

## **Integrating TMS, EEG and MRI as an approach for studying brain connectivity**

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## **Abstract**

The human brain is a complex network in which hundreds of brain regions are interconnected via thousands of axonal pathways. The capability of such a complex system emerges from specific interactions among smaller entities, a set of events that can be described by the activation of interconnections between brain areas. Studies that focus on brain connectivity have the aim of understanding and modelling brain function, taking into account the spatio-temporal dynamics of neural communication between brain regions. The majority of current knowledge regarding brain connectivity has been obtained from stand-alone neuroimaging methods. Nevertheless, the use of a multi-modal approach seems to be a powerful way to investigate effective brain connectivity, overcoming the limitations of unimodal approaches. In this review, we will present the advantages of an integrative approach in which transcranial magnetic stimulation-electroencephalography coregistration is combined with magnetic resonance imaging methods to explore effective neural interactions. Moreover, we will describe possible implementations of the integrative approach in open- and closed-loop frameworks where real-time brain activity becomes a contributor to the study of cognitive brain networks.

## **Brain networks and how to study their organization**

In recent years, the concept of networks (Sporns, 2011) has been adopted to define several complex systems in almost all fields, such as economics, politics and biology. In neuroscience, the term “network” implies several system properties that accurately characterize the complexity of brain connectivity; these properties include highly structured connectivity patterns, multiscale organization and non-linear dynamics. On a large scale, the brain’s composite “wiring diagram forms a network of hundreds of brain regions and thousands of white matter axonal pathways interconnecting those regions” (from van den Heuvel & Sporns, 2011) (see Sporns, 2011, 2013). Brain function emerges from the activation of these pathways, which can be dynamically reconfigured according to contingent demands. Such flexibility underlies the brain’s ability to sustain cognitive functions and to adapt and adjust to changing environments (Bassett and Sporns, 2017).

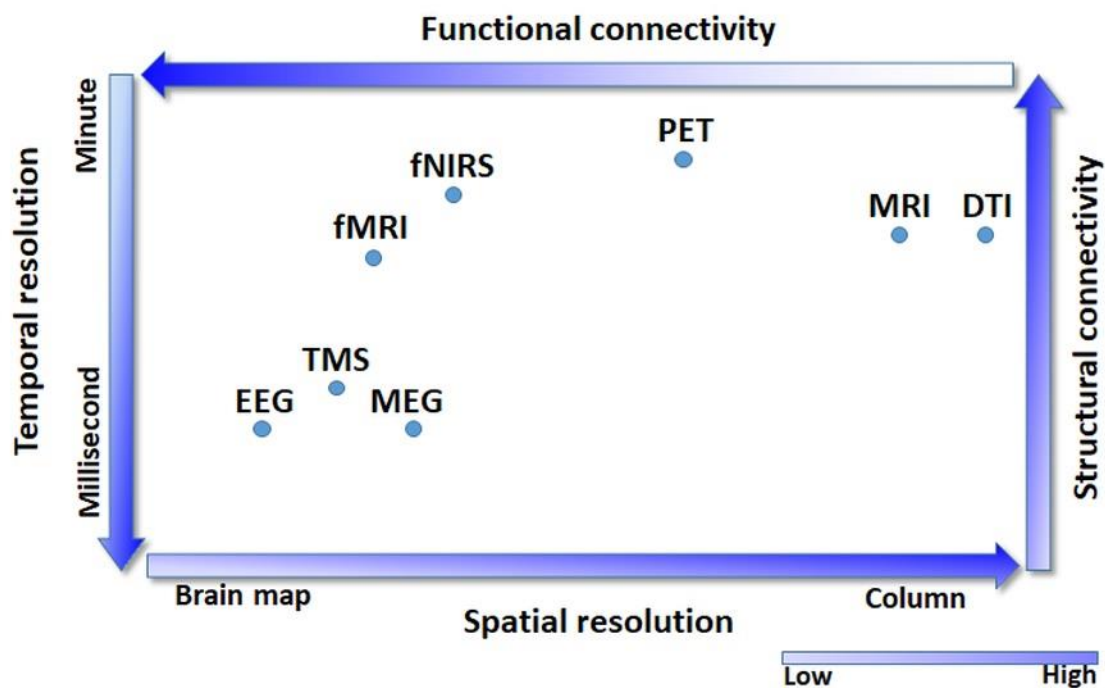
Neuroscience researchers have expressed great interest in exploring the dynamics of brain network connectivity. The field of brain connectivity can be referred to as “connectomics”, an area of research that aims to provide comprehensive maps of all neural connections within the nervous system. These neural maps incorporate several levels and include the following: structural descriptions of connectivity, i.e., structural elements and connections forming the human brain (Lang, Tomé, Keck, Górriz-Sáez, and Puntinet, 2012); functional descriptions of connectivity, i.e., the statistical correlations between distinct brain regions in terms of information processing (Friston, Frith, Liddle, and Frackowiak, 1993); and effective descriptions of connectivity, i.e., the description of the causal influences that given neural units exert over other neural units (see Box 1). As a general example, brain connectivity may be compared to the organization of a city: neighbourhoods, which represent regions, are connected by streets, which represent structural connectivity. Such architecture influences how people move around the city and intermingle with each other, representing functional connectivity. Notably, the directionality of human interactions in the city can also be described, as some people influence the behaviour of other people, representing the effective connectivity among elements of the network. This analogy highlights that the relationship between structure and function is dynamic because it is arranged based on contingent demands, which can be represented by a person acting toward goals in a changing environment (see Friston, Frith, Liddle, and Frackowiak, 1993; Sporns and Betzel, 2016).

Therefore, active connections are determined by many factors, such as the type of information, the amount of processing required, the state of the subject, any previous experience, and the complex relations among these elements. Thus, understanding how dynamical neuronal patterns give rise to human brain functions is one of the most intriguing and prevalent questions in neuroscience. Nonetheless, at present, researchers have not achieved a complete understanding of how the intricate structural architecture of the brain sustains functional brain dynamics.

In recent studies, brain connectivity has been investigated mainly by means of stand-alone neuroimaging methods. The principal instruments of investigation are structural magnetic resonance imaging (MRI), functional MRI, diffusion tensor imaging (DTI), computational tractography, positron emission tomography, functional near-infrared spectroscopy, transcranial magnetic stimulation (TMS), electroencephalography (EEG), and magnetoencephalography. Most of these neuroimaging methods address functional and effective connectivity, while MRI and DTI address structural connectivity (see Figure 1). However, each individual neuroimaging technique has both strengths and weaknesses. One possibility for addressing these weaknesses is to combine techniques, such that it becomes feasible to merge information and overcome some of the limitations of individual techniques.

Here, we outline what we consider to be a promising approach for studying connectomics. This approach takes advantage of the integration of TMS, EEG and MRI and imitates the strategy of the nervous system, relying single-element interactions to produce a complex behaviour. We believe that TMS and EEG steered by MRI information might incorporate the complexity of the phenomenon into a whole if used in a method-comprehensive context. First, we will briefly describe the state of art of the TMS-EEG coregistration approach (Bortoletto, Veniero, Thut, and Miniussi, 2015; Daskalakis, Farzan, Radhu, and Fitzgerald, 2012; Ilmoniemi and Kičić, 2010; Miniussi and Thut, 2010; Tremblay and others, 2019) and then emphasize the opportunity offered by the integration of TMS-EEG with MRI. Last, we will contemplate future scenarios in which TMS-EEG (or based on the integration type: EEG-TMS) and online measures of activity could be used in an open-/closed-loop approach to explore and modify neural activity in the brain. This integrative approach holds remarkable promise as a probe to elucidate basic mechanisms in both the normal and pathological brain.

Thus, it opens new opportunities for integrative neuroscience to be used for diagnostic and therapeutic purposes.



**Figure 1.** Connectivity can be represented based on the resolution that a given method occupies in this space. This figure shows the spatial and temporal resolution of the principal neuroimaging methods used to study brain connectivity. However, it is not merely the spatial and temporal selectivity that make neuroimaging a useful experimental approach; it is the ability of each single method to also define functional or structural connectivity. An ideal approach would integrate some of these techniques, covering a larger area of the figure space. fNIRS, functional near-infrared spectroscopy; PET, positron emission tomography; fMRI, functional magnetic resonance imaging; MRI, magnetic resonance imaging; DTI, diffusion tensor imaging; EEG, electroencephalography; TMS, transcranial magnetic stimulation; MEG, magnetoencephalography.

### Features of TMS-EEG integration

In the last two decades, the combination of single-pulse TMS and EEG, i.e., TMS-EEG, has been proposed as an ideal tool to investigate cortical excitability and effective connectivity in normal (Rogasch and Fitzgerald, 2013; Siebner and others, 2009; Thut and Pascual-Leone, 2010) and pathological brains (e.g., Bagattini and others, 2019; Darmani and Ziemann, 2019;

Massimini, Ferrarelli, Sarasso, and Tononi, 2012; Ragazzoni and others, 2017; Sarasso and others, 2014; Trevizol and Blumberger, 2019) (see Box 2). Using EEG to track the activity induced by TMS, which propagates directly or indirectly to anatomically and functionally connected regions (Bonato, Miniussi, and Rossini, 2006; Ilmoniemi and others, 1997; Rogasch, Thomson, Daskalakis, and Fitzgerald, 2013; Voineskos and others, 2010), it is possible to investigate communication across networks at rest and during execution of cognitive tasks (Bortoletto, Veniero, Thut, and Miniussi, 2015). The spreading of activity that reaches connected areas, including those that are spatially distant (Komssi and others, 2002), depends on the underlying intra- and inter-hemispheric structural pathways and on the parameters of the induced electric field. When the induced electric field is stronger, the TMS-induced spreading is also more robust (Nieminen, Koponen, and Ilmoniemi, 2015). Given that the strength of the effect of TMS depends on the coil geometry and stimulation parameters, such as the position and orientation of the coil, which affects the depolarization of the neurons (Casarotto and others, 2010; Komssi, Kähkönen, and Ilmoniemi, 2004), spreading can be used as a dependent variable.

The cortical response induced by the TMS can be evaluated as an evoked response that provides information on phase-locked oscillations to the TMS pulse (TMS-evoked potentials – TEPs) or as a total oscillatory response (also called event-related spectral perturbation), which captures both the phase-locked and non-phase-locked oscillations following TMS pulse (Pellicciari, Veniero, and Miniussi, 2017). The former provides measures of effective connectivity with high temporal resolution by analysing the amplitude and latencies of TEPs across the scalp or of the global field power (Hill, Rogasch, Fitzgerald, and Hoy, 2016, but see Conde and others 2019). The latter allows us to explore the impact of TMS in the frequency domain, which provides an opportunity to examine the functional specificity of brain rhythms in cognition (Thut and Miniussi, 2009). Although most studies have measured TMS-EEG after motor cortex stimulation, it is possible to observe the cortical response to TMS in other cortical areas (Bagattini and others, 2019; Rosanova and others, 2009). In all cases, an appropriate control condition should be employed when running connectivity protocols because TMS-EEG might also produce peripheral stimulation that can result in confounding cortical activation (Conde and others 2019).

Importantly, accumulated evidence has demonstrated that cortical responses recorded in different cortical areas with TMS-EEG have a high level of reproducibility and importantly can be used as measure sensitive to longitudinal changes (Casarotto and others, 2010; Farzan and others, 2010; Kerwin, Keller, Wu, Narayan, and Etkin, 2018; Lioumis, Kičić, Savolainen, Mäkelä, and Kähkönen, 2009). For instance, Casarotto and others (2010) evaluated the similarities/differences between pairs of TEPs recorded in the same/different stimulation conditions through a single-subject comparison. The obtained index (Divergence Index) was able to detect whether a change in the perturbation parameters occurred or not, proving that the obtained measures are sensitive to evaluating longitudinal changes.

Consequently, TMS-EEG measures may provide potential biomarkers in neurological (Koch and others, 2018; Koch, Martorana, and Caltagirone, 2019) and psychiatric diseases. Koch and others, 2018, have provided evidence of informative TMS-EEG measurements concerning the evaluation of Alzheimer's disease patients before and after a treatment protocol. The TMS-EEG signal indexed an increase in neural activity of the parietal cortex, measured as a change in amplitude of the global mean field power peaks and augmentation of brain oscillations in the beta band. It has also been possible to evaluate changes in the functional connections between the parietal cortex and medial frontal areas within the default mode network. Moreover, a recent review (Hui, Tremblay, and Daskalakis, 2019) reports evidence of the involvement of alterations in gamma oscillations in the prefrontal areas for depression and the potential of TMS-EEG to identify this as a reliable biomarker. Other studies (Colombo and others, 2019) have focused on the possibility of deriving an index from TMS-EEG, which may be helpful for supporting the diagnosis and valuation of clinical conditions (i.e., consciousness disorders).

### **Features of TMS-EEG and MRI integration**

As described in the previous section, TMS-EEG has a high temporal resolution that helps to infer effective connectivity. However, the spatial resolution is low both for localizing the target region and for estimating the sources of the TMS-induced responses. Therefore, TMS-EEG has often been integrated with MRI to improve the spatio-temporal information of brain network connectivity. The choice of proposing a methodologically integrated approach relies

on the possibility of establishing an efficient tool for exploring connectivity in a comprehensive scenario. The advantages of involving an integrated approach are several.

Many studies have integrated TMS-EEG with MRI to guide target location based on individual features. MRI information is of high importance when the target area is not the primary motor cortex, which is the only area that can be functionally localized with TMS using an objective method (i.e., motor evoked potential). In fact, using MRI to guide target location allows us to overcome the high interindividual variability of cortical areas. The spatial definition of the regions of interest by MRI is more accurate and can help provide precise constraints to TMS navigation (Ning, Makris, Camprodon, and Rathi, 2019). Ning and colleagues (2019) demonstrated a quantitative assessment of topographic precision and variability to identify cortical targets for neuromodulation. They described how several variables might impact the reliability of the targeting strategy, such as the data quality and the pre-processing. Moreover, the spatial resolution of TMS depends on several variables, such as coil geometry, coil orientation, pulse intensity, and head/brain anatomy. Individualized modelling or empirical assessments of the TMS-induced electric field may be an important additional step to maximize the efficacy of target and network modulation with TMS. Since a different architecture may correspond to differences in functional signal propagation, it would be possible through the combination of TMS-EEG and structural and functional maps to enhance the structural resolution and focus the stimulation to the interesting nodes with millimetric precision. Having the precise coordinates of the target areas derived from neuroimaging maps reveals a more accurate definition of the structural nodes and thus the opportunity to use spatial constraints for a more precise TMS-EEG connectivity evaluation.

The most common approach is to define the target based on anatomical landmarks. This approach is essential if the stimulation site is outside the primary motor, somatosensory or visual cortexes. Having the precise structural description of the target area reduced the inter- and intra-individual variability to the TMS-induced response. The anatomical landmarks derive from the MRI acquisition. The coil is positioned using the individual coordinates and monitored during the whole recording through a neuronavigation system. The anatomical landmarks used in neurophysiological measurements involved both anterior and posterior regions, i.e., prefrontal and parieto-occipital cortices (Gonzalez-Escamilla and others, 2018;



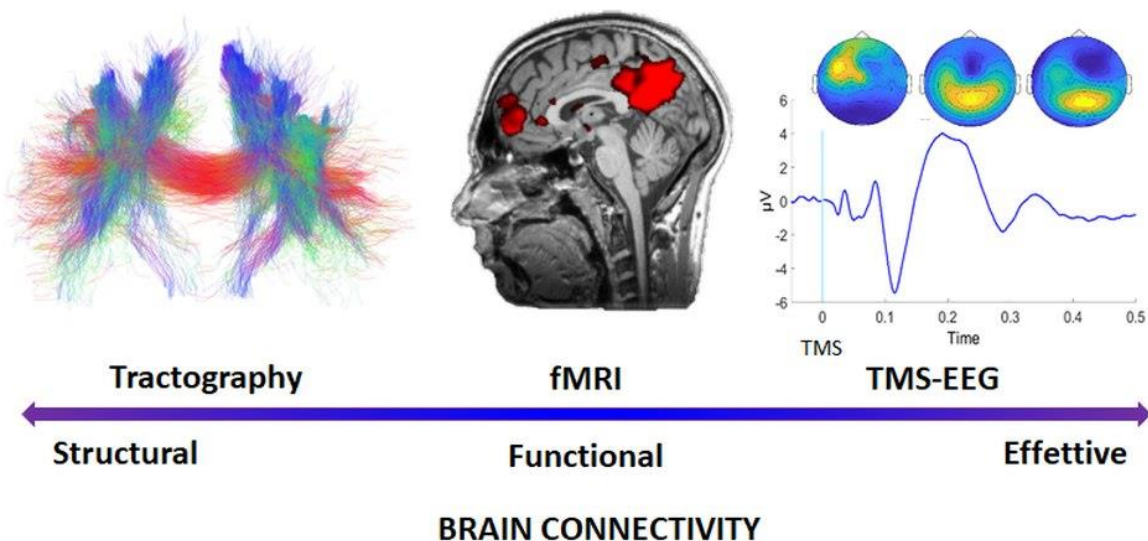
Mattavelli and others, 2019; Schauer and others, 2016; Vernet, Brem, Farzan, and Pascual-Leone, 2015).

Moreover, the target can be individuated based on cortical activity associated with a specific task. With this approach, the accuracy of individualizing the involved cortical area to a specific cognitive task is higher. The cortical coordinates, used for TMS-EEG, derive from the coregistration of the fMRI, used for detecting task-based activation, to the structural imaging of each individual. Even if the literature has identified the main (i.e., average) areas involved during any cognitive task, individuals have differences in structural and functional brain organization. In the presence of the activation maps, it is feasible to investigate such nodes with simultaneous TMS-EEG recordings, with a deeper precision in stimulating the target cortical node. Referring to the TMS-EEG literature, this approach is rare. Usually, individual anatomical landmarks are used based on previous fMRI studies (Kroczek, Gunter, Rysop, Friederici, and Hartwigsen, 2019; Pisoni, Romero Lauro, Vergallito, Maddaluno, and Bolognini, 2018). This approach is mainly used to inform stand-alone methods, such as TMS studies (Bolognini, Rossetti, Fusaro, Vallar, and Miniussi, 2014; Bolognini, Rossetti, Maravita, and Miniussi, 2011; Rocchi, Casula, Tocco, Berardelli, and Rothwell, 2016).

Interestingly, the target area can also be individuated based on MRI-based connectivity, with the possibility of investigating the relationship between structural indexes of anatomical connectivity and the temporal dynamics through TMS-EEG. The chance of implementing connectivity MRI measurements for both guiding and exploring the relation between the temporal dynamics and the underlying structural pathways represents a novel and promising approach for a better understanding of network activity (Figure 3). A great possibility for reaching this aim may correspond to integrating TMS-EEG with MRI, fMRI and DTI. The latter has great potential for describing how brain areas are connected to each other and thus to functions. Data analysis of the complex structural organization of the brain and the contributions of DTI provides quantitative information about the white matter of the brain. Through mathematical models called constrained spherical deconvolution (Tournier, Calamante, and Connelly, 2007), it is possible to estimate the distribution of fiber orientations and generate tractograms (Olivetti, Sharmin, and Avesani, 2016; Porro-Muñoz and others, 2015). They represent the structural connectome of the brain and can then be used as the underlying map to plan specific explorations of effective connectivity.

In fact, in an integrative scenario, functional regions of interest can be used to define the structural pathways underlying the functional network of interest. In this way, it would be possible to explore the functional dynamics of a target network, with its related anatomical connections. To reach this aim is crucial to extrapolate measures for both the structural and functional information and explore their relationship.

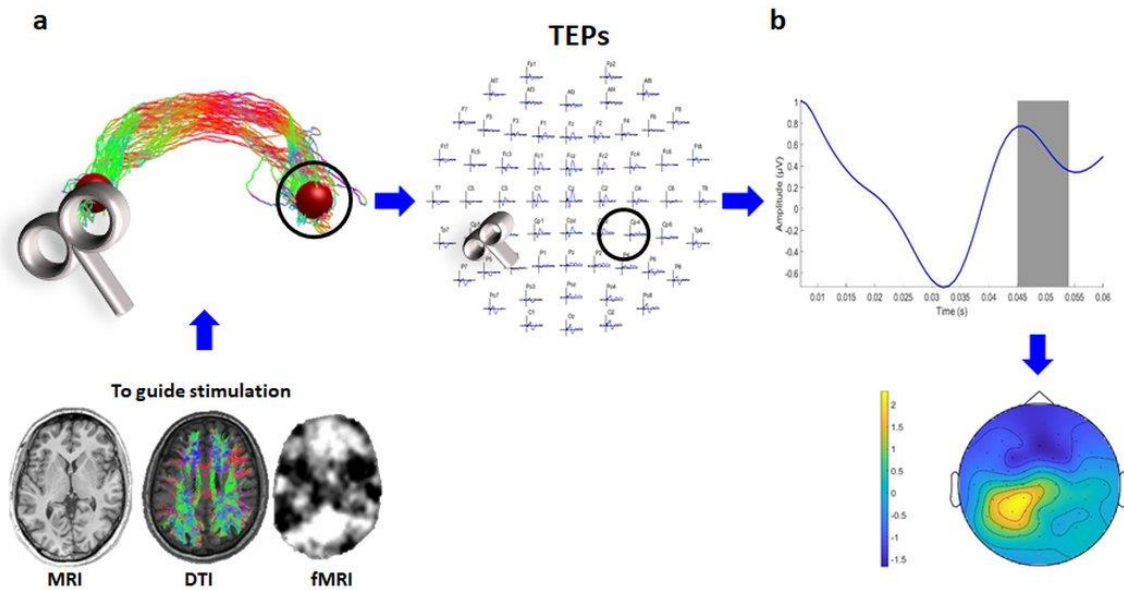
The integrated TMS-EEG-MRI approach is the most informative. However, a few technical limitations must be considered. The first challenge to face consists of finding a conjunction within all the different neuroimaging methods. The spatio-temporal characteristics, of each involved method, are different and thus lead us to focus on cortical information. Of course, this is an essential lack in the more in-depth comprehension of the dynamical integration at the basis of network processing. Another consequence of this issue is the restriction of the reconstruction of white matter pathways.



**Figure 2.** The scheme represents the three stand-alone methods [tractography (diffusion tensor imaging, DTI); functional magnetic resonance imaging, fMRI; and transcranial magnetic stimulation-electroencephalography coregistration, TMS-EEG] and the nature of the connectivity measure that can be provided by each method: structural, functional and effective, respectively. The integration of these neuroimaging methods can provide a more complete explanation of cortical connectivity if performed during the maximal information exchange between areas.

Through the proposed methodological combination of TMS-EEG and neuroimaging, it is feasible to increase the spatial constraints for a better explanation of the temporal dynamics. The high spatial resolution of MRI and tractograms can help clarify the architecture of the brain (Bullmore and Sporns, 2009). From the connection of each local neuronal community, it is possible to define a map of the brain architecture. “This means that all nodes of a large system are linked by relatively few intermediate steps. Most nodes maintain only a few direct connections, mostly within a clique of neighbours” (from Bullmore and Sporns, 2009). The resulting architectural map is the structural connection pattern of each node with other nodes. This is in line with the idea of combining structural and functional connectivity to provide constraints that inform effective connectivity (Seghier and Friston, 2013). The functionality of these nodes may be different based on their interactions and can be evaluated by TMS-EEG. Therefore, a different node rearrangement of the functional organization may correspond to different measurements. Hence, with the proposed TMS-EEG-MRI integrative approach, it will be feasible to provide strong and informed structural constraints to the exploration of neurophysiological signal propagation into the intricate brain architecture. At the same time, it will be feasible to study the prediction of the signal flow among the fibers drawn by tractography.

The topological organization of brain properties in terms of regional connectivity has been recently studied with graph theory (Bassett and Sporns, 2017; Bassett and Bullmore, 2006). Graph theory has helped clarify how human cognitive functions are linked to neuronal network structures by applying models called graphs, which describe brain connectivity. With the TMS-EEG-MRI approach, directed graphs are produced. The nodes are detectable by functional MRI, the edges are measurable by diffusion imaging, and the directions are tested by TMS-EEG.

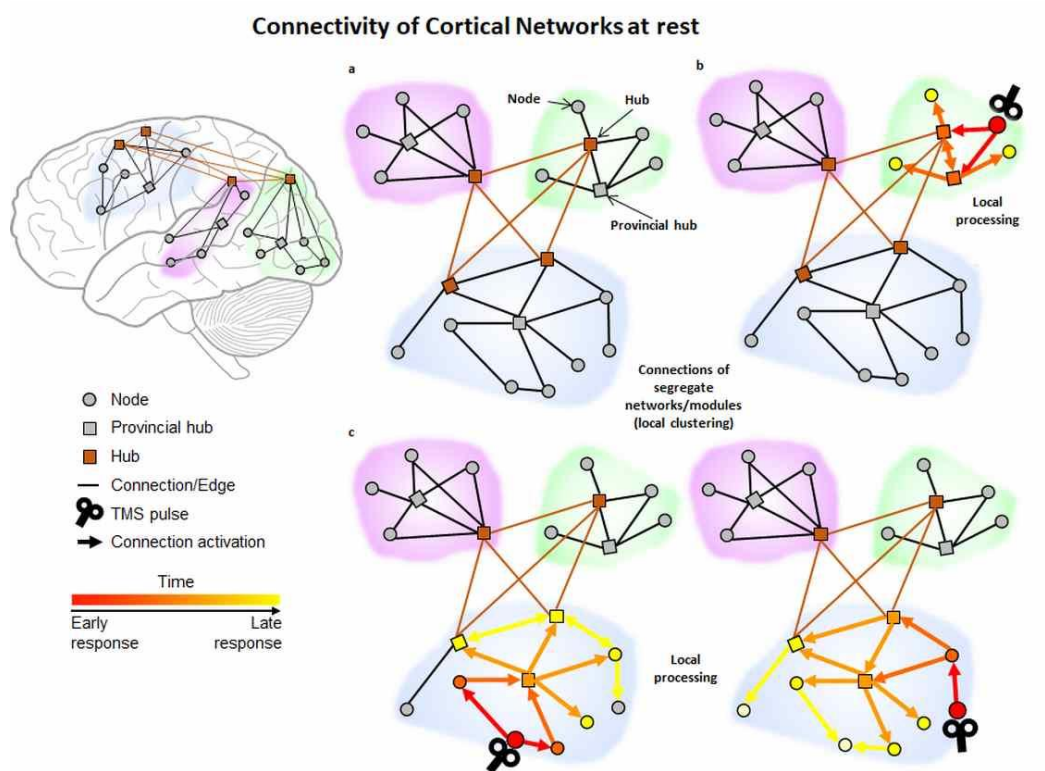


**Figure 3.** The image describes the possibility of integrating TMS-EEG and DTI with correlation analysis. a) The two cortical areas shown here (red spheres) are structurally connected by a direct path. In the proposed example, a single-pulse TMS is delivered over the left region of interest. b) The TMS-evoked potentials (TEPs) recorded in the area contralateral to the stimulated area, which is structurally connected (blue circle), are averaged, and the correlation of each time point with the size of the tractogram is evaluated. In the TEP graph, the significant component is overlaid with a grey bar; under the graph, the topographic correlation is illustrated.

### Mapping brain connectivity: network routing strategy

As we have described above, with the proposed TMS-EEG-MRI approach (or perhaps, considering the roles of the different methods, a more appropriate acronym would be MRI-TMS-EEG) it is feasible to obtain a detailed view of the *spatial* (MRI provides the structural pathways), *temporal* (EEG is able to measure the time course of the activity of the cortical areas) and *effective* (TMS gives the information about directionality) features of brain function at a macroscopic level (Figure 4). This approach increases the level of detail with which the causal links between brain architecture and dynamics can be examined, which could radically improve our understanding of brain connectivity (Avena-Koenigsberger, Misisic, and Sporns, 2017). This integrative approach may be useful in better understanding the communication of signals among nodes in complex networks. Determining how and which

spatio-temporal routes are used by the signal in each circumstance represents a feasible method for achieving deeper knowledge of the flow of information in the brain. This information flow may follow different routes (Avena-Koenigsberger and others, 2019). Some EEG signal components may follow short, direct pathways, while others may follow more complex routes, moving through more nodes of the same networks. Recently, there has been a strong interest in modelling networks (network neuroscience) to describe and predict their function. The topology of connectivity is able to shape the pattern of interaction between the elements of a system, which, in turn, regulates its global behaviour. Routing communication describes the possibility of two nodes of a network to communicate if they are joined by a path, where the length of the path is crucial for the efficacy of the communication. For example, in real neural systems, the number of synapses between systems is ideally minimized, considering that the risk of noise and the metabolic cost increase with the path length.



**Figure 4.** Integrating physiological evidence from TMS-EEG may improve network modelling to better describe signal propagation among the elements of a network. The image (from Bortoletto, Veniero, Thut, and Miniussi, 2015) “represents the modular organization of the

*brain network. (a) Nodes (grey circles), local hubs (grey squares) and rich-club hubs (red squares) are included, along with their short-range (black lines) and long-range (red lines) connections. (b) Coloured arrows represent the causal interactions between nodes and the latency of signal propagation from the TMS pulse. After TMS, the activation of the target area travels to other nodes of the same module through short-range connections. (c) When two lower-degree nodes of the same network are stimulated by TMS, the signal propagates within the same module. Different nodes (site-specific responses) are activated at first, followed eventually by the hubs connected to both initial sites (site-invariant responses)” (see Bortoletto, Veniero, Thut, and Miniussi, 2015).*

Although routing communication is efficient in describing communication for small networks, the issue becomes more delicate when we consider conditions that present a higher level of complexity. Modelling information processing among a large number of elements is challenging (Tadić, Andjelković, and Melnik, 2019). The difficulty in explaining the flow of information in the brain relies on the nature of the electrophysiological brain signal (Deslauriers-Gauthier and others, 2019). Different components of the signal may encode different information, which may follow several different routes, such that the overall information flow is spread across all the routes of each signal component. The integration of the functional dynamics is essential for explaining the mechanism that enables information to flow efficiently among different elements of a network through a complex topology (Deriche, 2016). Capitalizing on the strengths of the integrated TMS-EEG and MRI approach can help characterize the physiological basis of this information flow. Correlating spatially distributed physiological signals with structural pathways, within clear confines, has the potential to explain the relation between network dynamics and network topology. Furthermore, positive consequences may follow in the clinical domain. The proposed integrated approach will merit consideration as an instrument for sensitive quantification of previously subjective signal signatures (see Box 2).

### **EEG-informed systems: future implementations of EEG-TMS**



Thus far, we have described the advantages of implementing an integrative TMS-EEG approach for studying network activity. We will now detail the potential of using an informative EEG-TMS system, where the brain activity recorded by EEG drives stimulation in the exploration of brain dynamics. Neuroimaging methods have mainly investigated the brain in an “offline, open-loop” fashion using an a priori-defined stimulus protocol to dictate the input and its timing. Then, the outputs measured offline are used to modify protocols in subsequent experiments or to formulate theories. Although this approach has been truly productive (e.g., Romei, Gross, and Thut, 2010; Thut, Schyns, and Gross, 2011), it has not taken the brain into consideration as a fully active effector (Bergmann, 2018). In the offline, open-loop approach, the neurophysiological or behavioural responses are analysed after brain stimulation in an a posteriori procedure, which fails to take into consideration the state of the brain at the moment of the input.

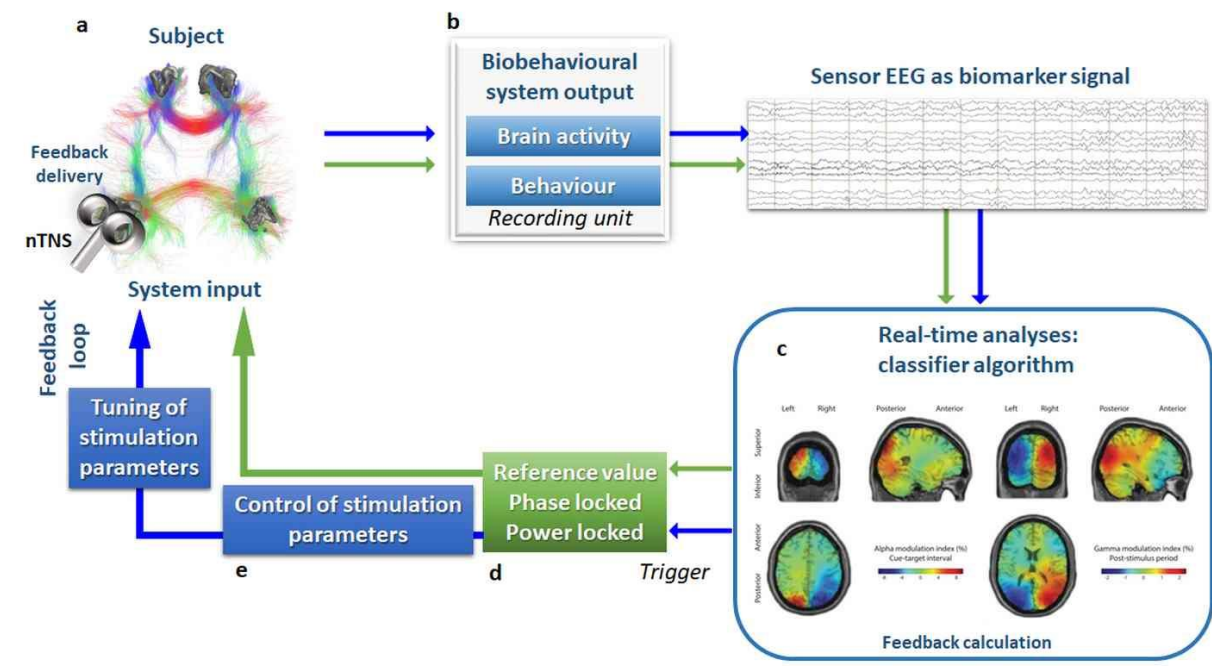
Broadly defined, a brain state can be considered the recurring set of activity of a neural population underlying a specific configuration over a defined time period and characterized by specific contingencies (Bergmann, Karabanov, Hartwigsen, Thielscher, and Siebner, 2016). Therefore, such a configuration relies on a specific neuronal population with excitatory/inhibitory circuits that can define the final output of the network. More specifically, the coordinated activity of such a neural population defines the extension of the network and characterizes the functional state. Therefore, defining the activity-dependent network configuration becomes a key element to characterize the principle of brain functioning for any given function (Zrenner, Belardinelli, Müller-Dahlhaus, and Ziemann, 2016; Zrenner, Desideri, Belardinelli, and Ziemann, 2018) underlying that the dynamic aspect is a key element to advance our understanding. The EEG-TMS guided by MRI provides new opportunities for studying the brain by designing stimulation protocols that are controlled in real time by the brain state itself via the EEG signal and thus creating an online, open-loop system (Karabanov, Thielscher, and Siebner, 2016) to test different specific configurations. The estimation of brain states may be reached in different ways, e.g., with a measure of frequency of an oscillation of interest (i.e., alpha oscillation) or with the instantaneous phase (Bergmann and others, 2012; Sauseng, Klimesch, Gerloff, and Hummel, 2009; Thut and others, 2011), although the estimation should take into consideration several aspects that may affect its accuracy. Stimulation intensity, coil geometry, coil orientation, and skull distance may

interfere in obtaining a clear cortical response. Moreover, it might be difficult to measure undisturbed brain states, given that the tool that we use to evaluate the state can contaminate the measured brain activity. Even single TMS pulses induce changes in cortical excitability; therefore, we might underestimate the dependence of a previous TMS pulse on the response modulation of a second single pulse (Pellicciari, Miniussi, Ferrari, Koch, and Bortoletto, 2016). Additionally, other technical issues are important to take into consideration, such as EEG impedance, which may affect the current density recorded, which would be distorted by the electric fields generated by TMS (Saturnino, Madsen, and Thielscher, 2019).

This approach can be developed even further when the system/network output is controlled using a closed-loop approach (Figure 5). Therefore, an evolution in testing brain functions is to use an online open- or closed-loop approach, where the brain activity informs the input or even controls the system (Bergmann, 2018; Zrenner, Belardinelli, Müller-Dahlhaus, and Ziemann, 2016).

As a standard definition, an open loop is a type of control system in which the input (here, the TMS pulse) to the brain is delivered at a predefined set point (here, a given brain state) and implies that the output (here, the brain response) has no “direct” influence (i.e., control) on the next input to the brain (i.e., influences of the input will be related only to the eventual reaching of the abovementioned set point by the brain state). Thus, we can manipulate the inputs based on the set point to be delivered at a given moment to obtain the desired/predicted effect on the output of the system. Therefore, the state of the brain is used to guide the stimulation (i.e., control signal), allowing an improvement in testing the brain response in specific conditions but without “direct” feedback to the system. The control action relates to the given TMS parameters applied in a given area identified by MRI.





**Figure 5.** Schematic of the combined EEG-TMS and MRI approach. a) After the definition of the anatomy of the system by means of tractograms derived from MRI, TMS can be navigated (nTMS) within the confines of the skull to accurately target TMS. b) The system output can be recorded by EEG (or other biobehavioural markers) and supplied to the different loops. The green arrows represent an open-loop approach, a type of control system in which the input to the brain is given at a predefined set point defined by real-time analyses and classifier algorithms (c), and the approach implies that the output has no “direct” influence on (i.e., control over) the next input to the brain. The blue arrows represent a closed-loop approach that uses feedback where a specific portion of the output signal (d) is fed back to the TMS controller (e) to induce a precise stimulation to drive a given state in the brain.

The other approach is a closed loop, which implies iteratively controlling the system state via a given signal with the additional purpose of reaching and maintaining a predefined set point. The aim is to reduce deviations from that set point by monitoring the parameter to provide feedback and adjusting the control signal (TMS) accordingly via a feedback loop.

The logic of open-/closed-loop systems can be explained using the brain-computer interface (BCI) approach, but in the EEG-TMS approach, the production of a given brain activity is driven by the EEG-TMS interaction and not by the subject. BCIs are systems that allow brain activity

(i.e., via EEG recordings) to be utilized to control external devices without using the natural motor cortico-spinal pathways (Mak and Wolpaw, 2009). A BCI uses brain activity to obtain the information that modulates the outputs and provides feedback to the subject for learning how to control the output. With EEG-TMS, the inputs (i.e., TMS pulses) to the brain are controlled by the brain state. However, the different element is that the subject is not asked to drive a device, the brain pattern is modulated by the TMS pulse, while the EEG drives features of that pulse.

Consequently, by these approaches using the EEG traces of a given brain state overlaid on MRI data, it is possible to iteratively adjust/decide TMS parameters. Examples include the timing and/or frequency, intensity, and stimulation site that will be used to test, suppress, facilitate, or even maintain that brain state with well-defined parameters by means of TMS (Bergmann, Karabanov, Hartwigsen, Thielscher, and Siebner, 2016; Thut and others, 2017). In short, we can reduce the reliance of experiments on stimulus-response statistics. Clearly, in order to develop an open-/closed-loop TMS-BCI, there must be a working understanding of the underlying neural response dependency (Panzeri, Safaai, De Feo, and Vato, 2016). With an open-loop EEG-TMS interface, we preferentially test the system in a given condition to establish how such a condition can determine the output. In a closed-loop EEG-TMS interface, the idea is that the feedback loop, via a controller, affects the system output. The former approach is ideal to study the system, and the latter approach is ideal to control it and define the consequences of a given state.

With such approaches, we are able to measure brain state values through the oscillatory activity of neuronal populations (Buzsáki and Draguhn, 2004), which occurs on various spatial and temporal scales and can be quantified by several measures, from which the most relevant in this context might be the phase and the relative frequency. In fact, fluctuations in a brain state can be described as a phase shift of a specific frequency band that defines the development of excitatory and inhibitory periods (Destexhe, Hughes, Rudolph, and Crunelli, 2007). For example, adopting EEG-TMS and MRI (Figure 5) in an open-loop design will allow us to evaluate changes in brain states through measures of neuronal synchronization, such as phase, thereby describing how a stimulated cortical area interacts with functionally and structurally connected areas. Triggering the TMS based on the phase component evoked by the previous pulse might guide the exploration of network connectivity based on the brain

states. This may be explained by the hypothesis of communication through coherence (CTC, Fries, 2005, 2015). Central to this hypothesis is that the modulation of oscillatory phase relationships among neuronal populations underlies communication. TMS pulses interact with oscillatory phases in terms of excitability, modifying the synchronization between oscillatory populations. The communication is facilitated when two oscillatory populations are aligned to their high excitability phases. Monitoring the phase time alignment of local rhythmic activity allows the temporal pattern of TMS inputs to be adjusted such that the exploration of cortical connectivity is conducted during the period of maximal information exchange.

### **Applications of open-loop and closed-loop systems**

Recent studies have focused on applying TMS to the motor cortex with an open-loop approach (Gharabaghi, Kraus, Leao, and others, 2014; Kraus and others, 2016; Meincke, Hewitt, Batsikadze, and Liebetanz, 2016; Raco, Bauer, Tharsan, and Gharabaghi, 2016; Royter and Gharabaghi, 2016; Zrenner, Belardinelli, Müller-Dahlhaus, and Ziemann, 2016; Zrenner, Desideri, Belardinelli, and Ziemann, 2018), obtaining evidence of the impact of brain states or activity on cortical excitability. Zrenner (2018) provides a detailed description of how real-time EEG can guide the exploration of corticospinal excitability through different phases of the endogenous sensorimotor mu-rhythm. The phase prediction was obtained with a fast Fourier transform. These studies opened new opportunities for adaptive TMS applications in the context of neurorehabilitation as a control system for neuromodulatory approaches (Zrenner, Belardinelli, Müller-Dahlhau, and Ziemann, 2016).

Moreover, the application of a closed-loop system has guided the controlled release of a treatment drug in a medical context. Yang and Shanechi (2016) used a closed-loop system to monitor the brain state via EEG and control the level of burst suppression (i.e., amount of reduction in bursts of increased electrical activity); this feedback approach was successfully used to regulate, in real time, the injection of an anaesthetic drug to keep the patient in a constant state.

Another study applied a closed-loop system during a protocol that is normally used in motor rehabilitation. In this work, Markovic, Dosen, Cipriani, Popovic, and Farina (2014) explored

the possibility of improving grasping by combining the electromyography (EMG) signal and artificial vision in a group of healthy subjects. The authors showed that using a closed-loop system, it was possible to improve the subject's motor performance.

In general, we might use an open-loop protocol with an adaptive method for exploring how the system's response sensitivity varies across stimulations or regions to define the "temporal dimension" of an effect. Based on the experimental aim, once we have established the parameters that increase or decrease the system sensitivity, we then design stimuli or experiments to estimate the model parameters. As efficiently as possible to increase or reduce the system sensitivity by a closed-loop approach to produce a reliable and repeatable performance.

Studying the brain is challenging because the relevant stimulus space is often a high-dimensional space (Mutanen, Nieminen, and Ilmoniemi, 2013), and neural responses are stochastic, meaning that repeated TMS of an area elicits variable responses. However, using an EEG-TMS open-loop approach, we can reduce the stimulus space. Moreover, any variations measured in this context should then be considered reflections of key physiological mechanisms in the workings of the brain (McDonnell and Ward, 2011; Panzeri, Harvey, Piasini, Latham, and Fellin, 2017).

Open-/closed-loop system implementation with EEG-TMS is still technically difficult. It is extremely important to take into consideration that the loop between the stimulus and the signal must be on the order of milliseconds due to the phase dependence of brain activity to avoid phase shift. This reasoning is valid for all kinds of latencies; consequently, it is important that the delay in signal processing, while it is transferred to the buffer, must have a sub-millisecond precision. Auspiciously, real-time processing of neural signals is becoming more feasible each day through the ever-increasing computational power of modern microprocessors. Other important obstacles with the concurrent EEG-TMS recording are the artifacts induced by the TMS pulse (approximately 5-10 msec) because of amplifier saturation (see Veniero, Bortoletto, and Miniussi, 2009). These artifacts can be approached with temporal interpolation, filtering, channel and/or epoch rejection and with algorithms, such as independent component analysis (ICA, Hyvarinen, 1999) or the source-estimate-utilizing noise-discarding (SOUND) algorithm (Mutanen, Metsomaa, Liljander, and Ilmoniemi, 2018). ICA algorithms are a feasible means of removing ocular artifacts and residual TMS-related

artifacts. The SOUND algorithm might rid the neurophysiological data of the remaining nonstationary disturbances. Moreover, compatible equipment already exists and is available for the combined EEG-TMS recording. Therefore, the most important focus should be on the development of a robust and efficient pipeline for online analysis via a classifier algorithm. There are BCI studies from other fields, as cited above, which may be helpful in inspiring the future of this approach and the needed analysis (e.g., Kothe and Makeig, 2013; [sccn.ucsd.edu/wiki/BCILAB](http://sccn.ucsd.edu/wiki/BCILAB)).

There is also a neural issue: while controlling a single neuron is a relatively straightforward problem that implies specific timing, the temporal dynamics can become highly complex at the network level. Therefore, we should take into account that physiological limitations are sometimes also present and arise from the constraints imposed by brain computation time. For example, the sensory systems have processing delays ranging between ~10 and 50 ms (e.g., somatosensory vs. visual) before the signal reaches the cortex. On the other hand, when TMS is delivered, it is conceivable that the response to stimulation extends to seconds as the signal is relayed through complex networks. Therefore, such delays should be considered, since they can be a key element in achieving an improved understanding of the temporal dynamics of the network.

## **Conclusions**

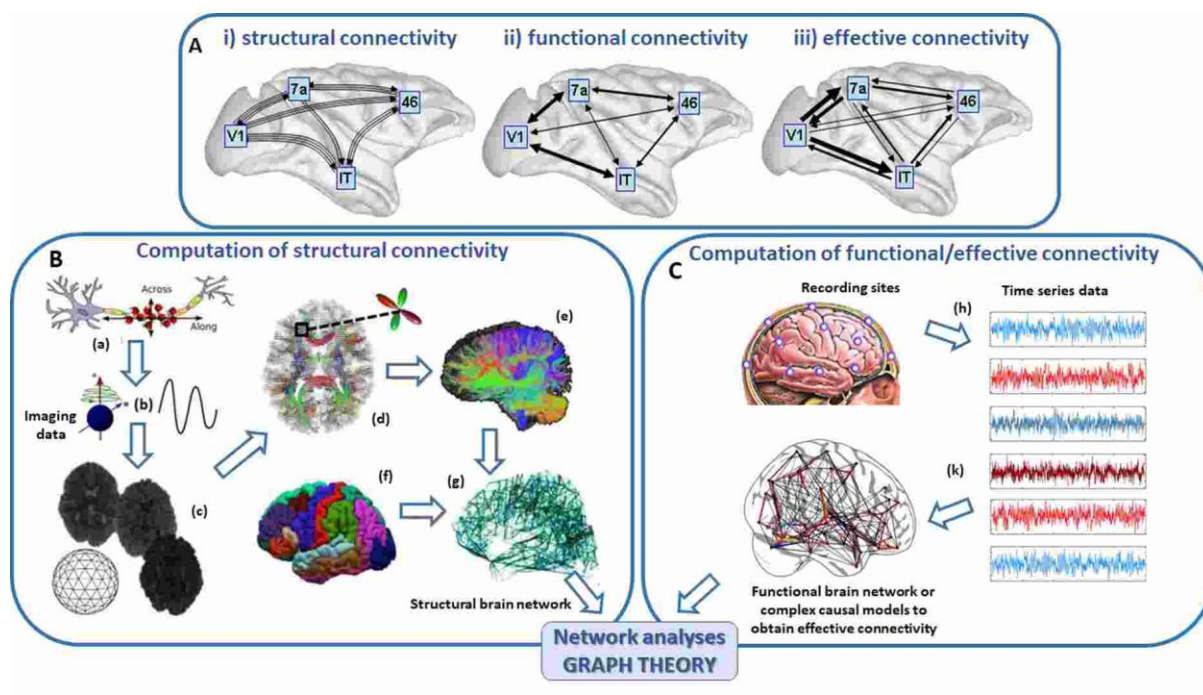
Studying brain connectivity is simultaneously interesting and challenging. Connectivity has a complex nature that requires a complex system to explore it. However, it is very important to invest in an integrative approach in the field of connectomics. The potential advantage of combining different methods is that it yields, a single, more complex instrument, which can provide more information that reduces variability in the data. In this way, it may be possible to reduce the gap between function and structure in the context of integrative neuroscience. Considering the open-/closed-loop scenarios, the TMS trigger is temporally guided by brain activity that becomes part of the experimental protocol in an adaptive approach, and it is spatially guided by the nodes estimated from neuroimaging. By adopting such a configuration, it is possible to study and manipulate the brain by guiding it through one direction or another. Therefore, with the open-/closed-loop setting, we will be able to explore the cognitive

architecture and test our hypothesis online. This approach describes a process where theory can be extracted from direct applications and overcomes the fundamental limits of indirectly testing our hypotheses by a correlative approach on a static “picture” of the brain. This approach provides an opportunity to non-invasively characterize in real time the causal dynamic relationships between brain cognitive architecture and neural responses, with the goal of understanding how diverse functions are integrated to produce complex behaviours.

**Box 1. Schematization of connectivity measures.**

In studying connectivity, the aim is to understand how neural elements exchange signals and influence each other. Connectivity can be defined from an anatomical (i), functional (ii) or effective (iii) viewpoint. Anatomical/structural (i) connectivity corresponds to the anatomical layout of axons and synaptic connections that determines which neural units can directly interact with each other (Friston, 1994). Considering that the total number of neocortical neurons is 15 to 32 billion (with inter-individual variation) and that each neuron has an average of 7000 synaptic connections (Herculano-Houzel, 2009; Pakkenberg and Gundersen, 1997; Walløe, Pakkenberg, and Fabricius, 2014), we can recognize that these are the anatomical constraints that limit which neural populations can link to form biological neural networks. Nevertheless, these wiring diagrams among neurons in the brain have a further grade of complexity that is defined by how “strong” or “direct” their interactions are. This increase in complexity is driven by communications between neurons and is defined by the “temporal correlations between spatially remote neurophysiological events”, or functional (ii) connectivity (Friston and others., 1993; Friston, Frith, and Frackowiak, 1993). Therefore, functional connectivity defines statistical dependencies and does not reveal the nature of the temporal correlation or allow the determination of instrumental interactions between regions, i.e., how well the activation in one node explains the activation in another and therefore what is the strength of the functional connection between the two, in a given state. Causal interactions are implemented in the study of effective (iii) connectivity, which is defined as the “causal influence that one neural system exerts over another either directly or indirectly” (Friston, Frith, and Frackowiak, 1993); thus, a causal model theoretically explains the initiation and direction of information flow.





**Figure Caption Box 1**

Representation of the modes of brain connectivity. A) Sketches at the top illustrate structural (i) connectivity (fiber pathways), functional (ii) connectivity (correlations), and effective (iii) connectivity (information flow) among four brain regions in the macaque cortex (adapted from Figure 1 of [http://www.scholarpedia.org/article/Brain\\_connectivity](http://www.scholarpedia.org/article/Brain_connectivity)).

B) Information flow of structural connectivity analysis by parcellation of the brain volume into coherent regions on the basis of structural or connectional features from magnetic resonance (MR) imaging. (a) Water molecules move faster along than across neuronal fiber. (b) Diffusion affects the electromagnetic waves radiated by precessing protons. (c) dMR imaging captures diffusion signals along different directions and forms images. (d) Fiber orientation distributions (FODs) are reconstructed from diffusion images. (e) Fiber tracts are simulated from FOD images. (f) The brain cortex is segmented into many regions using structural MR images. (g) Connectivity networks between cortex regions are constructed from fiber tracts (adapted from Li, Shi, and Toga, 2016).

C) Extraction of brain networks from empirical data follows node assignment by placement of sensors and/or recording sites from EEG, but the same can be obtained from magnetoencephalography (MEG), fMRI and PET data (part of the figure adapted from [thenassaeguardian.com](http://thenassaeguardian.com)); (h) recording of time series data to estimate coupling; (k)



construction of a connection matrix representing functional/effective networks. Obtaining measures of effective connectivity with neuroimaging techniques requires complex causal models, such as dynamic causal modelling, Granger causality, and information-theoretic methods (Friston, Moran, and Seth, 2013; Sporns, Chialvo, Kaiser, and Hilgetag, 2004), given that these techniques cannot address causality. Causality can be inferred through perturbation by non-invasive brain stimulation and EEG recording (Bortoletto, Veniero, Thut, and Miniussi, 2015).

## Box 2. Connectivity and neurological disorders

The study of brain connectivity improves our knowledge about the functioning of the brain in a healthy condition, and when a chronic or an acute event affects the nervous system, it opens the opportunity to understand brain connectivity more thoroughly. Neurological diseases, such as neurodegenerative pathologies, alter nodes and thereby cause the network connections to be altered. The consequences of neurological events have an impact on both the topology and functioning of the network, resulting in behavioural deficits in everyday life. TMS-EEG has been employed to evaluate altered connectivity in specific pathologies such as Alzheimer’s disease (Casarotto and others., 2011; Koch and others., 2018; Bagattini and others 2019) and as a tool for the early diagnosis of mild cognitive impairment (Julkunen and others., 2008; Bagattini and others 2019). Koch and others (2018) measured changes in precuneus connectivity after a TMS protocol among patients in the early stages of Alzheimer’s disease. These alterations in the connections were followed by selective improvement of episodic memory. The evidence described by the authors shows that TMS-EEG is able to measure effective connectivity and may represent an important tool for clinical diagnosis, with a secondary prospect of tracking recovery and rehabilitation (e.g., Ragazzoni and others., 2017).

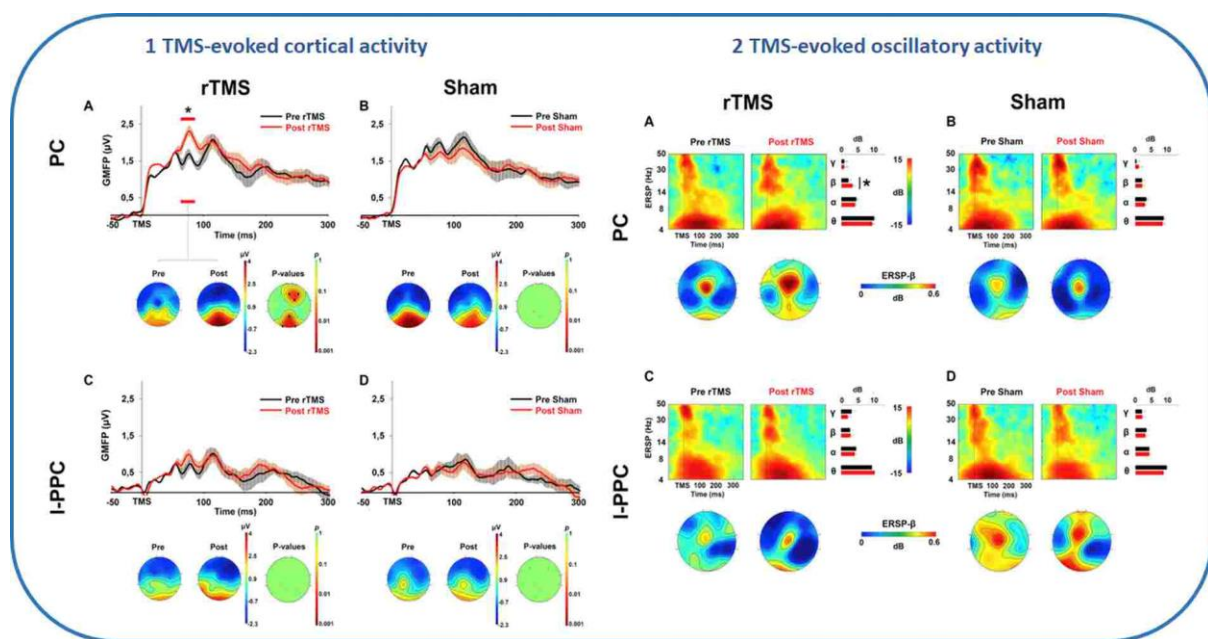


Figure Caption Box 2

Representation of TMS-evoked response (global mean field power, GMFP) and TMS-evoked oscillatory activity in patients with early-stage Alzheimer's disease (from Koch and others, 2018). The upper left panel (1 TMS-evoked cortical activity) shows that effective fronto-parietal connectivity increases after the real stimulation protocol. The black line (A, B) represents the GMFP measurement before the repetitive TMS (or sham) stimulation protocol. The single TMS stimulation pulse was delivered over the prefrontal cortex (PC). The increase in cortical activity starts at 60 ms and lasts until 90 ms. The red line represents the same measure recorded after a two-week treatment (repetitive TMS or sham) that consisted of forty trains of 20 Hz stimulation for 2 s per train, alternating with 28 s of no stimulation. No significant effects were detectable when the same analysis was conducted on the cortical response after single-pulse TMS over the posterior parietal cortex (I-PPC) (C, D).

The upper right panel (2 TMS-evoked oscillatory activity) shows the TMS-EEG response in the time-frequency domain. The results show an enhancement of beta activity in the PC in terms of spectral power after the real (A) and sham (B) stimulation protocols. As for the time domain, no significant effect was detectable when the same analysis was conducted on the I-PPC (C, D) (from Koch and others, 2018).

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