Hierarchical Functional Modularity in the Resting-State Human Brain

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Abstract: Functional magnetic resonance imaging (fMRI) studies have shown that anatomically distinct brain regions are functionally connected during the resting state. Basic topological properties in the brain functional connectivity (BFC) map have highlighted the BFC's small-world topology. Modularity, a more advanced topological property, has been hypothesized to be evolutionary advantageous, contributing to adaptive aspects of anatomical and functional brain connectivity. However, current definitions of modularity for complex networks focus on nonoverlapping clusters, and are seriously limited by disregarding inclusive relationships. Therefore, BFC's modularity has been mainly qualitatively investigated. Here, we introduce a new definition of modularity, based on a recently improved clustering measurement, which overcomes limitations of previous definitions, and apply it to the study of BFC in resting state fMRI of 53 healthy subjects. Results show hierarchical functional modularity in the brain. *Hum Brain Mapp* 30:2220–2231, 2009. © 2008 Wiley-Liss, Inc.

Key words: resting state functional MRI; complex network; modularity; small world

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INTRODUCTION

Functional magnetic resonance imaging (fMRI) techniques have been extensively used to highlight patterns of brain activations in subjects either performing a particular task [Eguíluz et al., 2005; Haynes and Rees, 2005, 2006], or at rest [Achard et al., 2006; Damoiseaux et al., 2006; DeLuca et al., 2006; Salvador et al., 2005, 2007, 2008]. Local changes in the blood-oxygen-level dependent (BOLD) signal detected by fMRI are commonly associated to local neuronal activation. Time- and frequency-based analyses of BOLD changes in resting-state fMRI have led to patterns of functionally connected brain regions. Such areas are characterized by highly correlated time courses, which can be detected by partial correlation analysis [Salvador et al., 2005] in the time-domain, or by Fourier- and wavelet-based analysis [Achard et al., 2006] in the frequency/scale domain. The outcome of such analyses is a complex brain functional connectivity (BFC) network describing functional connectivity of anatomically distinct brain regions: nodes in the network represent different anatomical regions, while edges highlight functional connectivity among them.

Previous studies have focused on basic properties of brain functional topology, proving that both functional and anatomical connectivity in the brain are characterized by small-world properties [Achard et al., 2006; Reijneveld et al., 2007; Rubinov et al., 2007; Salvador et al., 2005; Smit et al., 2007; Sporns and Honey, 2006]: this means that nodes in the network are highly clustered (i.e., the direct neighbors of a given node are more densely connected than what usually expected in similar, randomly generated, networks), and that the average minimal path connecting any pair of nodes is small compared to the network's size (in the same order of what is to be expected in similar, randomly generated, networks). These findings are an important step toward a better understanding of the BFC's topology, especially considering that the anatomical connections in the brain form a rather sparse network (in average 100 billion neurons, each connected to 7,000 other neurons [Drachman, 2005]). Recently, several real complex networks have been proved to present a scale-free topology [Adamic and Huberman, 2000; Barabási, 2005; Barabási and Albert, 1999; Eguíluz et al., 2005; Ravasz et al., 2002], whose main hallmark is a power law distribution for the degree correlations (probability of having k links in a given node). Analyses have shown that scale-free networks are robust against random failures, but easily destroyed by attacks targeting their hubs (i.e. highly connected nodes) [Albert et al., 2000]. Recent studies, performed both on anatomical and functional brain connectivity, have shown no sign of a scale-free topology [Achard et al. 2006; Reijneveld et al., 2007; Salvador et al., 2005], hypothesizing that a small-world topology might be more optimal in terms of information transfer, resilience to damages, and balance between local and global integration [Reijneveld et al., 2007]. Other studies have suggested that

while the resting-state BFC might be a small-world network, patterns of task-related activation might organize themselves into scale-free configurations [Eguíluz et al., 2005].

Besides brain research, the analysis of complex self-organizing networks is of high interest in diverse fields such as social sciences, molecular biology, business management, viral epidemiology, etc. [Barabási, 2005]. Modularity, a topological property of complex networks, has recently attracted the attention of researchers in several of these fields. There are two main reasons to investigate modularity: (1) dynamic systems can usually be represented by complex networks; thus, discovering modularity might be highly informative for the system being investigated (e.g., social communities within the social network, web pages related to a similar topic, etc.); (2) it has been suggested that modularity might be an ubiquitous property of self-organizing structures, from social communities [Hallinan, 2003] to metabolic reactions within a cell [Ravasz et al., 2002]: therefore, investigating its origins and mechanisms has a fundamental theoretical value.

A central question is which function could modularity have in the BFC network? Kaas [2000] observed that throughout evolution, an increase in brain size has mostly been associated with an increased number of neurons. A modularity structure would be the optimal solution to accommodate these extra neurons, simultaneously keeping both the average connections per neuron and the average length of connections. Bullinaria [2007] investigated several simulated artificial neural networks; the networks were allowed to evolve in order to optimize the response to a particular task: results showed that for several tasks, in which reduced interference was more important than computational power, modularity spontaneously emerged. This suggested that modularity might be the solution to allow efficient separate functionalities, without degrading the computational power. Studies on animals have indeed shown different anatomical and functional modules to be at work in the brain [Honey et al., 2007; Sporns and Kötter, 2004; Sporns et al., 2007].

With regard to modularity in the BFC, Eguíluz et al. [2005] reported that no sign of modularity was detectable in task-related activation patterns. In contrast, Salvador et al. [2005] did hint to the modularity of their functionally connected brain regions, in resting-state analysis, providing a qualitative analysis based on dendrogram and multi-dimensional scaling plots. A quantitative analysis can move beyond qualitative observations, but obviously requires a formal definition of modularity in complex networks.

Recently, several attempts have been made to obtain a formal definition. Ravasz et al. [2002] investigated modularity in metabolic networks, basing their definition upon that of clustering coefficient: intuitively, a node in a network has a high clustering coefficient (close to 1) if its direct neighbors are highly interconnected; close to zero otherwise. In their study, Ravasz et al. [2002] suggested

that a power law in the distribution of clustering coefficient with respect to the degree of connections might be a sign of modularity. Nevertheless, in a recent study, Soffer and Vázquez [2005] showed that the original definition of clustering coefficient is biased by the degree of connections: intuitively, the more connections a node has, the lower its clustering coefficient will be. After providing an unbiased definition, they suggested that prediction of modularity should not be based on the distribution of clustering coefficient, but rather be sought in the analysis of the degree correlations. Hallinan [2003] also based her definition on the original biased version of the clustering coefficient, investigating modularity in an internet community.

More recently, Newman and Girvan [2004] presented a divisive technique to identify communities in complex networks: their solution represented a breakthrough within the related scientific literature and was subsequently applied in diverse fields. Radicchi et al. [2004] improved on this method, by reducing the computational complexity associated with the algorithm. A further improvement was introduced by Newman [2006], with the definition of modularity matrix, whose eigenvectors can be used to separate a complex network into several communities. A common denominator of all these solutions is the nonoverlapping nature of the detected communities: the advantage of such approaches is that the resulting communities are easily identified. Nevertheless, brain modularity should not be restricted to a nonoverlapping partition of the functional connectivity map: one of the most important aspects of modularity we want to highlight is how different regions cluster together in larger modules, which then cluster again, climbing up through a hierarchical organization. An important step in this direction was taken by Palla et al. [2005]. In their work, they tried to overcome the major limitation of previous solutions, addressing the reality of complex networks and characterizing them by statistics of overlapping and nested communities. The higher flexibility in the description of the network presents, nevertheless, a drawback: the selection of the modules become more application dependent, and might need a thorough exploration through all the detected communities, or alternatively a threshold over the clustering of each community. For a more complete review of the advantages and disadvantages of the different methods, the reader is referred to Danon et al. [2005].

In this work, we applied the partial correlation method described in Salvador et al. [2005], in order to obtain the BFC network. Salvador et al. [2005] presented convincing results on their population (twelve subjects, imaged at the same center). As a first step, we validated their technique by reimplementing it and applying it on a larger population: remarkably consistent results proved the validity of Salvador's method, and provided us with a complex network of 90 nodes and 264 edges representing the BFC. We set up to investigate several topological properties of this network, with a particular focus on modularity. Starting from the suggestions given in Soffer and Vázquez [2005],

we introduced a new definition of modularity based on the unbiased formulation of clustering coefficient [Soffer and Vázquez, 2005] and capable of capturing overlapping and inclusive relationships among different clusters. We finally applied this new definition to the analysis of modularity in the BFC network.

MATERIAL AND METHOD VALIDATION

Subjects

Fifty-three healthy subjects were included in this study: the volunteers (17 males, 36 females; mean age = 41.28 years, standard deviation = 9.61 years, range = 21–64 years) were recruited within the large scale longitudinal multicenter cohort study NESDA (Netherlands Study of Depression and Anxiety, www.nesda.nl) and imaged at three different centers in The Netherlands (the Academic Medical Center, University of Amsterdam, Amsterdam, University Medical Center Groningen, and Leiden University Medical Center). For detailed information on the NESDA rationale, as well as the subject inclusion criteria, we refer to Penninx et al. [2008]. None of the subjects had a history of brain trauma and no abnormalities were found upon inspection of the subjects' structural images by a neuroradiologist. The study was approved by the Central Medical Ethical Committees of the three participating centers. Written informed consent was obtained from all subjects.

Data Acquisition and Preprocessing

Resting-state functional magnetic resonance images (fMRI) were acquired for each subject using a standardized protocol: each scanning session included 200 gradient-echo echo-planar imaging (EPI) volumes, acquired on a 3.0 T Achieva scanner (Philips Medical Systems, Best, The Netherlands). The following scan parameters were used: in Amsterdam and Leiden, repetition time (TR) = 2,300 ms, echo time (TE) = 30 ms, flip angle = 80°, 35 axial slices, no slice gap, in-plane voxel resolution = 2.3 mm², slice thickness = 3 mm; in Groningen: TR = 2,300 ms, TE = 28 ms, flip angle = 85°, 39 axial slices, no slice gap, in-plane voxel resolution = 3.45 mm², slice thickness = 3 mm.

Individual fMRI data preprocessing was carried out using FEAT.¹ The following steps were applied: motion correction using MCFLIRT [Smith et al., 2005], nonbrain removal using BET [Smith, 2002], grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50.0 s). Registration to high resolution structural and standard space (Montreal Neurological Institute (MNI)

¹FMRI Expert Analysis Tool, Version 5.90, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl).

template) images was carried out using FLIRT [Jenkinson and Smith, 2001; Jenkinson et al., 2002]. No spatial smoothing was applied, as previously suggested in Salvador et al. [2005].

Obtaining the BFC Network

The BFC network had to be extracted from our data, before any analysis of network's modularity could be performed. Salvador et al. [2005] introduced a new methodology, based on partial correlation analysis, to obtain such a network. Their results were promising, although somehow limited by the small cohort used in their analysis. To validate their approach, we attempted to reproduce their results on our population. Following the same procedure presented in Salvador et al. [2005], each fMRI volume was parceled into 90 anatomical regions (45 symmetric regions, see Supp. Info. Table I), according to the automatic anatomical labeling (AAL) template reported by Tzourio-Mazoyer et al. [2002]. Results were remarkably comparable to those obtained by Salvador et al. [2005], proving the robustness of the partial correlation analysis, and also suggesting that resting-state fMRI are intrinsically less subject to variability due to different acquisition centers. In the Appendix, we briefly report the results obtained by applying Salvador's methodology (see Supporting Information) to our population, presenting them in the same order as they were presented in Salvador et al. [2005]. The outcome of such analysis is a complex network (the BFC network) with 90 nodes and 264 edges (with an average number of links per node equal to $2 \times 264/90 = 5.87$), which we used to investigate brain functional modularity.

MODULARITY IN COMPLEX NETWORK: A NEW DEFINITION FOR QUANTITATIVE ANALYSIS

A classical definition of clustering coefficient for a node i is

$$C_i = \frac{2n}{k_i * (k_i - 1)}, \quad (1)$$

where k_i is the degree of connections of node i (i.e. number of its direct neighbors), and n is the number of direct connections between them. The global clustering coefficient for the network is obtained averaging the over all nodes.² In Ravasz et al. [2002], it was suggested that a power law distribution of $C(k)$ might be an indication of modularity within the network: the authors could successfully highlight a hierarchical modularity within a metabolic network.

²A different definition for a global clustering coefficient can be found in Newman [2003]: although originally given as a general property of a network, the definition of Newman can be applied locally to the subset of direct neighbors (for a given node), providing a different definition of local clustering coefficient.

However, it was recently showed that the definition of clustering coefficient given above is intrinsically biased by k [Soffer and Vázquez, 2005]: nodes with larger k tend to have smaller clustering coefficients. Therefore, changes in $C(k)$ related to changes in k might not be an optimal marker of modularity in a network. Figure 1A shows $C(k)$ distributions for 200 random networks: the variation of $C(k)$ is dependent on k , with larger deviations for smaller k s. The new definition given in Soffer and Vázquez [2005] solves this issue, by taking into account the degree of connectivity of the direct neighbors. Figure 1B shows that with the new definition of clustering coefficient the dependency of $C(k)$ over k is largely diminished, as one would expect for random networks. The unbiased clustering coefficient, however, is now uncorrelated from k : therefore, looking for a power law distribution in $C(k)$ as a marker for modularity might not provide informative results, as pointed out in Soffer and Vázquez [2005]. This raises the question: are there alternative ways of characterizing modularity in complex networks?

Soffer and Vázquez [2005] suggested that modularity can be predicted by analyzing the degree correlations of a network. Interestingly, a similar approach was followed by Eguíluz et al. [2005] to assess assortative mixing in task-related brain activation: in particular, they showed a positive correlation between a node's degree k and the average degree of its direct neighbors $\langle k_i \rangle$. In Figure 2, we show how the average over 200 random networks (blue line with error bars) does not present any correlation between k and $\langle k_i \rangle$ conversely, the network of interest for this study (BFC), shown in red, clearly presents a positive correlation (see the next section). The self-organizing principle behind the BFC seems to lead highly connected nodes to link predominantly to other highly connected nodes. In Stam and Reijneveld [2007], the authors showed that self-organization tends to generate assortative matrices in social networks, and disassortative matrices in technological and biological matrices. Our preliminary results suggest a more social-like organization for the human BFC network. Although the analysis of the degree correlations can highlight modularity in the network, it does not provide a quantitative measure.

The idea behind modularity can be intuitively grasped by considering Figure 3A: the hypothetical network presents three major modules. But how can one mathematically group nodes into different modules? Ideally, one would want to establish a distance measurement between any pair of nodes i and j , and subsequently apply a clustering algorithm. This is what Ravasz et al. [2002] set up to do in their study on metabolic networks: as a distance measure between two nodes i and j , they considered the topological overlap

$$O_T(i, j) = \frac{J_n(i, j)}{\min(k_i, k_j)}, \quad (2)$$

where $J_n(i, j)$ accounts for the total number of nodes to which both i and j are connected (plus 1 if i and j are

Figure 1.

A: Uncorrected clustering coefficient $C(k)$ versus degree correlations k in 200 random networks of 90 nodes (average degree per node $k = 6$). Each blue dot represents a node in one of the 200 random networks. The variation highlighted by the red error-bar plot indicates a correlation with k , not expected in

random networks: smaller k s have larger variations. **B:** When the unbiased definition of clustering coefficient is applied, the correlation between $C(k)$ and k vanishes, as expected for random networks. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 2.

Given a node of degree k , we evaluate the average degree $\langle k_i \rangle$ of its direct neighbors and plot it against k : averaging over 200 random networks (blue error-bar) does not show any correlation; conversely, the BFC network (in red) presents a clear trend, in which highly connected nodes tend to link to other highly connected nodes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

directly connected). Let us consider nodes A and B in Figure 3A: their degree of overlap according to Ravasz et al. [2002] is $2/2 = 1$. Although this definition moves towards a quantification of clustering for a given pair of nodes, it still lacks in specificity regarding different topologies of clusters. Intuitively, we perceive that nodes A and B are not as close as nodes E and F : nevertheless, applying Ravasz et al. [2002] gives us $O_T(E,F) = 3/3 = 1$; finally, if we consider nodes I and L , we obtain once again $O_T(I,L) = 3/3 = 1$. The reason behind this lack of specificity is that the overlap measure given in Ravasz et al. [2002] aims at discriminating nonoverlapping clusters, and therefore does not take into account the interconnections of common nodes both within and outside a given cluster.

We propose a new distance function which can take this effect into account. Consider again the pair (A,B) of Figure 3A: let us introduce an auxiliary node H directly connected to A , B , and all their direct neighbors (common and not) (Fig. 3B). It is now possible to apply the unbiased definition of clustering coefficient given by Soffer and Vázquez [2005; for a detailed description of the algorithm] to the auxiliary node H , determining $clust(A,B) = C_H$. For the different nodes previously examined, this procedure gives us $clust(A,B) = 0.8$, $clust(E,F) = 0.833$, and $clust(I,L) = 1$. $clust(A,B)$ is lower than $clust(E,F)$ because both C and D have available extra links that they use to link outside the cluster. Equivalently, $clust(E,F) < clust(I,L)$. Finally, we use $1-clust(A,B)$ as dissimilarity measure between A and B . Once dissimilarities have been evaluated for all possible pairs in the network, the classical average-linkage clustering algorithm [Eisen et al., 1998] can be applied.

The dendrogram associated with the clustering algorithm is shown in Figure 3C. The interpretation of such a dendrogram is different from the one given in Ravasz et al. [2002]. Let us consider the subgroup of nodes I and L : this is a 0-dissimilarity cluster indicating that nodes I , L , M , N (I , L , and their direct neighbors) are fully connected. Moving forward through the dendrogram, many other subgroups of the original set of nodes can be identified. One can order all these subsets according to their clustering coefficient, and subsequently highlight relationships of memberships between groups. In Figure 3D we report a list of clusters with clustering coefficient higher than 0.8, to illustrate the results of our method (different levels represent subsets of previous level): the cluster I , L , M , N (only enter in level 6) is a fully connected cluster. The cluster E , F , G , H (last entry at level 2) presents a lower clustering coefficient. These two clusters group together in a larger module at level 3, with a clustering coefficient of 0.92. When node D is also included (second entry in level 2), we obtain an even larger module of clustering coefficient 0.93. Thus, our method not only highlights the three major clusters, but also provides information over larger modules. As a comparison, the method presented in Ravasz et al. [2002] could only highlight the three major cluster (see Supp. Info. Fig. 1), without differentiating among them. We also tested the method proposed by Palla et al.

[2005], since their solution aims at identifying nonoverlapping communities. This method³ could only highlight the E , F , G , H and I , L , M , N modules, while nodes A , B , C , D were not included in any community. This is probably due to the definition of modularity used by Palla et al. [2005]: nodes belonging to the same community need to be connected by complete subgraphs of a particular dimension.

MODULARITY IN RESTING-STATE FUNCTIONAL CONNECTIVITY MAPS: RESULTS AND DISCUSSION

Starting from the BFC network obtained with the partial correlation method, we first investigated the small-world properties previously presented by Salvador et al. [2005]: by using the unbiased definition of clustering coefficient [Soffer and Vázquez, 2005], we obtained $C_{BFCn} = 0.18$; empirical investigation over 200 equivalent random networks (i.e. where equivalent means same number of nodes $n = 90$ and same average degree per node k) led to $C_{Rnd} = 0.08$. Similar analyses on the average minimal path among any pair within the networks led to $L_{BFCn} = 3.12$ and $L_{Rnd} = 2.53$. These results confirm the small-world topology for the BFC map, as previously reported in Salvador et al. [2005], even when the unbiased definition of clustering coefficient is applied. The remaining question is: can we detect modularity within the BFC map?

As previously explained, looking for a power law distribution of $C(k)$ might not produce informative results when the correct definition of clustering coefficient is used: Figure 4 shows no correlation over $C(k)$ for the BFC map. A first step to uncover modularity is the analysis of the degree correlations [Eguíluz et al., 2005; Soffer and Vázquez, 2005]. In Figure 2 (red diamonds and red line), we plot for each node in the network its degree of connection k versus the average degree of connection of its direct neighbors $\langle k_i \rangle$: the positive correlation suggests that highly connected nodes tend to connect mostly with other highly connected nodes. Experiments with 200 equivalent random networks (blue line in Fig. 2) showed no correlation, as expected. This first result suggests the presence of self-organized modularity in the BFC map, although it does not allow quantitative comparisons.

Finally, we applied our new definition of modularity, as described in the previous section. Figure 5A reports the dendrogram corresponding to the average-linkage clustering of the dissimilarity measurements for the BFC map: modules are clearly visible in certain areas of the brain, especially at low thresholds of dissimilarity (below 0.4). As a comparison, a dendrogram for an equivalent random network is shown (see Supp. Info. Fig. 2): the modularity is lost, and the few clusters which still are visible present higher degree of dissimilarity. Figure 5B shows histograms,

³The software described in Palla et al. [2005] is freely available at <http://www.cfindex.org/>.

based on clustering thresholds, for the BFC map and an average over 200 equivalent random networks: the average weighted clustering coefficient for the BFC network resulted in 0.53, while for random networks was 0.41, 12% less. Moreover, the BFC network presented a wide spectrum of clustering coefficients, largely above the 50% threshold, while in random networks the highest clustering coefficient was about 0.55. The improvements of our new definition of modularity can be appreciated by considering Figure 5C: similar histograms obtained by the definition given in Ravasz et al. [2002] no longer discriminate between the BFC map and the 200 equivalent random networks. This suggests that modularity within the BFC networks is mostly based on clusters which are not easily discernable by the previous definition of modularity.

How can we functionally and anatomically interpret the modules highlighted in the BFC map? The dendrogram in Figure 5A shows three major modules with low dissimilarity measures: the first highly modular cluster (denoted as 1) groups together brain areas of the medial temporal lobe with gray matter subcortical structures; a slightly enlarged cluster (denoted as 1*) also includes the middle temporal poles and the right putamen. The second modular cluster (denoted as 2) groups together different subcortical regions with frontal areas. Finally, the third modular cluster (denoted as 3) includes areas of the temporal and parietal lobe, and regions belonging to the premotor cortex; slightly enlarged clusters (denoted as 3* and 3**) also add frontal regions from the frontal and temporal lobes. A better insight in the hierarchical organization can be achieved by sorting the clusters according to their clustering coefficient, and highlight their inclusion relationships. Supporting Information Table III presents the results for clustering coefficients higher than 0.65 (the choice of the threshold was guided by the histograms of Fig. 5B). Interesting modules are the first one (at level 1), including submodules of medial temporal and gray matter subcortical structures; the second one (at level 1), involving mainly parietal- (pre) motor regions and temporal regions; and the eighth one (at level 1), showing the connection between frontal areas and subcortical regions. Finally, the last module of level 3 shows how temporal regions group together predominantly with parietal- (pre) motor regions to create larger functional modules.

Different ways of parceling the brain might lead to different results and conclusions, depending on which regions one is investigating. The atlas used in our study allowed us to draw conclusions on the functional connectivity of anatomically defined regions. The main advantage of studying modularity within an anatomical framework is that further investigations on pathological conditions might highlight significant differences in modularity for well-known brain areas, improving our understanding of the mechanisms behind a particular disorder. On the other hand, an atlas with a limited number of regions might prevent us to see underlying topological structures in the brain connectivity. The total number of regions included in

the major modules detected in our study represents ~43% of the total number of areas in the Tzourio-Mazoyer [Tzourio-Mazoyer et al., 2002]: should one conclude that modularity is not a global property of the brain, but rather limited to specific areas? Trying to address this question, we hypothesized that larger areas in the Tzourio-Mazoyer atlas would correspond to smaller cluster coefficients: intuitively, if modularity in the brain appears mostly at small scales, then a large area in the Tzourio-Mazoyer atlas might group different modules into one single label, preventing us to discover modularity in that particular area. Indeed, as shown in Figure 6, there is a significant ($P < 0.0002$) positive correlation between the volume of the different regions in the Tzourio-Mazoyer atlas and their corresponding dissimilarity measure as reported in Figure 5A. This finding suggests that modularity in the brain is mostly a small-scale phenomenon. Further studies should validate specific functionally oriented atlases, and use them to discriminate modularity in the entire brain.

Instead of parceling the brain, one could consider each voxel separately. The study of Eguíluz et al. [2005], based on voxel-wise analysis of task-related fMRI, highlighted a scale-free topology which was not identified in our study (see Supp. Info. Fig. 5), as well as in other studies based on the Tzourio-Mazoyer atlas. The drawback of voxel-wise analysis, however, is the difficulties in localizing the results, as well as a very high computational demand.

Regardless the particular atlas one might choose, an independent important issue is how to build up the final network. In this work, we have opted for an unweighted network obtained applying a threshold over the statistical significance of the partial correlation: given any pair of regions, an unweighted edge was drawn between the two if their partial correlation was significantly different than zero at a confidence level of 99%. A different approach would be to construct a network in which each edge is weighted according to the significance level of the partial correlation [see Stam et al., 2007] for a thorough discussion over this issue). When unweighted networks are used, as in this work, one has to investigate the stability of the final network as well, possibly repeating the analysis at different thresholds. Although not reported in this manuscript, we have performed the analysis at a confidence level of 95% as well [as originally presented in Salvador et al., 2005], obtaining similar results. Moreover, we would like to point out that the main goal of this study is to introduce a new definition of modularity, applicable on unweighted networks. Future research might focus on how to extend our definition to a more probabilistic domain, in which edges are weighted.

Finally, it is important to acknowledge the alternative techniques currently available for the detection of functional maps in fMRI data. In particular, methods based on probabilistic independent component analysis [pICA, Smith et al., 2004] have proved to be reliable. How do they relate to the work presented in this manuscript? The most common use of pICA analysis is to highlight spatially

independent maps which are highly coherent in time (or frequency) within a subject [DeLuca et al., 2006], either in task-related or resting-state fMRI. Improvements of pICA have led to concatenate and tensor pICA, for analyses across subjects and populations. An interesting study is the one presented by Damoiseaux et al. [2006]: the authors applied tensor pICA to highlight functional maps in resting-state fMRI, across a population of healthy subjects. Our work is not based on pICA: the functional maps have been detected using the technique presented by Salvador et al. [2005]. Upon that, we have elaborated a modularity analysis. A thorough comparison between the results found by Salvador et al. [2005] and those detected by other techniques [e.g. Damoiseaux et al., 2006] goes behind the scope of this manuscript. In general, both techniques highlight regions which are highly symmetrical and related to anatomical structures. Nevertheless, some discrepancies might still be present and one can reason over them. On one side, pICA is a more explorative technique since it does not make use of predefined atlases over which averaging the fMRI signal: the technique of Salvador et al. [2005], on the other side, is based on an anatomically defined atlas, and is therefore more restricted to the anatomy of the brain. As a consequence of its more explorative nature, tensor pICA usually provides a large set of independent components which then require a rather subjective selection. Attempts to solve this issue have recently been presented [see DeMartino et al., 2007] but a definitive solution has yet to be found. All current available techniques are based on precise mathematical assumptions and have therefore some limitations: a gold standard is still missing, although efforts are being made in this direction, for instance by trying to link functional connectivity to anatomical connectivity [Sporns et al., 2005].

CONCLUSIONS

To the best of our knowledge, this is the first study reporting modularity in resting-state functional connectivity, as well as a robust formal definition for quantitative analysis of modularity which takes into account overlapping and inclusive relationships among modules. The detection of modularity in complex networks is not a trivial task: thus, a formal definition which allows quantitative comparisons among networks is highly desirable. In this work, we have introduced a new methodology to assess modularity within complex networks: our novel approach, based on unbiased cluster coefficients, proved to be more specific in discriminating different cluster topologies than previous attempts. When applied to the BFC map, our definition allowed us to highlight areas of the brain with a hierarchical modular structure, whereas previous definitions could not discriminate between the BFC map and equivalent random networks. Our findings suggest that modularity in the brain is mostly detectable at small scales: even though limited by the given atlas, we could highlight

modularity between frontal, subcortical, parietal, and temporal regions of the brain, consistent with the notion of adaptive significance of modularity in complex neural systems. An attractive direction we are currently investigating is whether changes in brain modularity might be used as an early biomarker for different brain related diseases, such as Alzheimer disease and psychiatric disorders. Similar attempts have already been proposed, in the case of Alzheimer disease, focusing either on topological changes of cortical thickness [He et al., 2008] or changes in small-world properties of resting-state functional connectivity [Supekar et al., 2008]. Nevertheless, further investigations are needed to better characterize the effects that such diseases have on the brain functionalities.

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APPENDIX

Functionally Connected Regions

Supporting Information Table II reports the list of 264 significantly functionally correlated anatomical regions. Compared to Salvador et al. [2005], we could detect 188 more regions: this is due to the stronger statistical power of our analysis. Our population is composed of 53 subjects, while in Salvador et al. [2005] only 12 subjects were included. Remarkably, the regions with the highest significance (lowest P values) in Salvador et al. [2005] largely correspond to the most significant pairs highlighted in our study. Most of the detected pairs are intrahemispheric [75% in our study, 58% in Salvador et al., 2005], followed by interhemispheric symmetric pairs [17% in our study, 38% in Salvador et al., 2005], and interhemispheric asymmetric pairs [8% in our study, 4% in Salvador et al., 2005].

Functional Connectivity Versus Anatomical Distance

Salvador et al. [2005] showed that pairs with shorter anatomical distances tend to have higher degrees of partial

correlation: in particular, they showed that the coefficient r for partial correlation approximately follows an inverse square law $r \approx 1/D^2$. Moreover, they showed that the pairs deviating from this trend are mostly the interhemispheric symmetric ones. In Supporting Information Figure 3, we show analogous results drawn from our population: again, the similarities are remarkable, showing an inverse square law of $r \approx 60 \times 1/D^2$, and interhemispheric symmetric pairs deviating from this trend (circled diamonds in Supp. Info. Fig. 3).

Hierarchical Cluster Analysis

A final hierarchical cluster analysis run on the partial correlation coefficients proved once again the robustness of

the analysis reported in Salvador et al. [2005]. After transforming each partial correlation coefficient into a dissimilarity measurement ($d = 1 - |r|$), the linkage-average clustering algorithm [Eisen et al., 1998] was applied, and the corresponding dendrogram is shown in Supporting Information Figure 4. Detected clusters are very similar to those reported in Salvador et al. [2005], and correspond to well-defined brain areas.

Conclusions on the Partial Correlation Method

The methodology proposed by Salvador et al. [2005] proved to be robust: by applying it to a much larger cohort, imaged at different centers, we could confirm all of their findings.