TMS-EEG combined with Granger Causality – An innovative information flow approach over the full brain connectivity

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Orientadores:
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Prof. MD Hartwig Siebner, Danish Research Center for Magnetic Resonance (DRCMR), Hvidovre Hospital

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'Bobby Walker: I will win! Why? Because I have Faith! Courage! Enthusiasm!'

Bobbie Walker – The Company Man
A. Abstract

Brain connectivity is a ‘hot’ topic these days in neuroscience. One of its branches is the effective connectivity, which intends to offer an interpretation for the information flow across brain areas. Many techniques can be used, between which Granger Causality (GC) and transcranial magnetic stimulation in combination with electroencephalography (TMS-EEG) have a prominent position. On one hand GC allows an interpretation of direct connections among brain areas, being an explanatory approach over the data, where no assumptions regarding the behavior of the causal relations are needed. However, several issues affect the results of GC, since they have to be contained over the restrictions of the model (i.e. non-stationarity and colinearity) and they are highly affected by spurious causality making the statistical reliability tenuous. On the other hand, TMS is a brain stimulation technique that allows the depolarization of populations of neurons, creating a ‘wave’ of propagation over the brain, according to the place of stimulation and its physiological connections. When these waves are measured with an EEG system, a combination of TMS-EEG is made. Such technique can become a powerful tool in connectivity studies. Uniting these two powerful tools (GC and TMS-EEG) should allow a new and innovative approach to measure effective connectivity in the brain. However, as yet, no study was made coupling these two methods.

Therefore this project aims firstly to find answers in the GC approach for both its limitations and its parameters, allowing for the optimizations of the further TMS-EEG data analysis with GC. Secondly, and as a major goal, to create a Matlab toolbox (Effective Connectivity test - E Ct), that allows the compatible combination of GC with TMS-EEG, making possible to use it as a future tool for brain connectivity studies.

In the first phase, a comparison between methods of brain connectivity estimators was made (GC was compared with: Factor Modelling combined with GC, and with Transfer Entropy), showing that GC outperformed others. It was also taken into account and tested solutions for the non-stationarity and colinearity of the data over simulated data. Such procedure allowed to select specific GC parameters such as, number of ensemble trials, data length and number of variables. In a second part of the project, a TMS-EEG experiment was performed. Two conditions were recorded, a real TMS stimulation and a groundbreaking sham stimulation. The focus was on the resting state (rs) period in between the TMS pulses, because in a single pulse stimulation on the rs no changes in terms of connectivity were expected. Thus, this procedure allowed to validate the toolbox on recorded data by comparing such two conditions. An innovative pre-processing and statistical approach on the GC was implemented and validated, allowing the reduction of spurious connectivity. Considering the results, having in considerations the lack of subjects (only 6 subjects), they look promising since no big effect is seen (less than 9% of connections are significant) over the statistical analysis. All things considered, the toolbox, techniques here discussed and its premises, can be considered as a first step into measuring effective connectivity with a coupling of two techniques such as TMS-EEG and GC. In the future this might lead to a better understanding of the structure complexity and system dynamics of the brain.

Keywords

Effective Connectivity; Granger Causality; TMS-EEG; Time-series domain;
B. Resumo

Atualmente, no mundo das neurociências, a conectividade cerebral é um tema em destaque. Este conceito encontra-se dividido em conectividade estrutural (relações anatômicas entre estruturas cerebrais), conectividade funcional (dependências estatísticas entre estruturas cerebrais) e conectividade efetiva (relações de causalidade entre estruturas cerebrais). Esta tese debaterá fundamentalmente sobre o último destes conceitos, tentando oferecer uma interpretação para o fluxo de informações entre as áreas do cérebro. Muitas técnicas podem ser utilizadas na sua análise, entre os quais a Causalidade de Granger (GC) ou a estimulação magnética transtecnica em combinação com eletroencefalografia (TMS-EEG). Por um lado, a GC permite uma interpretação das ligações diretas dentro e fora das mesmas áreas cerebrais, sendo uma abordagem explicativa sobre os dados, onde não é necessária nenhuma hipótese sobre o comportamento das relações causais. No entanto, os resultados de GC são muito sensíveis, uma vez que dependem de sinais não-estacionários e não colineares, aspetos bastante presentes em sinais de eletroencefalografia (EEG). Desta forma, a qualidade dos resultados de causalidade irá sempre depender da qualidade do pré-processamento do sinal original, onde se tenta ao máximo reduzir o seu efeito tentando não alterar os padrões de conectividade artificialmente. Por outro lado, TMS é uma técnica que permite que a estimulação do cérebro através da despolarização de certas populações de neurónios, criando uma "onda" de propagação ao longo do cérebro, de acordo com o local de estimulação e as suas ligações fisiológicas. Estes sinais são medidos através de sistemas de EEG, fazendo de TMS-EEG uma poderosa ferramenta nos estudos de conectividade efetiva, uma vez que permite criar estados independentes da intenção consciente da pessoa, garantindo um acompanhamento abrangente da sua propagação. A combinação destas duas ferramentas poderosas (GC e TMS-EEG) permitirá uma abordagem inovadora no desenvolvimento do conhecimento relativo à conectividade efetiva no cérebro. É com essa intenção que esta tese foi desenvolvida, tendo criado uma ferramenta computacional que permita medir e inferir padrões de conectividade efetiva através da combinação de TMS-EEG com GC.

Sendo uma abordagem pioneira, a tese foi estruturada para que inicialmente se desenvolvessem garantias relativas a que uma redução nos efeitos ambíguos da GC, como a não-estacionaridade e não-colinearidade dos sinais de EEG, não afetasse a qualidade dos resultados de causalidade, ou que minimizasse a sua dependência dos métodos de pré-processamento. Portanto, este projeto visa, em primeiro lugar encontrar respostas na abordagem da GC, tanto para as suas limitações como para os seus parâmetros, permitindo que, posteriormente, houvesse uma otimização da análise de dados de TMS-EEG. Nesta primeira fase, os testes foram realizados com dados simulados. Só numa segunda fase é que, o objetivo principal a que esta tese se propunha, foi alcançado. Para isso foi criada uma toolbox em Matlab (Effective Connectivity test Toolbox - ECT) permitindo uma combinação compatível de GC com TMS-EEG. Esta projeto tentou validar esta toolbox para que se torne uma ferramenta futura para estudos de conectividade cerebral.

Sendo um pouco mais específico, na primeira fase, foi encetada uma comparação entre os métodos de estimadores de conectividade do cérebro. A tradicional implementação de GC foi comparada com um método inovador de combinar a modelação fatorial com a GC (FM-GC), e com duas aplicações de transferência de entropia (onde dois métodos de estimadores de entropia foram utilizados – Binning Estimator e k-Nearest Neighbor). Esta abordagem mostrou que, perante os dados simulados criados, a GC se adaptou melhor tanto ao ruído implementado no sistema, comprovando ser o método com maior
sensibilidade e especificidade. Provou-se também que para condições reais de EEG, nomeadamente número de pontos por trecho (512) e o número de ensaios (1 ensaio) a utilizar, GC verificou valores de falsos positivos menores comparativamente com os outros métodos. De seguida foram consideradas e testadas soluções que permitissem suavizar os efeitos da não-estacionariedade e colinearidade nos resultados da GC, tentando perceber novamente o desempenho deste método em dados simulados. Relativamente aos métodos de não-estacionariedade, o demean e o detrending foram implementados sendo que foi também analisada a capacidade de redução da presença de ‘raízes unitárias’, conduzindo à atenuação da não-estacionariedade, através do Augmented Dickey-Fuller (ADF). Este protocolo foi aplicado em dois tipos dados simulados – estacionários e não-estacionários. Foi também analisada a possibilidade de tornar os modelos autorregressivos (MVAR) mais estáveis através da conjugação de vários ensaios. Relativo ao primeiro teste pouca diferença foi verificada, no entanto conclui-se que inclusão desses métodos (demean & detrending) deveria ser introduzida na pipeline de pré-processamento. Relativamente à segunda etapa provou-se a eficácia de um aglomerar de ensaios sendo que o valor que otimizava essa estabilidade era de 5 ensaios. Por fim, testaram-se e debateram-se métodos que reduzissem a colinearidade e o overfitting do modelo. Relativo ao problema de colinearidade foi debatido, com base nas referências bibliográficas, que a implementação de uma solução para o problema inverso (encontrar matematicamente as fontes de sinal de EEG) seria necessário para remover essa ambiguidade. Sendo que a escolha recaiu sobre a análise de componentes independentes (ICA), assumindo que cada componente independente assegura o comportamento de uma fonte de sinal elétrico no cérebro. Relativamente ao overfitting verificou-se apenas, num sinal simulado de ERP, que com o acréscimo de sensores (variáveis) existe um aumentar de parâmetros que traduzem o overfitting como, os coeficientes de correlação (relação diretamente proporcional) ou as intensidades máximas de GC (que foram otimizadas para um número de sensores entre 15 e 20).

Na segunda parte do projeto, e de forma a responder ao objetivo principal, foi realizada uma experiência de TMS-EEG, que por um lado permitisse garantir dados realistas e provenientes dessa modalidade, como por outro que permitisse validar as mais-valias da toolbox ECt. Nesse âmbito, foram realizadas, a seis sujeitos, duas condições, uma estimulação real de TMS e uma inovadora estimulação sham, que foram repetidas cercas de 200 vezes. Em ambos os casos, o foco esteve no período de repouso (rs) entre os pulsos de TMS. Nestas condições, foi possível tentar validar a eficácia da ECT, pois no período de repouso após um pulso de TMS o comportamento de ambas as condições era suposto de ser idêntico, não se esperando mudanças ao nível da conectividade. Isto levou a formular a hipótese de que quando comparando as duas condições, estatisticamente os resultados não seriam significativamente diferentes. Colocada a hipótese, estruturou-se a toolbox em três pontos. O primeiro recaiu sobre os métodos de pré-processamento. Este abordou aspetos relativos ao tratamento dos dados de EEG recolhidos, onde se procedia a uma correção da baseline, procedia a uma redução da frequência de amostragem, se concatenavam os dados de todos os sujeitos para que posteriormente a abordagem de ICA fosse mais coerente. No segundo ponto debateu-se a aplicação do método da GC, onde se realizaram procedimentos como: estimar a ordem do modelo, estimar o modelo MVAR e, posteriormente, calcular os índices de GC. O último ponto incidiu sobre uma abordagem estatística inovadora de três níveis de análise. O primeiro pretendia validar os resultados de GC dentro de um sujeito e de uma das condições, através de uma análise estatística dos resultados de GC contra um surrogate (teste inverso de granger - RGT). O segundo nível pretendia comparar os resultados de GC entre condições dentro de um sujeito (Maximum permutation statistics). Por último, o terceiro nível tinha como objetivo comparar os valores de GC entre condições e sujeitos (t-test paralelo).
Os resultados permitiram, numa primeira fase, verificar que os métodos de pré-processamento permitiram a redução de conectividade espúria, uma vez que 10 em 12 (2 condições vezes 6 sujeitos) dos conjuntos de dados preservaram mais de 60% dos ensaios sobrevivendo às restrições impostas pelo modelo. Considerando os resultados estatísticos obtidos, e tendo em consideração a falta de sujeitos (apenas 6 indivíduos), eles parecem ser promissores já que não existe uma expressão significativa nas matrizes de causalidade quando comparadas no segundo nível de análise estatística (Comparação de um sujeito entre condições), onde apenas 9% das ligações possíveis foram estatisticamente significativas. Relativamente ao último nível de análise os resultados não mostram qualquer inferência significativa entre variáveis, muito provavelmente devido ao fraco poder estatístico (apenas 6 sujeitos) do procedimento realizado.

Para concluir, todos os aspetos considerados e discutidos nesta tese, relativos tanto a esta teoria como a esta toolbox podem e deveram ser consideradas como um primeiro passo, visto que este projeto visou criar uma base para o estudo da conectividade efetiva em protocolos de TMS. No futuro pode permitir abrir a porta à compreensão da complexidade da estrutura de causalidade e dinâmica do sistema cerebral.

**Palavras-chave**

Conectividade Efetiva; Causalidade de Granger; TMS-EEG; Séries temporais;
C. Acknowledgements

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### E. List of Abbreviations

- ADF: Augmented Dickey-Fuller test
- AIC: Akaike Information Criterion
- AR: Auto-Regressive Model
- BIC: Bayesian Information Criterion
- BIN: Binning Estimator
- CS: Covariance Stationary
- CGCI: Conditional Granger Causality Index
- CMAP: Compound Muscle Potentials
- DCM: Dynamical Causal Modelling
- dDTF: Direct Directed Transfer Function
- DTF: Directed Transfer Function
- EEG: Electroencephalography
- EPSP: Excitatory Postsynaptic Potential
- FDR: False Discovery Rate
- fMRI: Functional Magnetic Resonance Imaging
- GC: Granger Causality
- GCI: Granger Causality Index
- ICA: Independent Component Analysis
- IT: Information Theory
- LFP: Local Field Potentials
- LIN: Linear Estimator
- KPSS: Kwiatkowski–Phillips–Schmidt–Shin test
- MEG: Magnetoencephalographic
- MVAR: Multivariable Auto-Regressive Model
- NN: k-Nearest Neighbors Estimator
- NUE: Non-Uniform Embedding
- OLS: Ordinary Least Square
- PCA: Principal Component Analysis
- PDC: Partial Direct Coherence
- PLF: Phase locking factor
- rs: Resting State
- rTMS: Repetitive Transcranial Magnetic Stimulation
- TBI: Traumatic Brain Injury
- TE: Transfer Entropy
- TEP: Transcranial Magnetic Stimulation Evoked Potential
- TES: Transcranial Electric Stimulation
- TMS: Transcranial Magnetic Stimulation
- TMS-EEG: Modality that conjugate TMS with EEG
- MVAR: Multi-Variable Auto Regressive model
- UE: Uniform Embedding
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Chapter 1

1. Motivation

The human brain is a fundamental part of the human being especially in the coordination of the sensory, cognitive and resting functions. Many studies in neuroscience have aimed to determine the brain activity for a particular task (Jirsa 2007) or associated to a specific disease (Nejad et al. 2012). What these studies report is that the brain runs in a highly dynamical and complex way, connecting different structures within itself. This brain connectivity is a broad concept that can be generally divided into three categories: structural, functional and effective connectivity. The structural connectivity refers to the connections of brain regions via nerve fibers. The functional connectivity deals with the temporal interdependencies among the activity of brain regions. The effective connectivity characterizes the causal (directed) effects among brain regions.

Trying to understand the complexity of neural networks lead neuroscientists to build paradigms where brain states could be deliberately modified. This can be achieved by measuring brain activity over cognition/somatosensory related task (Vetter, Smith, and Muckli 2014; Wu et al. 2014), where a network is activated over the specificity of the task, or by modelling the brain activity over external stimulation (Transcranial Magnetic Stimulation – TMS, Transcranial direct-current stimulation – tDCS) (Nollet et al. 2003). Such non-invasive techniques are based on the depolarization of neuronal populations of the underlying cortical area where a coil (TMS) or electrodes (tDCS) are placed, as well as of functional activation of connected areas via cortico-cortical interactions. These methods are more relevant regarding studies of effective connectivity of cortico-cortical interactions once they are independent from human control over the networks activation/deactivations.

When TMS is used in combination with electroencephalography (EEG), the direct effect on the electrical responses of those neurons to the TMS pulse can be observed as TMS-evoked potentials (TEPs). Even though they are specific from each area undergoing stimulation (latencies and number of components), they can be recorded in brain areas which are far away from the stimulation point. This gives TMS-EEG a powerful advantage in the study of effective connectivity, due to its geographical distribution and type of data. Some studies have looked into the direct cortical effect of TMS comparing different groups of participants (i.e. healthy controls and patients suffering from disorders of consciousness) (Rosanova et al. 2012; Ragazzoni et al. 2013; Ferrarelli et al. 2010), looking for different patterns of effective connectivity. However, two major limitations of these studies can be pointed out. Firstly, the methods used yet can not reveal the propagation path of the TMS-induced cortical activation in the EEG response. Secondly, associated with TMS stimulation, the pulse discharge also generates both a somatosensory scalp response and a loud “click” sound which induces in the EEG measurement somatosensory-evoked (SEPs) and auditory-evoked potentials (AEPs). Both of SEPs and AEPs are not a result of direct cortical activation, on that sense they will distort the TEPs and consequently the way cortical activation is measured.

Regarding the first issue, this project will apply a new approach of effective connectivity in TMS-EEG data, using Granger Causality (GC) (Granger 1969). According to the Wiener Causality concept (Wiener 1956), if adding the past and present information of a system X to the past and present information of a system Y improves predicting the future state of system Y, it can be concluded that X is the cause of Y. Some years later, that concept was limited in its general definition to linear bivariate autoregressive models and also translated in a mathematical formulation for quantitative inferences by (Granger 1969) originating the
Granger Causality (GC). In 1982, Geweke proposed the most practical GC-based effective connectivity measure known as Granger Causality Index (GCI) (Geweke 1982). With it, it was also possible to build a tool that could distinguish correctly between direct and indirect causal links (Conditional Granger Causality Index (CGCI)) in the time domain (Ding M, Chen Y 2007), and the Direct Transfer Function (DTF), the Partial Direct Coherence (PDC) (Sameshima and Baccala 2001) and the Directed Direct Transfer Function (dDTF) in the frequency domain, where all are based on linear Multivariate Auto-Regressive (MVAR) models. The new combination that this project is aiming for is TMS-EEG with CGCI in the time-domain. On one hand CGCI will enable the track of information flow paths among nodes. On the other hand, TMS-EEG is a procedure that combines TMS with EEG, facilitating the location of the starting point in signal propagation, due to the TMS stimulation, that is then recorded by the EEG system. All together it forms a powerful tool in causality over conditioned brain states.

Accounting for the second issue, until now very few studies account for the influence of SEPs and AEPs in the seen results, and those who did used either conventional sham stimulation (only accounting for the AEPs) or a separate condition exploring SEPs with median nerve stimulation paradigms (Zanon et al. 2013). The use of conventional sham stimulation, moreover, does not result in a perceivable somatosensation due to a physical separation of the TMS coil from the scalp. The project proposal for that is to combined both influences and evaluate the remote evoked EEG responses derived from the target “behaviorally silent” scalp areas where TMS is commonly applied (i.e. parietal cortex), with the aim to generate resting state (rs) responses that match the multisensory components¹ present in real TMS stimulation. Such protocol is intended to explore to which extent the effective connectivity tool here proposed is reliable. This is due to the fact that sham and real condition effect over the effective connectivity should be theoretically similar on the so-called resting state period in between the TMS pulses.

The main aim of this master thesis project is to combine a method for effective connectivity that gives “causality” and directionality (GC) with a brain controlled stimulation (TMS-EEG) in one powerful tool (a Matlab toolbox) to be used further to map the brain networks. The work is structured in two main steps. The first one is related with a methodological approach, where it is provided to the reader answers on why was GC used and how did the project account for the GC limitations based on simulated data (i.e. Non-stationarity and Collinearity). The second step is related to applying GC to TMS-EEG data, and then compare different conditions (i.e. Real-TMS vs Sham-TMS) trying to obtain significant results that help validating such tool. This will be achieved if the theoretical point of view on the resting states between different TMS stimulation procedures is seen in the results. This step accounts for the innovative approach on measuring the sham condition of TMS. Hopefully this thesis will be a first step on future developments on how can influences after a TMS pulse be measured.

¹ Multisensory components meaning the patterns of information flow obtained throw GC.
2. Background

2.1. Brain Connectivity & Effective Connectivity

A complex system like the human body, could only be controlled and organized by a system that had a similar or higher order of complexity. That system is the Human Brain. Among all of that complexity, some things that have been concerning neuroscientists are (Swanson, 2003): How are brain areas connected? How is information going around the brain? And in which circumstances those connections happen? All of this merge towards asking how neurons and neural networks process information? And what is Brain Connectivity?

Brain connectivity refers to a pattern of anatomical links ("anatomical connectivity"), of statistical dependencies ("functional connectivity") or of causal interactions ("effective connectivity") between distinct units within a nervous system. The units correspond to individual neurons, neuronal populations, or anatomically segregated brain regions. Neural activity, and by extension neural codes, are constrained by connectivity. This patterns can be formed by structural links such as synapses or fiber pathways, or by statistical or causal relationships measured as cross-correlations, coherence, or information flow (Granger Causality or Dynamic Causal Models). Formally, brain connectivity patterns can be represented in graph or matrix format, see Figure 2.1-1. Structural brain connectivity forms a sparse and directed graph. Functional

![Figure 2.1-1 – Modes of brain connectivity. Top brain images illustrate structural connectivity (fiber pathways), functional connectivity (correlations), and effective connectivity (information flow) among four brain regions in macaque cortex, respectively. Matrices at the bottom show binary structural connections (left), symmetric mutual information (middle) and non-symmetric transfer entropy (right). (Honey et al. 2007).](image)
brain connectivity forms a full symmetric matrix, with each of the elements encoding statistically dependence among neurons, recording sites or voxels. Such matrices may be thresholded to yield binary undirected graphs, with the setting of the threshold controlling the degree of sparsity. Effective brain connectivity yields a full non-symmetric matrix inferring something on the directionality. This method attempts to extract networks of causal influences of one neural element over another (Valdes-Sosa et al. 2011). Reviews about the concept of connectivity, particularly in reference to effective connectivity, can be found in (Chicharro and Panzeri 2014; Valdes-Sosa et al. 2011). The interest of this can be related to network analysis techniques that allow the comparison of brain connectivity patterns, by using time-series data (e.g. TMS-EEG) where, by controlling the conditions of comparison, it would be possible to infer connectivity influences over different brain regions. Such issue will be the main line of this thesis².

This chapter will be used to give an overview of the background needed for this project. A logical path is built such that the reader can understand fully the content of this thesis. This will include a first approach on a summary on resting state brain networks, EEG, TMS and TMS-EEG. Afterwards an introduction on the principles for Brain connectivity estimators and Granger Causality will be made, mentioning pros and cons. It is going to be finished with a short sum up of what have been done in this field.

2.2. Resting state brain network

In recent years, there has been a growing interest in characterizing the functional network of the brain ‘at rest’. This so-called ‘resting state’ (rs) paradigm is believed to reflect intrinsic activity of the brain, which may reveal valuable information on how different brain areas communicate, while the subject is not performing an explicit task. It gather a variety of information, since it linked spontaneous – task independent – fluctuations in neural activity to diseases, cognitive decline, and disturbances in consciousness (van Diessen et al. 2015). Even though the interest in the ‘resting state’ has been associated with breakthroughs in functional magnetic resonance imaging (fMRI), such technique only provides an indirect measurement of brain activity and has a limited temporal resolution, e.g. Figure 2.2-1. Considering that part of the information processed in the brain at rest is encoded on time scales from milliseconds to seconds, a time scale that better suits techniques such as electroencephalography (EEG) and magnetoencephalography (MEG). EEG have been providing valuable information on deviant organization in the diseased brain, such as in Alzheimer’s disease, epilepsy, schizophrenia, multiple sclerosis, Parkinson’s disease, as well as in the healthy brain on topics as aging, gender differences and a healthy lifestyle, see (van Diessen et al. 2015) for a review.

Figure 2.2-1 – Resting-state fMRI cerebral activity in 71 healthy subjects aged from 19 to 80 years. Identification of the Default Mode Network (DMN). Adapted from Mevel et al. 2011.

² Especially in the analysis between real TMS condition vs sham TMS condition.
‘Resting state’ per se

Resting state (rs) is the state in which a subject is awake and not performing an explicit mental or physical task. Initially, the ‘resting state’ condition was commonly used in EEG research – besides event-related potential studies – to study patterns of brain activity. The early EEG studies, including the first EEG recordings performed by Berger in 1929, already shown such patterns of brain activity. However, ironically it was when Biswal and colleagues revealed a distinct fMRI pattern of interacting brain regions when not performing a task that the resting state condition became a research paradigm for the study of interconnectivity of brain regions (Biswal et al. 1995). Since then, many studies have identified sets of brain regions that share a common activation pattern during the resting state including the ‘default mode network’ and other so-called ‘resting state networks’. These resting state networks have been replicated and validated both in neuroimaging and neurophysiological studies, suggesting that resting state patterns of connectivity are the result of robust and specific intrinsic neural activity (van Diessen et al. 2015).

Methodological concerns

When performing a rs study, especially when performing connectivity measures, there are some aspects that should be taken into account. There are three distinct issues: the subject-related methodological issues, the analysis-related methodological choices and the connectivity measures used.

Firstly, performing a resting state might not be as straightforward as it seems; behavior during the experiment and the perception of a stimulus independent from the condition may vary greatly between

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3 Default Mode Network (DMN) - it is a network of brain regions that are active when the individual is not focused on the outside world and the brain is at wakeful rest. Also called the default network, default state network, or task-negative network, the DMN is characterized by coherent neuronal oscillations at a rate lower than 0.1 Hz (one every ten seconds) (Fair et al. 2009).

4 Resting state networks (rsfMRI or R-fMRI) - is a method of functional brain imaging that can be used to evaluate regional interactions that occur when a subject is not performing an explicit task. This resting brain activity is observed through changes in blood flow in the brain which creates what is referred to as a blood-oxygen-level dependent (BOLD) signal that can be measured using fMRI (Buckner 2013).
subjects despite similar instructions. This variations can be in terms of state of vigilance (constantly shifting between different levels of activation, leading to differences in spectral power and functional connectivity), eyes open vs eyes closed and external induced heterogeneity (van Diessen et al. 2015). All these effects need to be taken into account. Secondly, the measurement-related methodological issues should be taken carefully. In Figure 2.2-2, the reader can find a summary of such issues. Diessen also suggested ‘[…] Since the current literature is too diverse to provide an uniform methodological guideline, we suggest including different methodological approaches in resting state studies to better understand the influence of these approaches on study results’, an aspect that is going to be discussed in chapter 3 – Managing Granger Causality over EEG signals. Finally accounting for the influence of the connectivity measures chosen, (van Diessen et al. 2015), provides a good review stating that caution should be taken, regarding that some connectivity measures are more vulnerable to volume conduction5, which leads to unreliable connectivity values, and consequently, unreliable network estimations.

2.3. Electroencephalography - EEG

The brain generates electric current/charge that is maintained by billions of neurons. They are polarized by membrane transport proteins that pump ions across their membranes, a process that occurs constantly. These exchanges lead to periods when the neuron is in a resting potential or periods where there are a propagation of action potentials. The signal propagation can be yield by cascade phenomenon. Everything happens due to ions chain, where when ions of similar charge repel each other, they tend to push their neighbors, which then push their neighbors, leading to a wave of propagation. This is known as volume conduction, and as the reader will see ahead, is also one of the EEG drawbacks on Effective Connectivity. In order to measure those waves of propagation, it is necessary for the ions to reach the electrodes on the scalp, and then ions will start the cascade of dragging more ions and so forward (Tatum, W. O. et al. 2008).

The electric activity in the cortex has two components, the action potential and the excitatory postsynaptic potential (EPSP), see Figure 2.3-1 A). The action potential is presynaptic, axonal and generally

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5 Volume Conduction – is the propagation of electromagnetic field with a speed of light — $3 \cdot 10^{10}$ cm/s. For the distance of the order of centimeter the delay is roughly $3.3 \cdot 10^{-9}$ s. Such delay cannot be practically detected, and affect/smears the information of each EEG sensor (Broek et al. 1998).
not-measurable by EEG, on the other hand the EPSP is postsynaptic, dendritic and it is measurable with EEG. However, the electric potential generated by an individual neuron is far too small to be picked up by EEG - Figure 2.3-1 C). EEG activity therefore always reflects the summation of the synchronous activity of thousands or millions of neurons that have similar spatial orientation. If the cells do not have similar spatial orientation, their ions do not line up and consequently waves created in this way are not detected. Pyramidal neurons (Figure 2.3-1 B)), which are oriented tangentially to the scalp surface of the cortex are thought to produce the most EEG signal because they are well-aligned and fire together. Moreover, the scalp EEG activity shows oscillations at a variety of frequencies. Among them, there are Delta waves (up to 4 Hz), Theta waves (4Hz-7Hz), Alpha waves (7-14Hz), Beta waves (15Hz-30) and finally Gamma waves (above 32Hz) (Nunez PL et al. 1981).

All of these waves are known as brainwaves and they codify thoughts, emotions and behaviors, being dependent over the task, stimulation or behavioral situation to which the subject is insert. They work together in the sense that, in the brain there is a coupling of different brainwaves. Such coupling can be constraint to a specific brain area or can be much wider, according to the task specificity (Canolty et al. 2009). With this being said, frequency bands that represent brainwaves are much related with the understanding of the brain functions and connections. Many studies have been pointing out the relevance of these brainwaves, showing measures for coherence, cross-coupling frequencies among others, trying to infer over the brain paths of communications. However, in this thesis the specificity of the frequency will not be in focus, since the effective connectivity will be performed on the time-domain.

On the practical point of view, one thing that should be kept in mind is that, the signal recorded is affected by biological and external artifacts. To try to reduce those issues and obtain a noise free signal some considerations should be taken into account. Firstly, the signal has to be measured over the scalp with surface electrodes (made of AgCl) that maintain a very constant potential, and have high capacity. However, due to a very small amplitude, it is necessary to amplify it, in order to have a reasonable output. Secondly, it is necessary to reference the signal, which can be made by one of the three possibilities: reference electrode, average reference and Laplacian montage. Thirdly, EEG electrical signal detected along the scalp is much affected by biological artifacts with different amplitudes, such as eye-induced artifacts and / or muscle activation. There are also more artifacts, such as environmental artifacts that are mostly created by the surrounding environment of the recording process. Important to mention is also the problem of volume conduction, on the scalp which if not controlled for, will lead to smeared EEG information. However, we are going to discuss on how to solve this problem in section 3.4.3 and section 4.2, due to the challenging procedure of the pre-processing for effective connectivity, an acceptable answer is by solving the inverse problem. In order words by converting the sensor space into the source space.

Sensor space to source space

Understanding brain function requires sophisticated methods applicable to non-invasively measured bioelectric signals (EEG data). Those methods should avoid, as well as possible the contamination by artifacts or any type of perturbation that diminish the quality and veracity of the information. It was with this in mind, that the neuroscience community started to look into a different level of analysis, shifting the attention from the sensor space (where the EEG signal is recorded) to a cortical level. This is the so-called source level, and it tries to translate the dataset recorded over the localization of the brain sources beyond it, giving the activity over time for those sources. However, to achieve that is necessary to solve the inverse problem.
The source localization procedure works by first finding the scalp potentials that would result from hypothetical dipoles, or more generally from a current distribution inside the head – the forward problem, see Figure 2.3-2. Then, in conjunction with the actual EEG data measured at specified positions of (usually less than 100) electrodes on the scalp, it can be used to work back and estimate the sources that fit these measurements – the inverse problem. The accuracy with which a source can be located is affected by a number of factors including head-modelling errors, source-modelling errors and EEG noise (instrumental or biological). The two main categories of methods which were developed to solve the EEG inverse problem are mainly the parametric and the non-parametric methods. The main difference between the two is whether a fixed number of dipoles is assumed a priori or not, respectively. This is out of the scope of this thesis, nevertheless a review can be found in (Grech et al. 2008).

2.4. Transcranial Magnetic Stimulation - TMS

2.4.1. Physics and Biophysics of TMS

Electromagnetic induction, i.e., the induction of an electrical current in a circuit exposed to a changing field, is an old concept firstly introduced by Faraday in 1831:

\[
\varepsilon = \frac{\Delta \Phi_B}{\Delta t} \quad \text{where} \quad \Phi_B = \int_S B \cdot dS
\]

Equation 2.4-1

Figure 2.4-1 – Example of an average of two conditions MEPs in a specific time window (ms) where the stimulus is applied. Adapted from Tomasevic et al. 2014.
Connecting this principle with what was said above regarding electric brain activity, it could be hypothesized that it is possible to apply it in the human brain. The motor evoked potential (MEP – see Figure 2.4-1) comprises the proof of that concept. To obtain an MEP, a stimulus with a magnetic coil over the motor cortex is applied, and due to the central nervous system pathways, it is possible to induce a response in the peripheral muscles given an enough intensity of stimulation. The first approach using the electromagnetic induction principle to non-invasively stimulate the brain was made by Barker (Barker et al. 1985).

Basic physics principles

TMS creates a pulsed electric current induced by the time-varying magnetic field that can depolarize neurons. Although the actual pathways being investigated are not known, they incorporate the fastest conducting fibers which might include the pyramidal tracts (Corthout et al 2001). A magnetic field is generated by passing an electric current through a coil of wire, called magnetic coil – Figure 2.4-2 A), which is placed above the scalp – Figure 2.4-2 B). Due to Faraday Law, the magnetic pulse produced from a perpendicular electric current pulse will thus induce a current in an electrically conductive region (e.g. human brain). This current will be in intensity proportional to the magnetic field (Nollet et al. 2003), and can only be produced because the skull presents a low impedance to magnetic fields of this frequency. Flux lines around the coil represent the magnetic field and it is measured in Tesla (T).

In an homogenous medium, the magnetic field will induce, as can be seen in Figure 2.4-2 A), a parallel current that flows only parallel to the plane of the coil. There would be a difference in the tissues interaction with the magnetic field, according to the position relative to the coil. The loops near the coil will have a

Figure 2.4-3 – A) The strength of the electric field induced in a spherical volume conductor below a circular (left) and a figure-of-eight coil (right), in (Ilmoniemi et al. 1999). B) The comparison between the electric field created by two different coils, with a circular coil, and with a figure-of-eight coil (two circular coils), in (Nollet et al. 2003).
stronger current, while with the distance the strength falls. Due to this, TMS is only able to stimulate areas in the brain in a depth of 1-2cm in the cortex (Hess et al. 1987).

Most related with the problem of deepness and focality, is the specificity of the coil. According to those is possible to obtain different results. In that sense, magnetic coils may have different shapes Figure 2.4-3 A). Circular coils (usually with diameter of 5-10cm) induce a more widespread, less focal activation, while figure-of-eight shaped coils are more focal, producing maximal current at the intersection of the two round components as it can clearly be seen in Figure 2.4-3. Nevertheless, these coils have a lower induction than circular coils (Hallett 2007).

Comparing this technique with transcranial electrical stimulation (TES), it has been concluded that TMS has some advantages. One of the most significative is that it is not painful. Another one is that the electrical field induced with a coil decreases significantly less with the increasing distance of the propagation in the brain than a field induced by TES. This is due to the fact that electrical stimulation injects current into the body via a surface, while magnetic stimulation uses a pulse of magnetic field to cause the voltage difference between two points, triggering a natural process. This leads to lesser loss of ions on the process of activation/inhibition pathways (Nollet et al. 2003).

Biophysical approach

Since TMS was introduced, there has been a debate over which structures are participating in the propagation of the stimulus. Now it is known that there are two types of waves that conduct the signals, the ones that produce a direct activation (D-waves) and the ones that produce an indirect activation (I-waves) of the corticospinal tract. The D-waves conduct down the pyramidal system, which can be activated trans-synaptically only at higher intensities. In contrast, the lower threshold form of TMS, for instance over the hand area of M1, tend to commonly activate corticospinal neurons trans-synaptically in the pyramidal tract which activates I-waves. However, with higher intensities, both D-waves and I-waves are activated (Di Lazzaro, et al. 1998).

Using TMS, the brain can be briefly activated or briefly inhibited; in fact, likely both occur with each stimulus in different amounts and with different time courses. Such effect can be used to localize brain functions in both space and time. Many studies have been conducted but of most interest are the approaches in the motor system, mainly due to its practicability throw the measures of the Motor Evoked Potential (MEP) in the peripheral nerve. One of them is the study of (Wassermann et al 1992), which concludes that body parts, such as arm and leg, are completely separate, but there is overlapping of muscles in the same body.

Figure 2.4-4 – A) TMS Mapping of Upper Extremity Muscles in Right and Left Sides of One Normal Subject after Stimulation of Contralateral M1s, in (Wassermann et al. 1992). B) Activation maps based on TMS-evoked averaged EEG responses, in (Ilmoniemi et al. 1997).
part, Figure 2.4-4 A). Such study was performed with single pulses of TMS\(^6\). However, many other protocols exist, e.g. repetitive TMS protocols (rTMS) and the paired-pulse TMS. As a final comment, there is an inter-MEP variability, which the reason is not yet known. It might be related to ongoing activity differences between TMS pulses. Thus, many trials should be performed in order to obtain a reliable conclusion on the data obtained.

Another aspect of most interest is the capability of specific TMS pulses to influence the behavior of the brain in the medium/long run, the so-called induced plasticity. Very briefly, neuroplasticity, also known as brain plasticity, encompasses both synaptic plasticity and non-synaptic plasticity—it refers to changes in neural pathways and synapses due to changes in behavior, environment or neural processes. Depending on the type of TMS pulse and the area of stimulation, this effect can be more or less prominent. Regarding the single-pulse paradigm used during this thesis, Pellicciari (Pellicciari et al. 2015) studied the effects of several single TMS pulses, delivered at two different inter-trial intervals, on corticospinal excitability. The author found that Motor Evoked Potential (MEPs) significantly increased when the TMS pulses were delivered at both random and fixed inter-trial intervals, leading them to conclude that single TMS pulses induce cumulative changes in neural activity during a single pulse TMS stimulation, resulting in a motor cortical excitability increase. However, as other studies have reported, such effects are only transient stimulation-related effects in the amplitude (or frequency domains) following single-pulse TMS. Stamoulis (Stamoulis et al. 2011) presented novel results of cumulative effects of single-pulse TMS, at least in terms of phase changes in the EEG in a small number of healthy subjects. Although the exact mechanism of phase modulation by TMS is unclear, the author presented evidence that such modulation exists and results from the prolonged but not short term application of single-pulse TMS applied in the motor cortex, see Figure 2.4-5. These studies support the idea that between a real-TMS and a sham-TMS in the short term there are no differences in the resting state, in between pulses, of the brain networks.

\[\text{Figure 2.4-5} \] – Relative phase prior to and following TMS. Intensity levels are in radiants. (a) Max. Relative phase: pre-TMS. (b) Max. relative phase at the end of 5 min of single-pulse TMS. (c) Max. relative phase at the end of 25 min of single-pulse TMS. The relative phase increased almost uniformly across channels, though fronto-central/central and parietal channels had slightly higher relative phase changes, 25min after the start of TMS. Other variations appeared random. Although prior to or at the beginning of the TMS session clusters of channels had either positive or negative relative phases, indicating both spatial correlation/synchrony and de-correlation, at the end of the TMS session, almost all channels had positive relative phases, indicating correlation/synchrony. Adapted from (Stamoulis et al. 2011).

\(\text{Single pulse TMS} \) – is a pulse that occurs at a frequency over a time allowing for the recovery of rs in the brain.
2.4.2. TMS-EEG

By the analysis of the two modalities described above, is easy to understand that if implemented together these two techniques could give good insights in brain signal propagations and areas of activation - Figure 2.4-4 A). However, coupling them is very challenging, both in the technical and the analytic point of view. Still, with the combination of TMS and EEG, the evoked potentials generated by TMS (TMS-evoked potentials - TEPs) can be measured at the cortical level, which allow inferring cortical reactivity (functional and effective connectivity), differences in latencies and number of components depending on the area undergoing (Rosanova et al. 2012). Moreover, measures of effective connectivity can be derived from the identification of active EEG sources in the few hundreds of milliseconds after a TMS pulse, as a correlation tool among channels. This can be seen in Figure 2.4-4 B).

Methodological concerns

EEG measures voltage differences from different sites on the scalp. These voltage differences are set up by transmembrane currents, mainly postsynaptic potentials of apical dendrites of large pyramidal cells. Allowing this modality to easily record the changes after a TMS pulse. There are important concerns that should be taken into account: the ones related with the non-cortical signal recorded on the EEG, the ones related with the on-line phase and the ones related to the data cleaning (Giambattistelli & Tomasevic et al. 2014; Vernet et al 2014).

Starting by the first one this can be disentangled by performing a sham measurement. When a TMS pulse is discharged, the coil generates both a loud “click” sound and a somatosensory scalp sensation which, when combined with EEG, can induce somatosensory-evoked (SEPs) and auditory evoked potentials (AEPs) that are not a result of direct cortical activation. To disentangle the direct cortical effect of TMS and the somatosensory and auditory responses is necessary to control them, even more when comparing different conditions. So far, only conventional sham stimulation (only accounting for the AEPs) or a separate condition exploring SEPs with median nerve stimulation paradigms, have been used in (Ragazzoni et al. 2013; Zanon et al. 2013). For the AEPs sham usually is performed by using a “noise masking”7, in combination with a thin layer of foam placed (reducing the bone conduction of the sound) underneath the coil, which can damp both the scalp sensation of the discharge and the sound perception. However, its effectiveness varies across participant (Braack 2013). Other solutions may arise by placing the coil on top of a Plexiglas or wood cube, allowing for the click sound to still be present, and the coil be physically distanced from the scalp, so that no cortical stimulation is being yield. However, with such method the somatosensory response is lost (Rossi et al. 2007). An innovative proposal will be made in section 4.2.

Regarding the second one, the equipment for TMS-EEG must be specific to this purpose. In that way, the EEG cap has to be compatible with TMS having for instance specific electrodes, low impedance, coil vibration and click attenuators and amplifiers that can cope with the magnetic artifact. The challenges on data analysis occur when the TMS-related artifacts become stronger than the brain activity. The principal causes to that are: pulse artifact, the decay artifact, the muscular artifact8, the 50Hz artifact and the recharge artifact. It is difficult to eliminate some of them such as the pulse artifact or the muscular artifact. Others are simple to solve, such as the 50Hz or the recharge artifact (50Hz notch filter and shift the recharge data point,

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7 "Noise masking" – consists of a constant auditory input (white or broadband noise) provided through soundproof in ear headphones during the TMS experiment.

8 Muscular artefact – Originated by the stimulation of the facial muscles by the TMS, which will influence the EEG signals.
respectively). To correct for the pulse artifact, one should consider to only start the analysis after the artifact (after 30ms) or even the attempt on using Independent Component Analysis (ICA). Also, it is important to reject epochs due to eye movement, muscle activity and epochs containing EEG amplitudes greater than 100μV. However, since this project will focus mainly on the resting state period, after a TMS pulse, and just before the next pulse, the majority of the artifacts such as: pulse artifact, the muscular artifact and the recharge artifact will not impact on the analysed data.

2.5. Brain connectivity estimators

To study the effective connectivity it is necessary to find mathematical tools, such as brain connectivity estimators, that provide reliable results. Their main task is to find patterns in the brain activity. As a mathematical procedure, they usually evaluate connectivity from brain activity time series such as EEG or Local field potentials (LFP), with an impact on the directed influences. Among estimators of connectivity, there are linear or non-linear and bivariate or multivariate measures. In the panoply of different estimators we have five main groups: the classic measures (i.e. coherence or correlation), the phase synchronization (i.e. phase locking), the parameter-based models (i.e. granger’s theory) (Granger 1969; Sameshima and Baccala 2001; Bressler and Seth 2011), the information theory models (i.e. transfer entropy) (Schreiber 2000) and the dynamic causal models (Penny et al. 2004). Each of these methods have their strength, however they also vary in their effectiveness. This review, will provide an overview over the parameter-based model, also known as Granger Causality (GC).

Regarding the difference between linear and nonlinear estimators, Quiroga (Quiroga et al. 2005) showed that nonlinear measures require long stationary segments of signals, are prone to systematic errors and, above all, are very sensitive to noise. The author conclude that, by means of surrogate data, the nonlinearity in EEG is the exception rather than a rule. Plus, it was shown in (Winterhalder et al. 2005) that linear method perform quite well also for non-linear signals. Regarding the second comparison between bivariate (system where only two channels are considered) and multivariate models (system where multiple channels are considered), it was demonstrated in (Kuś & Kamiński 2004) that in case of interrelated system of channels, greater than two, bivariate methods supply misleading information.

Yet, to implement reliable metrics with an appropriate large-scale analyses it is necessary to impose specific constraints on connectivity metrics (Khadem and Hossein-Zadeh 2014):

- The metric must be robust to nuisance factors inherent to EEG signals noise and linear mixing. External, biological, and instrument noise can result both in reduced detection of connectivity or in detection of false positives;
- Large-scale all-to-all network analyses easily entail millions of estimations of a given metric. To be realistically applicable in such conditions, metrics need to be computationally efficient;
- The number of a priori parameters should be limited not only to reduce the cost of determining appropriate values for each signal pair but also to reduce the possibility of erroneous results caused by inappropriate parameter choice.

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9 Surrogates data – by constructing virtual data from the experimental data it is possible to assess the statistical significance of metric values (Dolan and Spano 2001)
2.5.1. Granger causality

Basic Theory

GC defined the causality relationship on two principles: the cause happens prior to its effects; and the cause has unique information about the future values of its effects. Thus GC is based on predictability and precedence. A variable \( X \) is said to \( G \)-cause a variable \( Y \) if the past of \( X \) contains information that helps predict the future of \( Y \) over and above information already in the past of \( Y \). With this being said GC test the following hypothesis for identification of causal effect of \( X \) on \( Y \):

\[
p(Y(t + 1) \in A|U(t)) \neq p(Y(t + 1) \in A|U_{a}(t))
\]

Equation 2.5-1

where \( A \) is an arbitrary non-empty set, \( U(t) \) and \( U_{a}(t) \) denote all the information until time \( t \) in the entire universe\(^{10} \) and the modified universe in which \( X \) is excluded, respectively (Barnett and Seth 2014a). The measures based on Granger causality principle are: Granger Causality Index (GCI), Directed Transfer Function (DTF) and Partial Directed Coherence (PDC). These measures are defined in the framework of Multivariate Autoregressive Model. For a given multivariate time series \( U(n) \in \mathbb{R}^M \) with \( L \) number of samples \( (n = 1, \ldots, L) \), a strictly-causal multivariate autoregressive (MVAR) model of order \( p \) is defined as:

\[
\begin{bmatrix}
U_1(n) \\
\vdots \\
U_M(n)
\end{bmatrix} = \sum_{k=1}^{p} A_k \begin{bmatrix}
U_1(n-k) \\
\vdots \\
U_M(n-k)
\end{bmatrix} + \begin{bmatrix}
\varepsilon_1(n) \\
\vdots \\
\varepsilon_M(n)
\end{bmatrix}
\]

Equation 2.5-2

where \( \begin{bmatrix}
\varepsilon_1(n) \\
\vdots \\
\varepsilon_M(n)
\end{bmatrix} \) is the residual matrix, a \( n \)-dimensional stochastic process normally distributed real valued with a zero-mean (white noise vector) and a diagonal covariance \( \Sigma = \langle \varepsilon \varepsilon^T \rangle = \text{diag}\{\lambda^2_{kk}\} \) operator. \( M \) denotes the number of channels and the \( n \times n \) real-valued matrix \( A_k \) is the regression coefficients. The regression coefficients represent the predictable structure of the data, the residuals the unpredictable. For a valid GC the VAR coefficients \( (A_k) \) must be:

- **Square summable**, i.e. \( \sum_{r=1}^{p} \| A_r \|^2 < \infty \);
- **Stable**, means that these coefficients define a covariance-stationary process, or if the characteristic polynomial is invertible on the unit disc \( |z| \leq 1 \) in the complex plane (Spectral radius of VAR as: \( \rho(a) \equiv \max_{|z|=1} | (z^{-1}) \) and for stability \( \rho(a) < 1 \);

Methodological approach

To obtain the GCI (Granger Causality Index) (considering the conditional way) is necessary to measure logarithm of the covariance matrix of the full and of the partial system:

\[
F_{Y \rightarrow X|Z} = \ln \left( \frac{\Sigma_{XX}}{\Sigma_{XX|A}} \right)
\]

Equation 2.5-3

The bigger GCI the better we can infer that “there is a linear causal information flow from \( Y \) to \( X \)”. But to get such values a combination of step is needed. Based on the logical system of (Barnett and Seth 2014a) MVGC toolbox, a 4-step process is needed, see Figure A-0-4. First it is necessary to determine the model order,

\(^{10} \)Universe of all channels
which can be done by the Akaike Information Criterion (AIC) or the Bayesian Information Criterion (BIC). Then it is necessary to estimate the VAR model. This is done by a standard ordinary least squares (OLS). After estimating the VAR model, the autocovariance matrix is generated. This step allows to check for errors in the data (e.g. stability of the covariance matrix). Finally, the Granger causality index is estimated, followed by statistical analysis.

The Granger causality calculation is estimated in the conditional case (the time domain). To understand the conditional case, let suppose that the universe \( U \) of known recorded variables splits into three jointly distributed (i.e. inter-dependent) multivariate processes \( U_n = (X_n, Y_n, Z_n) \), and we wish to eliminate any joint effect of \( Z \) on the inference of the GC from \( Y \) to \( X \). The VAR(p) \((p(Y(t + 1) \in A|U(t) \neq p(Y(t + 1) \in A|U_{-\alpha}(t)\)) splits in the three multivariate processes given the next full and reduced regression for \( X_i \):

\[
X_i = \sum_{k=1}^{p} A_{ix,i \cdot X_{n-k}} X_{n-k} + \sum_{k=1}^{p} A_{ix,i \cdot Y_{n-k}} Y_{n-k} + \sum_{k=1}^{p} A_{ix,i \cdot Z_{n-k}} Z_{n-k} + \varepsilon_{X_i}(n) \tag{2.5-4}
\]

\[
X_i = \sum_{k=1}^{p} A_{ix,i \cdot X_{n-k}} X_{n-k} + \sum_{k=1}^{p} A_{ix,i \cdot Z_{n-k}} Z_{n-k} + \varepsilon_{X_i}(n) \tag{2.5-5}
\]

The \( F(Y \rightarrow X_i | Z \in \ln \Sigma' XX \Sigma' XX) \) theory provides a natural framework for the analyses of parametric data modelling. Thus, the likelihood ratio statistic is an appropriate comparative measure for nested models of the form \( X_i = \sum_{k=1}^{p} A_{ix,i \cdot X_{n-k}} X_{n-k} + \sum_{k=1}^{p} A_{ix,i \cdot Y_{n-k}} Y_{n-k} + \sum_{k=1}^{p} A_{ix,i \cdot Z_{n-k}} Z_{n-k} + \varepsilon_{X_i}(n) \) Equation 2.5-4 and \( X_i = \sum_{k=1}^{p} A_{ix,i \cdot X_{n-k}} X_{n-k} + \sum_{k=1}^{p} A_{ix,i \cdot Z_{n-k}} Z_{n-k} + \varepsilon_{X_i}(n) \) Equation 2.5-5 in this framework. To be precise it is a statistic test for the null hypothesis of zero causality given by: \( H_0: A_{XX,i} = \cdots = A_{XX,p} = 0 \). This motivate the definition of the GC statistic as the appropriate likelihood ratio. Now standard Maximum likelihood theory tells that the likelihood for a realization of length \( F(Y \rightarrow X_i | Z \in \ln \Sigma' XX \Sigma' XX) \) Equation 2.5-3 is proportional to \( | \Sigma | ^{-(m-p)/2} \), where \( | \Sigma | \), the generalized variance of the model, is the determinant of the residuals covariance matrix. Thus the (conditional, time-domain) G-causality from \( Y \) to \( X \) conditioned on \( Z \), equals to Equation 2.5.3, and it must be read as "the degree to which the past of \( Y \) helps to predict \( X \), over and above the degree to which \( X \) is already predicted by its own past and the past of \( Z \)."

A case of particular importance is that of pairwise-conditional GC. Given a universe \( U \) of variables comprising \( n \) (recorded) jointly distributed univariate processes \( U_{t0} \ldots U_{tn} \), it is often of interest to estimate the G-causalities between pairs of variables \( U_i, U_j, i \neq j \), given by:

\[
\zeta_{ij}(U) \equiv F_{Y \rightarrow X_{ij}} \tag{2.5-6}
\]

where the subscript \([ij]\) denotes omission of the \( i^{th} \) and \( j^{th} \) variables in the multivariate universe \( U \). Finally, it is necessary to establish the statistical significance of the estimated causality against the null hypothesis. Under the null hypothesis of zero causality, \( (m-p) \) \( F_{Y \rightarrow X_{ij}}(u) \), the GC estimator scaled by sample size has an asymptotic \( \chi^2(d) \) distribution. Under the alternative hypothesis the scaled estimator has an asymptotic non-central-\( \chi^2(d;\nu) \) distribution, with noncentrality parameter \( \nu = (m-p) \) \( F_{Y \rightarrow X_{ij}} \) equal to the scaled actual causality. Test the Type II and Type I error with the F-distribution. However, as the reader will see further one, in this project more powerful means to statistically infer significant results were developed.

Implementation Complications

The GC analysis has copulated some implementation complications. The reported problems with time series data likely come from the following causes (Barnett and Seth 2014a; Bressler and Seth 2011; Barnett and Seth 2011; Flamm et al. 2013; Omidvarnia et al. 2014b; Haufe et al. 2013):
• **Non-stationarity** – Usually EEG data is non-stationary, since its mean and standard deviation vary in time. So it is necessary to find ways to correct this issue and then test its accuracy. The principal stationary test is to verify if the spectral radius, of the estimated full VAR model, is less than one (stable). If this condition is not satisfied then the data might be non-stationary and the analyses cannot be prosecuted. Solutions to this issue are going to be discussed in the next chapter 3. Briefly, they can be based on pre-filtering and conversions of non-stationary data into stationary data, or by dividing it into overlapping/non-overlapping windows, which implies a tradeoff between likelihood of stationarity (shorter->better) and accuracy of model fit (longer->better), or by implementing detrending and demeaning;

• **Colinearity (Volume conduction)** – occurs when there are linear relationships between variables (i.e. between individual time series) in multivariate time series data. There are solutions to eliminate it, such as: to eliminate linear dependencies, possibly via a Principal Component Analyses(PCA), or factor model approach (Flamm et al. 2013), by a source reconstruction techniques (e.g. Independent Component Analyses (ICA)) or by filtering. Barnett suggested that one should filter (notch filter) data as little as possible, and only as far as is necessary to render non-stationary data into stationary. This because values empirically filtered of stationary time series data may severely compromise statistical inference of causalities estimated in sample. Haufe and his colleagues have been studied the effect of reducing this issue, by comparing different reconstruction tools of source space, and its effect on detecting true causality, by testing in simulated data, see section 3.4 - Colinearity, for a more complete insights.

• **Long-term memory** – is related with the fact that the auto-covariance does not decay exponentially;

• **Strong moving average component** – the data, although stationary, might contain a strong (and in particular a “slow”) moving average component, violating the condition, meaning that GC is very sensitive on noise (reduce sensitivity) and mixing (error type I);

• **Heteroscedasticity** – the variance of the residual terms does depend on the actual values of the process;

• **GC only models linear interactions** – Even though it can be an issue, the equivalence with transfer entropy for Gaussian variables (Barnett L., Barrett A.B., Seth A.K. 2009) meaning that linear VAR modeling is guaranteed to capture all the relevant variance for Gaussian data, and most of it for approximately Gaussian data. And also, even substantially nonlinear interactions that unfold over a small number of observations can sometimes be approximated by a (linear) VAR model with a large model order.

2.5.2. Emerging directions with brain connectivity estimators

Brain connectivity estimators have been studied especially in the fMRI modality. However, there is already some literature around the different types of implementation in EEG data. Still those works are very often more related with methodological proofs of concept, i.e. studies with simulated data, (Ding et al. 2000; Haufe et al. 2013; Barnett and Seth 2011) than properly studies on quantifying brain effective connectivity. There have been also some papers comparing methods (Silfverhuth et al. 2012). Silfverhuth compared measures with different theoretical backgrounds, i.e., information theory, parametric-modeling based or phase related. They conclude that PDC is better suited for detecting directionality and dealing with SNR. Though, they name a lot of limitations, especially the fact that they just test on simulated data.

At this point, is important to settle that brain connectivity estimators (connectivity analyses) can be arranged along a scale from model-free to model-based tools. On one side there are the Model-free approaches, as mutual information theory (TE), imposing the fewest assumptions on the data (being
challenging to estimate given the limited data and the lack of assumptions). At the other extreme, methods like dynamic causal models (DCM) (Friston K.J., Harrison L. 2003), specify detailed state-space models that describe how dynamics are coupled at the level of underlying mechanisms (the “state” equation), and how these dynamics manifest in observable data (the “observation” equation). By jointly inverting candidate models and finding the most likely (among a set of competitors), DCM can address claims about physical-causal mechanisms. Granger causality, occupies a middle ground position, where is verified a combination between a generic dynamical model (i.e. AR process) and a liberal structural model (i.e. no assumptions are made about the underlying structural connectivity). When comparing DCM with GC, the latest can be applied directly to any time-series to obtain a measure of coupling among empirically sampled neuronal systems. In addition, it can also be applied to larger networks, increasing its importance in the neuroscience field. A simple way to compare both is that GC can provide ‘data-driven’ hypotheses for subsequent testing by explicit specified DCMs, see Figure 2.5-1. Adding to that, it is possible to say that GC and DCM ask very different questions, where choosing one or the other (or both) depends on whether the investigator is interested in describing the data in terms of information flow (GC) or exposing the underlying physical-causal mechanism (DCM).

This thesis aims to point an emergent direction of applications: GC in a TMS-EEG paradigm. It is possible to find inspiring results in the papers of (Flamm et al. 2013) and (Barnett and Seth 2015). The paper from Flamm, implements a factor modeling coupled with GC\(^{11}\), allowing the model to choose the best suited variables to build the AR model (applies PCA). With this, they are able to apply it on an epilepsy seizure (a non-stationary situation), achieving results which are in agreement with the expected seizures patterns determined by experts. The work by Barnett and Seth, is an attractive opportunity to analyze G-causality in the framework of ARMA models with a steady-state, which augment standard AR models with a moving

\(^{11}\) See annex 1 for obtaining further knowledge on how the process is implemented.
average (MA) component. This could be very useful since ARMA models behave sensibly under both downsampling, additive noise and non-stationary moments. However, this thesis will not approach such models.

Even though it is out of the scope of this thesis, it is still important to mention the fact that GC of electrophysiological data offers the important advantage of spectral analysis; that is, G-causal influences can be tied to specific frequency bands (Geweke 1982). This is very useful when testing neurophysiological hypotheses that attribute specific functional roles for different neural oscillations. For example, recent applications to monkey electrophysiological data have revealed “top-down” G-causality influences in the alpha and beta ranges, and “bottom-up” influences in the gamma range, consistent with popular predictive processing frameworks band-limited G-causality directly in the frequency domain (Bressler and Richter 2015).
Chapter 3

3. Managing Granger Causality over EEG signals

3.1. Introduction

This chapter takes the reader through the major steps of choosing and implementing a method of effective connectivity over EEG signals. These stages include first a model comparison between Granger Causality methods and Transfer Entropy, then an analysis over the problem of non-stationarity and finally an analysis on the collinearity. During this chapter the various stages are described and the results are discussed, as the motivation for some of these experiments originate from previous results, and they lead to better interpretation of the following chapter (Chapter 4). In the end, a summary of all findings and main ideas are exposed regarding the content of this chapter.

3.2. Model Comparison – Granger Causality vs Transfer Entropy

The first goal of the project was to obtain an understanding of the potentialities of Granger causality and apprehend the comparative results against other effective connectivity measures. Thus, in this section the focus was on the basic implementation of GCI, concluding it with results comparisons with another theory for brain connectivity estimators – Factor Modelling approach with GCI (FM-GCI) and Transfer Entropy (TE). At this level, all the results are based on simulated data.

3.2.1. Methods

With the intention of evaluating the performance of GCI, a comparison was made between it, a Factor Modelling\(^\text{12}\) approach with Granger Causality Index (FM-GCI) (Flamm et al. 2013) and TE\(^\text{13}\) (with non-uniform embedding & different entropy estimators – Binning estimator and Nearest Neighbor estimator). The algorithm implemented was based on the toolbox MVGC (Barnett and Seth 2014b) for the GCI analysis and on the MuTE (Montalto, Faes, and Marinazzo 2014) for the TE analysis. Such implementation followed the principal theoretical rules of Granger causality. However, an exploratory approach over the parameters, such as number of trials and length of the window, was made in order to better understand the behavior of GC. This code, and all the algorithms for this project, were run in Matlab 2011a (Mathworks Inc., Natick, MA, USA). The pipeline for the GCI algorithm can be seen in Figure 4.2-3 and Figure A-0-4, where it is summed up how GCI was performed and tested against a statistical null distribution, and also can be read in section 4.2.4.

To achieve the comparison purpose, a 5-dimensional time-invariant strictly-causal MVAR-process plus a linear superposition of sparse uniformly distributed random sources with approximately 50% nonzero entries within a specific interval, according (Omidvarnia et al. 2014b), was simulated (see Annex 2). The parameters mentioned above (number of trials and length of the data) were varied to generate measures of comparison. For a value of ‘number of trials’ bigger than one, an ensemble mean was performed over each connections after all single trials had generate causality results.

\(^{12}\) A short summary of Factor Modelling approach on Granger Causality can be seen in Annex 1.

\(^{13}\) A short summary of Transfer Entropy theory can be seen in Annex 1.
Figure 3.2.1 – Demonstration of the diagrams of causality obtained by the three different methods implemented B-D). A) Label of true causalities (represented by a number ‘2’). B) Granger Causality Index. C) Factor Modelling in combination with Granger Causality Index. D1) Transfer Entropy (Non-Uniform embedding with binning estimator). D2) Transfer Entropy (Non-Uniform embedding with nearest neighbor estimator). In B) the left plot shows the values for GCI ‘from’ channels in the columns ‘to’ channels in the lines. The middle plot represents the respective p-values for the GCI, and finally the right plot represents the statistical significant values for the GCI. In C) there is a figure with 20 subplots. Each one of them represent the causality ‘from’ channels in columns ‘towards’ channels in lines. The x-axes is represents the value for the determinant of the latent variables (see Annex 2), and the y-axes is represents the p-value for the F-test ($H_0: \hat{A}_{ij}(m) = 0$ for all m). The circles plotted in turquoise define causal relations, whereas the circles in red do not define causal relations. For both figures D) there is a representation of the TE values averaged across trials, where the colors define the magnitude of those values. The interpretation for these plot is that channels in the lines ‘cause’ channels in columns. All of these results are made over the simulated data (mentioned in the text), have 2000 data points and here they are averaged over 10 trials/subjects.
Figure 3.2-2 – A) Variation of Sensitive (left) and Specificity (right) over length of data (x-axis), with the number of trials fixed (number of trials = 1) for the four methods: FM-GCI (red), GCI (green), TE with Binning Estimator (blue) and with Nearest-Neighbor (cyan). B) Variation of Sensitive (left) and Specificity (right) over number of trials (x-axis), with the length of data fixed (data points = 512), for the four methods: FM-GCI (red), GCI (green), TE with Binning Estimator (blue) and with Nearest-Neighbor (cyan).
3.2.2. Results

The results for the connectivity for the three methods are depicted in Figure 3.2-1, B) – Granger Causality Index, C) Factor Modelling with Granger Causality Index, D₁) and D₂) Transfer Entropy with Non-Uniform embedding with binning estimator and nearest neighbor estimator, respectively. In A) it is represented the label for the true connectivity generated with the simulated data. These results were generated with a 2s-data, with a sampling rate of 500Hz over 10 trials. For the GC models the lag in time (model order) was defined for \( p = 3 \), due to the order of lags from the simulated system.

The flow of information in the Granger Causality Index (GCI), regarding the simulation conditions, showed some imprecisions. On one hand, it did not display false negatives when comparing with the label (causal relations between 1→2, 1→3, 1→4, 4→5, and 5→4). On the other hand, both in the p-values and in the significance\(^{14}\) matrix it showed the presence of some false positives (naming for instance, 1→5, 2→3, 3→5). The connectivity matrices generated by the FM-GCI, present in C), showed results that lead to very few conclusions on inferring patterns of information flow. Mainly because, a majority of turquoise point were seen in that figure. They represented the causality over that channels combination, being in the conditions defined by FM-GCI threshold conditions - determinant of the latent variables > 0.05 (x-axes) and the p-value for the F-test < 0.05 (y-axes), see Annex 1. In contrast, the amount of magenta points was very low (only shown in 3→4 and 4→3), proving the lack of capacity of this model to detect true negatives. Regarding the application of TE to the simulated data, the results are depicted in D₁) and D₂). The color scale can be translated by: the hotter the color, the more significant is the causality between those two channels. As it can be read in the caption of Figure 3.2-1, the causality is represented from the channel in the row to the channel in the column (contrary of GCI and FM-GCI matrices). The results returned no false negatives. However, there was a distortion in the false positives, which was higher compared to GCI, leading to a smeared conclusion over the information flow for both transfer entropy estimators. Nevertheless these false positives, highly expressed, were obtain in lower values/intensities compared to the true connections.

In order to check for better parameters fit and to try to grasp an idea over the best-suited method to apply to real data, a more challenging situation was performed, by testing both the data length and number of trials. These results can be found in Figure 3.2-2. In A) it is depicted the variation of sensitive (left) and specificity (right) over length of data, with the number of trials fixed in ‘1’. On the long run (with the increase of data points) all four methods showed a sensitivity of one, proving that the more information the system had, the less false negatives they obtained. Of note, is the fact that FM-GCI and TE-NN were quite fast in reaching a value of sensitivity of 1 (less than 100 data points). This can be explained for FM-GCI by its easiness in separating components in such a small data set, leading to a higher probability of each component explaining causality. Still in A), on the right side, it is depicted the variation of specificity over the data length. They showed a general decrease for the methods based on the GCI, while in the ‘long-run’ the methods based on TE show an improvement of specificity values. The performance of the GCI methods can be explained due to the decrease of the ratio between the model order and the size of the data. In a way, it under-fit the model, increasing the possibility of obtaining connections that are causal related. For FM-GCI it was even more severe because this model reduces even more the number of variables in the system, allowing a less conservative explanation of the variance, due to the few amount of channels (over the 5 channels, only a 3 subset of channels are selected as principal components – channels, see Annex 2). Regarding the TE methods, they increased in the ‘long-run’ because they are based on theory of clustering points of the same class

\(^{14}\) Significance – Value that represent the statistical significance adjusted for multiple hypotheses, corrected with False Discovery Rate (FDR). A value of one becomes significant after the family-wise correction.
together. Therefore, the higher amount of these points, the higher the probability of fitting only truth connections in the model – this works for both TE methods.

In Figure 3.2-2 B), the number of trials changed for a fixed number of 512 data points (value chosen over the similarities of the characteristics of the real data). Both plots represented the average over the performance of each causality methods on each trial. The sensitivity - B) left plot - was the highest possible (i.e. equal to 1) for all models. Regarding the specificity - B) right plot - it was depicted in general a negative correlation between the value of specificity and the number of trials. This decrease was steeper for the GCI, showing that this method, even though it obtained the higher values for this data length, it was also the more inconstant over trials. Nevertheless, it showed that for short number of trials it had significant higher results (a value of 1 compared to values of 0.7 for the other methods). Again, FM-GCI showed weaknesses on getting high values of specificity. Regarding the TE methods they seem constant over time, which is according to what was expected, due to its way of clustering data points together be affected by the trials. Probably, a reason for the changeover trials was the presence of noise in the simulated data. These noise affected more the GCI methods than the TE, according to what it is depicted in Figure 3.2-2.

3.2.3. Discussion

As a first approach it was essential, for the following report, to test the GC against other methods of effective connectivity. Regarding the results obtained in the first approach (Figure 3.2-1), GCI proved to show better results, emblazing the effect of true influences. The result proved to remain across the other two tests (Figure 3.2-2), where GCI showed an higher consistency in the specificity, having in mind a specific set of parameters (number of trials = 1 and data length = 512).

GCI is a method that accounts for the past information in a linear way. It flashback the information for a specific number of times, model order $p$, allowing the flexibility and increase the performance. The results showed an heuristics approach trying to bounder the limits in the relations between the model order (constant) and the number of trials and data points. These relations was used as guideline further in the thesis (around 500 data points), also allowing to disregard the behavior of the model on the long run.

An important asset of this simulated data was the variation of noise over trials. It allowed the perception of how different methods handle the data. When comparing the number of trials being averaged, it is possible to see the effect of such noise in the outcome. For one trial, GC and TE (both methods) present results for the specificity of 1 and in the range of 0.6 to 0.7 respectively. However, when increasing the number of trials, it was shown a decrease of GC and TE (both methods), showing that both are affected by such noise. Being possible to conclude that the most suitable parameters for GC average results would be of 1 trial.

Considering the FM-GCI, this is a method proposed by Flamm (Flamm et al. 2013) and it tries to remove the linear cross-talk between signals, however by doing so it under-fit the model with potential explanation of the noise. Such effect was well shown on the results of specificity (many false positives detected). With this, it is argued that this technique only shows some interest, when applied in time-series with a bigger amount of variables. However, it might be a good solution for the issue of colinearity; it will not be used further on this paper. This because other methods accounting for such issue are more accurate and well-studied, such as ICA$^{15}$ (Delorme et al. 2011).

$^{15}$ICA – Minimized mutual information, using measures like maximum entropy, diving data in as many independent components as channels the original data.
According to (Vicente et al. 2011), TE even being a model-free approach showed some limitations. First, in contrast to model-based methods, the detection of effective connectivity via TE does not entail information on the type of interaction, leading to a high sensitivity for all types of dependencies between two time-series, on this way nuisance dependencies are detected, decreasing the specificity. Secondly, if the embedding parameters for the construction of the state space is not chosen correctly, there will be reported a detection of false positive, having this implications if the data is found to be non-stationary (because the same \( \tau \) will be chosen for the all interval). This was probably one of the causes of the TE performance. Thirdly, using a non-linear approach such as TE, in a linear model, proved to be outperformed by GC. Theoretically TE also shows some weaknesses, especially for BIN and NN. In first place, they are quite affected by very noisy measurements (Silfverhuth et al. 2012). Then regarding the BIN, this method requires a big amount of time series to offer worthy results (Montalto, Faes, and Marinazzo 2014). Also, this approach is more selective with respect to the target variables for each driver and less sensitive to the confounding effect of volume conduction. Regarding the NN, it is computationally costly and depends on several \( a \ priori \) parameters to be defined for each signal pair. In addition, TE requires a large amount of continuous data, which can be problematic in the case of task-related, transient connectivity. Finally, TE does not have a specific statistics behind it. All of these helped on choosing for causality analysis GC.

As emphasized in section 0, the results of brain connectivity estimators, especially GC, are much affected by the non-stationarity of the time-series and by the over-fitting of the model (colinearity). This section tried to disentangle those criterions from the original data, as to have a measure of standard comparison between different brain connectivity estimators. The simplification here made was intended to provide the model with data that could be compared easily. However, it might be argue that by testing a time-series in such a primitive model the causal results does not provide trustful information to real data. It raises two main limitations, once the simulated data does not truly represent a physiologic response of the brain. Firstly, the non-stationarity is not being accounted for, since the model simulated is linear. Secondly, the number of variables (only 5) avoid the over-fitting of the model and also the multi-colinearity. Issues widely present in an EEG data set. In the next sections more complex analyzing methods are going to be studied and discussed (3.3 & 3.4). Nevertheless, all things considered, Granger Causality prove to be the best suited method throw the different tests.

### 3.3. Non-stationarity

Any GC implementation as mentioned in section 2.5.1, has to be performed over stationary data. In order to be stationary, data should be stable. In other words, stability is represented by a covariance-stationary (CS) process where the mean and the variance of each variable cannot vary in time. Adding to that, it has to be an unit-root free process. Accounting for this, in this section, tools to reduce and remove the non-stationary over the data allowing a trustful GC analysis were tested.

#### 3.3.1. Methods

There are specific tools to detect the stability of the VAR model, where some tests control for the presence of 'unit roots' within the data, others supervise the spectral radius of the VAR model. Accounting for the first method, the intuition behind is to test if a variable is covariance-stationary (CS), testing if such variable does not exhibit a tendency to return to a constant (or deterministically trending) mean. The Augmented Dickey-Fuller (ADF) test identifies the absence of this condition (absence of a unit-root) (A. K. Seth 2010), if it verifies it, then the trial is non-stationary. The second analysis, based on the spectral radius, looks whether the
coefficients from the VAR model define a stable model. They do so, iff the characteristic polynomial is invertible on the unit disc |z|≤1 in the complex plane. In other words, the condition of stability is: $\rho(A)^{16}<1$, anticipating possible ill-conditioned VAR models that would fail in exponential decay of the autocovariance. In this part of the project, data was only tested for the presence of ‘unit-roots’. Once, testing for the condition of stability is an intrinsic process of the pipeline of GC analysis, it only indirectly test the approaches implemented in this section.

Two independent simulations were made to test for the non-stationarity. The first one was similar to the data shown in the previous section. In the second situation, data was simulated under a time-varying model, see Annex 2. Both models were first tested under a ‘no correction effect’ for non-stationarity. Secondly, it was applied, by this order, detrending and demeaning, see section 0. The detrend accounts for the removal of deterministic linear trends. Demeaning assures that, on a multi-trial level, the non-stationarity can be removed by subtracting the ensemble mean\(^{17}\), removing the variation of the mean during each trial. Similarly, the standard deviation can be controlled over the intra-trial variation by dividing each data point by its ensemble standard deviation. Finally, it was tested if the ensemble of trials would help increasing the stability of the VAR model. This was performed by applying the so called Vertical Regression (VR)\(^{18}\) approach, proposed by Ding (Ding et al. 2000). Such technique is mainly used when causal relations among variables may be changing over time, as after Event-Related Potentials (ERP) or Transcranial Event-Related Potentials (TEPs), but can also be helpful to reduce the non-stationary issue, by providing more data to the VAR model estimation. It simply clusters together a selected amount of trials and builds over them the VAR model, decreasing the variability that may affect each trial when estimating the VAR over the OLS. This allows a locally CS by decreasing the spectral radius. Such test was performed over a data corrected for non-stationarity after applying detrending and demeaning.

3.3.2. Results

The two simulated datasets where tested against non-stationarity. The results outline that, depending on the type of statistical test used, the results can be misleading. As it is depicted in Figure 3.3-1 A) and B) for the time-invariant data the ADF test showed no evident changes between applying, or not, methods for removing the non-stationarity. Due to that, another test was used to measure the efficacy of implementing detrending and demeaning. This method is named KPSS unit-root statistical test\(^{19}\) or Kwiatkowski–Phillips–Schmidt–Shin (KPSS) test, it was used to test the null hypothesis that an observable time series is stationary around a deterministic trend. The different results of detecting non-stationarity with the two different statistical methods can be compared in Figure 3.3-1. Of note is the fact that for the time-invariant data, the method of ADF (almost 100% of trials had non-stationary channels) was much more sensible than KPSS (less than 3% of trials had non-stationary channel). The inverse occurs for the time-varying data (Figure 3.3-1 C) and D)) where for ADF, no non-stationary channels over trials were seen, yet for KPSS there were around 50% of channels on those circumstances. This might have to do with the fact that ADF only looks for unit-roots, and on a

\(^{16}\) Where: $\rho(A) = \max_{\phi_A(z)=0} (|z|^{-1})$ is the spectral radius.

\(^{17}\) Ensemble mean – averaging the values for each variable at each time point across trials (Ding et al. 2000).

\(^{18}\) Vertical Regression (VR) – Combine information from different trials over the same on-set and same time period, in order to build only one VAR model. Allowing to provide the VAR model with more data over a short time period, making it more stable.

\(^{19}\) KPSS - intend to complement unit root tests, e.g. ADF. By testing both the unit root hypothesis and the stationarity hypothesis, one can distinguish series that appear to be stationary, that appear to have a unit root, and for which the data (or the tests) are not sufficiently informative to be sure whether they are stationary or integrated.
signal contaminated by additive noise/linearly mixed noise, which even stationary per se, its effect changes
the properties of the signal intensively, leading to unit-roots. However, when the signal is perturbed by a
variation in time the effect of the noise is not as relevant as it was before. Regarding the behavior of KPSS,
as a result of looking for stationarity, it detects constant patterns in the time-invariant simulated data and
variations of those patterns in the time-varying simulated data, regardless the additive noise.

The average over trials of the GC results for the simulated data (1st line of plots – time-invariant; 2nd line
of plots – time-varying) is depicted in Figure 3.3-2. This figure was intended to show the differences between
not applying or applying corrections (detrending and demeaning) for the non-stationarity on both the
simulated data (by comparing 1st column with 2nd column, respectively). The Figure shows what was
expected. Regarding the time-invariant data, there is a little effect over the output of the analysis, only with
a small variation on the p-values. However, when looking at the time-varying results (Figure 3.3-2 C and D)
, it is possible to detect variations on the p-values matrix between them. After controlling for the non-
stationarity, the p-value matrix in combination with the GC matrix is closer to the label matrix (Figure 3.3-2
E)).
The effect of ensemble together a specific amount of trials in order to alleviate the effect of non-stationarity when the data is already corrected for non-stationarity by the method mentioned above is depicted in Figure 3.3-2. On one hand, the ensemble trials do not affect the sensitivity, as it is always maximized. On the other hand, the specificity changes for both datasets. It is possible to notice a maxim when ensemble together five trials, being this number the one that optimizes the results. It is worth mention that the effect over both datasets is very alike. Albeit there is lag in amplitude between them, the behavior of the evolution over ensemble trials is very similar.

Figure 3.3-3 – Average over 200 trials of GC results for the time-invariant simulated data without and with Non-stationary correction, A) and B) respectively. GC results for the time-varying simulated data without and with Non-stationary correction, C) and D) respectively. E) Label of true causalities for the time-varying data (represented by a number ‘2’). For an interpretation of each of the different subplots see Figure 3.2-1.

Figure 3.3-2 – Variation of Sensitive (left) and Specificity (right) over the number of ensemble trials for the time-invariant (left) and time-varying (right), simulated data.

The effect of ensemble together a specific amount of trials in order to alleviate the effect of non-stationarity when the data is already corrected for non-stationarity by the method mentioned above is depicted in Figure 3.3-2. On one hand, the ensemble trials do not affect the sensitivity, as it is always maximized. On the other hand, the specificity changes for both datasets. It is possible to notice a maxim when ensemble together five trials, being this number the one that optimizes the results. It is worth mention that the effect over both datasets is very alike. Albeit there is lag in amplitude between them, the behavior of the evolution over ensemble trials is very similar.
3.3.3. Discussion

On this section, there were presented and tested solutions to solve the non-stationary issue. The steps took into considerations the progresses made by (A. K. Seth 2010; Barnett and Seth 2011; Barnett and Seth 2014b). As a general observation, it is possible to infer that detection of non-stationarity was achieved. The methods presented showed that KPSS enhance better the non-stationarity from the model 1 (time-invariant) vs model 2 (time-varying). This was expected regarding the fact that ADF compared with KPSS only look for the presence of unit-roots, while KPSS also search for other patterns of non-stationarity. However, both are affected by the superimposed noise, disturbing the behavior of the detection model, since no signs of change were seen after applying the non-stationarity corrections methods (detrending and demeaning). Albeit, those methods did not show differences, it still can be argued that, even though they may not improve the output of the model, they lead to a more suited MVAR model. Mainly, they allow the decrease of the spectral radius, preventing an ill-conditioned model. Therefore, the effect of applying those corrections leads to a better interpretation of the GC results, being an important step in the pipeline analysis.

Time series can often be rendered stationary through another transformation – the difference (A. K. Seth 2010). This involves using the first derivative of the time series rather than the raw values. Differenced time series are then tested for stationarity. If they are still non-stationary, the process can be iterated until they pass the test. While in computational point of view, this approach lead to faster and more stable analysis, it may obscure the interpretation of Granger results (which might reflect velocity or acceleration of change in activation rather than raw activation in itself). Therefore, in this project the decision was made as to not implement it. Instead the solution can be achieved by filtering, since it helps removing non-stationary, more specifically an high-pass filter, by removing the slow oscillations (0Hz – 2Hz) of the wave. However, this effect was not taken into account here because it belongs to the preprocessing of the data, and if performed as a tool to remove non-stationarity in itself, it could control the outcome of the model leading to erroneous conclusions. Even though (Florin et al. 2010; Barnett and Seth 2011) state that the VAR model is theoretical invariant to filtering, it is still necessary to adapt the model order in order to maintain the same GC result, and sometimes this means using an infinite model order. To avoid such a situation, in this project, filtering will be conducted before ICA, which controls such effect.

These analysis were intended to be a proof of concept as so, it helped to direct the future of the thesis. By this, it means that some of the results here studied are not going to be accounted further one. Starting by the fact that, according to the goals of this thesis, rapid variations of the signal are not studied, meaning that this project is not about TEP in TMS-EEG experiments. Therefore, the test on time-varying data was only shown to support the efficacy of detecting non-stationary data. Adding to that the different complexity between time-invariant and time-varying model (i.e. different dimension and different model order) diminished the precision in the comparison. Finally, on the scope of this thesis and regarding the VAR model, the pipeline only state that is necessary to obtain a stable VAR model. For that, it is necessary to check if the data is unit-root free, and use it as the only criterion for non-stationarity, avoiding losing more data than the required (by including more limitations of non-stationarity with KPSS). Thus, from now on, KPSS is disregard and the method to test for non-stationarity is the ADF.
3.4. Colinearity

The other major issue of the GC implementation on the neurophysiological data is the colinearity. It arises due to the volume conduction effect, leading to a smearing of the EEG signal across the sensors. In other words, strong linear relationships among variables emerge due to the signal spreading of the same brain source over different EEG sensors. In order to account for this, in this section, it is included an analysis that wanted to show how and to what extent overfitting influence the scale of the result, giving an heuristic on overfitting. Then there are discussed simple methods to remove some of the volume conduction effect by using a well-established inverse source reconstruction techniques.

3.4.1. Methods

Testing the colinearity might be challenging due to the necessity of, not only simulate sources that will activate the sensors (variables), but also because is necessary to simulate linearly mixed noise. In this project, it was studied the effect of increasing the number of channels in the dataset – searching for ‘how was GC influence by the over-fitting of the data’. An ERP simulated data was used\(^{20}\) to test it (Figure 3.4-1), where the amount of channel was varied. It was then checked how the maximum value of GC\(^{21}\) changed by the increasing amount of channels, and also how did the correlations coefficients average changed according to the size of the time-series. An higher correlation (absolute value near 1) indicated an higher colinearity.

3.4.2. Results

The reader can see on Figure 3.4-2 A) the increase of the mean correlation coefficient, which is mainly due to the increasing number of channels that showed a response over the unique simulated dipole. In other words, the increase on the number of channels, in response to the only simulated dipole (specific of the model), leads to an increase in the probability of two channels be correlated, and to the matter of Granger

\(^{20}\) It was downloaded from: http://www.cs.bris.ac.uk/~rafal/phasereset/, these scripts allow to generate simulated EEG signal composed of superposition of phasic peak and noise, where the signal is composed by one active source.

\(^{21}\) The GC values are obtained by implementing the best suited parameters tested before, and also using an sampling rate, length of the data and number of trials that will match the real data tested in the next chapter.
Causality, to be more colinear. However, such process showed only the behavior over one source, disregarding the influence of many sources. Also the fact that for few amount of channels, special for 5 and 10 channels, the variables chosen did not include channels with an high correlations among them, see Figure 3.4-3. Leading to lower colinearity among them, explaining in part the behavior of the regression for the lower amount of channels.

Figure 3.4-2 – A) Evolution of correlation coefficient over the increase of number of channels. B) Evolution of the maximum GCI value and the different between the maximum and the minimum GCI value, over the increase of number of channels.

Figure 3.4-3 – Correlations matrix across all channels for simulated EEG data with the following number of channels, n=5, n=10, n=15, n=20, n=25, n=30. The closer the value to 1 (white), the more correlated are those two channels. The diagonal shows the correlations between the same channels, leading to a correlation of 1.
Such limitations motivated to add another approach on how the overfitting could be measured. This was then studied throw the evolution of the maximum value of GC (maxGCI) and the difference between the maximum and minimum value of GC over the number of channels (diffGCI). Since the connectivity bound across channels within the model is constant regardless the number of variables, due to the standardize error and only one dipole, theoretically the maxGC and the diffGCI value can only be affected by overfitting the GC model with more data. The results can be seen in Figure 3.4-2 B). These showed a plateau where the behavior tends to be best suited, due to its higher values. Regardless the limitations of this approach, these findings lead to the idea that whenever there is an increase of the number of channels over a certain threshold there will be an overfitting of the model (since the GC max value is drastically reduced over models with more than 20 channels). The correlation coefficient (CC) as a function of the number of channels, Figure 3.4-3 A), supported it, since it had higher values in the interval [20 35] than in the interval [1 20].

3.4.3. Discussion

Even though this section addressed the colinearity issue, it was pointing more towards the effect of the number of channels on the dataset (overfitting of the model). The overfitting and colinearity are two problems dependent one from the other where, the higher the overfitting the higher the possibilities of colinearity to be present. According to (Haufe et al. 2013), this is due to the fact that the number of parameters of these models grow quadratically with the number of variables included. The insights that this section brought were related to obtain an heuristics that could help obtaining an idea of how many variables should be selected in order to reduce the effect of overfitting the model. Here, it was not proposed a comparison between methods that attend to solve the colinearity problem, essentially because there were many discussions in the literature that provide good insights on how to address that issue (mainly on how to perform the reconstruction of the source space - (Haufe et al. 2013; Ventouras et al. 2010; Winkler et al. 2014)). Firstly, the results obtained above are discussed. Then it is argued the choices made on the inverse problem solution, debating with the insights from the Haufe and Ventouras paper.

As mentioned above, there were limitations for the model chosen, i.e. regarding the number of dipoles and its influence over the sensor space. However, it is possible to argue that the only dipole present represent one brain area being activated. Thus, specific patterns were being activated according to that dipole, and that is depicted in Figure 3.4-3. With this in mind, the heuristics towards the number of variables in the dataset were settled between 15 and 20, being the values that showed an intermediary CC values and, more important than that, the values belonging to the maximum plateau of GC (Figure 3.4-3 A) and B), respectively. This window of variables was the best suited for a AR model and it is in consonance with papers in the field, regardless if talking about sensor or source space, as it is possible to verify in (Ding et al. 2000) (15 channels) and Weiss, 2010 (24 channels). This choice is understandable, since the AR model require a specific number of variables that allowed it not to be underfitted or overfitted (intermediary values).

Approaching now the inverse solutions issue, this is an area that is being in the hot-spot when talking about Granger Causality. Mainly due to the following dilemma: On one hand, it is necessary to apply source reconstruction to reduce the correlation between variables and the volume conduction effect, so to reduce the computational effort and to estimate a well suited MVAR model. But on the other hand, every source space diminishes the physiological interpretation of the causality among the variables, which can miss-lead conclusions on how the brain areas are communicating. In other words, it is a very sensitive topic. Firstly, the Information flow between brain areas is difficult to estimate from EEG measurements due to the presence of noise as well as due to volume conduction. According to (Haufe et al. 2013), it is necessary to reconstruct a source space, as a tool to reduce the impact of the smeared information in the sensor space.
However, he also suggests that it is a very sensitive topic. The work from Winkler points the same direction (Winkler et al. 2014). He investigate which methods were bests suited to reveal Granger causal links between the power of brain oscillations and experimental variables, where GC was obtained from sensor directly, from spatial filtering methods that do not optimize for Granger causality (Independent Component Analysis and Source Power Correlation), and from Granger Causal Power Analysis (GrangerCPA). The author found that computing Granger causality on channel-wise spectral power suffers from a poor signal-to-noise ratio due to volume conduction, while all three multivariate approaches alleviate such issue (Winkler et al. 2014).

The author goes further stating that the sources are less correlated than the sensor time series, since the sources reconstruction tries to undo the mixing caused by volume conduction. However, this step will never be perfect, and there will always be a residual correlation. This is why one should use methods ‘robust’ to volume conduction such as the phase-slope index or the time-inversion scheme, even in source space (which will be discuss in section Error! Reference source not found.. 2 - Error! Reference source not found.). In addition, he concluded that the problem with correlated time series is not so much the ill-conditioning of the VAR model, but the misleading conclusion about connectivity that could be made from such a model.

Understanding all of that, in this thesis, regarding the inverse solution, the option was made to adjust a method that was easily/promptly computed, that adjusts the data by removing the majority of the colinearity and that was already acceptable in the scientific community. Thus, the method applied was the FastICA, since it is a statistical technique used for solving the Blind Source or Signal Separation (BSS) problem. In (Ventouras et al. 2010), the author concluded that a careful observation of morphological characteristics (such as power spectrum and topographic behavior) of the obtained Independent Components (ICs) and definition of distinct groups of ICs based on those characteristics, proved to be quite helpful in elucidating the true brain sources. And according to (Winkler et al. 2014) it also proves to be an option on the analysis of information flow over the independent components. As a first approach in this field (TMS-EEG with GC), the choice felt on the ICA, due to be one of the more generic methods, which allowed me to focus on the main goal of the project – finding an approach able to infer influences among brain areas on TMS-EEG data.

3.5. Summary

Briefly summing up the experiments from this chapter, it was learned that the GC is the best suited tool to cope with information flow over a dataset when compared with FM-GC and different approaches of TE. This method best works with a specific set of parameters: number of trials 1 and a data length of 512 points. Regarding the issue of non-stationarity, and accounting for the goals of this thesis, to test for non-stationarity an ADF test will be used, after applying to the dataset solutions for non-stationarity, specifically: detrending and demean. Finally accounting for the over-fitting problem, the number of variables should be settled within the interval of 15 to 20 variables. Relatively to the colinearity issue, and accounting for the need of a solution to the inverse problem, an ICA will be applied.

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22 GrangerCPA – a method that directly optimizes spatial filters to extract sources and the power dynamics of which maximally Granger causes a given target variable.
Chapter 4

4. Granger Causality applied to TMS-EEG data

4.1. Introduction

This chapter takes the reader through all the different experiments that were conducted regarding the possibilities of detecting patterns of connectivity on resting states periods in between TMS pulse. The main goal is to validate a tool of GC analysis by analyzing resting state connectivity over the brain in different situations (real TMS & sham TMS), measured on the source-level of EEG. Here it is shown how to conduct such an analysis and how to discuss similarities on the resting state information flow, in between TMS stimulations, for real and sham TMS. On first subchapter, it is mentioned the methodological approach needed for such analysis, explaining the TMS-EEG pre-processing steps, the GC implementation and the Statistical Analysis. On the second subchapter, there are presented the results for the comparison on the resting state over the conditions. Then an argument is made accounting for the methodology used and the results found.

4.2. Methodological approach

The project had the main goal of showing an adequate procedure for making the TMS-EEG data suitable for being analyzed with Granger Causality and compared statistically. As reported in chapter 2 - Background, Granger Causality shows some nuances depending on characteristics of the signal such as Non-stationarity or Colinearity or even on the amount of data provided (overfitting of the model). Here the goal was trying to optimize such process indicating a step-by-step procedure (pre-processing and GC analysis) that accounts for such issues, and then implementing statistical analysis performed on different levels. The procedure was implemented throw the creation of a Matlab toolbox, named Effective Connectivity Test Toolbox (ECT), able to work with TMS-EEG data.

![Figure 4.2-1](image)

Figure 4.2-1 – Scheme of the experiment design. Each red bar represents the TMS stimulation which is followed by a TEP. Afterwards it is depicted the resting state period which is the period of interest. These trials were repeated around 200 times. For both conditions the layout was similar.
4.2.1. Data acquisition

The data analyzed was recorded on 6 subject (2 males and 4 females, aged between 18 and 35 years old). The study design comprised a single experiment containing a MR scan and a TMS-EEG examination over superior parietal gyrus. Where the structural MRI (T1-weighted imaging sequence) of the brain was obtained to identify the position of the TMS hotspots (the medial third of the superior parietal gyrus) in the frameless stereotactic Neuronavigation system as an overlay in each individual T1-weighted image.

The data was recorded under the supervision of my supervisor Virginia Conde and it included, a 10 minutes resting state eeg, followed by a preparation of a TMS-EEG set up (achieving a good contact between electrodes and the scalp with a 10-20 EEG cap), where two different condition where tested. Firstly, a real TMS over superior parietal gyrus (hotspot) with noise masking and optimal coil orientation (around 200 pulses) was performed, see Figure 4.2-2. This condition provided with the standard hotspot specific TEP components (Figure 4.2-1), which might be a compound of TMS-induced cortical activation, SEPs, and AEPs. Secondly, a realistic somato-auditory sham stimulation was performed: an electrical cutaneous stimulation with simultaneous “click” sound over the hotspot (around 200 pulses) with sound masking. This condition allowed to mimic both the somatosensation as well as the auditory perception derived from real TMS pulses with no TMS-induced cortical activation. TMS-induced cortical activation was prevented by the physical separation of the coil from the scalp (around 3 cm), see Figure 4.2-2 B).

4.2.2. Effective Connectivity Test Toolbox (ECT)

In order to analyze the acquired data it was necessary to build a tool that could couple the TMS-EEG data, the Granger Causality analysis and the Statistics Inferences, being necessary to create Effective Connectivity Test Toolbox (ECT). ECT is a toolbox mainly thought to perform Granger Causality on the time-domain. It
follows a one-direction flow (gray arrows), as it is possible to see in Figure 4.2-3. The ideology of this toolbox was to take advantage of the work done by Seth and Barnett (Barnett and Seth 2014b), and implement it in a way that TMS-EEG data could be easily run accounting for some knowledge by the user.

The toolbox was design in three different stages – Pre-processing, Granger Causality analysis in the time-domain and Statistical analysis, see Figure 4.2-3. The first one was intended to: firstly remove the artifacts due to TMS stimulations, other TMS artifacts and also the EEG artifacts; secondly to generate independent components that can be interpreted has sources – see section 4.2.3 & Figure A-0-3 (for the scheme of the pipeline). The second step mainly included all the processing steps to perform GC. In the end it obtained the Granger causality in the conditional mode for the time-domain – see section 4.2.4 & Figure A-0-4 for the scheme of the pipeline. Finally, the last stage was envisioned to allow the performance of the statistical analysis – see section 4.2.5 & Figure A-0-5 for the scheme of the pipeline. These methods gathered the information of the following papers: (Barnett and Seth 2011; Barrett et al. 2012; A. K. Seth 2010; Haufe, Nikulin, and Nolte 2012; Haufe et al. 2013; Ding et al. 2000).

4.2.3. TMS-EEG pre-processing

Before analyzing the data with GC, pre-processing steps were required (see Figure A-0-3). Firstly, and just after loading the data (all subjects are loaded together) into Matlab, a baseline was defined to correct for the slow oscillations of the EEG signal over time, across trials within each subject. This was settled to be all the segmented that was later used (2 sec, applied to all trial). Then using the pipeline for the preprocessing (see Figure A-0-3), the data was segmented into epochs of two second that were too far away from the TMS pulse to show some direct influence from it, representing only a resting state period after a TMS stimulation. Those trials, baseline corrected, were concatenated together building a continuous signal. The signal was checked as to have a difference between triggers of at least 3 seconds, in order to prevent the concatenation from having data from the TEP. This procedure was performed over all subjects, so as an outcome of the
concatenation process: it was obtained a time-series for all subjects and all conditions, allowing to estimate the same source-space for both the real and the sham conditions. The next step was to check, by visual inspections, if there were channels that showed odd behavior in the power spectrum density (PSD)\textsuperscript{23}, Figure 4.2-4 C). Filtering was performed after PSD step. A band-pass was implemented (1Hz-125Hz), both for eliminating the slow oscillations and to control the aliasing effect that may raise when downsampling. In addition, a notch filter at 50Hz was applied to remove the main-electricity line-noise. Both filters implemented were a second order Butterworth filter. Then the data was downsampled to a sampling rate of 250Hz, this prevented spurious effects of information flow due to a very low SNR, and decreased the computational effort (speed and memory). These procedures took into account the advices given by (Barnett and Seth 2011). Sensor-space data was then source modeled using FastICA (using the function ‘ericam’ from \textit{EEGLAB} (Delorme and Makeig 2004)), obtaining all the independent components (Oja 2006). To conclude the preprocessing steps, a visual inspection was performed by me and by an expert, selecting only the components that had a normal behavior for PSD, in alpha and beta bands, and also by looking at the localizations of those components. All this procedure was implemented for all subjects, see Figure A-0-6. It lead to a selection of 16 variables in the context of this experiment. This value was within the interval mention above in section 3.4 for the number of adequate channels.

\textit{Figure 4.2-4 – A) Data plotted across all trials on only one channel, in green it is represented the triggers of the dataset. B) Data plotted across all trials for all-time-series in the 63 channels. C) Represents the contribution of each frequency to the PSD. Where it can be seen a huge movement in the alpha boundaries. The bold green line show the miss-behavior of the channel removed. On the yy axes in A) & B) it is depicted the amplitude of the EEG (mV).}

\textsuperscript{23}In this part of the experiment, one channel was removed.
4.2.4. GC implementation

The implementation of GC was constructed with all the information acquired on the last chapter (chapter 3), as to optimize the information flow results. The philosophy of the implementation was to reduce the computer effort, and to increase the reliability of the GC implementation. To do so, the code was based on the MVGC toolbox (Barnett and Seth 2014b), adapt to run on TMS-EEG data, see Figure A-0-4.

The data was recorded with a high time resolution, having for that matter a huge sampling rate (5000Hz), which was downsampled on the pre-processing approach. However, in order to perform GC (even after downsampling – see section 4.2.3), it was necessary to achieve a stationarity process. As the reader could read in section 3.3, this was made by applying a process of detrending (removal of trending) and demeaning, where both the ensemble mean and standard deviation are controlled. Data was then checked for the presence of unit-roots, as a tool for rule out non-stationarity trials, using Augmented Dickey-Fuller Test. In this test, if half of the channels per trial showed evidences of an unit-root, then those trials were removed from the following analysis. With such method, it was also pretended to remove potentially ill-condition MVAR models.

Following, an optimized model order was obtained accounting for the data being used. This process was conducted by estimating the order that minimize the variance accounted for by the model, against the number of coefficients to be estimated – using the method of Baysian information criterion (BIC)\textsuperscript{24}.

The next step was to build a MVAR model using ordinary least squares (OLS). For this step, some parameters needed to be estimated. Among them there was the amount of trials to agglomerate (5 – due to section 3.3) and the model order $p$ estimated in the previous step. As an output of this step, the AR coefficients and the covariance of the residues were obtained. To test its quality and behavior two measures were calculated. In first place, a consistence test was performed, which is represented by the ratio of the correlation vector of the real data with the correlation vector of simulated data generated via the MVAR model (Ding et al. 2000). This value should have been over than 80%, otherwise the trials lack of truth.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Figure 4.2-5 – iterative result over the AIC($p$) – blue and BIC($p$) – green, by selecting a model order ($p$) from 1 to 25. The minimum value will define the most suitable model order. The model order varied across subject between $p=9$ and $p=13$.}
\end{figure}

\textsuperscript{24} $BIC(p) = \ln(\det(\Sigma)) + \frac{\ln(T)p^2}{n}$, where $\Sigma$ is the determinant of the covariance, $T$ number of points, $p$ model order and $n$ number of variables.
representation over the MVAR model. The second measure was the radius of the unit-circle. This allowed to check for stability of the model. If it was higher than a specific value (0.994), the agglomerate of trials was not stable meaning that they were non-stationary or they were a very collinear group trials (unlikely due to performing ICA). The trials, where one (or both) of these condition was not achieved, were disregard.

Following, it was obtained the Yule-Walker reverse solution (autocovariance solution) – this step allowed, in the long run, the autocovariance sequence to decays exponentially with lag k, the rate of decay being just the spectral radius. The autocovariance sequence $G$ according to the respective VAR model was calculated (to as many lags as it took to decay below the numerical tolerance level, or to a number of lags specified, if it overcame that limit, such trials were eliminated from the analysis). This routine did a lot of error checking (e.g. checking for non-stationarity, collinearity), making the analysis more robust.

After obtaining the autocovariance solution, the process was inverted and with the resultant matrix of AR coefficients, the Granger Causality Index (GCI) was obtained (Time-domain). This was based on the Yule-Walker equation, and with that information, the likelihood ratio of the diagonal of the covariance matrix of the residues for the restrict model over the residues for the full model was analyzed. With that process both the $p$-value\footnote{Statistic $p$-value for GC - in theory the GC index tends to be distributed by an F-distribution in small samples, since it has a fatter tail than the corresponding [chi$^2$] distribution. However, for a multivariate predictee ($|n_{x}| > 1$) it is not appropriate; the usual F-test for a nested linear regression demands the Granger form statistic $|F = (RSS_{\text{reduced}} - RSS_{\text{full}}) / RSS_{\text{null}}|$.} and significance\footnote{Significance – Returns significance (0 or 1) of statistics based on p-values. It implements a family-wise by specifying a multiple hypotheses test adjustment – 'FDR' (false discovery rate).} values were also obtained. The last step was obtained when all the GCI, $p$-value and significance were plotted, showing graphical results for this implementation.

All these process were repeated (excepting the estimation of the model order) in order to build a null-hypothesis (surrogate) to test the data against it. This surrogate data, called time-reversed or RGT (Reversed Granger Testing), allowed the first level analysis for statistical power of the results, see section 4.2.5.

4.2.5. Statistical analysis

It was necessary to establish the statistical significance of the estimated causality against the null hypothesis. As was said in the background, under the null hypothesis of zero causality, $(m - p) F_{X \rightarrow Y} | Z(u)$, the GC estimator scaled by sample size in a univariate model has an asymptotic F-test distribution, testing in this way at a significant level of $\alpha=0.05$ if the causality is significantly different from zero. These results were obtained in a first phase, when the GC analysis was performed. However, such analysis only tested differences of the GC values against zero, without taking into considerations the effect of the uncorrelated noise and linearly mixed noise\footnote{Uncorrelated noise only weakly affects the detection of Granger-causal directionality, whereas linearly mixed noise causes a large fraction of false positives for standard Granger-causality metrics (Vinck et al. 2015).}.

With that in mind, a different approach was conducted to find significant results, see Figure A-0-5. This approach was based on the surrogate data, more specifically on the reversed granger testing (RGT) (Haufe, 2013). Where simply the time-series $x(t)$ is inverted in time, becoming $x(-t)$. This surrogate test proved to be well suited because it shared weak asymmetries (spectral densities, cross-covariance) with the original data but reduced in a big extent the strong asymmetries (temporal ordering, that leads to causal relations). In order to test this surrogate against the data, a two-sample paired t-test was performed (due to GC being
approximate Gaussian, through the log transform), where it was tested if the $G_{\text{net}}^{RGT}$ connections were significantly different from $G_{\text{net}}^{\text{AGT}}$. Then those values were converted to z-scores and corrected with a Bonferroni test for a $p$-value of 5%. This test represented the first level analysis of the statistical performance, where the main issue was to point out significant values of direction flow of information.

Yet, the main goal was to try to achieve a comparable result between conditions (in first instance – resting state during a real vs resting state during a sham TMS experience), leading the development of a second level analysis. Such analyzed required the construction of a null distribution entitled maximum permutation statistics, with the purpose of testing significant differences between conditions. Such analysis was performed has it follows: Firstly, a difference of the expected value of each connection of the condition 1 minus the expected value of each connection condition 2 was obtained. Then two surrogate groups were created. In both of them trials from condition 1 and condition 2, in the same number, were randomly selected and shuffled. Then similar as before, the difference between groups of the expected value of each connection was obtained, where the labels were completely randomized. Afterwards, a distribution was built with the maximum value of that difference. The procedure was repeated a $n$ number of permutation ($n=1000$). With such distribution the score at the 95th percentile of the distribution was computed, those values of the real difference between conditions bigger than that value were considered significant values. Whatever connections were in that significant level were reported as connections where the causality significantly changes from condition 1 to condition 2. Those relevant connections were then compared to the topoplots of the independent components in order to check for physiological meaning.

A third level analysis was implemented in other to check for significance results across subjects. It was implemented by a one-sample paired-sample t-test between conditions across subjects. The values introduced to the model were the averaged values of the $G_{\text{net}}$ for each condition for each subject, and it was tested if the difference between means across groups were significantly different from zero on a level of $p$-value = 0.05. To emphasize that in such test the interest was not in whether any particular GC value was statistically significant, but in whether GC values differ between conditions. In other to account for family wise errors (FWE), it was performed a false discovery rate (FDR) procedure.

### 4.3. Results

#### 4.3.1. Methodologic Implementations (1st level approach)

In this section there are shown results: firstly, for the pre-processing and its capacity of reducing the colinearity allowing a good model consistency; secondly for the first level analysis, which tested the significance of the causality measures against surrogate.

In general, all plots in Figure 4.3-1 point towards the efficiency of the methodological approach on performing GC. Firstly, cross-correlation matrix is depicted in Figure 4.3-1 A), where the reader can denote the prominence of dark colors (lower correlation). Such results were possible due to the application of the

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28 $G_{\text{net}}$ – This represent the ‘net’ information flows, so if $G_{ij}$ is the Granger score the flow from $i$ to $j$ and $G_{ji}$ is the flow from $j$ to $i$, then the $G_{\text{net}}(i,j) = G_{ij} - G_{ji}$, and all statistics were made for that quantity. In such a way the ‘connectivity matrix’ became antisymmetric, meaning that the bottom part of the diagonal was symmetric when compared to the upper part of the diagonal.

29 FDR – is a statistical method used in multiple hypothesis testing to correct for multiple comparisons. In a list of findings (i.e. studies where the null-hypotheses are rejected), FDR procedures are designed to control the expected proportion of incorrectly rejected null hypotheses (false positives). FDR is somewhat less conservative/more powerful method for correcting for multiple comparisons than methods like Bonferroni correction.
FastICA, which helped, as it was seen in section 3.4, to diminish the effort of the model to perform the VAR model estimation over collinear and overfitted data. Plus, it allowed to construct, with the Independent components (ICs), an approximation of what can be interpreted as a source space. Regarding Figure 4.3-1 B), an high consistency is seen. Reminding that only dots above 80% of consistency were plotted, it is depicted in this figure that over each five trials the majority of dots is being plotted. This means that by coupling 5 trials together, after the data being preprocessed with ECT toolbox, the majority of the data is suitable to generate a VAR model. These results are confirmed in Figure 4.3-1 C), where a percentage across all subjects/all conditions is depicted. The pre-processing lead to 10 out of 12 of dataset preserved\(^{30}\) more than 60% of the trials and 6 out of 12 datasets preserved more than 80% of the trials. This lead to the conclusion that, not only the preprocessing was successfully performed, as also the datasets were well recorded, minimizing the risk of miss-performing the GC analysis, increasing the efficiency of the model.

Figure 4.3-2 depicts the 1st level analysis of the statistical package of the ECT. These results showed how significant connections from ‘true’ data were when compared to a surrogate (the colorful squares plotted were the only ones where causality is significantly different from zero). These plots were anti-symmetric, meaning that the upper part of the matrix is symmetric to the lower part of the matrix. For instance, the connection of channel 4 with channel 15, in the lower part of matrix, was plotted as red (Figure 4.3-2 A)), and it was plotted as blue in the upper part of the matrix. When comparing plot A) with plot B) it

![Figure 4.3-1](image)

**Figure 4.3-1** – A) Matrix of Cross-Correlation between all channels of one subject after the preprocessing. B) Plot showing each model ‘consistency’ for the MVAR model obtained at each set of trials (each red dot), for one subject, one condition. C) Percentage of the trials kept in the model per subject per condition (each odd number represents the real TMS stimulation and each even number represents the sham condition of each subject) (Subj.1 – 1,2; Subj.2 – 3,4; Subj.3 – 5,6; Subj.4 – 7,8; Subj.5 – 9,10; Subj.6 – 11,12).

\(^{30}\) As trials that respect the model restrictions, see section 4.2.4.
is possible to see the differences on causal connections between the real TMS and the sham conditions, over one subject. In a visual inspection both look relatively identical, where in the sham condition, more connections seem to be present. As an early comparison, it looks that the causality did not vary extremely among the conditions. However, this is going to be further study on the next statistical levels of analysis, with a further approach on the physiology. Of interest, was the fact that the magnitude of the squares plotted that match between conditions was quite similar. Even though they did not represent the magnitude of the connections in itself, they showed a higher level of trust for the same causal connections. A small note, to explain that the diagonal even though it was colored in blue, it had a non-define value of causality, due to the lack of interest of detecting the causality between the one channel and itself. The result on this level of statistical analysis were shown in z-scores. This made it easier to compare the results of different experiments and subjects, in which the degrees of freedom of the t-distribution could have been different. The transformation was performed per $z = \text{cdf}_{-1}^{-1}( \text{cdf}_t(t,v))$, where cdf is the cumulative distribution function (cdf) of Students t-distribution with $v$ degrees of freedom and $\text{cdf}_{-1}^{-1}$ is the inverse of the cdf of the univariate standard normal distribution. Throughout the 1st level analysis, connections were reported as significant if the p-value associated with a z-score (corrected for the testing of multiple entries of a connectivity matrix using the Bonferroni method) fell below 0.05. In this experiment, this was the case for z-scores with absolute values greater than 3.322 (corresponding to an alpha value of 4.464e-4).

In conclusion, assessing the results obtaining throw this first approach of the ECt toolbox on TMS-EEG data, they opened good perspectives on the positive effect of this methodology implementation. This is going to be further investigated in the next section with the 2nd and 3rd level analysis, where a bridge with the physiology was made.
4.3.2. Comparison real TMS vs sham TMS (rs in between TMS pulses across conditions)

The main goal of this thesis was to build a tool for measuring causality over the time-domain analysis of TMS-EEG data, being a first approach on the topic. This section provide results that support the capacities of such tool (results for the 2nd and 3rd level analysis), showing also the weakness that will be necessary to be improved in further projects.

In Figure 4.3-3 A) there are present the results for the 2nd level analysis for one subject. On this figure is possible to note a majority of non-significant connections (being shown with the white color). For this subject, around 8.9%\(^{31}\) of the possible connections showed significant results, in other words it means that only 10 out of 112 possible connections were relevant. Having in mind the results seen in Figure A-0-7, it is possible to understand that such percentage never overcome the 9% excepting for 1 subject. Maximum permutation surrogate created a restrict control for the pseudo-‘false positives’, giving only the outcome that was expressed with an higher amplitude in terms of GC values (the more significant connections). Again, the intensity of the GC values cannot be taken as a measure of intensity of the information flow between components (sources), since it only represented a statistical value. Nevertheless the signal of these values and their significance between connections allowed an interpretation of how the brain is behaving in terms of information flow.

While looking at Figure 4.3-3 A) (2nd level analysis) the reader should disregard the information obtained with the 1st level analysis. There were two reasons for that. Firstly, due to the lack amount of true

\[ \text{numb}_{\text{channel Active}} \times \frac{\text{numb}_{\text{channel Total}} - \text{numb}_{\text{channel Total}}}{\text{numb}_{\text{channel Total}}} \times 100 \]

\(^{31}\) This percentage can be obtain throw the following expression:
GC values obtained after testing against the surrogate (RGT), which, if used, would decrease the statistical power of the 2nd level analysis approach, by reducing the efficacy of the maximum permutation test. Secondly, due to the fact that the results, even that in small scale, changed between condition (when compared against the surrogate - RGT), then they should not be used as meaningful tools for the pre-knowledge of the 2nd level analysis. Otherwise, the model would work on comparing results against information that was already eliminated by the 1st level analysis.

Even though a physiologic interpretation is still premature, it was possible to infer the behavior of the model over the TMS-EEG dataset. This, by comparing two different conditions (real vs sham) over the 2nd level analysis. Taking the ‘matrix of connectivity’ of the Figure 4.3-3 A) as an example, the model inferred information patterns over the sources in the following way: 1→7, 1→15, 6→15, 10→15, 14→15 and 1←10, 1←14, 2←15, 7←12, 9←14. In order to facilitate the physiologic understanding, a schematic map of the information flow over the independent components over the 2nd level was made, see Figure 4.3-4. The topoplot, represented the map of the activation of one component (result of the ICA), and the arrows showed the information flow between those sources (ICs). Albeit this scheme showed only a static average of connectivity across all trials, due to the way the toolbox uses the TMS-EEG data, it was possible to detect patterns of causal effect across the sources. Such patterns, according with the results for the subject being analyzed, would allow the following hypothetical physiologic interpretation. The occipital areas would be connected among each other and with parietal lateral areas (i.e. 1←10, 2←15), and vice-versa (i.e.
The occipital areas would also be driving a flow of information towards more medial areas of the brain in the medial left temporal lobe (i.e. 1→7). The medial temporal would also drive connections between itself (i.e. 9→14) and across the occipital areas (i.e. 14→15). These results were corrected for weak asymmetries yet, they only represented the effect of the connectivity analysis over one subject. A visual inspection was made to the results across the other subjects, see Figure A-0-7. It indicated that even though similarities can be spotted, it was not conclusive that the behavior was identical and further analysis were done.

Figure 4.3-5 – Plots for the 3rd level Analysis. A) Matrix of the z-scores without FWE correction, showing the differences between all subjects of condition - real TMS, against condition - sham TMS. The information flow from the source in the column to the source in the line is coded by ‘reddish’ colours, an information flow from the source in the ‘line’ to the source in the ‘column’ is coded by an ‘blueish’ colour, where basically it inverts the caption in the plot. Another aspect, its the fact that this matrix is antisymmetric. B) This plot represents the mask over the z-scores matrix showing the significance values over the z-score matrix without a FWE correction. Where ‘0’ represent a significant connections, and ‘1’ represents a non-significant connections. C) Results of p-values across all the causal connections after a FWE.
It was with that motivation that a 3rd level analysis was projected and made. In Figure 4.3-5, it was possible to inspect the results obtained for an one-sample paired-sample t-test between conditions across subjects. In Figure 4.3-5 A) there are plotted the z-scores for such statistical test without a correction of a FWE. The values that exceed the z-score of 1.96 (α=0.05) were plotted as significant connectivity between sources in a binary form. These results can be depicted in Figure 4.3-5 B). Of notice, is the fact that in such figure the interest was only to qualify a significant connectivity among the sources, being ignored the direction of the flow. In these two plots it is possible to verify that the patterns seen in the 2nd level analysis for the subject used to exemplified the analysis (Figure 4.3-3 & Figure 4.3-4), suffer some changes. For instance, only one connections was similar, i.e. 10→15. This observation suggested that the results of an individual analysis could mislead conclusions and could induce in error.

However, in order to verify if the plot of Figure 4.3-5 B) had enough statistical power, an analysis with the data controlled for multiple comparisons (FWE correction) was performed using FDR. Such results were reported in Figure 4.3-5 C). They showed a no-effect of significant connections among all sources, where none of them was able to survive the conservative test. Even though it was not shown, a similar test was perform with the Bonferroni (a less conservative FWE analysis) and the results were similar. The main reason for such result could have been the lack of statistical power to perform a t-test. Usually a test should be performed with 15-20 subjects, and in this experiment only 6 subjects were used.

Summing up, these results showed how different interpretations each of the statistical level of analysis gave and how they depend on the type of conclusion the researcher wants to ask. The first level, only validate the results of the GC on the dataset chosen. The second level allows the comparison on those GC values across different conditions, never forgetting that the conclusion should be very well argue due to subject heterogeneity. And finally the 3rd level analysis which, if with enough statistical power, provide an overview across the population of study over the GC values.

4.4. Discussion

In this thesis, it was proposed a procedure for deriving influence relations in high-dimensional TMS-EEG time series using a specific preprocessing, followed by Granger causality and powerful statistical analysis. In this section it is going to be discussed the methodology proposed in section 4.2 which produced the results of section 4.3.1, followed by a discussion of its application to a TMS-EEG recordings (based section 4.3.2).

4.4.1. Methodology

Building the most suitable methodology for a GC pipeline was in focus during this project, due to that some aspects are discussed here. To start, it is important for the reader to understand that the pre-processing step here implemented do not represent ‘the best’ approach and it should only be considered as a guideline and not a ‘recept’ of pre-processing for connectivity measures in TMS-EEG data. There are two main reasons for that. Firstly because there is yet no right answer for the following question: ‘What is the most suited pre-processing pipeline for connectivity inferences?’ as it can be conclusive in (Haufe et al. 2013; Barnett and Seth 2011; A. K. Seth 2010). Thus, the procedure implemented was made following the advices of Haufe, Barnett and Seth, by accomplishing the requirements of a standart pre-processing stage, i.e. removing artifacts (filtering and ICA) and reducing the unnecessary information (downsampling and filtering), allowing the performance of granger causality analysis. Secondly, it was not optimized since no further studies were developed, comparing how different pre-processing parameters influence the outcome of the information flow in simulated and real data. However, the propose of this thesis was never that one.
To stress that in the references mentioned above, the core pre-processing steps are filtering, downsampling and source reconstruction. As seen in section 3.3, filtering and downsampling play an important role in the increase of the signal-to-noise ratio, eliminating non-physiologic variations of the data and reducing the non-stationarity. However, this has to be made in a cautious way (see section 3.3.3) as not to remove/change data in a way that the measures of causality would be irreversibly unclear or would mislead conclusions (Barnett and Seth 2011). Relatively to the source reconstruction, the issue is mainly debated in by Haufe (Haufe, 2013), and was early discuss in section 3.4.3. Generically, it allows to overcome three main issues. Firstly, on the physiological point of view it helps diminished the volume conduction effect, which severely limits the neurophysiological interpretability of sensor-space connectivity measures. Also, it allows to reduce the computational effort, helping to overcame mathematical problems when computing the GC values (i.e. eliminating the non-positive define matrix of the covariance matrix – due to higher colinearity among data variables). Finally, it helps obtaining the independency of the choice of the reference, which as shown in (Haufe et al. 2013), controls the flux of information according to the specific selection. Even though it is a simplify version of source reconstruction, i.e. the ICA and the ICs, it is possible to assume that each component represent an independent behavior of the brain, in a specific brain region. Besides, it also helps removing many artifacts sources on the logic that certain artifacts (e.g. stereotyped eye and muscle artifacts) might be temporally independent from neural activities (A. K. Seth 2010; Delorme and Makeig 2004). In addition to that, it is a subtractive method, being compatible with G-causality analysis. With all these information and considering section 3.4.3, in this first approach it was intuitive to apply ICA as the source reconstruction technique.

Regarding the GC implementation, the majority of things to be discussed were already approached on section 3. There are still some points to make. Firstly regarding the model order of the VAR model. In such procedure, the BIC approach was chosen as the method to optimize the model order value because it compensates for the large number of data points commonly found in neural datasets, where others such as AIC do not. Secondly, the model order obtained varied between p=9 and p=13. Such values were enclosed in the usual values found in the references: (Omidvarnia et al. 2014b) p=5, (Flamm et al. 2013) p=8 and (Barrett et al. 2012) p=20. Even though it was constant across the group of ensemble trials, across subjects the model order varied. These variations were not significant according to the physiologic interpretation of the statistical analysis further in the process, mainly because the study was been made over a resting state period. By definition, the model order or p defines the resolution of the system in which the model finds information to predict the causality, and in such period (rs) the behavior of the brain is mainly constant in a scale of 1x10^-5s (100ms). Since the sampling frequency was 250Hz, and considering the model order between 9 and 13 (lower and upper limit of model orders obtained), the time window of causality analysis (the temporal resolution) varied between 36ms and 52ms (time window = p/fs), respectively. This proves that in such conditions, big changes due to the different windows of causality analysis were not expected, even though they had different ‘p’ values. Thus, it was privileged the quality of the estimation rather than the time window similarities across subjects. However, in further studies an approach analyzing the 95th-percentil of the values obtained for the model order can be made to standardize the value of p across subjects. Finally, the model order was obtain throw an iteration process and in cases where the model order specified by the minimal BIC was too large to permit feasible computation, or in cases where the BIC does not reach a clear minimum over the range tested, a smaller model order could have been chosen on condition that the BIC shows no further substantial decreases at higher orders. However, in real data this was not verified.

Regarding now the GC analysis implementation complications (see section 2.5.1), the results seen showed that the ECT avoid the majority of such issues. Apart from the non-stationarity and colinearity, already discussed above, the ECT got around the long-term memory by making a thorough error-checking,
detecting if there were any auto-covariance that did not decay exponentially, if so then the ensemble trials was ignored from the further analysis. Such auto-covariance decay, also removed any dependence from the actual values of the process by the residual term. Secondly, it dealt with the strong moving average component, by applying detrend and demean, being these procedure similar to the non-stationarity. Only regarding the linearity of the GC model, ECoT did not provide a solution *per se* (Barnett and Seth 2014b). However, its implication will be discussed further on in section 5.1.

Regarding the statistical analysis, all three levels will be discussed in detail in the next subsections.

4.4.2. Application

The main goal of the project was to construct a method able to perform an effective connectivity analysis over a TMS-EEG suitable dataset, not only to measure the respective information flow (1st level of analysis), but also to compare different conditions (2nd level and 3rd level analysis). Being a first approach on GC analysis over the TMS-EEG protocol, it was necessary to find a simple procedure that would validate the computational feasibility and in addition that could, in the future, become a powerful prove over the physiological behavior of the brain. As pointed above in section 2 and section 4.2.1, this was tried by using a single pulse protocol, over an area, superior parietal gyrus, which would create a measure of comparison between the resting state periods of realTMS vs shamTMS. The expected result was to see no difference, since, as Stamoulis (Stamoulis et al. 2011) showed with the modulation of phase, there should only be a change in the behavior of the brain over a prolonged period after the application of single-pulse TMS but not in the short term period (period tested in this experiment). The results seen point towards such conclusion, however some aspect should be considered: in a first stage the issues that affect the pre-processing quality and the connectivity measures in itself, and secondly the statistical power of the 2nd and 3rd levels.

Firstly, the results depicted in Figure 4.3-1 attend to work as a quality control of the methodology implemented, both regarding the pre-processing (i.e. evaluating the cross-correlation) and the GC implementation (i.e. the consistency and the number of surviving trials). These result tried to support the computational feasibility of implementing the influence analysis over a real dataset of TMS-EEG. These results support that this approach was able to deal and obtain influences over high-dimensional recordings, helping to support part of the main goal of the project. Note that regarding the cross-correlation matrix, it was only made a visual inspection over the quality of removing the colinearity with ICA, having no further interest in the GC analysis.

Worth saying is the fact that, due to consistency test and to others error checking (i.e. autocovariance test, see section 4.2.4), there are differences in the amount of final GC matrices across subjects or across conditions (i.e. number of trials kept in the model). Two options could had been made, or subsample the GC matrix obtained across subjects allowing that every subject/condition had the same number of matrices (same number of trials ensemble kept) or to apply the statistics in the different sized data. The second option was chosen. The influence of such issue was that, during the 2nd and 3rd statistical analysis, the comparison was made across groups with different number of GC matrix. This was not a limitation, since the statistical approaches account for asymmetries across data groups (differences in the size of the conditions groups).

The 1st level analysis was made to highlight the significant connections among channels. To do so, it used a surrogate data, the RGT. According to the results, this analysis can be seen as a 'signal-to-noise' test, since it looked where the strong asymmetries were relevant compared to the weak asymmetries. In other words, it tried to explain statistically which connections were more significant (i.e. the signal) when the non-causal characteristics of the signal were removed (i.e. the noise). Such test helped to identify the significant causality among the interaction between channels, avoiding the spurious connectivity caused by volume
conduction in the GC. While doing so it can be interpreted as reducing the ‘noise’ and enhancing the ‘signal’. In (Haufe et al. 2013), the author showed that RGT is the most appropriate surrogate to alleviate the influence of weak asymmetries on the result of any causal measure, while maintaining, or even amplifying, the contribution of strong asymmetries. Multivariate methods, e.g. EEG experiment, are commonly employed due to the fact that the inclusion of more time series helps to rule out indirect connectivity between channels that are caused by a common confounder. However, that argument does not apply to EEG data, where all causal confounders contribute to all channels due to source mixing. Accounting for this, such tool is powerful to determinate the strongest connectivity within the network, not only working by itself, but also with the help of a source reconstruction procedure (in this case ICA).

On the 2\textsuperscript{nd} level analysis, the need to test significant differences between conditions was achieved. To do so a surrogate was build, more specifically the maximum permutation surrogate. This approach was used because it maintained the statistical measures of the variables within the condition (i.e. mean and standard deviation) eliminating the effect of the true connectivity by introducing a shuffling procedure. In order words, by randomizing equally from each conditions, the variability of the built dataset was similar to the original’s one, allowing for a powerful comparison with a null distribution (surrogate). Also is important to take in consideration the number of permutation ‘n=1000’, that accounted for the random effect of selecting trials. As said above (see section 4.2.5), the relevance of the 2\textsuperscript{nd} level of statistical analysis, was not the intensity of the connections between sources but instead the significance and signal of the connections. The reason for that is mainly because on the physiologically point of view it would be extremely ambitious, and maybe even wrongly, to interpret the efficiency of an influence compared to another on, based on the intensity of statistical values, even though they were family wised corrected. This level of analysis was only used on the real datasets, because its theoretic effect was already predictable by its statistical nature so, it was not necessary to prove it experimental with a pilot study (testing it on simulated data). In order words, on the theoretical point of view, the maximum permutation test supports its quality on the capacity to test real data against a suitable surrogate, where the null distribution (the surrogate) is obtained by reducing the connections among variables by eliminating its temporal order.

Accounting now for the 3\textsuperscript{rd} level analysis, this analysis intended to show an effect of significant differences across subjects when comparing across different conditions with all subjects. The results were inconclusive and any physiology analysis at this moment would be precipitated. This because, even though the results showed no effect between conditions across subjects, which was the null-hypothesis, they were based only on six experimental subjects. Statistically speaking, such number of subjects does not generate a powerful statistical comparison, guiding to misleading conclusion. The effect of increasing the subject quantity could change drastically, or not at all, the results seen. Another reason might be due to the non-similarity of ensemble trials across time within the subjects and across subjects. Meaning that the rs period could produce different responses over the causality between different ensemble trials. This will be further pointed out as a limitation in section 5.1. However, the way the model worked in such a preliminary stage lead to higher hopes on the way the model can be useful for connectivity studies with TMS-EEG. Relatively to the statistical method chosen, the t-test fitted well the comparison of GC values, since they are a ratio that is logoritzimized. Due to that, they are approximated to the Gaussian, making it a perfect math to a t-test.

Important to mention is also the capacity that ECt has to deal with EEG, more specifically with brain resting state. Regarding the capacity to pre-process the data and to implement the GC analysis, it was achieved with success. However, other issues arise, more precisely, the assume linearity of the data and the issues related with the experimental produce, for instance the subject intra-variability. This will be further discussed in section 5.1.
Finally, it is important to emphasize that, one should be cautious using the term causality regarding the connections between channels/sources, instead it should be considered to apply the term influence. This because, the reductions of all noise, weak asymmetries and recorded artifacts are never perfect, and there is always components in the GC values that do not perfectly explain a true causal effect. Since those components may be blurred, one should use a less powerful term to address to the information flow.

4.5. Summary

Summing up on the achievements of this chapter, a methodologic approach was proposed to perform GC analysis over TMS-EEG datasets. Firstly it was explained how the data was recorded over two different conditions across 6 subjects. Then a construction of a toolbox, the Effective Connectivity test toolbox (ECT), was made and explained. On this chapter it was exposed the pre-processing and the GC implementation that were made with the pre-knowledge acquired in chapter 3. Then the statistical approach was presented, where an innovative approach of three levels of analysis was made.

When tested, this methodologic approach proved to give highly consistent and successful results, where 10/12 of dataset preserved more than 60% of the original ensemble of trials. The quality of this toolbox was also discussed in terms of its physiognomy and its technical details, such as model order and implementation complications.

The statistical levels of analysis showed first the effect of comparing a surrogate with the true GC values (1st level), being a suitable method to implement in order to address for true influences on the GC matrix within one condition. Then the 2nd level analysis attempted to search for differences between conditions within the subject, by implementing a maximum permutation analysis. It showed little differences between the conditions, which was expected. Finally, the third level tried to search for significant differences across the subject over conditions. It showed no effect, but has it was discuss, the main reason was due to lack of statistical power.
Chapter 5

5. Conclusions

5.1. Limitations

During the project there were some limitations that had to be considered and others that conditioned it. This section gives an overlook over them dividing it in two categories: experimental and methodology limitations.

Starting by the experimental limitations, one limitation was to measure and to record a systematic and identical resting state (rs) across subjects. According to what the reader can see in section 2.2, measuring connectivity over a rs is challenging and depends on the specific instructions given to the subject for the rs period, since they might affect the default mode network (van Diessen et al. 2015). Although, spontaneous thoughts (the hardest to control) have no major effect on changing the rs networks across time or subject, since they are produced in the same brain areas, there were many other issues that need to be controlled for. A list of those issues with the proposed solution found to limit their induced heterogeneity is now exposed. Firstly, in order to avoid introducing variability on the rs over conditions, the order of the experimental conditions was identical across subjects, the same for its duration. Secondly, regarding the eyes opened vs eyes closed, it was chosen to instruct the subjects ‘to keep their eyes opened with normal blink periods and to fixate them over a point in the wall during the all experiment’, as to avoid the dominated alpha band effect over the all brain (if in a eyes closed situation) and the muscular artifact, respectively. Thirdly, regarding the state of vigilance (four different states can be studied: wake, sleep, sleep deprivations and ‘drowsiness’) the subject was instructed to be awake, as to be coherent to the topic above. However, there are things that regulate the vigilance state and so regulate the network behavior, that were not controlled (i.e. caffeine intake and tiredness). Finally, the posture adopted (instructions to be sited straight) was also important as to avoid changes in the EEG amplitude. All these confounders were minimized as the reader can see above, however the rs always has a variability component.

Regarding now the methodology limitations, the first issue to consider was the exact relation between duration of recording and stability in EEG rs, in connectivity measures. Such relations are complex, meaning that the epoch length influence the connectivity measure over the rs period (van Diessen et al. 2015). As so, and following the directives of the GC analysis, the solution was found by admitting that, the solution for removing the non-stationarity, would fit best the model (see section 3.3). Thus, cautions were made as taking the same size length in all epochs and number of ensemble trials together to overcome such issue (ensemble 5 trials and obtained the causality over the 2s period with a 250Hz sampling frequency).

The second issue to consider was the fact that the inspection over the EEG and its power spectrum analysis was made by a visual inspection. To reduce its influence, it was made with the help of an expert, being that the recurrent procedure in EEG analysis.

Thirdly, regarding the ICA analysis there are two points that can be made. In first place, the FastICA generates theoretical identical results but still different ICs each time it is performed, due to a dependency of a random generator procedure in its algorithm, in the whitening part of the procedure and also in the rotation and separation of sources by maximizing their non-Gaussianity. The solution to obtain the same ICs across subjects was to agglomerate them in a single concatenated dataset, as to obtain similar ICs. However, to do so it was necessary to process all subjects at the same time, which conditioned the computational processing speed and restricted the configurations of datasets, that had to be composed by the full number
of subjects in study. In second place, the FastICA applied to the sensor-space EEG, as discussed in section 3.4.3, managed to obtain a source-space out of the original data. However, as stated in (Jonmohamadi et al. 2014), ICA has been extensively used for component extraction of event related potentials (ERPs) and for data cleaning, all procedures where a major event/activity is present. Meaning that when applied in a rs paradigm it has some troubles one distinguish the weak from the strong sources. In addition, it can only extract sources that are combined linearly. Both points were taken into account before chosing the ICA. Firstly, regarding the ability to separate and/or obtain weak sources in a rs, it did not have a major effect in the amount and quality of the ICs, nevertheless its interpretability remains a little ambiguous (each ICs is a tomographic map of one component). Secondly, the linearity across sources was assumed since the beginning not only for the ICA but also for the GC specifications as mentioned in section 2.5.

A short mention to the volume conduction effect. It influences the amplitudes of electric field measured on scalp and therefore affects the GC values on the time-domain. However, the situation is different if we consider phases of the signals. Both DTF and PDC\(^ {32} \) are based on the detection of phase differences between channels, they take zero value when there are no phase differences (Seth, Barrett, and Barnett 2015). This means that both DTF and PDC are not influenced by volume conduction. Nevertheless the goal of the project was to infer general influences (i.e. influences that encompassed all frequencies). Even though it was a limitation, it was mainly reduced by the FastICA approach (see section 3.4.3 and section 4.2.3), where a full elimination of its effect would be utopic in the EEG level (Haufe et al. 2013).

A final thing to have in mind is that the 2\(^ {nd} \) and 3\(^ {rd} \) level analysis did not account for alleviation of the weak asymmetries, considering only the comparison between conditions over both types of asymmetries. According to Haufe (Haufe et al. 2013), this can be seen as a huge limitations, when intending to address for significance of the GC values. However, when comparing different conditions within or across subjects, it is expected that those subjects share the same weak asymmetries, since the protocol was identical, which lead to present the results in such a way. Nevertheless, one thing is to expect, another thing is to be positive about, and so this point remains an open for discussion.

5.2. Future developments

Being a new methodologic approach to analyse information flow across brain regions with TMS-EEG data, this project will arise many questions in connectivity studies. However, in this section the main focus is to point out further development of the toolbox analyse and its finesse.

Firstly, regarding the pre-processing, further studies on the source-modelling can be done. Considering the paper by Haufe (Haufe et al. 2013), where a comparison over the abilities of the employed inverse method to recover the spatial distribution of the sources were made, the author suggested that artifacts of volume conduction persist regardless the source estimates. Thus, the application of GC over reconstructed sources, even testing against surrogates (i.e. RGT), might lead to the detection of spurious connectivity regardless of the inverse method used. However, a new approach proposed by Jonmohamadi (Jonmohamadi et al. 2014) compared Beamformer (one of the techniques used by Haufe) with an innovative approach - a source-space independent component analysis (Source-space ICA - ssICA)\(^ {33} \) for separation, tomography, and

\(^ {32} \) A further explanation of DTF and PDC can be found in Annex 1.

\(^ {33} \) Source-space ICA – is based on the application of singular value decomposition and ICA on the neuroelectrical signals from all brain voxels obtained post minimum-variance beamforming of sensor-space EEG. It is a tool for spatiotemporal reconstruction of EEG and MEG sources. It provides a tomographic map for each identified component, where the tomographic maps are obtained by back-projection of the ICA mixing coefficients.
time-course reconstruction of EEG. According to the author ssICA is superior to the minimum-variance beamforming in the reconstruction of multiple weak and strong sources, as ssICA allows weak sources to be identified and reconstructed in the presence of stronger sources. With this said, it will be interesting to see its effect over the connectivity analysis, comparing the results of information flow on simulated data and real data by using the ECT, in order to see if it will improve the preprocessing of the TMS-EEG data, by reducing the spurious connectivity.

Being a toolbox that deals with TMS-EEG data and, knowing the potentialities of such technique in terms of influence the brains states (see section 0), it makes also sense to covet working with data segments more complex than the resting state. In other words, the TMS, as mentioned above, generates ‘waves’ of information flux over the brain, such ‘waves’ patterns and directions are unknown and may lead to important discoveries in consciences studies and in traumatic brain injuries studies (Rosanova et al. 2012; Ferrarelli et al. 2010). Having this in mind, it will be interesting to study the evolution of the information flow over/after the TMS pulse, in the TEP segment. As already argued, to find solutions to such question it is necessary to find answers to the non-stationary problem (see section 3.3), and to obtain a time-resolution of the system in the same scale as the changes in the TEP (around 5-10 ms). The most traditional solution would be the ‘vertical regression’, which consist in dividing the data into (overlapping or non-overlapping) windows, on the logic that shorter windows are more likely to be approximately stationary. This implies a trade-off between likelihood of stationarity (shorter is better) and accuracy of model fit (longer is better). An advantage of windowing, assuming there is sufficient data (enough number of trials), is that time-varying G-causality can be analysed (Ding et al. 2000). This was already implemented on the toolbox, however preliminary experiments indicated that hardly the time resolution will increase below 40 ms due to the stability conditions. To overcome such issue, two other options can be considered to perform time-variant MVAR models: the generalized recursive least square (RLS) (Möller et al. 2001; Hesse et al. 2003) and a Kalman filter technique – the general linear Kalman filter (GLKF) (Milde et al. 2010; Barnett and Seth 2015). The first method is based on the adaptive recursive fit of a VAR model with time-dependent, which also takes into consideration a set of EEG epochs as a whole. In contrast to short-window techniques, the multi-trial RLS algorithm involves the information of the actual past of the signal, whereby the influence of the past decreases exponentially with the time distance to the actual samples. Thus, adaptive filter algorithms enable the fit of VAR models with an arbitrary order. The latter one (GLKF) is based on an extension of the state-space model for a multivariate time series to a matrix-state-space model for multi-trial multivariate time series. From a methodological point of view, the RLS minimizes the prediction errors, whereas the Kalman filter minimizes the error of the estimated parameters by choosing a so-called Kalman gain matrix. GLKF showed motivating results when compared to RLS since it had no dimensional restrictions (RLS failed to perform in higher than 24 channels). Kalman filters also have the advantage of allowing more control over the parameter process covariance matrices and the estimation of the prediction error covariance matrices. According to (Milde et al. 2010) GLKF also improves the speed of the parameter estimation, compared with the RLS. The implementation of such techniques should be one of the next step in order to achieve studies that deal with the dramatically fast TEP, behaviour and spread, over the brain.

A powerful feature of GC is that it may be decomposed in a natural way by frequency. The resulting spectral G-causality integrates the time-domain causality previously introduced (see section 2.5.1), which may thus be considered an average overall frequencies of the spectral causality. In the frequency domain a very similar definition holds for GC as in the time domain. Let I and J be two disjoint subsets of V. Then GC conditioned on iff the following condition holds: \( A_{ij}(f) \gg 0 \), for some frequency \( f \). The importance of the frequency-domain analysis reflects at least over two aspects. Firstly, if spectral causalities are required, values outside the frequency range of interest may simply be ignored, while appropriate time-domain
causalities may be obtained by averaging spectral causalities over the frequency range \( \mathcal{B} \) of prior interest to obtain band-limited GC\(^{34} \). This procedure can be taken as a filtering procedure, where a fraction of the GC is obtained, however is particularly different from the filters mentioned above in section 3.3 and section 4.2.3, since it is not used to improve the stationarity of the dataset as seen before (Barnett and Seth 2011). Secondly, it might be used to address research questions that intend to show the behavior of specific frequencies on connecting different brain areas. Some studies using the frequency GC domain have already been made (Omidvarnia et al. 2014a; Barrett et al. 2012), where they overcome the limitation of simply infer correlations and cross-correlations, and jump into conclusions of information flows due to frequencies influences among the all spectrum considered. Thus, the frequency domain should be seen as a future implementation of the ECT toolbox.

5.3. Final Standings

Regarding the proposed goal ‘to combine a method for effective connectivity that gives causality and directionality (GC) with a brain controlled stimulation (TMS-EEG) in one powerful tool (a Matlab toolbox) to be used further to map the brain networks’ it was achieved considering, however that it was a first step that will need further developments. In a first phase, a comprehensive analysis over simulated data helped to point the direction to a GC analysis and its best suited parameters and approaches regarding the non-stationarity and colinearity. Then on the second part of the project, by comparing two situation of supposed identical connectivity (real vs sham TMS conditions on rs) over 6 subjects, it was possible to infer the effect of the implemented toolbox over TMS-EEG data. However, due to statistical and experimental limitations, a firm conclusion cannot yet be made regarding the truly physiological interpretation of the results seen. Such issues are here discussed and will be developed in the future.

All things considered, this thesis goal was achieved in the sense that a colligation between TMS-EEG and GC was achieved and might lead in the future to fruitful results in terms of effective connectivity analyze. The toolbox built (ECT), with its statistical environment here discussed, can enable new valuable insights into the neuronal processing of such data. It can be expected that with improved measurement and analysis, mentioned in the above section, deeper investigations into the dependencies between structure complexity and system dynamics of brain networks can be made providing more detailed knowledge of organization in the human brain.

\(^{34} \) Band-limited GC—it follows the following expression in the conditional version: \( \mathcal{F}_{Y \rightarrow X | Z} = \frac{1}{\mu(\mathcal{B})} \int_{\mathcal{B}} f_{Y \rightarrow X | Z}(\lambda)d\lambda \); where \( \mu(\mathcal{B}) \equiv \int_{\mathcal{B}} d\lambda \) is the measure (length) of \( \mathcal{B} \), \( \lambda \) is the frequency of integration and \( f_{Y \rightarrow X | Z} \) is the spectral GC from \( Y \) to \( X \) under the system \( Z \).
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Annex

Annex 1 – Other Brain Connectivity Estimator Theories

Factor Modeling with Granger Causality

This theory tries to bring the benefits of Factor Modelling as a tool to help simplifying the dataset and afterwards apply it to GC, this theory was used by Flamm in 2013, (Flamm et al. 2013). Factor Modelling is useful for the analysis and forecasting of high-dimensional time series, when the single time series show similarities or a kind of co-movement (colinearity). The idea of factor models is to separate the n-dimensional observations \( x(n) \) into a part representing the co-movement \( \chi(n) \) (also referred to as latent variables) and a part representing the noise of the data \( \eta(n) \).

First, PCA is used to separate the observations into the latent variables (explaining the co-movement) and the noise. It is assumed that the causal structure of the observations is reflected in the causality structure of the latent variables. Second, for a fixed \( i \) and \( j \) we analyze the conditional Granger causality relation \( \chi^2 \implies x_i|\chi_j \), given a fixed channel selection \( I \supseteq i, j \). Third, it is performed an analysis for all admissible channel selections \( I \supseteq i, j \) and derive an heuristic statement for the influence from \( \chi_i \) to \( \chi_j \), condensing the information of all sub-systems.

In detail, firstly the PCA is applied on the observations \( x(n) \) in order to obtain the factor loading matrix \( \Lambda \) and the static factors \( z \). The dimension of the static factors \( q \) is determined via a Scree plot. In this graphical method the principal components are sorted according to their explanation of the total variance of the data in descending order. Usually some kind of bending point can be observed in this graphical representation, which divides the principal components into important and unimportant ones. This values should be settled around 0.13.

Afterwards the \( i, j, I \) (\( l \) – represent the subsystem choosed with a size of \( q \)) is fixed. The straight-forward application of the approach of the factor modelling approaches yields two problems. While in theory it is easily distinguish regular and singular matrices \( \Lambda_1 \) in:

\[
\Lambda_1 a(z) \Lambda_1^{-1} \chi_l(n) = \Lambda_1 \epsilon(n)
\]

Equation A-0-1

where \( \Lambda \) is termed factor loading matrix, \( \chi_l \) are the latent variables, \( a(z) \) is the AR process and \( \epsilon \) is the residues associated to the model; by considering the determinant, the estimator \( \Lambda_1 \) will typically yield \( \det(\Lambda_1) \neq 0 \). The causality relations drawn from systems with very small values of \( |\det(\Lambda_1)| \) are not meaningful, which is due \( a(z) \) in \( M_{2q+2} \chi_l(n) a(z) = M_{2q} \epsilon(n) \),

Equation A-0-1, cannot be computed reliably. The term \( |\det(\Lambda_1)| \) is a measure for the similarity of the selected channels. Therefore it is only considered a channel selections \( l \) with \( |\det(\Lambda_1)| \) exceeding a threshold \( t \). This threshold is chosen empirically in order to yield reasonable results (setted as \( t=0.05 \)).

A similar challenge arises in the estimation of \( \hat{\Lambda}_l(z) \) (which has a finite order now). In theory the Granger Causal Index, \( \hat{\Lambda}_l \), if it is equal to ‘0’, \( \hat{\Lambda}_l(m)=0 \) \( \forall m \) (where ‘\( m \)’ represent the lag in time, and has a maxime value equal to the model order ‘\( p \)’), signifies that \( \chi_l \) is Granger non-causal for \( \chi_i \). However, typically in estimation \( \hat{\Lambda}_l(m) \neq 0 \), so it has to statistically be tested whether the polynomial coefficients \( \hat{\Lambda}_l(m) \) (for all lags \( m \)) are
significantly jointly different from zero. For this purpose, the Flamm and affiliates use an F-test 
\( H_0: \hat{A}_i(m)=0 \quad \forall \ m \), which is implemented with the MVGC toolbox. They consider the p-value of the test as a 
measure for Granger causality: rejection of \( H_0 \) (\( p < 0.05 \)) signifies Granger causality, acceptance means non-
causality. To sum up, for each channel selection \( I \) (for fixed \( i, j \)) two values were obtained: \(| \text{det}(\hat{A}_i) | \) as a 
similarity measure of the channels in \( I \) and the p-value as an indicator for the causality from \( \chi_i \) to \( \chi_j \).

As a global influence statement from \( \chi_i \) to \( \chi_j \), the different conditional causality statements based on 
distinct channel selections \( I \) are condensed into a single one. For this purpose Flamm propose an intuitive 
rule: if more than 95% of the statements for distinct channel selections match, it is conclude a global influence 
statement. In other words: if \( \chi_i \rightarrow \chi_j | \chi_i \) for 95% of \( I \) with \( | \text{det}(\hat{A}_i) | > \tau \), then \( \chi_i \) influences \( \chi_j \). On the other hand, if \( \chi_i \rightarrow \chi_j | \chi_i \) for more than 5% of \( I \) with \( | \text{det}(\hat{A}_i) | > \tau \), then \( \chi_i \) does not influence \( \chi_j \). Finally, as the causality 
structures of the observations and the latent variables are equal by assumption, \( \chi_i \) influences \( \chi_j \) if \( \chi_i \) influences \( \chi_j \). The analog reasoning holds in case of non-influence. More details in (Flamm et al. 2013).

Transfer Entropy

Transfer Entropy (TE) (Schreiber 2000) is a non-parametric static method, measuring the amount of directed 
(time-asymmetric) transfer of information between two random processes. It belongs to “information theory 
(IT)” class and is model-free. It states that, if a signal \( X \) causes a signal \( Y \), then the probability density of the 
future of \( Y \) conditioned on its own past should be different from the probability density of the future of \( Y 
conditioned on the past of both \( X \) and \( Y \) (Vicente et al. 2011; Lobier et al. 2014; Kawasaki et al. 2014). In IT 
the uncertainty of a variable is quantified by Shannon Entropy:

\[
(X) = - \sum x p(x) \log p(x) \tag{A-0-2}
\]

\( TE \) from a signal \( X \) to a signal \( Y \) can therefore also be expressed:

\[
TE_{X \rightarrow Y} = H(Y(t)|Y(t')) - H(Y(t)\mid Y(t'),X(t')) \tag{A-0-3}
\]

Then to compute \( TE \), entropy rate (\( h_1 \)) is calculated using the current observation values \( x_t \) and \( y_t \) and 
the time-shifted (\( \tau \)) observation value \( x_{t+\tau} \) as follows:

\[
h_1 = - \sum x_{t+\tau} x_t y_t p(x_{t+\tau},x_t,y_t) \log_2 p(x_{t+\tau}|x_t,y_t) \tag{A-0-4}
\]

If it is assumed that the 2 systems are independent, the time-shifted observation value \( x_{t+\tau} \) of system \( X \) is 
independent of the current observation value of the other electrode, \( y_t \). Therefore, the entropy rate (\( h_2 \)) is 
defined as:

\[
h_2 = - \sum x_{t+\tau} x_t y_t p(x_{t+\tau},x_t,y_t) \log_2 p(x_{t+\tau}|x_t) \tag{A-0-5}
\]

The TE from system \( Y \) to \( X \) is defined as the difference between \( h_2 \) and \( h_1 \) as follows:

\[
TE_{X \rightarrow Y} = h_2 - h_1 = \sum x_{t+\tau} x_t y_t p(x_{t+\tau},x_t,y_t) \log_2 p(x_{t+\tau}|x_t) \tag{A-0-6}
\]

In the case of multiple interacting processes (Montalto, Faes, and Marinazzo 2014), we obtain an extension 
of the original TE measure proposed for pair-wise systems:

\[
TE_{Y \rightarrow X|Z} = \sum y_{t+\tau} y_t x_t z_t p(y_{t+\tau},y_t,x_t,z_t) \log_2 p(y_{t+\tau}|y_t,x_t,z_t) \tag{A-0-7}
\]
Note that the TE can be seen as a difference of two conditional entropies (CE), or equivalently as a sum of four Shannon entropies:

\[
TE_{Y\rightarrow X|Z} = H(y_{t+\tau}|y_t, z_t) - H(y_{t+\tau}|y_t, x_t, z_t) = H(y_{t+\tau}, y_t, z_t) - H(y_{t+\tau}, y_t, x_t, z_t) + H(y_t, x_t, z_t)
\]

\[\text{Equation A-0-8}\]

TE does not have a meaningful upper bound (i.e., there is no value associated with ‘full’ connectivity). Current practical TE implementations report statistical significance values obtained from comparisons either between conditions or between real and surrogate data (Dolan and Spano 2001) rather than theoretical statistical hypothesis. TE, as other methods of brain estimators, is affected by very noisy measurements (Silfverhuth et al. 2012).

Transfer entropy is implemented in a multivariate time series, where it is necessary to proceed by first reconstructing the systems past states and then run the entropy estimators which evaluate the probability distribution functions.

The first critical issue is how to approximate the n-dimensional variables in order to represent the past of the processes. The main idea is to reconstruct the past of the whole system represented by the processes \(X, Y, Z\) with reference to the present of the destination process \(Y\), in order to obtain a vector \(\{V_n^Y, V_n^X, V_n^Z\}\) containing the most significant past variables to explain the present of the destination. Once \(V\) is computed TE is evaluated as Equation A-0-8. There are two ways of doing this. By using an uniform embedding (UE) and non-uniform embedding (NUE). According to (Montalto, Faes, and Marinazzo 2014), Figure A-0-1, is possible to see the lack of performance of UE for a multivariate non uniform system in separating the true from the false direction. So in this study the focus is on NUE. This method is based on the progressive selection: an iterative selection of the most informative terms through the optimization. The optimization criterion is based on the maximum relevance and minimum redundancy for candidate selection, so that the resulting embedding vector \(\{V_n^Y, V_n^X, V_n^Z\}\) includes only the components of \(y_t, x_t, z_t\) which contribute most to the description of \(y_{t+\tau}\).

After having determinate the most informative terms it is necessary to apply the estimation of TE. There are different approaches to evaluate the probability distribution function which constitutes the basis for TE in multivariate systems: Linear estimator (LIN), Binning estimator (BIN) and the k-Nearest Neighbor (NN). For the same reason as for the UE, LIN is not used in this thesis. Regarding the BIN it consists of coarse-graining the observed dynamics using Q quantization levels, and then computing entropies by approximating

\[
\text{Figure A-0-1} \text{ – TE values versus the number of significant realizations on a non-linear simulated system. For the NUE and UE with LIN, BIN and NN, in (Montalto, Faes, and Marinazzo 2014).}\n\]
probability distributions with the frequencies of occurrence of the quantized values. The transfer entropy is estimated as:

$$H(V_\xi) = -\sum_{V_\xi \in A^d} p(V_\xi) \log(p(V_\xi))$$  \hspace{1cm} \text{Equation A-0-9}$$

where the sum is extended over all vectors found in the available realization of the quantized series, and the probabilities $p(V_\xi)$ are estimated for each hypercube simply as the fraction of quantized vectors $V_\xi$ falling into the hypercube (i.e., the frequency of occurrence of $V_\xi$ within $A^d$). NN exploits the statistics of distances between neighboring data points in the embedding space to estimate the entropy term. Is optimal for the selection of candidates in a non-uniform embedding approach. TE based on the NN estimator is given by:

$$TE_{Y\rightarrow X|Z} = \psi(k)+<\psi(N_{\nu_1}v_1^Z + 1) - \psi(N_\nu + 1) >$$  \hspace{1cm} \text{Equation A-0-10}$$

where $N_{\nu_1}v_1^Z$, $N_\nu v_1^Z$ and $N_\nu$ are the number of points whose distance from $[V_n^I V_n^Z]$, $[V_n^I v_1^Z]$ and $V_1$, respectively, are strictly less than the distance from its $k$-th neighbor, and $< >$ denotes average over all $n$.

The TE is calculated as just described with the help of the toolbox MuTE, (Montalto, Faes, and Marinazzo 2014). This toolbox starts by normalizing the data and performing quantization when needed. Then it

$$TE_{Y\rightarrow X|Z}=yt+\tau yt, xt, zt, yt+\tau yt, xt, zt log2p(yt+\tau yt, xt, zt yt+\tau yt, xt)$$  \hspace{1cm} \text{Equation A-0-7}, CE2 accounting for the present state of the target series conditioned to the past

$$TE_{Y\rightarrow X|Z}=yt+\tau yt, xt, zt, yt+\tau yt, xt, zt log2p(yt+\tau yt, xt, zt yt+\tau yt, xt)$$  \hspace{1cm} \text{Equation A-0-7}, CE1 accounts for the present state of the target series conditioned to the past including the past of the target series and of the all other series except the driver. Such a vector is obtained subtracting the candidates belonging to the driver series from the set of candidates evaluated in the previous

$$TE_{Y\rightarrow X|Z}=yt+\tau yt, xt, zt, yt+\tau yt, xt, zt log2p(yt+\tau yt, xt, zt yt+\tau yt, xt)$$  \hspace{1cm} \text{Equation A-0-7} is performed given the finally result for the entropy.

Others GC measures: DTF and PDC

With the GC is also possible to perform frequency connectivity analysis. These methods are the directed transfer function (DTF) (Blinowska 1991) and the partial direct coherence (PDC) (Sameshima and Baccala 2001). DTF was first introduced in the form of:

$$DTF_{j=i}(f) = \frac{|H_{ij}(f)|^2}{\sum_{m=1}^{M}|H_{jm}(f)|^2}$$  \hspace{1cm} \text{Equation A-0-11}$$

where $H_{ij}(f)$ is an element of a transfer matrix of MVAR model. DTF describes causal influence of channel $j$ on channel $i$ at frequency $f$. The above equation (7) defines a normalized version of DTF, which takes values from 0 to 1 producing a ratio between the inflow from channel $j$ to channel $i$ to all the inflows to channel $i$. Other measures can income from DTF such as direct DTF which measure the direct connections. Regarding the PDC, it has the following form:

$$P_{ij}(f) = \frac{A_{ij}(f)}{\sqrt{a_{j}^*(f)a_{j}(f)}}$$  \hspace{1cm} \text{Equation A-0-12}$$

In the above equation, $A_{ij}(f)$ is an element of $A(f)$, where $a_{j}(f)$ is $j$-th column of $A(f)$ and the asterisk denotes the transpose and complex conjugate operation. Although it is a function operating in the frequency domain, the dependence of $A(f)$ on the frequency has not a direct correspondence to the power spectrum. PDC is
normalized to show a ratio between the outflows from channel $j$ to channel $i$ to all the outflows from the source channel $j$, so it emphasizes rather the sinks, not the sources.

Annex 2 – Simulated data

Here the reader can find details for the two simulated data used in the section 3.2 3.2 Model Comparison – Granger Causality vs Transfer Entropy (Time-invariant dataset) and section 3.3 Non-stationarity (Time-varying dataset). Both are based on the work of (Omidvarnia et al. 2014b).

For the time-invariant dataset, the model is a 5-dimensional time-invariant strictly-causal MVAR-process plus a linear superposition of sparse uniformly distributed random sources with approximately 50% nonzero entries within the interval $[0, 1]$, given by:

$$x(n) = y(n) + Vs(n)$$  \hspace{1cm} \text{Equation A-0-13}

where $y(n)$ is a strictly-causal MVAR model of order 3 with 5 channels and $x(n)$ is its distorted version with some confounding instantaneous interferences between channels defined by $V^{35}$. a time-constant random mixing matrix and $s(n)$, the intermittent interactions between channels given as a 6-channel sparse uniformly distributed random matrix with 50% nonzero entries. The distorted matrix $x(n)$ is finally used for connectivity analysis. The MVAR process $y = [y_1, y_2, y_3, y_4, y_5]^T$ is expressed as:

$$y(n) = \begin{cases} 
  y_1(n) = 0.95\sqrt{2}y_1(n - 1) - 0.9025y_1(n - 2) + 10w_1(n) \\
  y_2(n) = 0.5y_1(n - 2) + 5w_2(n) \\
  y_3(n) = -0.4y_1(n - 3) + w_3(n) \\
  y_4(n) = -0.5(n - 2) + 0.25\sqrt{2}y_4(n - 1) + 0.25\sqrt{2}y_5(n - 1) + 1.5w_4(n) \\
  y_5(n) = -0.25\sqrt{2}y_4(n - 1) + 0.25\sqrt{2}y_5(n - 1) + 2w_5(n)
\end{cases}$$  \hspace{1cm} \text{Equation A-0-14}

where $w = [w_1, w_2, w_3, w_4, w_5]^T$ is a normally distributed white noise vector with different variances for its entries.

For the time-varying dataset, the model is a 3-dimensional time-varying strictly-causal MVAR-process plus a linear superposition of sparse uniformly distributed random sources with approximately 50% nonzero entries within the interval $[0, 1]$ given by Equation A-0-14. Where $V$ and $s(n)$ follow the same characteristics as in the previous model. The MVAR process $y = [y_1, y_2, y_3]^T$ is expressed as:

$^{35}$ Matrix $V$ is a weighting matrix whose element in the $ij$ position represents the random interaction between the $i^{th}$ and $j^{th}$ component of $s(n)$. The elements were selected from the interval $[0, 1]$ through a uniformly distributed pseudorandom generator.
\[ y(n) = \begin{cases} 
\ y_1(n) = 0.59y_1(n - 1) - 0.20y_1(n - 2) + b(n)y_2(n - 1) + c(n)y_3(n - 1) + w_1(n) \\
\ y_2(n) = 1.58y_2(n - 2) - 0.96y_2(n - 2) + w_2(n) \\
\ y_3(n) = 0.60y_3(n - 3) - 0.91y_3(n - 2) + w_3(n) 
\end{cases} \]

where \( w = [w_1, w_2, w_3]^T \) is a normally distributed white noise vector with different variances for its entries.

The time course of the parameters \( b(n) \) and \( c(n) \) which denote the intensity of the influences are depicted in Figure A-0-2.

\[ \text{Figure A-0-2 – The parameters of the strength of directed influence: } b(n) \text{ denotes the strength of the influence between } y_1 \text{ and } y_2, \text{ and } c(n) \text{ that between } y_3 \text{ and } y_1. \text{ In the xx axes it is represented the data point 'n', and in the yy axes its amplitude} \]
Annex 3 – Figures

Figure A-0-3 – Scheme of Stage 1 of ECT Toolbox – Preprocessing of the Data.
Figure A-0-4 – Scheme of Stage 2 of ECT Toolbox – Granger Causality Analysis
Figure A-0-5 – Scheme of stage 3 of ECT toolbox – Statistical Inference.
Figure A-0-6 – A) Power spectrum densities of the ICA components for the all concatenated time-series. B) Topoplots with the maps of the ICA components for the all concatenated time-series. These two outputs allowed the visual checking for the most relevant independent components present in the time-series.
On the left side it is possible to see the results of the 2nd Level Analysis of 5 out of 6 subjects. Subject 1 shows an activity of 3.57% of the possible combinations, Subject 2 – 2.68%, Subject 4 – 8.93%, Subject 5 – 13.39%, Subject 6 – 0.89%. This percentage is obtained through the following formula: \[ \frac{\text{numb_channels}_{\text{active}}}{\text{numb_channels}_{\text{total}} \times (\text{numb_channels}_{\text{total}} - \text{numb_channels}_{\text{total}})} \times 100. \]

The information flow from the source in the column to the source in the line is coded by reddish colours, an information flow from the source in the line to the source in the column is coded by a blueish colour. Each of the subjects has to plot as in Figure 4.3.3. On the right side it is possible to see the histogram of a 1000 permutation of the null distribution on the maximal permutation statistics of a shuffle of both of the two condition. In green, it is represented the 95%-percentile that is used as threshold for relevance of the difference between condition rs on real TMS stimulation and rs on sham TMS stimulation, seen in the respective plot.
Figure A-0-8 – Representation of the topographic maps plotting the independent components (ICs) that represent cortical responses.