UNIVERSITA' DEGLI STUDI DEL MOLISE

DIPARTIMENTO DI MEDICINA E SCIENZE DELLA SALUTE "VINCENZO TIBERIO"

"*RESTING STATE FMRI OF THE CEREBELLAR LOBES IN PATIENTS WITH MULTIPLE SCLEROSIS: A SEED-BASED ANALYSIS*"

DOTTORATO DI RICERCA IN MEDICINA TRASLAZIONALE E CLINICA

XXXII CICLO

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2018/2019

"Non possiamo fare grandi cose su questa Terra,

solo piccole cose con grande amore"

Madre Teresa di Calcutta

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Abstract - English

Introduction: Anatomical, clinical and imaging findings suggest that the anterior and posterior cerebellar lobes are engaged in motor control and in cognitive functions, respectively.

The scope of this thesis: The material presented in this thesis provides new insights concerning the role of the cerebellum and its functional alterations in multiple sclerosis, with the application of functional MRI.

The aim of our work is to focus on the different contributions of the two lobes of the cerebellum at rest, in order to better understand their different involvement in motor and cognitive functions.

In the current study, we used resting-state functional magnetic resonance imaging (rs-fMRI) to study subjects with Multiple Sclerosis (MS) while they are at rest.

Resting state functional connectivity (rsFC) of the sensori-motor and the cognitive lobes of the cerebellum and their correlation with clinical variables were investigated.

Methods: We enrolled 119 patients (28 males, aged 38.9±10.1 years, mean±SD), including 91 relapsing remitting and 28 secondary progressive MS, and 42 age- and gender-matched healthy subjects (HS, 13 males, aged 35.6±11.3 years). Subjects underwent a 3T MRI, including T13D, T2w and resting state functional MRI. Patients were evaluated by the Expanded Disease Status Score (EDSS) and MS functional composite scale. We used EDSS, 9-Hole Peg Test (9-HPT) and 25 Timed Foot Walking Test (25-TFWT) as measures of motor impairment and Paced Auditory Serial Addition Test (PASAT) as measure of cognitive functions. In each subject, cerebellum was parcelled into smCb (lobules I–V and VIII) and cCb (lobules VI–VII, IX-X) via the Spatially Unbiased Infratentorial Toolbox (SPM). Data were analyzed via FSL. Voxel-wise rsFC was calculated using smCb and cCb as seeds. Correlations and group differences

were non-parametrically computed (Randomize, FDR corrected at $p<.05$). Age, lesion load, grey and white matter volumes were included as covariates of no interest.

Results: Compared to HS, in MS patients rsFC between smCb and precentral gyrus, operculum and basal ganglia was decreased, while rsFC between smCb and superior parietal and prefrontal cortices, cuneus and thalamus was increased. As well, rsFC between cCb and prefrontal gyrus, lateral temporal cortex, precuneus, insula and vermis was decreased, whereas rsFC between cCb and pre- and post-central gyri, occipital and mesial temporal cortices, precuneus and thalamus was increased. Both EDSS and 9-HPT negatively correlated with rsFC of smCb with precuneus, lateral parietal cortex and post-central gyrus; 25-TFWT negatively correlated wth rsFC between smCb and the right post-central gyrus and left precuneus. PASAT positively correlated with rsFC of cCb with superior and middle frontal gyri, superior parietal and lateral occipital cortices, caudate nucleus and cerebellum.

Conclusions: Cerebellar rsFC with supratentorial brain areas is altered in MS. The correlations between cerebellar rsFC and clinical scales indicate that the higher the rsFC the lower the clinical disability, in terms of both motor impairment and cognitive decline, suggesting a compensatory role of the increased cerebellar rsFC.

Abstract - Italiano

Introduzione: Il cervelletto è noto per essere coinvolto sia nelle funzioni motorie che in quelle cognitive del cervello, più in particolare esso presenta una suddivisione interna in parte anteriore e parte posteriore, che si differenziano per il loro diverso coinvolgimento nelle funzioni sensorimotorie e in quelle prettamente cognitive, rispettivamente.

Scopo: Studiare la connettività funzionale in condizioni di resting state, ovvero di veglia rilassata, dei lobuli cerebellari coinvolti nelle funzioni sensorimotorie e di quelli coinvolti nelle

funzioni cognitive, correlando con i dati clinici e con il danno strutturale, in pazienti con Sclerosi Multipla (SM).

Metodi: Sono stati inclusi nello studio 119 pazienti con MS (28 maschi, età 38.9±10.1 anni, media±SD), di cui 91 Relapsing Remitting e 28 Secondary Progressive, e 42 soggetti di controllo, della stessa età e sesso (13 maschi, età 35.6±11.3 anni).

I soggetti sono stati sottoposti a Risonanza Magnetica 3T, includendo sequenze come T13D, T2 ed EPI. Gli stessi sono stati esaminati con una batteria di test clinici, quali Expanded Disease Status Score (EDSS) e la scala funzionale della SM. I test EDSS, 9-Hole Peg Test (9-HPT) e 25 Timed Foot Walking Test (25-TFWT) sono stati usati come misura di disabilità motoria, mentre il Paced Auditory Serial Addition Test (PASAT) come misura di disfunzione cognitiva. Il cervelletto di ciascun soggetto è stato parcellizzato in una parte sensorimotoria (smCb), dato dai lobuli I–V and VIII, e in una parte cognitiva (cCb) data dai lobuli VI–VII, IX-X, con lo Spatially Unbiased Infratentorial Toolbox (SPM12). La connettività strutturale è stata studiata calcolando il volume del smCb e del cCb. I dati funzionali sono stati analizzati con FSL toolbox. La connettività funzionale in resting state (rsFC), a livello dei voxel, è stata analizzata prendendo come regioni di interesse il cervelletto sensorimotorio e quello cognitivo. Infine sono state calcolate le correlazioni con i dati clinici tramite test non parametrici (FSL Randomize, FDR corretto se $p<0.05$). L'età, il carico lesionale, il volume di materia bianca e materia grigia sono stati inclusi come covariate di non interesse.

Risultati: I pazienti con SM, rispetto al gruppo di controllo, hanno presentato una rsFC diminuita tra il smCb e giro precentrale, opercolo e basalganglia, e una rsFC aumentata con corteccia parietale superiore, corteccia prefrontale, cuneo e talamo. La rsFC del cCb è risultata diminuita nel giro prefrontale, corteccia temporale laterale, precuneo, insula e verme del cervelletto, mentre è aumentata nel giro precentrale, postcentrale, corteccia temporale mesiale

e occipitale, precuneo e talamo. L'EDSS e il 9-HPT correlano negativamente con l'rsFC dell'smCb nel precuneo, corteccia parietale laterale e giro postcentrale. Il PASAT correla positivamente con l'rsFC del cCb nel giro frontale superiore e medio, corteccia parietale superiore, corteccia occipitale laterale, nucleo caudato. I pazienti presentano atrofia cerebellare sia del smCb che del cCb.

Conclusioni: La connettività funzionale del cervelletto, in condizioni di resting state, è risultata alterata con le aree sopratentoriali nei pazienti con sclerosi multipla. Le correlazioni con i dati clinici indicano che tale alterazione funzionale è associata ad una disabilità clinica minore, sia in termini di disabilità motoria che di declino cognitivo; ciò suggerisce un ruolo compensativo della rsFC cerebellare aumentata.

Extended summary

The first chapter covers the theory of magnetic resonance imaging, including the theoretical description of nuclear magnetic resonance. Functional magnetic resonance and the pre-processing steps needed for the analysis are further described. Resting state fMRI and its advantages in respect to task-related design are listed.

The second chapter is concerned primarily with the disease we focus on, Multiple Sclerosis, and the role of the cerebellum in this disorder. An outline of the main aspects of the pathology and the state of the art about the relation of the cerebellum in MS are described.

In the third chapter are described the materials and the methods implemented in our study; the main software tools and imaging techniques are briefly illustrated. Aspects of the experimental protocol are also reported. This chapter also illustrates the results from our fMRI study in patients with Multiple Sclerosis.

In the fourth chapter we discuss the results found from our experimental study, answer to our experimental question and interpret them, relating our findings with previous studies.

Keywords: resting state functional magnetic resonance imaging; functional connectivity; cerebellar lobes; Multiple Sclerosis.

Introduction

Magnetic Resonance Imaging (MRI) has revolutionized the diagnosis and studying of neurological disease, with both conventional and advanced methods. MRI has changed radically the approach to Multiple sclerosis (MS), an immune-mediated disease of the central nervous system characterized by inflammatory and degenerative processes (Owens; Ciccarelli et al.).

In MS, one major site of disease pathology is the cerebellum (Mormina et al.). It is highly connected with other brain regions and receives input from most areas of the cerebral cortex via the cortico-ponto-cerebellar pathway and projects back to the cerebral cortex via the dentato-rubro-thalamo-cortical pathway (Brodal e Bjaalie; Glickstein e Doron).

Given the early and severe involvement of the cerebellum in MS (Parmar et al.), it is conceivable that patients with MS may show altered patterns of disrupted cerebellar functional connectivity. As a matter of fact, cerebellar FC is altered in MS patients and correlates with clinical impairment (Sbardella et al.; Tona et al.). As well, functional connections between cerebellar areas and cerebral cortex vary according to the degree of disability, being higher in patients with low disability and lower in patients with severe disability (Tommasin et al.). A relationship between cerebellar FC rearrangements and cognitive performances has been also described in progressive MS patients (Cocozza et al.).

According to its function, the cerebellum may be divided in two main domains: the sensorimotor cerebellum (smCb), which is constituted by the anterior lobe (lobules I-V) plus lobule VIII, and the cognitive cerebellum (cCb), which includes lobules of posterior lobe (VI-X) except for VIII (Stoodley e Schmahmann).

Motor and cognitive disability in MS have an important impact on the quality of life and life expectancy. Therefore, it is of great impact in the life of MS patients the assessment and

comprehension of the mechanisms that determine this kind of disability, as they may be targets for therapeutic intervention, either pharmacological or rehabilitative.

CHAPTER 1: Magnetic Resonance Imaging

1.1 Nuclear Magnetic Resonance

MRI is one of the most powerful methods to investigate structure and dynamics of biological matter and is based on the phenomenon of nuclear magnetic resonance (NMR).

NMR employs a static magnetic field (e.g. in clinical use today they range 0.5-3T) and electromagnetic (EM) waves in the radio-frequency range between 1-300 MHz.

Some nuclei, which have a property called "spin", show a nuclear resonance to the radiofrequency RM. Spin is a fundamental property of nature and it comes in multiples of 1/2. Spin can be positive or negative. Protons, electrons and neutrons possess spins. Individual unpaired electrons, protons and neutrons each possess a spin of 1/2, but if two particles have opposite spin, they will have a net spin equal to zero, because the opposite signs pair up and the charge will cancel out.

Hydrogen (H^1) is the most important isotope for the phenomenon of NMR, because of two reasons: it possesses a net spin, and it is very copious in human body.

In an external magnetic field B_0 along the z-axis the spinning proton causes a torque, which let it precess around the z-axis at a stable angle (Figure 1). The precession frequency of the spin, known as the Larmor frequency, is $\omega = \gamma * B_0$, where γ is the gyromagnetic ratio, different for each atom, and B_0 is the magnetic field. For the hydrogen, the gyromagnetic ratio is $\gamma = 42.58$ MHz/T.

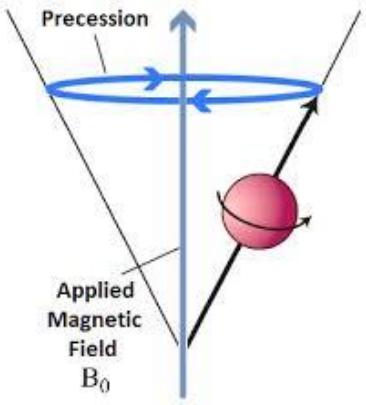

Figure 1: The spinning proton causes a precession movement, due to an applied magnetic field B_0 at a stable angle (extracted from *Physics of MRI*).

In presence of this external magnetic field, the hydrogen nucleus has only two possible energy states: the parallel one and the antiparallel one: the majority of the spins align in the parallel way, so that a net magnetization vector along the positive direction of the external field is created (Figure 2).

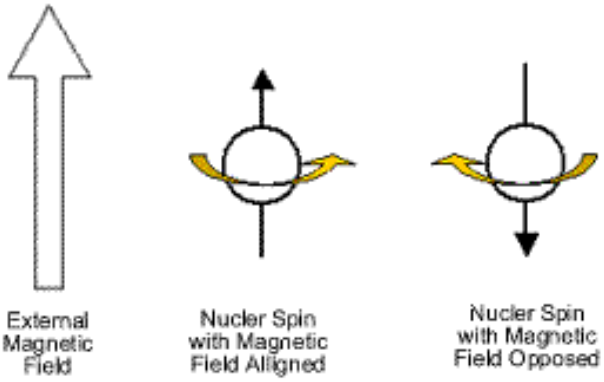

Figure 2: In the external magnetic field, some nuclear spins align in the parallel way of the magnetic field, some others in the antiparallel way (extracted from quizlet.com).

Then a radio-frequency (RF) pulse at the Larmor frequency, perpendicular to the main magnetic field (B_0), is sent. Individual spins begin to precess in phase, as will the net magnetization vector. As the RF pulse continues, some of the spins in the lower energy state absorb energy from the RF field and make a transition into the higher energy state. This has the effect of "tipping" the net magnetization M_z toward the transverse plane, and a transversal component

(M_{xy}) of the net magnetization is induced. If the energy is sufficient, it will produce a 90-degree flip of the net magnetization, as illustrated in the figure 3.

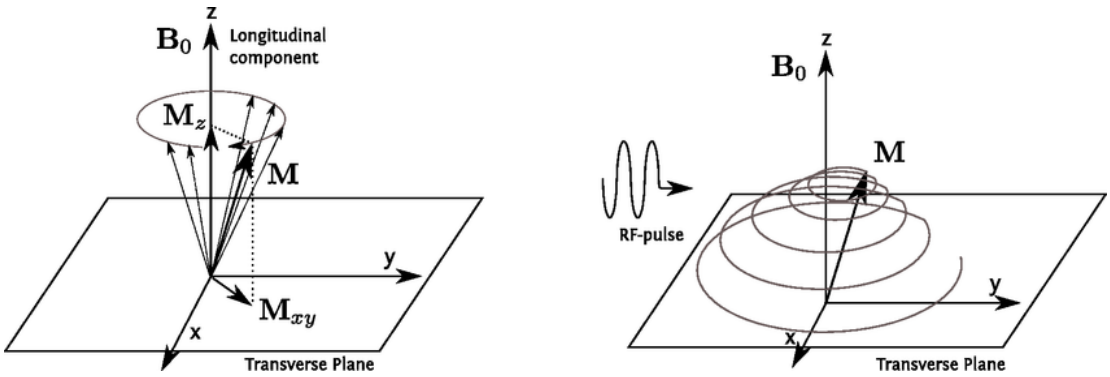

Figure 3: The net magnetization M has two components: the longitudinal component M_z and the transverse component M_{xy}. As the radiofrequency impulse begins, the net magnetization starts to precess along the z axis (extracted from Ghosh e Deriche).

The decay time constant of the net magnetization vector is T2. The spins start to flip back to the low-energy state, the longitudinal magnetization goes back to its original state. The time constant of the longitudinal relaxation is T1. This longitudinal relaxation causes the MR signal to decay in time, the so-called "free-induction decay" (FID) signal (Figure 4). Because of local field inhomogenities, the FID signal actually decays faster and the time constant T2* describes this enhanced decay (Pozzo).

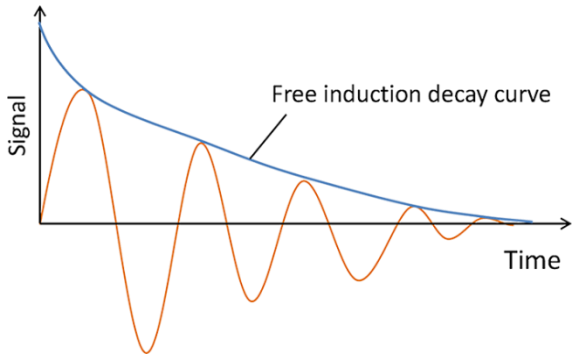

Figure 4: When the RF impulse fails, the longitudinal magnetization goes back to its original state, generating the so-colled free-induction decay (FID) signal.

1.2 From NMR to MRI

To obtain a three-dimensional image, three additional gradient fields are needed: the slice-selection gradient (to select the slice in the z-direction), the frequency-encoding gradient (to encode the x-axis), and the phase-encoding gradient (to encode the y-axis).

Typically, the FID signal is not measured, but rather its "echo", the signal peak induced in the coil after a time to echo (TE), which is generated by a second RF pulse applied at time TE/2.

The time interval between two successive 90 degrees pulses is called the repetition time (TR).

1.3 MRI sequences for brain imaging

The most common structural MRI sequences are T1-weighted, T2-weighted and FLAIR (Fluid Attenuated Inversion Recovery). T1-weighted sequences are characterized by having shorter TE and TR times, in respect to T2-weighted sequences. Cerebrospinal fluid (CSF) can be used to easily differentiate T1 from T2 images: it is dark on T1, and bright on T2.

FLAIR sequences adopt longer TE and TR times than T2, suppressing the CSF signal, and it is highly sensitive to pathology. Proton density (PD) images reflect the actual density of protons and have long TR ad short TE times, it is indicated for joint disease. Double inversion recovery (DIR) sequences suppress signals of cerebrospinal fluid and white matter, it has high signal of multiple sclerosis lesions. Diffusion Weighted Imaging (DWI) sequences detect the random

movements of water protons in the brain, it has a relatively low resolution but is extremely sensitive for detecting acute stroke.

In general, T1 sequence is used for the definition of anatomical structures and segmentation/parcelling of cortical and subcortical structures. T2, FLAIR, PD and DIR are commonly used for white matter lesions localization and characterization. DWI/DTI are employed for white matter fibres assessment and for structural connectivity between different brain areas.

1.4 Functional Magnetic Resonance Imaging and BOLD effect

Functional Magnetic Resonance Imaging (fMRI) is a non-invasive powerful imaging technique that can be used for studying brain functions in vivo. The technique used in fMRI is the blood-oxygen-level-dependent (BOLD) contrast.

The BOLD is the acronym of Blood Oxygenation Level Dependent and was first used by Ogawa in 1990 (Ogawa e Lee): it allows to localize local cerebral changes of hematic oxygenation during a physiological stimulation.

Ogawa showed that with magnetic field B_0 >1.5T and T2 weighted images, a signal dependent by the hemoglobin oxygenation level can be obtained, and that this is correlated with the ongoing neural activity. The BOLD signal is based on the physiological changes of the blood magnetic properties, and in particular on the difference of oxyhemoglobin (Hb) and deoxyhemoglobin (dHb) concentration. Neurons during a task consume oxygen and therefore the blood flow increases in order to provide blood full of oxygenated hemoblogin (Figure 5). This has a magnetic susceptibility more than 20% greater than deoxyhemoglobin. With an increase of oxyhemoglobin, the T2 weighted MR signal raises and the BOLD signal can be extracted.

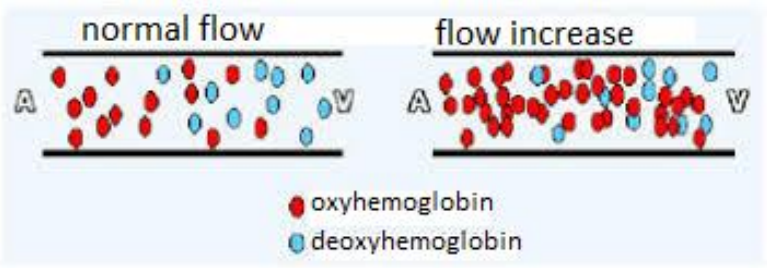

Figure 5: Normal blood flow in absence of task has an homogenic concentration of oxyhemoglobin and deoxyhemoglobin (A); during a task, neurons absorbe more oxygen and, in response of this phaenomenon, the blood flow increases the concentration of oxygenated hemoblogin (B) (extracted from https://www.slideshare.net/jdtomines/f-mri).

The regional BOLD response generated by the activation of a brain area is known as the Hemodynamic Response Function (HRF) (Figure 6). The HRF typically has a small initial dip, followed by a tall peak, and then a variable post-stimulus undershoot. The initial dip is caused by oxygen extraction by the arterial capillaries and an increase of dHb. The following peak represents the cerebral blood flow increase due to the immediate metabolic needs: the ratio Hb/dHb increases with an increase of MR signal. The post-stimulus undershoot is probably due to a continuous oxygen consumption, which causes a dHb reduction and a signal decrease.

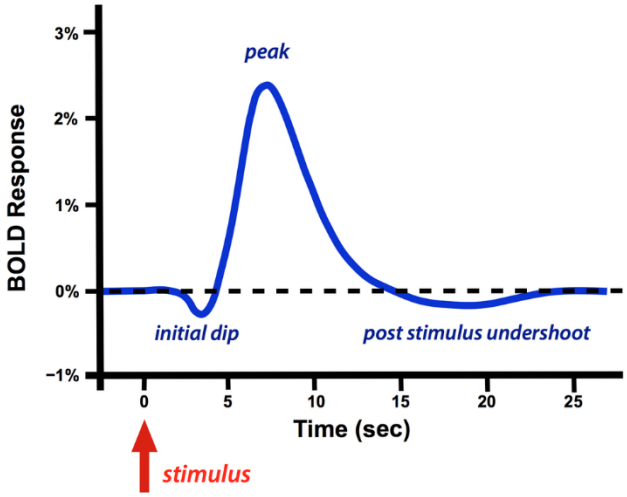

Figure 6: The Hemodynamic Response Function represents the regional BOLD response generated by the activation of a certain brain area: it starts with a small initial dip, followed by a tall peak, representing the increasing cerebral blood flow (extracted from MRIquestion.com).

The BOLD signal extraction is the basis of fMRI, which is a neuro-imaging technique developed in the last twenty years to study our brain and its functioning. FMRI has become an essential technique to study neurological and neurodegenerative diseases such as Alzheimer's, Parkinson's, Multiple Sclerosis and Lateral Amyotrophic Sclerosis. With fMRI, indeed, blood flow changes can be studied and therefore the correlated neural activity. This technique does not need contrast agent, but it only uses magnetic properties of hemoglobin, so it is a non-invasive method to explore our brain activity.

To capture the rapid physiological changes underlying the BOLD signal, images should be acquired fast, at the same rate as the physiological processes unfold. The most common imaging sequence in fMRI is the echo planar imaging (EPI) sequence (Mansfield), in which one slice can be acquired in 20–100 ms. The drawback of this fast scanning is the very low spatial resolution. Therefore, the functional data are registered on high-resolution structural images.

fMRI experiments can be divided in task-related experiments and resting-state experiments: the first measure brain activity during a task performance, the second evaluate the interaction between different brain regions without the execution of any specific task, in a rest condition (rs-fMRI) (Mormina et al.).

1.5 Resting state fMRI

Resting state fMRI (rs-fMRI) is the acquisition of the BOLD signal in the Resting state condition, in which the patient is not stimulated with any motor or cognitive task, he is asked to rest and not thinking about something special, not falling asleep and to stay with closed eyes.

The concept behind resting-state fMRI is that when the brain is "free-wheeling" (not involved in any kind of task), correlations in slowly fluctuating spontaneous brain activity tend to reflect the intrinsic functional networks of the brain (Biswal et al.).

These spontaneous fluctuations range from 0.01 to 1 Hz, and in the past they were thought to be only a random signal, a noise, which researchers were not interested in. Bharat Biswal (Biswal et al.) was the first researcher who used fMRI to study how different regions of the brain communicate while the brain is at rest and not performing any active task.

Recent findings demonstrate that brain fluctuations at resting condition are not randomly produced (Krishnan et al.) and nowadays fMRI at rest is considered one of the most powerful technique to study our brain and to shed a light in still unknown outcome of neurological diseases.

1.6 fMRI pre-processing

Several pre-processing steps are needed to proceed with images analysis to reduce artifacts and noise and to perform spatial transformations. SPM12 software (http://www.fil.ion.ucl.ac.uk/spm Wellcome Department of Imaging Neuroscience, London) is the most used tool to analyze both structural images and functional data:

- slice timing correction;
- motion correction;
- coregistration;
- spatial smoothing.
- normalization;

- Slice timing correction: scanning a whole brain volume is made by acquiring single slices at different time points. Slice timing correction is therefore needed to solve this problem. Between two slices there is a delay, which depends of the TR of the scanner and is generally 1.5 to 3.0

sec. Methods to correct this delay are different: temporal interpolation with linear method, Spline or sinc function. Using interpolation is useful to realign the hemodynamic responses of all the slices.

- Motion correction: The main error source in functional acquisitions is the head motion, which can be limited with systems immobilizing the head. Coregistration is the process that allows the spatially realignment of the brain volumes to one volume of reference. Coregistration is made by rigid transformations (three rotations and three translations) to overlap the volumes. Algorithms of transformations estimate the rotation and translation parameters; then with spatial interpolation the volumes are resampled.

- Coregistration: functional data are very blurred images, with very low spatial resolution; structural data have high spatial resolution, that allows the identification of anatomical details (Figure 7). Coregistration is therefore needed to align the functional images to the structural images, in order to anatomically localize the activation and to obtain a spatial correspondence between the two images.

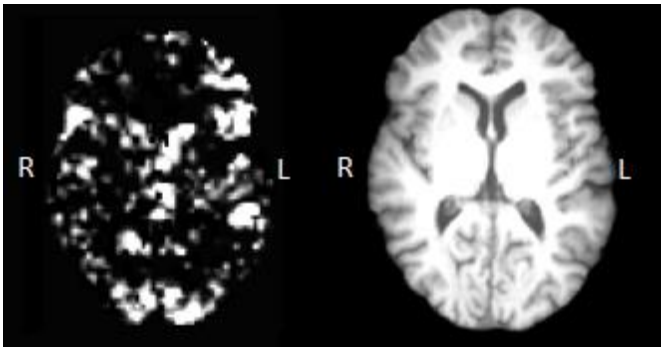

Figure 7: image of a functional activation map, with low spatial resolution (left), and a structural image (right), with high spatial resolution.

- Spatial smoothing: smoothing procedure is equivalent to set the value of every voxel with the average of the nearest voxels, in order to blur the images. This is necessary to remove the spatial

high frequencies, which are not of interest for the hemodynamic response. The most used filter is the Gaussian filter, which has a normal kernel. Its spatial dimension is named Width, and the Full Width at Half Maximum (FWHM) (Figure 8) defines how data are smoothed. Typical values of FWHM in fMRI are 6 to 10 mm (Figure 9).

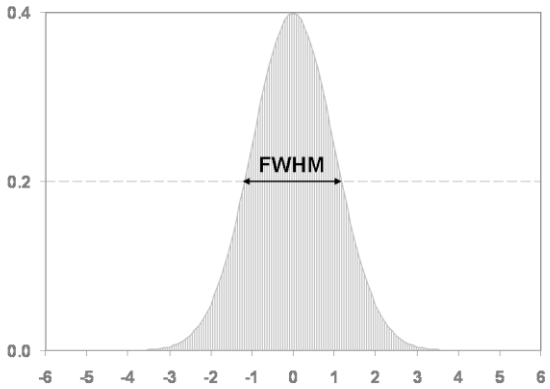

Figure 8: Gaussian filter for spatial smoothing, needed to remove the spatial high frequencies

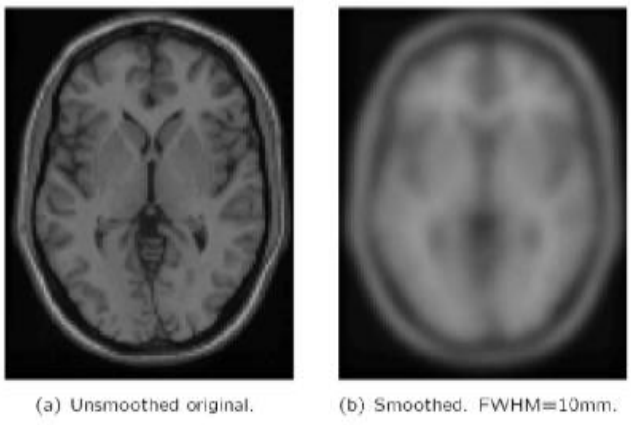

Figure 9: Unsmoothed T1 image (a) and smoothed T1 images after a smoothing with a FWHM=10mm (b).

- Normalization: this a procedure to compensate the morphological variability between different brains and to normalize them in a standard space, which is a stereotaxic space; the most used space is the Montreal Neurological Institute (MNI) atlas (Figure 10), made by averaging 152 MRI scan of normal subjects. Normalization is needed to compare brains between them, make

statistical inference and to localize brain areas with atlas coordinates. With normalization, thought, the spatial resolution decreases.

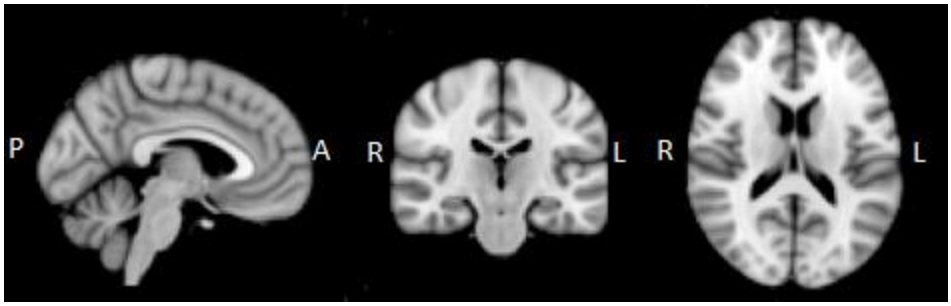

Figure 10: Montreal Neurological Institute standard brain atlas. In the figure, the sagital, the coronal and the axial views are shown in radiological convention (Right side is on the left of the image).

1.7 Functional connectivity and analysis methods

Resting state functional connectivity (rsFC) traditionally defined as the temporal dependency between spatially remote brain areas (Friston et al.; Lee et al.) and it is recently utilized as a marker of ongoing neural activity.

Rs-fMRI experiments can be analyzed using two major approaches: the seed-based approach and Independent Component Analysis (ICA).

In the first approach a region of interest, named "seed", is selected and the corresponding time-series are extracted, then the voxels with correlated activation are assumed to be functionally connected to the chosen seed. Seed-based analysis is able to quantify the amplitude of low frequency fluctuations (ALFF), which represent the average power of the low frequency fluctuations in the range (0.01–0.08 Hz).

The result is a map of brain voxels significantly correlated with the chosen seed ROI (Golestani e Goodyear) (Figure 11)

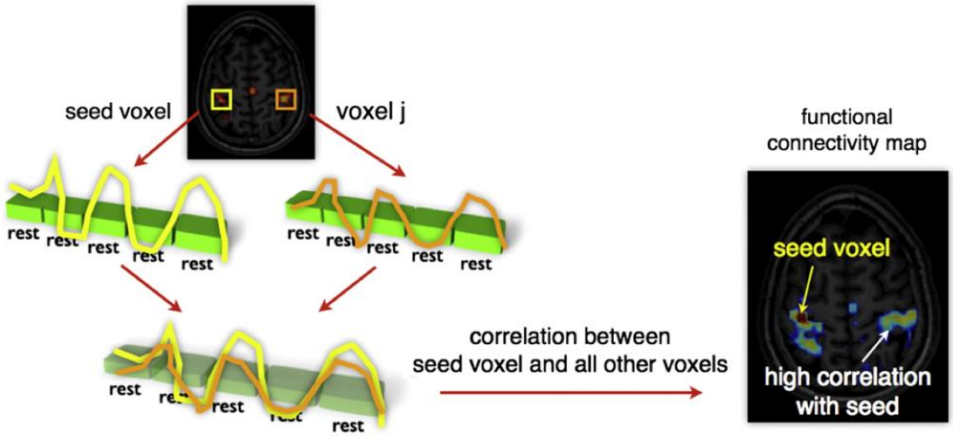

Figure 11: Seed-based FC. To find brain areas that are functionally connected with the seed region, the time series of the seed is extracted and correlated with the time series of the voxel of the whole brain. A significant correlation between the time series of the seed and voxel j is reflecting a significant level of FC between these regions. The result is a functional connectivity map that reflects the regions that show a high level of functional connectivity with the selected seed region (van den Heuvel e Hulshoff Pol).

Seed-based correlation is a powerful and effective tool in identifying the brain areas that show correlated activity during the resting state. Areas which are correlated with the seed region depend on the way the seeds are defined (Cole et al.). Seeds can be chosen based on the location of activity during a task (Biswal et al.; Xiong et al.), or using anatomical images as a guide (Di Martino et al.; Taylor et al.), based on standardized coordinates (Maldjian et al.).

The second method is a mathematical algorithm, without a priori seeds: it divides the multivariate signal into additive sub-components, which are statistically independent from each other. The so-called data-driven methods like principal component analysis (PCA) (Friston et al.) and independent component analysis (ICA) (Beckmann et al.; De Luca et al.) were introduced as FC analysis methods to discover connectivity patterns across brain areas.

In this study, we used a seed-based approach, because the a priori thesis was to discriminate the different role of two cerebellar areas in brain functional connectivity.

1.8 Advantages and limitations of rs-fMRI functional connectivity

Studying functional connectivity at rest is a useful tool to investigate the functional organization of our brain and has a lot of advantages (Reza Daliri): first of all it avoids any kind of problem or difficulty of the subject in performing the task. The acquisition protocol is quite standard and is parameter-free: it avoids all the problems in interpreting the results on the basis of the input parameters. Furthermore, asking no voluntary task to the subject may be an advantage in cases where the subject active interaction is compromised. FC reflects the intrinsic general organization of the brain and not the involvement in one specific task.

Rs-fMRI can study a broader sample of patients in different diseases, because in task-related design, most of the patients could not do the experiment correctly in fMRI scanner.

Further, the results in different studies demonstrate that the signal-to-noise ratio in resting state is better than task-based approaches.

On the other hand, this technique has its limitations, which are relative to the fMRI technique itself. One of the first is the low spatial and temporal resolution of fMRI data: a high resolution would allow a better localization of the activation areas.

To study a specific area or brain network, such as auditory network, a specific design with task-state has to be defined.

Some subjects can fall asleep during the resting state scanning: it is still not clear if there are individual differences in brain activity across sleep state and wake state and controlling this is difficult. Another problem is the impossibility to control the state of mind of the patient: it is actually impossible to know if the subject is thinking about something special or just resting during the scanning.

In the future the optimization of single-subject FC analysis might provide a more accurate method to detect FC alterations, which can be used as a non-invasive biomarker of the pathology (Arbabshirani et al.).

CHAPTER 2: Multiple Sclerosis and role of cerebellum

2.1 Multiple sclerosis pathology

MS is characterized by the development of an inflammatory reaction mainly supported by autoreactive T lymphocytes directed against myelin antigens such as myelin basic protein (MBP) and other proteins forming the myelin sheath.

Hallmarks of MS pathology are cortical and spinal atrophy, demyelination and axonal damage which can cause structural and functional disconnection among cerebral areas and alterations in neuronal synchronization.

Though, MS pathology is characterized not only by macroscopic WM plaques but the pathological damage involves also the normal appearing brain tissue. Normal-appearing white matter (NAWM) has been defined in 1979 as the macroscopically normal WM, distant at least 1 cm away from a plaque's edge (Allen e McKeown) and presenting deep microscopic alterations of myelin: gliosis, demyelination, small round cell and macrophage infiltration can be present in NAWM.

Further, MS pathology is also characterized by a loss of axons, with a decrease in density, ranging from 12 to 42% (Evangelou et al.; Bjartmar et al.). It is still not clear if this phenomenon is due to Wallerian degeneration consequent to axonal transection within the plaque, or if it is based on a primitive degenerative basis. Cortical demyelination is already present in early MS, although more frequently observed in patients with progressive forms of MS.

The clinical outcome of MS pathology can, in summary, be explained as the combination of two fundamental processes: one *inflammatory*, determining the formation of plaques of demyelination, and another *degenerative* process, which accumulates over time throughout the brain tissue

2.2 Cerebellum anatomy

Cerebellum is part of the Central Nervous System, it is located in posterior cranial fossa and is separated from the overlying cerebrum by a layer of dura mater (Figure 12). In humans, cerebellum has a key role in movement control, but recently a new involvement in cognitive functions, such as attention, memory and language has been shown. The cerebellum receives input from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine-tune motor activity (Fine et al.). Cerebellar damage produces disorders in fine movement, equilibrium, posture and motor learning.

Anatomically cerebellum is composed by a central vermis and two lateral expansions, named cerebellar emispheres. Three lobes can be distinguished within the cerebellum: the anterior lobe, the posterior lobe and the flocculonodular lobe. Within the lobes, ten lobules can be distinguished: lobules I through V, grouped together as the anterior lobe, lobules VI through IX, grouped together as the posterior lobe, and lobule X, known as the flocculonodular lobe.

Cerebellar surface exhibits many sulci, which delimit lobes and lobules. Lobule VII is subdivided into Crus I, Crus II, VIIA and VIIB; lobule VIII is subdivided into VIIIA and VIIIB. Cerebellum exhibits a tightly folded layer of gray matter, named cerebellar cortex, which has an irregular shape because of many transversal sulci. Within the cerebellar cortex, four deep nuclei are embedded in the white matter, and a fluid-filled ventricle is located in the middle.

Cerebellar cortex surface is half of brain surface, but it has a higher neurons density in respect of the whole brain, although it is 10% of the total brain weight.

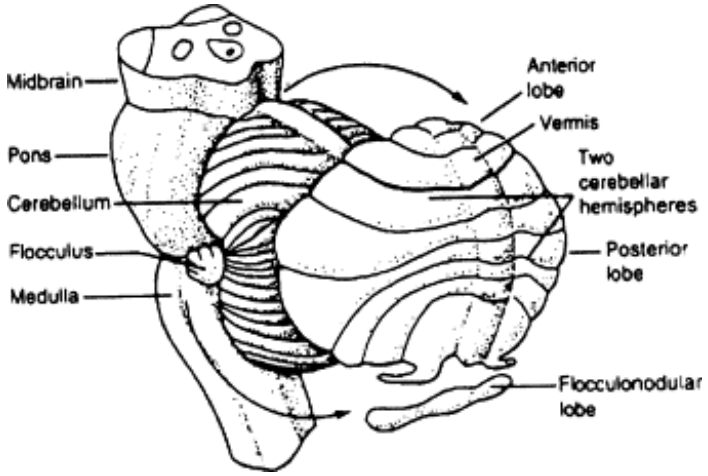

Figure 12: cerebellum anatomy (extracted from Lai).

2.3 Involvement of the cerebellum in multiple sclerosis: clinical aspects

Recently MS has been investigated more deeply with fMRI studies (Pantano et al.; Rocca et al.), which showed different patterns of brain activation in patients, when compared to healthy subjects.

Only in the past few years, the cerebellum has been established to have an essential role in the pathology of multiple sclerosis: it has been shown to have an important involvement not only in various sensory-motor networks, but also in cognitive-behavioural processes, domains primarily affected in patients with MS (Mormina et al.; Parmar et al.).

Cerebellar damage contributes significantly to clinical disability in multiple sclerosis (Wilkins). Balance deficit and motor disfunction are common symptoms related to atrophy of both the cerebellum and spinal cord in MS (Prosperini, Petsas, et al.). Clinical aspects of multiple sclerosis pathology are also limb, gait, and truncal ataxia, which are dependent on cerebellar structural damage (Wilkins; Swingler e Compston).

According to its function, the cerebellum may be divided in two main domains: the sensorimotor cerebellum (smCb), which is constituted by the anterior lobe (lobules I-V) plus lobule VIII, and the cognitive cerebellum (cCb), which includes lobules of posterior lobe (VI-X) except for VIII (Stoodley e Schmahmann).

2.4 Involvement of the cerebellum in multiple sclerosis: MRI aspects

As aforementioned, WM lesion play a key role in MS, in particular, cerebellar lesions lead to important motor dysfunctions in fine motor and coordination tasks (Davie et al.; Compston e Coles). At the same time, evidence from magnetic resonance imaging (MRI) demonstrated that also neuropsychological functions, such as attention, working memory and verbal fluency are altered in MS patients with cerebellar lesions (Sarica et al.). Indeed higher cerebellar leukocortical lesion load has been linked to poorer Paced Auditory Serial Addition Test (PASAT) performance (Damasceno et al.), demonstrating that cerebellar T2-lesions contribute to cognitive impairment.

Further, MS is commonly characterized by cerebellar atrophy, which has been associated with disability and cognitive decline (De Stefano et al.; Filippi et al.). MRI studies have shown a reduction of total cerebellar volume in MS patients when compared to healthy subjects, but the correlation with global measure such as the Expanded Disease Status Scale (EDSS) has been found to be moderate or not significant (Davie et al.; Ramasamy et al.; Weier et al.; Mesaros et al.).

Diffusion tensor imaging (DTI) assesses the microstructural integrity of WM tracts, examining the directionality of water diffusion across the brain (Basser). DTI allows to evaluate the evolution and the extension of tissue damage. In patients with MS, DTI abnormalities were found in NAWM (Gallo et al.). Furthermore, recent DTI studies showed a relationship between

WM tracts integrity and GM atrophy, explored with Tract-based spatial statistics (TBSS) (Bodini et al.). These findings suggest that structural damage of WM tracts may simulate a mechanism of disconnection between different GM areas (Dineen et al.). As a matter of fact, cerebellar functional connectivity (FC) is altered in MS patients and correlate with clinical impairment (Sbardella et al.; Tona et al.).

As well, functional connectivity (FC) between cerebellar areas and frontoparietal cortex varies according to the degree of disability, being higher in patients with low disability and lower in patients with severe disability (Tommasin et al.). A relationship between cerebellar FC rearrangements and cognitive performance has been also described in progressive MS patients (Cocozza et al.). In fact, the cerebellum presents a functional compartmentalization, which has its representation on cortical areas (Konczak e Timmann). FMRI studies showed an association between attention-task performances and increased activity in the posterior cerebellar lobe (Cerasa et al.), and, in particular, between lobules VI and VII and language, memory and executive function tasks (Cocozza et al.; Stoodley e Schmahmann). Furthermore, lobule VIII was found to be activated during hand/arm and lower limb movement (Buckner et al.; Mottolese et al.).

Neuroimaging studies demonstrated a correlation between reduced postural control and the location and the extent of the damage of infratentorial structures, cerebellar connections, and supratentorial associative bundles (Prosperini, Kouleridou, et al.; Preziosa et al.; Prosperini, Sbardella, et al.; Tona et al.).

Task-based fMRI studies showed how cerebellar FC and dysfunctions are related: e.g. Saini and colleagues showed a loss of crossed cerebello-cortical connectivity paired with a gain of ipsilateral cerebello-cortical connectivity in MS patients, in comparison with healthy controls, during a simple motor task (Saini). Further, altered cerebellar activation and cerebello-cortical

connectivity has also been observed in cognitively impaired patients during Paced Visual Serial Adding Test (PVSAT) task (Cerasa et al.).

Taken together, all these recent findings confirm the cerebellum as one of the major sites of MS pathology, and an interesting target of future fMRI studies.

CHAPTER 3: Experimental study

3.1 Aim

Although the role of altered cerebellar FC has been already investigated in MS (Mormina et al.; Sbardella et al.; Tona et al.; Cocozza et al.), FC changes of the two distinct cerebellar domains, i.e. sensorimotor and cognitive, remain to be explored. We hypothesized that both smCb and cCb functionally reorganize in response to MS-related damage, in order to preserve respectively motor and cognitive functions. Therefore, we expected to find altered FC between each of the two cerebellar compartments and different cortical and subcortical regions, as well as a correlation between possible FC changes and clinical scores related to either motor or cognitive impairment.

The aim of our work was to focus on the different contribution of functional disruption of both smCb and cCb in MS, in order to better understand the relationship between cerebellar functional changes and both motor and cognitive impairment.

3.2 Methods

Population

For this cross-sectional study, patients were recruited at the MS center of S. Andrea Hospital (Rome, Italy). A group of healthy subjects (HS) was selected among university personnel and included as control group. After a complete description of the study, patients and HS signed written informed consent prior to participation. Study protocol was approved by the institutional ethical committee (Policlinico Umberto I, Sapienza University of Rome, Italy).

Patients' inclusion criteria were: diagnosis of MS according to revised McDonald criteria (Thompson et al.), clinical history available on medical record; ability to sign written informed consent. Exclusion criteria were: use of steroids and introduction or modification of any

medication in the previous month; beginning of any disease-modifying or symptomatic treatment 3 months prior to enrollment; any relevant concomitant diseases; contraindications to MRI.

Patients were clinically evaluated, including Expanded Disability Status Scale (EDSS), 9 Hole Peg Test (9-HPT) with both dominant and non-dominant hands, 25-Timed Foot Walk Test (25-TFWT), Paced Auditory Serial Addition Test (PASAT) at 2 and 3 seconds. Single values of 9HPT and PASAT were obtained by averaging outcomes respectively from either dominant and non-dominant hand performances or 2 and 3 seconds performances.

MRI Protocol

All participants were scanned on a 3T MRI scanner (12-channel head coil for parallel imaging, Verio, Siemens AG) (Figure 13). The protocol included a high resolution 3-dimensional T1-weighted MPRAGE (T13D) with 176 sagittal slices, 1-mm-thickness slice, no gap (TR = 1900 ms, TE = 2.93 ms, flip angle = 9°, matrix = 256 × 256, FOV = 260 mm); dual-echo, proton density (PD) and T2-weighted images (TR = 3320 ms, TE = 10/103 ms, FOV = 220 mm, 384 × 384 matrix, 25 slices of 4-mm thickness, 30% gap); BOLD single-shot echo-planar images (TR = 3000 ms, TE = 30 ms, flip angle = 89°, 64 × 64 matrix, 50 slices, no gap, 140 volumes, acquisition time = 7 min), subjects were instructed to close their eyes and stay awake, without falling asleep during rs-fMRI acquisitions.

Figure 13: 3T RM Scanner, Verio, Siemens AG, 12-channel head coil

Structural MRI

Preprocessing of structural and functional images was performed with FSL version 4.0.1 (FMRIB's Software Library http://fsl.fmrib.ox.ac.uk/fsl).

Anatomical images. White matter (WM) lesions were segmented on PD images, by an experienced observer, unaware of patient's identity, using a semi-automated technique with Jim 5.0 software (Xinapse System, Leicester, UK; http://www.xinapse.com). From the obtained lesion mask, lesion load (T2LL) was calculated by summing lesion volumes over the brain, while infratentorial T2LL was calculated by summing lesion volumes within the cerebellum.

T13D images were bias-corrected (FSL FAST) and lesion-filled (Battaglini et al.), non-brain substance were removed from head (FSL BET) and the resulting brain was segmented into grey matter (GM), WM and cerebrospinal fluid (CSF).

Cerebellar parcellization. To calculate cerebellar lobe volumes on the T13D MRI images, we used The Spatially Unbiased Infratentorial toolbox (SUIT) version 3.2 (Diedrichsen et al.),

implemented in Statistical Parametric Mapping software (SPM12; http://www.fil.ion.ucl.ac.uk/spm) running under MATLAB R2012.

SUIT is a high-resolution atlas template of the human cerebellum and brainstem, spatially unbiased, which through automated non-linear normalization methods, provides a more accurate inter-subject alignment than current whole-brain methods (Diedrichsen et al.).

The following steps were performed with SUIT: (1) extraction of each subject's cerebellum from T13D anatomical image; (2) normalization of the isolated cerebellum to the SUIT atlas template space using the affine transformation matrix and non-linear flow field; (3) re-slicing of the cerebellum in order to preserve the volume of cerebellar lobules into the SUIT atlas. Lastly, the obtained SUIT atlas was realigned back to the native subject space.

For each subject we computed 28 lobular volumes (Figure 14). Finally, volume of smCb was calculated as sum of lobules I–V and VIII, as well as, volume of smCb was calculated as sum of lobules VI–VII, IX-X (Stoodley e Schmahmann) (Figure 15).

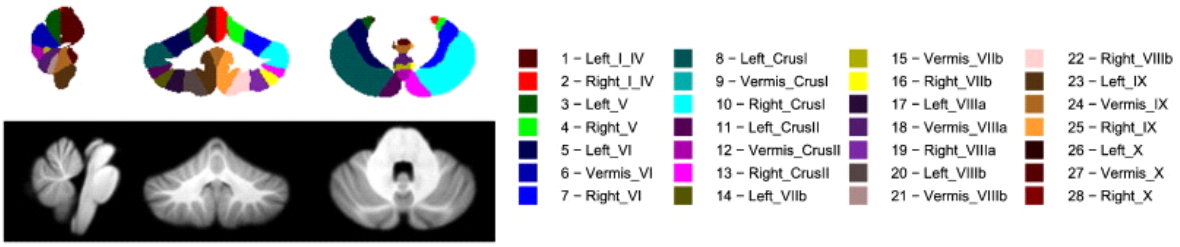

Figure 14: SUIT segmentation of the 28 cerebellar lobules

Figure 15: the sensori-motor cerebellar lobe (yellow) and the cognitive lobe (light blue), which are used as seed for the rsFC analysis.

Functional MRI

Preprocessing of functional data included the following steps: removal of the first 3 volumes from the 140 functional volumes to allow the signal to reach equilibrium; spatial smoothing at 5 mm Full-Width-at-Half-Maximum Gaussian kernel; movement removal with ICA-AROMA (Pruim et al.); application of a band-pass filter [0.008-0.09 Hz] to exclude physiological artifacts; correction for WM and CSF signals that were extracted and used as nuisance covariates.

A two-step procedure of linear/non-linear registration was implemented: with FLIRT (Jenkinson et al.) we linearly registered the T13D and the cerebellar images on the functional images for the following seed-based analysis; then the T13D and the correlations maps were non-linearly registered from subject space onto MNI standard space with FNIRT (http://www.fmrib.ox.ac.uk/dataseets/techrep/tr07ja2/tr07ja2.pdf).

We performed a seed-based analysis using FSL FEAT (FMRI Expert Analysis Tool). In subject space, the mean time series of smCb and cCb were extracted from each subject and used as seeds in the analysis. Voxel-wise maps of FC were calculated between seeds and the rest of the brain, at individual participant-level.

3.3 Statistical analysis

Statistical analysis of demographic characteristics. Student's t test was used to compare age and $\chi 2$ test to compare gender between patients and HS.

Statistical analysis of structural imaging features. Student's t test was used to test group differences in terms of volume of smCb, cCb and total GM, WM, between patients and HS.

Statistical analysis of seed-based functional maps. Maps of smCb and cCb FC were compared between groups at voxel-wise level via non-parametric tests (FSL Randomise, 5000 permutations) including age, GM and WM volumes as covariates of no interest. Significance was set if $p<0.05$ after false discovery rate (FDR) correction; minimum cluster extent was set to 100 voxels.

Relationships between cerebellar FC and both clinical and MRI variables. Voxel-wise correlation between smCb FC and either EDSS, 9HPT or 25-TFWT, as well as between cCb FC and PASAT, was performed non-parametrically (FSL Randomise, 5000 permutation) using two different designs. Design 1 included age, GM and WM volumes, and total T2LL, as covariates of no interest. In design 2 we excluded total T2LL and added the infratentorial T2LL and cerebellar lobe volumes as covariates of no interest, to take into account the influence of structural cerebellar damage on cerebellar FC changes.

3.4 Results

Demographics and clinical characteristics

For this study, 119 patients with MS (28 males and 91 females) with age 38.9±10.1 years, disease duration of 8.63±8.08 years, median EDSS of 2.0 and range of [0.0, 7.5] were included. Among patients, 91 were diagnosed with relapsing remitting and 28 with secondary progressive MS forms.

Patients on average scored 33.2±1.6 at PASAT test, 20.8±7.9 s at 9HPT and 6.6±5.7 s at 25-TFWT. Mean PASAT score of patients was lower than the normative value (48.2±9.8), while mean 9HPT and 25-TFWT scores were comparable to normative values (19.0±2.2 and 4.4±1.0, respectively) (Benedict et al.). Demographic and clinical characteristics of MS patients are summarized in Table 1.

Table 1: Demographic and clinical characteristics of the MS patients

Characteristics	MS patients
Number of subjects (RR /SP)	119 (91/28)
Age (years)	38.9 (10.1)
Gender (female/male)	91/28
Disease duration (years)	8.63 (8.08)
EDSS	2.0 [0.0, 7.5]*
PASAT	33.19 (1.64)
9-HPT (s)	20.85 (7.99)
25-TFWT (s)	6.61 (5.7)

Data are shown as means (standard deviation). Abbreviations: RR: Relapsing-Remitting, SP: Secondary Progressive, EDSS: Expanded Disability Scale Status, PASAT: Paced Auditory Serial Addition Test, 9-HPT: Nine-Hole Peg Test, 25-TFWT: 25-Timed Foot Waling test, NA: Not Applicable. *: Median [range].

HS group included forty-two healthy subjects (13 males and 29 females) with age 35.6±11.3 years. No significant differences in age or gender were present between patients and HS.

Structural MRI. Patients' T2LL and infratentorial T2LL were 6.15±6.42 mm^3 and 0.32±0.36 mm^3, respectively. Compared to HS, patients showed significant lower volumes of total GM, WM and smCb and cCb volumes. Structural MRI parameters are reported in Table 2.

Table 2: Structural brain features of the MS patients and healthy controls

Characteristics	MS patients	Healthy controls	T value	P value
smCb Volume [ml]	42.2 (6.3)	45.8 (8.8)	-2.46	0.01
cCb Volume [ml]	66.21 (9.6)	73.8 (10.2)	-4.17	1E-4
GM Volume [mm3]	7.63E5 (4.45E4)	7.94E5 (5.24E4)	-3.44	1E−3
WM Volume [mm3]	7.46E5 (3.74E4)	7.76E5 (6.79E4)	-3.46	1E−3
T2LL [mm3]	6.15 (6.42)	NA	NA	NA
Infratentorial T2LL [mm3]	0.32 (0.36)	NA	NA	NA

Data are shown as means (standard deviation). Abbreviations: smCb: sensori-motor cerebellum, cCb: cognitive Cerebellum, GM: Gray Matter, WM: White Matter T2LL: T2 Lesion Load, NA: Not Applicable, Statistics are two-Samples T-Test, two tailed, significance values for $p<0.05$.

Seed-based rs Functional Connectivity: sensori-motor cerebellar lobe

Compared with HS, MS patients showed increased rsFC between the smCb lobe and prefrontal and superior parietal cortices, cuneus, thalamus and cerebellum, bilaterally Compared with HS, MS patients had decreased rsFC between the smCb lobe and precentral gyrus, operculum and basal ganglia, bilaterally (Figure 16).

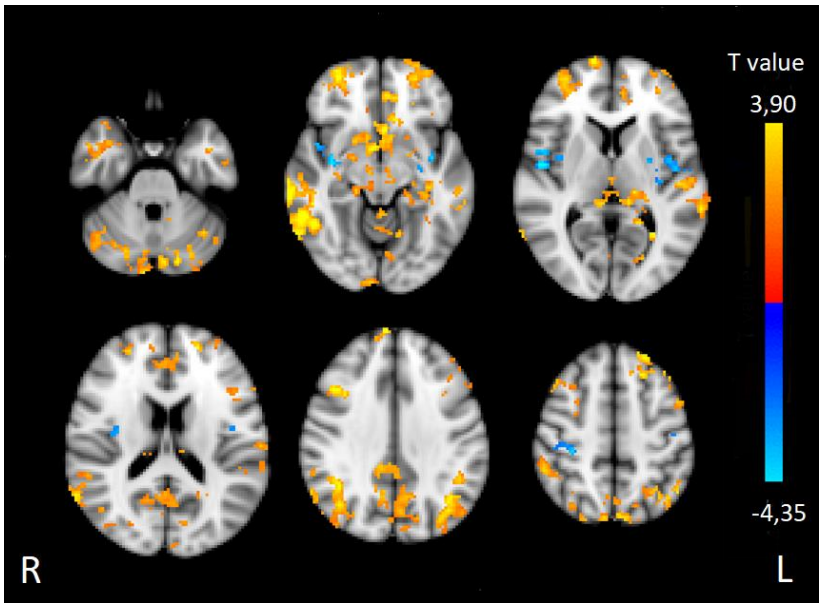

Figure 16: ***Sensori-motor seed.*** *Comparison of resting state functional connectivity (rsFC) of sensorimotor cerebellum maps between patients and controls. The color bar represents the t statistic of differences in functional connectivity between the two groups. Positive values (red colors) indicate areas where rsFC was significantly higher in patients than in controls, and negative values (blue colors) indicate areas where rsFC was significantly lower in patients than in controls. Statistical significance was reached if $p<0.05$.*

Seed-based rs Functional Connectivity: cognitive cerebellar lobe

Relative to controls, MS patients showed significantly enhanced rsFC between the cCb lobe and precentral and postcentral gyri bilaterally, left precuneus, occipital and mesial temporal cortex bilaterally and right thalamus. Patients had decreased rsFC between the cCb lobe and prefrontal gyrus and lateral temporal cortex bilaterally, right precuneus, right insula and vermis. (Figure 17)

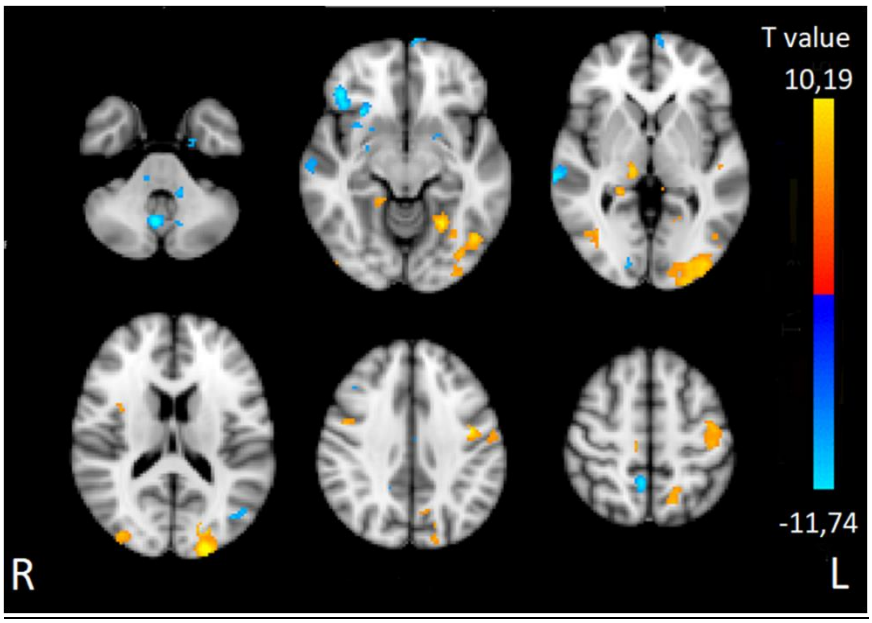

Figure 17**: *Cognitive seed***: *Comparison of resting state functional connectivity (rsFC) of sensorimotor cerebellum maps between patients and controls. The color bar represents the t statistic of differences in functional connectivity between the two groups. Positive values (red colors) indicate areas where rsFC was significantly higher in patients than in controls, and negative values (blue colors) indicate areas where rsFC was significantly lower in patients than in controls. Statistical significance was reached if $p<0.05$.*

Correlation Analysis

smCb FC versus clinical motor scores. In patients, design 1, (correcting for total T2LL) showed that FC between smCb and the right thalamus, left caudate, bilateral insula, precuneus and precentral gyrus correlated negatively with EDSS (Figure 18A). As well, FC between smCb and the precuneus, superior parietal gyrus, posterior cingulum, and thalamus, bilaterally, correlated negatively with 9-HPT (Figure 18B). Lastly, FC between smCb and the right post-central gyrus and left precuneus correlated negatively with 25-TFWT (Figure 18C). After correcting for structural cerebellar damage (design 2), results did not change.

cCb FC versus PASAT scores. In patients, design 1 showed that FC between cCb and the lobule V, right mesial temporal cortex and left parieto-occipital cortex positively correlated with

PASAT (Figure 18D). By applying design 2, no cluster of significant correlation between cCb FC and PASAT were found since no cerebral area survived the cluster threshold of 100 voxels.

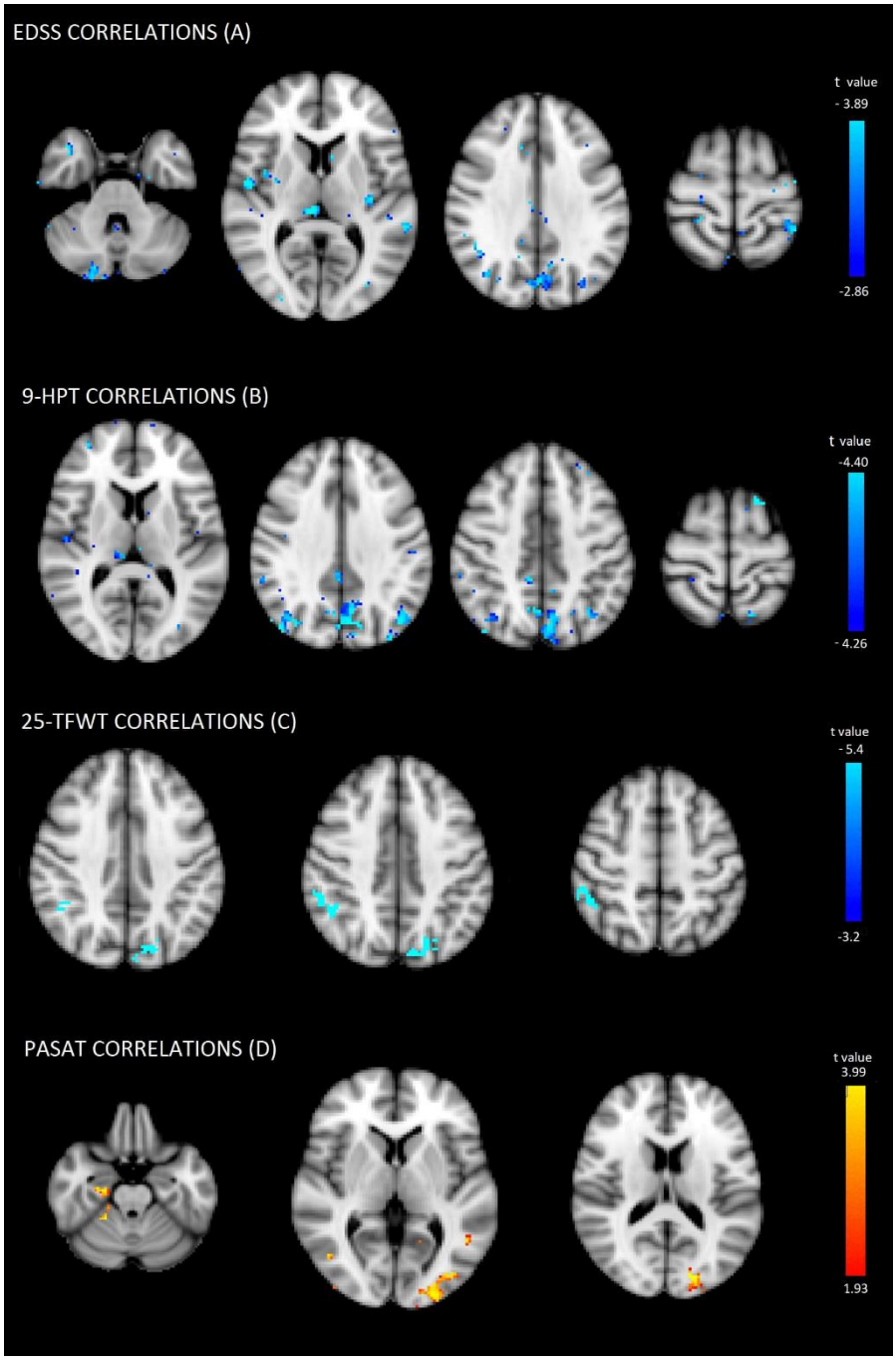

Figure 18: ***Correlations maps****: Resting state functional connectivity (FC) of sensori-motor cerebellum was negatively correlated with EDSS in thalamus, cuneus, precentral gyrus bilaterally and right*

operculum (A), with 9-HPT in cuneus, superior parietal gyrus, posterior cingulum, bilaterally and right thalamus (B), and with 25-TFWT in the right post-central gyrus and left precuneus (C). FC of cognitive cerebellum was positively correlated with PASAT in Lobule V, right mesial temporal cortex and left parieto-occipital cortex (D). Positive correlations are shown in yellow-red color, and negative correlations are shown in light-blue color. The color bar represents the t statistic of correlation between FC of cerebellar seeds and clinical data. Significant correlation was reached if p< 0.05 (FDR corrected).

CHAPTER 4: Discussion

In this study we investigated the FC of the sensorimotor and cognitive cerebellar domains in a large cohort of MS patients with various levels of disability and cognitive impairment in order to assess whether the two neocerebellar circuits are differently disrupted, whether cerebellar functional alterations correlate with both motor and cognitive clinical status, and, lastly, whether cerebellar FC changes are independent from cerebellar structural damage. We mapped voxel-wise FC of cerebellar lobules involved in sensorimotor abilities, grouped together in the smCb, and that of cerebellar lobules involved in cognitive functions, grouped together in the cCb. To the best of our knowledge, this is the first study exploring the role of functional connectivity of the cerebellum, considering distinctly these two functional subdivisions, in MS. The main finding of this study was that FC changes in the two cerebellar domains occur in patients with MS and correlate with both motor and cognitive performance. In particular smCb and cCb show different patterns of functional reorganization, including both areas of increased and areas of decreased resting state FC, supporting the existence of distinct pathways of cerebellum-cortex circuits. These results are in keeping with those of previous fMRI studies investigating the dentate nucleus FC in relapsing remitting MS patients (Sbardella et al.; Tona et al.). Sbardella et al. described greater FC between the dentate nucleus and some cortical areas, located in the frontal and parietal lobes (Sbardella et al.). Tona et al. found lower dentate FC with the thalamus and caudate nucleus and increased dentate FC with some clusters in the cerebellum, pons, amygdala and orbitofrontal cortex (Tona et al.). In paediatric MS patients, Cirillo et al. (Cirillo et al.) described reduced dentate FC with the caudate nuclei and thalamus as well as increased dentate FC with the precentral and postcentral gyri. However, cerebellar functional abnormalities reported in those studies relate to the whole neocerebellum since the

dentate nucleus is the main output station that receives from the entire neocerebellar cortex and contains output channels involved in both motor and cognitive functions (Bernard et al.).

Altered FC of smCb and cCb with supratentorial brain areas could reflect a functional reorganization of cerebellar functional connections following disease-related structural or functional changes occurring within the cerebellum itself. The cerebellar structure is frequently and severely damaged in MS patients (Davie et al.) and local functional abnormalities have also been demonstrated (Dogonowski et al.). By calculating regional homogeneity of BOLD signal fluctuations as a measure of local cerebellar FC, indeed, Dogonowski et al. described a decreased FC in the left lobules V and VI in MS patients relative to healthy controls that could represent a disintegration of local processing in the cerebellum in MS (Dogonowski et al.).

In our study, correlations between cerebellar FC and clinical scales indicate that the higher the FC, the lower the clinical disability, in terms of both motor impairment and cognitive decline. We found that patients with higher FC had lower EDSS, 9HPT and 25-TFWT scores. The correlation with PASAT was coherent with the previous result: the better the performance at PASAT, the higher the FC. These results suggest a compensatory role of the increased cerebellar FC.

The adaptive role of functional cerebellar changes in limiting the clinical consequences of structural damage in patients with relapsing-remitting MS and mild disability has been also suggested by a task-related fMRI study (Rocca et al.). In that study, Rocca et al. described increased FC between the cerebellum and the primary motor cortex during the execution of a simple motor task, which inversely correlated with the severity of structural damage of the underlying WM tracts.

Our results are in keeping with those reported by Sbardella et al. (Sbardella et al.) in a smaller cohort of MS patients showing that increased FC of the dentate nucleus with supratentorial brain areas correlated with lower clinical disability and better PASAT performance.

Accordingly, Tona (Tona et al.) reported that functional disconnection between the dentate and caudate nuclei is associated with worse balance performance in MS patients, suggesting a role of cerebellar disconnection in postural deficit in MS. More recently, cerebellar FC has been investigated by placing seeds at the level of four sites of the posterior cerebellum, namely lobule VI, Crus I, Crus II, and lobule VIIb to explore the relationship with the cognitive function in patients with progressive MS, thus with higher disability and lesion burden with respect to patients who we studied (Cocozza et al.). They found an inverse correlation between increased FC in the right precentral gyrus and cognitive scores that suggested mechanisms of maladaptive plasticity. Conversely, in our study, the association between low FC and high levels of physical and cognitive disability suggests exhaustion of compensatory mechanisms of neuroplasticity with disease progression and damage accumulation.

Lastly, we found that only correlation between cCb FC and PASAT scores was influenced by the severity of cerebellar structural damage, whereas correlations between smCb FC and EDSS, 9HPT and 25-TFWT scores were not. This finding suggests that the severity of structural damage of the cerebellum, commonly detected in MS (Mormina et al.; Parmar et al.), is not sufficient to explain the disability severity levels, since adaptive mechanisms of neuroplasticity may contribute to determine the clinical status. Similarly, Cocozza and colleagues (Cocozza et al.) described cerebellar FC changes that were partially independent from cerebellar structural damage in their sample of patients with progressive MS. Taken together, these results converge in highlighting the role of functional rearrangement of cerebellar connections in modulating the clinical effects of cerebellar damage in MS.

Our study is not without limitations. First, we used a cross-sectional design, including both relapsing remitting and secondary progressive subtypes of MS. By doing that, however, we obtained a sample with a large variability in terms of disability, making it possible to analyze the relationships between functional connectivity and clinical impairment. Second, we tested

the cognitive impairment by means of the PASAT alone, but it has been proved to be an effective tool to detect cognitive impairment in multiple sclerosis (López-Góngora et al.). Longitudinal studies, considering also specific MS phenotypes, are needed to confirm our results and to define how the FC changes contribute to the clinical outcome over time.

Conclusion

In conclusion, our study separately investigated functional abnormalities of the sensorimotor and cognitive cerebellum in a large series of MS patients and demonstrated that different areas of the cerebral cortex are disconnected from the two functional cerebellar subdivisions. Our study confirms the key role of the cerebellum in the pathophysiology of MS and extend the current knowledge about cerebellar involvement in both motor and non-motor functions. The congruent association between high FC of both smCb and cCb with low levels of physical and cognitive disability suggests that functional changes of cerebellar connectivity represent an adaptive mechanism able to limit the effects of cerebellar structural damage on clinical conditions. Overall our results strengthen the general concept that adaptive plasticity is a finite process that occurs in the early phases of MS and becomes exhausted as the disease progresses (De Giglio et al.; Schoonheim et al.). Lastly, the finding that cerebellar functional changes of the smCb are independent from cerebellar lesion burden and atrophy confirms the complex relationship existing between functional changes and structural damage and support the importance of plasticity and reorganization capabilities of cerebellar functional connections. It is those mechanisms that worth further investigation for targeted intervention, in order to change positively the course of disability in MS.

Conflict of Interests

Gabriele Pasqua, Silvia Tommasin, Komal Bharti, Claudia Piervincenzi have nothing to disclose.

Nikolaos Petsas received speaker fees from Biogen and mission support from Genzyme and Novartis.

Serena Ruggieri received fee as speaking honoraria from Teva, Merck Serono, Biogen, travel grant from Biogen, Merck Serono, fee as advisory board consultant from Merck Serono and Novartis.

Carlo Pozzilli received consulting and lecture fees from Sanofi-Aventis, Biogen Idec, Bayer Schering, Merck Serono, and Novartis. He also received research funding from Novartis, Sanofi-Aventis, Merck Serono, and Bayer Schering.

Patrizia Pantano has received founding for travel from Novartis, Genzyme and Bracco and speaker honoraria from Biogen.

Abbreviations

25-TFWT = 25 Timed Foot Walking Test

3D-T1 = Three dimensional T1-weighted

9-HPT = 9 Hole Peg Test

ALFF = Amplitude of Low Frequency Fluctuations

BET = Brain Extraction Tool

BOLD = Blood Oxygen Level-Dependent

cCb = Cognitive Cerebellum

CSF = Cerebrospinal Fluid

dHb = Deoxyhemoglobin

EDSS = Expanded Disability Status Scale

EPI = Echo Planar imaging

FA = Flip Angle

FC = Functional Connectivity

FDR = False Discovery Rate

FID = Free Induction Decay

FIRST = FMRIB's Integrated Registration and segmentation tool

FLIRT = FMRIB's Linear Image Registration Tool

fMRI = Functional Magnetic Resonance Imaging

FOV = Field Of View

FSL = FMRIB Software Library

FWE = Family-Wise Error

FWHM = Full Width at Half Maximum

GM = Grey Matter

HDR = Hemodynamic Response

HF = High Frequency

Hb = Hemoglobin

HRF Hemodynamic Response Function

HS = Healthy Subjects

ICA = Independent Component Analysis

LF = Low Frequency

MNI = Montreal Neurological Institute

MRI = Magnetic Resonance Imaging

MS = Multiple Sclerosis

NA = not applicable

PASAT = Paced Auditory Serial Addition Test

PD = Proton Density

PET = Positron Emission Tomography

RF = Radio Frequency

ROI = Region of Interest

RR = Relapsing Remitting

RS = resting state

rs fMRI = resting state fMRI

smCb = sensorimotor Cerebellum

SNR = Signal-to-noise Ratio

SP = Secondary Progressive

SPM = Statistical Parametric Mapping

SUIT = Spatially Unbiased Infratentorial Toolbox

T2LL = T2 Lesion Load

TE = Time to Echo

TR = Repetition Time

WM = White Matter

Acknowledgement

For of all, I would like to express my gratitude to Prof. Patrizia Pantano for the continuous support of my Ph.D study and related research.

This study could not be completed without the effort and cooperation of our colleagues, so I thank my labmates: Silvia Tommasin and Nikolaos Petsas, for helping me in writing my thesis; Costanza Giannì, Komal Bharti, Claudia Piervincenzi, Serena Ruggieri, Sankalp Tikoo, Sara Pietracupa, prof. Carlo Pozzilli for their support in my work.

I would like to thank my tutor Prof. Giuseppe Lembo, my colleagues Luigi Pavone, Fabio Sebastiano, for giving me this opportunity.

Last, but not least, I want to thank my family, my friends and life-mates: Nina Tirozzi, Anna Donadio, Angelo Cozzoni, Giada Mascio, Mariangela Lionetti, Federica Perretta.

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