Persistent patterns of interconnection in time-varying cortical networks estimated from high-resolution EEG recordings in humans during a simple motor act

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Abstract

In this work, a novel approach based on the estimate of time-varying graph indices is proposed in order to capture the basic schemes of communication within the functional brain networks during a simple motor act. To achieve this, we used a cascade of computational tools able to estimate first the electrical activity of the cortical surface by using high-resolution EEG techniques. From the cortical signals of different regions of interests we estimated the time-varying functional connectivity patterns by means of the adaptive partial directed coherence. The time-varying connectivity estimation returns a series of networks evolving during the examined task which can be summarized and interpreted with the aid of mathematical indices based on graph theory. The combination of all these methods is demonstrated on a set of high-resolution EEG data recorded from a group of healthy subjects performing a simple foot movement. It can be anticipated that the combination of the time-varying connectivity with the theoretical graph analysis is able to reveal precious information about the interconnections of the cerebral network as the significant persistence of mutual links and three-node motifs.

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(Some figures in this article are in colour only in the electronic version)

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1. Introduction

The extraction of relevant features from brain connectivity patterns is an open challenging topic, since often the estimated cerebral networks have a relative large size and complex structure. Recently, it was realized that functional connectivity networks estimated from the actual brain-imaging technologies (MEG, fMRI and EEG) can be analysed with the tools of graph theory (Stam 2004, Salvador *et al* 2005, Micheloyannis *et al* 2006). Beyond the investigation of the network main global features, such as the presence of a small-world behaviour (Watts and Strogatz 1998, Strogatz 2001, Sporns *et al* 2004, Sporns and Zwi 2004), and of scale-free degree distributions (Barabási and Albert 1999, Jeong *et al* 2000, Amaral *et al* 2000), the understanding of the basic structural elements composing brain networks would reveal important information about the basic type of communication among different cerebral areas. In particular, a study of *link reciprocity*, i.e. the degree of mutual interaction between couples of nodes (Garlaschelli and Loffredo 2004), and the investigation of recurring patterns of interconnections, known in the literature as *motifs* (Milo *et al* 2002), represent two adequate candidates for the analysis of cerebral networks.

Since brain connectivity is often modulated by rapid changes in time and frequency, the necessity to detect and study such dynamics has been recently underlined (Boccaletti *et al* 2006). In this regard, the use of an adaptive multivariate auto-regressive (aMVAR) model for the estimation of the time-varying cortical connectivity is particularly effective. In fact, it characterizes at the same time the evolving direction and spectral properties of the interaction between different brain signals and requires only one model to be estimated from all the time series (Ding *et al* 2000, Moeller *et al* 2001). In addition to this, the recent developments in the field of the high-resolution EEG techniques (Babiloni *et al* 2000) allow us to estimate accurately the electrical activity in particular regions of interests (ROIs) of the human cortex, giving the opportunity to go beyond the raw EEG signals that return lower quality results (since in this case the nodes within the network represent electrodes on the scalp, which might have indirect links with the cortical areas beneath them).

In this work, we consider time-varying cortical networks constructed from a set of high-resolution EEG recordings in a group of normal subjects performing a task consisting of a foot movement. We present an analysis of the time evolution of the networks in terms of link reciprocity and presence of three-node-directed motifs during the task performance. In particular, the main questions we want to address are as follows.

(1) How do the mutual interactions evolve in a normal subject during the foot movement?
(2) What are the privileged building blocks of the cortical network that significantly occur during the task performance?
(3) Does any persistent pattern of interconnections appear within the temporal period of interest?

2. Materials and methods

Five voluntary and healthy subjects participated in the study (age, 26–32 years; five males). They had no personal history of neurological or psychiatric disorder, and they were free from medications, alcohol or drugs abuse. The informed consent signature was obtained from each subject after the explanation of the study, which was approved by the local institutional ethics committee. For the EEG data acquisition, subjects were comfortably seated on a reclining chair in an electrically shielded and dimly lit room and they were asked to perform a dorsal flexion of their right foot (each subject was right-footed). The movement task was repeated

every 8 s, in a self-paced manner and 200 single trials were recorded by using 200 Hz of sampling frequency. A 96-channel system (BrainAmp, Brainproducts GmbH, Germany) was used to record EEG and EMG electrical potentials by means of an electrode cap and surface electrodes, respectively. The electrode cap was built accordingly to an extension of the 10–20 international system to 64 channels. Structural MRIs of the subject's head were taken with a Siemens 1.5 T Vision Magnetom MR system (Germany). Three-dimensional electrode positions were obtained by using a photogrammetric localization (Photomodeler, Eos Systems Inc., Canada) with respect to anatomic landmarks: nasion and the two pre-auricular points. Trained neurologists visually inspected EEG data and trials containing artefacts were rejected. Subsequently, they were baseline adjusted and low-pass filtered at 45 Hz.

2.1. Estimation of the cortical activity

High-resolution EEG technologies have been developed to enhance the poor spatial information content of the EEG activity (Le and Gevins 1993, Gevins *et al* 1994, Nunez 1995). Principally, this technique involves the use of a large number of scalp electrodes (64–256), realistic MRI-constructed subject's head models (Babiloni *et al* 1997, 2000) and spatial de-convolution estimations, which are commonly computed by solving a linear inverse problem based on boundary-element (BEM) mathematics (Grave de Peralta Menendez and Gonzalez Andino 1999). In this work, the cortical activity from EEG recordings was estimated by using a realistic head model whose cortical surface consisted of about 5000 uniformly-disposed dipoles. Estimation of the current density strength for each dipole was computed by solving the linear inverse problem according to techniques described in previous papers (Babiloni *et al* 2005, Astolfi *et al* 2006). By using the passage through the Tailairach coordinates system, 16 ROIs were then obtained by segmentation of the Brodmann areas on the accurate cortical model utilized for each subject. Bilateral ROIs considered in this analysis are the primary motor areas for foot (MF) and the proper supplementary motor area (SM), the lateral pre-motor area (6), the cingulated motor area (CM) and the associative area (7), the frontal areas 9, 8 and the inferior parietal area 40. According to high-resolution EEG techniques, electrical activity was estimated in these 16 Brodmann areas of interest for this task. In order to inspect the brain dynamics during the execution of the foot movement, a time segment of 2 s centred on the onset detected by a tibial EMG was analysed. In fact, the most interesting cerebral processes subserving the movement are actually thought to occur within such an interval.

2.2. Time-varying connectivity

The partial directed coherence or PDC (Baccalà and Sameshima 2001) is a spectral measure used to determine the directed influences between any given pair of signals in a multivariate data set. As recently stressed in Kus *et al* (2004), the multivariate approach avoids the problem of the estimation of spurious functional links, which is very common with conventional bivariate approaches like, for instance, the ordinary coherence. The PDC is obtained from a unique MVAR model estimated on the entire set of trials, according to the method proposed by Ding *et al* (2000). MVAR estimators have been already applied to cortical waveforms estimated from high-resolution EEG recordings in order to achieve static connectivity networks during motor tasks in normal subjects and spinal cord injured patients and during cognitive tasks (Babiloni *et al* 2005, Astolfi *et al* 2005, 2007, De Vico Fallani *et al* 2007b). In order to capture the cerebral network dynamics, a time-varying formulation of PDC based on an adaptive MVAR (aMVAR) model is employed in this study. Time-dependent parameter matrices were

estimated by means of the recursive least squares (RLS) algorithm with the forgetting factor, as described in Moeller *et al* (2001) and Hesse *et al* (2003). Time-varying PDC allowed for the observation of transient influences among different cerebral regions during the execution of the experimental task and provided the evolving patterns of connectivity in particular frequency ranges. The rough connectivity estimation returns a fully connected weighted and asymmetric matrix, representing the Granger-causal influences (Granger 1969) among all the cortical regions of interest. In this work, we considered unweighted networks with a common number of connections that resulted statistically different from a rest period (see the appendix).

2.3. Graph analysis: reciprocity and motifs

Application of graph theory to small networks is rather new when compared to standard applications of such theory in the biological context. However, the need for the use of graph analysis applied on small cerebral networks has been recently thoroughly discussed (Hilgetag *et al* 2000, Stam *et al* 2006a, 2006b). Although the application of graph theory to 28 raw EEG signals has been already addressed (Micheloyannis *et al* 2006), we want to stress here that the opportunity to deal with the cortical activity allows representing nodes as particular Brodmann areas on the cortex (Babiloni *et al* 2005, De Vico Fallani *et al* 2007a). The use of raw EEG signals instead returns less powerful results since nodes within the network represent electrodes on the scalp, which could have indirect links with the cortical areas beneath them.

The first analysis we perform is that of *link reciprocity*. Reciprocity reflects the tendency, in a directed network, of vertex pairs to form mutual connections between each other (Wasserman and Faust 1994). Here we compute, as a function of time, the correlation coefficient index ρ proposed by Garlaschelli and Loffredo (2004) that measures whether double links (with opposite directions) occur between vertex pairs more or less often than expected by chance. The correlation coefficient can be rewritten as a function of the time variable t as follows:

$$\rho(t) = \frac{r(t) - c}{1 - c}. \tag{1}$$

In this formula, $r(t)$ is the ratio of the number of links pointing in both directions to the total number of links at the time t, while c is the connection density, which equals the average probability of finding a reciprocal link between two connected vertices in a random network. As a measure of reciprocity, $\rho(t)$ is an absolute quantity which directly allows one to distinguish, at each time, between reciprocal ($\rho(t) > 0$) and anti-reciprocal ($\rho(t) < 0$) networks, with mutual links occurring more or less often than random, respectively. The neutral or areciprocal case corresponds to $\rho(t) = 0$. Note that if all links occur in reciprocal pairs, one has $\rho(t) = 1$ as expected.

The second analysis we perform consists in finding the *statistically significant motifs* in the network evolution. By motif, it is usually meant a small connected graph of M vertices and a set of edges (maximally $M^2 - M$, for directed graphs), forming a subgraph of a larger network with $N > M$ nodes. For each N, there are a limited number of distinct motifs. For $N = 3$, 4 and 5, the corresponding numbers of directed motifs are 13, 199 and 9364 respectively (Harary and Palmer 1973). In this work, we focus on directed motifs with $N = 3$. The 13 different three-node-directed motifs are shown in figure 1.

Counting how many times a motif appears in a given network yields a frequency spectrum that contains important information on the network basic building blocks. Introducing the time variable, a time-varying motif spectrum can be represented as a three-dimensional histogram, which illustrates the evolving participation of different motifs within the functional network
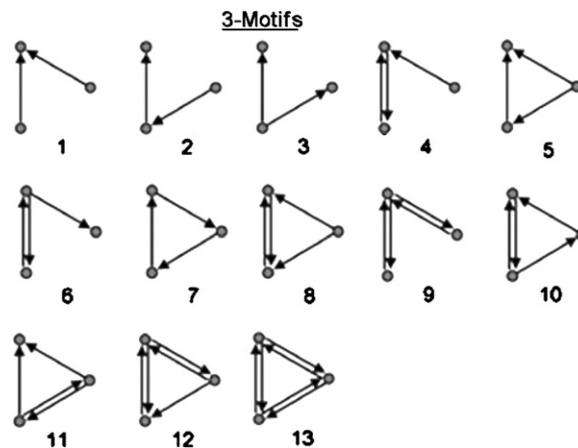
Figure 1. Representation and numeration of all the possible directed motifs with three nodes (3-motifs).

at each time instant. All the indexes computed[8] for cortical networks were standardized by considering their Z-score with respect to the distribution obtained from an ensemble of 100 random graphs. Random graphs were generated from the cerebral network (separately for each subject, band and time sample) by maintaining the same number of nodes and links, and randomly (with uniform probability) shuffling the connections in order to destroy the cortical relationships present in real networks. By means of this procedure, we can look at those motifs within the cortical networks that occur at a frequency significantly higher than in random graphs (Milo *et al* 2002).

3. Results

Figure 2(*a*) presents a superimposition of the electrode montage with the actual head structures for a representative subject as an example for the different steps involved in the estimation of the high-resolution EEG signals obtained in this study. The locations of the ROIs are illustrated on the left hemisphere of the cortex together with their estimated temporal activity. As described above, the use of the time-varying PDC on the cortical waveforms obtained from EEG signals returns a cortical network for each selected time sample and frequency. In this study, we focused the analysis on two particular spectral ranges related to the movement, namely the Alpha and Beta bands. In fact, these frequency bands have been suggested as most responsive to the preparation and execution of a simple limb movement (Pfurtscheller and Lopes da Silva 1999). In figure 2(*b*) the time-varying functional networks extracted at three particular instants are illustrated for the representative subject. The Granger causality from an area X to Y is represented by an arrow between the nodes X and Y. The colour of the arrow indicates if the particular link changed (light) or not (dark) its direction in at least one of the three instants.

The reciprocity indices were gathered from the cortical network of all the subjects in each time instant. In particular, we calculated the correlation coefficient ρ that indicates the level of links reciprocity of the networks estimated with respect to a random reorganization of the actual connections. In figure 3(*a*), the average profile of the correlation coefficient $\rho(t)$ is shown for

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[8] The motifs detection algorithms that we used are available in Matlab format at http://www.indiana.edu/~cortex/CCNL.html (Sporns 2002).
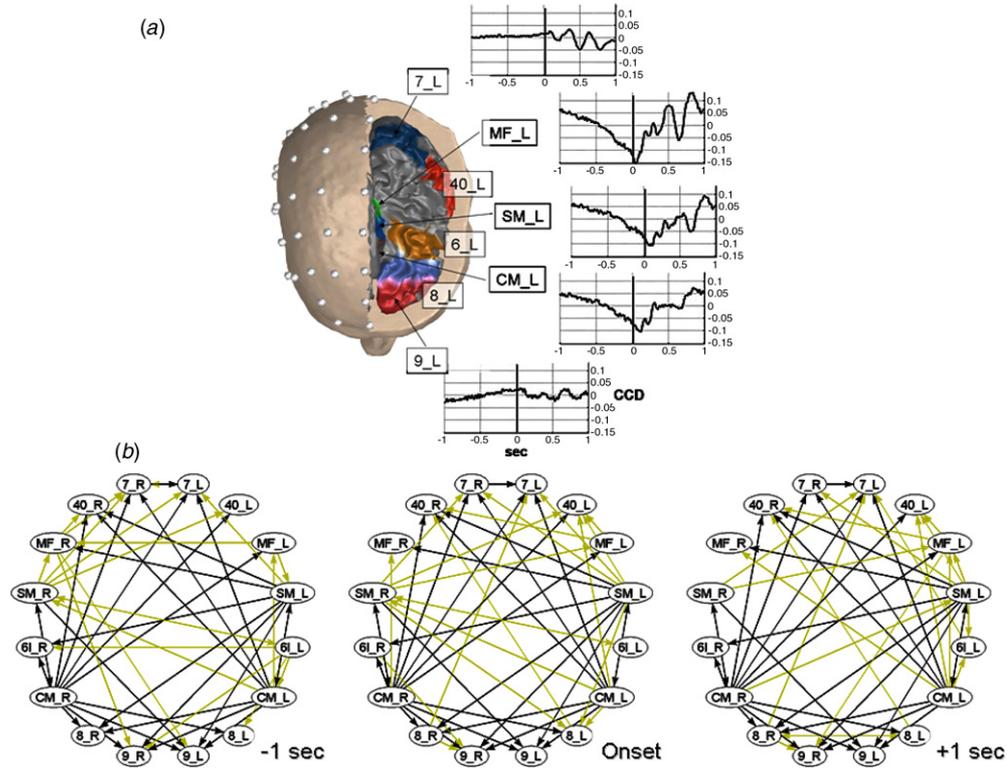
Figure 2. (*a*) Realistic head model for the representative subject. On the right hemisphere of the scalp, the positions of the electrodes are depicted as white little spheres. On the left hemisphere of the cortex, all the cortical regions of interest are displayed and opportunely labelled. The trial-averaged waveforms for a particular subset of areas (7_L, MF_L, SM_L, CM_L, 9_L) are illustrated. (*b*) Functional networks of the subject in the Beta frequency band during three representative instants (−1 s, onset, +1 s) of the task performance. Dark arrows represent the functional links that persist in all the three instants, while the light arrows represent those flows that changed direction in at least one instant.

the representative Beta frequency band. The overall presence of mutual links in the cortical networks is always higher with respect to random ($\rho > 0$). However, a different behaviour can be found between the preparation and the execution of the movement. In particular, during the movement preparation the reciprocity of the cortical networks moves from a relative high reciprocal state ($\rho > 0.25$) to a lower ($\rho < 0.17$) level as revealed by the negative slope of $\rho(t)$ for $-1 < t < 0$ s. Instead, during the movement execution the average trend of $\rho(t)$ for $0 < t < 1$ s constantly remains in the low reciprocal state reached in proximity of the onset ($0.15 < \rho < 0.2$). In figure 3(*b*), the level of reciprocity of all the possible connections within the cortical network is illustrated for the same band and during the entire period of interest. The level of grey codes the number of subjects that actually hold a particular reciprocal link identified by the values at the ordinates. In table 1, the correspondence between the *y*-values and the bilateral link can be deduced by inspecting the values of the symmetric adjacency matrix. The presence of continuous horizontal lines indicates a sort of 'persistence' of particular reciprocal connections which can also remain active during the entire task performance, as for the cingulate motor areas (CM_L and CM_R) with the ipsi-lateral supplementary motor areas (SM_L and SM_R), respectively. In such a case, at least three subjects present these persistent
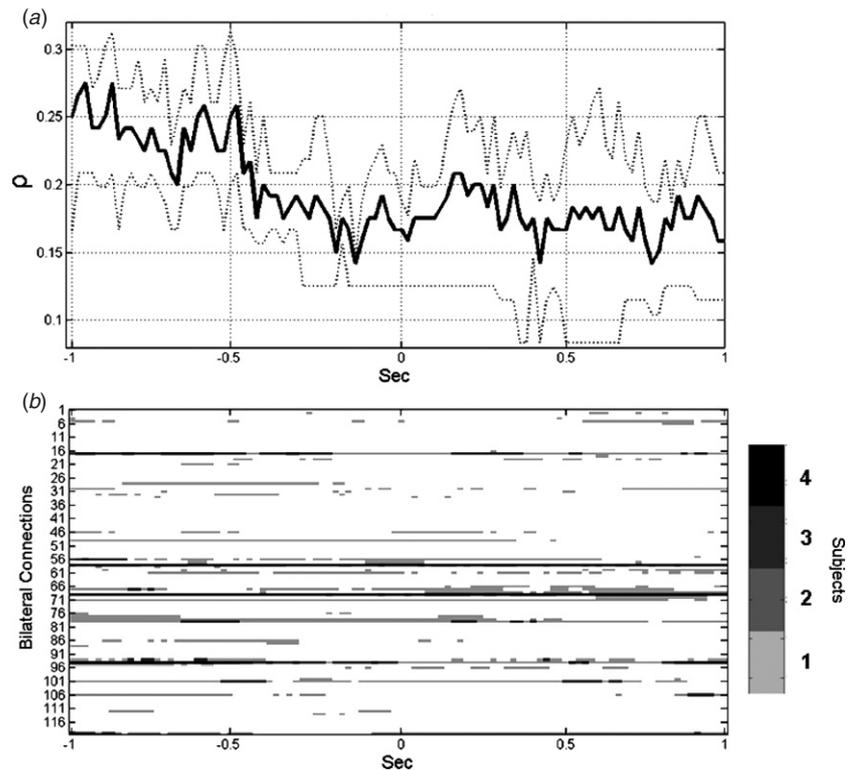
Figure 3. (*a*) Group-averaged time-varying 'reciprocity' during the period of interest in the Beta band. On *y*-axes is the correlation coefficient ρ, while time in seconds is on *x*-axes. Dotted lines represent the 25th and 75th percentiles. (*b*) Representation for the persistence of the bilateral connections within the cortical network during the task performance. On *y*-axes are all the 120 possible reciprocal connections, while time in seconds is on *x*-axes. The colour of the line corresponding to a particular link codes the number of subjects that actually hold such a connection.

connections. Analogous results were observed in the Alpha band, whose data are not shown here.

The level of involvement for the basic building blocks within the time-varying functional networks estimated was analysed by means of the motifs' spectra. The occurrences of all the 3-motifs' classes were recorded from the evolving cortical networks of each subject in the Beta band. Figure 4(*a*) shows the average time-varying motifs spectra by means of the respective histograms (seen from above). On the ordinates, all the possible 13 motifs with three nodes are listed (the respective sub-graph structures are shown in figure 1), while on the abscissas time in seconds is displayed; the greyscale codes the average count for the motifs' occurrences of the experimental group. Successively, the contrast with the random networks was addressed for every subject by calculating the *Z*-score of all the three motifs' occurrences in each temporal instant. By means of this procedure, we can look at these motifs within the cortical networks that occur with a significant frequency than in random graphs. Figure 4(*b*) shows the average *Z* values for the time-varying motifs' spectra according to the conventions used for the previous figure. The significant ($p \ll 0.01$) role of two types of building blocks (the third and eleventh called 'single-input' and 'uplinked-mutual-dyad', respectively) is revealed by the persistent high *Z* values observed during the entire period analysed. Moreover, the

Table 1. Numeration of all the possible reciprocal connections among the ROIs within the cortical network.

	9-L	9-R	8-L	8-R	CM-L	CM-R	6L-L	6L-R	SM-L	SM-R	MF-L	MF-R	40-L	40-R	7-L	7-R
9-L	–	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
9-R	–	–	16	17	18	19	20	21	22	23	24	25	26	27	28	29
8-L	–	–	–	30	31	32	33	34	35	36	37	38	39	40	41	42
8-R	–	–	–	–	43	44	45	46	47	48	49	50	51	52	53	54
CM-L	–	–	–	–	–	55	56	57	58[a]	59	60	61	62	63	64	65
CM-R	–	–	–	–	–	–	66	67	68	69[a]	70	71	72	73	74	75
6L-L	–	–	–	–	–	–	–	76	77	78	79	80	81	82	83	84
6L-R	–	–	–	–	–	–	–	–	85	86	87	88	89	90	91	92
SM-L	–	–	–	–	–	–	–	–	–	93	94	95	96	97	98	99
SM-R	–	–	–	–	–	–	–	–	–	–	100	101	102	103	104	105
MF-L	–	–	–	–	–	–	–	–	–	–	–	106	107	108	109	110
MF-R	–	–	–	–	–	–	–	–	–	–	–	–	111	112	113	114
40-L	–	–	–	–	–	–	–	–	–	–	–	–	–	115	116	117
40-R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	118	119
7-L	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	120
7-R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Persistent connections.

fifth and sixth 3-motifs show an increasing and decreasing involvement, respectively. In fact, during the movement preparation (from about −1 to 0 s) the significant ($p < 0.05$) presence of the sixth motif (called 'feedback-with-one-mutual-dyad') is revealed by Z values >1.96, while a rapid decaying in the zone of non-significance occurs during the execution. A contrary behaviour can be observed for the fifth motif (called 'feed-forward-loop'), which instead tends to significantly ($p < 0.05$) appear during the movement execution (from about 0 to +1 s). Analogous results were observed in the Alpha band, whose data are not shown here.

4. Discussion

Many of the anatomical and functional networks of the human brain have been shown to share non-trivial properties related to their complex organization. The large part of the studies showed interesting evidences related to the global features of the networks inspected. Among these, the 'small-world' property (Strogatz 2001, Stam 2004, Salvador *et al* 2005, Sporns and Zwi 2004) of short paths between any two nodes and highly clustered connections and the 'scale-free' characteristic of a few nodes with many more connections than the average node has (Barabási and Albert 1999, Jeong *et al* 2000) represented the most important and frequent outcomes. However, the necessity to capture simpler mechanisms such as the presence of basic building blocks (i.e. motifs) and mutual interactions (i.e. reciprocity) within the cerebral network represents another interesting aspect that has been poorly inspected. Moreover, even if the study of brain connectivity by means of graph theory has already provided some clues in understanding some cortical functions (Tononi *et al* 1994, Sporns *et al* 2004, Stam *et al* 2006a), several aspects still remain unclear. Among these, the absence of a computational model able to study how the brain dynamics can be affected by a time-varying connectivity as well as the negligence of considering asymmetric couplings make up real obstacles to the study of the cerebral interactions. In order to overcome this limitation, the possibility of tracking the time evolution of the functional connectivity patterns by means of the adaptive partial directed coherence could represent a major breakthrough in the analysis of EEG and MEG data. In this study, the indices related to the link reciprocity and three-motifs spectrum have
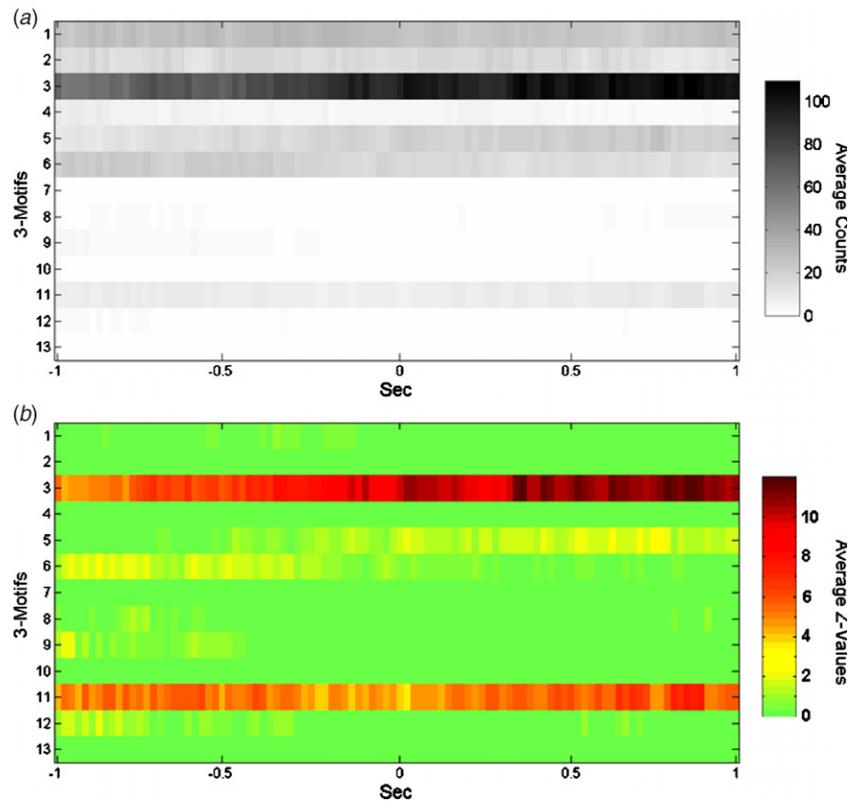
Figure 4. (*a*) Representation for the group average of the time-varying 3-motifs spectra in the Beta band. On *y*-axes all the 13 possible directed 3-motifs are listed, while time in seconds is displayed on *x*-axes. The colour codes the average count of the motifs' occurrences. (*b*) Contrast between the cortical and random time 3-motifs spectra. Same previous conventions for the axes, while the colour now codes the group average of the *Z*-values obtained.

been applied to the time-varying cortical functional networks estimated in a group of healthy subjects during a simple foot movement. In order to limit the analysis and the discussion of the results, we presented the results obtained mainly in the Beta frequency band that together with the Alpha band is known to be involved mainly in the cortical activity involved in the preparation and the execution of simple motor acts (Pfurtscheller and Lopes da Silva 1999). However, it must be noted that the methodology presented here is not limited to a particular frequency band or a particular set of ROIs, and can be adapted to investigate both cognitive and motor tasks in all the desired spectral content.

The analysis of the average time-varying reciprocity index revealed the significant presence of mutual links within the cortical networks during the entire period analysed. In particular, during the preparation (from about -1 to 0 s) of the movement in the Beta frequency band the functional network moved from a high ($\rho > 0.25$) to a lower ($\rho < 0.17$) reciprocal state. This aspect emphasizes the role of the early preparation in which a higher level of mutual exchange of information is required to speed up the cortical process in expectation of the execution. Although the cortical networks seem not much correlated during their maximum state of reciprocity, this result does not diverge too much from other empirical results obtained for networks of neuron classes in which values ranged around 0.17–0.18 (Garlaschelli and

Loffredo 2004). Moreover, by tracking the evolving involvement of each single reciprocal connection it was possible to observe their 'persistence' during the entire period of interest. Interestingly, the permanence of stable mutual links seems a peculiar characteristic of the functional networks estimated, which instead cannot be observed in any sequence of random graphs. In particular, the persistent bilateral links between the cingulate motor areas (CM left and right) and the supplementary motor areas (SM left and right) in the Beta band reveal a novel aspect of such a connection that anyway was expected in a self-paced modality of movement generation, as under our experimental condition (Gerloff *et al* 1998).

In brain networks, motifs are those basic building blocks consisting of a set of cortical areas and pathways that can potentially engage in different patterns of interactions depending on their degree of activation, the surrounding neural context (Sporns and Kötter 2004). In general, motifs occur in distinct motif classes that can be distinguished according to the size (N) of the motif, equal to the number of nodes, and the number and pattern of interconnections. In this study we considered the simpler case of $N = 3$ (i.e. 3-motifs), as the ROIs involved are not so numerous. The analysis of the average time-varying spectra of the 3-motifs revealed the basic rules governing the structure of the complex networks estimated during the task performance in the Beta frequency band. We compared the 3-motif properties of real brain networks with those of random networks, and we identified some motif classes that occurred more frequently during particular stages of the movement. Of particular interest is the involvement of the feed-forward-loop motif that tends to significantly ($p < 0.01$) increase during the proper movement execution (from about 0 to +1 s). This type of building block is known to play an important functional role in information processing. In fact, one possible function of this circuit is to activate output only if the input signal is persistent and to allow a rapid deactivation when the input goes off (Shen-Orr *et al* 2002). In the cortical context, a possible interpretation of such a motif would make a particular ROI act as a 'switch' for the communication between the others two ROIs composing the triad. Another interesting aspect was revealed by the significant ($p \ll 0.01$) 'persistence' of the single-input motif that represented the highest recurrent pattern of interconnections within the cortical network during the entire evolution of the foot movement. The main function of this motif is known to involve the 'activation' of several parallel pathways by a single activator (Shen-Orr *et al* 2002). Thus, since the single input only differs from the feed-forward-loop motif for the lack of a functional link between the two areas activated, we can claim that the privileged scheme of communication within the functional networks estimated consists in a parallel activation from a particular ROI of two other distinct areas, whose communication seems to increase significantly only during the proper movement execution.

On the base of these experimental results obtained from the application of theoretical graph indices to the time-varying connectivity networks estimated by using advanced high-resolution EEG, we have possible answers to the experimental questions posed in section 1.

In particular,

(1) The global level of mutual interaction in the cortical networks of healthy subjects changes from a high reciprocal state during the early preparation of the foot movement to a lower level of reciprocity persisting until the end of the period considered. This aspect emphasizes the role of the early preparation in which a high level of mutual exchange of information is required to speed up the cortical process in expectation of the movement execution.

(2) Among the several building blocks that significantly occurred, of particular interest is the involvement of the feed-forward-loop motif that tends to increase significantly during the proper movement execution. In the cortical context, this type of 3-motif plays an

important functional role in information processing making a particular ROI act as a 'switch' for the communication between the others two ROIs composing the triad.

(3) The permanence of particular schemes of interconnections seems a peculiar characteristic of the cortical networks estimated that cannot be observed in any sequence of random graphs. The persistent bilateral links between the cingulate motor areas and the supplementary motor areas in the Beta band reveal a novel aspect of the dynamics of such connections in a self-paced task. Moreover, the single-input 3-motif is significantly present during the entire temporal period assuring a persistent functional substrate that characterizes the preparation and the execution of the foot movement.

Acknowledgments

Appendix

In order to consider only the task-related connections, we adopted a filtering procedure based on a statistical contrast with a rest period. In each trial, a rest period of 2 s preceding the movement was considered as a means of contrast (from -4 to -2 s before the onset). For each time sample, the different connections' intensities were collected in order to build for each pair of ROIs a distribution of values characterizing such a rest period. In order to test the significance of the connections estimated within the period of interest, a threshold range was then extracted from the values of the rest distribution by considering a percentile of 0.01 and 0.99, respectively, for the lowest and highest edges. Significant functional links are those whose intensity falls outside such a range.

Then, in order to analyse the topology of the cortical networks estimated we moved to the respective unweighted networks by just considering the presence (1) or the absence (0) of the significant functional links. In particular, since we are comparing cortical networks that belong to different subjects, frequency bands and time samples, a common number of connections (or density) has been considered in each unweighted graph. In fact, when seeking a common behaviour among different networks, the graph indices that evaluate their architecture could suffer in robustness when they apply to graphs with different densities. Moreover, it can be proved that, in a graph with 16 nodes, a density of 0.2 is able to emphasize its global and local characteristics (De Vico Fallani, data not shown here). Hence, in this study we considered 48 connections (i.e. density = 0.2) for each network obtained by removing the weakest links from each weighted graph.

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