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Catarina Dinis Fernandes

Dissertação

Mestrado Integrado em Engenharia Biomédica e Biofísica

Perfil em Radiações em Diagnóstico e Terapia

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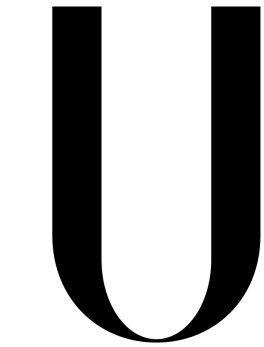

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Orientador externo: Professor Christian Schwarzbauer

Orientador interno: Professor Alexandre Andrade

2013

Declaration

I hereby declare that the work presented in this thesis is my own and has not been accepted in any previous application for a degree. It is a record of work, which has been carried out by myself at the Aberdeen Biomedical Imaging Centre, University of Aberdeen. Any contribution from other workers has been acknowledged in the text. All quotations have been distinguished by quotation marks and all sources of information specifically acknowledged.

Catarina Dinis Fernandes

September 2013

Para ser grande, sê inteiro,

Nada teu exagera ou exclui.

Sê todo em cada coisa.

Põe quanto és no mínimo que fazes.

Assim em cada lago a lua toda

Brilha, porque alta vive.

Ricardo Reis, 14-2-1933

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Resumo

O ser humano desde sempre se sentiu fascinado pelo estudo do seu próprio corpo assim como das suas propriedades funcionais. Do desejo de compreender e explorar o corpo humano surgiram então técnicas que permitem o seu estudo de modo não invasivo. Entre as primeiras técnicas de imagiologia encontram-se os Raios-X, a tomografia axial computadorizada (TAC) e a terapia por emissão de positrões (PET: do inglês "Positron Emission Therapy"). Contudo, todas elas utilizam radiação ionizante, e como tal surgiu o desejo de desenvolver novas metodologias igualmente não invasivas mas que por seu lado não utilizem qualquer tipo de radiação ionizante.

Entre estas técnicas encontra-se a imagiologia por ressonância magnética (MRI: do inglês "Magnetic Resonance Imaging") que pode ser utilizada para estudar as estruturas anatómicas mas também os seus mecanismos funcionais através da aplicação da técnica de ressonância magnética funcional (fMRI: do inglês "functional magnetic resonance imaging).

Contrariamente às técnicas que utilizam radiação ionizante, a imagiologia por ressonância magnética tira partido do facto de o ser humano ser maioritariamente constituído por água. Um ser humano adulto é por norma constituído por cerca de 70 – 80% de água (H_2O) o que se reflecte numa grande abundância de protões – núcleo ^1H. Quando submetidos a um forte campo magnético, o momento magnético destas partículas tende a alinhar-se de acordo com a direcção do campo magnético externo (B_0). Após alinhados os protões são então submetidos a um pulso de radiofrequência (com frequência igual à frequência de Larmor destas partículas) que é absorvido e modifica o momento magnético (i.e. *Spin*) dos protões. Quando este pulso é desligado, o *spin* dos protões retorna ao equilíbrio termodinâmico, de acordo com a direcção do campo magnético B_0, emitindo energia sob a forma de radiofrequência (RF). Estes mecanismos de relaxação diferem consoante o conteúdo em água dos tecidos e são estes que permitem a identificação da sua estrutura. Gradientes de campo magnético são tamb ém utilizados de modo a criar ligeiras diferenças no campo magnético que permitem a codificação do sinal com informação espacial.

A imagiologia por ressonância magnética faz, nos dias de hoje, parte da rotina hospitalar providenciando imagens com grande precisão e resolução anatómica.

Todavia a informação estrutural nem sempre é suficiente para estudar patologias que não exibem diferenças anatómicas, tais como depressão ou esquizofrenia. Surge então a ressonância magnética funcional, que utiliza o nível de oxigenação do sangue (BOLD: do ingles "Blood-oxygenation level dependent") como uma medida indirecta de activação neuronal. Através da utilização desta técnica é então possível mapear zonas cerebrais responsáveis pelo processamento de sinais como por exemplo estímulos visuais, tácteis ou auditivos. A título de exemplo, temos o estudo de doenças como o autismo ou até mesmo de distúrbios de consciência. A nível clínico a ressonância magnética funcional é utilizada para mapear funções críticas como por exemplo a fala, o movimento, o planeamento de tarefas, etc. Esta técnica oferece aos profissionais de saúde a chance de desenvolver um melhor planeamento cirúrgico sendo que é também aplicada no planeamento de tratamentos de radioterapia a nível cerebral com o intuito de mapear funcionalmente o cérebro e detectar os efeitos que tumores, AVC e lesões cerebrais possam ter ao nível da re-estruturação das suas funções.

Até muito recentemente a grande maioria da informação disponível acerca da conectividade anatómica cerebral era estritamente proveniente de estudos efectuados em primatas, recorrendo ao uso de técnicas extremamente invasivas (Felleman, Van Essen 1991, Jones, Powell 1970, Mesulam 2000, Ungerleider, Haxby 1994) assim como do estudo de lesões em casos humanos (ex: (Geschwind 1965)).

Frinston (Friston *et al*. 1993) utilizando PET e Biswal (Biswal *et al*. 1995) através do uso de fMRI foram os primeiros a identificar que para além das ligações anatómicas entre diferentes estruturas cerebrais é também possível identificar ligações funcionais entre regiões que à primeira vista parecem não ter qualquer tipo de ligação. À técnica que usa MRI no estudo da conectividade funcional foi dado o nome de conectividade funcional de ressonância magnética (fcMRI: do ingles "Functional connectivity MRI"). Esta utiliza ressonância magnética funcional e as oscilações de baixa frequência ao nível do sinal BOLD em cada voxel para estabelecer correlações. Com base na ideia de que duas zonas se podem dizer funcionalmente relacionadas se estas se encontram a operar no mesmo processo, é portanto possível assumir que as variações no seu sinal BOLD serão bastante semelhantes exibindo uma alta correlação. A título de exemplo vejamos duas regiões do córtex motor primário, localizadas em hemisférios opostos, e que contudo apresentam sinais BOLD altamente

correlacionados. Com esta ideia em mente foi então desenvolvido o conceito de redes* funcionais que são usualmente estudadas durante períodos de repouso†. Exactamente durante esta condição foi verificada a existência de uma rede funcional extremamente consistente entre indivíduos, e mesmo entre diferentes estados como durante o sono ou anestesia. A esta rede foi dado o nome de "Default-mode network" (Raichle et al. 2001) sendo que esta inclui regiões do córtex posterior cingulado, precuneus e do córtex prefrontal medial. A "default-mode network" é a rede mais estudada, mas para além desta existem outras redes tal como a rede visual, a auditiva, a de controle executivo, a de atenção, entre outras. Estas redes encontram-se frequentemente interrompidas ou modificadas em casos de doença. Os projectos descritos no âmbito desta dissertação focam-se no estudo destas redes bem como das suas propriedades em casos de doença (distúrbios de consciência, AVC) e durante a performance de actividade física. A fim de estudar estas redes funcionais foram utilizados diferentes métodos para o cálculo da conectividade funcional. Entre os mais reconhecidos métodos de cálculo de conectividade funcional encontram-se a análise com base numa região de interesse‡, a análise através do estudo da independência entre componentes§ bem como métodos que permitem o cálculo da conectividade cerebral a nível global**. Os métodos que utilizam uma região de interesse focam-se no cálculo da conectividade entre esta região e o resto do cérebro através do uso de medidas de correlação. O segundo método mencionado separa as várias redes neuronais com base na máximizacao da sua independência estatística. Por último, os metodos de análise global calculam a correlação das série temporal de cada voxel com todos os outros voxeis do cerebro. A contribuição da autora para os estudos descritos ao longo desta dissertacao focou-se no uso de duas destas técnicas – "seed-based analysis" e "wGBC"- no cálculo da conectividade cerebral em cada um dos diferentes projectos.

No primeiro projecto, descrito no capítulo 3 desta dissertação, são apresentadas vários paradigmas que em conjunto com o uso de ressonância magnética funcional, foram desenhados para detectar consciência e percepção em doentes que sofrem de distúrbios de consciência. Estes paradigmas foram testados num grupo de voluntários saudáveis de modo a verificar se são adequados ou se necessitam de ser optimizados. A autora foi então responsável por executar uma análise individual e de grupo da activação induzida pela

* do inglês: "networks"
† do inglês: "resting state"
‡ do inglês: "seed-based analysis"
§ do inglês: "ICA"
** do inglês: "wGBC"

execução destes mesmos paradigmas. O desenvolvimento de paradigmas adequados a estes pacientes, combinadas com o uso de fMRI vem complementar e melhorar o diagnóstico e prognóstico destes doentes.

No capítulo 4 desta dissertação a autora focou-se na análise da conectividade functional em pacientes que foram diagnosticados com um pequeno AVC, com enxaquecas e com TIAs[††]. Este procedimento utilizou técnicas de cálculo da conectividade com regiões de interesse e medidas globais de conectividade funcional. O objectivo deste estudo é uma vez mais averiguar se a inclusão de uma sequência de conectividade functional poderá facilitar o diagnóstico destes doentes bem como o seu prognóstico.

No quinto capítulo a autora foca-se no estudo das diferenças induzidas ao nível da conectividade funcional por uma única sessão de exercício físico. São uma vez mais utilizadas técnicas de cálculo da conectividade com regiões de interesse bem como outros métodos implementados por outros investigadores do departamento.

É também incluído nesta dissertação um capítulo no qual foram analisadas as propriedades destas redes neuronais ao nível de uma população saudável. É importante que tanto as condições de aquisição dos dados de ressonância magnética funcional como as metodologias de análise estejam bem estabelecidas para que os dados provenientes de diferentes estudos sejam comparáveis e para que possamos estabelecer de forma fiável conclusões acerca de populações saudáveis e doentes. O conceito de repouso é ainda muito variável, particularmente quando é apenas pedido aos participantes que permaneçam calmos e imóveis. Certos estudos requerem que os participantes permaneçam de olhos fechados, outros de olhos abertos e outros ainda que fixem uma imagem projectada num ecrã. Uma grande variabilidade de estados podem ser originados com este design experimental, sendo que estes vão desde o simples devaneio em torno de um assunto, que por qualquer razão se encontra mais fortemente em mente, ou até mesmo o adormecer. Com o objecto de estudar estas variações, o capítulo 6 foca-se na investigação da conectividade cerebral resultante de duas diferentes situações bem como da sua variabilidade. Neste capítulo a autora procurou estudar a reprodutibilidade e confiança destas redes funcionais cerebrais quando é pedido aos participantes que executem uma tarefa de baixo requerimento cognitivo. A análise foi executada através do cálculo da correlação entre séries temporais bem como da sua análise

[††] do inglês: “Transient Ischaemic Attack”

estatística, utilizando medidas como o coeficiente de correlação intra-classes, que fornece uma estimativa de reprodutibilidade entre diferentes medições.

Deste trabalho resultaram uma apresentação oral e a apresentação de um poster. Os resultados foram no geral positivos mas em alguns casos bastante ambíguos. As mais recentes publicações evidenciam o interesse em estudar não só a distribuição espacial destas redes como também as suas propriedades temporais que se parecem evidenciar como extremamente dinâmicas. Como tal fica aqui aberto o caminho para a continuação da exploração das redes funcionais cerebrais bem como da sua variabilidade.

Numa nota final, consideramos importante salientar que o vasto estudo da conectividade cerebral assim como o dos seus mecanismos é ainda uma área de investigação com pouco mais de uma década e com um ainda longo caminho a percorrer.

Abstract

Conventional functional magnetic resonance imaging (fMRI) is used to measure small fluctuations in the blood oxygenation level dependent (BOLD) signal resulting from neural activation due to an external stimulus or task. Nonetheless, this imaging technique can also be applied to the study of functional connectivity in the human brain. Since it was first acknowledged that BOLD signal fluctuations also occur during resting periods that increased attention has been directed to the investigation of brain behaviour during this particular state. There is still an on-going debate as to whether these fluctuations actually reflect neuronal baseline activity or are just the result of physiological metabolism and therefore independent o neuronal function. Also, can this resting state activity be truly called a "baseline" for comparisons? Moreover, functional connectivity has identified several networks, of which the default mode network is the most robust. This network is believed to have a great importance in brain awareness and cognition.

Further research is crucial to correctly understand these events and also to create a standardised methodology to perform the resting state fMRI acquisitions. The RESTATE (Resting State Techniques) project arises from the need to comprehend and correctly interpret the measured low frequency BOLD oscillations during resting periods. With this longitudinal study, comprising a baseline and a follow-up scan, we aim to assess the implications of using a low cognitive level paradigm upon the reproducibility of the data during functional connectivity analysis.

Key Words

Magnetic Resonance Imaging (MRI); Functional Magnetic Resonance Imaging (fMRI), Functional connectivity Magnetic Resonance Imaging (fcMRI); Resting State fMRI; Seed-based analysis; Reliability and Consistency.

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List of Abbreviations

3D	Three Dimensional
Aud	Primary auditory cortex
BOLD	Blood Oxygen-level Dependent
BA	Brodmann Area
CSF	Cerebrospinal Fluid
DMN	Default-mode Network
DoC	Disorders of Consciousness
DWI	Diffusion Weighted Imaging
EPI	Echo Planar Imaging
FC	Functional Connectivity
fcMRI	Functional Connectivity Magnetic Resonance Imaging
FEF	Frontal eye fields
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FWE	Family wise error
FWHM	Full width at half maximum
GE	Gradient Echo
GM	Grey matter
HF	Hippocampal formation
ICA	Independent Component Analysis
ICC	Intraclass Correlation Coefficient
IPS	Intraparietal sulcus
LatPar	lateral parietal cortex
LIS	Locked-in-Syndrome

M1	Primary Motor Cortex
MCS	Minimally Conscious State
Mot	Primary motor cortex
mPFC	medial Prefrontal cortex
MRI	Magnetic Resonance Imaging
MT	Medial temporal area
NBS	Network Based Statistic
PCC	Posterior cingulate cortex
RF	Radiofrequency
ROI	Region of Interest
rs-fcMRI	Resting state Functional connectivity Magnetic Resonance Imaging
RSN	Resting state networks
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
SE	Spin Echo
SnPM	Statistical non-Parametric Mapping
SPM	Statistical Parametric Mapping
TE	Echo Time
TIA	Transient Ischaemic Attacks
TR	Repetition Time
V1	Primary Visual Cortex
Vis	Primary visual cortex
VS	Vegetative State
wGBC	Weighted Global Brain Connectivity
WM	White matter

Chapter 1

Introduction

1.1 Rationale

Research has extensively been developed during the last decade using functional connectivity MRI. This method has proved to efficiently distinguish between healthy and diseased populations and has also provided us with a greater insight into brain mechanisms. Particularly during resting periods, this methodology has raised the interest of researchers and practitioners since it does not require the performance of a particular task. We therefore focus our attention into the different analytical methodologies to perform functional connectivity analysis using different populations and study designs.

1.2 Project Aims

Resting state fMRI has found large application in the study of pathological states where the intrinsic activity was found to be related to the severity of the disease. Regardless of the still early stages of development, the clinical applications of this technique demonstrate great promise. The aim of this dissertation is to investigate functional connectivity in pathological cases as well as connectivity differences arising from exercise.

Nevertheless, the concept of resting state is complex and probably elusive (Morcom, Fletcher 2007a) and up until today there is no established methodology for resting state fMRI studies.

It is therefore expected that the reliability of functional connectivity during resting state is affected due to inter-subject and inter-session variability.

Several papers have been published studying the reliability and consistency of resting state fMRI measurements (Wang *et al*. 2011, Fiecas *et al*. 2013, Yan *et al*. 2009, Guo *et al.* 2012, Shehzad *et al*. 2009, Patriat *et al*. 2013) and networks such as the Default-mode network (DMN) (Greicius *et al*. 2003, Fox *et al*. 2005, Fransson 2005), when using conditions such as eyes open, eyes closed and passive visual preprocessing tasks. To our knowledge, no study has been published investigating these statistical parameters in the presence of a low cognitive demand task. With the RESTATE study we aim to quantify possible differences and their statistical significance between the two conditions, subjects and scans.

1.3 Methodology

Every study described during the course of this dissertation resulted from the interest to study functional connectivity under different conditions and diseases. All of them went through a process of study design, ethical approval, recruitment and data acquisition.

The author took part in the RESTATE study design, execution and analysis. The following general methodology was applied to all separate studies described in this dissertation.

1. The study started with a literature review of what has been previously done in order to design a suitable study protocol. The literature review provides important clues on how to efficiently outline the purpose of the study regarding what has been previously investigated and what sort of contributions can be achieved to provide better knowledge towards the work of researchers and the general public.

2. A proposal had to be written to the ethical committee in charge of the area in order to obtain ethical approval for the study. In the particular case of the RESTATE study it was designed as an amendment to the ethics application previously submitted for the TIA cfMRI study.

3. After ethical approval a proposal had to be submitted to the Research and Development (R&D) department and approved before the study could officially start.

4. The recruitment started after approval of the R&D. Patient/volunteers were carefully screened to ensure that they were suitable for the specific research.

5. Data management is of increased importance with studies involving patients. For the studies involving NHS stroke patients all information had to be stored on the main site file at the Aberdeen Biomedical Imaging Centre as well as in a site file located at the Stroke-Unit of the Aberdeen Royal Infirmary (NHS Grampian).

6. At last, the structural scans were screened by a radiologist to identify any possible accidental findings and if these existed they were reported to the participant as well as to their General Practitioner (GP). The data was then processed to obtain the functional connectivity results.

1.4 Thesis Outline

This dissertation features a series of connectivity studies developed at the Aberdeen Biomedical Imaging Centre during a period of 9 months. The methodology used to perform functional connectivity MRI analysis is common to all of the separate studies even though the their aims and outcomes vary. Therefore, this dissertation is outlined in a way so that each chapter represents a specific study. The particular purpose, aims, methods and results of each study are discussed in each of these chapters. The studies are organised chronologically as the work was developed.

- **Chapter 1.** *Introduction* – This chapter is a brief overview of the structure used throughout the dissertation as well as of the work developed.

- **Chapter 2.** *Background* – In order to contextualize the work developed this chapter includes an introduction to MRI, resting state techniques and functional connectivity methodologies. The various scanning procedures used in the studies are also described in this chapter.

- **Chapter 3**. *DoC study* - The first study focuses on Disorders of Consciousness (DoC) and how the use of new imaging techniques such as fMRI can help in the assessment of these patients. The contribution of the author to this study was in ascertaining the validity of the paradigms designed to assess consciousness in DoC patients. In order to do so, the author used an in-house built method to quantify the activation induced by the paradigms in a group of healthy volunteers. This analysis was performed to test the paradigm and use the results as a point for improvement.

- **Chapter 4**. *TIA cfMRI study* -The second project is focused on the study of stroke patients presenting at the Aberdeen Royal Infirmary and who are given an uncertain diagnosis. This study aims to ascertain the advantages of including a functional MRI sequence into the already established neurovascular MRI protocol.

- **Chapter 5.** *PECON study*- The third study was designed to be an integrating part of the TIA cfMRI study by assessing the effects of an acute session of exercise in functional connectivity using a group of healthy volunteers.

- **Chapter 6.** *RESTATE study* **-** The fourth and last study chapter describes the RESTATE study, which focuses on the study of resting state fMRI acquisition techniques and the investigation of their reliability and reproducibility. The study was designed and put into practice during the course of this internship and this chapter provides a description of the whole process from ethical approval, volunteer recruitment, scanning procedure and equipment, cognitive assessment, the paradigm used, data analysis and results.

- **Chapter** 7. *Closing Remarks* – This last chapter describes the achievements accomplished during the course of the internship and their contribution to future research.

Chapter 2

Background

2.1 Magnetic Resonance Imaging

Magnetic Resonance (MR) is an imaging method that uses protons and their magnetic properties to generate an image. This modality takes advantage of the great abundance of the hydrogen nucleus in tissue in the form of water (each water molecule containing two hydrogen nuclei/protons) and particularly in the human body constituting up to 70% to 90% of most tissues. Moreover, the presence of a single positively charged proton in each hydrogen nucleus gives it a relatively large magnetic moment. When exposed to a strong static magnetic field (B_0), the majority of the magnetic moments (or spins) of the hydrogen nuclei will align parallel to the static field. The use of a short oscillating magnetic field at the Larmor frequency (i.e. RF pulse) results in the precession of the net magnetization towards the xy plane. The xy-magnetization will straight after start to diphase, along with the regrowth of the magnetisation in the direction of the external magnetic field (z-direction). Magnetic field gradients are used to cause the nuclei at different locations to precess at different speeds providing us with spatial information. Severe alterations in the tissue water content as well as in their magnetic properties can be found in cases of disease or injuries.

MR can not only be used to obtain structural anatomical images and investigate pathologies, but it can also be applied to study organ function, the chemical composition of tissues and to provide an insight into brain activity.

2.2 Imaging Techniques

2.2.1 Noise in functional magnetic resonance imaging sequences

The noise characteristics of data obtained using functional magnetic resonance imaging (fMRI) can pose a great challenge for the analysis process. The signal under focus represents less than 2% or 3% of the total BOLD (Blood oxygenation level dependent) response, reflecting a very small effect size. The majority of the signal is dominated by physiological noise (Kruger, Glover 2001) and scanner drift (Bianciardi *et al*. 2009). The noise present in the data has several sources that can be broken down into true noise and unaccounted-for-signal. True noise results from thermal motion of electrons residing inside the bore of the magnet or within the equipment that is used to collect the raw data. A second source of true noise is related with brain physiology. The observed noise in the fMRI data is also the result of various other contributions that can be described as unaccounted-for signal. Head motion, scanner drift, and uncontrolled cognitive activity on the part of the subject are some of the sources for this type of noise. Scanner drift is the result of slow but constant changes in the strength of the magnetic field inside the bore over the course of the scanning session and is modelled during data analysis. The subject under study should ideally lie very still in the scanner since even small movements of the head position can cause movement artefacts. Although these are accounted for and corrected during the preprocessing steps, large head movements can be problematic to correct. Physiological fluctuations resulting from heartbeat and respiration can be corrected by monitoring and recording these and use them as nuisance variables (regressors in the GLM model) during data analysis. Regarding the spontaneous low-frequency BOLD fluctuations, unrelated to the paradigm, and due to unconstrained cognitive activity, these are impossible to correct due to its unpredictable nature regarding time and location and are currently a relevant topic under extensive study.

2.2.2 Echo-Planar Imaging – EPI

Echo planar imaging (EPI) is one of the most used imaging sequences finding application in diffusion, perfusion and functional magnetic resonance imaging (fMRI). The methodology behind this sequence involves the acquisition of all k-space lines in one repetition time and using a single radio-frequency excitation. It provides us with images with decreased motion

artefacts with a reduced imaging time, providing us with the ability to image rapid physiological processes of the human body.

2.2.3 T_1 –weighted images

T_1-weighted images, usually known as 'anatomical scans', have very good contrast enabling a great distinction between the different tissue boundaries. In this particular sequence fluids are usually displayed as dark, water-based tissues have a mid-grey colour and fat-based tissues will be displayed very brightly. The studies described during the course of this dissertation use an ultrafast spoiled gradient echo (GE) sequence: T1-3D TFE (Turbo Field Echo), with a small flip-angle (8˚) and a very short repetition time (TR = 8.2 ms). This sequence uses an optimised k-space filling procedure to reduce the acquisition time. However, the drawback of a small flip angle and very short TR is poor T1-weighting. Therefore this GE pulse sequence uses an initial 180 degrees inversion pulse to prepare the magnetization and provide contrast enhancement before starting acquiring data.

2.2.4 T_2 –weighted images

T_2-weighted images acquired using a spin echo sequence will require a long TR and echo time (TE), making them more time-consumable than T_1-weighted images. When using this type of sequence the fluids will appear very bright and both water- and fat-based tissues will be displayed on the middle of the grey scale. This is one of the preferred sequences used for pathological scans since it enables the easy distinction of collections of abnormal fluid. Long T_2s will provide more signal and thus images obtained using this combination will be brighter than using short T_2s. T_2^*-weighted images are closely related to T_2-weighted ones, having basically the same contrast. The main difference is due to susceptibility effects responsible for creating field inhomogeneities that speed the transverse relaxation decay. SE sequences can correct for this effect but GE cannot. So GE sequences will result in images with a combined effect of T_2 and magnetic field inhomogeneity, being this relaxation known as T_2^*. For the TIA cfMRI study the acquired images were T_2^*-weighted using a GE sequence (T2W_FFE – Steady state Free precession).

2.2.5 Neurovascular protocol

The consultant neuroradiologist on the site, Dr. Arnab Rana, originally developed the neurovascular protocol used in the TIA cfMRI study.

The local minor-stroke MRI protocol includes:

1) T_1 structural image;

2) T_2* weighted sequence;

3) Fluid attenuation inversion recovery (FLAIR) pulse sequence;

4) Diffusion-weighted imaging (DWI) sequence.

For the purpose of the TIA cfMRI study an Echo-Planar Imaging functional MRI sequence was also included in the scanning protocol. Each of these sequences has a specific diagnostic value, even though they complement each others.

The T_1 structural image is used to identify bleeding, tumours and other structural abnormalities such as cortical laminar necrosis. The T_2* gradient echo structural image will exhibit an enhanced contrast in the presence of microbleeds therefore is used to confirm intracerebral haematomas. If a heaematoma is diagnosed then the use of blood thinning drugs is not recommended to prevent a potential stroke. The FLAIR sequence is included in this protocol once the inversion recovery nulls the CSF signal resulting in an easier identification of infarcts at the cortical surface compared to the standard T_2SE. This sequence also shows infarcts that are older than the hyper-acute lesion. Finally, DWI will identify infarcted regions as diffusion restriction.

Apart from the structural and the functional sequences, all of the other sequences are acquired in an axial plane. The reason behind this choice is related to an easier diagnose using this particular plane when compared with the coronal or sagittal.

The neurovascular protocol parameters are as follows:

A gradient-echo echo-planar sequence (EPI) was used to obtain the functional images (used in the posterior functional connectivity analysis) with an acquisition time of 10 minutes (30ms echo time; 2s repetition time; 78˚ flip angle; 96×96 matrix size; 240×240 mm^2 field of view; 32 slices; 3.5 mm slice thickness; 1 mm inter-slice gap; SENSE parallel imaging method with two-fold acceleration; 300 dynamic scans; 4 dummy scans). A high-resolution T1-weighted structural scan was also obtained in 5 minutes and 58 seconds, using fast three-dimensional gradient-echo imaging (8.2s repetition time; 3.8ms echo time; 8˚ flip angle; 240×240×125 matrix size; 240×240×160 mm^3 field of view; 1.0×1.0×1.0 mm^3 voxel size). A T_2*-weighted image was acquired with 706 ms repetition time; 16.11ms echo time; 18˚ flip angle; 232×229×131 matrix size; 230×131×182 mm^3 field of view; 4.50×4.5×4.5 mm^3 voxel size; 24 slices; slice thickness 4.5 mm. The parameters used to obtain the FLAIR images are 11000ms repetition time; 125 ms echo time; 120˚ refocusing pulse; 0.65×0.87 mm^2 voxel size; 29 slices; 4 mm slice thickness; Finally, a diffusion-weighted imaging (DWI) scan was acquired in 1 min and 30 s (b value of 1000; 152×152 matrix size; 230x230 mm^2 field of view; 24 slices; 4.5 mm slice thickness; 1 mm inter-slice gap; SENSE parallel imaging method with two-fold acceleration).

2.3 Resting state fMRI

During periods of resting wakefulness the human brain presents spontaneous, low frequency, fluctuations of the blood oxygenation level dependent (BOLD) signal. In 1995 Biswal and colleagues(Biswal et al. 1995) published the results of a study that focused on explaining the meaning of these oscillations. The authors first identified a region of interest in the left somatosensory cortex by using a traditional fMRI experimental design during which the subjects were performing bilateral finger tapping. The same subjects were posteriorly scanned during a period of rest, without performing any sort of cognitive, motor or language task. The results reflected high correlation between the seed region in the left somatosensory cortex and the homologous areas in the contralateral hemisphere.

One of the major motivations for studying spontaneous activity in the brain focuses in understanding brain energy metabolism systems. The brain represents only 2% of total body mass but is responsible for the consumption of 20% of body's energy (Raichle, Mintun 2006). Task related increases in neuronal metabolism are usually small (<5%) when compared with its large resting energy consumption(Raichle, Mintun 2006).

The first studies identifying the presence of spatial patterns with coherent signal fluctuations in the human brain were performed in the late 90's and early 2000 using both fMRI (Biswal et al. 1995, Lowe, Mock & Sorenson 1998) and positron emission tomography (PET) (Friston *et al*. 1993, Shulman *et al*. 1997, Raichle *et al*. 2001a). These patterns have been named "intrinsic connectivity networks" (Seeley et al. 2007) or "resting state networks" (RSNs) (Greicius et al. 2003). These RSN are located in grey matter regions and several studies have suggested the importance of some of these networks supporting core perceptual and cognitive processes therefore strengthening the hypothesis that these reflect functional systems with intrinsic energy demands. The neuron population enclosed within each networks is thought to be firing together with a common functional purpose. The patterns displayed by RSN are reliable and reproducible across a range of analysis techniques, conscious states and both in an individual subject and group level (Greicius, Menon 2004, Damoiseaux et al. 2006, Shehzad et al. 2009).

These BOLD signal fluctuations are intrinsically generated by the brain and do not happen as a result of an input from the outside environment (e.g. being asked to perform a task) or an output (e.g. performing the task). Therefore, fMRI studies of spontaneous activity attempt to

minimize changes in sensory input and ask the participants to refrain from making any specific cognitive task. This scanning protocol has been commonly named 'resting state fMRI' since subjects are usually instructed to simply lie in the scanner and refrain from falling asleep. However, there is still some debate on whether the low frequency BOLD fluctuations observed during 'resting state' represent true intrinsic neuronal activity or are just the result of 'mind wandering' and conscious mentation (Morcom, Fletcher 2007b).

Up until today, most of the studies performed seem to suggest that although spontaneous individual behaviour is likely to contribute to resting state BOLD fluctuations, this is unlikely to be the main source of the signal. Supporting this hypothesis are the studies reporting similar spatial location of BOLD correlations across different behavioural states, including different resting conditions, task performance, sleep and even anaesthesia. Furthermore brain activity evoked by the performance of a task seems to be distinct from and only superimposed on the underlying spontaneous activity. A 2006 study performed by Nir and colleagues (Nir et al. 2006) reported that spontaneous cognition, such as mental imagery, results in patterns of neuronal activity in visual regions that are distinct from the patterns observed in spontaneous activity. These studies support the idea that unconstrained behaviour experienced by the participants inside the scanner will result in BOLD modulations that are in addition to, and not the source of, spontaneous coherent BOLD fluctuations. Therefore one can divide the spontaneous BOLD oscillations into two different components: resulting from unconstrained behaviour and the intrinsic activity underlying the first and persisting across different states (Fox, Raichle 2007).

Two very important data analysis issues should be considered when studying resting state data: how to account for non-neuronal noise and how to identify spatial patterns of spontaneous activity. As mentioned in the previous section, non-neuronal components (e.g. cardiac and respiratory activity) can be measured during data acquisition and removed from data through linear regression. Independent component analysis is a technique that can be used to isolate noise sources from the BOLD data itself, along with the regression of signals that are common to all the voxels (e.g. the global signal) or signals from regions that are likely to have a high degree of physiological noise compared to the amount of neuronal activity (e.g. ventricles or white matter).

In the early days of resting state analysis the attention was focused on how this state could potentially represent a baseline for comparisons with activation studies. The

acknowledgement of a true baseline would provide a new tool for block-design fMRI experiments as well as the correction of the influence that this baseline might have on the signal under study. However the lack of agreement on whether 'resting state' represents a true neuronal baseline or just a physiological response has moved the current functional connectivity studies to focus on the dynamic properties of these low frequency oscillations which can provide new clues to the mechanisms underlying brain function.

2.3.1 Resting state networks

The low frequency BOLD fluctuations observed during the 'resting state' (i.e., in the absence of an external stimulation task) and described in the previous section were not only found to be reproducible but also to show temporal correlations between different areas of the brain. To these distant regions that are thus hypothesised to be working on the same process, we give the name of resting state networks. The most studied and reproducible network is the default-mode network (DMN) that is thought to be involved in memory consolidation and keeping a certain level of awareness even when resting.

The first evidence for the default-mode hypothesis came from a PET study carried out by Raichle *et al*. in 2001(Raichle et al. 2001a). In this study the volunteers were asked to rest quietly with their eyes closed. It was found that consistent regions of the brain were active at rest but decreased their activity when cognitive tasks were performed. The authors then suggested the existence of an organised, baseline default mode network (DMN) of brain function. Not long after, Greicius *et al*., 2003(Greicius et al. 2003)firstly identified the DMN using functional MRI. A study performed by the same team also revealed that even thought this network is affected by the performance of cognitive tasks (presenting a decreased magnitude) its activity persists throughout both experimental and rest periods if the experiment is not sufficiently challenging(Greicius et al. 2004).

Numerous studies(Greicius et al. 2003, Shulman et al. 1997, Mazoyer et al. 2001)hypothesise the existence of two large opposing networks in the brain: the "task-negative" including the DMN and the "task-positive" composed by networks involving attention and task-based systems such as the somatosensory or visual.

Several other resting state networks have been identified up until today and investigation suggests the existence of at least 6 consistent networks: Visual, Auditory, Dorsal and ventral attention, default-mode network, somatosensory network and frontoparietal network. Although there is no agreement on the literature regarding neither the names nor their divisions the existence of significantly agreeable resting state networks is already an established fact. Once again, these patterns of activated regions are consistent across subjects, states of cognitive development, degrees of consciousness, to some degree even across species, and under pharmacological manipulation.

2.4 fMRI analysis

After data acquisition and before model estimation the data has to be preprocessed. During this stage the images are realigned with each other, the functional scans are co-registered to the structural image and there is usually also a normalization step that ensures that all the brains are in the same image space. **Figure 2.1** summarises the most common preprocessing pipeline procedure.

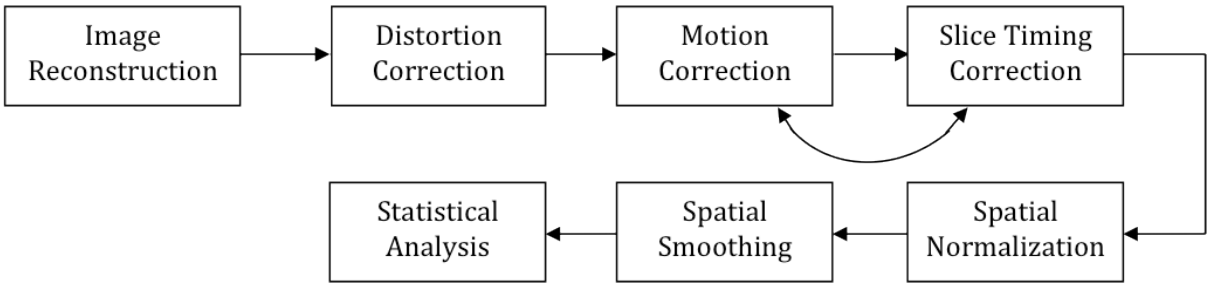

Figure 2.1 - Schematics of the fMRI preprocessing pipeline

We designate as 'native space' the original coordinate system as the images were acquired from the MRI scanner. The brain of different individuals will not necessarily line up in the native space due to different brain sizes, and even the same individual will have his or her brain in a different position during different scans. Therefore there is a need for a standardised/stereotactic space to register the images with. The studies performed and described on the forthcoming chapters will use the MNI (Montreal Neurological Institute) stereotactic coordinate space by default.

Motion correction (realignment and reslice) is another step that has to be performed during fMRI data analysis. The studies described in this dissertation used a rigid-body spatial transformation model (with six-parameters), which assumes that the effects of motions do not change the shape of the brain, just its position and orientation.

Slice timing correction is a process that is not implemented in all studies. Nonetheless, slice timing correction is important and it is used because different slices are acquired in sequence therefore the BOLD signal is sampled at different time points in different parts of the brain. One would ideally like to have the signal for the whole brain at the same time point. This correction method works at the voxel level examining the time course and shifting it by a

small amount and using interpolation with the points actually sampled in order to obtain the time course we would have if we sampled all the voxels at the same time.

Normalization of data with the presence of lesions can prove to be difficult due to existence of areas with missing signal. This can result in substantial lack of information to perform a correct spatial normalization. The standard way to deal with this problem is to use cost function masking, in which a portion of the image (in this case the area with the lesion) is excluded from the cost function computation during registration. For the particular case of the patients involved in the fcMRI study of a stroke population (Chapter 3) the participants only presented with small lesions such as minor ischaemic attacks. As it has been described before, the normalization of standard stroke lesions would use a lesion masking procedure, however, the lesions in the dataset of chapter 3 are of a relatively small size and have proven not to interfere with the normalisation of the data.

As mentioned before artifacts should always be corrected during data preprocessing. When carrying out connectivity analysis one has to be extremely careful with artifacts such as head motion since these can cause spurious connections between regions of the brain. The typical nuisance trends considered to be removed from the resting state fMRI data include the six motion parameters, the average signal from white matter regions, ventricular signal, global mean signal, and physiologic signals such as the heart rate and respiration (if available) (Cohen et al. 2008, Fox et al. 2005). Opinions differ on the use of global signal regression and whether this step should or not be implemented. The main reason supporting the use of global signal regression is related to information on non-neuronal signal artifacts (such as components related to motion and respiration) that are encompassed in this variable. Nevertheless recent studies discourage the use of this variable as a regressor. A more detailed overview about this topic can found in the Chapter 5 of this dissertation.

When using spatial smoothing we assure a more homogeneous signal since the data in one voxel is averaged with its neighbours. It is important to avoid using too much smoothing otherwise most of the information can also be lost. It is a fine compromise and the usual values for smoothing range between a Gaussian kernel of 4 and 8 mm.

The last step of fMRI analysis is the statistical testing of the results obtained with the aim of testing/validating a hypothesis. This procedure usually involves the use of t-tests and will provide a valid method to verify if for e.g. two conditions elicit a different brain response or not.

2.5 Functional connectivity MRI (fcMRI)

The analysis of brain connectivity can be divided into three types: Anatomical connectivity (AC), Functional Connectivity (FC) and Effective Connectivity (EC). Anatomical connectivity, as the name implies, is the study of the anatomical connections between different brain regions. On the other side, functional connectivity assumes the possibility of an anatomical pathway but is mainly focused on the study of temporal correlations in BOLD fluctuations between different brain areas. Finally, effective connectivity is the specific study of causal influence between at least 2 regions, and the direct and/or indirect influence that these regions exert upon each other (Varsou, Macleod & Schwarzbauer 2013). Apart from their fundamental differences these types of connectivity are usually found to interact with each other. Hence the measured connectivity may be the result of a combination between anatomical, effective and functional networks. The studies in this dissertation are based on functional connectivity and therefore any future references on brain connectivity will relate to this specific type of connectivity.

As mentioned before, functional connectivity targets the study of temporal correlations between different brain areas in the fMRI data. This is a non-invasive technique used to study the large-scale neural networks in the brain (Bullmore, Sporns 2009, Salvador et al. 2005, Zhang, Raichle 2010).

Low-frequency spontaneous oscillations in the BOLD signal are correlated over time between regions within the same working brain systems. **Figure 2.2** depicts the BOLD time course of an example subject measure in (*top*) both left (green) and right (red) primary motor cortex where can clearly be seen a pattern of correlated fluctuations; and (*bottom*) the absence of correlation between motor (green) and visual (blue) regions.

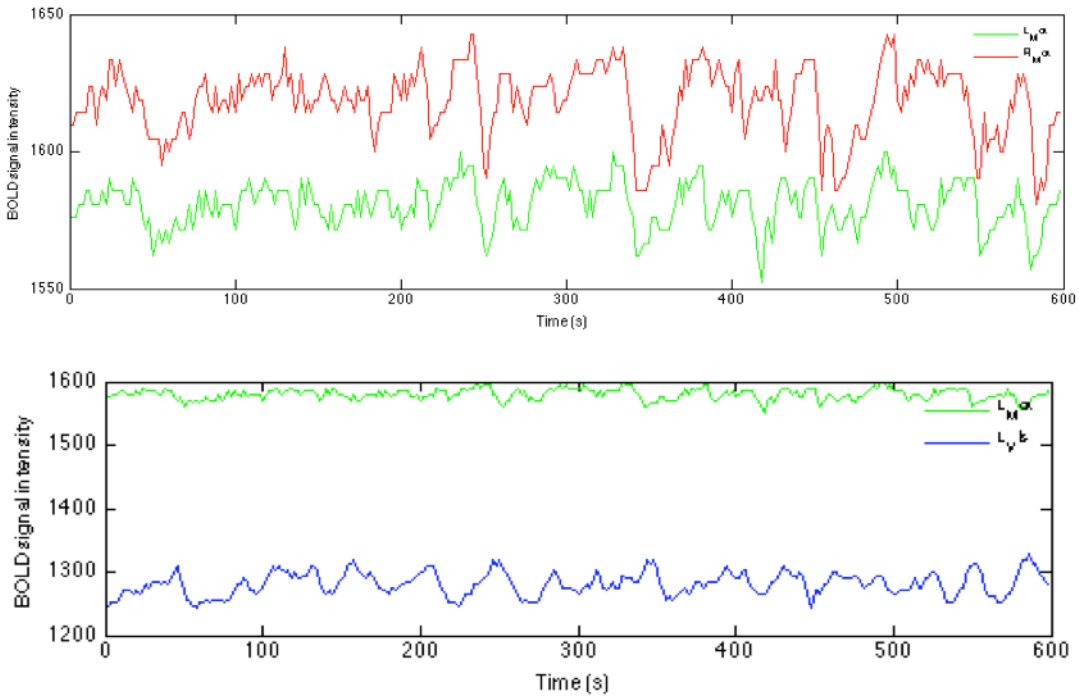

Figure 2.2 - BOLD time course of an example subject: *top* – time course of two regions located in the left (green) and right (red) primary motor cortex; *bottom* – time course of two regions located in the left (green) primary motor cortex and the left (blue) primary visual cortex.

Functional connectivity MRI applied during 'resting state' has proved to be a good segregation method between health and diseased population. Resting state fMRI has also shown that neuronal network patterns change with increasing age(Meunier et al. 2009) and it has just started to be used in the study of the neonatal and fetal development (Schöpf et al. 2012). On the other hand, the resting state networks on their own have also provided clues regarding brain function. The DMN has shown to be affected by ageing (Damoiseaux et al. 2008) and disrupted in several neuropsychiatric disorders such as mild cognitive impairment (Sorg et al. 2007), Alzheimer's disease (Greicius et al. 2004, He et al. 2009), schizophrenia (Calhoun, Eichele & Pearlson 2009, Zhou et al. 2007), depression (Greicius et al. 2007), and autism (Kennedy, Redcay & Courchesne 2006, Müller et al. 2011). Regarding autism spectrum disorders the study of resting state activity has shown that multiple networks are affected by this condition presenting each presenting some sort of dysfunction. Depressive disorder studies show some evidence for abnormal hyperconnectivity, moreover the study published by Perrin *et al.* (Perrin et al. 2012) analysed depression patients before and after electroconvulsive therapy (ECT) treatment and reported a decreased connectivity in the left dorsolateral prefrontal cortex after treatment. The patients included in the study had reported improvement in symptom intensity after the ECT treatment, which was found to be correlated

with the decreased connectivity pattern identified. Patients suffering from Alzheimer's exhibit abnormal neuronal patterns as well as Parkinson's (Wu et al. 2009) (connectivity disrupted in motor areas), epilepsy and multiple sclerosis.

Some of the most widely used methodologies to investigate functional connectivity in fMRI data include seed-based analysis, independent component analysis (ICA) and graph theory methods. Graph measures were not used in any of the studies described in this dissertation so they will not be described any further. The two forthcoming sections (Section 2.5.1 and Section 2.5.2) will give a brief overview of the theory behind seed-based and weighted global connectivity measures as well as of their strengths and weaknesses. Section 2.5.3 will focus on independent component analysis.

It is important to note that subtle changes in the analytic approach of resting state data, for example using slightly different spatial seeds in seed-based correlation, or altering the model order dimensionality estimation in ICA, can have a significant impact on the spatial characteristics of the RSNs identified.

2.5.1 Seed-correlation analysis

Seed-based correlation mapping is a methodology based in extracting the BOLD signal time course from a "seed" region of interest (ROI). The time courses from all the other voxels in the brain are also extracted and a correlation measure is computed between these and the seed's time course (**Figure 2.3**). The signal from the seed region is either averaged before computation of the correlation measure or after by averaging the correlation values of the voxels inside the ROI. The Pearson product-moment correlation method is the most widely used measure of functional connectivity. Nevertheless, when using this method one has to carefully remove the non-neuronal contributions (such as head movement or any other confounding variables) insofar as is possible to ensure that the calculated correlations truly reflect neuronal activity. In addition, low pass filtering of the data should be performed under the assumption of temporal sample independence, to address the high noise content of fMRI data. This step attempts to minimise artificial correlation emanating from noise processes such as synchronised cardiac and respiratory signals (Birn et al. 2006). The physiological noise resulting from respiratory and cardiac function is concentrated at relatively high frequencies (>0.1Hz) (Cordes et al. 2001a, Lowe, Mock & Sorenson 1998, Thomas,

Harshman & Menon 2002) while scanner drift is localised to frequencies bellow 0.01Hz. Signal resulting from spontaneous neuronal activity is mostly confined to frequencies between 0.01Hz – 0.1Hz (Biswal et al. 1995, Cordes et al. 2001a, Demirci et al. 2009, Salvador et al. 2005). Therefore the majority of spontaneous BOLD studies perform low-pass filtering of the data at a cut-off of 0.08 or 0.1Hz.

The primary advantage of seed correlation analysis (SCA) over other methods is that this approach provides straightforward, sensitive and easy to interpret results, showing the network of regions most strongly functionally connected with the seed voxel or region of interest. SCA is an attractive approach for many researchers due to its inherent simplicity, sensitivity and ease of interpretation. In 2009 Shehzad *et al*. (Shehzad *et al*. 2009) published a study with an assessment of the test-retest reliability of the most widely used connectivity measures and SCA has proved to provide moderate to high reliability in identifying RSN connectivity relationships.

Nonetheless, this method requires *a priori* assumptions regarding seed location, which in turn can be considered to bias the connectivity findings towards specific, smaller or overlapping sub-systems, rather than the larger, distinct networks. Moreover, the fundamental problem resides in the fact that there are as many possible networks to be derived, as there are possible seeds. Some seeds are regarded as being very reliable (e.g. posterior cingulate cortex) in determining specific networks (e.g. DMN). However small changes in the seed coordinates can prove to completely distort the resulting connectivity map.

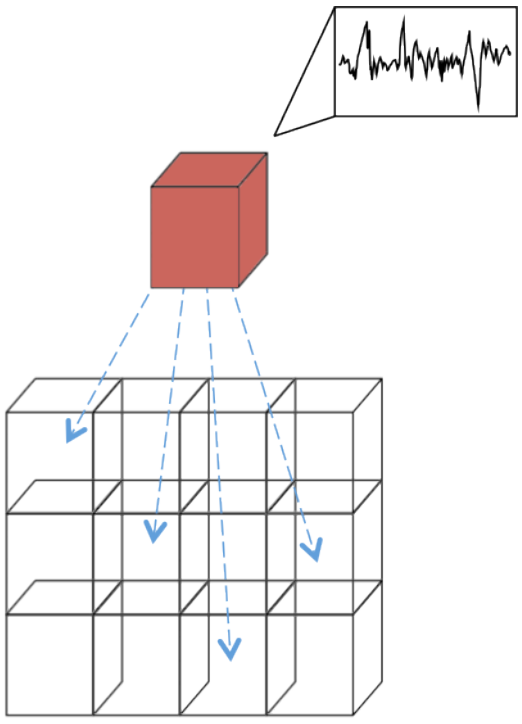

Figure 2.3 - Schematic of the principles of seed-based functional connectivity analysis

2.5.2 Weighted Global Connectivity (wGBC)

Alongside the increasing power of computation as well as the desire to study how the brain is intrinsically correlated, new methods have just recently started to be applied to the study of brain connectivity. One of them is a voxel-wise whole brain analysis, developed by Cole and Schneider (Cole, Schneider 2007), and first applied to the whole brain by Cole, Pathal and Schneider (Cole, Pathak & Schneider 2010), named weighted Global Brain Connectivity (wGBC). This method calculates a correlation measure (usually Pearson's) between each voxel with all other voxels in the brain, creating a similarity matrix. Since the matrix is symmetric at its' diagonal, each voxel's wGBC value can be obtained either through column or row averaging of the correlation values. Prior to averaging across correlation values, a Fisher's Z-transform is applied to the previously calculated Pearson's r-correlation values. After averaging, these values are converted back to r-values and back to the image space. This routine is repeated for all other grey matter voxels resulting in a wGBC image with the averaged correlation value of each voxel for each subject.

The primary source of neuronal signal is within grey matter voxels and so in order to avoid contamination with white matter connectivity a binary mask for the grey matter is applied. For similar reasons the smoothing process is only applied after the wGBC maps have been created. This method has the great advantage of being very easy to implement and interpret as well as the strength of not requiring any arbitrary user-defined parameters.

2.5.3 Independent Component Analysis (ICA)

Independent Component Analysis (ICA) is a methodology used as a means of separating distinct sources from one another based on the assumption of statistical independence between them. It was first introduced by Comon in 1994 (Comon 1994) and since then it has been extensively applied in many different fields. One application of ICA methods is precisely to the analysis of fMRI data, and the first publication regarding the use of ICA in fMRI dates from 1998(McKeown et al. 1998). ICA is a data driven method that can be used to interpret fMRI data without the need for further input. It is also very sensitive in detecting group differences and removing sources related to motion. As mentioned before, ICA analysis eliminates the need to specify explicit seed locations since this method works through spatial correlation. However, there is a loss in specificity compared to a single well-

defined seed of interest, resulting in a more complex interpretation of the results. While ICA requires fewer *a priori* assumptions it still compels the user to manually select the number of components in which to divide the signal (noise, physiological signals, neuronal components, etc). The user is responsible for choosing which of the components are noise and which are the components of interest. The choice of the model order and the number of components for ICA decomposition are likely to introduce other types of variability in the final outputs such as network breakdown.

Chapter 3

Assessing conscience with the aid of functional Magnetic Resonance Imaging (fMRI). The Disorders of Consciousness (DoC) study

Chapter Summary

An increasing awareness has been given to the assessment of consciousness. Common diagnostic guidelines are widely used for patients suffering from Disorders of Consciousness (DoC) and behavioural assessment is still the most commonly used method to establish conscious awareness in patients who sustain severe brain damage. New protocols using Functional Magnetic Resonance Imaging (fMRI) together with paradigms are being specifically designed to provide evidence of awareness and volition. This new approach may provide profound insights into altered states of consciousness. The aim of this study was to design fMRI paradigms that could more accurately predict awareness and diagnose patients according to their correct level of consciousness.

A group of paradigms specifically designed for this study were subjected to analysis to assess the response triggered in healthy volunteers. The author was responsible for performing data analysis of the fMRI data to localise and quantify the activation induced by the paradigm in a group 21 of healthy subjects. The results from this study seem to suggest that most of the paradigms are fitted for the use in DoC patients even though minor corrections might need to be taken into account.

3.1 Introduction

Our knowledge about consciousness is still very limited and mostly based on external assessment methods. Nevertheless when faced with unresponsive patients, who have usually sustained severe brain damage, these methods are often insufficient. Several problems arise regarding the definition and assessment of consciousness. The lack of understanding on what consciousness is creates even more difficulties to the development of treatments and methodologies to restore it.

Behavioural assessment is still the most widely used method to establish conscious awareness in patients suffering from disorders of consciousness (DoC). These guidelines acknowledge 3 levels of conscience: Coma, Vegetative State (VS) and Minimally Conscious State (MCS), ranging from the most unconscious to the most aware respectively. Nonetheless these behavioural assessment methods are prone to error resulting in diagnostic uncertainty.

Meanwhile, the implementation of new imaging techniques together with quantitative assessment methods is providing additional information and a new nosology is gradually being created including more levels of consciousness. Functional magnetic resonance imaging together with paradigms/tasks specifically designed to provide evidence of awareness and volition had a great impact among practitioners in the field. This technology was able to correctly diagnose patients as locked-in when previously, based on behavioural assessment, they were incorrectly diagnosed as being in a vegetative state (Owen, Coleman 2008). These studies are performed with the use of active paradigms that involve following commands, where the patients are instructed to imagine a well-defined sensorimotor- or cognitive-mental task. It has also been the case when faced with large evidences of possible volition and consciousness, that these tasks can also be used as a “yes” or “no” answer. The questions are usually personal and upon correct answers these can be used to prove patient’s volitional neural activation, therefore providing proof of awareness. New active paradigms are being developed such as “look at a screen and silently name the objects as they appear” (resulting in a language network activation) (Hirsch, Moreno 2011), “move the hand” (resulting in premotor cortex activation)(Andres Bekinschtein et al. 2011)and “imagine swimming” (resulting in supplementary motor area activation) (Laureys, Schiff 2012).

Paradigm development for DoC patients can be very challenging and time consuming as the ‘tasks’ have to be very specific but not too complex. If the paradigm is too complex the

patient most likely will not be able to execute it and so no activations (or dispersed activation) will be seen. On the other hand, we know that areas such as fingertips and lips have a big representation in the cortex (Homunculus – Penfield, 1951. *Epilepsy and the Functional Anatomy of the Human Brain)*. Thus there is a requirement for very specific stimulation so that awareness and volition can be correctly verified. Nonetheless, the absence of command related fMRI activation does not permit the extrapolation of conclusions regarding consciousness. A literature review was elaborated on some of the most relevant publications regarding this subject. A compilation of these publications can be found in the Appendices A **Section 3.1**.

This chapter reflects the efforts made to develop suitable paradigms to study consciousness in DoC patients. An in-house built method entitled "Heat map" analysis was used to localise and quantify the activation induced by the paradigms in a group of healthy volunteers. The results were posteriorly used to identify which paradigms are more likely to be successful in ascertaining consciousness and which ones still need to be improved.

3.2 Material and Methods

3.2.1 Participants

To test the paradigms developed at the Aberdeen Biomedical Imaging Centre a group of 21 healthy volunteers was scanned. This group consisted of 7 females and 14 males, with a mean age of 33 years old.

3.2.2 Measurements

Six stimulation paradigms were used to assess the patients. These included a language comprehension task, a tennis task, a tactile stimulation both on the left and right hand, a visual stimulation both using a flickering checkerboard and the use of a spinning spiral.

TABLE 3.1 – PARADIGM DESCRIPTION FROM MRS SUSA MERZ DOCUMENTATION

Stimulation	On	Off	Ncycles	Duration	Notes/Description
Tennis	30s	30s	5	300s	On phase was started by instructing the participant with a 3s audio: 'Please start to imagine playing tennis now' Off phase was started by instructing the participant with a 1.5 s audio: 'You can now relax'
Language Comprehension	30s	30s	6	360s	Participant heard descriptions of objects (e.g. 'long yellow fruit') during the On phase, no stimulation was given during the Off phase
Tactile (left)	16s	16s	5	160s	One of the researchers went into the scanner room and squeesed the participant's left hand during the On phase, no stimulation was given during the Off phase. Participants could see On-Off phase and remaining duration of each phase on projection
Tactile (right)	16s	16s	5	160s	Same setup as the tactile left paradigm, for the participant's right hand
Checkerboard	16s	16s	5	160s	On phase: Flickering Checkerboard Off phase: grey projection of equal average light intensity

Spirals	16s	16s	6	192s	On phase: visual stimulation with a turning spiral Off phase: visual stimulation with a scrambled version of the spiral image, no movement
Resting state	60 0s	0s	1	600s	No external stimulation. Participants were instructed to relax.

Total duration of paradigms: 30 min 40 sec

3.2.3 Imaging Methods

All the data was acquired using the 3T Philips Achieva MRI scanner at the Aberdeen Biomedical Imaging Centre. A structural T1 was acquired as well as seven standard EPI sequences, including one resting state and six stimulation paradigms designed to evaluate the level of consciousness. The structural T1 was acquired using a 3D Ultrafast gradient echo sequence with the following parameters: TR = 6.490 ms; slice thickness = 1.3 mm; 122 slices with no gap; scan resolution (x,y) = (184, 184); FOV (mm) = [240 240 158.6]. The functional MRI was acquired using a gradient echo planar imaging technique with a TR = 2000 ms; TE= 30 ms; 32 slices; slice thickness = 3.5 mm; scan resolution (x , y)=(96,96); TR = 2s, FOV (mm) = [240 143 240];

3.2.4 Image Analysis

Preprocessing and Modeling

Mrs Susa Merz had previously preprocessed and modelled the fMRI data according to the corresponding task fMRI model. **Section 7** from the Appendices A includes more information regarding the researchers and their contributions to each project.

The fMRI data was pre-processed using the statistical parametric mapping software package (SPM8)[7]. The preprocessing steps included realignment, co-registration, segmentation, normalization, reslicing to 2x2x2mm, and spatial smoothing using a 8-mm FWHM Gaussian isotropic kernel. The data was posteriorly modelled using a block design specific for each paradigm, and using the second-level analysis of fMRI data implemented on the SPM software. The onset periods and duration used in the modelling of each paradigm can be extrapolated based on the data presented in **Table 3.1**. The movement parameters obtained

[7] Statistical Parametric Mapping Software (SPM8); www.fil.ion.ucl.ac.uk/spm

during preprocessing were regressed out of the data. The canonical HRF was the only basis function used to model the data (no derivatives). A contrast was created to study the activations induced by the task. The activation maps result from the estimation of the model parameters. This procedure results in 6 activation maps for each subject.

Heat map analysis

All of the single subject activation maps obtained for each of the 21 subjects and for each task were compared using an in-house built script that generates a heat map. This method is a tool to perform a group-level analysis. This script first transforms each activation map into a binary map. Then, and separately for each paradigm, the script operates in a voxel-scale, comparing all the correspondent voxels from each subject and weighting which are the most consistently activated brain voxels for all the subjects. Each voxel starts with the value zero and its value is incremented by one for each consistent activation (on a group-level comparison). For each paradigm, the final result is a map of the most consistently activated regions for all the subjects - heat map.

3.3 Results

The results from the heat map analysis for all the 6 paradigms are displayed in the following pages. During the analysis process some of the volunteers exhibited abnormal or non-significant activations (regarding the activation maps). Those who displayed positive activation at a lower threshold (lower than p=0.05) were included in the heat map analysis with the activation map obtained for p=0.05. Volunteers that failed to present activation at a lower threshold were excluded from the heat map analysis. In the following table a summary of the exclusion performed for each paradigm is presented.

TABLE 3.2 – BRIEF DESCRIPTION OF PARTICIPANT EXCLUSION AND REASONS

Paradigms	Participants excluded	Reason
Language Comprehension	No participants excluded	-
Checkerboard	No participants excluded	-
Tactile (left)	No participants excluded	-
Tactile (right)	Volunteer 5, Volunteer 6	No activation, No activation
Spirals	Volunteer 6, Volunteer 13	No activation, No activation
Tennis	Volunteer 5, Volunteer 8	Spread activation, No activation

As shown in table **Table 3.2** the same volunteers were consistently excluded for all the paradigms. The reason for exclusion was in some cases due to widespread activation but most of the times due to no significant activation, not even when lowering the threshold of significance. Both Volunteer 5 and 6 showed consistent problems with the paradigms as well as Volunteer 13 that also showed a very small activation for the tactile stimulation of the left and right hand.

The heat maps obtained for each paradigm are displayed in the next pages using the display software Freesurfer (http://surfer.nmr.mgh.harvard.edu/).

Tactile left and right hand

This is one of the most basic paradigms used to test functional activation. It does not tell us much about the level of consciousness or awareness since the motor pathways may be intact while the rest isn't. By stimulating the right and left hand one would expect activation of the primary and secondary somatosensory cortices in the contralateral sensory areas. The primary

somatosensory cortex (S1) is located in the post central gyrus and the secondary somatosensory cortex (S2) is located in the parietal operculum on the ceiling of the lateral sulcus. Sensory stimulation to only one side of the body will induce a bilateral response in the S2 but a more localised on S1. Both heat maps (**Figure 3.1**) show activation on the paradigms' contralateral motor region as well as a shallow activation on the ipsilateral S2.

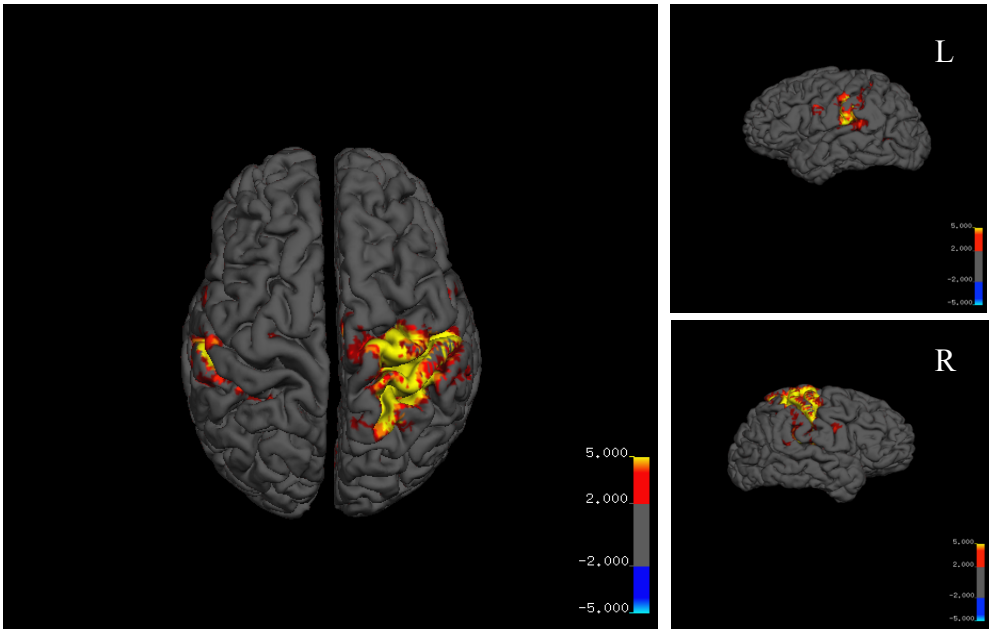

Figure 3.1 - Heat map resulting from the tactile stimulation of the left hand

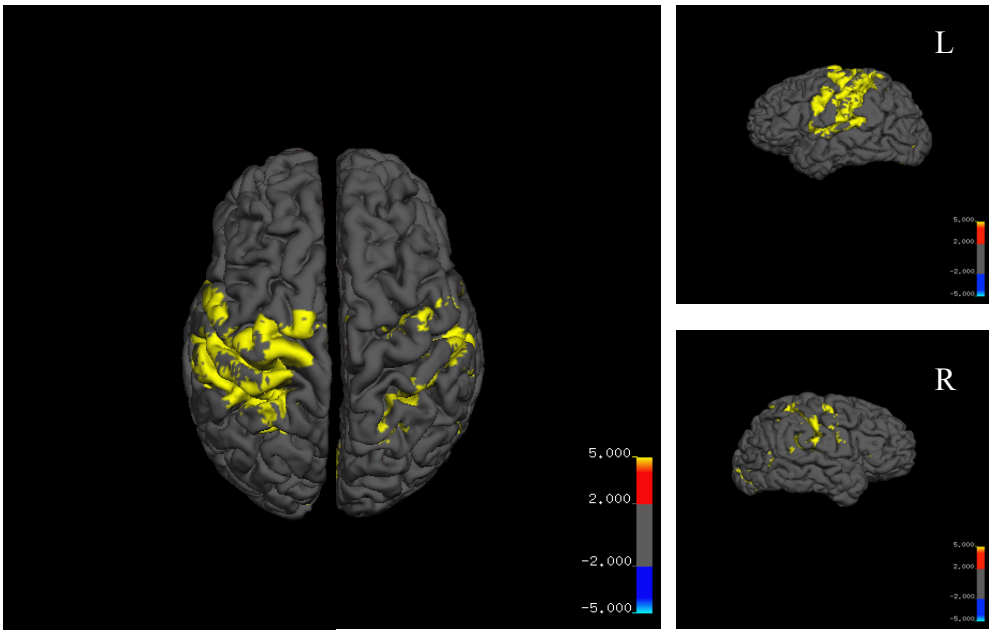

Figure 3.2 - Heat map resulting from the tactile stimulation of the right hand

Language Comprehension task

The great majority of the population exhibits left hemispheric language dominance (Rasmussen, Milner 1977, Springer et al. 1999). The language processing centres are mainly situated in the left hemisphere being that the right hemisphere analogues are also involved in processing this information even though it is to a lesser extent. This paradigm is expected to generate activation in the Wernicke's and eventually Broca's areas. Wernicke's area is responsible for written and spoken language comprehension and is located on the posterior section of the superior temporal gyrus in the dominant cerebral hemisphere.

As it can be seen in **Figure 3.3** the language comprehension paradigm elicited a clearly larger functional activated area on the left hemisphere when compared with its' analogue. The activation is present in both the Wernicke's and Broca's areas. Right hemisphere activation can also be seen over the temporal lobe, which can be due to auditory stimulation. Small bilateral activation over the occipital lobe is most likely due to visual stimulation from the paradigm presentation.

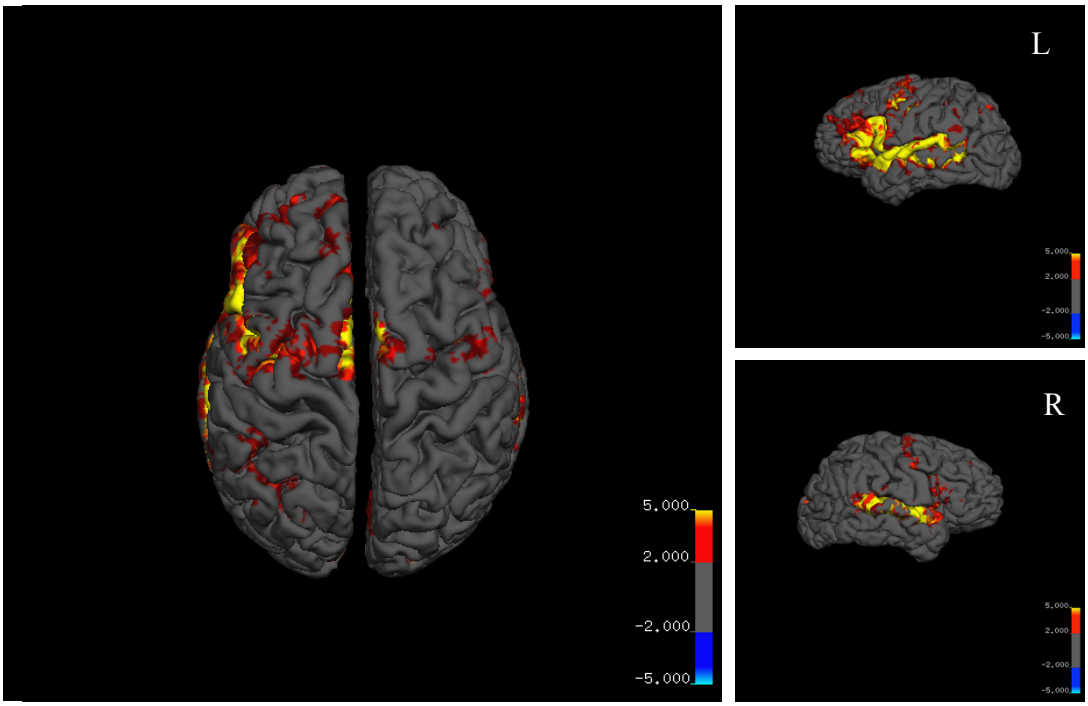

Figure 3.3 - Heat map resulting from the language comprehension paradigm

Tennis Task

For the subjects performing the tennis paradigm one should expect activation of the supplementary motor area (SMA). This region is a part of the primate cerebral cortex, situated in the midline surface of the hemisphere, anterior to leg representation on the primary motor cortex. The SMA contributes to the control of movement. **Figure 3.4** shows the activation maps resulting from the performance of this paradigm. Besides activation in the SMA, activated regions of the secondary motor cortex and the posterior parietal association cortex were also found. Most of the patients exhibited a larger activation on the left hemisphere since they were right handed. Activation of the frontal lobes reflects involvement of the executive frontal centres in the performance of this task.

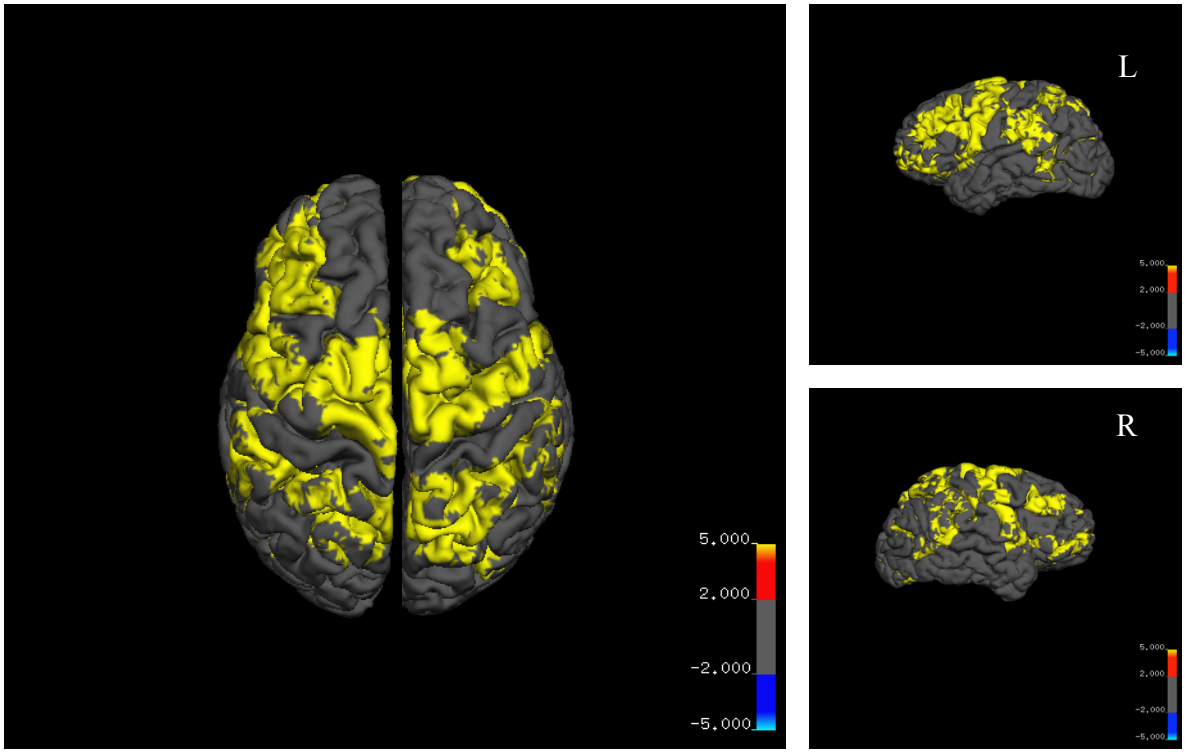

Figure 3.4 - Heat map resulting from the tennis paradigm

Spirals

The visual stimulus of spinning spirals will induce activation of the primary visual cortex (V1). This cortical area is responsible for processing the visual information and is located in the occipital lobe. In **Figure 3.5** it is displayed the heat map resulting of the presentation of this paradigm showing a large activation of the occipital lobe in the primary visual cortex region. Even though this is not clear in the image there is also residual bilateral activation due to auditory stimulation on the temporal lobe.

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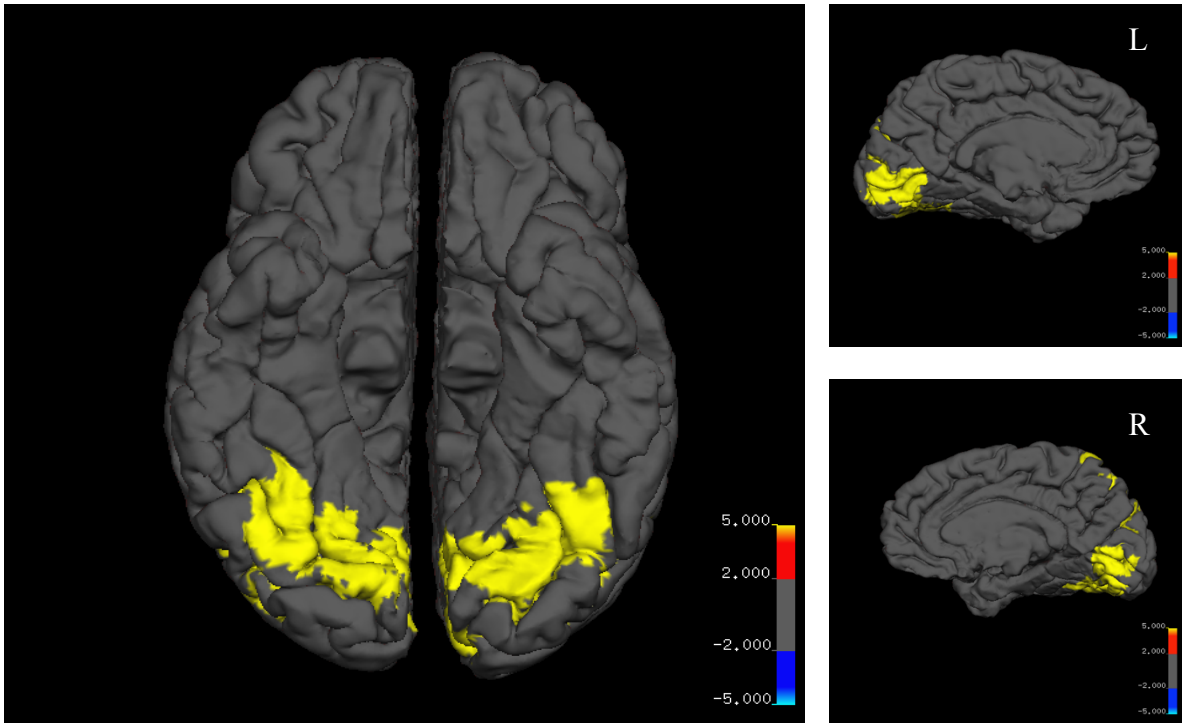

Figure 3.5 - Heat map resulting from the spinning spirals paradigm

Checkerboard

The visual checkerboard paradigm – **Figure 3.6** – is, concurrently with the spinning spirals, going to induce activation in the V1 (Brodmann area 17). Both these paradigms have similar activation maps. However the spinning spirals induce a larger and stronger area of activation when compared to the checkerboard

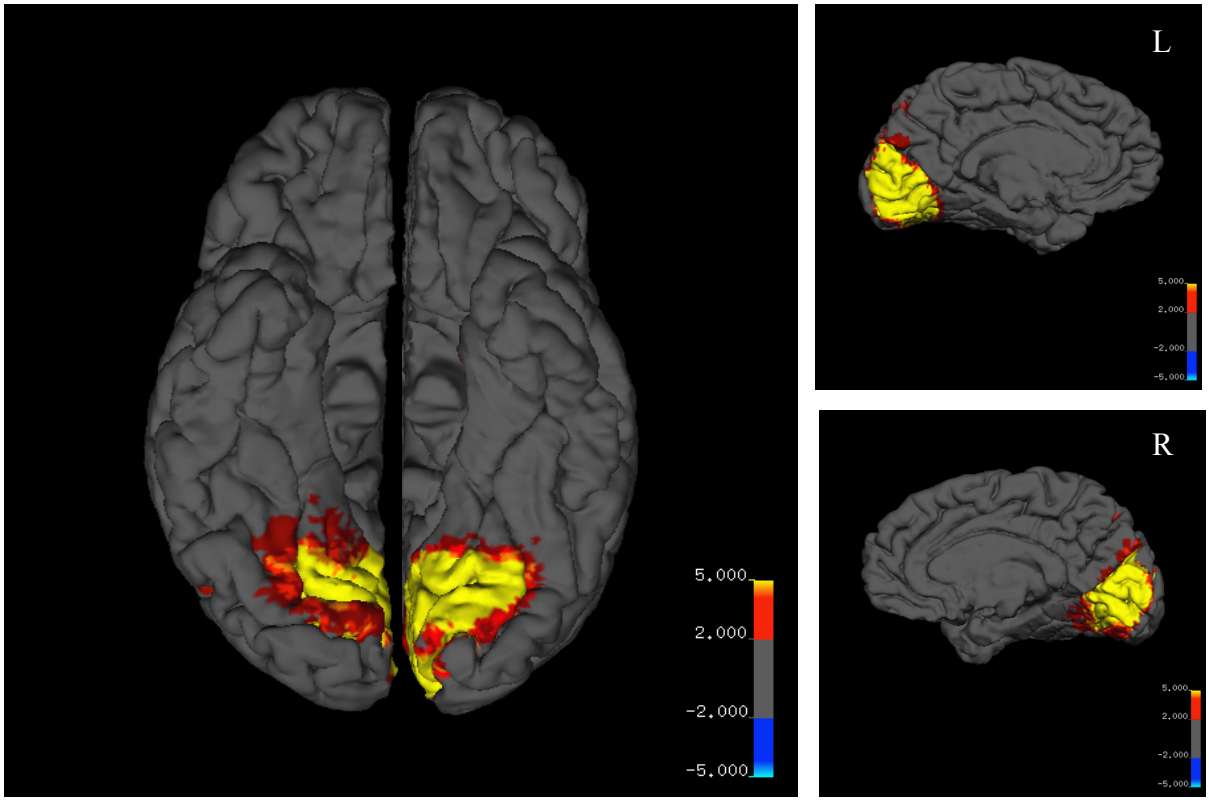

Figure 3.6 - Heat map resulting from the checkerboard paradigm

3.4 Discussion

These results provide evidence that the group of healthy participants recruited for the study show some degree of between-subject variability regarding the paradigm response. **Table 3.2** provides some of the reasons for exclusion from the heat map group-analysis. Some participants failed to elicit a significantly positive activation regarding their activation maps. Particularly for paradigms such as the tennis imagination task it is clear that the activations are quite broad and will hardly be reproducible or useful in the case of DoC patients. Hence, it is important to design paradigms that provide an accurate response so these results cannot be mistaken for random brain activity.

Some of the healthy volunteers did not present significant differences in brain activity when faced with a well-established visual paradigm (e.g. spinning spirals) or a tactile paradigm, which eventually resulted in the removal of certain participants from the analysis. These results are a clear evidence for the necessity of using more precise paradigms that would either recruit more attention or elicit a larger brain response. The spirals paradigm might need to be re-designed for increased differences between on- and off-phase. On the other hand, the tennis task might just be too complex to elicit a consistent response. Moreover, the broad variety in activation maps for this particular task might be the result of different subjects having a different perception of playing tennis, whether they have already played the sport or not. It would also be interesting to investigate if the heat maps (i.e. activity) maps increase their specificity for a larger sample/population size. The recruitment of healthy volunteers is on going, and has reached a total of 47 participants to date.

Further improvement was posteriorly made to the pre-existing paradigms in order to improve their specificity. Meanwhile the DoC patients have started to be scanned and the paradigms seem to provide specific and resilient activations. A particular patient displayed activation only on one hemisphere for the language comprehension task. Therefore the paradigm was specifically adapted to verify his hearing capacities with a single stimulation to the right and to the left ear. This is an important process in identifying which paradigms are more likely to induce a response. It is crucial that these paradigms are extensively tested on healthy volunteers before they are used to ascertain consciousness in DoC patients.

3.5 Conclusion

The results are in agreement with the activation one would expect to elicit with the execution of these paradigms in healthy volunteers. The lack of power of the tennis paradigm in eliciting a significant activation on a single-subject level might be an evidence that this task is not suitable designed to be applied to DoC patients, particularly when the analysis of these subjects is performed in a single subject-level. Nonetheless, and apart from the tennis task, all other paradigms seem to be reproducible and able to elicit a significant activation at their respective functional area.

Chapter 4

Diagnostic dilemma: longitudinal assessment of transient ischaemic attacks, minor stroke and migraines with functional connectivity MRI

Chapter Summary

Patients referred to the Neurovascular clinic present with motor, sensory, visual, and speech symptoms and also, less likely, with headaches. These patients constitute a very heterogeneous group and the consultants base their differential diagnosis on the examination and the background history of the patient. The diagnosis can be of a minor stroke, a transient ischaemic attack (TIA), migraine or other. TIA and migraines exhibit very similar clinical manifestations to a minor stroke. While an ischaemic minor stroke is a clinical syndrome characterised by focal neurologic deficit definition of TIA remains a matter of debate.

The most up-to date definition of TIAs according to the American Stroke Association (ASA) is built on a tissue-based classification rather than the previous time based definition. Based on the new guidelines, TIAs are now considered to be brief episodes of neurological dysfunction, which are caused by temporary focal cerebral ischaemia without acute infarction on imaging (Easton et al. 2009). **Table 4.1** summarises the main characteristics of the aforementioned conditions.

TIA and minor stroke are two conditions that still present a diagnostic dilemma. CT is commonly used for the assessment of cerebral ischaemia but be insufficient in diagnosing these particular conditions. Alternatively MRI with combined diffusion and perfusion is

superior to CT in the diagnosis of cerebral ischaemia, with a probability of detecting an acute ischaemic lesion in about 60-70% of the patients. The differentiation between TIAs and migraines is based on the strong history accompanying migraines patients while TIA symptoms resemble the common stroke symptoms including risk factors such as high blood pressure and cholesterol levels. Patients that present a diagnostic uncertainty are referred to diagnostic investigations using the MRI scanner. Both TIA and minor stroke patients should be given the same preventive treatment due to the high risk of recurrence of a proper ischaemic attack.

Nevertheless up to 40% of patients with symptoms suggestive of minor stroke may have a clear MRI scan. This could be due to a small-sized lesion which is undetectable on the DWI or a long period of time between symptom onset and scan. For these patients, this result means an on-going diagnostic uncertainty, particularly if they are experiencing persisting physical or psychological symptoms. The diagnosis of minor stroke also poses significant challenges from a clinical point of view with lesions often small and, at times, difficult to detect during diagnostic investigations. The large variability regarding lesion location and size amongst different minor stroke patients requires the development of a suitable method comparing a single subject against a set of healthy controls. Functional connectivity magnetic resonance imaging (fcMRI) may provide a mean of detecting connectivity changes associated with these conditions. fcMRI can have the significant potential to improve the accuracy of diagnosis as well as the prognostic of these patients.

The aim of this study is to assess the diagnostic potential of the integration of a functional connectivity MRI sequence into the current vascular protocol for stroke patients. This type of connectivity measure could potentially function as a tool to distinguish migraines from TIA’s and help in the assessment of minor stroke patients.

4.1 Introduction

Patients presenting with transient stroke like symptoms and a restricted diffusion lesion on MRI are categorised as having suffered from a brain infarction, and present a very high risk of stroke recurrence. Transient ischaemic attack (**Figure 4.3**) and minor stroke (**Figure 4.2**) are a common condition that should be seen as a warning for a more severe event (acute stroke). An early diagnosis and the introduction of secondary prevention may help prevent further events. These events, often of short duration, present a diagnostic dilemma, with a possible diagnosis of minor stroke or, in absence of lesions, a transient ischaemic attack (TIA) or migraine.

Since the establishment of resting state fMRI techniques as a valid research tool this technique has been used to study the rearrangement of networks, particularly in disease (Sorg et al. 2007, Greicius et al. 2004, He et al. 2009, Calhoun, Eichele & Pearlson 2009, Zhou et al. 2007, Greicius et al. 2007). Resting state techniques could be a valuable tool in studying the cortical reorganisation of neural networks in stroke survivors (Traversa et al. 1997). Resting state techniques are also easier to implement among stroke patients than a conventional fMRI protocol because no physical effort is required.

TABLE 4.1 – SUMMARY OF THE MOST COMMON DIAGNOSIS AND THEIR PROGNOSTIC

Diagnosis	Lesion	Prognostic
Minor Stroke	Lesion	The 10-year recurrence rate of major strokes was 14% [8]
TIA	No Lesion	10% risk of a serious recurrence taking place within the first 90 days[9]
Migraine	No Lesion	Migraine population appears to have a higher risk to develop an ischaemic stroke [10]

[8] Massimiliano Prencipe *et al*, 1998. Long-term prognosis after a minor stroke, 10-Year Mortality and Major Stroke Recurrence Rates in a Hospital-Based Cohort. *Stroke*

[9] Philippe Couillard *et al,* 2009. Predicting recurrent stroke after minor stroke and transient ischemic attack. *Expert reviews.*

[10] Mahyar Etminan et al, 2005, Risk of ischaemic stroke in people with migraine: systematic review and meta-analysis of observational studies. *BMJ*

The acquisition time is yet another constrain since stroke patients are hardly suitable to remain still for long periods of time inside the scanner without moving. Once again resting state fMRI is easier to perform since regular acquisition times are around 5-10min.

Lesions locations as well as the cognitive performance are different for every stroke patient. This creates difficulties in performing a proper group analysis without averaging all the differences between patients. As mentioned previously a suitable method for the comparison of a single subject with a group of healthy controls has yet to be developed.

The literature on the implementation of functional connectivity among stroke patients is scarce. Some of the most relevant papers in the field were reviewed in the paper by Varsou O. *et al.* (Varsou, Macleod & Schwarzbauer 2013) and will be highlighted in this dissertation for the purpose of contextualization.

A study performed by Carter *et al.* in 2010 (Carter *et al.* 2010) demonstrated a significant association between impaired performance and disrupted inter-hemispheric functional connectivity. The same was not observed for intra-hemispheric connectivity. Another study performed in 2011, by Park *et al.* (Park et al. 2011) sought to identify longitudinal changes that take place in the functional connectivity of the ipsilateral primary motor cortex over a period of six months. The patients involved in this study showed increased functional connectivity with the ipsilateral regions and diminished connectivity with contralateral areas.

With the aim of elucidating the functional changes occurring during the post-stroke period in the motor network, Wang *et al*. (Wang et al. 2010) performed resting state fMRI at five consecutive time-points during a period of 12 months. In this study it was reported a random and less efficient re-arrangement of the functional connections, whereas the ipsilateral motor cortex and contralesional cerebellum presented increased functional connectivity over time.

A study by He *et al.* published in 2007 (He *et al*. 2007) assessed longitudinal changes in functional connectivity of the dorsal and ventral frontoparietal attention networks during the acute and chronic post-stroke stages as well as the relationship with spatial neglect symptoms, verified after stroke. Functional connectivity was disrupted in both networks during the acute period, even though one of the networks was affected by structural damages. The patients that reported impaired performance were also the ones who presented with decreased connectivity. In 2012 Carter *et al.* (Carter *et al.* 2012) published a study on the somatomotor network and the resulting functional connectivity after an episode of structural

damage to the corticospinal tract. This sort of lesion was found to be associated with a decrease in interhemispheric connectivity. Diaschisis is the impact that a focal brain lesion can have in distant areas structurally intact, but connected to the primary site of injury, and it has been reported to happen in some stroke studies (Nomura *et al*. 2010). Post-stroke deficit has been associated with alterations in connectivity and the relationship between the breakdown of interhemispheric links and impaired neuromotor function. Restoration of connectivity in motor areas affected by the stroke and within the affected hemisphere is an important indicator for recovery. Therefore the assessment of connectivity patterns in minor stroke and TIA patients can provide further information on networks reorganization and re-wiring after these lesions.

Migraine with aura is a subtype of migraine characterised by transient focal neurology. Patients suffering from this condition experience a range of different symptoms shortly before the headache onset, including visual, sensory, speech and motor symptoms. These early symptoms are called prodome. Due to the resemblance of this symptomatology with the one experienced by stroke patients, a great number of migraine patients that experience aura are given an initial differential diagnosis of cerebral ischaemia. For this reason, magnetic resonance imaging is usually part of the routine diagnosis.

The patients analysed for this study were scanned during their interictal period (i.e. in between attacks) and therefore not experiencing any symptoms. **Figure 4.1** shows the scan of one illustrative patient.

Recent neuroimaging studies report pain mechanisms that also trigger visual and auditory symptoms. Photophobia in migraine patients has been addressed in several recent neuroimaging studies, mainly using PET (Denuelle et al. 2011, Boulloche et al. 2010) and task fMRI (Hougaard et al. 2013). These have reported malfunctions in visual networks among migraine patients upon the use of luminous stimulations. Studies have also reported structural abnormalities (Granziera *et al.,* 2006) thought to be the result of cortical hyperexcitability of migraineurs. Hence, the current hypothesis is for a cortical hyperexcitability among this population.

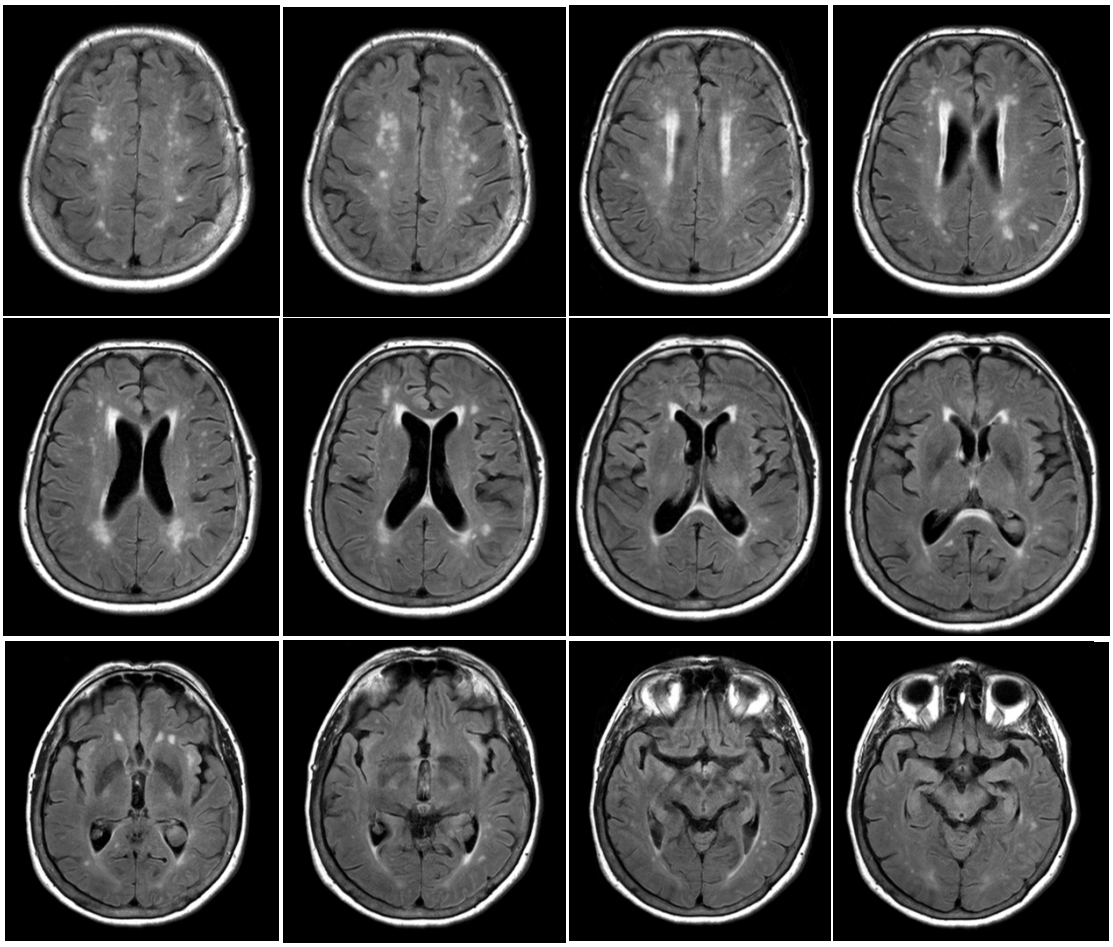

Figure 4.1 - Migraines. Female, 71yo, right-handed, presenting with sensory symptoms. MoCA score of 29. Strong presence of WMH with a Scheltens score of 55, and a history of migraines. Initial and final diagnose of migraine. FLAIR image.

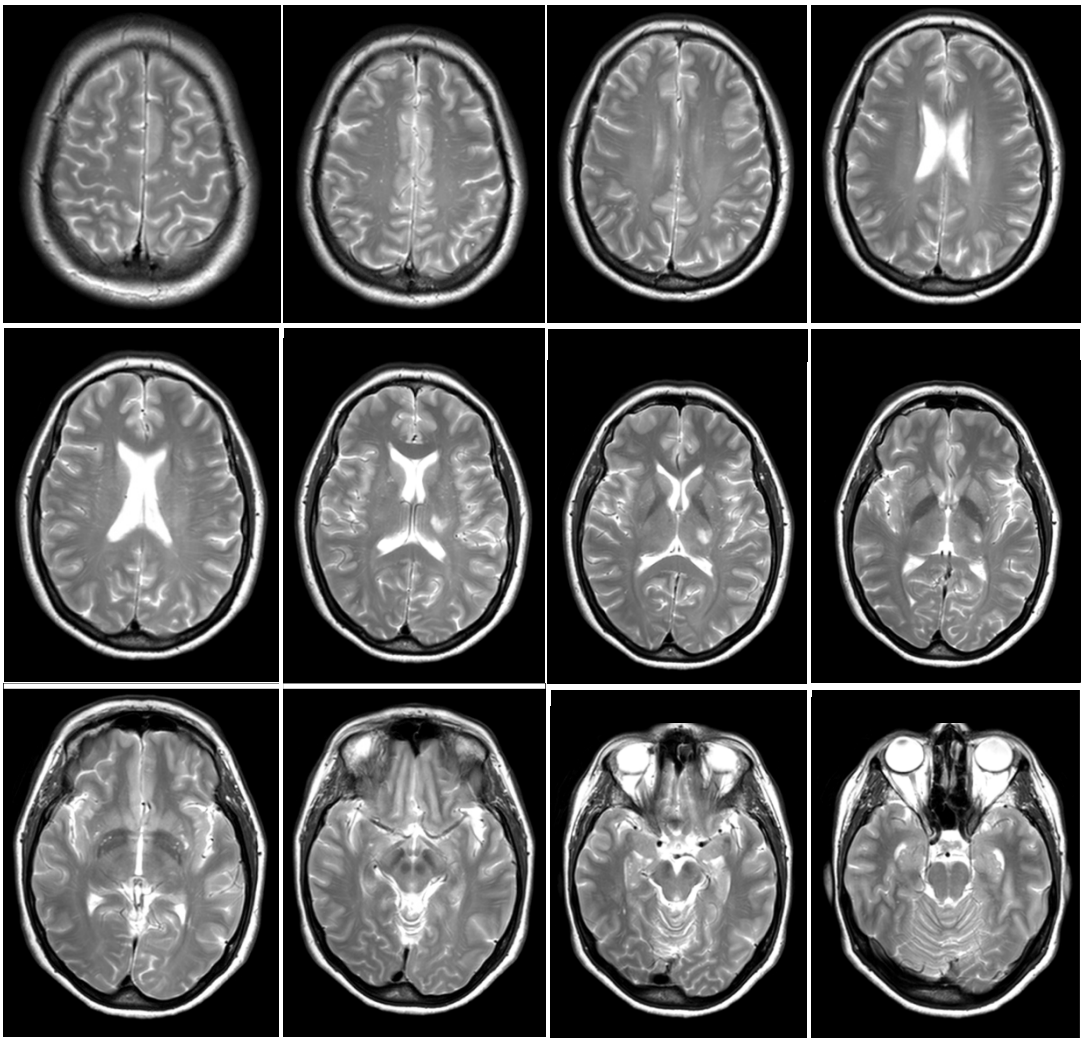

Figure 4.2 - Minor Stroke. Female, 45 yo, right-handed, sensory and motor symptoms on the left side. Hypertension. MoCA score of 30. Presented with single right acute ischaemic lesion. Initial and final diagnose of minor stroke. Lacunar infarct in the posterior limp of the right internal capsule. Schelten's score of 34. T2* enhanced image.

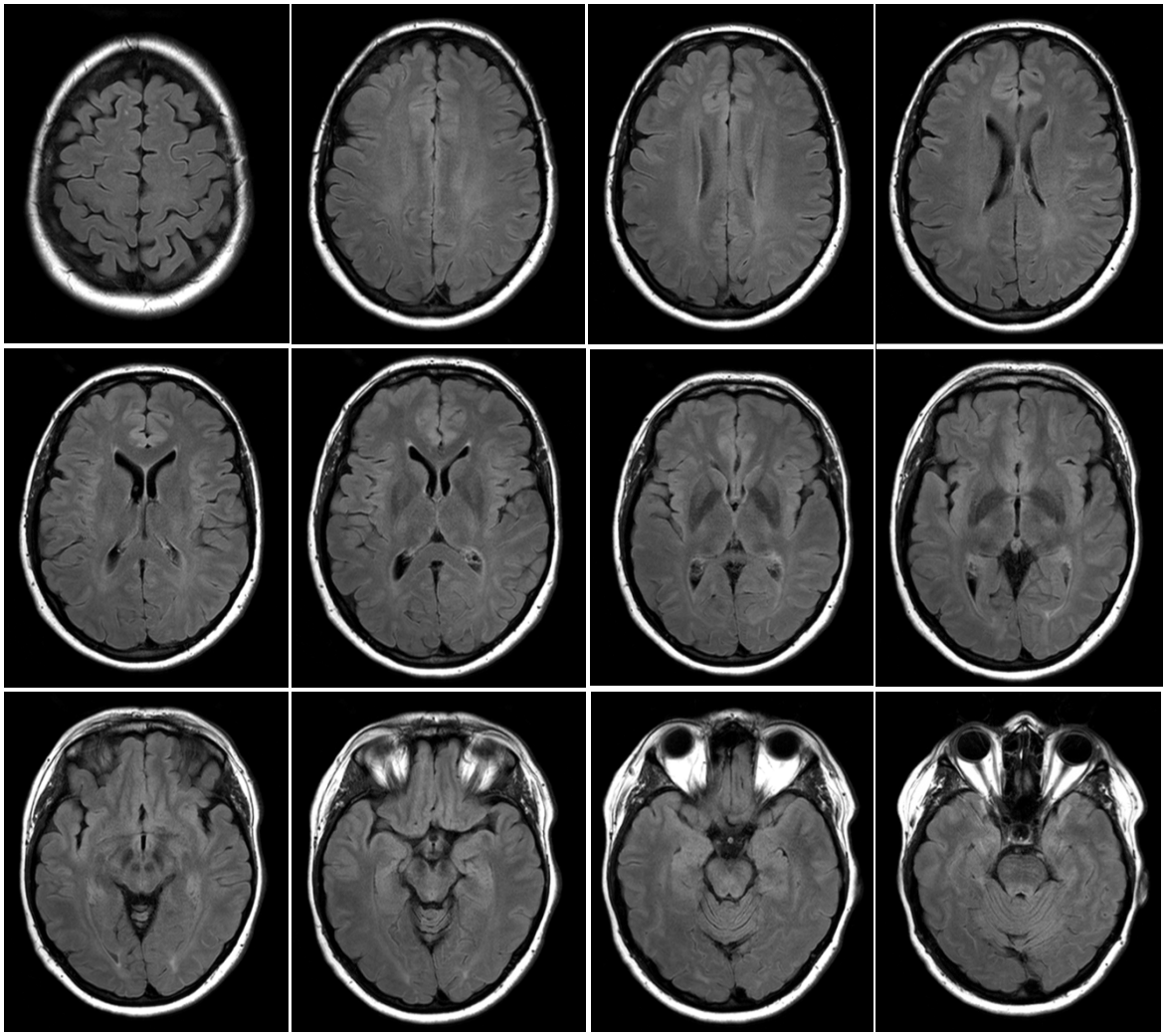

Figure 4.3 - TIA. Female, 49 yo, right–handed, sensory and speech symptoms on the right side. No history of migraines. MoCA score of 29. Symptoms duration were < 24h.

4.2 Material and Methods

4.2.1 Participants

To the present date, 86 patients took part in the TIA cfMRI study and all of them were subjected to a cognitive assessment and their white matter hyperintensities were scored using the Scheltens scoring method (the MoCA cognitive assessment form as well as the white matter hyperintensities can be found in the Appendices B). The participants age ranges from 21 to 82 yo, with a mean age of 50.37 yo, SD = 12.395. The study includes 46 males and 40 females and the most common symptoms are sensory and motor. More information regarding this population can be found in the Appendices A **Section 4.1**.

The minor stroke patients used for the analysis were carefully selected from the patient population. A group of eight patients diagnosed with a single ischaemic lesion were originally included in the analysis. Two of them were retrospectively excluded due to an inconclusive diagnosis and a lesion located outside the grey matter. This population includes 4 males and 2 females with a mean age of 46.3 years, and SD = 22.71. This group of patients all presented with WMH and had an average Scheltens score of 34.17, SD = 14.72. The most common symptoms were motor and sensory. A summary of all the information regarding these participants can be found in the Appendices A **Section 4.2**. **Table 4.2** includes information regarding lesion size and location for every patient.

TABLE 4.2 –INFORMATION REGARDING LESION SIZE AND LOCATION FOR MINOR STROKE PATIENTS

	Lesion location	Lesion biggest size [mm]
Patient 1	Posterior part of the right putamen	11.67
Patient 2	Left tail of the caudate nucleus	2.15
Patient 3	Lateral part of the hand knob of the precentral gyrus	7.42
Patient 4	Right thalamus	2.90
Patient 5	Left putamen	14.21
Patient 6	Right thalamus	18.23

Also included in the analysis were 13 migraine patients (diagnosed as migraine with aura), 7 males and 6 females, with a mean age of 39.17 yo, SD=11.20, and mean Scheltens score of 24.50, SD=4.83. More information about the demographics of these patients can be found in **Section 4.3** of the Appendices.

The volunteers used for the comparison with migraine patients consisted of 7 males and 7 females, with a mean age of 30.93yo and SD=7.09 (**Section 4.4** of the Appendices A).

4.2.2 Imaging Methods

According to what has been mentioned in Morcom *et al*., 2007 (Morcom, Fletcher 2007b) and Varsou O. *et al.* (Varsou, Macleod & Schwarzbauer 2013) longitudinal studies would provide us with a better insight on the neuronal re-arrangements that take place after stroke. According to this idea a follow-up scan was performed 30 ± 5 days after the baseline scan.

The patients were scanned using the clinical research 3T Philips Achieva X-series MRI scanner located within the Aberdeen Biomedical Imaging Centre (ABIC). The imaging parameters can be found in **Chapter 2**, section *2.2.5 Neurovascular protocol.* A low cognitive level paradigm was used during the resting state sequence (see **Chapter 6,** section *6.2 Materials and Methods* for a more detailed description of the paradigm).

4.2.3 Image Analysis

A more detailed explanation about the theoretical assumptions behind seed-based and wGBC methods can be found in **Chapter 2** – Background.

Weighted Global Brain Connectivity (wGBC)

A first analysis of the data was performed using wGBC methods. The goal was to investigate how this method behaves in the case of anatomical brain lesions and to verify if wGBC is able to correctly identify possible functional connectivity abnormalities in TIAs, which present no anatomical alteration in a regular DWI scan. It is of prime importance to assess the sensitivity and efficiency of the applied wGBC method. The behaviour of wGBC in

identifying reduced (or none at all) functional connectivity in the lesion voxels was explored using the Minor-Stroke data. This technique was also implemented using migraine patients on a comparison with healthy volunteers. wGBC was calculated for each subject using the CHART method, stage 1, (a detailed description of the method can be found at Perrin, Merz *et al*., 2012) developed at ABIC. All the data was resampled to a 3x3x3 voxel size in order to approximate DWI voxel resolution (1.53mm).

Lesion mask

Due to the inability of performing group comparisons with a single subject against a set of healthy volunteers, the hemisphere contralateral to the ischaemic lesion was used for this analysis as a basis for comparison in each subject. Diffusion Weighted Imaging (DWI) is recognised as the preferred sequence to identify the lesions (**Figure 4.7**). The patients included in this first type of analysis presented with ischaemic lesions ranging between 2.15mm and 14.21mm.

Manual segmentation was used to create a binary mask of each lesion based on the diffusion weighted imaging (DWI) scan of every patient (**Figure 4.8**). The mask was then flipped at the sagittal midline with the aim of creating the seed mask on the opposite hemisphere. Due to hemispheric symmetry the maps computed with each of the seeds can be used as a control for each other (e.g. healthy hemisphere VS lesion hemisphere). These masks were used as a region of interest to compute the functional connectivity maps.

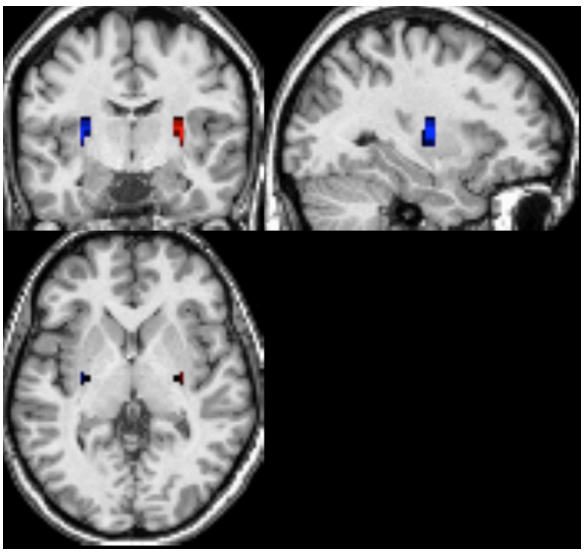

Figure 4.4 - Lesion mask and opposite hemisphere seed mask overlaid on top of the normalised structural T1

Pre-processing

The fMRI data was pre-processed using the statistical parametric mapping software package (SPM8). The data was realigned, slice timing corrected, co-registered, segmented, normalised, resliced to 3x3x3mm, and spatially smoothed using a 8-mm FWHM Gaussian isotropic kernel. It was also low-pass filtered at 0.1-Hz, and baseline corrected. A binary mask was created based on the SPM8 grey matter tissue probability map, including all voxels with a probability greater than 0.3. A total of 33,985 voxels from the functional imaging time series were within the grey matter binary mask.

Functional connectivity analysis

A seed-based connectivity mapping method combined with non-linear parametric statistics used according to the procedure described in J.S. Perrin, Merz *et al*. (Perrin et al. 2012) was applied to the pre-processed data, in order to compute the functional connectivity maps between region of interest in the lesion site and the remaining voxels in the brain. The same procedure was used to calculate the functional connectivity maps with the seed in the opposite hemisphere.

Non-parametric tests were utilised to check for significant differences between connectivity maps obtained with the two different seeds. Subjects were grouped according to the hemisphere of the lesion and functional connectivity was compared within each group (i.e. right and left hemisphere). Individual statistical analysis was finally performed between both maps for each subject.

Statistical Testing

The nonparametric permutation testing (SnPM) toolbox for MATLAB (http://go.warwick.ac.uk/tenichols/snpm) was used for the statistical analysis. The tests were corrected for multiple comparisons with a whole-brain FWE correction ($p < 0.05$). Subjects were grouped according to the hemisphere of the lesion (i.e. right and left hemisphere) and functional connectivity was compared within each group using a non-parametric Paired T-test. Functional connectivity maps of each subject (for both the seed on the lesion and on the opposite hemisphere) were compared individually using a two sample T-test for 2 conditions.

ICA analysis

Mr Michael Stringer (see **Section 7**, Appendices A) performed an ICA analysis of the data using the group ICA functional toolbox (GIFT version 2.0e; http://mialab.mrn.org/software/gift/). The control and patient data were analysed separately using the Infomax algorithm. Using the minimum description length (MDL) information theoretic criterion the number of components was estimated to be 38 for the migraine patients and 41 for the control group. In order to back-reconstruct the individual subject components the GICA3 algorithm was applied. An established set of templates available online (http://findlab.stanford.edu/research) was used to identify which was the component with the highest spatial correlation for each of the relevant networks (primary auditory, primary visual and high-level visual network).

The SnPM toolbox was used to carry out the statistical testing between the selected networks from the patient and control group. The comparison was performed with the use of a two-sample *t*-test with 5000 permutations and a family-wise error (FWE) correction at 5% ($P < 0.05$). Variance smoothing was also applied, matching the smoothing used in pre-processing, with FWHM set to 8 mm.

4.3 Results

i. Migraines

The study of the migraines patients was focused over the connectivity map resulting from the use of a seed in the right temporal lobe. Additionally a wGBC analysis was also performed.

ICA analysis

The ICA analysis has found a cluster of hyperconnectivity in the right auditory cortex of migraine patients.

Seed-based analysis

A seed was placed over the right auditory cortex, in the temporal lobe. The MNI coordinates are [69, -28, 13] and the seed was comprised of a cube, one voxel in each direction from the coordinate centre, accounting for a total of 7 voxels. This seed was chosen after the ICA methodology has identified this region as being hyperconnected in migraine patients. Non-parametric statistics were used for the comparison between the connectivity maps of both groups (migraine and healthy volunteers). It was used a two-sample T-test, with 10,000 permutations after smoothing with an 8-mm Gaussian kernel. No significant results were found between both groups for a FWE $p<0.05$.

wGBC

wGBC was used to compare migraine patients with healthy volunteers. Performing a two-sample T-test on differences between the wGBC maps for migraine patients and volunteers, using kernel with an FWHM of 8mm, resulted in a significantly different ($p<0.05$) cluster of 3 voxels in the MNI coordinates [-27, -55, 10]. Using an in-house script – written by Christian Schwarzbauer – the Brodmann area (BA) and anatomical location of these clusters was identified.

T-Threshold: 4.2

MinClusterSize: 1

MinPerc: 1.000000e-05

TABLE 4.3 –INFORMATION REGARDING CLUSTERS LOCATION – WGBC METHOD

Cluster	Coordinates (MNI)	Voxels	Location
#1	[-26 -54 8] mm	3vox	BA19.L: 3vox (0.2% overlap) PCN.L (Parietal): 1vox (0.1% overlap)
#2	[-21 -49 10] mm	1vox	BA17.L: 1vox (0.2% overlap) PCN.L(Parietal): 1vox (0.1% overlap)
#3	[-9 -31 19] mm	1vox	-

The regions mentioned above show increased connectivity in migraine patients when compared with healthy volunteers. These are all situated on the left hemisphere representing the primary and secondary visual cortex. BA17 is the Brodmann area equivalent to the Primary visual cortex (V1) responsible for processing information about static and moving objects as well as pattern recognition. BA19 is part of the associative visual cortex (V3), which along with BA18 is involved in feature-extraction, shape recognition, and visual attention.[11] Increased activation is also present in the left precuneus over the parietal lobe, which is thought to be involved in episodic memory, visuo-spatial processing and aspects related with consciousness and self.

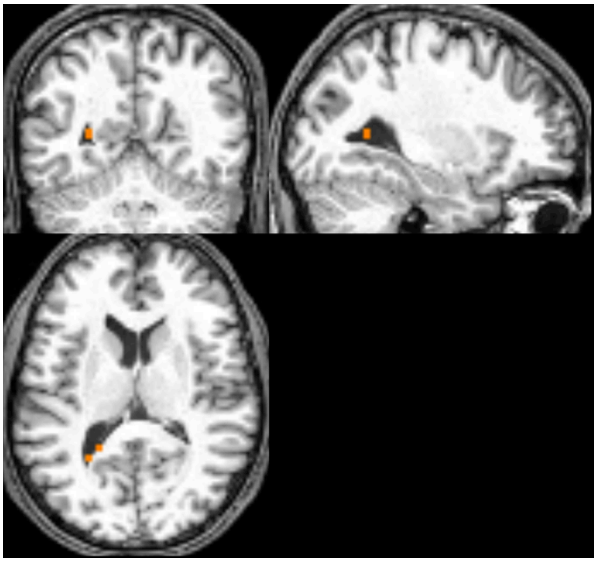

Figure 4.5 - Increased connectivity for migraines patients when compared with healthy volunteers using wGBC

[11] Lloyd 2007. What do Brodmann areas do? or: Scanning the neurocracy"

ii. Minor Stroke

wGBC

A map of whole brain connectivity was plotted for each subject using the aforementioned wGBC methodologies. No significant differences were found between healthy participants and minor-stroke patients.

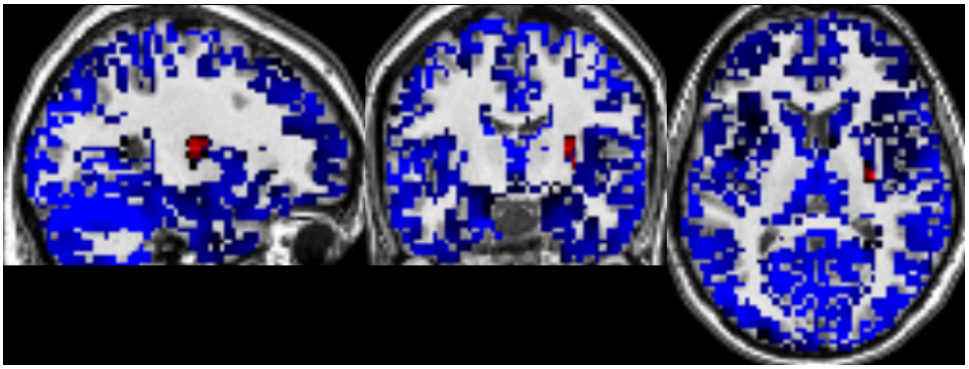

Figure 4.6 - Weighted global connectivity map of one example subject. The lesion is also represented in red even though it has not been used for the computation of WGBC maps

Seed-based analysis

Significant differences were not detected either between the connectivity maps of individual subjects, or within each group.

The results from the functional connectivity analysis obtained with seed based methods and weighted global connectivity proved to be inconclusive. Nevertheless, the use of ICA methodologies resulted in significant differences found between patients and healthy population. These results offer a possible explanation for the null results using wGBC and seed based analysis.

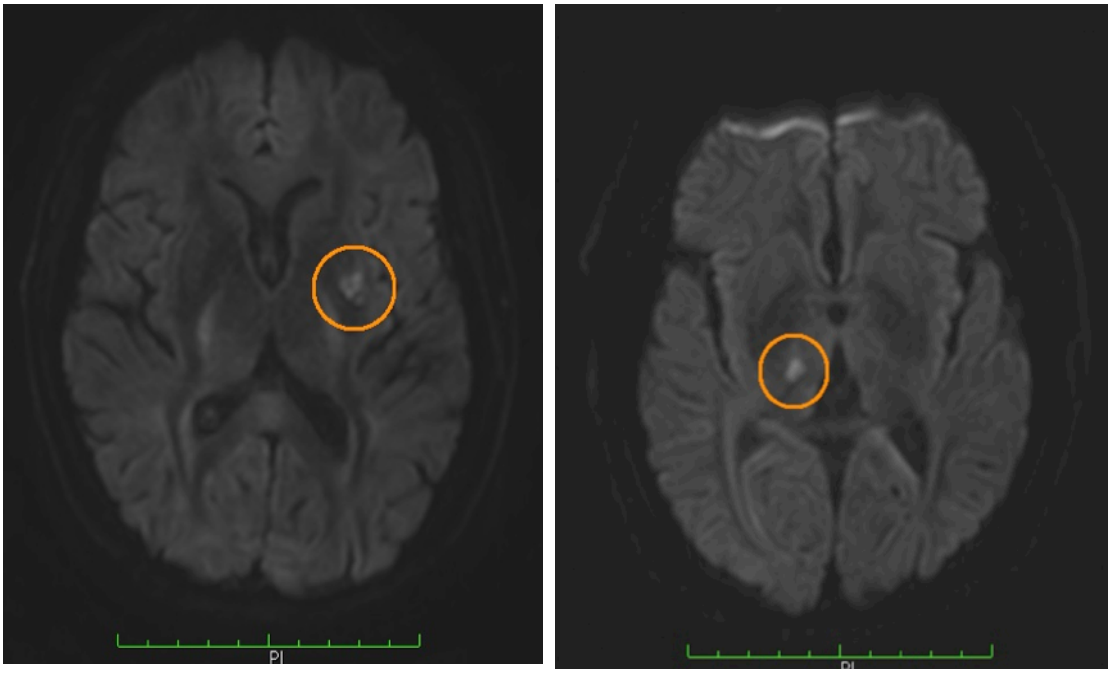

Figure 4.7 - Diffusion Weighted Images (DWI) presenting with lesions from two representative patients

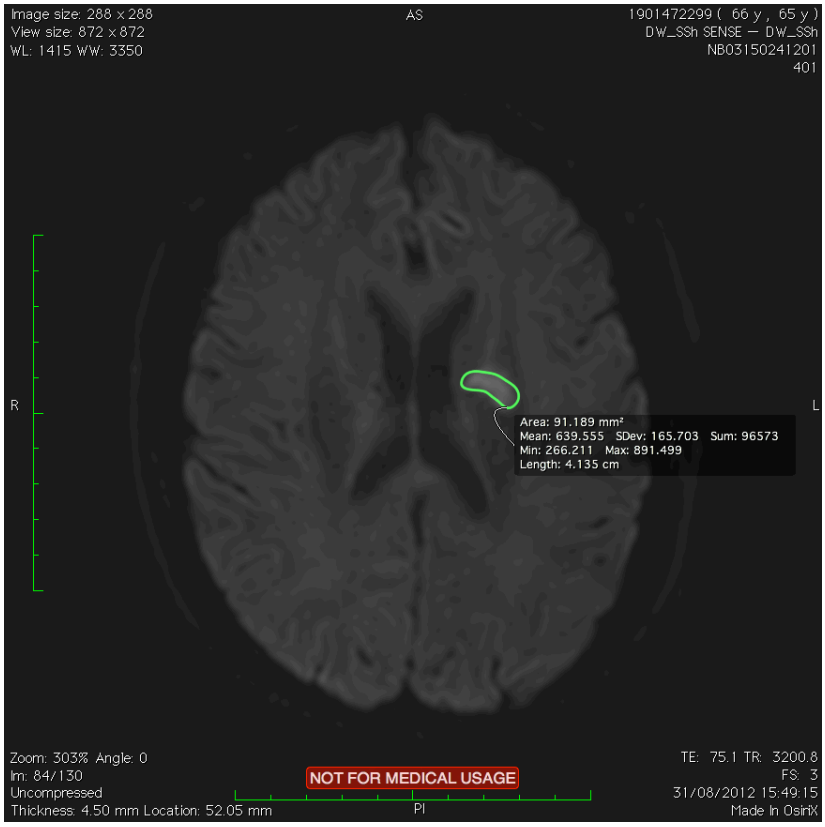

Figure 4.8. Lesion mask drawn on top of the diffusion weighted imaging scans

iii. Transient Ischaemic Attack (TIA)

Transient ischaemic attacks have proven to be extremely difficult to analyse with the methodologies available nowadays. The initial focus of this study was to study this particular condition. The number of TIA patients available was not enough to provide significant results either using ICA or wGBC methods. Migraine and minor stroke patients have proven to be more fruitful and therefore have been studied to a greater extend detail than TIA patients. Besides the small sample size, these patients are all very different in their essence therefore it is not surprising that the analysis did not provide any significant differences. The recruitment of more patients with this symptomatology and the development of methodologies for the comparison of single subject with a group of healthy volunteers will enable a further study of such an enigmatic condition.

4.4 Discussion

Migraine

Weighted global connectivity was able to correctly identify changes in the primary visual cortex in migraine patients, subscribing previous literature (del Rio, Linera 2004) that describes altered mechanisms of visual and pain processing. The use of ICA has proven to be more efficient identifying regions of altered connectivity than seed-based methodologies. The ICA methodology not only has identified regions of hyperconnectivity in the primary visual cortex (BA 18) and high-level visual networks (BA 17) but has also reported the same condition in the auditory association cortex (BA 22). These findings provide a valid explanation for the general presentation of phonophobia and photophobia in migraine with aura patients, even between attacks.

These results also support the idea of cortical spreading depression (CSD), a condition during which there is a malfunction on the visual cortex, which tends to spread to other brain regions. fMRI studies have been carried out during the period of typical aura in migraine revealing multiple neurovascular events in the occipital cortex within a single attack(Cao et al. 1999, Hadjikhani et al. 2001). These neurovascular alterations closely resemble CSD with an initial hyperaemia (lasting for 3 ~ 4.5min) followed by a mild hypoperfusion (lasting for 1~2h), an attenuated response to visual activation, and the first affected area is the first to recover. A cortical event such as CSD is able to activate trigeminal vascular system being this system comprised of sensory fibres of the ophthalmic division of the trigeminal nerve that innervate the blood vessels of the dura mater. The activation of the trigeminal fibres is involved in the mechanism of pain and leads to an inflammatory process in the dura mater – neurogenic inflammation.

To our knowledge it has not yet been published an original research study reporting disrupted functional connectivity among visual and auditory networks, while using a population comprising only migraineurs with aura during their interictal period.

Minor Stroke

Comparison between contralateral and ipsilateral connectivity maps was expected to result in decreased connectivity when the seed was placed over the lesion. However, at this stage, no significant differences were observed to justify this assumption. These preliminary results provide further support for the importance of data re-sampling and the possible incompatibility of seed-based methods when dealing with small lesions such as ischaemic minor strokes. On the other hand, ICA analysis identified increased connectivity in sensorimotor components on the hemisphere opposite to the lesion.

Furthermore, due to the differences reported using the ICA methodology one can hypothesize that the changes in connectivity do not occur within the lesion itself but in other brain regions that change their connectivity in order to compensate for the disruption. Brain plasticity is a well-known phenomenon happening among stroke survivors. These results encourage a further analysis of a possible network re-arrangement after such a disabling occurrence. One must assume that the tissue within the infarcted region is poorly or even no longer functioning. For this reason, it is only logical to assume that other surrounding brain regions, provided that the patients are offered a suitable rehabilitation treatment, take over the functions of the no longer existing tissue.

4.5 Conclusion

The findings regarding migraine patients are in complete agreement with what has been previously reported in the literature, and provide a plausible explanation for the symptoms experienced by patients suffering from migraine with aura. These broadly acknowledge the involvement of visual mechanisms during prodome and also provide new evidences for some of the auditory symptoms experienced by migraine patients.

The results for minor-stroke patients are not what had been initially hypothesised but instead they seem to suggest that the changes in functional connectivity occurring among minor stroke survivals do not happen within the lesion itself but instead in the surrounding areas using plasticity mechanisms.

Transient ischaemic attacks patients were the primary goal of this study. Nonetheless, and due to small sample size and lack of common symptomatology for all the participants the comparisons did not result in any measurable differences between their connectivity and the one of a group of healthy volunteers. This is a complicate condition and grouping patients with different symptoms within a single group might result in averaging out of the actual effect. This analysis should be repeated when a larger number of TIA patients are available.

Chapter 5

Physical exercise & brain connectivity. The physical exercise connectivity (PECON) study

Chapter Summary

Recent studies have proved that exercise does have an effect on functional brain connectivity and more specifically into resting state networks right after a training session (Taubert et al. 2011, Pereira et al. 2007, Voss et al. 2010, Voelcker-Rehage, Godde & Staudinger 2010). The fronto-parietal brain networks seem to be responsible for containing the information regarding acquired motor skills. Nevertheless how these networks experience changes and evolve throughout motor training is still unclear. It is also unknown whether long-term changes in RSN can be induced by motor training as well as if these changes overlap with reported structural alterations. It is a fact that the changes in functional connectivity are measurable after a long-term intervention, namely in fronto-parietal networks. After physical activity this particular network has been reported to present with increased connectivity. The aim of the PECON study is to explore whether a single session of acute exercise temporarily changes the connectivity between different brain regions.

A wide range of different connectivity measures has been used including seed-based analysis, ICA, cluster analysis, Network Based Statistic (NBS) methods and graph theory. The author performed data analysis using seed-based methodologies and the results from the implementation of this method will be described in this chapter. These will also be compared with other significant findings using different methodologies.

5.1 Introduction

The idea of a constantly changing brain was firstly suggested by the psychologist William James in the late nineteen century. In his book entitled The *Principles of Psychology* he developed the idea that nervous tissue has a great degree of plasticity, unlike the current thinking at the time.

It was only in the second half of the twentieth century that researchers began to explore neuroplasticity events happening in older adults who had suffered massive strokes and who were still able to regain function. Current research also targets the rewiring process that seems to happen when brain tissue experiences learning, memorisation or even a traumatic injury, involving the reorganization of pathways and the creation of new connections.

Physical exercise has been reported to induce not only structural but also functional plasticity. Nonetheless, the study of how functional connectivity is affected by exercise is still a fairly new branch of research. Several studies have reported neuroplasticity due to motor training sessions as a reflex of morphological adaptation of the human brain, although no linear relationship has been shown yet between anatomical changes and the amount of practice. They do however report increased functional connectivity immediately after a training session, while the structural changes induced by training are usually only visible after several weeks (Colcombe et al. 2006, Ruscheweyh et al. 2011). However, some studies have reported rapid changes of brain structure happening during very early stages of learning in specific motor and parietal regions (Driemeyer et al. 2008, Taubert et al. 2010). The structural changes are found mainly in frontal areas such as the anterior cingulate cortex (ACC), along with increased volume of the temporal lobe (Colcombe et al. 2006) and the hippocampus (Erickson et al. 2011). Structural changes correlated with functional connectivity changes in the prefrontal and supplementary-motor areas have also been reported to happen within white matter fibre structure and into grey matter. Hippocampal and medial temporal lobe volumes are larger in higher-fit adults, and it has been established that physical activity training increases hippocampal perfusion. Furthermore, aerobic exercise training has been reported to increase the size of the anterior hippocampus, leading to improvements in spatial memory. Erickson *et al.* (Erickson et al. 2010) provided evidences that performing aerobic exercise training increased the hippocampal volume, reversing age-related loss in volume in late adulthood. The reported hippocampal volume changes seem to

be sensitive to the duration of cardiovascular activity (Erickson et al. 2011). Nonetheless, the functional meaning of these structural changes is not yet fully understood.

Coordination and planning of complex motor skills is controlled by fronto-parietal brain networks (Rizzolatti, Luppino 2001). Other fMRI studies (Hallett, Grafman 1997; Halsband, Lange 2006; Pascual-Leone, Grafman & Hallett 1995) have found that prefrontal, premotor, supplementary motor and parietal brain areas are recruited when acquired motor skills are executed. It has been shown increased fronto-parietal network connectivity just after two motor skill-learning sessions (Taubert et al. 2011). These effects progressed accordingly with subject performance. Within the DMN the connectivity over parietal regions was demonstrated to be increased by the performance of a finger sequence task during a learning period of 4 weeks (Ma et al. 2011) or by 6 weeks of learning a whole-body balance task (Taubert et al. 2011). Changes were detected within fronto-parietal areas of the DMN even after a short session of 11minutes of visuo-motor stimulation inside the scanner using a block design (Albert, Robertson & Miall 2009). Voss *et al.* on her 2010 (Voss et al. 2010) study showed increased functional connectivity within frontal and parietal regions after a 6-month stretching intervention with older adults. Fewer changes happen after the initial learning period, however other motor and frontal areas show a continuous increase across 5/6 weeks of practicing a complex task. After cessation of motor learning, changes seem to be persistent for at least for 4 weeks (Scholz et al. 2009). It has been hypothesised that learning a challenging motor task leads to long-lasting changes in functional resting state networks and the corresponding cortical and sub-cortical brain structures (Taubert et al. 2011). However, structural changes seem to return to baseline levels after a period of 8-16 weeks (Draganski et al. 2004, Driemeyer et al. 2008). For this reason, longitudinal studies have been the preferred design to study training-induced changes in both brain structure and function.

Longitudinal brain studies revealed a great degree of training-induced structural plasticity in the adult human brain. For example, three months of juggling training resulted in profound changes in the parietal grey matter structure (Draganski et al. 2004). Training-induced changes have also been found in the parietal white matter microstructure after six weeks of juggling training (Scholz et al. 2009). One year of walking (Voss et al. 2012) increased functional connectivity between aspects of the frontal, posterior, and temporal cortices within the default-mode network and a frontal executive network. This study also showed that exercise duration and intensity is another extremely important factor for the successful observation of brain changes. A study performed in older adults using resistance training only

revealed benefits of exercise when it was practiced at least twice a week, whereas exercise once a week revealed no effect (Liu-Ambrose et al. 2012).

It has also been acknowledged that not all types of exercise will produce measurable changes in brain function and structure. No positive effects on cognitive functions are expected for physical activity with very low metabolic or cognitive demands.

The PECON study is a brain imaging study that aims to investigate the effects of an acute exercise session of one hour on functional connectivity. With this in mind this project was designed to provide us with a better understanding of the underlying neuronal networks involved and to posteriorly apply the insights about the mechanisms involved to develop exercise-rehabilitation programs.

5.2 Material and Methods

5.2.1 Participants

This study included a total of 13 participants, all male, right-handed, healthy and fit. The mean age was of 28yo with SD=5.45. All of them regularly practice exercise such as running, cycling and football. One of the participants had to withdraw from the study due to a physical lesion. More information regarding the demographics of the volunteers can be found in the Appendices A, **Section 6.1**.

5.2.2 Study Design

Dr Ourania Varsou was responsible for the study design and data acquisition (Section 7, Appendices A). All of the participants were asked to abstain from caffeine and alcohol at least 24h before the scanning sessions. All of them were subjected to the Montreal cognitive assessment (MoCA). The volunteers were assessed in three separate occasions. The individual maximum oxygen uptake (VO_2max) and maximum heart rate (HR_{max}) were determined during static cycling at their first visit. Their second visit was designed to provide familiarisation to the task and the equipment and to avoid confounding factors. During this visit the volunteers were asked to exercise at 50% of their VO_2max Over a one hour period using a static bicycle. The third and last visit comprised of a baseline MRI scan, cycling for one hour at 50% of their VO_2max, resting for a period of at least 30 minutes and then a follow-up scan. The resting period after exercise ensures that all the physiological parameters return to baseline (e.g. heart rate, etc) and therefore the changes in BOLD signal will not be a result of physiological instability.

5.2.3 Imaging Methods

A structural T_1 was acquired when the volunteer performed the first scan and two fMRI scans were acquired before and after exercising. Both T_1 and fMRI sequences were performed using the same scanning parameters as the ones used in the neurovascular protocol (see **Chapter 2**, section *2.2.5 Neurovascular Protocol*).

5.2.4 Measurements

The CO2 levels of every participant were monitored during the whole scanning procedure. During the resting state acquisition a low level cognitive paradigm was used to keep the volunteers focused. More information about the paradigm can be found in **Chapter 6**, section *6.2 Materials and Methods*.

5.2.5 Image Analysis

Pre-processing

The fMRI data was pre-processed using the statistical parametric mapping software package (SPM8).[12] The data was realigned, slice timing corrected, co-registered, segmented, normalised, resliced to 3x3x3mm, and spatially smoothed using a 8-mm FWHM Gaussian isotropic kernel. It was also low-pass filtered at 0.1-Hz, and baseline corrected. A binary mask was created based on the SPM8 grey matter tissue probability map, including all voxels with a probability greater than 0.3. A second preprocessing was performed using exactly the same parameters but the data was instead resliced 4x4x4. The second preprocessing was performed in order to enable a proper comparison with the NBC results (which have been resliced at 4x4x4).

Seeds

Table 5.1 outlays the coordinates of the seed regions used to perform the seed-based analysis of the data. Published literature reports structural changes in the hippocampus, therefore one can hypothesise that there should also exist underlying functional differences. In order to study functional connectivity in this structure it was included a separate group of ROI located in this particular part of the brain. The right cerebellum seed without referencing in **Table 5.1** resulted from the application of a clustering method to this data - performed by Mr. Alex Ing (**Section 7**, Appendices A). This method discovered a cluster of significant differences (FWE corrected $p<0.05$) in these coordinates.

[12] Statistical Parametric Mapping Software (SPM8); www.fil.ion.ucl.ac.uk/spm

Functional connectivity analysis

A seed-based approach according to the procedure described in Perrin, Merz *et al*. (Perrin et al. 2012) was the method used to calculate the functional connectivity maps. This procedure was applied to the pre-processed data, in order to compute the functional connectivity maps between the regions of interest and all the other voxels of the brain. This procedure was performed for scans before and after exercise.

Statistical analysis

Non-linear parametric statistics were used to test the results for significant differences between the functional maps computed before and after the exercise session. SnPM was used in combination with paired T-tests, FWHM = 8mm, 4096 permutations and a FWE corrected $p<0.05$.

TABLE 5.1 – REGIONS OF INTEREST USED FOR SEED BASED CONNECTIVITY ANALYSIS

Network	SEED	MNI coordinates
Default-mode Network	Posterior cingulate cortex	[0, -53, 26] [13]
Motor Network	Primary motor cortex	[-32, -30, 68] [14]
Auditory network	Primary auditory cortex	[-40, -22, 8] [15]
Prefrontal-limbic Network	Bilateral Amygdala	[22, -6, -16] [-22. -6, -16] [16]
Sensorimotor Network	Cerebellum, lobule VI	[32, -52, -28] [17]
	Left Cerebellum (foot)	[-17, -33 -26] [18]
	Right Cerebellum (foot)	[14, -34 -26] [18]
	Right Cerebellum	[46, -63, -46]
	Left Hippocampus (I)	[-27, -24, 9] [19]
	Left Hippocampus (II)	[-18, -16, -18] [20]
Executive control Network	Bilateral DLPFC	[44, 36, 20] [-44, 36, 20] [21]
	Right anterior prefrontal cortex (raPFC)	[32, 40, 28] [22]
Fronto-parietal Network	Right inferior parietal sulcus (rIPS)	[25, -62, 53] [22]

[13] Perrin *et al*., 2012. Electroconvulsive therapy reduces frontal cortical connectivity in severe depressive disorder. *PNAS*

[14] Shahabeddin Vahdat *et al*., 2011. Functionally specific changes in resting state sensorimotor networks after motor learning. *J Neurosci.*

5.3 Results

The seed region located in the right cerebellum at the MNI coordinates [46,-63,-46], has resulted in a cluster of significantly decreased connectivity following exercise (FWE $p<0.05$, with a t-threshold=5.21) between the left parietal lobe (MNI [-50, -24, 42]) and the right cerebellum.

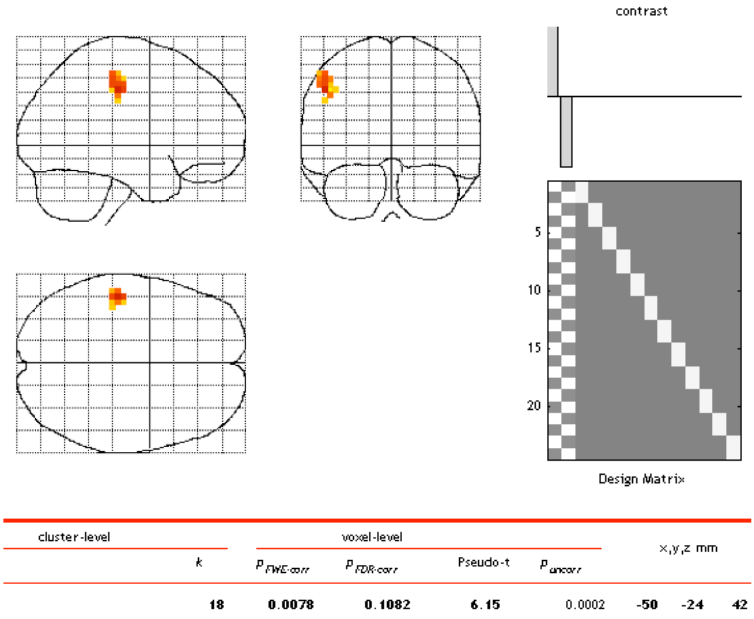

Figure 5.1 - Glass brain and design matrix resulting from the statistical testing of the connectivity maps resulting from the use of a right cerebellum ROI. Group A - scans pre-exercise; Group B - scans post-exercise.

[15] Eckert MA *et al*., 2008. A cross-modal system linking primary auditory and visual cortices: Evidence from intrinsic fMRI connectivity analysis. *Hum Brain Map*

[16] JN Pannekoek *et al.,* 2013. Aberrant limbic and salience network resting state functional connectivity in panic disorder without comorbidity. *Journal of Affective Disorders*

[17] Shahabeddin Vahdat *et al*., 2011. Functionally specific changes in resting state sensorimotor networks after motor learning. *J Neurosci*

[18] Buckner *et al*., 2011. The organization of the human cerebellum estimated by intrinsic functional connectivity. *J Neurophysiol*

[19] K Duncan *et al*., 2009. Distinct memory signatures in the hippocampus: Intentional States distinguish match & mismatch enhancement signals. *J Neurosci.*

[20] A Viard, *et al*., 2007. Hippocampal activation for autobiographical memories over the entire lifetime in healthy aged subjects: An fMRI study. *Cereb Cortex*

[21] Seeley WW *et al*., 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci*

[22] Voss *et al*. 2010. Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Frontiers in Aging Neuroscience*

Using an in-house built script - written by Christian Schwarzbauer – the Brodmann areas (BA) and anatomical regions present in this cluster were identified.[23]

T-Threshold: 5.2
MinClusterSize: 1
MinPerc: 1.000000e-04

TABLE 5.2 – INFORMATION REGARDING CLUSTER LOCATION – RIGHT CEREBELLUM SEED

Cluster	Coordinates (MNI)	Voxels	Location
#1	[-49 -25 46] mm	18vox	BA48.L: 1vox (0.1% overlap) BA4.L: 2vox (0.7% overlap) BA3.L: 8vox (4.3% overlap) BA2.L: 7vox (6.0% overlap) PoCG.L(Parietal): 7vox (1.4% overlap) IPL.L(Parietal): 9vox (3.0% overlap) SMG.L(Parietal): 2vox (1.3% overlap)

The anatomical locations mentioned above are the left Postcentral gyrus (PoCG), left supramarginal gyrus (SMG) and left inferior parietal gyri.

Apart from the above finding, the use of the other seeds reported in **Table 5.1** did not result in any significant differences between the first scan (before exercise) and the scan acquired after the exercise session. However, it will also be presented the results that by one reason or another did not reach significance. Hence, the following results are obtained with a Family Wise Error (FWE) correction but using more lenient p-thresholds.

The seed placed in the left hippocampus with MNI coordinates [-27, -24, 9] resulted in a cluster of 30 voxels of decreased connectivity after exercise between this region and mainly the left cerebellum and the cerebellar vermis (FWE p<0.5 k=30 MNI [-3, -64, -44]).

```
Threshold: 3.7
MinClusterSize: 1
MinPerc: 1.000000e-04
```

TABLE 5.3 - INFORMATION REGARDING CLUSTER LOCATION – LEFT HIPPOCAMPUS SEED

Cluster	Coordinates (MNI)	Voxels	Location
#1	[-4 -63 -44] mm	18vox	C8.L(Cerebellum): 18vox (5.4% overlap) C9.L(Cerebellum): 7vox (4.0% overlap) V9(Vermis): 1vox (2.0% overlap)

[23] Both SnPM and the Brodmann area script report the results in MNI coordinates. The slight difference between both values is due to the fact that one gives the information about the peak voxel (SnPM) and the other about the centre of mass of the cluster

#2	[-18 -70 -34] mm	12vox	C1.L(Cerebellum): 10vox (1.3% overlap) C2.L(Cerebellum): 1vox (0.2% overlap) C6.L(Cerebellum): 1vox (0.2% overlap)
#3	[-10 -63 -7] mm	12vox	BA18.L: 10vox (0.7% overlap) LIN.L(Occipital): 11vox C6.L(Cerebellum): 1vox
#4	[6 -56 -46] mm	2vox	-

The anatomical location mentioned above is the left lingual gyrus (LIN.L), **Figure 5.2.**

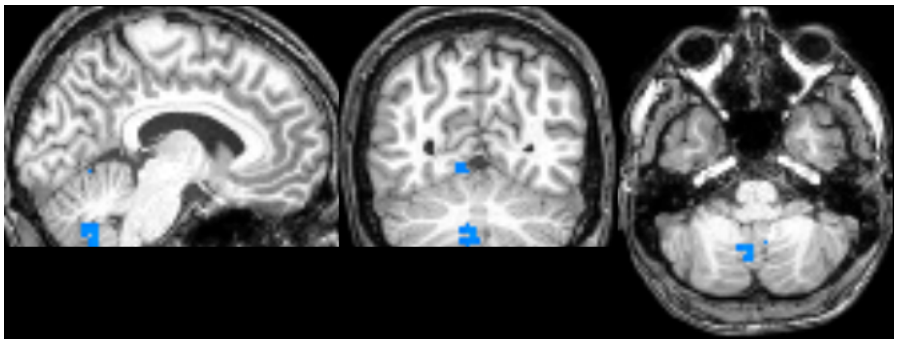

Figure 5.2 - Significant decreases in functional connectivity after exercise between the hippocampus and the cerebellar vermis

On the other hand, the left amygdala shows increased connectivity with the right cerebellum after exercise (FWE p<0.5 k=3 [30, -91, -23]).

The foot coordinates extracted from Buckner *et al.* (Buckner et al. 2011) provided the following results for an uncorrected p=0.01:

- For the left cerebellum seed, decreased connectivity was observed after exercise with a cluster (k=19 voxels) in the left parietal lobe with MNI coordinates of [-66 -16 25];

- For the right cerebellum seed, decreased connectivity was observed with 2 clusters in the right parietal lobe (k=29vox) with MNI coordinates of [15 2 -14] and (k=16vox) [33 -13 19];

At last, the PCC seed tested with a FEW p<0.2 resulted in a cluster (k=12vox) of decreased connectivity after exercise with the left parietal lobe with MNI coordinates of [-51 -34 52]. These changes are within BA 40 and BA 2 (**Figure 5.3**).

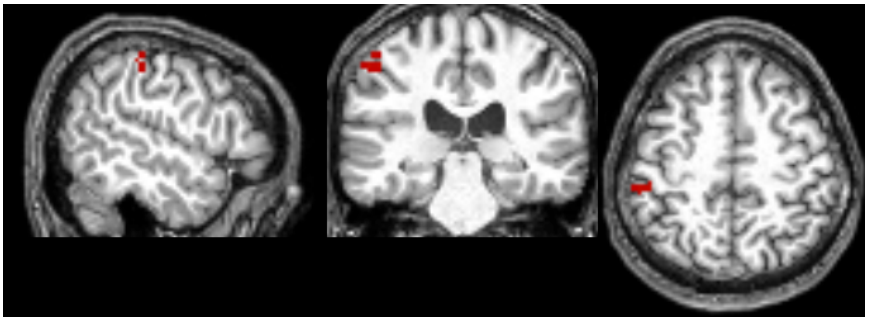

Figure 5.3 - Decreased functional connectivity between the PCC and the left parietal lobe

5.4 Discussion

Our results strongly agree with the hypothesis that exercise has an effect on functional connectivity. Moreover, they offer proof that these changes not only occur after a longitudinal intervention but also after a single acute session of exercise. Upon the second scanning session the physiological parameters of the participants had already returned to baseline, therefore it is extremely unlikely that the BOLD fluctuations observed during this period are the result of these physiological changes.

Previous literature on the effects of physical exercise has mainly reported increases in connectivity, namely in the fronto-parietal networks. Surprisingly, our results reflect exactly the opposite, with decreases in connectivity being more evident between cerebellar structures and the left parietal cortex. Other methodologies were combined with seed-based methods in order to gain further information about the measured connectivity changes. An ICA analysis using GIFT and FSL extracted several components from the data and found a cluster (k=14vox) of significantly increased connectivity after exercise in the cerebellum. This component highly correlated with the basal ganglia network extracted from the paper published by Allen *et al*. (Allen et al. 2011). Graph measurements resulted in increased connectivity within the frontal lobes. Performing this analysis with the 160 Dosenbach regions resulted in increases in the right ventromedial prefrontal cortex, in the right dorsolateral prefrontal cortex, in the right superior frontal gyrus and the ventromedial prefrontal cortex. Increased local efficiency was verified in the cerebellum as well as a decrease in path length within the same structure. The data was further analysed using a Network Based Statistics (NBS) Toolbox (Zalesky, Fornito & Bullmore 2010)for MATLAB. The results from the implementation of this method reflect decreases in connectivity between the cerebellum and the rest of the brain following physical activity.

In concordance with the seed-based results, most of these methodologies seem to point towards decreased connectivity between cerebellar regions and other brain structures. These results are by no means surprising due to the role that the cerebellum is known to play in motor coordination and motor learning. All of the participants were already somehow familiar with exercise activities, therefore cerebellar connectivity differences are most likely to be the product of the exercise task.

One of the most relevant and significant results reflects decreased connectivity between the right cerebellum and the left parietal cortex. Regions such as BA 2 and BA 3, which have presented with decreased connectivity, are part of the primary somatosensory cortex. On the other hand, BA 4 is the area where the primary motor cortex is located. The results are in agreement with areas that would normally be required while performing physical exercise, and one can argue that the functional changes are still the remaining of the functional and structural mechanisms active during the acute session of physical activity.

Concurrently to what has been previously mentioned in the literature it is reported activity between the cerebellum and the contralateral parietal lobe. However the relationship seems to be bilateral, with the stronger links found to be towards the opposite hemisphere. The seeds extracted from Buckner *et al.* (Buckner et al. 2011) were not above thresholding for significance, however they illustrate the connectivity that seems to exist between structures from the same hemisphere. The NBS methodology has found a vast network between the right cerebellum and parietal regions. The contralateral regions were more highly connected to the cerebellar structure however connections to the right parietal cortex were also present.

The vermis has integrative functions and is the structure uniting both cerebellar bodies. It is responsible for the transmission of sensory and motor information to and from higher processing areas. Our results point towards the idea of a functional network that connects the cerebellum with hippocampal structures. Several changes have been reported in the literature reflecting hippocampal volume changes following exercise and related with improvement of spatial memory. Therefore it is not surprising that it have also been found functional changes within this structure. The amygdala, part of the limbic system and responsible for memory processing and emotions, presents with decreased connectivity to the cerebellum, along with what has been reported for the hippocampus. The hypothesis is that these structures reported changes due to memory retrieval mechanism, since all the participants went through a familiarisation session on the bicycle before the day when they had to perform the acute session of exercise. Once again, the changes found within the DMN were related with the left parietal cortex, namely with the primary somatosensory cortex.

All of the differences found seem to be focused in regions within the cerebellum, primary motor and somatosensory cortices. These regions appear to be less connected to the rest of the brain after the exercise session.

Overall, these results strengthen the hypothesis that exercise is capable of inducing measurable functional brain connectivity changes. It is remarkable that even after such a short session of exercise significant differences in brain functional connectivity can be found. However, these findings are hard to interpret and further studies will be necessary to fully perceive the mechanisms involved in these functional changes. Future studies should focus on the functional changes and try to assess for how long they last, their extensiveness and the influence of the exercise type.

Apart from the seed-based methods all the other analysis methodologies described in this section were implemented by other researchers working within this project. Mr. Michael Stringer performed the ICA analysis; the graph measures were computed by Mr. Joel Parkinson, and finally the clustering methods and NBS analysis was carried out by Mr. Alex Ing. More information about their contributions can be found in **Section 7**, Appendices A.

5.5 Conclusion

The goal of this study was to investigate whether it would be able to find significant functional changes induced by a single session of acute exercise. Using different methodologies to study functional connectivity it has been found that there are indeed significant functional differences resulting from a single session of exercise. Each method seems to be more accurate in identifying specific functional changes, nonetheless all of them seem to report differences focused in regions within the cerebellum, primary motor and somatosensory cortices. As a result of a short session of exercise all these regions appear as hypoconnected to the rest of the brain.

These results have not been previously reported in the literature and they are encouraging towards the idea of measurable exercise-induced functional brain connectivity changes.

Chapter 6

What is rest? A methodological approach to resting state functional MRI acquisitions

Chapter Summary

Up until today there is no established methodology for resting state fMRI studies. In most of the typical studies using functional connectivity "resting state fMRI" the subjects are instructed to lay very still, usually with their eyes open, to relax and not to think about anything in particular. Thus, a wide variance in brain activity is expected due to the fact that different subjects will be thinking about different things and some of them may even fall asleep. Therefore reliably identifying neuronal intrinsic activity in the human brain in the absence of a specific task can prove to be extremely problematic. The aim of this project is to develop a protocol for resting state fMRI acquisitions that will provide us measurements with less variability by using a low cognitive level paradigm to keep the attention of the volunteer focused. It is expected that by using this paradigm it can be induced a more unified response, boosting the power of the methodology, and decreasing the variability of the analysed statistical parameters. Based on the paper from Morcom and Fletcher (Morcom, Fletcher 2007b) it is anticipated that the inclusion of this task will decrease the within group variability and therefore improve the sensitivity of the between-group comparison.

6.1 Introduction

Patterns of FC observed at rest have been shown to resemble those elicited by more traditional task-based paradigms or derived directly from task-data (Hutchison et al. 2013, Biswal et al. 1995, Calhoun, Eichele & Pearlson 2009, Fox et al. 2005, Laird et al. 2011, Smith et al. 2009, Vincent et al. 2007). It has been established that the same functional networks are cohesively active during a multitude of tasks as well as rest(Smith et al. 2009). On the other hand, rigorous task demands have also been shown to modulate functional connectivity inside the RSN(Fransson 2005, Hampson et al. 2006, Harrison et al. 2007, Kelly et al. 2008). Complex cognitive processes such as working memory require the reallocation of neural resources from default-mode brain regions to lateral prefrontal regions. McKiernan *et al*. in 2003(McKiernan et al. 2003) demonstrated that this reallocation of resources increases with an increase in task difficulty. Nonetheless the study by Greicius *et al*, 2004 (Greicius et al. 2004) suggests that not all tasks are sufficiently engaging to disrupt the default-mode network.

The lack of consistency in the methodology used by different research labs to perform 'resting state' fMRI acquisitions makes it nearly impossible to accurately study the variability among the identified network patterns. A relatively small change in the study design can have a large impact on how these networks are represented and how reliably these will be identified. Given the unconstrained nature of 'resting state' there is the emerging need for an agreement among researchers regarding the protocol used to study such a stage. Accordingly, the reliability of resting state measurements, and the factors that may modulate them, need to be thoroughly examined.

A study design using eyes-closed is considered to be one valid experimental way to switch the brain into its resting state activity (Raichle 2010, Logothetis et al. 2009). While this might be regarded as experimentally valid and sufficient, it may, however, prove insufficient when considering the input from the remaining senses, such as audition, that cannot be shut down completely. According to Logothetis *et al.* (Logothetis et al. 2009) a fixating cross requires the eyes to be open which implies an additional effort of fixation and therefore cannot be considered an appropriate method for measuring the resting state activity. Nonetheless, the study performed in 2013 by Patriat *et al.* (Patriat et al. 2013) analysed the effect of eyes open, eyes closed and the fixation of a cross on resting state fMRI reliability. The results supported

the fixation condition as the one providing better reliability. Beyond these contradictory results Logothetis raised some important questions "What should be considered as reflecting the intrinsic, spontaneous activity of the brain? What is the optimal experimental protocol for studying such activity? If no optimal protocol exists, and external sensory stimulation of at least some modalities is unavoidable, then what should be the strategy to assess the effects of the stimulated areas on the higher association cortices that might – at the outset – appear to be unrelated to the sensory stimulus?".

A study carried out by Smith *et al*. (Smith et al. 2009) compared two groups of data, one with true resting state and another with a large database of activation studies to test the hypothesis that the set of functional networks seen in resting state data closely matches the set derived from thousands of different activation conditions. The authors showed a strikingly strong correlation between the networks derived from both groups with ICA with 20 components (and later with 70 components). These included the visual, default-mode network, cerebellum, sensorimotor, auditory, executive control and frontoparietal networks. The quality of the correspondence between the derived activation networks and resting state fMRI is particularly compelling given the fundamentally different nature of the data feeding into these two analysis.

The methodology chosen to analyse the 'resting-sate' data can also be acknowledged as a factor for variability. Different seed locations when using region of interest methods, or different number of component decomposition using ICA can be responsible for slight variations in the patterns of the RSN identified. Despite the fundamental differences between these two methodologies, a 2012 study (Rosazza et al. 2012) showed that the results of seed-based analysis and ICA are significantly similar in a group of healthy subjects.

Another factor of variability is the scanning time. A typical acquisition in humans includes a single scan of approximately 5-10 minutes using a repetition time (TR) in the range of 2-3 s that allows for whole-brain coverage with standard imaging sequences. It has been suggested that correlation values within and between ICNs stabilise within 4-5min of data (Van Dijk et al. 2010), implying that most studies are adequately sampling the network activity despite relatively few data points. However the identification of an optimal duration of a resting fMRI session (and the possible need for multiple sessions) is an open question (Cole, Smith & Beckmann 2010).

Several recent studies analysing the stability of RSN patterns through various sleep states indicate that the correlation patterns are relatively stable, except for a slight weakening during deep sleep. Even though they are designated RSN, the fMRI acquisitions are not usually collected during a proper 'resting state'. Most of the studies incorporate passive visual stimulation, instructed or self-initiated changes in mental state or focus, or occur immediately following some other experimental manipulation, and therefore cannot be described as involving true, stimulus-unguided rest. All these factors affect reliability and have an impact even in the within-subject variability through different scanning sessions. Within-subject FC has been shown to vary considerably, even between different scans within the same imaging session (Honey et al. 2008, Liu et al. 2009, Shehzad et al. 2009, Van Dijk et al. 2010). Therefore the most recent studies have focused on finding the condition that provides the more reliable and reproducible measurements

Reliability of RS-fMRI

Test-retest reproducibility and inter-subject variability studies suggest that RSNs can be detected reliably across imaging sessions (Shehzad et al. 2009) and across different subjects (Damoiseaux et al. 2006, Shehzad et al. 2009) though there may be some source of variability between subjects. In the study published by Chou *et al.* (Chou et al. 2012) the reproducibility of RS-fMRI during a period of one year was assessed, resulting in an intraclass correlation coefficient (ICC) of >0.60 for >70% of the functional networks examined.

In general, the reliability and stability of resting state functional connectivity MRI is critical to establish the network patterns present during normal development and aging so that deviations from these healthy states can be assigned with certainty to a particular condition or disease. As mentioned before, it has been suggested that many neuropsychiatric diseases disconnect brain areas belonging to the DMN. However, the potential use of the DMN as a functional imaging marker for individuals at risk with these diseases requires that the components of the DMN are reproducible over time in healthy individuals. Thus, studies like the one carried out by Meindl *et al.* (Meindl et al. 2010), assessing the reliability of the DMN over time in healthy volunteers, are of extreme importance. The most reproducible areas were found to be the anterior and posterior cingulated gyrus being the DMN patterns generally reproducible in healthy young subjects.

Furthermore, in the publications by Varsou *et al.* (Varsou, Macleod & Schwarzbauer 2013) and Perrin *et al.* (Perrin et al. 2012) it has been suggested that the inclusion of a low cognitive level paradigm may help reduce the variability in the RSN patterns identified and also within and between subjects. If the inclusion of this low level cognitive paradigm causes minimal impact/disruption of the RSN patterns the RSN patterns then it is expected that the connectivity maps obtained during rest will be replicable during this simple task.

Clinical applications

Rs-fMRI has provided many interesting insights on RSNs in the healthy brain and in multiple disease states. Potential clinical applications at the single subject level have been demonstrated on some studies. Literature available on group-level studies is rather limited.

- Identification of patients with Alzheimer Disease (Wang et al. 2007, Damoiseaux 2012, Agosta et al. 2012)
- Surgical planning in patients with epilepsy (Liu et al. 2009, Stufflebeam et al. 2011)
- Psychiatric diseases such as major depressive disorder and schizophrenia (Perrin et al. 2012, Venkataraman et al. 2012)
- Patients with autism spectrum disorders (von dem Hagen et al. 2013, Jones et al. 2010)
- Attention deficit/hyperactivity disorder (Fair et al. 2010)

Literature review

During the course of this study a literature review regarding resting state fMRI reliability and reproducibility was conducted using electronic bibliographic databases, online journals and the websites of key organisations. This review used the following bibliographic databases: a) Web of Knowledge; b) Medline (via Ovid) – including studies since 1946; c) Embase (via Ovid) – including studies since 1974; and d) Scopus. The search mainly focused on articles published in the last 5/10 years. Natural language searching was used combined with Boolean operators to establish the relationship between different search terms. Whenever necessary more than one term for the same concept was used (e.g. fMRI, functional MRI and functional

resonance imaging) to strengthen the search. The keywords for this literature search were “resting state”, “magnetic resonance imaging”, “task”, “reproducibility” and “reliability”.

6.2 Material and Methods

6.2.1 The RESTATE study

This is a pilot / feasibility neuroimaging longitudinal study comprising of 13 healthy volunteers. There were two scanning sessions: a baseline scan and a follow up after 30 ± 5 days. The goal of this study is to compare the reliability of functional connectivity estimates in two different resting conditions: true resting state (RS) and while performing a low cognitive level paradigm (LCP). The participants are asked to always perform the task with their dominant hand. There are several variables that can contribute to increased variability in the results (such as gender, age, social background, etc) therefore the aim was to make the population as homogeneous as possible in order to minimise the variability resulting from possible confounds. Each scanning session lasted for a total of 45 minutes including 10 minutes of true resting state and another 10 minutes of the low cognitive demand paradigm.

Problem

It is not uncommon that the measurements resulting from a single subject present a very high standard deviation. This is ultimately reflected in an increased difficulty to compare subjects and conditions with certainty. In these cases it is virtually impossible to be sure that what is being observed is in fact a true effect and not just random signal.

Questions

This study was designed to answer the following questions:

a) *Does a low cognitive demand paradigm reduce the within group variability?*
b) *Does a low cognitive demand paradigm offer a better baseline for the comparison with the follow-up scan and stroke patients?*

6.2.2 Participants

- *Demographic information*

Thirteen participants were originally included in the study. One of them was excluded due to the non-attendance to the second scanning session. The twelve participants included for further analysis were all right-handed (mean age 28,83 ± 4.687). Seven of the participants were females (58.3%) and five of them were males (41.7%). The participants were all age and sex matched with the stroke patients.

For this cohort the number of days between baseline and follow-up scans was of 29.83 ± 3.271 days, with a range from minimum to maximum of 27 – 35 days. Further information regarding the demographic statistics can be found in **Section 6.1** from the Appendices A.

- *Medical Conditions*

On the first selection process all of the selected volunteers stated to be healthy. However, when reporting their medical history at the time of their first scan two of the participants acknowledged having medical conditions that should be taken into account. One of the participants reported suffering from depression and another from migraines. Some less severe conditions such as hyperlipidaemia, hypothyroidism and ulcerative colitis were also reported. Even though the participant suffering from migraines was not under any medication the volunteer suffering from depression acknowledged to be currently taking antidepressant tablets. These participants were still included in the study since they were considered to be representative of the normal population.

- *Ethics*

The RESTATE study was approved by the North of Scotland Research Ethics Service (NOSRES), and written informed consent of all the participants was obtained prior to participation in the study.

6.2.3 Paradigm

The low cognitive demand paradigm was designed by Dr Ourania Varsou and Mr Gordon Buchan using the Presentation software (more information can be found in **Section 7**, Appendices A). This task consists on the presentation of alternating pictures of buildings and landscapes (**Figure 6.1**) where the participant is asked to press the dominant hand index finger button every time an image of a building is presented. The button handle has a specific design so that the patient/volunteer can rest his/her hand on the chest and the action of pressing the button becomes more comfortable. The participants were shown 200 pictures - 100 landscapes and 100 buildings – centred in the middle of the screen and presented every 2.9 seconds.

The presentation has a background image of which 70% is grey, to avoid white flashes between images, inducing a more pleasant, neutral and calm reaction to the paradigm. Based on previous literature, the clinical psychologist in the Department Dr Jennifer Perrin, carefully chose the pictures used in this paradigm. Each time the button is pressed a feedback response – "Button pressed" – is presented on the screen. The feedback message is designed to be simple and to avoid triggering any competitive behaviour.

To perform the visual paradigm a screen and a projector have been positioned on the back of the scanner bore. The head coil has an incorporated mirror that allows the visualization of the screen and each volunteer is given a button to press every time a building is shown.

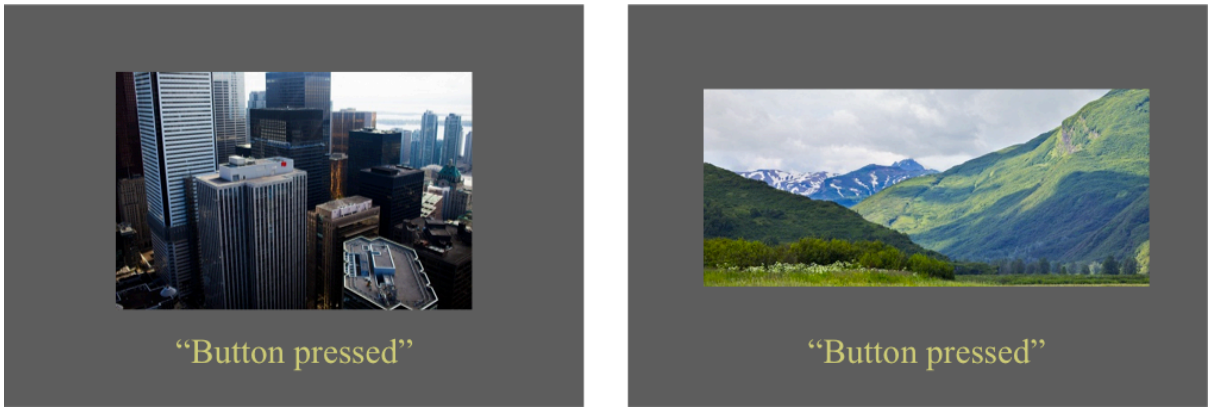

Figure 6.1 - Low cognitive paradigm presented to the participants with pictures of a) buildings and b) landscapes. Once the button was pressed a message saying "Button pressed" would be displayed

6.2.4 Imaging Methods

The participants were scanned two times with the neurovascular protocol (Baseline and Follow-up) plus the two fMRI resting conditions: Resting state (RS) and Low Cognitive Paradigm (LCP). During the RS sequence volunteers were asked to relax and the radio was switched off. The follow-up scan was acquired in a period of 30 ± 5 days from the baseline scan. The full MRI protocol can be found in Appendices B.

In order to avoid habituation or prediction regarding the scanning sequence a random number generator script was used to create the order of the scanning procedure for each volunteer and each scanning session.

TABLE 6.1 – SCANNING ORDER FOR BOTH BASELINE ANDFOLLOW-UP SCANS

	Scanning order	
Volunteers	**Baseline**	**Follow-up**
1	Low cognitive/ RS	Low cognitive/ RS
2	RS/Low cognitive	RS/Low cognitive
3	RS/Low cognitive	RS/Low cognitive
4	Low cognitive/RS	Low cognitive/RS
5	RS/Low cognitive	RS/Low cognitive
6	RS/Low cognitive	RS/Low cognitive
7	Low cognitive/RS	Low cognitive/RS
8	Low cognitive/RS	Low cognitive/RS
9	RS/Low cognitive	RS/Low cognitive
10	Low cognitive/RS	Low cognitive/RS
11	Low cognitive/RS	Low cognitive/RS
12	RS/Low cognitive	RS/Low cognitive
13	RS/Low cognitive	RS/Low cognitive

The participants were asked to fill a research form that included questions such as sex, age, medical conditions and current regular medications. Upon agreement of the volunteer a Montreal cognitive assessment test (MoCA) would also be performed by the researcher. The scans were posteriorly checked by a radiologist to make sure that no medical problems were

found. A white matter hyperintensity visual scoring was also performed based on the local Scheltens protocol developed by Professor Alison Murray. This protocol classifies the hyperintensities in four categories: deep white matter hyperintensities (DWMHs), grey matter hyperintensities (GMHs), infra-tentorial foci of hyperintensities (ITFHs) and periventricular hyperintensities (PVHs). The differences between the local and original protocols are in terms of DWMH and GMH classifications and the scaling points of PVH. In addition, the internal capsule has been incorporated into the DWMH section instead of the GMH which was originally used. In the local protocol, FLAIR images are used to score the PVH and DWMH, while scoring of the GMHs and ITFHs is performed using T2-weighted images. WMH present in MR images as visible changes within white matter structures. These are thought to be due to hypoxic episodes related to hypoperfusion of vulnerable deep white matter, secondary to hypertension, diabetes, and other vessel diseases. A copy of the Research form and information about the local Scheltens protocol can be found in the Appendices B.

6.2.5 Image Analysis

Preprocessing

The data was preprocessed using the statistical parametric mapping software package for MATLAB (SPM8; www.fil.ion.ucl.ac.uk/spm). Preprocessing was performed according to the standard steps including realignment and reslicing, slice timing correction, co-registration, segmentation, normalization to the MNI space, reslicing to 3 x 3 x 3 mm, and spatial smoothing with an 8-mm FWHM Gaussian isotropic kernel.

A binary mask was created using the SPM8 tissue probabilistic map for grey matter (spm/spm8/apriori/gray.nii) to segment the grey matter tissue of each subject, including all the voxels with a grey matter probability greater than 0.3. The time courses of all grey matter voxels included inside the binary mask were extracted from the imaging data and stored in an array of N= 33, 985 time course vector.

The segmentation step also provided a probabilistic map of the white matter (spm/spm8/apriori/white.nii) and cerebrospinal fluid (CSF) (spm/spm8/apriori/csf.nii) for each subject. These maps were thresholded to ensure 30% of tissue type probability and used to create a tissue-averaged time-course for each subject, which was posteriorly used as a confounding variable to be regressed out of the data. In order to perform this step the thresholded masks were applied to the time series of each individual and a mean time serie was calculated by averaging across all voxels within the mask. This is a common step used to avoid the discovery of false correlations related with white matter or cerebrospinal fluid signal that are not the main signal of interest (i.e. grey matter signal) and therefore are considered to be nuisance variables (Di Martino et al. 2008, Shehzad et al. 2009, Fiecas et al. 2013, Patriat et al. 2013).

Templates

Unlike most of the previously published reliability analysis literature it has been decided to use a set of templates instead of a region of interest (ROI) approach. The templates used in this analysis were derived by *Allen et al.* in her 2011 paper (Allen et al. 2011). This study used Independent Component Analysis (ICA) to analyse the data from 603 healthy adolescents and adults with a mean age of 23.4 years, ranging from 12 -71 years. The fMRI data was analysed using a 75-component group independent component analysis (GICA), and

based on visual inspection and power spectra 28 components were identified as Resting state Networks (for a detailed description of the component selection see Section 2 of (Allen et al. 2011)). The unthresholded t-maps for 28 resting state components including 7 different networks are available online (http://mialab.mrn.org/data/index.html) .For display purposes the maps where thresholded at $t_c > \mu_c + 4\sigma_c$. (see Appendix B of (Allen et al. 2011)). The RSN are divided into groups according to their anatomical and functional properties including the basal ganglia (BG), auditory (AUD), sensorimotor (MOT), visual (VIS), default-mode (DMN), attentional (ATTN), and frontal (FRONT) networks (**Figure 6.2**).

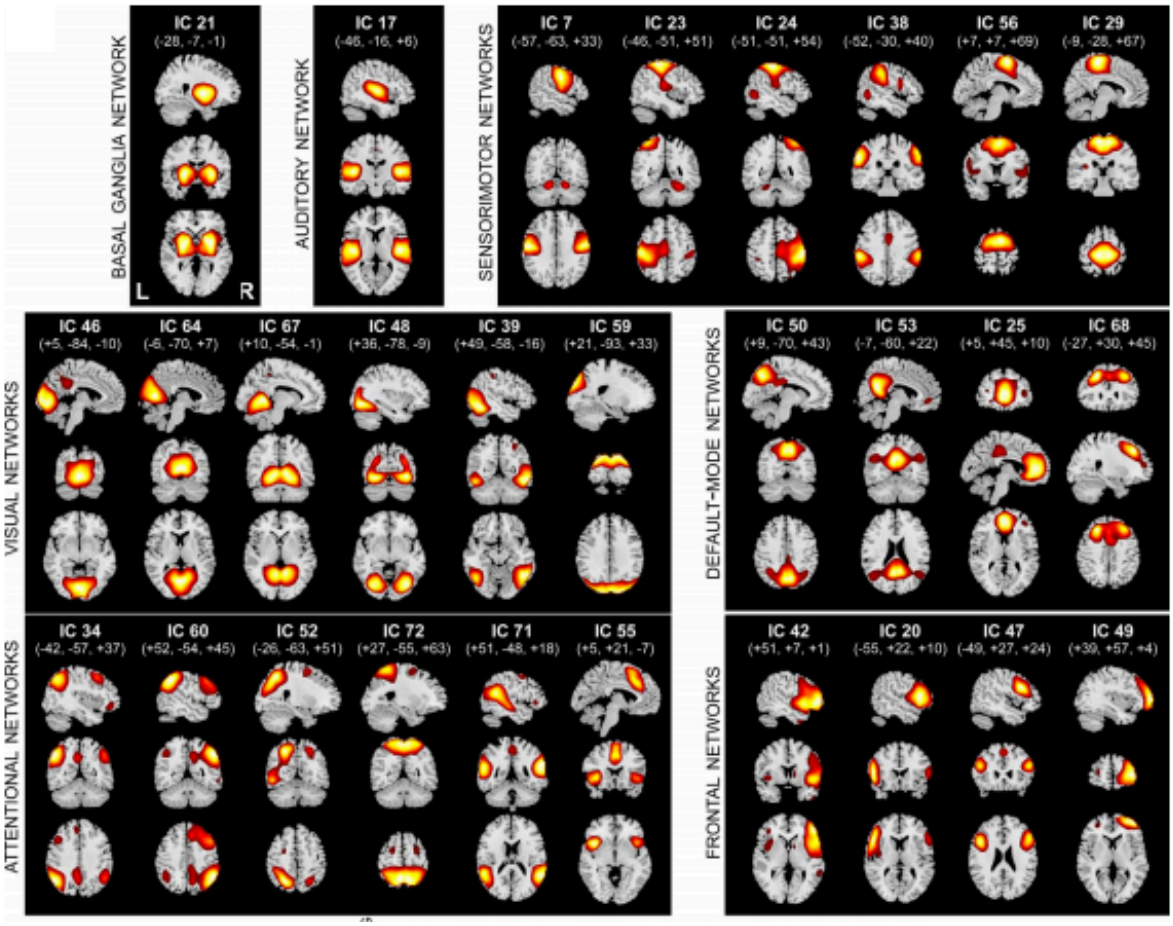

Figure 6.2 - 28 components identified as RSN in the Allen *et al*., 2011 paper

TABLE 6.2 – THRESHOLDS FOR THE T-MAPS OF EACH COMPONENT FROM THE ALLEN *ET AL*, PAPER

Component number	Resting – State Network	t-statistic threshold
7	Sensorimotor (MOT)	21.48
17	Auditory (AUD)	28.62
20	Frontal (FRONT)	26.55
21	Basal Ganglia (BG)	33.00
23	Sensorimotor (MOT)	20.61
24	Sensorimotor (MOT)	20.05
25	Default-mode (DMN)	31.81
29	Sensorimotor (MOT)	30.19
34	Attentional (ATTN)	34.08
38	Sensorimotor (MOT)	35.55
39	Visual (VIS)	37.36
42	Frontal (FRONT)	26.49
46	Visual (VIS)	17.71
47	Frontal (FRONT)	41.69
48	Visual (VIS)	28.44
49	Frontal (FRONT)	29.02
50	Default-mode (DMN)	31.29
52	Attentional (ATTN)	37.22
53	Default-mode (DMN)	32.64
55	Attentional (ATTN)	47.00
56	Sensorimotor (MOT)	31.64
59	Visual (VIS)	43.92
60	Attentional (ATTN)	29.70
64	Visual (VIS)	24.99
67	Visual (VIS)	25.25
68	Default-mode (DMN)	45.33
71	Attentional (ATTN)	37.80
72	Attentional (ATTN)	23.27

The single BG component shows activation mainly focused in the putamen and pallidum (Robinson et al. 2009, Ystad et al. 2010). The auditory component, IC 17, represents large parts of the auditory system, including the superior temporal gyrus, superior temporal sulcus and middle temporal gyrus all with bilateral activation(Seifritz et al. 2002, Specht, Reul 2003).

In order to create a mask/template of each network the thresholds corresponding to the display of the t-map for each component are needed. The author herself provided the threshold values through e-mail correspondence. The values used to create the binary masks for each of the components can be found in the **Table 6.2**.

Data analysis

Only the voxels included inside both the RSN template and the grey matter mask were selected for further analysis.

Temporal low-pass filtering

According to previous literature (Biswal et al. 1995, Fox, Raichle 2007, Cordes et al. 2001b) resting state data fMRI should be filtered to exclude physiological components and non-neuronal sources as well as to improve the signal-to-noise ratio (Van Dijk et al. 2010). Therefore a 10^{th} order low-pass Butterworth filter with a cutoff frequency of 0.1Hz was applied to each time course vector. The filter coefficients were computed using the MATLAB "butter" function and applied using the "filtfilt" function as described in(Perrin et al. 2012).

Baseline Correction

Linear regression was used to perform a linear baseline correction of each time course vector for the confounding factors. For this purpose and according to the standard analysis procedure used in the Department, a second order cosine basis set was applied, consisting of the two vectors with the components $x_i = \cos(i.t/T.\ \pi)$ with t = 1, 2, …, T and i = 1, 2; $T = 300$ is the number of time points. In addition to the two cosine basis vectors, the six realignment vectors generated by SPM8 during the realignment preprocessing routine were included as covariates to correct for movement-related global signal changes simultaneously. The

averaged time course for the white matter and CSF were also included as nuisance regressors to correct for a potential contamination of the signal with sources that do not originate in the grey matter. These regions are also thought to include high proportions of noise related to cardiac and respiratory signals.

The linear regression coefficients were calculated in MATLAB for each time course vector v according to $\beta = (X'.X)^{-1}.X'.\ v$, where X is the 11 x T design matrix that consisted of a constant column vector of ones followed by the two cosine basis vectors, the six realignment vectors and the two averaged time courses (white matter and CSF). The corrected time course vector was calculated for each time course according to $v_c = v - X.\ \beta$.

Global signal regression was not performed in this dataset. This process consists of regressing out the average time course of the entire brain with the aim of improving the specificity of correlations and reducing noise. The decision to not include this step followed after consulting the published literature (Cole, Smith & Beckmann 2010, Murphy et al. 2009, Liang et al. 2012, Weissenbacher et al. 2009, Saad et al. 2012, Song et al. 2012) suggesting that the inclusion of this step tends to reduce the overall reliability, induce false negative correlations and affect group results. The paper by Liang *et al*. (Liang et al. 2012)also found that greater reliability was observed for Pearson-correlation-based brain networks without performing global signal removal.

Functional connectivity analysis

The seeds used for computation of the correlation maps were extracted from the paper published by Patriat *et al.* (Patriat et al. 2013). **Table 6.3** outlays the seeds and their corresponding MNI coordinates. The seeds consisted in a cube with the central voxel in the coordinate and one voxel in each direction accounting for a total of 7 voxels.

TABLE 6.3 – SEED REGIONS USED FOR THE SEED-BASED CONNECTIVITY ANALYSIS

Network	Seed	MNI Coordinates
Default-mode Network	PCC	[0, -53, 26]
	mPFC	[0, 52, -6]
	left LatPar	[-48, -62, 36]
	Right LatPar	[46, -62, 32]
	left HF	[-24, -22, -20]
	right HF	[24, -20, -22]
Dorsal Attention Network	left FEF	[-38, -4, 48}
	right FEF	[40, -4, 48]
	left MT	[-56, -60, -2]
	right MT	[54, -58, -4]
	left IPS	[-24, -58, 52]
	right IPS	[22, -58, 54]
Auditory Network	left Aud	[-43, -26, 12]
	right Aud	[43, -26, 12]
Motor Network	left Mot	[-36, -25, 57]
	right Mot	[36, -25, 57]
Visual Network	left Vis	[-30, -88, 0]
	right Vis	[30, -88, 0]

PCC, posterior cingulate cortex; mPFC, medial prefrontal cortex; LatPar, lateral parietal cortex; HF, hippocampal formation; FEF, frontal eye fields; MT, medial temporal area; IPS, intraparietal sulcus; Aud, primary auditory cortex; Mot, primary motor cortex; Vis, primary visual cortex.

The seed-based analysis was performed using Pearson correlation measures and the CHART method stage 2 according to what has been described in the previous chapters and following the methodology applied in the paper by Perrin *et al*. (Perrin et al. 2012).

Averaged time-course

The time-course of the voxels of interest for each template was averaged independently for each subject and condition in order to create a vector of N=1 x T where T = 300 time points.

Correlation Coefficient

Correlation coefficients can be used to investigate how close is the relationship between two variables. This measure is based on the sum of products about the mean of the two variables (x and y). The distance of the point (x_i and y_i) to the axis represents the deviations from the mean. This value can be positive or negative and so their product will either be positive or negative according to the sort of relationship that exists between the variables (positive or negative correlation). When plotting the variables, and if the two are not related, it will result in a scattered diagram with roughly the same number of points in each of the sections, resulting in a sum equal to zero – no correlation.

In order to obtain a dimensionless coefficient one can divide the sum of products by the square roots of the sum of squares of both variables (x and y). This is denoted by *r*, the Pearson correlation coefficient. Assuming there are *n=1,2,..i* pairs of observations denoted by (x_i,y_i) then *r* is given by

$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{(\sum(x_i - \bar{x})^2)(\sum(y_i - \bar{y})^2)}}$$

$$= \frac{\sum x_i y_i - \frac{(\sum x_i)(\sum y_i)}{n}}{\sqrt{\left(\sum x_i^2 - \frac{(\sum x_i)^2}{n}\right)\left(\sum y_i^2 - \frac{(\sum y_i)^2}{n}\right)}}$$

However this correlation coefficient only accounts for linear relationships between the two variables. Therefore the two variables under study can be related in a non-linear way and thus the correlation coefficient will be equal to zero. This shows the importance of plotting the data and not relying on statistics only.

The Spearman correlation coefficient is the nonparametric version of the Pearson parametric test. It is specifically used under the assumption of ranked variables instead of raw scores. Unlike the Pearson correlation coefficient which only results in a perfect correlation of the two variables are related in a linear fashion, the Spearman correlation will be perfect for any two variables X and Y which are related by any monotonic function. The nonparametric characteristic of this measure also means that the Spearman correlation does not require any previous knowledge about the joint probability distribution of the variables under study.

Similarity matrix

With the aim of studying the stability within each network it was calculated a similarity matrix between all the voxels included inside of the template for each network. A Bravis Pearson product moment correlation (commonly known as Correlation Coefficient measure) was used. This procedure was followed by a Fisher Z-transform. The N x N similarity matrix is symmetrical on its diagonal being the unique values equal to S = N x (N-1) /2, where N is the number of voxels inside of the template. Extracting only the unique correlations (UC) from the similarity matrix results in a vector of size S for both the Resting state and Paradigm in the Baseline scan and the Follow up. Under the assumption of reproducibility these vectors were used to calculate a further correlation measure between the unique correlation vector (UC) resulting from the same condition but comparing the 2 different scanning times – intersession variability.

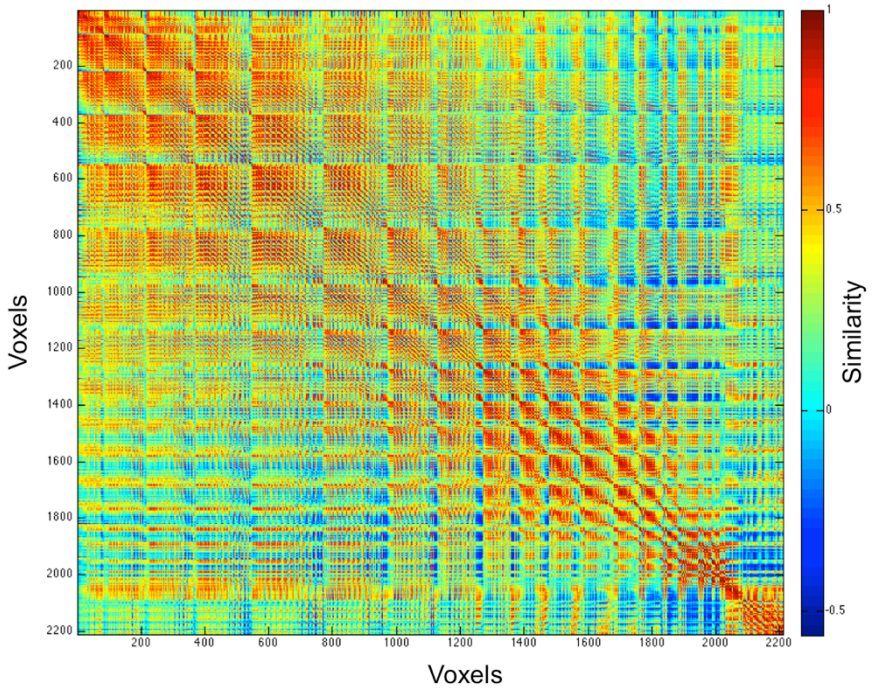

Figure 6.3 - Similarity matrix resulting from the computation of Pearson's correlation of all the voxels within the template

Ideally if it was possible to reach 100% reproducibility the two vectors would have a perfect correlation between each other. The correlation measures used were a Pearson and Spearman correlation coefficients resulting in a single value of correlation per subject and condition.

The averaged time course of the template (with size 1 x T with T =1, 2…300 time points) was calculated based on the mean of the time course of all voxels. A Pearson correlation coefficient was computed between the averaged time course and all the voxel included in the template generating a more localised comparison without averaging out the differences. These values were converted back to the image space in order to obtain maps of correlation within the template.

Intraclass Correlation Coefficient

Intraclass Correlation Coefficient (ICC) is a common measure of test-retest reliability (TRT) firstly introduced by Shrout and Fleiss in 1979(Shrout, Fleiss 1979) to measure inter-rater reliability. This measure has been extensively used to assess the test-retest reliability of fMRI activations and connectivity (Braun et al. 2012, Li et al. 2012, Shehzad et al. 2009, Thomason et al. 2011, Zuo et al. 2010, Patriat et al. 2013). Greater reliability will be expressed by higher ICC values while lower ICC values reflect low reliability between different measures.

Supporting the choice of using templates of RSN instead of a whole brain analysis is the finding reported by Shehzad *et al*. (Shehzad et al. 2009) that significant differences in ICC can be found depending on the significance of the connection. Assuming the study of the connectivity values of two unconnected regions these should be approximately zero throughout all subjects and scans, possibly with slight variations due to noise. Consequently, the between subjects variance will be approximately the same as the within-subjects variance resulting in an ICC value of zero. If a non-zero ICC value is found for a non-significant connection this suggests the presence of consistent noise within a subject that is reliably different from another subject. In reality it is extremely unlikely that two areas are totally unconnected, however the ICC values of most weakly connected areas will be more influenced by noise (Patriat et al. 2013), therefore the study of well-defined networks should in principle give us more reliable information than whole brain analysis.

For each unique correlation of the similarity matrix it was created one 12 x *n* matrix, using the Z-transformed correlation values (according to the procedure performed in(Patriat et al. 2013) and (Shehzad et al. 2009)) for the 12 participants and *n* = 2 scans - Baseline and Follow-up. This process was repeated for all of the 28 components.

The ICC values were calculated using a public MATLAB script developed by the University of Wisconsin – Madison[24]. A one-way ANOVA with random subject effects was used to compute the between-subject mean square (BMS) and within-subject mean square (WMS). The ICC values were subsequently calculated according to the equation proposed by Shrout and Fleiss, 1979, where k is the number of repeated measurements per voxel (here, $k = 2$):

$$ICC = \frac{BMS - WMS}{BMS + (k-1)WMS}$$

This is the model widely used to in fMRI reliability testing (Shehzad et al. 2009, Telesford et al. 2010, Zuo et al. 2010, Braun et al. 2012, Wang et al. 2011, Liang et al. 2012, Schwarz, McGonigle 2011, Song et al. 2012, Guo et al. 2012, Gorgolewski et al. 2013, Patriat et al. 2013). Once all the scans have occurred at different times there is nothing intrinsic about the label of "scan one" for the scans of each subject. To further access the network variability the ICC values were averaged within each of the 28 components. ICC values range from 0 to 1 with a value close to 1 representing a reliable measure with low within-subject variance relative to between subject variance. In agreement with what has been considered in previous studies (Liang et al. 2012, Liao et al. 2013) the test-retest (TRT) reliability was assessed in a component-wise manner according with the classifying criteria of ICC values (Sampat et al. 2006) that states that values <0.4 indicate low reliability; 0.4 < ICC < 0.6 indicates fair reliability; 0.6 < ICC < 0.75 indicates good reliability and 0.75< ICC < 1.0 indicates excellent reliability.

[24] Birn Laboratory, Department of Psychology: http://birnlab.psychiatry.wisc.edu/

Statistical analysis

Statistical analysis of the data was performed using three different softwares:

→ SnPM was used for the comparison of the seed-based connectivity maps using paired T-tests. The statistical testing was performed using a FWHM of 8mm and 4096 permutations;

→ SPSS[25] was used to perform paired T-tests of the UC and ICC values obtained;

→ FSL was the software chosen to perform the nonparametric statistical paired T-tests between the correlation maps of all the subjects for each scanning session and condition due to its easier implementation and automisation of the procedure.

[25] IBM SPSS Statistics: http://www-01.ibm.com/software/uk/analytics/spss/products/statistics/

6.3 Results

A record was kept on the amount of times the participants pressed the button and in which situation (buildings or landscapes). Taking into account that the paradigm consisted in 100 pictures of buildings and 100 pictures of landscapes and that these are healthy volunteers one would expect that the button would be pressed 100 times for buildings and 0 times for landscapes according to what has been asked the participants to do. The actual statistics are as follows: *Scan1* - number of times the button was pressed for buildings was of 99.25 ± 1.288 times with median 100; *Scan2* - the participants pressed the button for buildings 95.08 ± 16.093 times with a median of 100.

For all the participants the movement parameters were carefully plotted and analysed. The movement present in the data was very small, being currently accepted that values of movement in this range can be easily corrected by the regression of the movement parameters from the model. For the purpose of this study it has been considered excessive head motion as any of the translational parameters presented > 2 mm or rotational parameters > 2degrees. A comparison between the movement parameters resulting from one of the RESTATE volunteers and one DoC patient can be found in the Appendices A **Section 6.3**.

Statistical Analysis

Pearson correlation coefficient between the Schelten score reported for Scan 1 and for Scan 2 was significant ($p<0.05$) meaning that both sessions reported the same results. No significant correlation was found between the Scheltens score and the MoCA test neither between Scheltens and sex. Curiously, in this population it is reported an inverse correlation between age and Scheltens score. This fact can be explained by the healthier profile or our older participants when compared with the younger ones.

The number of times that the button is pressed in each session is normally distributed for a significance of 0.001 according to the Kolmogorov-Smirnov good-fit parametric test. (see Appendices A **Section 6.1**)

Seed-based correlation

Seed-based connectivity maps were computed for the scans acquired under the resting state condition as well as the paradigm. These maps were posteriorly compared between sessions (e.g. Baseline: Resting state vs Follow-up: Resting state) in order to study the intersession reliability when using seed-based methods. The resting state condition did not provide any significant differences between the two sets of connectivity maps for a FWE corrected of $p<0.05$. On the other hand the Paradigm maps were significantly different for the seed regions of the left primary auditory cortex (1 voxel, MNI [33,41,37]), right frontal eye fields (2 voxels MNI [24, -64,64] & 3 voxels MNI [-39,-55,25]), the left intraparietal sulcus (4 voxels MNI [36,-10,67]) and the right intraparietal sulcus (1 voxel MNI [15,-79,-32]). All the differences pointed towards increased connectivity in all these regions during the baseline scan when compared with the follow-up.

Network statistical analysis

The Spearman correlation consistently provided lower values of correlation for the subject average of the UC correlations. From each subject resulted a set of 2 correlation values, one per condition (Paradigm and Resting state). For the purpose of displaying the results in a more concise manner the values of correlation for each subject were averaged per component as can be seen in **Table 6.4** and the resulting plots **Figure 6.4** and **Figure 6.5.**

Both the Kolmogorov-Smirnov and the Shapiro-Wilk normality tests proved that the values of correlation for Pearson and Spearman tests are normally distributed.

A paired T-test was used to compare the Pearson correlation values of each subject within the same component and between Paradigm and Resting state. On a component level there were significant differences (considering for a FWE $p<0.05$) in two visual components – component 39 and 48. Averaging the results of all the components within each network the DMN, attention network, frontal networks and visual network present no significant differences between both conditions, however in the sensorimotor networks the differences are significant at $p<0.05$. Again, and using the Spearman correlation values this time, a paired T-test was performed on a component level showing significant differences in the visual components 39 and 48 for the correlation values between both conditions for a $p<0.05$. On a network level no significant differences were found between the conditions for a $p<0.05$. The

auditory and basal ganglia networks did provide any significant differences ($p<0.05$) in the correlation values obtained for both conditions.

TABLE 6.4 - UNIQUE CORRELATION VALUES FOR EACH COMPONENT AND AVERAGED PER SUBJECT

		UC correlation *averaged per subject*			
		Paradigm		**Resting State**	
		Pearson	Spearman's	Pearson	Spearman's
DMN	**Component 25**	0.7964	0.7276	0.8052	0.7409
	Component 50	0.8651	0.8247	0.8603	0.8133
	Component 53	0.8590	0.8171	0.8733	0.8329
	Component 68	0.8167	0.7655	0.8277	0.7794
Auditory N.	**Component 17**	0.7686	0.7260	0.7912	0.7530
Attentional Networks	**Component 34**	0.8420	0.8008	0.8559	0.8170
	Component 52	0.8395	0.7888	0.8303	0.7836
	Component 55	0.8090	0.7445	0.8153	0.7568
	Component 60	0.8208	0.7769	0.8301	0.7822
	Component 71	0.8240	0.7686	0.8333	0.7872
	Component 72	0.8026	0.7501	0.8044	0.7543
Frontal networks	**Component 20**	0.8428	0.7947	0.8380	0.7791
	Component 42	0.8033	0.7506	0.8041	0.7478
	Component 47	0.8415	0.7898	0.8429	0.7849
	Component 49	0.7966	0.7248	0.8304	0.7585
Basal ganglia N.	**Component 21**	0.7282	0.6141	0.7448	0.6394
Sensorimotor Networks	**Component 7**	0.8164	0.7655	0.8310	0.7849
	Component 23	0.8227	0.7738	0.8208	0.7714
	Component 24	0.8047	0.7597	0.8150	0.7662
	Component 29	0.7643	0.7120	0.7868	0.7461
	Component 38	0.8411	0.7971	0.8541	0.8163
	Component 56	0.7675	0.7170	0.7795	0.7196
Visual networks	**Component 39**	0.8541	0.8125	0.7905	0.7326
	Component 46	0.8914	0.8767	0.8552	0.8332
	Component 48	0.8830	0.8600	0.8061	0.7717
	Component 59	0.8730	0.8358	0.8651	0.8365
	Component 64	0.8507	0.8174	0.8697	0.8517
	Component 67	0.8249	0.7617	0.8255	0.7695

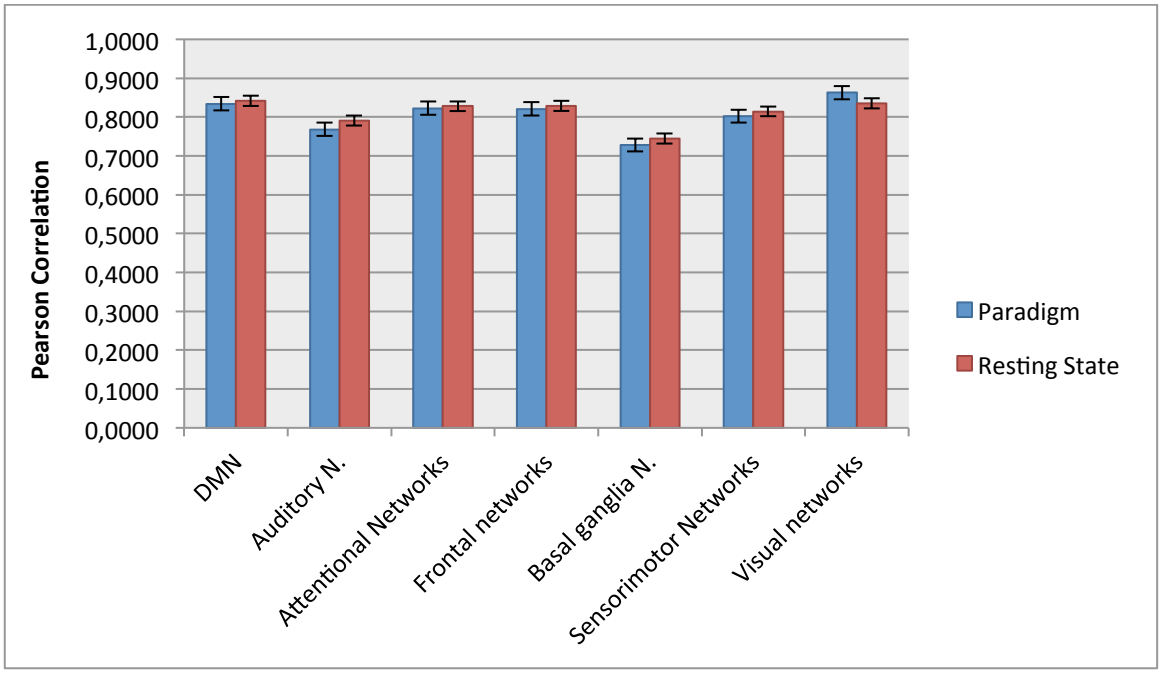

Figure 6.4 – Plot of the Pearson correlation coefficient obtained for the unique correlations and statistical error bars

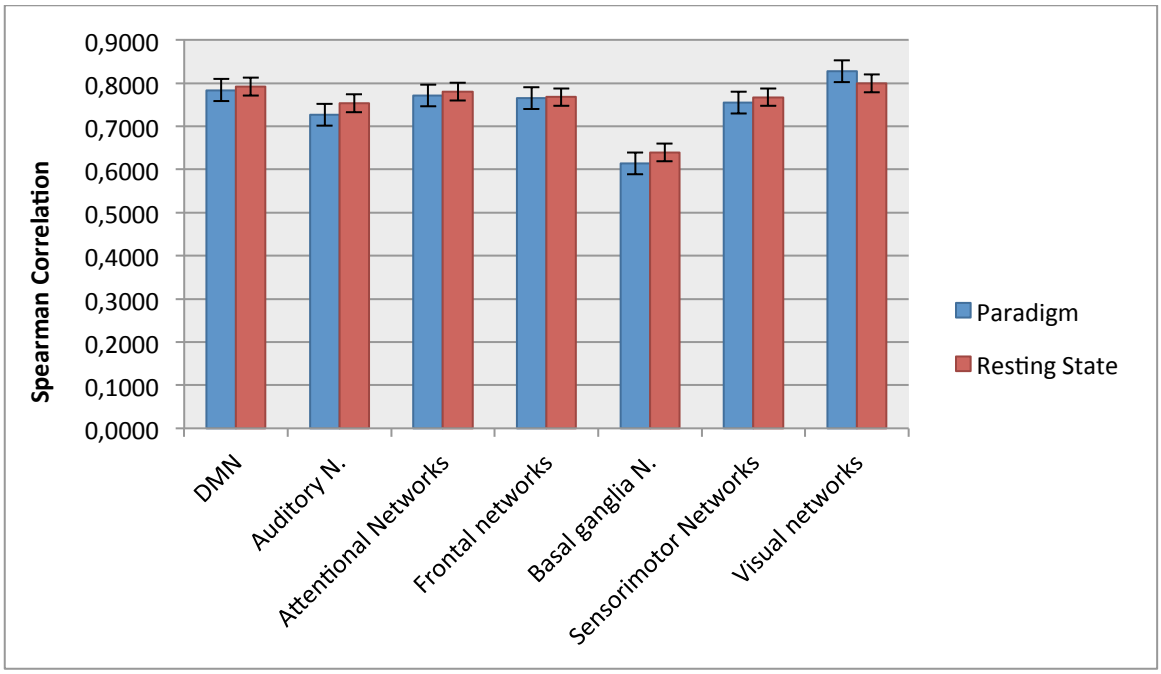

Figure 6.5 - Plot of the Spearman correlation coefficient obtained for the unique correlations and statistical error bars

ICC Results

Table 6.5 shows the results of the average values of ICC for each component. Both the Kolmogorov-Smirnov and the Shapiro-Wilk normality tests proved that the ICC values obtained for both resting state and paradigm are normally distributed. A paired T-test was used to compare the average ICC values between Resting state and Paradigm. For a $p<0.05$ this test demonstrated that there were significant differences between both conditions values.

TABLE 6.5 - ICC VALUES AVERAGED WITHIN EACH COMPONENT

		Average ICC	
		Resting State	**Paradigm**
DMN	**Component 25**	0.3578	0.3557
	Component 50	0.6040	0.6045
	Component 53	0.6171	0.4979
	Component 68	0.5270	0.4954
Auditory N.	**Component 17**	0.5132	0.4284
Attentional Networks	**Component 34**	0.5437	0.5038
	Component 52	0.5849	0.5111
	Component 55	0.5231	0.4752
	Component 60	0.5733	0.5521
	Component 71	0.5842	0.5362
	Component 72	0.5534	0.5207
Frontal networks	**Component 20**	0.5027	0.4380
	Component 42	0.4698	0.4577
	Component 47	0.5137	0.4838
	Component 49	0.4924	0.5169
Basal ganglia N.	**Component 21**	0.4196	0.3610
Sensorimotor Networks	**Component 7**	0.5003	0.4764
	Component 23	0.4910	0.4612
	Component 24	0.4899	0.4599
	Component 29	0.4913	0.4596
	Component 38	0.5326	0.4919
	Component 56	0.4122	0.4704
Visual networks	**Component 39**	0.5116	0.5038
	Component 46	0.5144	0.5471
	Component 48	0.4718	0.5346
	Component 59	0.5591	0.5903
	Component 64	0.5897	0.5439
	Component 67	0.5191	0.4962

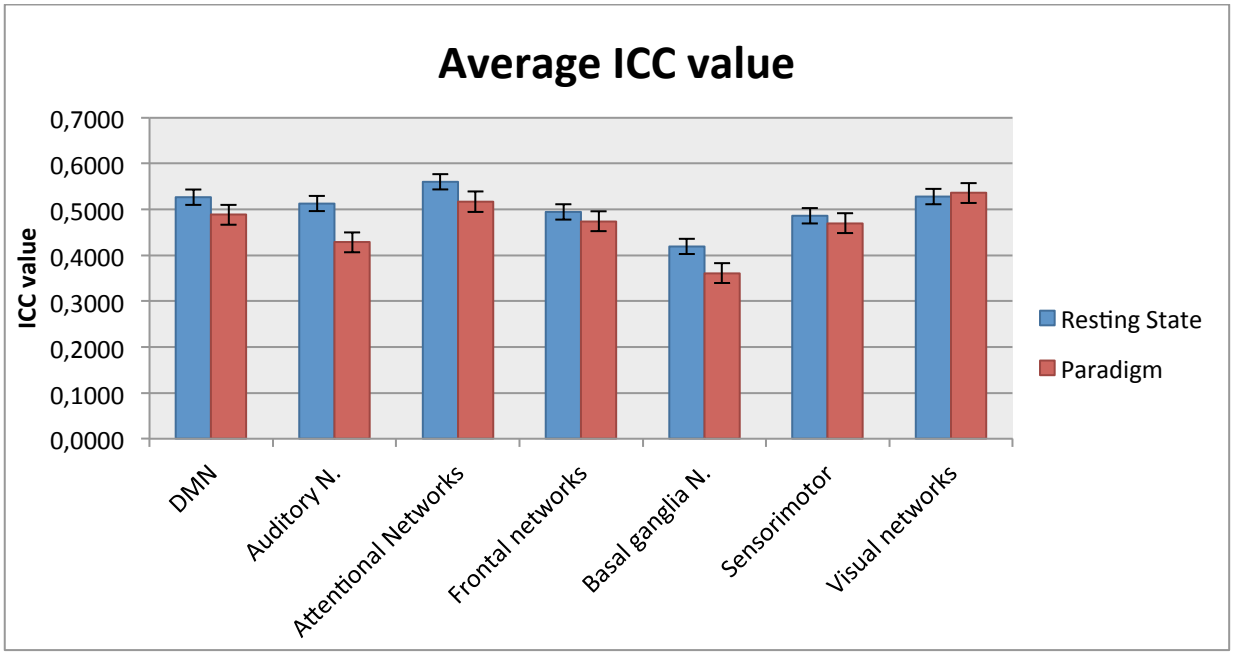

Figure 6.6 – Plot of the average ICC values for each network and statistical error bars

From visual inspection from the graphic (**Figure 6.6**) it can be seen that for all the networks, with exception of the visual, the resting state condition showed higher values of intraclass correlation coefficient - reflecting a lower intersession variance (Baseline vs Follow-up) for this particular condition. The visual network, as being the one most stimulated by the paradigm, did show a small increase in stability under this condition when compared with the true resting state.

The ICC values are mainly in the range between 0.4 and 0.6 reflecting fair reliability (0.4< ICC <0.6) for the detection of these networks.

Correlation maps

The correlation maps resulting for both scanning procedures and scanning session were compared using non-parametric statistics and a paired T-test, 10000 permutations at a significance level of $p<0.05$. This comparison comprised of a normal voxelwise comparison, and a cluster check on the voxelwise results and a threshold free cluster enhancement. Whenever the results were positive for significant differences these were reported using FSL view. Unlike MRIcron used in the previous studies FSLview displays the images according to the radiological convention (i.e. left is right) and uses the MNI coordinates as the stereotaxic space.

TABLE 6.6 – CLUSTERS OF SIGNIFICANT DIFFERENCES BETWEEN THE CORRELATION MAPS FROM BASELINE AND FOLLOW-UP

	Paradigm comparison		
Cluster analysis	Component	Vox	Coordinates
Increases in Baseline	24	1	[24,30,19]
	49	10	[24,59,19]
Increases in Follow-up	53	1	[33,19,20]
	71	1	[9,38,7]

	Resting state comparison		
Cluster analysis	Component	Vox	Coordinates
Increases in Baseline	-	-	-
	-	-	-
Increases in Follow-up	20	1	[43,47,27]
	47	4	[45,44,25]
		1	[44,45,27]

Components 20, 47 and 49 are all part of the frontal networks, while component 24 is included in the sensorimotor network, component 53 on the default-mode network and component 71 in the attentional networks. The cluster of 10 voxels presenting increases in the Baseline session compared to the Follow-up, during the Paradigm condition, is mainly included in the right Brodmann area (BA) 11. One of the voxels is part of the right BA 25 and all of the 10 voxels are included in the right anterior cingulate and paracingulate gyri.

Appendices **Section 6.2** includes a table with the results from the comparison of the correlation maps obtained for both conditions during the same scan (i.e. Baseline: Resting state vs Paradigm). The differences are significant in number and size but appear to be consistent for the first and second scan.

Individual analysis

Figure 6.7 is illustrative of two patients with a relatively high load of WMH. The white matter hyperintensities presented beneath are visualised in a FLAIR sequence, on which the WMH appear as hyperintense regions in the white matter. A higher load of WMHs is usually correlated with the development of vascular diseases such as stroke and multiple sclerosis. The volunteers included in this study had a Scheltens score between 5- 25. In this particular study it has been found that the volunteers who suffered from migraines and depression where the ones that presented with the highest score of WMH.

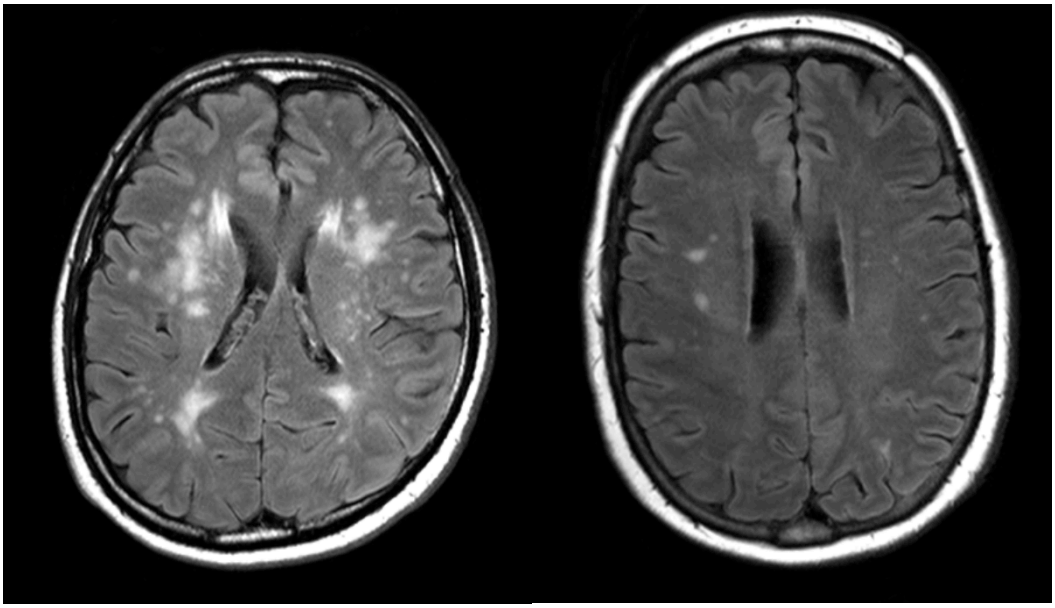

Figure 6.7 WMH in a) Patient with a very high Scheltens score 53; and b) a patient with Scheltens score of 38.

Incidental Findings

Even though no medically relevant incidental findings were discovered during the course of this study some participants did present incidental findings (**Figure 6.8**) as well as normal variations (**Figure 6.9**). This finding in such a small population (13 individuals) supports the potential interest in developing a study involving a larger sample of healthy subjects. This study would target the investigation of how many incidental findings can be found in the regular healthy population used for control purposes, what sort of findings are more predominant and how medically relevant are they.

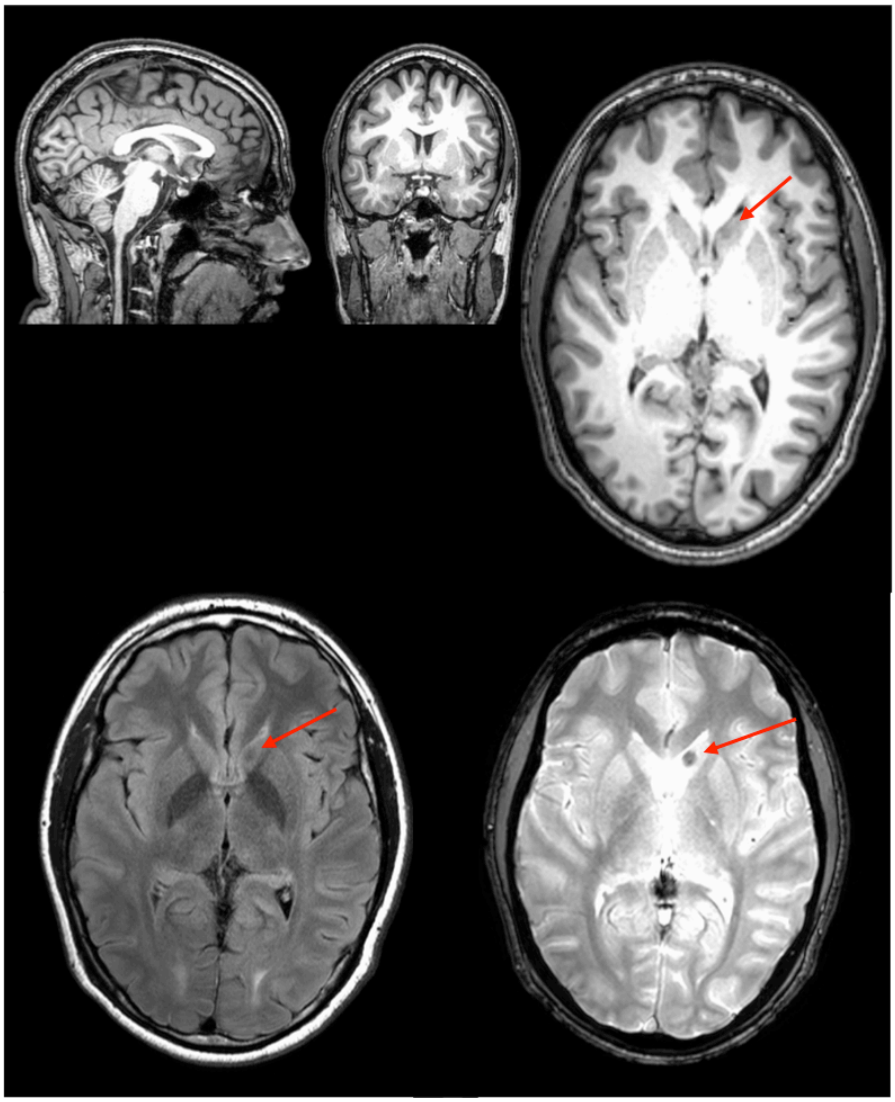

Figure 6.8 - Incidental finding of a 6mm calcified cyst in the right caudate nucleus.

Normal variations

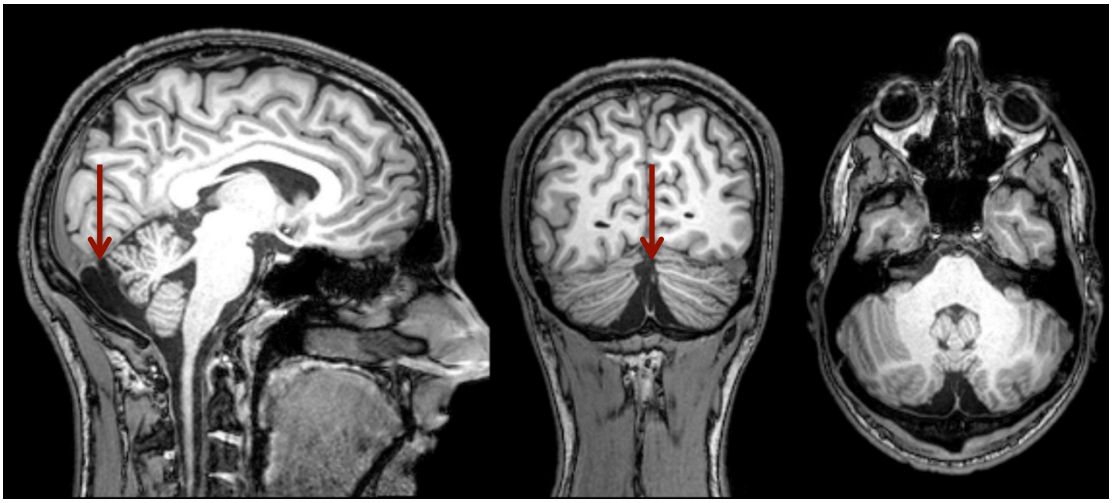

Figure 6.9 - Proeminent cisterna magna

ICA analysis

Mr Michael Stringer (more information on **Section 7**, Appendices A) performed ICA analysis of the data from both sessions and conditions. The components were extracted from one ICA session and compared between baseline and follow-up. No significant differences were detected in either case using a 2-sample Paired T-test with FWE $p<0.05$. The comparison between both conditions in session 1 and session 2 only resulted in a small cluster (3voxels and 2 voxels) of significant differences for FWE $p<0.05$ in two components of the visual network.

6.4 Discussion

Seed-based analysis

The results from the seed-based analysis support the idea that there is a considerable degree of reproducibility between scans for both conditions. However, contradicting out initial hypothesis, the results seem to suggest that the resting state condition is more stable and reproducible than the low-cognitive paradigm.

Our results point for greater connectivity patterns in regions such as the left and right intraparietal sulcus. Both regions are included in component 71 part of the attentional networks of the Allen template. This component also overlaps the alerting system(Fan et al. 2005). Seed-based connectivity maps for these seeds (L_IPS & R_IPS) show increased connectivity during baseline paradigm when compared with the same condition during follow-up. This behavior can be explained as the result of the baseline scan being the first contact the participants had with the task and therefore they are more focused and attentive comparative to the follow-up session. The differences observed in visual regions (L_FEF and R_FEF) are also interesting. There is increased connectivity in these regions during the baseline scan when compared with the follow-up. Increased connectivity in visual regions during paradigm when compared with resting state is somehow expected due to the performance of the task. As a result it can once again be assumed that during the first scan the participants were dedicating more attention to the task than when they performed it for the second time.

Moreover, it would be interesting to see how patterns of functional connectivity change during a third scanning session. This could give us further knowledge about the reproducibility of resting state and about the fluctuations observed during the execution of the paradigm.

The results obtained for the resting state condition do allow us to conclude that this condition is totally reproducible, but only that the similarity between the resulting maps cannot the denied. It is also important to stress that these small but significant differences can also be the product of other numerous factors. They can result of a small population size, random variations, etc. Therefore it is important to study other parameters (e.g. within group variability) that might give us a more into depth overview on what is actually happening between both scanning sessions.

Correlation maps

Most of the differences observed for the same condition and between the two sessions (Baseline vs Follow-up) are very small (~1 voxel), which can result not from significant inter-session differences but to be rather justified by other factors such as natural variance between sessions.

Component 23 and 24 are part of the left and right sensorimotor networks, situated in the vicinity of the central sulcus. Component 24 presented increases during baseline when compared to follow-up for the paradigm condition. Since all of the participants had the task button on their right hand, it can be hypothesised that the contralateral (left) somatomotor hemisphere (component 23) is more consistent and homogeneous during the two scanning sessions while executing the paradigm. The reported finding in component 24 is small (1 voxel) and can be acknowledged as being the result of inter-session variation. The same component and coordinate is also increased (1 voxel) in the baseline session during the execution of the paradigm when compared with the RS condition in the same session. This comparison also resulted in increases in component 23 during paradigm when compared to RS (1 voxel), that can be accepted as being the result of the performance of the task. These differences are strikingly small, proving that the execution of the paradigm does not seem to elude significantly greater activation of the contralateral somatomotor cortex when compared with the resting condition. Component 38, a bilateral component also part of the sensorimotor networks, is clearly the one from this network that is mostly affected by the execution of the paradigm. In a comparison between rest and paradigm, for both sessions, component 38 was the component that presented with greater increases during the execution of the task (Baseline: 7 voxels + 2 voxels & Follow-up: 9 voxels + 1 voxel).

Component 39, 46, 48, 59, 64, 67 are all part of the visual networks. The Allen templates have been created based on resting state fMRI acquisitions where the subjects were instructed to lay with eyes open fixating on a cross. During the resting state scan the volunteers were instructed to lay still and relax, giving them the option on whether they would like to keep their eyes open or closed. It has been found a higher correlation with the average time course during resting state for component 46 (178 voxels in the Baseline scan & 214 voxels in the Follow-up), component 59 (142 voxels in the Baseline scan & 264 in the Follow-up) and component 64 (59 voxels in the Baseline scan & 752 voxels in the Follow-up). Components 39, 48, 61 and 67 also report increased connectivity during resting state when compared with

paradigm for both scanning sessions. These results suggest that the behaviour of the visual network is much more similar to the template during resting state than during a period when there is visual stimulation. In some cases the same components reported increased correlation with the averaged time course in both conditions, however in different regions. As an example, component 59 shows an increase of 142 voxels [27,7,21] during RS and an increase of two clusters of 56 voxels [12,12,56] and 53 voxels [38,9,25] clusters during the paradigm, all during the first scanning session. Nonetheless the same behaviour is observed for the follow-up scan.

Component 49 comprises of the right prefrontal cortex, which is thought to be involved in executive functions. It was found a cluster (9 voxels) of increased connectivity during the first time performing the paradigm. This cluster is mainly included in BA 11, the orbitofrontal area, which is involved in planning, reasoning and decision-making. All of the voxels are included in the right ACC, structure that once again plays a role in decision-making. Hence, it can be hypothesised that the higher correlation values within the network when first executing the task (Baseline: Paradigm) and when compared with the second time they perform the task (Follow-up: Paradigm) – **Table 6.6** – are due to this being the first contact with the paradigm. During the second session the participants were already familiar with the task and might have been more comfortable and less focused while performing it. Both components 47 (bilateral) and 49 are part of the frontal networks and these are also the components that present with increased connectivity during the execution of the paradigm. The frontal networks are usually implied in decision making and mentalizing as well as mediating executive, memory and language functions (Koechlin, Ody & Kouneiher 2003, Koechlin, Summerfield 2007)hence their involvement during the execution of the paradigm, even though being minimal, is not surprising.

Component 71, which is part of the attentional networks and is focused at the temporo-parietal junction, comprises the intraparietal sulcus. This area is thought to be related with perceptual-motor coordination (for directing eye movements and reaching) as well as visual attention. The functional relevance of this sulcus for visual-motor tasks comprising target selections for arm and eye movements, object manipulation and visuo-spatial attention has been established through animal and human studies (Grefkes, Fink 2005). This same component appears to be consistently more correlated (for both baseline and follow-up) and working in a more homogeneous way when the subjects are under the resting state condition than when they are performing the task (**Table 6.6** and Appendices A **Section 6.2**). The

increased clusters in the baseline session (when compared with RS) are mainly focused on the parahippocampal gyrus, a region that plays a role in memory encoding and retrieval. The increased clusters in the follow-up session (when compared with RS) are mainly located in the right (36vox) and left (10vox) thalamus. This structure has the important role of relaying both motor and sensory signals to the cerebral cortex and also regulates consciousness, sleep and alertness. Therefore, increased correlation during resting state may be related with a mechanism working to maintain awareness during a passive period and also the retrieval of memories related with the execution of the task or maybe even just daydreaming. The comparison of the paradigm between the first and second scan shows a small increase (only 1 voxel) in this component during session 2. Once again, during the task performance it can be assumed that there would be a constant flux of information from sensory stimuli towards the cortex and therefore involvement of the thalamus.

Component 53, one of the most representative of the default-mode network, presents increases during resting state when compared with the paradigm for the Baseline session. A direct comparison between RS and LCP during the first scanning session reported a cluster of 16 voxels [21,21,18], one of 2 voxels [34,24,11] and 1 voxel [17,26,10] of increases in this component during RS. The same behaviour was not found to happen during the follow-up session. However, when looking at the results provided by the comparison of Baseline: Paradigm vs Follow-up: Paradigm it is found a small increase during the follow-up session for this same default-mode component. Therefore it can be hypothesised that when executing the task for the second time the participants no longer devoted a great amount of attention as when they first executed the paradigm.

Component 55, comprising the anterior cingulate and insular cortex has been reported to be active during demanding tasks and conflict processing (Ridderinkhof et al. 2004, Klein et al. 2007, Eichele et al. 2008). Nonetheless this component is never found during our analysis reflecting no significant differences between both conditions (Paradigm and Resting state). Therefore it can infered that the implementation of this low cognitive level paradigm did not cause activation of higher processing mechanisms.

Overall, the differences found between the correlation maps for the two different conditions within the same scanning session are significant in number as well as in size. These differences are hard to explain and to account for. Nonetheless, they also appear to be consistent on the first and the second scan.

ICC

The results from the ICC calculations are in good agreement with the ICC results usually reported in the literature. These seem to establish that resting state generally provides more reproducible data than the one obtained with the implementation of the low cognitive-demand paradigm. The differences between both conditions are not totally obvious since statistical testing proved that the differences on a network level are mostly not significant between the results obtained for both conditions. On the other hand, the use of the low-cognitive demand task seems to result in better reproducibility among visual networks.

ICA

The ICA results seem to agree with the findings provided by the use of correlation measures. Only two small clusters of significant differences have been identified, both in visual network components. These results lead to the conclusion that the visual network seem to be the one that is mostly affected by the execution of the paradigm.

Reliability

Assuming that if the results seen in the first session are not just due to random noise then they should be reproducible under the same conditions in a follow-up scan. However, if the statistical testing does not find any significant differences for the same condition between both sessions one cannot state that there are no differences, but only that the null hypothesis cannot be denied. Then one should pose the question of "why do we not see any differences?". It might be that the variance within the group is greater and so it masks the actual effect. In this case it is important to test the within-group (between subjects) variability. Thus, the Pearson and Spearman correlation of the unique correlation values at a subject level have been studied. Curiously, once again only component 39 and 48, both visual network components, reported significant differences. From out analysis it seems that both conditions (Resting state and Paradigm) are in fact quite similar, producing analogous results. One can also say that these results show a certain degree of reproducibility reporting the same differences in both baseline and follow-up. Moreover, our results seem to be in accordance with what has been previously reported by Patriat *et al.* (Patriat et al. 2013) when comparing three different resting conditions during three different time points. This study reported that

the differences in reliability and consistency between different resting conditions are relatively small in effect size but with results that were in fact significant.

Further investigation should be carried out to confidently establish these conclusions. It is required more in-depth knowledge about the underlying operation of resting state networks as well as their reliability in order to properly understand these functional mechanisms. Albeit a great number of research papers have been published studying the reliability and reproducibility of these networks no consistent methodology for resting state acquisition has been implemented yet. Nonetheless, our results seem to suggest that most of these networks are strongly reproducible even when using different study protocols. This finding is encouraging since it supports extrapolation of conclusions and combination of results arising from different studies.

Limitations

The recently published paper by Hutchison *et al.* (Hutchison et al. 2013) mentioned the relevance of having a long scanning session, ideally longer than the 10 minutes that have been performed for this study.

Another issue is related to the series time filtering. During our functional connectivity processing it has been used the conventional time filtering, applying only a low-pass Butterworth filter with f = 0.1Hz. However, a recent study published by Davey *et al.* (Davey et al. 2013) suggests that the use of conventional temporal filtering to exclude the high noise content in fMRI data may induce sample dependence. Moreover, the paper by Liang *et al.* (Liang et al. 2012) studied different correlation metrics and preprocessing factors and their effect on functional networks, revealing that the brain networks derived in the 0.027-0.072 Hz band exhibited greater reliability than those in the 0.01 – 0.027 Hz band.

Another possible limitation is regarding the white matter and CSF time series masking and regression. The white matter and CSF masks used to extract the average BOLD signal from these regions are most commonly thresholded between 0.8 and 0.9, unlike the thresholding of 0.3 that has been performed. This might have resulted in the averaging and posterior exclusion of signal that was not actually part of these brain regions.

At last, nowadays the tools commonly applied to perform functional connectivity analysis are based on the assumption that there is statistical interdependence of signals between distinct brain regions. However some literature has proposed that it might not be true and that quantifying changes in functional connectivity metrics over time may provide greater insight into fundamental properties of brain networks (Hutchison et al. 2013).

On a last note it would have also been interesting to study the relationship between the connectivity strength of our network connections and their reliability as some previous studies have done (Patriat et al. 2013).

Future Directions

Current techniques to perform functional connectivity analysis implicitly assume that the relationships are constant throughout the scanning period. Dynamic functional connectivity seems to be the future of functional connectivity analysis. Recent studies are now uncovering the variability within functional connectivity measures, unveiling flexible connections between regions that are frequently treated as separate and antagonistic (Allen et al. 2011). Future studies should focus on the investigation of the temporal trends in functional connectivity which may provide an even more accurate mechanism to differentiate between healthy and disease.

On the other hand, considerations about what is too much movement are still not clear. While some studies use high values of movement, such as 2mm, as a threshold, other report that even fine movements can somehow modify the resulting networks from connectivity analysis. While most of our participants remained still throughout most of the scanning session with an average of 0.5 mm of translational movement and 0.3 degrees of rotation, some spikes of movement were identified, some of them reaching almost 1mm of translation and 1degree of rotation. It has also been verified that is during the resting state condition that the participants most move. Studies have demonstrated that subject motion produces substantial changes in the timecourse of resting state fcMRI (Power et al. 2012). Therefore it is hereby suggested the development of a correction mechanism to extract the spikes of the data in order to avoid the misidentification of what in reality are just spurious correlations.

6.5 Conclusion

The aim of this study was to longitudinally evaluate the reliability and reproducibility of functional networks during a true resting state and while executing a low cognitive demand paradigm. Our initial assumption was that the use of a low cognitive demanding paradigm would provide increase stability and reliability when compared with true resting state. Our analysis seems to reflect that both conditions are in fact quite similar, producing identical results. The ICC analysis seems to suggest that resting state provides in general more reproducible data than when using the low cognitive demand task. However, the use of this task did improve the reproducibility among visual network components.

Moreover, these results show a good degree of network reproducibility during a longitudinal approach.

In summary these findings are very encouraging, supporting the comparison and combination of results arising from studies performed under different study protocols.

Chapter 7

Closing Remarks

Functional connectivity studies are broadening our knowledge about the anatomo-functional organization of the human brain. The last decades saw major developments towards the concept of brain plasticity with innumerous publications regarding this subject. This idea challenges the previously established notion that each brain area was hyper-specialised and only responsible for processing specific cognitive functions. Research is nowadays walking towards a more interactive and dynamic concept of networks (Bressler, Menon 2010) introducing the notion that plasticity is the fundamental property characterizing brain function (Pascual-Leone, Grafman & Hallett 1995) and that the functional behaviour of the human brain can be essentially changed through the use and development of this capacity.

Current research, namely using resting state fMRI, has been providing us with clues on brain dynamical properties as well as regarding the intrinsic mechanisms of reshaping functional networks as one experiences extreme conditions such as trauma, lesion, diseases and even just regular exercise (Lang et al. 2012, Voelcker-Rehage, Niemann 2013). Therefore, the application of functional connectivity techniques can shed some light over this intriguing subject and how this brain capacity can also be implemented as, for e.g. a therapy.

The last decade has seen the rise of new and powerful imaging sequences and analysis techniques as the computing power has also increased exponentially. As it has been proposed by van Dijk *et al*. (Van Dijk et al. 2010), we strongly believe in the benefits of combinating fcMRI with HARDI techniques to study human connectomics. Future studies should work towards the combination of these different techniques and methodologies in order to achieve further knowledge about brain structure and its still enigmatic functional mechanisms.

ACHIEVEMENTS

During the course of this internship the author had the chance to be integrated within a multidisciplinary research team as well as to deal with NHS professionals, with patients as well as with a wide range of materials and techniques. The author was involved in five different studies during the course of this internship, ranging from exercise to disorders of consciousness, which reflects the wide range of research projects currently being developed at ABIC. The immense growth experienced in the past year was both professional and personal.

During this period the author has not only received medical training that makes her now qualified to perform cognitive assessments on both healthy volunteers and patients, but she has also underwent a Good Clinical Practice (GCP) course, important to any researcher who is in the process of creating his own study. Moreover, the author still had the chance to be involved in every step of the creation and development of a new study, managing all the study data in a site file, dealing with ethics and with both patients and healthy population. During this process the author was also taught on how to prepare the MRI scanner for our specific study as well as to deal with the Presentation software. On a regular basis the author would volunteer to test new MRI sequences and study protocols specially designed for the different projects currently being carried out at the ABIC. These involve heart, musculoskeletal and brain research. It was also during this internship that the author had the chance to audit an MRI deeper course for the master in Biomedical sciences, which proved to be extremely fruitful. Moreover, the author had the chance to deal with researchers developing new MRI techniques, such as Fast field-cycling MRI.

The SINAPSE program – Scottish Imaging Network: A platform for scientific excellence – is a platform that aims to connect researchers carrying out medical imaging studies all over Scottish territory. The author presented a poster on the SINAPSE Student Day at Dundee University, in April 2013.

On the 12th of June 2013, as part of the Postgraduate Radiology Symposium, the author gave a presentation to an audience of radiology students and NHS professionals about the current research and methods being applied in the TIA cfMRI stroke study. The author was also involved in a lesson provided to 4th year medical students about MRI. On the 10th of September, and as part of the S6 Biology day at the University of Aberdeen, the author

worked as a demonstrator for secondary advanced higher biology students. Recently the author has also became a postgraduate student ambassador for the University of Aberdeen.

During this time the author underwent training and became a STEM (Science, Technology, Engineering and Mathematics) ambassador, carrying out several public engagement and scientific communication activities. These include teaching and sparkling the interest of children, young students and higher levels as well as the general public about science, MRI and the research carried out at the Aberdeen Biomedical Centre. These events include the use of hands-on experiments and material that was developed on-site (e.g. RF coils, 3D anatomic models, etc). The author visited a school with the activity "Lab-in-a-Lorry" including hands-on physical experiments about light, resonance, among others. The author co-organised and took part in events during the Aberdeen May Festival 2013 (http://www.abdn.ac.uk/mayfestival/) with an activity that took place at the Satrosphere Science Centre a part of the physics project "Explore your Universe: Familly fun day", entitled "Meet the Researcher: Magnetise your Brain". More recently, and after a science basking event at the local Waterstone's book shop, the author took part in Techfest - Festival of Science, Technology, Engineering and Mathematics (http://www.techfestsetpoint.org.uk/tis/). During this festival the author has co-organised and took part in the Doors open Day, event during which the general public gets to know about the research projects currently being developed at the University and to do an interactive tour of the facilities used to carry out scientific research. Another edition of the event "Magnetise your Brain" happened on the 19th of September 2013 at Satrosphere Science Centre, as part of the Techfest.

In brief, this internship has given the author the chance to get to know new imaging analysis methods and equipment as well as to closely relate the scientific research to the clinical environment.

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APPENDICES A

- **Section 3.1**
  DoC Literature Review

Study	Number	Diagnosis	Ethiology	Duration	Active Task	Main Findings
1. Assessing Consciousness **Owen *et al.* (2006)**	1	VS	TBI	5m	Motor and spatial mental imagery	Activation of the supplementary motor area for motor task. Activation of the parahippocampal gyrus, posterior parietal and premotor cortex for spatial task
Monti *et al.*, (2010a)	54	23 VS 31 MCS	32 TBI 22 NTBI	22m	Motor and spatial mental imagery	Activation of supplementary motor area for motor task in 4VS and 1 MCS. Activation of the parahippocampal gyrus for spatial task in 3 VS and 1 MCS
Rodriguez Moreno *et al.* (2010)	10/17 included	3 VS 5 MCS 1 EMCS	5 TBI 45 NTBI	20m	Silently picture naming	Activation of the left superior temporal, inferior frontal and pre-supplementary motor are in 1 VS, 2MCS, 1LIS, 1 EMCS
Bekinschtein *et al.* (2011)	5/43 included	VS	4 TBI 1 TBI-anoxic	10d	Motor task	Activation of the contralateral dorsal premotor cortex in 2 VS
Bardin *et al.* (2011)	6/7 included	5 MCS 1 LIS	4 TBI 2NTBI	6m – 3y	Motor mental imagery	Activation of the supplementary motor area in 2 MCS and 1 LIS
2. Assessing communication **Monti *et al.* (2010a)**	1/54 included	VS	TBI	1m – 25y	Yes (motor mental imagery) or No (spatial mental imagery) autobiographical questions	Correct responses in expected brain regions in 5 out of 6 questions
Bardin *et al.* (2011)	4/7 included	3 MCS 1 LIS	2 TBI 2 NTBI	6m – 3y	Binary and multiple choices tasks	Incorrect responses in expected brain regions in 1 MCS

VS: vegetative state; MCS: minimally conscious state; EMCS: emerged from minimally conscious state; LIS: locked-in-syndrome; d: days: m: months; y: years; TBI: traumatic brain injury; NTBI: non-traumatic brain injury;

- # Section 4.1

TIA cfMRI stroke population demographics

Sex					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	46	53.5	53.5	53.5
	Female	40	46.5	46.5	100.0
	Total	86	100.0	100.0	

Motor symptoms					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	41	47.7	47.7	47.7
	No	45	52.3	52.3	100.0
	Total	86	100.0	100.0	

Sensory symptoms					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	55	64.0	64.0	64.0
	No	31	36.0	36.0	100.0
	Total	86	100.0	100.0	

Speech-related symptoms					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	30	34.9	34.9	34.9
	No	56	65.1	65.1	100.0
	Total	86	100.0	100.0	

Visual symptoms					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	18	20.9	20.9	20.9
	No	68	79.1	79.1	100.0
	Total	86	100.0	100.0	

Headache					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	29	33.7	33.7	33.7
	No	57	66.3	66.3	100.0
	Total	86	100.0	100.0	

Other symptoms					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	13	15.1	15.1	15.1
	No	73	84.9	84.9	100.0
	Total	86	100.0	100.0	

Affected side					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Right	32	37.2	37.2	37.2
	Left	43	50.0	50.0	87.2
	Both	1	1.2	1.2	88.4
	Neither	10	11.6	11.6	100.0
	Total	86	100.0	100.0	

- ## Section 4.2
  Information regarding the minor stroke patients

	Age	Gender	Presenting complain	WMH	Scheltens score
atient 1	30	Female	Motor / Sensory	Yes	22
Patient 2	31	Male	Motor / Sensory / Headache	Yes	26
Patient 3	25	Male	Motor / Speech	Yes	21
Patient 4	82	Male	Sensory	Yes	43
Patient 5	65	Male	Speech	Yes	59
Patient 6	45	Female	Motor / Sensory	Yes	34

- ## Section 4.3
  Information regarding the migraine patients

	Sex	Age	Symptoms	Scheltens score
Patient 1	male	50	Motor, Sensory, Speech, Headache	29
Patient 2	female	21	Motor, Headache	23
Patient 3	male	46	Motor, Sensory, Visual	31
Patient 4	female	31	Motor, Speech, Headache	15
Patient 5	male	33	Sensory, Speech, Headache	26
Patient 6	female	55	Visual	25
Patient 7	male	31	Motor, Speech, Headache	21
Patient 8	male	40	Motor, Sensory, Visual, Headache	29
Patient 9	female	51	Sensory, Speech, Headache	27
Patient 10	male	50	Sensory, Speech, Visual, Headach	28
Patient 11	female	25	Motor, Headache	18
Patient 12	female	37	Motor, Speech	22
Patient 13	male	47	Sensory, Visual, Headache	28

- **Section 4.4**
  Information regarding the healthy volunteers

ID	Sex	Age
Control 1	female	32
Control 2	female	21
Control 3	male	31
Control 4	male	31
Control 5	male	29
Control 6	male	45
Control 7	female	39
Control 8	female	37
Control 9	male	38
Control 10	male	32
Control 11	female	29
Control 12	female	21
Control 13	male	24
Control 14	female	24

- **Section 5.1**
  Demographics from the PECON study participants

ID	Sex	Age	Sports practiced	MoCA	HR	sBP	dBP	Height	Weight	BMI	HRmax	VO2max
1	male	26	Football, Running	30	86	110	70	171	80	27	192	37
2	male	25	Running, Cycling, Football	30	82	122	80	180	73	23	189	43
3	male	26	Running	30	75	125	80	177	73	23	180	37
4	male	33	Running, Cycling	27	68	110	70	186	71	21	186	51
5	male	33	Running, Cycling	27	82	122	70	181	74	23	172	40
6	male	27	Running	30	60	110	70	188	67	19	179	38
7	male	22	Running	29	84	118	70	185	74	22	184	45
8	male	25	Running	28	84	110	70	170	67	23	182	42
9	male	39	Running, Yoga	29	71	118	78	181	83	25	175	33
10	male	34	Cycling	30	81	118	80	184	80	24	187	50
11	male	29	Running	30	64	122	80	170	67	23	169	38
12	male	19	Running	30	86	122	80	178	75	24	192	38
13	male	26	Running	30	77	118	70	187	73	21	167	38

sBP - sistolic blood pressure (mmHg); dBP - Diastolic blood pressure (mmHg); BMI - Body mass index (kg/m2);
Hrmax - Maximum heart rate (beats/min); VO2max - Maximum oxygen uptake (ml/kg/min)
NOTE: Participant in red pulled out of the study due to injury

- # Section 6.1

RESTATE Statistics

Statistics					
		Age (years)	Sex	Dominant hand	Occupation
N	Valid	12	12	12	12
	Missing	0	0	0	0
Mean		28.83	1.58	1.00	2.00
Std. Error of Mean		1.353	.149	.000	.000
Median		28.00	2.00	1.00	2.00
Std. Deviation		4.687	.515	.000	.000
Variance		21.970	.265	.000	.000
Minimum		23	1	1	2
Maximum		37	2	1	2
Percentiles	25	24.50	1.00	1.00	2.00
	50	28.00	2.00	1.00	2.00
	75	33.50	2.00	1.00	2.00

Sex					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	5	41.7	41.7	41.7
	Female	7	58.3	58.3	100.0
	Total	12	100.0	100.0	

Age (years)					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	23	1	8.3	8.3	8.3
	24	2	16.7	16.7	25.0
	26	2	16.7	16.7	41.7
	27	1	8.3	8.3	50.0
	29	2	16.7	16.7	66.7
	32	1	8.3	8.3	75.0
	34	1	8.3	8.3	83.3
	35	1	8.3	8.3	91.7
	37	1	8.3	8.3	100.0
	Total	12	100.0	100.0	

Days from baseline to follow-up MRI scan					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	27	2	16.7	16.7	16.7
	28	6	50.0	50.0	66.7
	31	1	8.3	8.3	75.0
	35	3	25.0	25.0	100.0
	Total	12	100.0	100.0	

Number of times button was pressed for buildings (scan 1)					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	96	1	8.3	8.3	8.3
	98	2	16.7	16.7	25.0
	99	1	8.3	8.3	33.3
	100	8	66.7	66.7	100.0
	Total	12	100.0	100.0	

Number of times button was pressed for buildings (scan 2)					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	44	1	8.3	8.3	8.3
	99	3	25.0	25.0	33.3
	100	8	66.7	66.7	100.0
	Total	12	100.0	100.0	

- **Section 6.2**

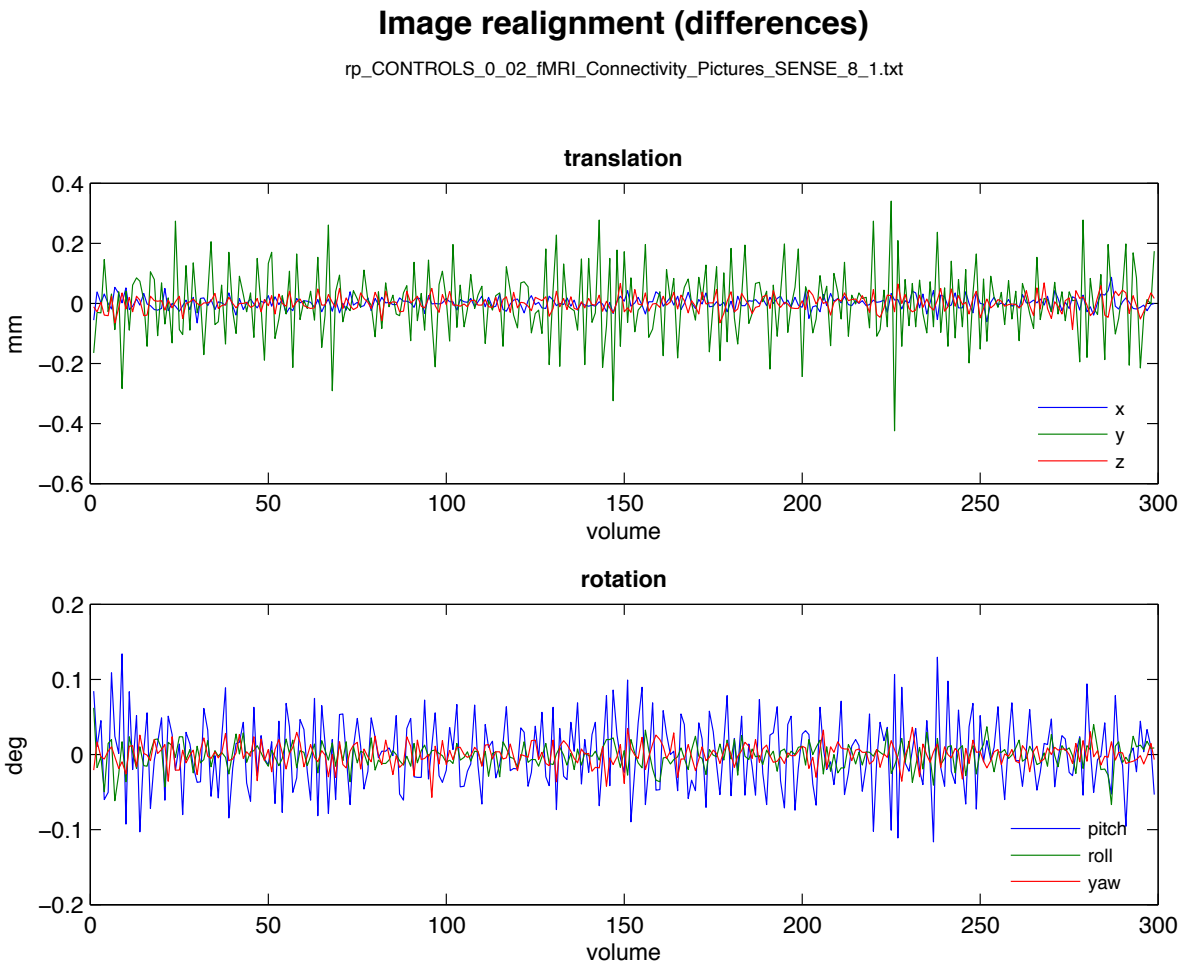
Differences resulting from a) Paired T-test of the same condition in the two scanning sessions (e.g. Baseline: Resting state vs Follow-up: Resting State); b) Paired T-test of the two different conditions in the same scanning session (e.g. Baseline: Resting State vs Paradigm).

	Baseline comparison			
Voxelwise analysis	component	vox	coordinates	Network
Increases during Resting state	21	34	[15,45,15]	Basal ganglia
	46	178	[27,10,24]	Visual
		1	[19,14,19]	
	48	12	[31,16,14]	Visual
		1	[19,11,27]	
		1	[32,11,26]	
		1	[29,9,26]	
		1	[30,9,24]	
	52	15	[38,21,38]	Attentional
		3	[40,23,36]	
		2	[34,19,39]	
	53	16	[21,21,18]	Default-mode
		2	[34,24,11]	
		1	[17,26,10]	
	59	142	[27,7,21]	Visual
	64	59	[27,7,20]	Visual
	67	45	[21,15,21]	Visual
		26	[30,14,19]	
	71	7	[8,35,11]	Attentional
		2	[42,12,19]	
		1	[7,37,10]	
		1	[9,40,7]	
Increases during the Paradigm	23	1	[17,17,8]	Sensorimotor
	24	1	[24,30,19]	Sensorimotor
	34	2	[40,52,11]	Attentional
	38	7	[46,22,25]	Sensorimotor
		2	[42,21,26]	
	39	15	[11,11,24]	Visual
		9	[16,25,10]	
		7	[17,20,11]	
		7	[37,22,11]	
		6	[17,14,13]	
	47	1	[25,52,29]	Frontal
		1	[26,50,28]	
	48	3	[40,19,13]	Visual
	52	28	[38,10,25]	Attentional
	59	56	[12,12,56]	Visual
		35	[38,9,25]	
	67	3	[12,17,14]	Visual
		2	[39,22,11]	
	71	2	[7,24,30]	Attentional

	Follow-up comparison			
Voxelwise analysis	component	vox	coordinates	Network
Increases during Resting state	21	1	[16,45,15]	Basal ganglia
		1	[24,34,12]	
		1	[27,33,12]	
	39	40	[9,18,17]	Visual
		3	[45,15,18]	
		1	[13,23,36]	
	42	22	[7,37,12]	Frontal
		1	[15,37,11]	
	46	214	[27,14,21]	Visual
		1	[19,14,19]	
	48	26	[31,16,14]	Visual
		12	[32,11,26]	
		6	[19,11,27]	
		2	[20,16,15]	
		1	[30,9,24]	
	59	264	[26,7,20]	Visual
	64	752	[24,11,15]	Visual
		18	[35,19,15]	
		3	[34,15,19]	
		2	[19,14,19]	
		1	[17,17,19]	
		1	[34,17,18]	
	67	195	[30,15,22]	Visual
	71	43	[9,12,18]	Attentional
		15	[43,11,20]	
		3	[12,24,20]	
		3	[7,35,12]	
		1	[13,17,22]	
Increases during the Paradigm	34	1	[27,27,32]	Attentional
	38	9	[6,20,29]	Sensorimotor
		1	[9,22,28]	
	39	3	[16,24,11]	Visual
		2	[37,17,11]	
	46	14	[30,12,7]	Visual
		3	[24,11,9]	
		1	[26,12,9]	
	59	43	[12,11,28]	Visual
		13	[41,11,25]	
		2	[38,10,29]	
		1	[38,9,26]	
	67	13	[29,12,7]	Visual
		7	[24,11,8]	
		7	[17,15,7]	

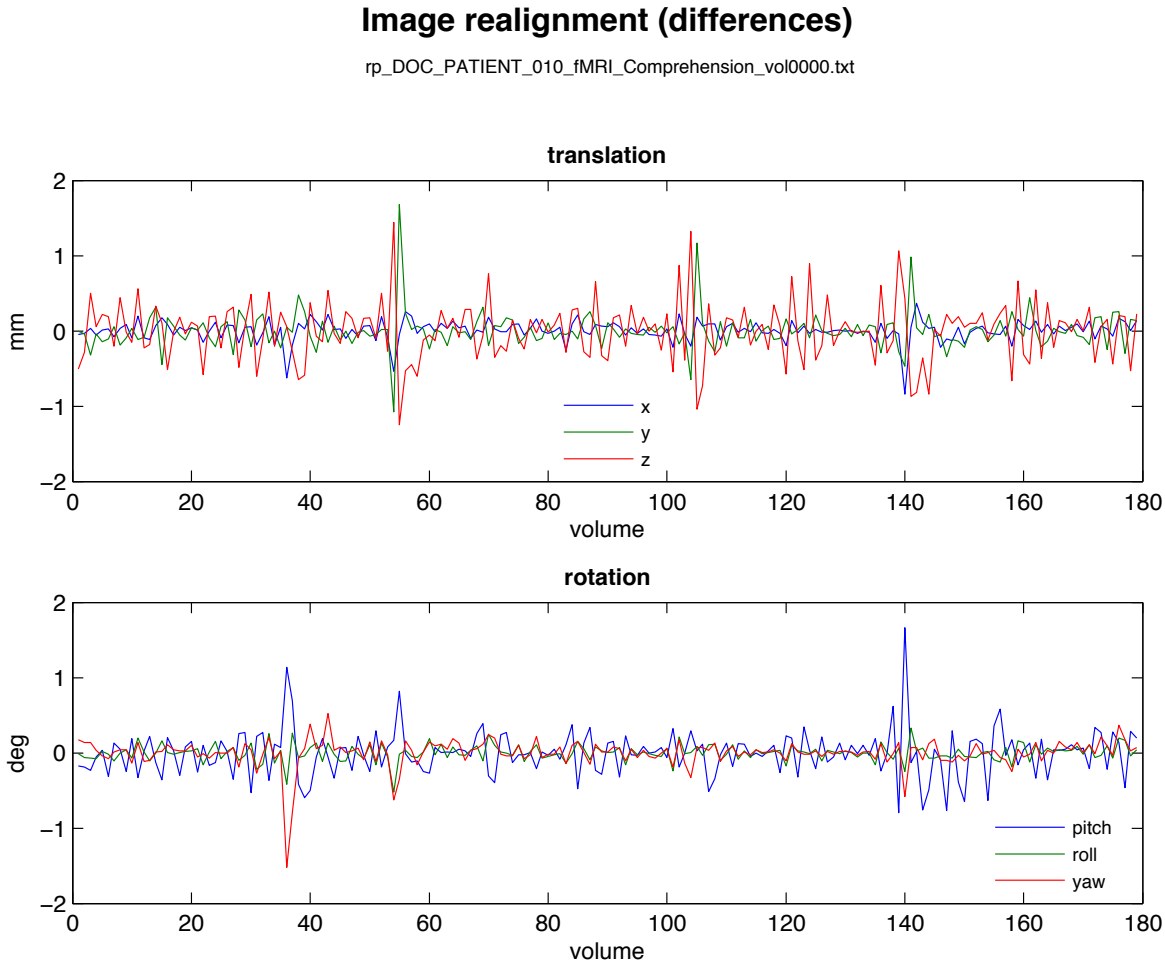
- # Section 6.3

Movement parameters:
RESTATE volunteer



- **Section 6.3 (cont)**

Movement parameters:
DoC patient



• Section 7

Contributions

List of people who contributed to the projects described along this dissertation. All contributions resulted from people currently working at the Aberdeen Biomedical Imaging Centre with the following positions:

Professor Christian Schwarzbauer, *Chair in Neuroimaging*
Dr Mary Joan Macleod, *Senior Lecturer*
Mr Gordon Buchan, *Research Technician*
Mrs Susa Merz, *PhD Student*
Dr Ourania Varsou, *PhD Student*
Mr Michael Stringer, *PhD Student*
Mr Joel Parkinson, *PhD Student*
Mr Alex Ing, *PhD student*
Miss Catarina Dinis Fernandes, *Research Assistant*

. **Chapter 3 – DoC study**

Mrs Susa Merz, under the supervision of Professor Christian Schwarzbauer, was responsible for data acquisition, preprocessing and modelling of the fMRI data as well as development of the "Heat map" method. She was also involved in the design and optimisation of the paradigms. Dr. Ourania Varsou was involved with patient recruitment.

. **Chapter 4 – TIA cfMRI study**

Dr Ourania Varsou was involved in study design along with Dr Mary Joan Macleod and Professor Christian Schwarzbauer. Dr Varsou performed patient recruitment, assessment and data acquisition. She was also responsible white matter hyperintensities scoring. Mr Gordon Buchan provided assistance during scanning.

. Chapter 5 – PECON study

Dr Ourania Varsou, under the supervision of Professor Christian Schwarzbauer, designed the study and was responsible for the recruitment and data acquisition. Mr Gordon Buchan provided assistance during scanning. Mr Alex Ing used Network Based Statistic methods in the

analysis of the data, Mr Michael Stringer analysed the data using and ICA methodology and Mr Joel Parkinson with the use of Graph analysis.

. **Chapter 6 – RESTATE study**

Dr Ourania Varsou along with Miss Catarina Fernandes, under the supervision of Professor Christian Schwarzbauer, were responsible for the study design, advertisement and recruitment. Both have also performed data acquisition with the aid of Mr Gordon Buchan during the step up the scanner. Data analysis was performed by Miss Catarina Fernandes along with Mr Michael Stringer who was responsible for ICA analysis.

APPENDICES B

1) RESTATE Study MRI protocol
2) Montreal Cognitive Assessment
3) White matter hyperintensities scoring protocol

1) RESTATE study MRI protocol

Principal Investigator 1: Prof. Christian Schwarzbauer
Contact Details: Ext – 8361
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Principal Investigator 2: Dr Mary-Joan Macleod – Ward 39 (Acute Stroke Unit)
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PROJECT CONTACTS
Students: Ourania Varsou – o.varsou@abdn.ac.uk – bleep 4203
Michael Stringer – r01mss12@abdn.ac.uk
Catarina Fernades – c.dinisfernandes.50@aberdeen.ac.uk

PREPARATION

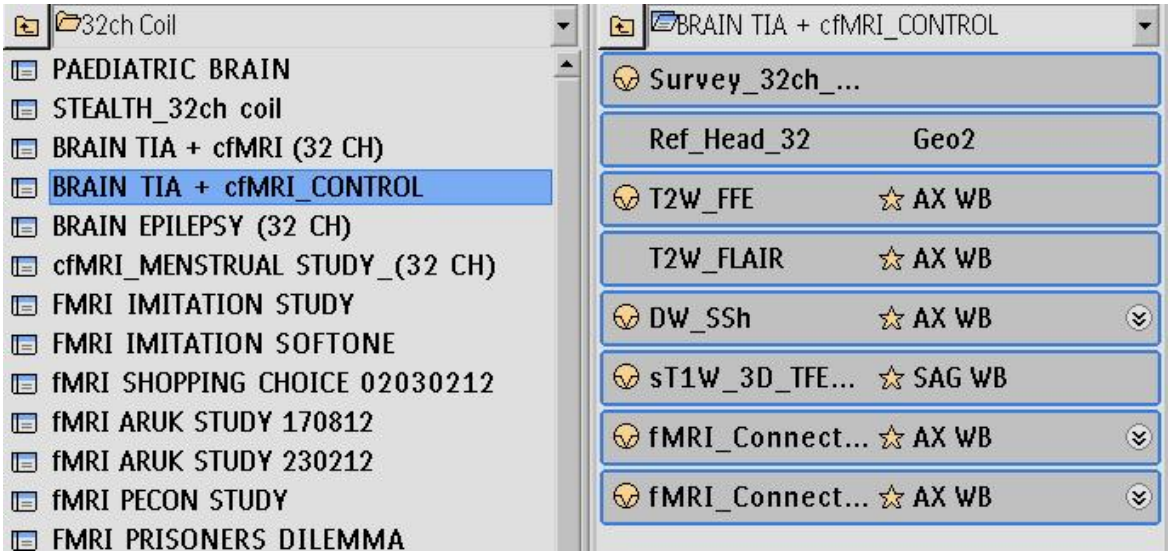
The scanning session lasts **45 minutes**

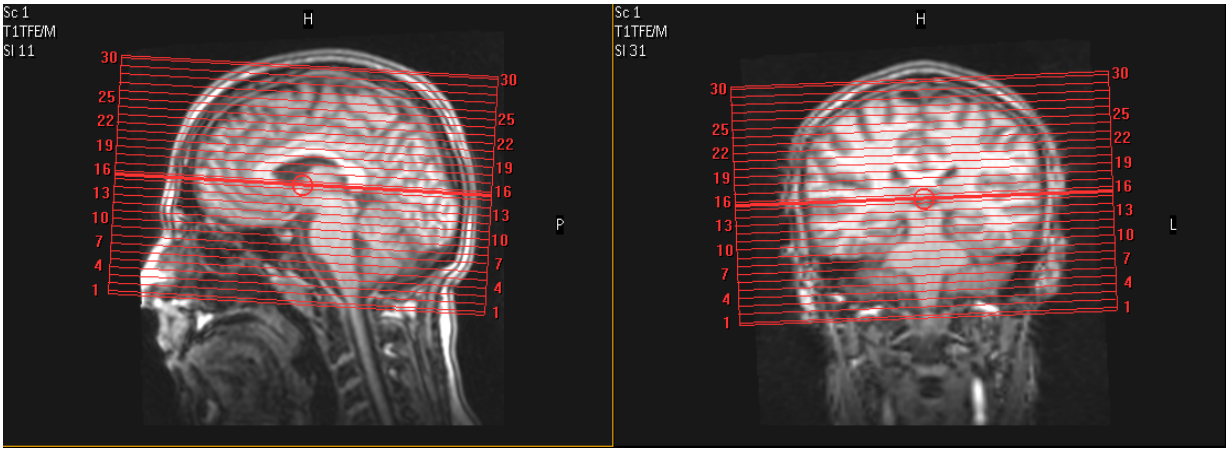
- Set up the backward facing coil mirror
- Give the right hand index finger button to the participant in their dominant hand
- Both connectivity fMRI sequences are done in a resting state with the radio switched off
- One of the connectivity fMRI sequences has a low level cognitive paradigm using alternating pictures of buildings and landscapes (paradigm saved in the **RESTATE** folder of the presentation library in the shared network that is accessible in the stimulus delivery PC; pathway: X:\Imaging\Projects\Presentation_Library\RESTATE)
- The participants have to press the right index finger button only when they see a picture of a building

SCAN PROTOCOL

32ch head coil

- Protocol – Abdn – 32ch Coil – Brain TIA + cfMRI_CONTROL (Research)





ADMINISTRATION

- Ensure "Auto-push" is disabled
- Archive all the data **except fMRI** – DVD (All & NHS)
  EWS1 workstation.
- All data sent to the "X" Drive
- Patient details added to the Daily Log and the appropriate study database
- All study data logged in the work book
- Request form filed in the study box folder

All data transferred into "X" drive must be under ID no.

- System
- Advanced Tools
  - Research
  - dbimexp (database image export tool))
- Select patient
- Highlight all sequences except DWI & survey
  - **T2W_FFE**
  - **T1W_3D**
  - **T2W_FLAIR**
  - **fMRI_Connectivity_Resting_State**
  - **fMRI_Connectivity_Pictures**
- Browse
- My computer
- Abic projects
  - TIA_cfMRI folder
  - TIA controls_RESTATE subfolder
  - Create new folder e.g. Controls_0_01
- Click OK
- Check NIFTI has been selected
- Proceed
- Windows explorer – check data has been exported

TO TRANSFER DWI DATA:

- Patient
- Administration
- Select patient – select DWI data
- Disk files
- Browse
- Temp file – create folder
  OK – proceed

Once data has been transferred, please copy and paste folder from the temp drive into the "X" drive and the corresponding patient folder.

2) Montreal Cognitive Assessment

NHS GRAMPIAN & UNIVERSITY OF ABERDEEN

fcMRI Stroke Study

Please complete sections **1** to **5** by marking the appropriate box with an **X** or by writing the answer in the space provided

Your answers will be treated in the strictest confidence

Thank you for your help with this study
This is very much appreciated

Volunteer version 3: November 2012

A member of the research team to complete sections 6 to 8

fcMRI Stroke Study

Volunteer's trial number:

Section 1: Volunteer Information					
1a	Sex:	Male☐	Female☐		
1b	Age *(years)*:				
1c	Dominant hand:	Right☐	Left☐		
1d	Occupation:	Retired☐	Employed☐	Unemployed☐	Registered disabled☐

Section 2: Medical Conditions			
2a	High blood pressure:	Yes☐	No☐
2b	High cholesterol:	Yes☐	No☐
2c	Diabetes:	Yes☐	No☐
2d	Ischaemic heart disease:	Yes☐	No☐
2e	Previous TIA / stroke:	Yes☐	No☐
2f	Depression:	Yes☐	No☐
2g	Migraines:	Yes☐	No☐
2h	Please list any other medical conditions:		

Section 3: Current Regular Medications			
3a	High blood pressure treatment:	Yes☐	No☐
3b	High cholesterol treatment:	Yes☐	No☐
3c	High blood sugar treatment:	Yes☐	No☐
3d	Blood thinning treatment:	Yes☐	No☐
3e	Antidepressant(s):	Yes☐	No☐
3f	Please list any other regular medications:		

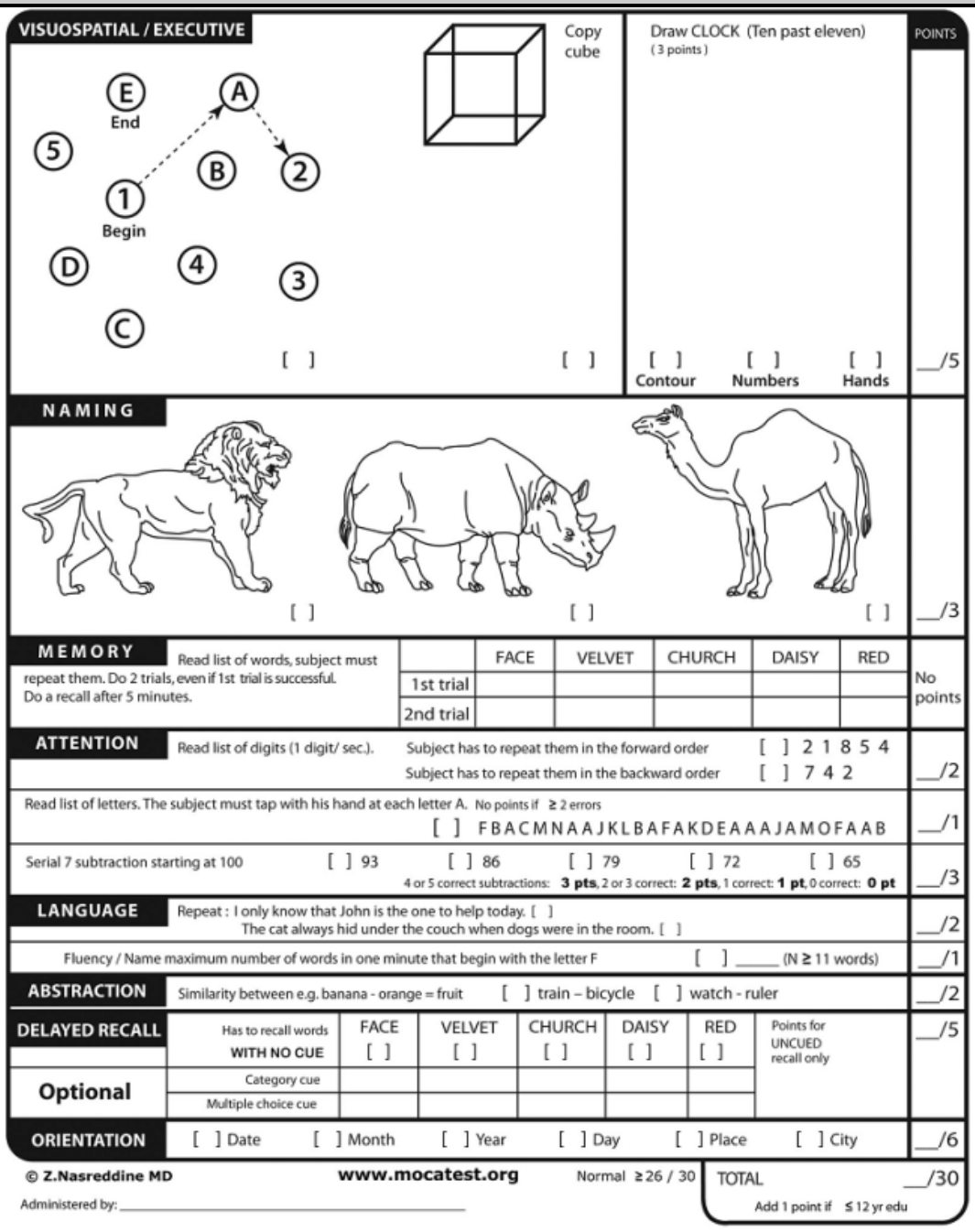
Section 4: Registered GP Practice	
4a	Current GP Practice:
4b	Address:

Section 5: Declaration
I confirm that I have read and understand this form. I have answered all questions to the best of my knowledge.
Volunteer's name:
Volunteer's signature:
Date:

Section 6: General Information

6a	Volunteer's trial number:
6b	Date form completed *(dd/mm/yyyy)*:
6c	Person completing form *(please print name & sign)*:

Section 7: Montreal Cognitive Assessment (MOCA) Version 7.1



Section 8: MRI Scan			
8a	Scan date *(dd/mm/yyyy)*:		
8b	Presence of white matter hyperintensities:	Yes☐	No☐
8c	Presence of ischaemic lesion(s):	Yes☐	No☐
8d	Clarification of ischaemic lesion(s):		
8e	Presence of non-stroke lesion(s):	Yes☐	No☐
8f	Clarification of non-stroke lesion(s):		

3) White matter hyperintensities scoring protocol

Scheltens Scoring Scale

Participant's trial number	
Date of scan	
Name of scorer	
Date of scoring	

White matter hyperintensities	Score (0-6)
Frontal	
Parietal	
Temporal	
Occipital	
Internal capsule	

0=normal **1**=<3mm, n<5
2=<3mm, n>6 **3**=4-10mm, n<5
4=4-10mm, n>6 **5**=>11mm, n>1
6=confluent

Total _____ (max 30)

Grey matter hyperintensities	Score (0-6)
Caudate nucleus	
Putamen	
Globus pallidus	
Thalamus	
Hippocampus	

Score as above

Total _____ (max 30)

Infra-tentorial foci of hyperintensity	Score (0-6)
Cerebellum	
Midbrain	
Pons	
Medulla	

Score as above

Total _____ (max 24)

Periventricular white matter lesions	Score (0-3)
None (normal)	
Frontal horns	
Bodies	
Occipital horns	

0=None
1=Pencil thin lining
2=Smooth halo
3=Large confluent

Total _____ (max 9)

Total score	**.....(max 93)**

Additional findings