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Modelling a Multiplex Brain Network by Local Transfer Entropy

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Abstract

In this work we report on a systematic study of the causal relations in information transfer mechanisms between brain regions under resting condition. The 1000 Functional Connectomes Beijing Zang dataset was used, which includes brain functional images of 180 healthy individuals. We first characterize the information transfer mechanisms by means of Transfer Entropy concepts and, on this basis, propose a set of indexes concerning the whole functional brain network in the frame of a multilayer description. By exploring the influence of a set of states in two given regions at time t (A_t ; B_t) over the state of one of them at a following time step (B_{t+1}), a series of time-dependent events can be observed pointing to four kinds of significant interactions, namely:

- (de)activation in the same state (ActS);
- (de)activation in the opposite state (ActO);
- turn off in the same state (TfS);
- turn off in the opposite state (TfO).

This leads to four specific rules and to a directional multilayer network based upon four interaction matrices, one for each rule. By hierarchical clustering methods the four rules can be reduced to two sharing some similarities with positive and negative functional connectivity. The global architecture of the four interactions and the features of single nodes were initially explored under stationary conditions. The information transfer mechanisms on the ensuing functional network were studied by specific indexes describing in a multilayer frame the effects of the network structure in several dynamical processes. The healthy subjects database was used to carefully calibrate and validate the proposed approach, whose final aim remains the detection of clinical differences among individuals, as well as among different cognitive states.

1. INTRODUCTION

Recently developed multivariate statistical methods allow to model brain fMRI signals in a large-scale network where brain regions can be associated on the basis of their functional behaviour. The classical measure used in such a context is the functional connectivity, defined as the statistical dependence, usually calculated by temporal correlation, between spatially remote regions¹. The above approach, however, has some weak sides since: 1) it is a symmetric measure and the influences among regions appear biunivocal, which impairs to explore causal relations; 2) it assumes a steady relationship between brain regions, while recent evidences^{2,3} show that a time-varying measure provides a much more detailed information. Dealing with the first point Friston¹ introduced the Effective Connectivity (EC) proposing the Dynamic Causal Modeling (DCM) as a tool to extrapolate causal interactions⁴. Other methods refer to Granger Causal Analysis⁵ and Dynamical Graphics⁶. Concerning the second point, several approaches were proposed, among which the time-windows correlation² and the Co-Activation Patterns (CAP) analysis³. More recent works give a more complete picture of brain dynamics combining the causal analysis and the time-varying with a detailed characterization of different states in different subjects⁷.

Another relevant issue in the study of interacting brain regions is the assumption that functional connectivity values can be split into positive and negative^{8,9,10,11}, corresponding to in-phase and anti-phase signal dependence, respectively. As for the negative functional connectivity, although the pre-processing methods (e.g. Global Signal Regression, GSR) could insert spurious anti-correlation in fMRI data¹², significant anti-phase signal dependences were found by different methodologies: intracranial electrophysiological measurements^{9,13}, magnetoencephalographic (MEG) recordings¹⁴, as well as DCM¹⁵. Moreover, within the fMRI functional connectivity analysis, changes of anti-correlations have been related to some phenotypic and clinical features as working memory¹⁶, aging¹⁶, schizophrenia¹⁷ and NMDA receptors^{8,18}.

In the present paper we report on the information transfer mechanisms proposing a new estimate of causal relations by Transfer Entropy (TE) values between brain regions from fMRI data under resting condition. TE allows to reckon the influence of the state of two agents (nodes) at a given time over the state of one of them at a following time¹⁹. Since the reciprocal influence between the two agents is not symmetrical a directional relation can be estimated. In addition, taking advantage of the methods described by Lizier *et al.*²⁰, different kinds of influence (or transfer mechanism) between brain regions could be singled out and evaluated by a data-driven statistical analysis. It should be noticed that different information transfer processes can influence one another so that the ongoing brain activity cannot be estimated just by summing up, as if they were independent, the effects of each process. The multiplex network analysis provides a useful tool to explore many complex features of networks^{21,22} by modelling different kinds of relationships between nodes as different single-layer networks. In other words, a multiplex network is generated by

several kinds of interactions among the same set of nodes (e.g. people using different social media at the same time) and was successfully used in several contexts like diffusion processes²³, information and epidemic spreading²⁴, etc.. Thus, we decided to use a set of indexes to extract dynamical information on the whole functional brain network, in the frame of a multiplex (multi-layer) description. In summary, we propose to estimate and characterize functional causal interactions among brain regions on the basis of the TE and Multiplex Network concepts. A detailed account of the data pre-processing and TE values reckoning is provided in section 3.1; the interaction rules among brain regions are characterized in section 3.2; and the multiplex network approach in section 3.3. For a synthetic overview of the paper workflow see Suppl. Mat. 1.

2. MATERIALS AND METHODS

2.1 Data collection and pre-processing

The 1000 Functional Connectomes Classic collection, Beijing Zang dataset, was used (http://fcon_1000.projects.nitrc.org/indi/retro/BeijingEnhanced.html). The database includes 180 brain functional images of healthy individuals acquired in a resting-state condition at the Beijing Normal University in China, the study was approved by the Institutional Review Board of Beijing Normal University Imaging Center for Brain Research. The Functional images were acquired with a 3.0 T Siemens scanner and the following characteristics: 240 EPI volumes; repetition time, 2000 ms; echo time, 30 ms; slices, 33; thickness, 3 mm; gap, 0.6 mm; field of view, 200x200 2 mm; resolution, 64x64; flip angle, 90). The corresponding anatomical images, including a T1-weighted sagittal three-dimensional magnetization prepared rapid gradient echo (MPRAGE) sequence, was acquired covering the entire brain: 128 slices, TR (repetition time)= 2530ms, TE (echo time) = 3.39ms, slice thickness= 1.33 mm, flip angle= 7, inversion time= 1100 ms, FOV= 256x256 mm, and in-plane resolution = 256x192. The pre-processing step was performed as follows: the functional images were oriented to the twentieth scan, realigned and co-registered to the T1 image. Both the functional and the anatomical images were normalized to standard space (EPI image in Montreal Neurological Institute coordinates) using the normalization parameters of the T1 image. Then, a spatial gaussian filter was used (4x4x4 mm), the motion parameters were regressed out and a band-pass filtering in the range 0.008-0.09 Hz used. Afterward, the images were corrected by the anatomical CompCorr method²⁵. SPM8 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK) and the Functional Connectivity Toolbox (CONN) were used in the previous steps, on a MATLAB R2010b platform.

The images of each subject were divided into 90 ROIs by the automatic anatomical labeling²⁶ and from each ROI the time series were extracted. The short names used to identify the ROIs throughout this paper are listed in the Suppl. Mat. 2. The Framewise Displacement (FD) method²⁷ was used to check the movement-linked variability in single scans. Movements larger than 0.2 mm were taken as an indication of bad scan and a temporal mask from the preceding to the following two scans (spanning 8 sec) was also considered. Following Power *et al.*²⁷ bad scans should be scrubbed off from the analysis. Although this operation does not affect the brain regions showing synchronous activation, however the dynamics among regions is influenced, and we decided to remove the subject if bad scans > 30 (lasting more than 60 sec). As a result, 23 subjects were filtered out.

2.2 Causal interactions estimate

In order to pick up significant events, we filtered the z-score normalized time-series by four increasing thresholds of standard deviation fractions^{28,29}: (+/-)0.25; (+/-)0.50; (+/-)0.75; (+/-)1. In such a way, the signal complexity was reduced to single events (T) in which (because of the symmetric threshold) three possibilities are considered: 1; -1; 0, whether the signal is above the positive threshold, below the negative threshold, or in between the positive and negative thresholds, respectively (see Fig. 1). Moreover, the three above conditions point to possibly significant associated events: activation (1); deactivation (-1); null-activation (0). Notice that in the state of rest (null-activation), not only activation but also deactivation are possibly significant events. Thus, to account for the anti-correlated interactions^{8,9,10,11}, we distinguish the null-activation (0) from the deactivation (-1).

To elucidate time-dependent interactions, we need the state of a node (B) at a given time step (n), and at the following step (n+1). We also need the state of another node (A) at the time step n, maybe interacting with the state of (B) : $A_n(\text{state}) + B_n(\text{state}) \rightarrow B_{n+1}(\text{state})$, where the arrow indicates the time flow and (state) defines the activity condition (1, -1, 0) of the node. Given the 3 possible conditions (0, 1 and -1) and the 3-time dependent states (A_n , B_n and B_{n+1}), $3^3 = 27$ possible combinations describe the interaction among the two nodes. Hereafter, we call "rules" such combinations of interactions.

The TE estimate described by the following equation¹⁹ has been used to quantify the significant probability of each combination:

$$T_{A \rightarrow B} = \sum P(B_{n+1}; A_n^{(k)}; B_n^{(l)}) \ln \frac{P(B_{n+1}|A_n^{(k)}; B_n^{(l)})}{P(B_{n+1}|B_n^{(k)})} \quad (1)$$

where the state of nodes A and B is specified by: n = the current time step; n+1 = the following time step; k and l = number of time steps before n related to the event P(A) and P(B), respectively. In our analysis the k and l parameters were set to 1. However, since the repetition time for the dataset is equal to 2 sec, the time between two consecutive steps of the time series vector corresponds to 2 sec. In Fig. 2 a concise scheme of the method is reported. The TE

method deals with the difference (or Kullback-Leibler divergence³⁰) between the interaction: $P(A_{n+1}|A_n^{(k)}; B_n^{(l)})$; and the non-interaction hypotheses as described by a general Markov process: $P(A_{n+1}|A_n^{(k)})$. Thus, if the interaction hypothesis is verified, a positive value is assigned to that rule and all negative values are set to 0, assuming they indicate a misinformation transfer³¹. Among the 27 interactions also indicated as "rules", the not significant ones were filtered out by a series of statistical tests after reshuffling the original preprocessed BOLD signals for each subject. Thus, since only the time-sequences are randomized, the marginal frequency of events in each brain region is unchanged, and the local TE could be re-calculated for each brain region combination, for all subjects and rules. (more details in the Suppl. Mat. 3). The correlation among the significant rules was explored by a hierarchical clustering method³².

2.3 Network Analysis

The significant rules can be represented by a series of not symmetrical networks, where links are not reciprocal ($A \rightarrow B \neq A \leftarrow B$). Accordingly, any interaction between nodes (ROIs) is described for each subject by a weighted and directional network ($W = \{w_{ij}\}$) where links are represented by weight (w) and direction. In such a frame, the nodes are characterized by the sum of the associated weights: $s_i = \sum_j w_{ij}$, where w_{ij} is the weight of a link between nodes i and j . This quantity is called *node strength*. Moreover, due to a possible asymmetry between mutual interactions, *in-strength* and *out-strength* can be separately calculated by summing the weights of in- and out- connections (i.e., inverting subscripts i and j), and the total sum is defined *total-strength*. This can be also applied to directional binary networks (values = 1 or 0), producing in-, out-, and total-strength degrees.

2.3.1 Multiplex Networks

In the case of a multiplex network the classical metrics of the network theory applies to each single-layer network included in the multiplex. Thus, for a global description, the introduction of new network indexes is required: a) in a system including N nodes and M layers, each layer α (with $\alpha = 1, \dots, M$) is associated to an adjacency matrix $A^\alpha = a_{ij}^\alpha$, where $a_{ij}^\alpha = 1$ if nodes i and j are connected by a link in the α layer; b) in a weighted network (W) each $a_{ij}^\alpha \neq 0$, is associated to a weight w ; c) in a directional network links are from i to j . A multiplex network can be specified by the vector of adjacency matrices of the M layers as $W = \{W^{[1]}, \dots, W^{[M]}\}$, where $W^{[\alpha]} = w_{ij}^{[\alpha]}$ and we can define the strength of node i by the vector: $s_i = \{s_i^{[1]}, \dots, s_i^{[M]}\}$

where $i = 1, \dots, N$, and the strength of node i in layer α as: $s_i^{[\alpha]} = \sum_j w_{ij}^{[\alpha]}$. As an alternative, a multiplex network is obtained by summing up all (or a set of) layers calculating the weighted aggregated overlapping adjacency matrix: $O^w = o_{ij}^w$, where $o_{ij}^w = \sum_\alpha w_{ij}^{[\alpha]}$. In this case the strength of node i , is calculated by the weighted overlapping degree: $o_i^w = \sum_j o_{ij}^w = \sum_\alpha s_i^{[\alpha]}$. The directional version of this formalism is obtained by splitting the node strength into the corresponding in-, out- and total-strength. In summary, we introduce two ways to describe a multiplex network:

- the vector of adjacency matrices, W ;
- the weighted aggregated overlapping adjacency matrix, O^w .

The W contains all the information of the multiplex network preserving any difference between layers and allows to calculate any structure formed by the combination of all (or set of) layers. However, its combinatorial nature needs a huge computing time. The second approach, on the other hand, loses the architecture of the layers and, although faster, may miss the structural features of the network. To characterize the whole brain functional network we used both methods, comparing the results in each particular condition.

2.3.2 Network dynamics

The influence of the network structure on dynamical processes can be estimated by appropriate indexes in the framework of the analysis of reciprocity^{33,34,35} (RI) and of subgraphs^{36,37}. The RI estimates the occurrence of mutual relationships among node pairs looking at the number of reciprocal links normalized to the total number of links³³. In the case of weighted networks, however, the number of links (different for each connection type) cannot be easily calculated and, among the proposals introduced to face the problem, we chose the reciprocity method described in Squartini *et al.*³⁴. An extension to a multiplex directional network³⁵ introduced the notion of multireciprocity, i.e. the tendency of links in one layer to be reciprocated by links in another layer. In summary, RI values normalized to a null-model network in the range $[-1, +1]$ (see the Statistical Analysis Section below) indicate:

- reciprocate connections (RI > 0);
- reciprocation avoidance (RI < 0);
- independent behaviour (RI = 0).

Concerning subgraphs, the recent introduction of graphlets³⁶, suggests the following definition of a subgraph: a combination of in- and out- links within a set of connected nodes where double links are not allowed. Notice that different combinations of in- and out- nodes can draw the same geometric pattern, called isomorphic, to be counted only once (see Fig. 3, panel A). According to Onnela³⁷ we characterize a subgraph by two indexes: Intensity (I) and Coherence (C). The I index is defined as the geometric mean of the subgraph links:

$$I_g = (\prod_{(i,j) \in l_g} w_{i,j})^{1/|l_g|} \quad (2)$$

Where g is a subgraph; w_{ij} is the weight of g between node i and j ; l_g is the set of links in g and $|l_g|$ is their number. In such a way, only triads formed by non-zero links are taken into account (a link = 0 would nullify the product). Moreover, a continuum set of low intensity values in a subgraph indicates a possible noise effect of link weights. The C index is calculated as the ratio between the geometric and arithmetic average of subgraph weights and checks the similarity of weight values within a subgraph. Since high level of I could be due by a single link, C indicates whether a whole set of subgraph links participate to the I value. Note that we use C instead of Q used by Onnela *et al.*³⁷ in order to distinguish the Q index of modularity described below. The Intensity (I) and Coherence (C) indexes were used in this paper to quantify the magnitude as well as the statistical significance of each subgraph (see also Suppl. Mat. 4). In our work we studied the geometry of closed 3-nodes subgraphs in a directional network. Concerning the trajectories of subgraphs, we adopted the classification proposed by Takaguchi and Yoshida³⁸ where two kinds of subgraphs are considered: Cycle and Flux triads (Fig. 3, panel B):

- Cycle triads is a subgraph of three connected nodes having non-zero weight in both in- and out-connections, namely a directional cycle;
- Flux triads is a connected subgraph in which one node has non-zero weight in both in- and out-connections, and the other two nodes only have in- and out- non-zero connections, respectively.

To characterize triads in a multiplex network we adopted the definitions reported by Battiston *et al.*²² (Fig. 3, panel C). In addition, a 0th order triad is referenced by its type (Cycle or Flux) associated to the belonging layer. For example: Flux ActS or Cycle O. In the case of 1st and 2nd order Cycle subgraphs, the names are derived by the sequence of layers in the Cycle. For example: Cycle ActS/TfO/ActS, Cycle ActO/ActS/TfS., etc. Fig. 3, panel D provides a schematic picture of the functional characterization of nodes and links.

2.3.3 Centrality and Modularity Analyses

In the Centrality analysis³⁹ nodes can be sorted by specific features accounting for interactions within the whole system. The node degree is used to characterize node centrality and, in a directional and weighted network, the corresponding metrics. Thus, in-, out- and total- strength indicate nodes endowed with a major in-Flux or out-Flux. In particular, we defined as central the nodes having a value higher than, at least, the third quartile of its distribution (>75%). Nodes with strength values higher than the interquartile distance of 85% and 95% were also considered as peculiar cases. In a multilayer description, besides the study of the node degree distribution in each layer, the distribution of central nodes across different layers was reckoned according to the following definitions:

- Local central node: if present in not more than 25% of the total layers;
- Intermediate multiplex central nodes: if present in the range between 25% and 75% of the total layers;
- Multiplex central nodes: if present in more than 75% of total layers.

In binary and bidirectional networks a widely used definition of Modularity^{40,41} accounts for nodes grouped into sets densely connected internally among each other through the Q index. In this way, a node can be included in a finite number of communities.. The difference between the fraction of links in a community and the expected fraction of links in a null model is estimated as follows:

$$Q = \frac{1}{2m} \sum_{ij} [A_{i,j} - \frac{k_i * k_j}{2m}] \delta_{ci,cj} \quad (3)$$

where A_{ij} is the link between nodes i and j in the adjacency matrix A ; k_i and k_j are the node degrees of nodes i and j ; m is the total number of links in the network and $\delta_{ci,cj}$ is the Kronecker delta symbol, where ci and cj are the labels of the community to which nodes i and j are assigned. In equation (3) the term A_{ij} refers to the real network, and the term $\frac{k_i * k_j}{2m}$ is the probability of an edge between nodes i and j in a random graph. In a directional network a different approach is needed, and Newman updated the Q estimate to account for the direction of links⁴¹. Thus, the second term in equation (3) is defined as: $\frac{k_i^{in} * k_j^{out}}{m}$, where k_i^{in} is the in- degree of node i , k_j^{out} is the out- degree of node j , and m the total number of links. The random model for directional network refers to the probability distribution of nodes i and j being directly linked. In weighted networks the fraction of edges is substituted by the fraction of weights in a community. In equation (3) the matrix W_{ij} is inserted as an adjacency weighted matrix and the $\frac{s_i^{in} * s_j^{out}}{m_w}$ as a null model, where s_i^{in} and s_j^{out} indicate the in- and out-strength on nodes i and j , respectively, and m_w is the sum of the whole set of weights in the network.

2.3.4 Reckoning Network Indexes

For the calculation of the previously described indexes an original script available upon request from one of the authors (F.P.) was designed, except for the random models and the modularity analysis, where we used scripts from the Brain Connectivity Toolbox⁴² (BCT). The global indexes were calculated for each subject separately, while the local (centrality) and the modularity analysis were carried out over mean matrices across all the subjects. For the randomized

models, an extended version of the Maslov and Sneppen⁴³ method was used, in which the weighted directional network is randomized preserving the -in strength distribution. However, since fully connected matrices are obtained from averaged networks, in the modularity analysis a combinatorial weight randomization was used. In all cases random models refer to averages of 100 randomizations.

3. RESULTS

3.1 Statistical characterization of interaction rules

Different interaction rules show different patterns of TE values summed over all subjects as a function of the signal threshold: the rules showing an increasing and a decreasing trend are indicated in the top and bottom panels of Fig. 4, respectively. It is worth noting that the Mean Squared Error (MSE) analysis shows an increasing trend in the same twelve rules (Fig. 4 and 5, top panel) while the remaining rules show a decreasing, flat and fluctuating mixed behaviour (Fig. 5, bottom panel). A series of t-test for independent samples (right tail) were performed to compare real and random local TE at the highest signal threshold (1 S.D.) for the twelve rules in Fig. 4, top panel. The ANOVA analysis shows a significant effect of rules ($p < 0.0001$) at the higher signal threshold (1 S.D.) and in Fig. 6 the post-hoc analysis confirms a significant increasing of local TE for eight of the above mentioned twelve rules. Thus, eight rules appear to share all the assumptions indicated in the statistical methods (see also Suppl. Mat. 3) and we restricted the analysis to those rules, where at least two of the previously described features, namely increasing trend of local TE and MSE at increasing threshold, actually appeared. Moreover, the result could be replicated under all the explored conditions for the following four rules:

- $A_n(1) + B_n(0) \rightarrow B_{n+1}(1)$;
- $A_n(-1) + B_n(0) \rightarrow B_{n+1}(-1)$;
- $A_n(1) + B_n(-1) \rightarrow B_{n+1}(0)$;
- $A_n(-1) + B_n(1) \rightarrow B_{n+1}(0)$.

The remaining four rules, namely:

- $A_n(1) + B_n(0) \rightarrow B_{n+1}(-1)$;
- $A_n(-1) + B_n(0) \rightarrow B_{n+1}(1)$;
- $A_n(1) + B_n(1) \rightarrow B_{n+1}(0)$;
- $A_n(-1) + B_n(-1) \rightarrow B_{n+1}(0)$;

showed a higher variability. To remove any potential source of noise we excluded from any further analyses 49 subjects (corresponding to the 31% of the database) since they did not produce significant (t-test independent sample) results in the distribution study reported in Table 1. A final relevant observation concerns the following rules:

- $A_n(1) + B_n(-1) \rightarrow B_{n+1}(-1)$;
- $A_n(-1) + B_n(1) \rightarrow B_{n+1}(1)$;

characterized by an increasing trend in the MSE, not significant results in the right tailed although significant in the left tailed comparison test (not shown). Thus, the decrease of their local TE at increasing thresholds indicates a lower probability of expression in the time vector than in the random counterpart.

3.2 Classification of interaction rules

In order to characterize the eight rules selected in Table 1, we explored their interaction pattern shown by the correlation matrix in Fig. 7 (left panel), from which the following considerations emerge: 1) Rules having a similar functional meaning, although with a different node state, appear more related among each other. This points to dynamical processes operating in the frame of different sets of states; 2) positive and negative relations may exist among particular sets of rules. Fig. 7 (right panel) shows a hierarchical classification splitting the eight rules in two groups at the first level which are further split into four pairs of rules, matching the anti-correlated rules of the previous analysis. Just one of the two related forms in each pair is discussed below.

- G1) $A_n(1) + B_n(0) \rightarrow B_{n+1}(1)$ and $A_n(1) + B_n(-1) \rightarrow B_{n+1}(0)$ have, respectively, the functional meaning of activation and "turn off". "Turn off" is used to distinguish de-activation (-1) from null-activation (0) in a process in which the states of A and B are shared: in the former case A (de-) activates B in its own state, while in the latter A turns off B if in the opposite state.

- G2) $A_n(1) + B_n(0) \rightarrow B_{n+1}(-1)$ and $A_n(1) + B_n(1) \rightarrow B_{n+1}(0)$ have, respectively, the functional meaning of (de-) activation and "turn off" when the states of A and B are opposite. In the former case A (de-)activates B in the opposite state, while in the latter A turns off B if in the same state.

In particular, concerning the logical relations: one pair of rules (G1) concern a transition of B to the same state of A ($0 \rightarrow$ state A); another pair (G2) describe a transition of B to the opposite state of A ($0 \rightarrow$ state non-A).

Table 2 shows the grouping of rules in line with the above sketched functional criteria:

- ActS = (de-)activation in the same state;
- ActO = (de-)activation in the opposite state;
- TfS = turn off in the same state;
- TfO = turn off in the opposite state.

It is noticeable that the G1 and G2 rules refer, respectively, to the positive and negative functional connectivity and tend to be expressed in different and non-overlapping set of links.

3.3 The multiplex network

The four matrices ActS, ActO, TfS and TfO correspond to the main four layers in the vector of adjacency matrices of a multiplex network, hereafter defined as α -hierarchical level. We extended the analysis to two matrices calculated as weighted, aggregated, overlapping adjacency matrices from those in the α -hierarchical level, namely S matrix (Same state, combination of ActS and TfS), and O (Opposite state, combination of ActO and TfO). The new vector of adjacency matrices was defined in a β -hierarchical level. Finally, a T matrix was calculated as the weighted aggregated overlapping adjacency matrix from S and O matrices. The corresponding vector of adjacency matrices consists of a single matrix formed by a linear combination of the first four layers which defines a γ -level hierarchical. Fig. 8 contains a global representation of the multiplex network including the above-mentioned hierarchical levels.

In Table 3 the characteristic indexes of each layer in the considered hierarchical levels are reported. The T matrix, containing the whole set of weights, appears almost completely connected, while the TfS and TfO show the lowest (and almost the same) values. Similar results are found for the connection density. Consequently, the S rule has higher values in both weights and connection density as compared the O rule.

3.3.1 Reciprocity and Multireciprocity

As a first global feature of the multiplex network we looked at the Reciprocity Index (RI). Table 4 shows the clear difference with the random model ($p < 0.001$) reached for all matrices but for ActS and ActO, as well as for their interactions in the vector of adjacency matrices. In detail, for the α -hierarchical level both TfS and TfO show negative values, indicating an asymmetric behaviour. Similar results are detected for the interacting matrices ActS/TfS and ActO/TfO, while opposite results hold for the interacting TfS/TfO, as well as ActS/TfO and ActO/TfS. An asymmetric behaviour is also found in the β -hierarchical level matrices S and O, but the opposite behaviour for their interaction S/O, as well as for their sum in the γ -hierarchical level matrix T. As for the subgraph analysis, a summary of the whole set of possible subgraphs at various hierarchical levels are provided in Suppl. Mat. 4.

3.3.2 Subgraph analysis

Looking at the combination of different patterns of links in the Cycle and Flux triads, the following number of subgraphs in the three hierarchical levels were observed:

- α -hierarchical level: 4 Cycle and 4 Flux (0th order), 12 Cycle and 36 Flux (1st order), 8 Cycle and 24 Flux (2nd order);
- β -hierarchical level: 2 Cycle and 2 Flux (0th order), 2 Cycle and 6 Flux (1st order);
- γ -hierarchical level: 1 Cycle and 1 Flux (0th order).

Fig. 9 shows that the whole set of significant subgraphs for both I and C indexes are of the Flux type belonging to the α -hierarchical level, and most of them fall in the 1st and 2nd order. These subgraphs share the same interaction rule acting on the sink (source to sink: Acts; middle to sink: TfS), but differ in the middle way, alternating the two opposite rules: ActO and TfO. Other less stable (not significant C) Flux triads act with the same rules: Acts, ActO, TfO. In the case of the β -hierarchical level, both S and O make a Flux subgraph, while their interaction produces a Cycle subgraph. However, no subgraphs reach a clear significance in both I and C, in this hierarchical level. In table 5 a complete summary of the above results is reported.

3.3.3 Centrality features

The Centrality Index of each layer shows different patterns of different brain areas (nodes) as a function of their in- out-connection weights. In what follows each layer will be separately considered in each hierarchical level. Centrality of ActS and TfS rules are inserted in Fig. 10, in red circle (ActS) and yellow circle (TfS). Regarding the first one, most of the out-strength and in-strength central nodes fall in the vision related brain regions (calcarine, lingual and fusiform) and limbic/DMN areas; the total strength confirms the previous brain areas as the main nodes of this rules. Conversely, central nodes of the TfS rule have a well distinct pattern of in- and out-strength central nodes, mainly centred in the sub-cortical regions of basal ganglia (caudate, putamen, pallidum) and limbic (amygdala). Other hubs of in-strength are found in motor related areas (SMA and middle cingulum) and in the frontal superior gyrus, while out-strength hubs cover a sparse cortical area including temporal, frontal and limbic brain regions.

The ActO and TfO rules are inserted in Fig. 10 in blue circle and green circle, respectively. Central nodes of ActO cover almost the whole area of the dorso-lateral pre-frontal cortex and the inferior parietal gyrus (parietal inferior, angular, supramarginal). However, in the in-strength also sensory-motor (SMA, insula), sub-cortical (pallidum and putamen) and DMN (cingulum posterior) regions appear as central nodes. The TfO rule has an intermediate pattern between the TfS and the ActO, having the cingulate cortex (anterior and posterior) and sub-cortical regions (thalamus, pallidum, caudate, putamen) as the main in-strength central nodes, and the fronto-parietal cortex as out-strength central nodes.

In the β -hierarchical level, Fig. 11 (orange circle: S; violet circle: O) confirms as central regions the sensory-limbic related areas and the fronto-parietal network for S and O rules, respectively. However, less evident appears the sub-cortical action of the TfS and TfO components since the weights of these last rules are covered by those of the ActS and ActO rules. More intriguing is the result at the higher hierarchical level of the T matrix (Fig. 11, black circle), where the main inward nodes are detected in a sparse brain region (sub-cortical, motor and attention networks), and the same holds for the outward ones (sensory-related areas, limbic and sub-cortical regions).

In conclusion, looking at the results of the α -hierarchical level, several nodes appear in more than one layer: intermediate multiplex nodes (two layers). In particular, the basal ganglia regions (caudate, putamen, pallidum and thalamus) appear to be shared among the TfS and TfO rules (TfS and TfO) as inward central nodes, as well as in the total strength. Thus, these sub-cortical regions appear as a possible intermediate station for the modulation of the Turn Off rules. Moreover, the ActO and TfO rules together cover most of the prefrontal and parietal cortex for outward connections. As a matter of fact, the O rule seems having a major role in these brain areas. Finally, the ActS and TfS rules share the cingulum middle, the parahippocampus and the temporal superior gyrus as out- and in-strength, respectively for the former and the second ones. Again, these last results are confirmed by the central nodes found in S rules of the β -hierarchical level. No central nodes are found between the ActS and ActO rules. In Fig. 10 and 11 a summary of local and intermediate central nodes is reported within the corresponding layer. A more detailed account of single connections is included in Suppl. Mat. 6.

3.3.4 Modularity

When compared to the random model, only ActS and S rules show a significant increment of Q values (Table 6). In both rules the same three main modules were found (Table 7): the first one referring to the visual cortex and its process-related areas; the second one including the Default Mode Network (DMN) and limbic regions; the last one covering most of the Task Positive Network (TPN) and including dorso-lateral prefrontal cortex, parietal cortex, basal-ganglia, auditory cortex and sensory-motor cortex.

A particular left/right asymmetry is detected in the caudate: left and right split in DMN/limbic and in the TPN module, respectively. We hypothesize that the caudate tend to get connected with both modules, as a sort of bridging node. Note that since this analysis concerns the averaged matrices, the networks obtained is fully connected, and we used the combinatorial randomization.

4. DISCUSSION

In order to explore causal influences between brain areas a data-driven approach is proposed using fMRI data under resting state and taking advantage of the Transfer Entropy (TE) concepts. The brain areas (ROIs) used in this paper and their short names, as well a flow chart of the adopted analytical strategy, are included in the Suppl. Mat. 1 and 2. As compared to previous works in the field^{1,5,6}, we describe a new way to estimate the functional brain causal interactions by TE parameters somehow similar to the Granger Causality^{5,44}. At difference with the latter method, however, we use a non-parametric approach and avoid a whole set of a priori statistical assumptions. Another and most popular method is the Dynamic Causal Modeling¹ (DCM), based on a series of differential equation arranged in a hemodynamic model⁴⁵, a recent updating of which has been proposed for the resting state condition⁴. The above-mentioned models, however, do not consider the different kinds of brain interactions although a recent work of Zhou *et al.*¹⁵ focused on negative interactions looking at feed-back (negative) and feed-forward (positive) connections. In our approach the distinction between positive and negative functional connectivity is a leading idea and our results, although preliminary, could open the door to a reliable detection of clinical differences among individuals in different cognitive states.

4.1 Transfer Entropy (TE) Parameters

The TE estimate was initially proposed by Vicente *et al.*⁴⁶ to characterize brain causal interactions. In our case we use a discrete time-series analysis where continuous values in a time-series are converted into a finite number of discrete events by threshold filtering the amplitude of BOLD signals. This allows the detection of cerebral states closer to the temporal resolution of the data acquisition with no (or few) assumptions and no substantial waste of information. In this respect Tagliazucchi *et al.*²⁹ showed that only considering spontaneous events the resting-state network can be recovered by a continuous signal fluctuations method. In addition, Petridou *et al.*⁴⁷ deconvolving a hemodynamic response function in the resting state, clarified how spontaneous events may contribute to the correlation strength and the power spectra of slow spontaneous fluctuations. We found four different kinds of interactions among brain areas, namely:

- (de)activation in the same state (ActS);
- (de)activation in the opposite state (ActO);
- turn off in the same state (TfS);
- turn off in the opposite state (TfO).

Hierarchical clustering analysis showed that such interactions can be reduced to two sets sharing similarities with positive and negative functional connectivity and in which two alternative mechanisms are active: (de)activation and turn-off. A particular modulation of brain areas emerges including not only activations and de-activations but also a subtle mechanism of turning off. The individual variability of the observed events was checked by a statistical analysis

on each subject. All positive interactions were validated on the whole data set, while the negative ones seem less accurate. This agrees with the higher variability of anti-correlations in 0-lag correlations estimates of brain connectivity¹¹. A possible explanation is the different synchronization timing for the negative connectivity^{14,48}. A more speculative proposal invokes a faster transfer mechanism associated to negative interactions. Exploring the variability changes as a function of different levels of time-lag connectivity would provide additional evidence to that.

Four rules show a lower probability trend as compared to the random model:

- $A_n(1) + B_n(1) \rightarrow B_{n+1}(1)$;
- $A_n(-1) + B_n(-1) \rightarrow B_{n+1}(-1)$;
- $A_n(1) + B_n(-1) \rightarrow B_{n+1}(-1)$;
- $A_n(-1) + B_n(1) \rightarrow B_{n+1}(1)$.

Such rules can be reduced to two couples: in the first couple the target node (B) assumes the same state of the previous time step if the source node (A) falls in the same state; in the second couple, the target node (B) assumes the same state of the previous time step if the source node (A) falls in the opposite state. Thus, both couples can represent a state preservation process, since they maintain the state in time given an equal or opposite state of the acting node (A). We may argue that such combinations are less favoured than others and that an underlying mechanism stands in the way of this dynamics. It seems that brain regions can be characterized by single and unique events, in agreement with previous findings^{29,47}. However, it must be considered that only few peaks survive at the highest threshold level, so a persistent value of the same state over time could anyway be less likely. As an alternative, an effect due to the reshuffling/randomizing method cannot be excluded.

4.2 Functional interpretation of the rules

Assuming that brain states can be estimated by the fluctuating combination of node connections⁵⁰ defining a functional connectivity state or meta-state, a particular cognitive condition could be characterized by a finite set of meta-states^{2,3} where different connectivity patterns alternate^{51,52}. As a matter of fact, several evidences showed how these dynamics draw a phase transition between more or less integrated/segregated network structure⁵³. Brain dynamics are usually defined in terms of meta-stability⁵⁴ or multi-stability⁵⁵ in the dynamical system frame. Both terms point to a fluctuating dynamic between different sets of synchronized states as a possible explanation of functional flexible (in)stability, (see Breakspear 2017⁵⁵ for a general review). In such a frame, the four rules we found provide an exhaustive description of the network dynamic: activation rules change the node states from 0 to 1 (or -1); the turn off rules gets back to 0 the node state. Both the ActS and TfS rules promote some uniform node states: the former one changes the target node to the same state of the source, the latter turns off the target node if in the opposite state of the source. Conversely, the ActO and TfO rules promote a process inducing the activation of an opposite state than the source node and turning off nodes with the same state. In other words, we have a double classification for such rules: a) a simpler process of changing the target node (activate or turn off), and b) a more comprehensive process pointing to uniformity or differentiation within the system, namely to a converging or diverging state among nodes (S or O). Thus, a particular synchronization of action rules within a given geometrical network could generate different dynamical patterns. Moreover, each change of nodes' state can be driven by the network connection itself, without necessarily needing an external or internal disturbance. In brief, these rules can describe a self-sustained dynamic, following an initial condition and consequential to external or internal stimuli. Assuming that the state of co-activated brain regions can be driven by causal interactions (ActS) while the mutual de-activation can be maintained by the ActO rule, both ActS and ActO may share a similar physiological interpretation^{8,9,56}. Conversely, the turn off rules do not seem to overlap any of the functional measures in use.

From a neurophysiological point of view, such interactions could be associated to two functions: 1) turning off a co-activated set of brain regions; 2) enhancing the excitability of brain regions. The former function arises when a particular action has to be suppressed, the latter function puts the node in the condition to be re-changed in an alternate state (1 or -1 cannot be changed without passing from 0). Thus, the rule has both abilities: turning off a functional state of a brain region and enhancing the excitability of the region towards interactions. Such a mechanism could be related to the inhibition of a tonic depolarization, for example the action of a GABAergic interneuron. However, these considerations remain speculative at this stage and to check the above sketched dynamical features appropriate simulations should be performed. e.g. in some agent-based simulation environment⁵⁷. As for the DCM estimation, the bivariate correlation appears the most natural method to study the hypothetical relation between the brain states transition and the causal brain interaction⁷. The CAP approach³, using a discrete method to analyse the BOLD signal, would be our favoured one.

4.3 Network analysis

A multilayer network was analysed on the asymmetric adjacency matrix of each subject. This matrix describes the network of the corresponding interaction rule and accounts for the 8100 possible interactions between the 90 brain regions. Three hierarchical levels have been considered as indicated by the hierarchical clustering analysis (Fig. 7), as well as all the possible inter-layer relations. It appears that the most significant features characterize the lower hierarchical level only, while merging the information in a higher hierarchical level seems to mitigate (or dilute) the specific trait of the single rule network in the lower hierarchical level. The four basics rules show peculiar features characterizing each of them in a specific manner. As a former comment it can be noted that the brain networks show a

not reciprocal nature, most marked in the turn off rules. The same is obtained for interacting matrices in the same kind of process (ActS and TfS, ActO and TfO), indicating a preferential unidirectional flow of interactions within the same dynamics. However, reciprocal connections arise between opposite interaction processes, pointing to a possible feed-back mechanism. Similar relations are found for the higher hierarchical level S and O, while in the T matrix the network lost the single layers detail becoming almost reciprocal.

4.3.1 Subgraphs

In dealing with graphs a main problem for us was the lack of knowledge concerning the real timing of events in the triads. Although we know that rules tend to be close to each other within a subgraph, we ignore whether the links work together (are synchronous), or in sequence. In other words, the links from the source node could be synchronous, while the connection from the middle to the sink node could work afterwards. However, we can infer the dynamics by studying the logical relations among rules: for example, on the sink node cannot insist at the same time two links endowed with the Turn Off (TfS / TfO) and the Activation (ActS / ActO) rules, because they need a different state on the node (0 for the Acts and 1 or -1 for TfS). Thus, a particular combination of links can be inferred, and the relative dynamics predicted.

The proposed dynamics of the 1st order subgraphs are reported in Fig.12. The three cases in the top right panel of the figure point to the same (de-)activated state between the source and the sink node. At the same time, the last two (bottom right panel) result in the same null (0) state. We asked whether these properties are shared by other combinations of subgraphs and, by the same procedure on the other 1st order subgraphs (left panel), we checked that these triads are unique. Thus, taking into consideration the possible logical temporization, we hypothesize two general dynamics: 1) a switch two pathway for the same target with different temporization (direct and indirect pathway); 2) a reinforce pathway to have a synergic action on the sink node.

In the case of the 2nd order subgraphs, a more complex situation emerges including the remarkable conditions depicted in Fig.13, in which: the source node seems to operate by two alternative ways on the sink node (activated or turned off) modulating an intermediate reaction in the indirect pathway (top left panel). Moreover, a whole series of fine modulations seems to occur along the direct and indirect pathways characterizing most of the information transfer mechanisms of the considered phenomena (top right and bottom panels). As a summary conclusion, it appears that the brain dynamics promotes a non-competitive behaviour in both first and second order subgraphs. This is quite reasonable in order to overcome possible conflicting situations. Moreover, the mechanisms are arranged in a hierarchical way: in the first order (two layers) only the congruent triads, for the direct and indirect pathways, are promoted; in the second order a possible compensatory mechanism (feed-forward) appears. This suggests that the complexity of the brain dynamics emerges when more than one connection type is considered. However, merging the rules in the higher hierarchical levels may lose the specific information on the interaction of single layers and hide significant results.

At our knowledge, we report for the first time a subgraph analysis in a multiplex network derived from an fMRI dataset. Other papers studied a multiplex brain network using MagnetoEncephaloGraphy (MEG)^{58,59}, but due to the quite different approaches we did not try a critical comparison with those results. In the works carried out using fMRI datasets, not in a multiplex perspective, the close triangle motifs are lacking, and the open motifs are detected as significant subgraphs⁶⁰. The same was found in a classical paper on anatomical connectivity by Sporn and Kotter⁶¹. In our work no triads are detected in the single layers (but for a trend in the case of ActS). Thus, it is possible to consider that the open motifs could characterize the single layer, while the triad motifs may arise only when more than one layer are taken into consideration. Furthermore, we did not find significant triangle subgraphs at higher hierarchical levels and hypothesize that in a general estimation of only directional connections the role of the possibly different connection pathways is hidden. In the same way, in the anatomical connectivity a functional characterization of connections is lacking, and thus important information about the system is lost. It is worth mentioning that a comparison between anatomical and functional connectivity in a multiplex approach has been only recently proposed⁶².

4.3.2 Cerebral circuits

On the basis of our results, the following general considerations concerning a possible cerebral circuit can be drawn:

- the ActS rule can play a major role in the intra-module cortico-cortical connections and in carrying perturbing stimuli both external (vision) and internal (limbic / DMN areas);
- the fronto-parietal network and the DMN show a reciprocal, but non-overlapping, deactivation dynamics using the ActO rule;
- sub-cortical regions (mainly basal ganglia) are connected with large cortical networks using the turn off rules (TfS and TfO) and appear as possible intermediate stations of brain cortex modulation.

A schematic overview describing this circuit is reported in Fig. 14.

The analysis of the brain functional multiplex network allows a comprehensive comparison with previous works.

As for the ActS rule, the DMN/limbic and vision related areas are the main outward and inward nodes. At the same time, the modularity analysis shows a significant subdivision of the network into more connected modules. These results point to a possible recursive nature of that rule.

In line with analysis of single links (Suppl. Mat. 6), the ActS rule shows: 1) a connection to the same contralateral brain region mutual and non-mutual; 2) mutual bidirectional and non-mutual connections with non-homologous contralateral brain regions. Although some bidirectional connections were detected, the reciprocity analysis did not find any

significant reciprocal connection. However, the ActS remains the only rule having a small trend in this direction, to be possibly ascribed to the cohesive nature of the rule. The non-homologous brain regions fall mainly in the same functional or structural network, e.g.: a) DMN $\rightarrow$ frontal medial and medial orbitalis with anterior cingulum, posterior cingulum to precuneus; b) Cingulum cortex $\rightarrow$ middle to anterior cingulum; c) Limbic $\rightarrow$ hippocampus to parahippocampus; d) Vision $\rightarrow$ calcarine and lingual, middle occipital gyrus to fusiform. Accordingly, the modularity analysis shows three main clusters: vision, DMN/limbic and sparse regions of TPN. A last consideration concerns the possible role of the ActS rule as source of internal/external perturbation. If the experimental design deals with the resting state, it should not be surprising that the main ActS nodes fall in that state. As a matter of fact, subjects are open-eyes and free to perceive visual stimuli. Moreover, since they are not engaged in any task, other main perturbations may arise from internal stimuli. The main nodes fall, in fact, in the processing visual perception, as well as in the limbic and DMN brain networks, commonly activated in the rest condition⁶³.

It is quite interesting that the ActO rule appears associated to the pre-frontal brain regions which, according to previous evidence, are the main hubs of an anticorrelated network¹¹. These regions, have been recently associated to a causal interaction suppressing the DMN activity⁶⁴ while previous studies identified, by a DCM approach¹⁵, the dorsal attention network (superior parietal lobe, FEF and inferior frontal gyrus) and the salience network (anterior cingulate, anterior insula and prefrontal cortex) as inhibitory nodes on the DMN, but not vice versa. In such a context, we found that the same prefrontal and parietal regions appear as a main inward/outward station, endowed with both active and passive roles.

Studying in deep the single connections, in the TPN/DMN competitive behaviour a large amount of connections arise from the DMN hubs (frontal medial orbitalis and cingulate posterior) and involve a number of fronto-parietal brain regions (frontal inferior, SMA, insula, parietal inferior and supramarginal), while the lateral frontal cortex (frontal middle and inferior) deactivates the medial part of the same lobe. Similar results are produced by studies of causal connectivity estimated by Granger Causality⁵. Although, in general, different results may be due to different causal estimation methods, the Granger Causality, a data-driven approach based upon time-delay signals, is more in line with our method.

By our results the ActO rule is not characterized by a reciprocal connection (as shown by the multi-reciprocity analysis), indicating a low probability of bi-directional connections. In this regard, we hypothesize that the mutual deactivations are expressed by different hubs of TPN and DMN connecting different non-hub regions, in terms of crossing but not overlapping structures. This architecture would be in line with the model proposed by the network analysis of the (static) negative brain functional connectivity¹¹.

Finally, taking into consideration both turn off rules (TfS and TfO), the basal ganglia circuit appears as the most involved in the modulatory interactions above. According to structural and functional evidence about basal ganglia in human and non-human primates^{65,66}, the main cortico-striatal connections are located in the prefrontal, motor and limbic cortical areas. In our results the putamen is modulated by the motor related areas (SMA, pre-central, rolandic operculum) using the TfS rules, and by the limbic (rectus) and the DMN (cingulum posterior, precuneus, angular gyrus) using the TfO. As for the caudate, a high number of connections point to the medial/orbital frontal regions of the DMN (anterior cingulate, the frontal superior medial and medial orbitalis gyrus) and the limbic network (parahippocampus, the olfactory cortex and the rectus) only with the TfS rule.

It is worth remembering that the basal ganglia are topographically differentiated on the basis of their cortical projections in the following way⁶⁷: the most rostral and ventral part are connected to the medial and orbital frontal regions; the central and dorsolateral parts of putamen take connection with the primary and supplementary motor cortices; the dorsal caudate is connected to the dorsal prefrontal and parietal regions, while the posterior part of the caudate with the posterior lobe (including temporal, parietal and occipital cortex). Recent functional studies in humans confirmed these structural evidences using fMRI data⁶⁸.

In our work we could not distinguish the anatomical subdivision of putamen and caudate and check the precise anatomical location of the connections. However, the cortical topographic differences resulting from our data were obtained by measuring different kinds of connections. Thus, it is not possible to exclude that cortico-striatal functional connections could depend upon different kinds of dynamical interactions. The detailed mapping of such interactions will be a quite demanding experimental and modelling endeavour.

4.5 Limitation and future prospects

The major limitations of our study concern:

- the low time resolution of fMRI data⁶⁹. Such data, in fact, have no well-defined time resolution, and spurious results can be easily obtained. Fast fMRI acquisition⁷⁰ would be a possible solution to the problem.
- the resting state paradigm: In such an experimental condition subjects are not checked for a particular request, and less robust results can be obtained. In order to overcome this point, in a further step of our study comparison between different states will be explored.
- the spatial resolution. As a matter of fact, we used a not-so-recent atlas²⁶ (AAL) to compare our results with our previous work. Taking into consideration that different brain atlas with different spatial resolution could give different results, an in-depth analysis of the causal connectivity as a function of spatial resolution is also in our research plans.

All in all, our model stems from a dataset of healthy subjects without a specific phenotypic characterization and in a resting state condition. Then, a systematic evaluation of possible different brain connectivity patterns would be precious in different experimental conditions, as well as within different phenotype subjects. Also, in a resting state condition a recent work⁷ found a possible implication of a non-stationary dynamics in effective (directional) connectivity, and in such a context exploration of dynamical events by a Transfer Entropy approach remains of extreme interest.

5. CONCLUSION

We first introduced a new method to estimate effective connectivity from brain functional data and found, by a data-driven statistical method, four kinds of significant brain interactions. Then, we characterized the brain network properties at both dynamical and structural levels. Finally, the complex interaction patterns of brain regions were studied in the frame of a multiplex network analysis.

The results show some peculiar anatomical and functional properties of the brain interactions suggesting possible neurophysiological underlying mechanisms.

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Author Contributions

F.P.: designed the model and the computational framework, analyzed the data and wrote the manuscript.

A.C.: verified the analytical methods, supervised the project and helped the draft of manuscript.

All authors discussed the results and contributed to the final manuscript.

Additional Information

Compliance with Ethical Standards

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Competing Interests:

The authors declare the absence of any financial and non-financial competing interests.

Figures' captions

Figure 1 Flow chart of brain signal processing.

Brain signals of each subject were submitted, after pre-processing, to the following procedure:

- Z-score normalization of time-series;
- thresholding by four symmetrical thresholds;
- discretization of signal values in: 1, -1 and 0.

Thus, each signal is redefined as a dynamic process involving: activation (1), de-activation (-1) and null-activation (0).

Figure 2 Characterization of dyadic interactions.

Given the state value of two ROIs (A, B) at the time step (n) and the state of one of them (B) at the following time step (n+1), a causal relation from A to B can be estimated by the Transfer Entropy (TE) through the calculation of:

- $P(A_n)$, marginal frequency of A in a particular state (1, -1 or 0) at the time step n;
- $P(B_n)$ and $P(B_{n+1})$, marginal frequencies of B in a given state (1, -1 or 0) at the time step n and at the time step n+1;
- $P(A_n; B_n)$, joint frequency of A and B in a particular state at the time step n ;
- $P(B_{n+1} | B_n)$, conditional frequency of B in a particular state at the time step t=n+1 taking into account the marginal frequency $P(B_n)$;

- $P(B_{n+1} | B_n; A_n)$, conditional frequency of B in a particular state at the time step t = n+1 taking into account the joint probability $P(A_n; B_n)$;

- $\ln \frac{P(B_{n+1} | A_n; B_n)}{P(B_{n+1} | B_n)}$: a direct comparison between the causal hypothesis $P(B_{n+1} | A_n; B_n)$ and the null-hypothesis $P(B_{n+1} | B_n)$.

Taking into account the three possible states (1, -1 and 0) and the three positions (A_n , B_n and B_{n+1}) a total of 27 combination rules are possible.

Figure 3 Triadic motifs and subgraphs.

Panel A: the whole set of motifs within three nodes. Panel B: the Cycle (top) and Flux (bottom) subgraphs with the corresponding isophorms. Panel C: the three orders of Flux subgraphs in the multiplex network:

- 0th order having all links in the same layer (M^1);
- 1st order with two links shared in a same layer (M^1) and one in another layer (M^2);
- 2nd order in which each link belongs to different layers (M^1 , M^2 and M^3).

Panel D: Functional characterization of nodes and links in a Flux subgraph (Top); Models of Flux subgraphs of 1st and 2nd order used in this paper (Bottom). For more details see the text and Suppl. Mat. 4.

Figure 4 Sums of local TE in the interaction rules at increasing signal thresholds.

The sums include the individual values of the subjects in the database (see the Methods). Blue, red, yellow and magenta colours refer to 0.25, 0.50, 0.75 and 1.00 S.D. threshold values, respectively.

Top panel: rules with an increasing trend at increasing thresholds.

Bottom panel: rules with mixed trends at increasing thresholds.

Figure 5 Mean Squared Error (MSE) of local TE as a function of signal thresholds.

For the meaning of columns and colours and the sequence of rules, see figure 4. Top panel: rules with an increasing trend at increasing thresholds. Bottom panel: rules with mixed trend at increasing thresholds.

Figure 6 Post-hoc analysis of ANOVA one way.

The distribution of local TE in the 27 different rules (see the text for details) shows that the first eight rules form a cluster independent from the others.

Figure 7 Classification of functional rules.

Left panel: correlation matrix among rules (Pearson C.I. scale on the right axis). Right panel: dendrogram produced by a hierarchical cluster analysis. In the hierarchical structure symmetrical rules are clustered:

- a) in the same group in the first level: (101, -10-1), (1-10, -110) (10-1, -101), (110, -1-10);
- b) again in two groups in the second level [(101, -10-1), (1-10, -110)] ; [(10-1, -101), (110, -1-10)].

Rules grouped in the second level appear anti-correlated in the correlation analysis of the left panel).

Figure 8 Schematic overview of the multiplex network defined in this paper.

The four α -hierarchical level matrices (ActS, TfS, ActO and TfO) are in the central core; the β -hierarchical level matrices (S and O) on the left and the right side of the core, respectively, and the γ -hierarchical level matrix (T) on the bottom side. The interactions among single-layers of the vector of adjacency matrices at the α - and β -hierarchical level, are indicated by blue and red dashed double arrow, respectively. The weighted aggregated overlapping adjacency matrices to the higher hierarchical level are indicated by green (from the α - to β -hierarchical level) and orange (from the β - to γ -hierarchical level) arrows.

Figure 9 Significant Flux subgraphs of different order.

Top left: The Flux subgraph in the ActS layer appear different from random (Flux ActS= I: 1654.2 ± 190.6 ; p-value: $6 \cdot 10^{-4}$ but the coherence does not reach a clear significance (0.03), indicating a low stability;

Top right, middle left: Flux subgraphs within ActS and ActO interacting layers appear quite robust, as well as ActS and TfO. ActS and TfS Flux shows a significant I but not C.

Bottom left and right: As for the 2th order subgraph in the α -hierarchical level, most of the Flux combinations show a significant Intensity (I). However, only two reach significance for Coherence (C): Flux ActO/TfS/ActS and Flux TfO/TfS/ActS. See the text for further comments.

Figure 10 Central nodes in the α -hierarchical level of the multiplex network.

The coloured circles are associated to the rules at the α -hierarchical level: red = ActS; orange = TfS; blue = ActO; green = TfO, and in each circle the brain regions acting as local central nodes are reported. The intermediate multiplex central nodes are in the intersections. In bold are the outward nodes.

Notice that: a) the basal ganglia regions are shared by the TfS and TfO rules; b) the fronto-parietal cortex falls within the ActO and TfO rules; c) a sparse number of brain regions covering the middle cingulum, the parahippocampus and the superior temporal gyrus, are found in the ActS and TfS rules.

Figure 11 Central nodes in the β - and γ -hierarchical level of the multiplex network.

The coloured circles are associated to the rules as follows: orange = S (β); violet = O (β); black = T (γ), and in each circle the brain regions acting as local central nodes are reported. In the intersections are the brain regions acting as intermediate multiplex central nodes. The asterisks indicate overlapping brain regions between the intermediate (β) and the highest (γ) hierarchical level. In bold are the outward nodes.

Notice that: a) only in the black circle are localized brain regions that do not appear in the intermediate hierarchical level; b) overlapping brain regions of the α -level disappear in the β -level.

Figure 12 Dynamics of 1st order subgraphs.

Source, middle and sink nodes are in red, blue and green colour, respectively. From the source node two pathways can be characterized: an indirect pathway (i.p.) passing through the middle node, and a direct pathway (d.p.) going directly to the sink node. The time-dependent changes of the nodes state are reported for both indirect (i.p.) and direct pathways (d.p.). The triads in the left and right sections are not isophorms. For the difference between top and bottom panels see the text. Links can work in a synchronous or sequential way depending upon the relative state of the interacting nodes.

Figure 13 Dynamics of 2nd order subgraphs.

Source, middle and sink nodes are in red, blue and green colour, respectively.

- Panel A: on the sink node the ActS rule applies on the direct pathway, and the TfS on the indirect pathway. The middle node is modulated by an opposite rule: ActO and TfO for the ActO/TfS/ActS and the TfO/TfS/ActS triads, respectively.

- Panel B: the ActS/TfO/ActS and ActS/ActO/TfO, are symmetrical Flux triads in which the same mechanism operates: indirect pathway (de-)activates the sink node in the opposite state than the source, while the direct pathway turn off the sink node if in the same state of the source. The same occurs, although with inverted pathways, for the Flux ActS/TfO/ActO, in which the indirect way turns off the sink node if in the same state of the source, while the direct way changes its state to the opposite than the source.

- Panel C: the Flux TfO/ActS/ActO operates as a competition solving mechanism: since the sink node is subjected to the competing rules ActS and ActO, the source to middle pathway solves the impasse turning off the ActS rule and promoting the opposite state. It is worth to note that also in this case the symmetrical form promoting the ActS rules is not significant.

Figure 14 Minimal scheme of brain circuits.

Integrating the single nodes link information and the centrality analysis within the network modularity architecture, different patterns of brain areas are associated to a specific rule (for details see Centrality analysis). Red line = ActS; orange line = TfS; blue line = ActO; violet line = TfO.

Tables

Interaction Rules	% of subjects
$A_n(1) + B_n(0) \rightarrow B_{n+1}(1)$	100
$A_n(-1) + B_n(0) \rightarrow B_{n+1}(-1)$	100
$A_n(1) + B_n(-1) \rightarrow B_{n+1}(0)$	100
$A_n(-1) + B_n(1) \rightarrow B_{n+1}(0)$	100
$A_n(1) + B_n(0) \rightarrow B_{n+1}(-1)$	97
$A_n(-1) + B_n(0) \rightarrow B_{n+1}(1)$	97
$A_n(1) + B_n(1) \rightarrow B_{n+1}(0)$	83
$A_n(-1) + B_n(-1) \rightarrow B_{n+1}(0)$	79
$A_n(1) + B_n(1) \rightarrow B_{n+1}(1)$	0
$A_n(-1) + B_n(-1) \rightarrow B_{n+1}(-1)$	0
$A_n(1) + B_n(-1) \rightarrow B_{n+1}(-1)$	0
$A_n(-1) + B_n(1) \rightarrow B_{n+1}(1)$	0

Table 1 Distribution of significant local TE among subjects.

Fraction of 109 subjects with the same results for the different rules, $p < 0.0001$ Bonferroni corrected (157 repetitions).

Function	G1	G2
(De-) Activation	$A_n(1) + B_n(0) \rightarrow B_{n+1}(1)$ $A_n(-1) + B_n(0) \rightarrow B_{n+1}(-1)$ ActS	$A_n(1) + B_n(0) \rightarrow B_{n+1}(-1)$ $A_n(-1) + B_n(0) \rightarrow B_{n+1}(1)$ ActO
Turn off	$A_n(1) + B_n(-1) \rightarrow B_{n+1}(0)$ $A_n(-1) + B_n(1) \rightarrow B_{n+1}(0)$ TfS	$A_n(1) + B_n(1) \rightarrow B_{n+1}(0)$ $A_n(-1) + B_n(-1) \rightarrow B_{n+1}(0)$ TfO

Table 2 Proposed functional classification of the rules.

The significant rules show a specific nested structure and possible functional role as defined by the four relations: (de-)activation, turn off, same state and opposite state. The two-by-two combination of these features (see the text) point to a functional process described by an opposite interaction matrix.

Hierarchical level	Layer	Weight	Connection Density
α	ActS	56.1 ± 3.5	0.64 ± 0.02
α	TfS	26 ± 1.1	0.57 ± 0.02
α	ActO	40.5 ± 2.8	0.56 ± 0.02
α	TfO	25.9 ± 1.2	0.55 ± 0.01
β	S	82.2 ± 3.6	0.83 ± 0.01
β	O	66.4 ± 3.2	0.76 ± 0.01
γ	T	148.6 ± 4.4	0.99 ± 0.002

Table 3 Descriptive statistics of layers in a multiplex network.

The Interaction Rules refer to the layers in the multiplex network represented in Fig. 8. Weights and Connection density values are given as averages $\pm$ S.D.

Hierarchical level	Layer	RI (mean $\pm$ S.D.)
α	ActS	0.01 ± 0.03
α	TfS	$-0.08 \pm 0.02^*$
α	ActO	-0.01 ± 0.03
α	TfO	$-0.08 \pm 0.02^*$
α	ActS/TfS	$-0.05 \pm 0.02^*$

α	ActO/TfO	-0.03±0.02*
α	ActS/TfO	0.08±0.02*
α	ActO/TfS	0.13±0.02*
α	ActS/ActO	-0.01±0.03
α	TfS/TfO	0.05±0.02*
β	S	-0.06±0.04*
β	O	-0.05±0.04*
β	S/O	0.09±0.03*
γ	T	0.03±0.02*

Table 4 Reciprocity Index (RI) in the Multiplex network.

Asterisks indicate significant values (p-value<0.05, Bonferroni corrected).

Hierarchical level	Order	Subgraph	Intensity (I)		Coherence (C)
			mean±S.D.	p-value	p-value
α	1 st	Cycle ActS/TfO/ActS	1094.7±80.0	0.030	n.s.
α	1 st	Flux ActO/ActO/ActS	1012.0±83.6	4*10 ⁻⁶ *	0.001*
α	1 st	Flux ActO/ActO/TfS	726.4±64.2	0.01	0.05
α	1 st	Flux ActO/ActS/ActO	1077.4±89.7	3*10 ⁻⁷ *	5*10 ⁻⁴ *
α	1 st	Flux ActS/ActO/ActO	1082.6±89.5	3*10 ⁻¹³ *	5*10 ⁻⁵ *
α	1 st	Flux ActS/ActS/TfS	1136.6±90.6	0.03	n.s.
α	1 st	Flux ActS/TfO/TfO	806.4±50.4	8*10 ⁻¹¹ *	2*10 ⁻⁴ *
α	1 st	Flux ActS/TfS/TfS	855.3±58.0	1*10 ⁻⁴ *	0.04
α	1 st	Flux TfO/ActS/TfO	795.0±50.3	0.02	0.02
α	1 st	Flux TfS/ActS/TfS	838.3±57.3	0.03	n.s.
α	1 st	Flux TfS/TfS/ActO	754.0±60.2	0.01	n.s.

α	2 nd	Cycle ActO/TfS/ActS	918.3±44.0	0.0006*	n.s.
α	2 nd	Cycle ActS/TfO/TfS	797.5±44.3	0.02*	n.s.
α	2 nd	Cycle ActS/TfS/ActO	918.3±41.8	0.0003*	n.s.
α	2 nd	Cycle TfS/TfO/ActS	798.0±43.2	0.02	n.s.
α	2 nd	Flux ActO/ActS/TfO	902.7±46.2	1*10 ⁻⁵ *	0.005
α	2 nd	Flux ActO/TfS/ActS	889.6±43.4	4*10 ⁻⁶ *	0.002*
α	2 nd	Flux ActS/ActO/TfO	893.7±47.2	2*10 ⁻⁸ *	0.003
α	2 nd	Flux ActS/TfO/ActO	886.6±46.7	1*10 ⁻⁵ *	0.02
α	2 nd	Flux TfO/ActS/ActO	896.2±44.8	0.0006*	0.01
α	2 nd	Flux TfO/TfS/ActS	779±43.5	2*10 ⁻⁵ *	0.002*

β	0 th	Flux S	3870.5±252.9	0.03	n.s.
β	0 th	Flux O	2877±129.5	0.01	n.s.
β	1 st	Cycle S/O/S	3330.2±117.4	0.02	n.s.

Table 5 Intensity and Coherence values of Subgraphs at different hierarchical levels.

Asterisks on the p-values indicate significance after Bonferroni correction - see the text for details.

Hierarchical level	Rule	Q
α	ActS	0.11*
α	TfS	0.004
α	ActO	0.007
α	TfO	1*10 ⁻¹⁶
β	S	0.07*
β	O	0.003
γ	T	0.004

Table 6. Q index comparison between static and dynamic modularity. Only ActS and S rules show a significant increment of Q values (p<0.001) when compared to the random model.

Module 1 – visual	Module 2 – DMN/Limbic	Module 3 – TPN & Other
V1	F1	PRE
Q	F1O	F2O
LING	OC	F3OP
O1	F1M	F3T
O2	F1MO	F3O
O3	GR	RO
FUSI	ACIN	SMA
	PCIN	IN
	HIP	MCIN
	PHIP	AMYG
	AG	POST
	PQ	P1
	T2	P2
	T2P	SMG
	T3	PCL
	CAU (L)	PUT
		PAL
		THA
		HES
		T1
		T1P
		CAU (R)

Table 7 Modules of ActS rule.

The network includes three main clusters: the visual cortex; the limbic/DMN; the sparse brain regions belonging to the TPN. Unless explicitly indicated, all modules refer to both (L/R) hemispheres.

Figures

Figure 1

Flow chart of brain signal processing. Brain signals of each subject were submitted, after pre-processing, to the following procedure: - Z-score normalization of time-series; - thresholding by four symmetrical thresholds; - discretization of signal values in: 1, -1 and 0. Thus, each signal is redefined as a dynamic process involving: activation (1), de-activation (-1) and null-activation (0).

Figure 2

Characterization of dyadic interactions. Given the state value of two ROIs (A , B) at the time step (n) and the state of one of them (B) at the following time step (n+1), a causal relation from A to B can be estimated by the Transfer Entropy (TE) through the calculation of: - $P(A_n)$, marginal frequency of A in a particular state (1, -1 or 0) at the time step n; - $P(B_n)$ and $P(B_{n+1})$, marginal frequencies of B in a given state (1, -1 or 0) at the time step n and at the time step n+1; - $P(A_n; B_n)$, joint frequency of A and B in a particular state at the time step n ; - $P(B_{n+1} | B_n)$, conditional frequency of B in a particular state at the time step t=n+1 taking into account the marginal frequency $P(B_n)$; - $P(B_{n+1} | B_n; A_n)$, conditional frequency of B in a particular state at the time step t = n+1 taking into account the joint probability $P(A_n; B_n)$; - $\ln(P(B_{n+1} | A_n; B_n) / (P(B_{n+1} | B_n)))$: a direct comparison between the causal hypothesis $P(B_{n+1} | A_n; B_n)$ and the null-hypothesis $P(B_{n+1} | B_n)$. Taking into account the three possible states (1, -1 and 0) and the three positions (A_n , B_n and B_{n+1}) a total of 27 combination rules are possible.

Figure 3

Triadic motifs and subgraphs. Panel A: the whole set of motifs within three nodes. Panel B: the Cycle (top) and Flux (bottom) subgraphs with the corresponding isophorms. Panel C: the three orders of Flux subgraphs in the multiplex network: - 0th order having all links in the same layer (M1); - 1st order with two links shared in a same layer (M1) and one in another layer (M2); - 2nd order in which each link belongs to different layers (M1, M2 and M3). Panel D: Functional characterization of nodes and links in a Flux subgraph (Top); Models of Flux subgraphs of 1st and 2nd order used in this paper (Bottom). For more details see the text and Suppl. Mat. 4.

Figure 4

Sums of local TE in the interaction rules at increasing signal thresholds. The sums include the individual values of the subjects in the database (see the Methods). Blue, red, yellow and magenta colours refer to 0.25, 0.50, 0.75 and 1.00 S.D. threshold values, respectively. Top panel: rules with an increasing trend at increasing thresholds. Bottom panel: rules with mixed trends at increasing thresholds.

Figure 5

Mean Squared Error (MSE) of local TE as a function of signal thresholds. For the meaning of columns and colours and the sequence of rules, see figure 4. Top panel: rules with an increasing trend at increasing thresholds. Bottom panel: rules with mixed trend at increasing thresholds.

Figure 6

Post-hoc analysis of ANOVA one way. The distribution of local TE in the 27 different rules (see the text for details) shows that the first eight rules form a cluster independent from the others.

Figure 7

Classification of functional rules. Left panel: correlation matrix among rules (Pearson C.I. scale on the right axis). Right panel: dendrogram produced by a hierarchical cluster analysis. In the hierarchical structure symmetrical rules are clustered: - a) in the same group in the first level: (101, -10-1), (1-10, -110) (10-1, -101), (110, -1-10); - b) again in two groups in the second level [(101, -10-1), (1-10, -110)] ; [(10-1, -101), (110, -1-10)]. Rules grouped in the second level appear anti-correlated in the correlation analysis of the left panel).

Figure 8

Schematic overview of the multiplex network defined in this paper. The four α -hierarchical level matrices (ActS, TfS, ActO and TfO) are in the central core; the β -hierarchical level matrices (S and O) on the left and the right side of the core, respectively, and the γ -hierarchical level matrix (T) on the bottom side. The interactions among single-layers of the vector of adjacency matrices at the α - and β -hierarchical level, are indicated by blue and red dashed double arrow, respectively. The weighted aggregated overlapping adjacency matrices to the higher hierarchical level are indicated by green (from the α - to β -hierarchical level) and orange (from the β - to γ -hierarchical level) arrows.

Figure 9

Significant Flux subgraphs of different order. Top left: The Flux subgraph in the ActS layer appear different from random (Flux ActS= I: 1654.2±190.6; p-value: 6*10⁻⁴ but the coherence does not reach a clear significance (0.03), indicating a low stability; Top right, middle left: Flux subgraphs within ActS and ActO interacting layers appear quite robust, as well as ActS and TfO. ActS and TfS Flux shows a significant I but not C. Bottom left and right: As for the 2th order subgraph in the α-hierarchical level, most of the Flux combinations show a significant Intensity (I). However, only two reach significance for Coherence (C): Flux ActO/TfS/ActS and Flux TfO/TfS/ActS. See the text for further comments.

Figure 10

Central nodes in the α -hierarchical level of the multiplex network. The coloured circles are associated to the rules at the α -hierarchical level: red = ActS; orange = TfS; blue = ActO; green = TfO, and in each circle the brain regions acting as local central nodes are reported. The intermediate multiplex central nodes are in the intersections. In bold are the outward nodes. Notice that: a) the basal ganglia regions are shared by the TfS and TfO rules; b) the fronto-parietal cortex falls within the ActS and TfO rules; c) a sparse number of brain regions covering the middle cingulum, the parahippocampus and the superior temporal gyrus, are found in the ActS and TfS rules.

Figure 11

Central nodes in the β - and γ -hierarchical level of the multiplex network. The coloured circles are associated to the rules as follows: orange = S (β) ; violet = O (β) ; black = T (γ), and in each circle the brain regions acting as local central nodes are reported. In the intersections are the brain regions acting as intermediate multiplex central nodes. The asterisks indicate overlapping brain regions between the intermediate (β) and the highest (γ) hierarchical level. In bold are the outward nodes. Notice that: a) only in the black circle are localized brain regions that do not appear in the intermediate hierarchical level; b) overlapping brain regions of the α -level disappear in the β - level.

Figure 12

Dynamics of 1st order subgraphs. Source, middle and sink nodes are in red, blue and green colour, respectively. From the source node two pathways can be characterized: an indirect pathway (i.p.) passing through the middle node, and a direct pathway (d.p.) going directly to the sink node. The time-dependent changes of the nodes state are reported for both indirect (i.p.) and direct pathways (d.p.). The triads in the left and right sections are not isophorms. For the difference between top and bottom panels see the text. Links can work in a synchronous or sequential way depending upon the relative state of the interacting nodes.

Figure 13

Dynamics of 2nd order subgraphs. Source, middle and sink nodes are in red, blue and green colour, respectively. - Panel A: on the sink node the ActS rule applies on the direct pathway, and the TfS on the indirect pathway. The middle node is modulated by an opposite rule: ActO and TfO for the ActO/TfS/ActS and the TfO/TfS/ActS triads, respectively. - Panel B: the ActS/TfO/ActS and Acts/ActO/TfO, are symmetrical Flux triads in which the same mechanism operates: indirect pathway (de-)activates the sink node in the opposite state than the source, while the direct pathway turn off the sink node if in the same state of the source. The same occurs, although with inverted pathways, for the Flux ActS/TfO/ActO, in which the indirect way turns off the sink node if in the same state of the source, while the direct way changes its state to the opposite than the source. - Panel C: the Flux TfO/ActS/ActO operates as a competition solving mechanism: since the sink node is subjected to the competing rules ActS and ActO, the source to middle pathway solves the impasse turning off the ActS rule and promoting the opposite state. It is worth to note that also in this case the symmetrical form promoting the ActS rules is not significant.

Figure 14

Minimal scheme of brain circuits.

Supplementary Files

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