analysis (ICA), which is a statistical technique that separates a set of signals into independent uncorrelated and non-Gaussian spatio-temporal components, wherein the ICA decomposition was calculated using the single-subject ICA plug-in that corresponded to a C++ implementation of the fast-ICA algorithm. For each subject, the initial dimensions of the functional data-set were reduced from 300 (number of time points) to 50 using the principal-component analysis (PCA) technique; for each subject, the 50 independent components were estimated using the fast-ICA method. Two criteria were used to select the components that most closely matched the default-mode network.

1. Only components with a signal frequency in the 0.01–0.1 Hz range were included.
2. A spatial template of the default mode network was used to select the best-fit of the remaining low-frequency components.

In particular, we spatially correlated all the components with a default mode mask. This mask contained the posterior parietal cortex (BA 7), frontal pole (BA 10) and occipitoparietal junction (BA 39), as well as the posterior cingulate and precuneus. The component that spatially correlated most significantly with the template was selected as the default mode component. The group components were calculated as random effects maps. A two-sample t test was used to compare the healthy subject and patient group maps. Significant clusters of resting state activity for the two sample t tests were determined by using a ($p<0.05$) threshold corrected at the whole-brain level using the False Discovery Rate.[5] In order to quantify the degree of impoverishment in the resting state, we used various procedures. First, we compared the observed resting state activity maps with the reference template: a spatial correlation index was obtained providing the spatial template to the ICA plug-in in Brain Voyager (Brain Innovation, Maastricht, The Netherlands). Second, we examined the maps and then split them, by visual inspection, in specific neural functional connectivity networks. Third, to better characterise the asymmetry of the resting state activity we also computed a lateralisation index, using the formula: (right –left) / (right + left), where "right" denotes the active voxels situated to the right of the commissural sagittal plane, and "left" denotes the active voxels situated to the left of the commissural sagittal plane; the index can vary from –1 to 1, where 1 indicates a total right lateralisation, –1 total left and 0 total symmetry. See Supplementary Online Material for full details.

In patients, DMNs were partially impaired compared with the healthy controls. On qualitative analysis, the more severe the clinical condition, the more impaired was the DMN. In the male patient (fig. S1b), the DMN was impaired, showing only the dorsolateral prefrontal cortex

(DLPFC), anterior cingulate cortex (ACC), posterior parietal/temporoparietal junctional (PP/TPJ; correlation with template = 0.283, lateralisation = 0.04). In the same patient the visual RS network (V1 and extrastriate visual cortices), dorsal attentional circuit and motor circuit (mainly bilateral MI) were present. In the young female (fig. S1c) the posterior CC precuneus and left PP/TPJ junctional cortices were present (the parietal component was excluded from comparative analysis (healthy subject vs patient group maps) due to a valve artefact), but the DLPFC, ventromedial PFC (VMPFC) and ACC were not (correlation with template = 0.271, lateralisation = 0.06). The eldest patient (fig. S1d), who lacked both consciousness and vigilance, displayed only a small portion of VMPFC and precuneus, but with active temporal cortex areas (likely part of the auditory–phonological system; correlation with template = 0.192, lateralisation = 0.03). Globally, patients (vs healthy controls) showed a reduced connectivity in several right-sided areas, but a greater connectivity of left areas (see fig 1, table S1). The controls displayed the expected RS components: DMN (correlation with template = 0.46 (0.08) (mean and SE) and a lateralisation = 0.062 (0.008) (mean and SE)), visual, dorsal attentional, motor and linguistic.

Apparently, as the Disability Rating Scale (DRS) worsens, the correlation with the template and number of subnetworks diminish, which is indicative of a greater lack of consciousness.

As far as the lateralisation index is concerned, in two patients it was below average (of controls) and in one patient it was average (combined lateralisation index: 0.043 (0.007), mean and SE, ie, below average). Overall, the lateralisation index seems to be the most variable parameter.

Although no meaningful correlation analysis is possible with only three patients, the resting state activity and level of consciousness (as measured on the DRS clinical scale) can be descriptively matched. This suggests that consciousness may require a fully intact and integrated DMN, that is fully intact anterior and posterior components, even in the presence of other operating RS networks (as hinted at by a study on the infant brain[6]). Some PVS patients are known to exhibit signs of associative cortex activation, without any external signs of awareness, particularly those with prevailing theta or slow alpha EEG background activity.[7 8] However, activity in these areas is not, by itself, indicative of conscious awareness.[2] However, recent studies suggest that patients in PVS retain not only islands of preserved function but also full-blown self-awareness, which for some reason cannot be made explicit to others.[9] Complex and incompletely reliable activation paradigms have been developed and tested in order to ascertain this possibility. At least one patient was detailed who, in the authors' opinion, fulfilled their criteria for being fully self-aware.[10] We suggest that an intact DMN may act as a "neural marker"[9] of patients who, although unable to move or speak, retain self-awareness. Admittedly, more work is needed before any certain conclusion can be drawn.

The lateralisation of findings in patients versus the controls is noteworthy (see also Damoiseaux *et al*.[11]). Fully conscious, healthy controls displayed a dominant right-sided connectivity. The importance of the right hemisphere in the processes of self-awareness has been highlighted in clinical and neuroimaging studies.[12–14] The reduced right-hemisphere connectivity in our PVS cases further supports this role and shows for the first time the need to consider laterality in the study of DMN. The reason for the left lateralisation in patients remains speculative. One hypothesis is that each hemisphere tends to cause a decrease in the activation of the other one, and this asymmetry of activation is mediated by the corpus callosum,[15] since the right hemisphere network could play a crucial role in the self-awareness component of awake subjects,[16] in which our speculative explanation is that the reduced awareness in PVS subjects could have a reduced activation of the right hemisphere regarding awake subjects that may result in contralateral dishinibition, with subsequent connectivity clustering in the left hemisphere. Moreover, both hemispheres are activated by the sensory input processed by the "aroused" brain of the PVS subjects. Therefore, the PVS brain connectivity differs from the connectivity of the anaesthetised brain,[17] in which the arousal system is impaired.

These data cast doubts on the contention that monkeys display an equivalent DMN to humans.[17] In humans, the DMN may have to be intact in order for the subject to be fully conscious. The presence of a supposedly similar DMN in an anaesthetised monkey can be most parsimoniously explained in evolutionary terms, that is human traits may evolve by

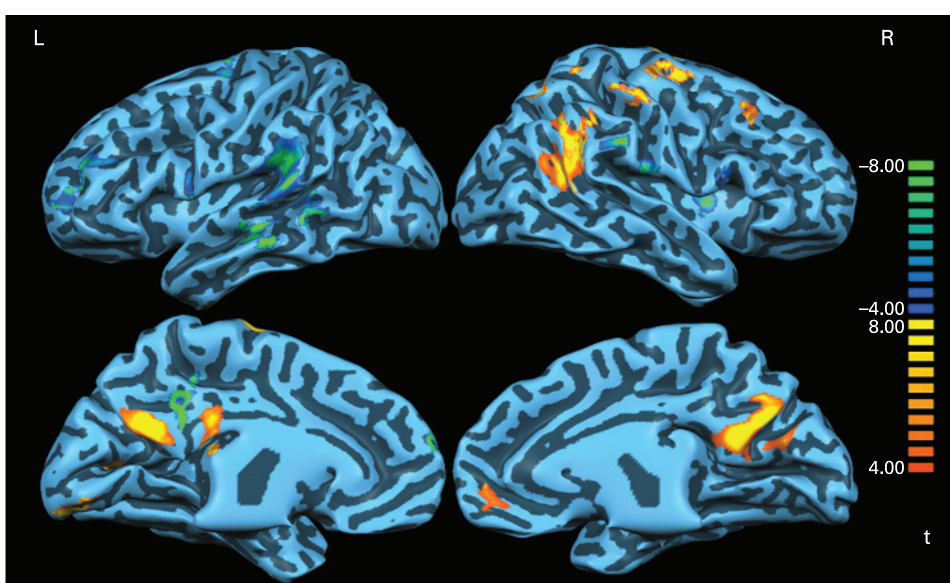

Figure 1 Results for the healthy subjects' default mode network (DMN) minus the patients' DMN. Yellow to red areas indicate a significant prevalence of connectivity in the healthy subject; vice versa, blue to green areas show a significant prevalence of connectivity in the persistent vegetative state patients (two sample t test: $p<0.05$, false discovery rate corrected, 3D brain reconstruction obtained with Brainvoyager QX 1.9; Brain Innovation, Maastricht, The Netherlands).

 J Neurol Neurosurg Psychiatry 2009;**80**:429–431. doi:10.1136/jnnp.2007.142349

modifying pre-existing systems that support cognitive functions present in a wide array of primate species.

In conclusion, these data point to a fully integrated, right-predominant DMN as a marker of conscious awareness, which we tentatively propose as a surrogate of self-awareness.

Acknowledgements: This study was supported by Regione Piemonte, Ricerca Scientifica Applicata, Settore Scienze della Vita, 2006.

Competing interests: None.

Ethics approval: Ethics approval was provided by the Ethical Committee of the Department of Psychology, University of Turin.

Patient consent: Obtained.

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