Department of Psychiatry and Clinical Psychobiology

Functional, structural and metabolic remodeling related to cognitive recovery in ischemic stroke patients

Rosalia Dacosta-Aguayo

Department of Psychiatry and Clinical Psychobiology

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Thesis presented by

Rosalia Dacosta-Aguayo,

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Supervised by

Dr. Maria Mataró Serrat (University of Barcelona)

Dr. Manuel Graña Romay (University of the Basque Country)

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Dr. MARIA MATARO SERRAT, associated Professor at the University of Barcelona, and Dr. MANUEL GRAÑA ROMAY, Professor at the University of the Basque Country,

CERTIFY that they have guided and supervised the doctoral thesis entitled: "**Functional, structural and metabolic remodeling related to cognitive recovery in ischemic stroke patients**" presented by Rosalia Dacosta-Aguayo. They hereby assert that this thesis fulfils the requirements to present her defense to be awarded the Degree of Doctor.

Signature,

Dra. Maria Mataró Serrat Dr. Manuel Graña Romay Rosalia Dacosta-Aguayo

This thesis has been undertaken in the Neuropsychology Group of the Department of Psychiatry and Clinical Psychobiology, Faculty of Medicine, University of Barcelona and in the Group of Computational Intelligence, Faculty of Informatics, University of the Basque Country.

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"*In summary, the coexistence of segregation and integration is indispensable for the proper functioning of large-scale neurocognitive networks. All coherent perceptual and cognitive states require the functional integration of very large numbers of neurons within the distributed system of the cerebral cortex…The capacity of the network to sustain high levels of both segregation and integration is crucial for its efficiency in cognition and behavior, and in an information-theoretic context it forms the origin of brain complexity."*

Olaf Sporns *Networks of the Brain*, p.190

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FOREWORD

This thesis, presented for the degree of Doctor by the University of Barcelona, is the result of different studies carried out over a three-year period at the Department of Psychiatry and Clinical Psychobiology of the University of Barcelona and at the Group of Computational Intelligence of the University of the Basque Country. During this period, I have obtained the degree of Master of Neuroscience, which is linked to the Doctorate in Biomedicine Programme (Quality Mention MCD2008-00023; Mention to Excellence MEE2011-0316) at the University of Barcelona.

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GLOSSARY OF ABBREVIATIONS

AAL: Automatic Anatomic Labeling

ACA: Anterior Cerebral Artery

ANCOVA: Analysis of the Covariance

ANOVA: Analysis of the Variance

BMI: Body mass index

BOLD: Blood-Oxygen Level-Dependent

BP: Blood Pressure

CHD: Coronary heart disease

Cho: Choline Compounds

Cr: Creatine

DMN: Default Mode Network

FC: Functional Connectivity

FSL: Free Software Library

FWE: Family wise error

FWE: Family Wise Error

Glx: Glutamate + Glutamine

GM: Grey Matter

GPT: Grooved Pegboard Test

HDL: High-density lipoprotein

Lac: Lactate

LDL: Low-density lipoprotein

MCA: Middle Cerebral Artery

mI: Myoinositol

MMSE: Mini Mental State Examination Test

NAAG: N-acetyl-aspartyl-glutamate

NBS: Network Based Statistics

NIHSS: National Institute of Health Stroke Scale

PCA: Posterior Cerebral Artery

pICA: Probabilistic Independent Component Analysis

ROI: Region of Interest

rs-fMRI: Resting State Functional Magnetic Resonance

RSN: Resting State Network

SFT: Semantic Fluency Test

SPM: Statistical Parametric Mapping

TBSS: Tract Based Spatial Statistics

TCh: Total Cholesterol

TFCE: Threshold-free cluster enhancement

TG: Triglycerides

TIA: Transient Ischemic Attack

TMTA: Trail Making Test, part A

VOI: Volume of Interest

WAIS: Wechsler Adults Intelligence Scale

WM: White Matter

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BACKGROUND

The main aim of the current PhD research was to identify functional, structural and metabolic changes of patients suffering from an ischemic stroke and to attempt to relate them with cognitive recovery by applying the most up-to-date neuroimaging techniques.

Much of our current knowledge in relation to cognitive brain function is based on the modular paradigm, in which brain areas are postulated to act as independent processors for specific complex cognitive function. This paradigm, although has helped to build much of the present understanding of disease pathophysiology, has serious limitations when applied to the explanation of clinical and cognitive dysfunctions after a neurological insult. In stroke, clinical and cognitive deficits are not always easily explicable by the lesion localization itself, lesions of some areas tend to have more severed effects than others and individual outcomes seem to be due more to residual anatomy than to lesion localization. This scenario makes difficult to predict which patient will recover and will reintegrate into society and which will be relegated to a life of disability.

Novel research utilizing probabilistic Independent Component Analysis, Diffusion Tensor Imaging, Spectroscopy Imaging, and Graph Theory Analysis has reported properties of the human brain as a complex network as well as has revealed that the contralesional hemisphere is not so "healthy" as assumed at first glance.

1. INTRODUCTION

1.1. Ischemic stroke

1.1.1. Epidemiology

Acute ischemic stroke is the second most common cause of death worldwide and a major cause of long-lasting disability in the elder population with a high socioeconomic burden (Murphy and Corbett, 2009; Gorelick et al., 2011; Carmichael, 2012). Among 30-day survivors of first-ever stroke, about half survive for 5 years; one-third remain disabled, and 1 in 7 are in permanent institutional care (Hankey et al., 2002). Eighty seven percent of strokes are ischemic, in which blood flow to the brain is reduced; the remaining thirty percent are hemorrhagic, in which vessel ruptures and blood accumulates in the brain (Gleichman and Carmichael, 2013). There is agreement that between 44% and 74% of patients present some degree of cognitive disturbance (Savva and Stephan 2010; Dolan et al., 2010). Vascular Cognitive Impairment (VCI) and Vascular Dementia (VaD) increases the morbidity, disability, and healthcare costs of the growing elderly population, and decreases their quality of life and survival (Knopman et al., 2003; Fitzpatrick et al., 2005; Hill et al., 2005; Sicras et al., 2005).

1.1.2. Vascular risk factors

Risk factors for stroke may be classified as modifiable and non modifiable (Rincon and Sacco, 2008). Modifiable risk factors for stroke include hypertension (HTN), diabetes mellitus (DM), dyslipidemia, cigarette smoking, obesity, alcohol abuse, physical inactivity, and atrial fibrillation (AF) (Leys et al., 2002).

a) Hypertension

Epidemiological studies have shown a continuous, positive, linear relationship between both systolic and diastolic blood pressure and the incidence of any subtype of ischemic or hemorrhagic stroke, at any age, and in both sexes (Wolf et al., 1991). Elevated blood pressure (BP) has been termed the silent assassin because many people are asymptomatic until major end-organ damage has developed (Rincon and Sacco, 2008). According to the Framingham study, which defined definite hypertension as systolic BP > 160 mm Hg or diastolic BP > 95mm Hg, or both, the age-adjusted relative risk of stroke among those with definitive HTN was 3.1 for men and 2.9 for women (Dannenberg et al., 1988). The risk of stroke doubles for every 7.5mm Hg increase in diastolic blood pressure (Gubitz and Sandercock, 2000; Wolf et al., 1991). Meta-analyses of randomized controlled trials confirm an approximate 30% to 40% stroke risk reduction with BP lowering (Yusuf et al., 2000; Lawes et al., 2004).

b) Diabetes

Diabetes is a condition characterized by the inability to metabolize glucose appropriately resulting in elevated fasting blood sugars. It is the result of either deficiency of insulin production (type I) or insensitivity or resistance to its effects (type II) (Rincon and Sacco, 2008). Insulin-dependent diabetics have an increased risk for stroke (Tuomilehto and Rastenyte, 1999) for 2 reasons: (i) an increased prevalence of atherosclerosis, and (ii) an increased prevalence of other independent risk factors, such as arterial hypertension, obesity, and hyperlipidemia (Goldstein et al., 2001). Case-control studies and prospective cohort studies have found an independent association between diabetes mellitus and ischemic stroke (Burchfiel et al., 1994; Goldstein et al., 2001; Kannel and McGee, 1979). High blood pressure is common in patients with type 2 diabetes (Goldstein et al., 2001) and contributes to increase the stroke risk (Leys et al., 2002).

c) Lipids

Abnormalities of serum lipids have been regarded more as risk factors for coronary artery disease than for cerebrovascular disease (Rincon and Sacco, 2008). The role of hypercholesterolemia as a risk factor for stroke remained a matter of debate until recently: in most observational studies there was no clear (Prospective Studies Collaboration, 1995) or only a weak (Wannamethee et al., 2000) association between total cholesterol (TCh) and stroke. However, three quarters of trials reported only fatal strokes (Hess et al., 2000) and did not take into account stroke subtypes; many patients with cerebral hemorrhages have normal or even low TCh levels (Giroud et al., 1995), and cerebral hemorrhages being more frequent in fatal strokes, the results may have been skewed towards lower TCh levels in stroke patients. Other reasons may be that (i) ischemic stroke subtypes influenced by TCh probably account for a minority of strokes; (ii) coronary death occurring in younger patients, and (iii) TCh levels decrease in the elderly. Actually, more recent studies taking into account stroke subtypes found an increased risk of non-hemorrhagic strokes when TCh, LDLc and triglycerides (TG) levels are increased, and when high density lipoprotein cholesterol (HDLc) levels are decreased, without any threshold (Lindenstrom et al., 1994).

d) Cigarette Smoking

Smoking has been determined as a biologically plausible independent determinant of stroke through epidemiological studies and remains as an independent predictor after controlling for known risk factors of stroke (Lightwood and Glantz, 1997). The effects of smoking are multifactorial, affecting both vessels and coagulation. Vascular effects of smoking are the consequence of reduced vessel distensibility and compliance, and increased arterial wall stiffness, leading to atheroma (Kool et al., 1993). Blood effects consist of increased fibrinogen levels, hematocrit and platelet aggregation, and decreased HDL levels (Cruickshank et al., 1989). Smoking doubles the risk of ischemic stroke (Shinton and Beevers, 1989). Approximately 25% of

adults are active cigarette smokers, but figures differ according to countries, age-categories, education and gender (Leys et al., 2002).

e) Obesity

Obesity (defined as a body mass index (BMI) ≥ 30 kg/m2) is independently associated with an increased risk of vascular disorders, including stroke (Walker et al., 1996). However, obesity is often associated with increased BP, DM and dyslipidemia. Abdominal obesity, rather than BMI or general obesity, is more closely related to stroke risk (Leys et al., 2002).

f) Alcohol abuse

Alcohol consumption has a direct dose-dependent effect in the risk of hemorrhagic stroke (Donahue et al., 1986). Chronic heavy drinkers and young adults with acute alcohol intoxication have an increased risk of cerebral ischemia (Hillbom and Kaste, 1978). However, cross sectional studies have suggested a possible protection with moderate alcohol consumption (Donahue et al., 1986). Some studies have suggested a J-shaped dose-response curve between alcohol intake and ischemic stroke risk, with protection for those drinking up to 2 drinks a day and an increased risk for those drinking more than 5 drinks a day (Hillbom and Kaste, 1978; Rodgers et al., 1993; Sacco et al., 1999). The deleterious effects of alcohol for stroke may occur through various mechanisms, including increasing arterial hypertension, hypercoagulable states, cardiac arrhythmias and reduced cerebral blood flow. However, a light alcohol intake increases HDL cholesterol, and endogenous tissue plasminogen activator and may act as a protector (Leys et al., 2002).

g) Physical inactivity

Lack of exercise may be an independent risk factor for stroke and CHD (Fletcher et al., 1992; Wannamethee and Shaper, 1992) as important as arterial hypertension or smoking. People who exercise have less risk factors. The risk of stroke in people who exercise depends on the intensity and regularity of exercise (Wannamethee and Shaper, 1992). Regular physical activity reduces the risk of premature death, and vascular diseases including stroke (Haheim et al., 1993). The benefits are apparent even for light-to-moderate activities, such as walking. The protection induced by physical activity may be mediated in part through its role in controlling various known risk factors for stroke, such as arterial hypertension, cardiovascular disease, diabetes, and body weight, reductions in plasma fibrinogen and platelet activity, and elevations in plasma tissue plasminogen activator activity and HDL concentrations (Williams, 1996).

1.1.3. Mechanisms

Ischemic stroke is classified into various categories according to the presumed mechanism of the focal brain injury and the type and localization of the vascular lesion. Based on the etiology, there are several etiologic classification systems (Amarenco et al., 2009; Ay, 2010; Gao et al., 2011). Classic categories have been defined as large-artery atherosclerotic infarction, which may be extracranial or intracranial; embolism from a cardiac source; small-vessel disease; other determined cause such as dissection, hypercoagulable states, or sickle cell disease; and infarcts of undetermined cause (Adams et al., 1993; Pandya et al., 2011) (Table I adapted from Chen et al., 2012).

Based on the location of the symptoms, the Oxford Community Stroke Project classification (OCSP, also known as the Bamford or Oxford classification) classifies ischemic stroke as total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI), or

posterior circulation infarct (POCI) (Pandya et al., 2011). TACI refers to the symptoms of a patient who clinically appears to have suffered from a total anterior circulation infarct, but who has not yet had any diagnostic imaging to confirm the diagnosis. It is diagnosed when it causes all three of the following symptoms: 1) higher dysfunction (dysphasia, visuospatial disturbances, decreased level of consciousness); 2) homonymous hemianopia; 3) motor and sensory defects (≥ 2/3 of face, arm, leg). PACI is a type of cerebral infarction affecting part of the anterior circulation supplying one side of the brain. Refers to the symptoms of a patient who clinically appears to have suffered from a partial anterior circulation infarct, but who has not yet had any diagnostic imaging to confirm the diagnosis. It is diagnosed by one of the following: 1) two of three features of higher dysfunction (dysphasia, visuospatial disturbances), homonymous hemianopia, motor and sensory defects (> 2/3 of face, arm, leg); 2) Higher dysfunction alone; 3) partial motor or sensory defect. If all of the above symptoms are present, a TACI is more likely. LACI is a type of stroke that results from occlusion of one of the penetrating arteries that provides blood to the brain's deep structures. Patients who present with symptoms of a lacunar stroke, but who have not yet had diagnostic imaging performed, may be described as suffering from LACI infarct. It is estimated that lacunar infarcts account for 25% of all ischemic strokes (Sacco et al., 2006). Lacunes are caused by occlusion of a single deep penetrating artery that arises directly from the constituents of the Circle of Willis, cerebellar arteries and basilar artery. The corresponding lesions occur in the deep nuclei of the brain (putamen, thalamus, caudate) as well as the pons or posterior limb of the internal capsule. They occur less commonly in the deep cerebral white matter, the anterior limb of the internal capsule and the cerebellum. Each of the 5 classical lacunar syndromes (Table 2) has a relatively distinct symptom complex. Symptoms may occur suddenly, progressively, or in a fluctuating manner.

Table 1. - Etiologic Classification Systems for ischemic stroke

Publication year	TOAST (1993)	CCS (2007)	A-S-C-O (2009)	CISS (2011)
Type of system	Causative	Causative and phenotypic	Phenotypic	Causative
Major subtypes	1. Large artery 2. Cardioembolism 3. Small vessel occlusion 4. Other determined etiology 5. Undetermined etiology	1. Supra-aortic large artery atherosclerosis 2. Cardio-aortic embolism 3. Small artery occlusion 4. Other causes 5. Undetermined causes	1. Atherosclerosis 2. Cardioembolism 3. Small vessel disease 4. Other causes	1. Large artery atherosclerosis 2. Cardiogenic stroke 3. Penetrating artery disease 4. Other etiologies 5. Undetermined etiology
Advantages	Worldwide use. Simple, logic and easy to use. Validation by independent groups. Predicting prognosis and risk of stroke recurrence.	Rules and criteria based on published. Evidence. Updated criteria to stratify cardioembolism into high- and low-risk groups. Web-based automated version available. Reducing the ratio of "undetermined" category	Integration of diagnostic evaluation into the level of confidence for subtype assignments. Clarified diagnostic criteria to identify or rule out a stroke etiology. Integration of noncausative factors into subtype assignments.	Incorporation of the etiology and underlying mechanism into stroke subclassification. Creating a new subtype of PAD. Large artery atherosclerosis further subclassified into four categories.
Disadvantages	Only moderate inter-rater reliability. Oversized "undetermined" etiology. Not fit to recent advances in diagnostic technology.	Depending on the availability of modern diagnostic technology Based on evidence from diverse studies Aortic arch atherosclerosis belonging to cardio-aortic embolism	Further reliability and validity data needed. Interpretation cautiously with the combination of causative and noncausative factors. Depending on the completeness of diagnostic tools. Too many phenotypic subtypes (n=625) for research studies. Too restrictive definition to diagnose atherosclerosis and small vessel disease	Lacking reliability and validity data Depends on the availability of brain and vascular imaging. Future imaging technology needed to verify the concept of PAD.

ASCO, atherosclerosis, small vessel disease, cardiac causes, other uncommon causes; CCS, Causative Classification of Stroke System; CISS, Chinese Ischemic Stroke Subclassification; PAD, penetrating artery disease; TOAST, Trial of ORG 10172 in acute stroke treatment. (Table adapted from Chen et al., 2012).

Table 2. - Five classical lacunar syndromes

Name	Location of Infarct	Presentation
Pure motor stroke/hemiparesis	Posterior limb of the internal capsule, basis pontis, corona radiata.	It is marked by hemiparesis or hemiplegia that typically affects the face, arm, or leg of one side. Dysarthria, dysphagia, and transient sensory symptoms may also be present.
Ataxic hemiparesis	Posterior limb of the internal capsule, basis pontis, and corona radiata, red nucleus, lentiform nucleus, SCA infarcts, ACA infarcts.	It displays a combination of cerebellar and motor symptoms, including weakness and clumsiness, on the ipsilateral side of the body. It usually affects the leg more than it does the arm; hence, it is known also as homolateral ataxia and crural paresis. The onset of symptoms is often over hours or days.
Dysarthria/clumsy hand	Basis pontis, anterior limb or genu of internal capsule, corona radiata, basal ganglia, thalamus, cerebral peduncle	The main symptoms are dysarthria and clumsiness (i.e., weakness) of the hand, which often are most prominent when the patient is writing.
Pure sensory stroke	contralateral thalamus), internal capsule, corona radiata, midbrain	Marked by persistent or transient numbness, tingling, pain, burning, or another unpleasant sensation on one side of the body.
Mixed sensorimotor stroke	Thalamus and adjacent posterior internal capsule, lateral pons.	This lacunar syndrome involves hemiparesis or hemiplegia with ipsilateral sensory impairment

During a stroke, oxygen- and energy-hungry neurons that are deprived of their normal metabolic substrates cease to function in seconds and show signs of structural damage (Murphy et al., 2008). Neurons are unable to maintain their normal transmembrane ionic gradients, resulting in an ion and water imbalance that leads to apoptotic and necrotic cell deaths cascades (Murphy et al., 2008). During this process, glutamate is released in large

amounts from neurons and astrocytes, causing cellular overload of calcium through its action on calcium-permeable NMDA receptors (Campos et al., 2011). As a result of cell deaths cascades, microglia activates (Patel et al., 2013). Once activated, microglia develop macrophage-like capabilities including phagocytosis, cytokine production, antigen presentation and the release of matrix metalloproteinases that weaken the blood brain barrier (Iadecola et al., 2011). As a result, peripheral leukocytes infiltrate into the brain and the brain environment is exposed to systemic responses that further exacerbate inflammation and brain damage (Patel et al., 2013) and the normal flow of information between the ischemic region and areas connected to it via fiber tracts is disrupted causing functional deafferentation (Haberg et al., 2009). This phenomenon, known as diaschisis (von Monakow 1914), leads to changes in electrical activity, cerebral blood flow and metabolism in non-ischemic areas highly connected to the ischemic lesion (Gold and Lauritzen 2002; Enager et al. 2004) and it is responsible for the clinical deficits exceeding the functional impairments expected from the location and size of the focal lesion (Seitz et al. 1999; Whishaw 2000). Although it seems clear that spontaneous and gradual return of some abilities mainly depends on brain plasticity in both ipsilesional and contralesional hemispheres (Lee and van Donkelaar, 1995; Seil, 1997; Steinberg and Augustine, 1997; Johansson, 2000; Hallett, 2001; Nelles, 2004; Hurtado et al., 2007), mechanisms underlying functional recovery after stroke have not been clarified so far. Some of the most relevant factors cited are: vascular repair, immunomodulation, endogenous neurogenesis (Bliss et al., 2010; Horie et al., 2011; Liu et al., 2008) and the rewiring of surviving brain circuits enabling the healthy brain to compensate for the loss of functionality corresponding to the damaged area (Benowitz and Carmichael, 2010; Dancause, 2006; Murphy and Corbett, 2009).

1.1.4. Vascular Cognitive Impairment

Vascular cognitive impairment (VCI) was introduced to comprise the heterogeneous group of cognitive disorders that share a presumed vascular cause and to include both dementia and cognitive impairment without dementia (Hachinski et al., 1993; Bowler et al., 1999; O'Brien et al., 2003; Moorhouse and Rockwood, 2008; Dong et al., 2014). It is a syndrome that includes a spectrum of cognitive severity ranging from vascular mild cognitive impairment (VaMCI) to dementia (Hachinski et al., 2006). VaMCI is defined by impairment in at least one cognitive domain with intact or mildly impaired instrumental activities of living (Gorelick et al., 2011). More than half of patients with VCI (57%) are VaMCI (Rockwood et al., 2000), while 40% with non disabling ischemic stroke had VaMCI (Tham et al., 2002). VaMCI can be further classified into four subtypes in line with the MCI diagnostic algorithm proposed by Petersen (2004): (i) amnestic single-domain (amnestic sd-MCI); (ii) non-amnestic single-domain (non-amnestic sd-MCI); (iii) amnestic multiple-domain (amnestic md-MCI); and (iv) non-amnestic multiple-domain (non-amnestic md-MCI) (Dong et al., 2014) (Table 3).

1.1.5. Neuropsychological assessment of VCI

VCI encompasses a large range of cognitive deficits, from relatively mild VCI no dementia to more severe vascular dementia, or combined cerebrovascular disease with other dementing conditions such as AD (Desmond, 2004) (Table 3). The pattern of VCI cognitive deficits may include all cognitive domains, but there is likely to be a preponderance of so-called "executive" dysfunction, such as slowed information processing, impairments in the ability to shift from one task to another, and deficits in the ability to hold and manipulate information (ie, working memory) (Garrett et al., 2004; Nyenhuis et al., 2004; Troyer et al.,

1998). Neuropsychological protocols must therefore be both sensitive to a wide range of abilities and especially attuned to the assessment of executive function. Timed executive function tests may be especially sensitive to VCI-related impairment because of the slowed information processing noted in this patient sample (Gorelick et al., 2011). The Neuropsychological Working Group was responsible for recommending test protocols that could be used in multicenter investigations of potential patients with VCI. Because different protocols serve different purposes, the working group produced 3 separate protocols one that required approximately 60 minutes, a second that required 30 minutes, and a third that required 5 minutes (Hachinski et al., 2006). The 60-minute protocol contains recommended tests in 4 domains: executive/activation, language, visuospatial and memory. The 30-minute protocol included tests within the 60-minute protocol and was designed as a clinical screening instrument for patients with suspected VCI (Table 4). Finally, the 5-minute protocol was designed for the use of nurses and physicians who need a quick screening in their office (Hachinski et al., 2006).

Table 3. – Vascular Cognitive Impairment

1. The term *VCI* characterizes all forms of cognitive deficits from VaD to MCI of vascular origin. 2. These criteria cannot be used for subjects who have an active diagnosis of drug or alcohol abuse/dependence. Subjects must be free of any type of substance for at least 3 months. 3. These criteria cannot be used for subjects with delirium.
Dementia
1. The diagnosis of dementia should be based on a decline in cognitive function from a prior baseline and a deficit in performance in 2 cognitive domains that are of sufficient severity to affect the subject's activities of daily living. 2. The diagnosis of dementia must be based on cognitive testing, and a minimum of 4 cognitive domains should be assessed: executive/attention, memory, language, and visuospatial functions. 3. The deficits in activities of daily living are independent of the motor/sensory sequelaes of the vascular event.
Probable VaD
1. There is cognitive impairment and imaging evidence of cerebrovascular disease and a. There is a clear temporal relationship between a vascular event (eg, clinical stroke) and onset of cognitive deficits, or b. There is a clear relationship in the severity and pattern of cognitive impairment and the presence of diffuse, subcortical cerebrovascular disease pathology (eg, as in CADASIL). 2. There is no history of gradually progressive cognitive deficits before or after the stroke that suggests the presence of a nonvascular neurodegenerative disorder.
Possible VaD
There is cognitive impairment and imaging evidence of cerebrovascular disease but 1. There is no clear relationship (temporal, severity, or cognitive pattern) between the vascular disease (eg, silent infarcts, subcortical small-vessel disease) and the cognitive impairment. 2. There is insufficient information for the diagnosis of VaD (eg, clinical symptoms suggest the presence of vascular disease, but no CT/MRI studies are available). 3. Severity of aphasia precludes proper cognitive assessment. However, patients with documented evidence of normal cognitive function (eg, annual cognitive evaluations) before the clinical event that caused aphasia *could* be classified as having probable VaD. 4. There is evidence of other neurodegenerative diseases or conditions in addition to cerebrovascular disease that may affect cognition, such as

a. A history of other neurodegenerative disorders (eg, Parkinson disease, progressive supranuclear palsy, dementia with Lewy bodies); b. The presence of Alzheimer disease biology is confirmed by biomarkers (eg, PET, CSF, amyloid ligands) or genetic studies (eg, *PS1* mutation); or c. A history of active cancer or psychiatric or metabolic disorders that may affect cognitive function.
VaMCI
1. VaMCI includes the 4 subtypes proposed for the classification of MCI: amnestic, amnestic plus other domains, nonamnestic single domain, and nonamnestic multiple domain. 2. The classification of VaMCI must be based on cognitive testing, and a minimum of 4 cognitive domains should be assessed: executive/attention, memory, language, and visuospatial functions. The classification should be based on an assumption of decline in cognitive function from a prior baseline and impairment in at least 1 cognitive domain. 3. Instrumental activities of daily living could be normal or mildly impaired, independent of the presence of motor/sensory symptoms.
Probable VaMCI
1. There is cognitive impairment and imaging evidence of cerebrovascular disease and a. There is a clear temporal relationship between a vascular event (eg, clinical stroke) and onset of cognitive deficits, or b. There is a clear relationship in the severity and pattern of cognitive impairment and the presence of diffuse, subcortical cerebrovascular disease pathology (eg, as in CADASIL). 2. There is no history of gradually progressive cognitive deficits before or after the stroke that suggests the presence of a nonvascular neurodegenerative disorder.
Possible VaMCI
There is cognitive impairment and imaging evidence of cerebrovascular disease but 1. There is no clear relationship (temporal, severity, or cognitive pattern) between the vascular disease (eg, silent infarcts, subcortical small-vessel disease) and onset of cognitive deficits. 2. There is insufficient information for the diagnosis of VaMCI (eg, clinical symptoms suggest the presence of vascular disease, but no CT/MRI studies are available). 3. Severity of aphasia precludes proper cognitive assessment. However, patients with documented evidence of normal cognitive function (eg, annual cognitive evaluations) before the clinical event that caused aphasia *could* be classified as having probable VaMCI. 4. There is evidence of other neurodegenerative diseases or conditions in addition to cerebrovascular disease that may affect cognition, such as a. A history of other neurodegenerative disorders (eg, Parkinson disease, progressive supranuclear palsy, dementia with Lewy bodies); b. The presence of Alzheimer disease biology is confirmed by biomarkers (eg, PET, CSF, amyloid ligands) or genetic studies (eg, *PS1* mutation); or c. A history of active cancer or psychiatric or metabolic disorders that may affect cognitive function.
Unstable VaMCI
Subjects with the diagnosis of probable or possible VaMCI whose symptoms revert to normal should be classified as having "unstable VaMCI."

VCI: vascular cognitive impairment; VaD: vascular dementia; MCI: mild cognitive impairment; CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CT/MRI: computed tomography/magnetic resonance imaging; PET: positron emission tomography; CSF, cerebrospinal fluid; and VaMCI, vascular mild cognitive impairment. (Table adapted from Gorelick et al., 2011).

Table 4. - Sixty minute protocol.

Executive/Activation
Animal Naming (semantic fluency)
Controlled Oral Word
Association Test
WAIS-III Digit Symbol-Coding
Trail making Test
List Learning Test Strategies
Future Use: Simple and choice Reaction Time
Language/Lexical Retrieval
Boston Naming Test 2nd Edition, Short Form
Visuospatial
Rey-Osterrieth Complex Figure Copy
Supplemental: Complex Figure Memory
Memory
Hopkins Verbal Learning
Test-Revised
Alternate: California Verbal
Learning Test-2
Supplemental: Boston Naming Test Recognition
Supplemental: Digit Symbol-Coding Incidental Learning
Neuropsychiatric Inventory
Questionnaire Version Center for Epidemiological Studies-Depression Scale
Other
Informant Questionnaire for Cognitive Decline in the Elderly, Short Form
MMSE

Table adapted from Hachinski et al., 2006.

1.2. Neuroimaging the recovery after stroke

After stroke, the brain tries to compensate for loss of function through the reorganization of neuronal networks (van Meer et al., 2010; van Meer et al., 2012; Bury and Jones 2002). This reorganization involves the bilateral recruitment of the lesional and the contralesional hemispheres (Gerloff et al., 2006; Lotze et al., 2006) in which functional (Cramer, 2008; Cramer and Crafton, 2006; Wahl and Schwab, 2014; Ward, 2004; Ward et al., 2003; Weiller et al, 1993; Seitz et al, 1998), structural (Liu et al., 2008; Buffon et al., 2005; Gerloff et al., 2006; Brus-Ramer et al., 2007; Dancause et al., 2005) and metabolic (Demougeot et al., 2001; Demougeot et al., 2003; Khang et al., 2009; Kobayashi et al., 2001; Muñoz-Maniega et al., 2008; Craciunas et al., 2013; Takatsuru et al., 2009; Takatsuru et al., 2013) changes take place.

Nowadays, many neuroimaging techniques are suitable to investigate structural, functional and metabolic changes subtending neuronal plasticity. However, none of them individually has a temporal and spatial resolution accurate enough to describe exhaustively brain activities. Therefore, an integrated approach constitutes the best way to achieve the goal (Landi and Rossini, 2010).

1.2.1. Functional Magnetic Resonance Imaging

Functional MRI (fMRI) has played an integral role in defining the neural substrates and mechanisms underlying recovery after brain disease such as stroke at the system level of the brain (Park et al., 2011). Cortical reorganization has been characterized by observation of changes in brain activation during motor recovery after stroke (Kim et al., 2006; Tombardi et al., 2004; Loubinoux et al., 2007; Ward et al., 2006; Nair et al., 2005; Ward et al., 2003).

During motor recovery, three main forms of motor reorganization have been described under motor tasks: (1) increased cortical excitability in cortical regions distant from, but connected to the stroke core within both hemispheres; (2) reduced lateralized activation; (3) somatotopic modifications within intact cortical regions (Wahl and Schwab, 2014). The first form of reorganization appears days after stroke and diminish within months post incident (Ward, 2004). The second form of reaction to stroke, reflects the increased activity in the contralesional hemisphere, which reduces the extent of interhemispheric balance (Weiller et al., 1993; Seitz et al., 1998). Reduced lateralized activation is a common brain response not only seen in stroke but also in other neurological contexts such as epilepsy, traumatic brain injury and multiple sclerosis (Cramer, 2008). Interpretation is that the contralesional hemisphere has to take over functions that were previously based in the ipsilesional hemisphere. Both phenomena, increased cortical excitability and reduced laterality, are related to spontaneous functional recovery (Cramer, 2008). Both are time dependent and decrease over months thereafter. This decrease is greater among stroke patients with stronger functional recovery while the persistent increased activity over both hemispheres is greatest in those patients with the poorest outcome (Ward et al., 2003; Cramer and Crafton, 2006). Finally, the third response to ischemic injury implies that intact cortical area within the periinfarct region reassign their functions to take over the function that have been affected or lost by the ischemic event. Some studies suggest that the largest degree of somatotopic reorganization is associated with very large stroke injuries (Cramer and Crafton, 2006).

Studies of motor recovery using resting state functional activity have paid attention mainly to changes in the resting-state functional connectivity (rsFC) of the ipsilesional primary sensorimotor cortex after stroke (Carter et al., 2010; Golestani et al., 2013; Park et al., 2011; Wang et al., 2010). In these studies it has been reported the following process of reorganization: (1) in the acute phase, rsFC is initially decreased and there is a reduced inter-

hemispheric rsFC (Carter et al., 2010; Golestani et al., 2013; Park et al., 2011; Wang et al., 2010; Carter et al., 2012); (2) rsFC is gradually increased during recovery; (3) rsFC is finally restored to near normal or above normal levels at 10 weeks after stroke onset and there is a reinstatement of the inter-hemispheric rsFC (van Meer et al., 2012).

1.2.2. Diffusion Weighted MRI

While fMRI outlines activated cortical networks and provides clues about functional connectivity among areas involved in new skills acquisition or post-lesion plastic brain reorganization, it does not provide full information about the anatomical substrates of such a rearrangement (Landi and Rossini, 2010). Diffusion tensor imaging (DTI) is a technique in neuroimaging that may be one of the most sensitive neuroimaging biomarkers of vascular damage (Crofts et al., 2011; Bucur et al., 2008; Burgmans et al., 2010). It is based upon the phenomenon of water diffusion known as Brownian motion. Molecular motion is affected by the properties of the medium in which it occurs and diffusion within biological tissues reflects both tissue structure and architecture at the microscopic level (Kubicki et al., 2002). Equal, or isotropic, diffusion occurs when a medium does not restrict molecular motion, as would be the case with cerebrospinal fluid. Skewed, or anisotropic, diffusion, seen in crystals and polymer films, is not equal in all directions. DTI measures diffusion properties and consequently allows spatial description of the medium under study (Kubicki et al., 2002). Taking advantage of the fact that diffusion is not uniform throughout the brain (differing between gray matter, white matter, and cerebrospinal fluid) researchers can employ DTI to evaluate tissue characteristics. The technique is particularly useful in the study of white matter tracts in the brain since the mobility of water is restricted perpendicular to the axons oriented along the fiber tracts (anisotropic diffusion). This is due to the concentric structure of multiple tightly packed myelin

membranes wrapped around the axon fibers. Although myelination is not essential for diffusion anisotropy of nerves (Beaulieu and Allen, 1994), myelin is generally assumed to be the major barrier to diffusion in white matter tracts (Kubicki et al., 2002). DTI allows the computation of indices as the mean diffusivity (MD), the fractional anisotropy (FA), the radial diffusivity (RD) and the axial diffusivity (AD). The MD measures the overall mean displacements of molecules and reflects the overall presence of obstacles to diffusion. FA provides information of the degree of directionality of water diffusion. RD is the average of the second and third eigenvalues and provides information about the character of the myelin. Decrease in RD has been related to increased myelination, and increase represents demyelination (Bennett et al., 2010; Hu et al., 2011; Keller and Just, 2009). AD is the principal eigenvalue and provides information about axon morphological changes such as changes in axonal density or caliber (Kumar et al., 2010; Kumar et al., 2012).

With cerebral ischemia, reductions in cerebral blood flow disrupt energy metabolism resulting in perturbation of ionic pumps and disruption of ionic homeostasis (Irving et al., 2001). Cytotoxic edema occurrence produces both a decrease in the extracellular volume fraction and changes in membrane permeability (Iadecola and Anrather, 2011). These events reduce fractional anisotropy (FA), a DTI-derived measure of white matter microstructure (Beaulieu, 2002, 2009) above and below the stroke placement, consistent with patterns of Wallerian and retrograde degeneration (Pierpaoli et al., 2001; Werring et al., 2000). Furthermore, reduced fractional anisotropy has been found to be associated with level of recovery in stroke patients. In a recent study conducted by Schaechter et al. (2009), they found that while poorly recovered patients had reduced FA in both corticospinal tracts relative to healthy controls, well-recovered stroke patients had elevated FA relative to controls in the same regions. This observation shows not only that white matter microstructure can be

apparently improved following stroke, but also that such changes occur not just in the stroke hemisphere but also in the contralesional hemisphere (Crofts et al., 2011).

Other studies of tract's FA asymmetries have concluded that the contralesional corticospinal tract may play a role in motor recovery after unilateral stroke (Brus-Ramer et al., 2007; Jankowska and Edgley 2006; Liu et al., 2008; Dancause et al., 2005). In these studies, larger interhemispheric asymmetries in FA for this anatomical region have been associated with reduced motor recovery (Schaechter et al., 2009; Jang et al., 2005; Lindenberg et al., 2010; Watanabe et al., 2001), reduced skill improvement in response to training (Thomalla et al., 2004) as well as motor dysfunction after stroke (Jang et al., 2005; Stinear et al., 2007). Although plastic changes in the contralesional hemisphere have already proved to play a role in motor recovery after stroke (Qiu et al., 2011; Carmichael, 2003; Buffon et al., 2005; Gerloff et al., 2006), this role remains still unclear from a cognitive point of view.

Human studies usually use FA (Pitkonen et al., 2012; Muñoz-Maniega et al., 2004; Wang et al., 2006; Bhagat et al., 2006) and Mean Diffusivity (MD) (Bhagat et al., 2008; Marks et al., 1999; Beaulieu et al., 1999) to study the recovery of the injured brain over time. Regarding FA values, microstructural integrity of normal-appearing WM improves during 1 and 2 years following ischemic stroke, achieving subsequent stabilization (Muñoz-Maniega et al., 2004). There is a growing interest in investigating WM microstructural mechanism underlying the FA change by analyzing other – more direct – diffusion metrics, such as axial (AD) and radial (RD) diffusivity. A recent study identified RD and AD decrease accompanying FA increase in areas surrounding the anterior cingulate cortex after 4-week integrative body-mind training, a form of mindfulness meditation (Leritz et al., 2010). Reduction in RD is usually interpreted as improved myelination, whereas reduction in AD is associated with axon morphological changes, such as changes in axonal density or caliber (Tang et al., 2012; Bennett et al., 2010).

1.2.3. Spectroscopy

Proton MR Spectroscopy (^{1}H-MRS) in stroke research is considered a sensitive tool for the assessment of metabolic abnormalities detected even in the absence of structural MRI changes (Kobayashi et al., 2001). Regarding gray matter tissue, the most frequently assessed metabolites with the ^{1}H-MRS technique are N-acetyl-aspartyl-glutamate (NAAG), Creatine (Cr), Choline compounds (Cho), Myo-inositol (mI), and Lactate (Lac). Between them, NAA contributes to the most prominent signal observed in the ^{1}H-MRS spectrum (Baslow, 2003) and has been used as a biochemical marker for assessing neuronal viability/integrity after cerebral ischemia (neural loss) (Demougeot et al., 2001; Demougeot et al., 2003). In the ischemic area, two drastic spectral changes are observed (1) within 24 hours after occlusion, levels of NAAG, Creatine, Choline compounds and Myoinositol decrease rapidly and severely (Zhang and Chopp., 2009; Yang et al., 2012; Parsons et al., 2000) whereas Lac, Glutamate and GABA increase and reach high levels (Houking et al., 1993; Takagi et al., 1993; Hertz et al., 2008; Khang et al., 2000; Kobayashi et al., 2001; Munoz Maniega et al., 2008); (2) In the subacute phase (more than one month after onset), Lac remains high, Glutamate and GABA normalize, and NAAG, Choline compounds and Cr remain decreased (Munoz-Maniega et al., 2008; Yang et al., 2012).

Although it is known that white matter is equally sensitive as gray matter to ischemia (Hertz et al., 2008), remarkably little information is available about metabolic parameters during and after ischemic insults covering white matter tissue (Hertz et al., 2008; Rosenzbeig and Carmichael, 2013). However, following severe incomplete global ischemia a large decrease in Phosphocreatine has been demonstrated in white matter, without any change in gray matter, and the increase in Lac appeared to be larger in white matter (Lesnick et al., 1986; Veriac et al., 1992).

Still, the impact of focal ischemia on metabolism in remote non-ischemic tissue has not been investigated and results are unconclusive. In one experiment of middle cerebral artery occlusion in rats conducted by Haberg et al (2009), it was found that during the acute phase, Glutamate and GABA concentrations were decreased in the contralesional hemisphere. The significant reduction in both Glutamate and GABA content was interpreted as a consequence of the acute ischemic depolarization and subsequent functional deafferentation of a significant number of both GABAergic and glutamatergic transhemispheric connections. This reduction would contribute to neuronal reshaping which may facilitate recovery by means of keeping metabolism activity low to allow hemispheric reorganization (Haberg et al., 2009). In other study with ischemic stroke patients conducted by Cirstea et al (2011), they found lower levels of NAAG and higher levels of mI in the contralesional hemisphere at 6 months after stroke. Levels of NAAG were correlated with time after stroke.

1.2.4. Graph Theory applied to Functional and Structural Connectivity

The ability to integrate information from different sensory systems is a fundamental property of the brain (Bolognini et al., 2013). The human brain is endowed with different neural mechanisms that evaluate whether there is a concordance of information arriving through different sensory channels or whether these same signals give rise to conflict and thus should be processed separately (Bolognini et al., 2013). Much of our knowledge regarding multisensory processing in the human brain is derived from brain imaging studies (Calvert, 2001; Driver and Noesselt, 2008). Novel research utilizing graph theory analysis based on a variety of neuroimaging techniques have consistently reported non-trivial topological properties of the human brain network, such as small-worldness, which reflect the nature of

efficient information transfer (Bullmore and Sporns 2009) and test resilience of networks to insult (Rubinov and Sporns 2010). Small-world network describes a configuration in which most nodes are not neighbors, but can be reached from every other node by a small number of steps tending to minimize wiring costs while supporting efficient processing of complex information (Menon, 2011). Other network properties include measures of local connectivity levels (such as the clustering and modularity of various nodes in the network), global integration (average path length between two nodes, which is indicative of how efficiently the network is connected) and measures of the degree of a node (number of edges connecting this node to the rest of the network). The combination of these attributes simultaneously promotes high segregation (specialization) and high integration within a modular architecture (Sporns, 2011). Aberration in these network metrics can result in general network failure, which affects network robustness as well as efficient information transfer, and can cause deficits in the access, engagement, and disengagement of large-scale networks (Dosenbach et al., 2008; Rubinov and Sporns, 2010).

Implicit in all neuroimaging studies of brain networks is the notion that dysfunctional nodes or edges result in aberrant signaling which can then propagate to the whole network or sub networks across the brain. Analysis of connectivity data from many studies examining various states of psychopathologies has found common aberrations in various network metrics. For example, when comparing structural data from schizophrenia patients and healthy controls, schizophrenia patients show a loss of small world architecture as measured by a significantly lower clustering coefficient, longer characteristic path lengths and dysfunctional central hubs (Bassett et al., 2008; van den Heuvel et al., 2010). Abnormalities in these network metrics were also reported when functional connectivity data from patients with Alzheimer's disease was compared to controls (Stam et al., 2007, 2009; Supekar et al., 2008; Sanz-Arigita et al., 2010). Reductions in network efficiency have been associated with

greater lesion load in patients with multiple sclerosis (He et al., 2009) and reductions in nodal degree have been associated with greater severity of local Amyloid deposition in patients with Alzheimer's disease (Buckner et al. 2009).

Using DTI tractography, Li et al. (2009) showed that higher intelligence quotient scores were associated with larger global efficiency in the brain networks, suggesting an association between the structural organization of the brain and intelligence performance. Gong et al. (2009) showed that the local efficiency of structural brain networks constructed from diffusion-weighted MRI decreases with age (from 19 to 85 years) and that there is also a shift of regional efficiency from the parietal and occipital to the frontal and temporal neocortex in older brains. Regarding to stroke patients, a major unsolved question in understanding the structural organization of the brain is how the damage of one specific region can not only have remote effects in other regions but which networks are the most sensitive and how their damage have implications in the patient prognosis and recovery. At this point, the ability to assess multiple networks at once and their interaction may be especially valuable since stroke patients show deficits in a wide range of cognitive functions being executive function, processing speed and memory the most aforementioned (O'Brien et al., 2003). These functions are known to be sub served by distributed and integrated neural networks (Leh et al., 2010). Crofts et al. (2011) found that changes in brain structure occurred in remote regions following focal damage and that there was a reduction of communicability not only in regions surrounding the lesions in the affected hemisphere but in homologous locations in the contralesional hemisphere suggesting disrupted information processing across widely distributed regions.

2. OBJECTIVE AND HYPOTHESIS

OBJECTIVES AND HYPOTHESIS

General Objective

The main objective of this thesis is to study neurobiological mechanisms of cognitive recovery after ischemic stroke. We addressed specifically to the role of the contralesional hemisphere in cognitive recovery after stroke from a functional, structural and metabolic point of view. We used functional (fMRI), structural (DWI), spectroscopy and graph theory approach applied to functional data, and neuropsychological testing to assess stroke recovery at three months.

Firstly we focused on whole brain resting-state functional connectivity patterns and structural correlates related to better and poorer cognitive recovery. This issue was investigated in papers I and II. Secondly we focused on metabolic imbalance and on DMN activity related to cognitive performance. This issue was investigated in papers III and IV. Finally, we made a technical approximation to automatic lesion segmentation's algorithm. This issue was investigated in paper V.

Specific objectives and hypotheses

Paper I

Objectives

1. To explore whether patients with good cognitive recovery showed differences in resting-state functional patterns of brain activity when compared to patients with poor cognitive recovery.
2. To determine whether such patterns were correlated with cognitive performance.
3. To assess the existence of prognostic factors for cognitive recovery

Hypothesis

We expected that:

1. Stroke patients will show changes relative to healthy controls in the RSNs, both in the vicinity of the lesion as well as in remote cortical areas in the injured and healthy hemisphere
2. One of the RSNs impaired in stroke patients with poor cognitive recovery will be the DMN, because it has already been associated with more successful performance in cognitive tasks.
3. Patients with poor and good cognitive recovery will show different functional connectivity patterns at three months after stroke.

Paper II

Objectives

1. To evaluate the effects of right hemispheric stroke on left hemispheric WM.
2. To investigate the microstructural mechanism underlying the eventual WM change
3. To assess the effect of structural changes on cognitive recovery of stroke patients at three months after stroke.

Hypothesis

We expected that:

1. Stroke patients with poor cognitive recovery will show more widespread WM disruption in the contralesional hemisphere.

2. Stroke patients with good cognitive recovery will have better contralesional FA integrity.
3. The better contralesional WM integrity will be related to the cognitive performance of stroke patients with good cognitive recovery.

Paper III

Objective

To examine metabolic changes in a single frontal region of white matter located in the contralesional healthy hemisphere and to associate those changes with functional, structural status and cognitive outcome.

Hypothesis

We expected that:

1. Patients will show metabolic, functional and structural alterations when compared to healthy controls.

2. Metabolite's concentrations will be related with functional, structural and cognitive measures in stroke patients.

Paper IV

Objectives

To investigate the alterations in the functional connectivity of the DMN in ischemic stroke patients during resting state condition, in order to assess more accurately DMN integration and segregation properties three months after ischemic stroke

Hypothesis

We expected that:

1. Individual seed-based functional connectivity of DMN regions will reveal important differences in resting-state functional connectivity between them
2. Those alterations will explain cognitive performance

Paper V

Objective

To perform lesion segmentation by means of Random Forest classifiers in different image acquisitions.

3. RESULTS

3.1. Study I

◆ Human Brain Mapping 00:00–00 (2014) ◆

Prognostic Value of Changes in Resting-State Functional Connectivity Patterns in Cognitive Recovery After Stroke: A 3T fMRI Pilot Study

R. Dacosta-Aguayo,[1,2] M. Graña,[2] A. Savio,[2] M. Fernández-Andújar,[1,3] M. Millán,[4] E. López-Cancio,[4] C. Cáceres,[4] N. Bargalló,[5,6] C. Garrido,[5] M. Barrios,[7] I. C. Clemente,[1,3] M. Hernández,[4] J. Munuera,[8] A. Dávalos,[4] T. Auer,[9] and M. Mataró[1]*

[1]*Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain*
[2]*Department of CCIA, Group of Computational Intelligence, University of the Basque Country UPV/EHU, San Sebastian, Spain*
[3]*Institute for Brain, Cognition and Behaviour (IR3C), Barcelona, Spain*
[4]*Department of Neurosciences, Hospital Germans Trias I Pujol, Universitat Autònoma de Barcelona, Badalona, Barcelona, Spain*
[5]*Diagnostic Center for Image, Clinic Hospital, Barcelona, Spain*
[6]*Imatge Platform of IDIBAPS, Barcelona, Spain*
[7]*Department of Methodology of Behavioral Sciences, University of Barcelona, Spain*
[8]*Institut de Diagnostic per la Imatge (IDI), Hospital Germans Trias I Pujol, Badalona, Barcelona, Spain*
[9]*MRC Cognition and Brain Sciences Unit, Cambridge, United Kingdom*

Abstract: Resting-state studies conducted with stroke patients are scarce. First objective was to explore whether patients with good cognitive recovery showed differences in resting-state functional patterns of brain activity when compared to patients with poor cognitive recovery. Second objective was to determine whether such patterns were correlated with cognitive performance. Third objective was to assess the existence of prognostic factors for cognitive recovery. Eighteen right-handed stroke patients and eighteen healthy controls were included in the study. Stroke patients were divided into two groups according to their cognitive improvement observed at three months after stroke. Probabilistic independent component analysis was used to identify resting-state brain activity patterns. The analysis identified six networks: frontal, fronto-temporal, default mode network, secondary visual, parietal, and basal ganglia. Stroke patients showed significant decrease in brain activity in parietal and basal ganglia networks and a widespread increase in brain activity in the remaining ones when compared with

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*Correspondence to: M. Mataró, Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Passeig de la Vall d'Hebron, 171, 08035 Barcelona, Spain. E-mail: mmataro@ub.edu

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healthy controls. When analyzed separately, patients with poor cognitive recovery ($n = 10$) showed the same pattern as the whole stroke patient group, while patients with good cognitive recovery ($n = 8$) showed increased activity only in the default mode network and fronto-temporal network, and decreased activity in the basal ganglia. We observe negative correlations between basal ganglia network activity and performance in Semantic Fluency test and Part A of the Trail Making Test for patients with poor cognitive recovery. A reverse pattern was observed between frontal network activity and the abovementioned tests for the same group. *Hum Brain Mapp 00:000–000, 2014.*

Key words: ischemic stroke; resting state; fMRI; probabilistic independent component analysis; interhemispheric balance; cognitive recovery

INTRODUCTION

Acute ischemic stroke is the second most common cause of death worldwide and a major cause of disability in the elder population [Gorelick et al., 2011]. Mechanisms underlying functional recovery after stroke have not been clarified so far. Some of the most relevant factors cited are: vascular repair, immunomodulation, endogenous neurogenesis [Bliss et al., 2010; Horie et al., 2011; Liu et al., 2008] and the rewiring of surviving brain circuits enabling the healthy brain to compensate for the loss of functionality corresponding to the damaged area [Benowitz and Carmichael, 2010; Dancause, 2006; Murphy and Corbett, 2009].

Functional imaging and stimulation studies in patients have shown a rewiring of the brain circuits after stroke which, at least in the first few weeks, indicates recruitment of both ipsi- and contralesional areas suggesting that this remapping is caused by local and long distant changes in axonal sprouting and dendritic arborization [Gonzalez et al., 2003].

Resting-state functional magnetic resonance imaging (rs-fMRI) demonstrates task unrelated brain networks, such as the default mode network (DMN), and networks of functionally related areas, such as the motor, visual, auditory, and attentional networks [Biswal et al., 2010, Buckner et al., 2009]. These resting state networks (RSNs) have shown a high reproducibility across subjects, time and research sites [Damoiseaux et al., 2006], and have been proved as surrogate biomarkers of neurological diseases (including schizophrenia, autism and Alzheimer's disease).

Few resting-state functional connectivity studies have been conducted with stroke patients so far, and most of them have focused on the study of motor recovery [Carter et al., 2010; Golestani et al., 2012; Park et al., 2011]. These studies have mainly investigated disruptions in interhemispheric resting-state functional connectivity of attentional and motor networks over a priori selected regions [Calautti et al., 2007; Corbetta et al., 2005; Cramer and Crafton, 2006; Muellbacher et al., 2002]. Therefore, they considered some networks while discarding others that may be equally important for the prognosis of stroke patients. These studies have shown that neuroplasticity occurs, so that focal injury may even result in interhemispheric changes. Some studies suggest that the restoration of perilesional networks, which have escaped irreversible damage, is the principal contribution to recovery, and that the role of the contralesional hemisphere is subsidiary, because it is recruited only when the left hemisphere is severely damaged [Heiss and Thiel, 2006]. However, fMRI studies with language tasks performed very early after the stroke event suggest that activation in the intact right hemisphere is related to the long-term outcome [Crinion and Leff, 2007].

The study reported in this paper investigates the resting-state functional connectivity patterns of the whole brain on functional MRI captured three months after a focal stroke event, using the probabilistic independent component analysis (pICA) approach [Beckmann et al., 2005]. pICA does not need a priori definition of a seed region, allowing unbiased exploration of the association between the RSNs and patient's cognitive improvement. Study hypotheses are (1) stroke patients will show changes relative to healthy controls in the RSNs, both in the vicinity of the lesion as well as in remote cortical areas in the injured and healthy hemisphere; (2) one of the RSNs impaired in stroke patients with poor cognitive recovery will be the DMN, because it has already been associated with more successful performance in cognitive tasks [Anticevic et al., 2012], and (3) patients with poor and good cognitive recovery will show different functional connectivity patterns at three months after stroke. To our knowledge, this is the first study describing the functional reorganization of brain activity patterns after stroke in relation to cognitive recovery.

MATERIALS AND METHODS

Participants

From September 2010 to May 2012, 26 patients were admitted to the acute stroke unit of the Germans Trias I Pujol University Hospital (Badalona, Spain). Eighteen of

them fulfilled the following criteria: (1) Right-handedness; (2) First focal ischemic stroke in the territories of the anterior, middle, or posterior cerebral arteries (ACA, MCA, PCA, respectively) without significant hemorrhagic transformation; (3) Age between 40 and 75 years; (4) Absence of severe aphasia (fourteenth scoring item of National Institute of Health Stroke Scale (NIHSS) ≤ 1); (5) Absence of alcohol or drug abuse, psychiatric comorbidities, or severe visual or hearing loss; (6) Absence of contraindications to undergo MRI. Eighteen healthy volunteers from the Barcelona Asymptomatic Intracranial Atherosclerosis study [López-Cancio et al., 2011; Miralbell et al., 2012] matched by age, sex, education, and handedness (Edinburgh Handedness Inventory [Olfield, 1971]) were recruited as the control group. None had a previous history of neurological or psychiatric diseases and brain scans were reported as normal. The study was approved by the ethics committee of the University of Barcelona. All participants received explanation of study procedures and gave their written consent to participate in the study, which was conducted according to the provisions of the Helsinki declaration.

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago), version 17.0 for Windows. The distributions of demographic variables were tested for normality by the Shapiro-Wilk test. We assessed group differences using parametric (t test) and nonparametric (Mann-Whitney test) independent sample tests for continuous variables and Chi-Square or Fisher's exact test for categorical variables. The threshold for two-sided statistical significance was set at $P < 0.05$.

Neuropsychological Assessment and Grouping Criteria Regarding Cognitive Recovery

Information about previous cognitive impairment was assessed by a trained neuropsychologist with the short version of the Spanish Informant Questionnaire on Cognitive Decline in the Elderly [Morales-González et al., 1992] and the Frontal Behavioral Inventory [Kertesz et al., 1997] on admission day. Premorbid Intelligence was estimated using the vocabulary subtest of Wechsler Adults Intelligence Scale (WAIS-III-R) [Wechsler, 1999] at three months poststroke. Patients underwent neuropsychological examinations both within 72 h after the stroke (acute phase) and after 3 months (subacute phase). We selected a test battery that covered a variety of possible cognitive manifestations of vascular brain injury. Attentional abilities were explored by the Digit Span Forward Test (WAIS-III-R) [Wechsler, 1999], the subtest of attention extracted from the Montreal Cognitive test [Nasreddine et al., 2005], and the Line Cancellation Test [Strauss, 2006]. Executive abilities were assessed with the Digit Span Backwards from WAIS-III-R [Wechsler, 1999], part B of Trail Making Test [Strauss, 2006], Phonological fluency (letter P) [Strauss, 2006], and Semantic fluency test (animals) [Strauss, 2006]. Language abilities were assessed listening to patient spontaneous speech (talking briefly about his/her health problems), and with the following tests: the repetition and understanding items extracted from the Mental Status Examination in Neurology [Strub and Black, 2000], the writing one sentence item extracted from the Mini Mental State Examination Test (MMSE) [Folstein, 1983], and the short version (15-items) of the Boston Naming Test [Kaplan et al., 1983]. Premotor abilities were assessed with Luria's sequences test, Rhythms subtest extracted from the Montreal Cognitive test [Nasreddine et al., 2005], and interference and inhibitory control subtest extracted from the Frontal Assessment Battery [Dubois et al., 2000]. Speed and visuomotor coordination were assessed with the part A of the Trail Making Test [Strauss, 2006] and the grooved pegboard test (GPT) [Ruff and Parker, 1993]. Neuropsychological examinations also included the MMSE [Folstein, 1983), as a global cognitive test and the Geriatric Depression Scale [Yesavage et al., 1982].

The neuropsychological examination at the acute phase was time-bound to 60 min. If the patient was fatigued, a pause was introduced. The second subacute cognitive examination lasted about 2 h. We only considered the scores of tests included in both examinations. Healthy controls received the same neuropsychological assessment as patients at the acute phase.

Stroke patients were split into two groups according to their level of cognitive recovery between acute and subacute phase by the following process. First, a paired t-test was conducted over the cognitive test scores to select the tests with overall significant patient improvement. Second, a subject was categorized as a good cognitive recovery patient if he/she had achieved a minimum improvement of 1.5SD of the scores in at least three of the selected tests. Some patients achieve cognitive normalization.

Lesion Analysis

Infarct depth (cortical, subcortical or both), laterality (left/right), and vascular territory involved were determined within the first 24 h employing computed tomography and/or magnetic resonance (MRI). Lesion volume was calculated in the subacute phase as the product of the three largest lesion diameters, along the three orthogonal axes, divided by 2 [Sims et al., 2009]. Maps of the lesion distribution for each stroke group are shown in Figure 1.

Image Analysis

fMRI acquisition

fMRI data were acquired in the subacute phase using a Siemens Magneto TIM Trio operating at 3 Tesla at the Image Platform of IDIBAPS, Centre de diagnostic per la Imatge from Hospital Clínic, Barcelona. We used a 32-channel phased-array head coil with foam padding and head phones to restrict head motion and scanner noise. Resting-state blood oxygen level-dependent data were acquired using an echo-planar imaging sequence (repetition time = 2 s; echo time = 29 ms; flip angle = 80°; in plane spatial resolution = 3 $\times$ 3 mm^2; field of view = 240 $\times$ 240 mm^2; slice thickness = 4

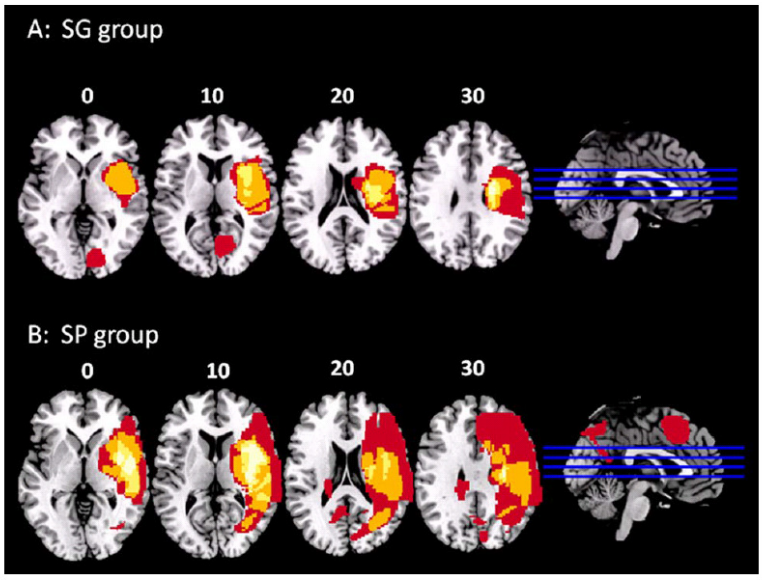

Figure 1.
A: Frequency distribution of the lesions for patients with good cognitive recovery. B: Frequency distribution of the lesions for patients with poor cognitive recovery. Images are depicted in radiological convention (R-L).

mm; number of slices = 32; number of volumes = 240; acquisition time = 8 min). Participants were instructed to lie still with their eyes closed but remaining awake.

fMRI preprocessing

The analysis was conducted using pICA as implemented in FSL 4.1.9 (FMRIB Center, Department of Clinical Neurology, University of Oxford, www.fmrib.ox.ac.uk/fsl). Data preprocessing consisted of the removal of the first 6 volumes to ensure saturation and adaptation of the subjects to the environment leaving 234 volumes for further analysis, removal of nonbrain structures using Brain Extraction Tool, motion correction using MCFLIRT, high-pass filtering with a frequency cut-off at 160 s, low-pass temporal filtering (5.6 s), spatial smoothing using a Gaussian kernel of full-width half-maximum of 5 mm, intensity normalization, and affine linear registration to the MNI152 standard template. Absolute head movement was below 1.5 mm for all subjects.

fMRI analysis

pICA identified fifty-one independent components. We discarded components representing known artifacts, such as motion, high-frequency noise, or venous pulsation [Beckmann et al., 2005; De Luca et al., 2006], components not located mainly in gray matter, and components not resulting in compact clusters [De Martino et al., 2007]. Finally, components of interest were selected by means of spatial correlation with freely available standard templates of RSNs (http://www.nitrc.org/projects/fcon_1000/) [Biswal et al., 2010], which left us with eighteen anatomically and functionally relevant RSNs.

Subject-specific statistical maps for the 18 RSNs were created using a dual regression procedure [Filippini et al., 2009] that involves spatial and temporal regression. Then, we estimated differences between the stroke and the healthy control group. The volumetric map of each RSN across subjects was collected into a 4D file to be evaluated for between-group differences using a nonparametric permutation test (5,000 permutations) [Nichols and Holmes, 2002]. For each RSN, the resulting statistical map was thresholded at $P = 0.05$ and corrected for Family Wise Errors (FWE) employing threshold-free cluster enhancement (TFCE). Only six RSNs showed significant between-group difference. Moreover, each of these networks was significantly correlated ($r > 0.45$) with one of the standard template RSNs [Biswal et al., 2010]. We labeled these networks as (1) frontal network ($r = 0.57$), (2) Fronto-Temporal network ($r = 0.57$); (3) DMN ($r = 0.55$); (4) secondary network ($r = 0.46$); (5) basal ganglia network ($r = 0.57$), and (6) parietal network ($r = 0.53$). Next, we

TABLE I. Demographic and clinical data

	Healthy controls ($n = 18$)	Stroke patients ($n = 18$)		SG ($n = 8$)	SP ($n = 10$)	(SG–SP)
Sociodemographic factors			*P*			*P*
Age (years)	62.61 ± 6.01	63.94 ± 8.26	0.583	61.50 ± 10.14	65.90 ± 6.26	0.274
Women	7 (38.88%)	5 (27.77%)	0.480	2 (25%)	3 (30%)	1.00[3]
Education (years)	7.33 ± 4.1	7.67 ± 4.24	0.812	6.63 ± 3.29	8.50 ± 4.88	0.367
Vocabulary subtest	37.78 ± 7.9	34.61 ± 11.34	0.337	35.25 ± 9.18	34.10 ± 13.29	0.838
Handedness (EHI)	95.56 ± 13.5	97.50 ± 4.29	0.564	97.50 ± 4.63	97.50 ± 4.25	1.000
GDS	2.17 ± 3.42	4.50 ± 4.09	0.072	4.50 ± 3.55	4.50 ± 4.67	1.000
Vascular risk factors						
Hypertension	8 (44.4%)	13 (72.22%)	0.317	5 (62.50%)	8 (80%)	0.608
Dyslipidemia	9 (50%)	10 (55.55%)	0.739	4 (50.00%)	6 (60%)	0.670
Diabetes mellitus	1 (5.55%)	7 (38.88%)	**0.041**	4 (50.00%)	3 (30%)	0.630
Smoking	6 (33.33%)	3 (16.66%)	0.443	1 (12.50%)	2 (20%)	1.000
Alcohol intake	9 (50%)	6 (33.33%)	0.317	1 (12.50%)	5 (50%)	0.152

Independent T-Test for continuous variables. Chi-Square test and Fisher's exact test for categorical variables. EHI: Edinburgh Handedness Inventory; GDS: Geriatric Depression Scale; *P*: *P* value for two group comparisons; SG: Stroke patients with good recovery; SP: Stroke patients with poor recovery; Alcohol intake. Diagnosis for a particular vascular risk factor was based in clinical history or use of medication for this particular condition at the time of the clinical assessment.

investigated whether these differences were more characteristic to patients with poor cognitive recovery than to patients with good cognitive recovery by means of separate comparison with the healthy control group.

We also entered cognitive scores of test showing significant acute-to-subacute difference (see Section "Neuropsychological assessment and grouping criteria regarding cognitive recovery") into the General Linear Model, Analysis of Covariance (ANCOVA), as covariates of interest to examine whether these cognitive scores showed association with between-group differences of brain activity at rest. All analyses were thresholded at $P = 0.05$ and corrected for FWE employing TFCE. Anatomical labeling of every result was performed with reference to the Harvard-Oxford cortical and subcortical structural atlases (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases).

RESULTS

Sample Characteristics

Demographic and clinical data are given in Table I. There was no significant difference between stroke patients and healthy controls, except for a higher frequency of diabetes in the stroke group. Since we did not focused on diabetes in this study, we considered it and its nonspecific effect(s) rather as a "confounding factor" regressing it out when performing group analyses. Table II contains stroke severity at baseline (NIHSS scale) and characteristics of the ischemic lesions (location, brain hemisphere, volume, and vascular territory). Most patients had lesions in the right hemisphere (15/18) and all infarcts were in the territory irrigated by the MCA with the exception of 2 infarcts located in the PCA territory. Lesions affected one or more of the following regions, ordered by number of subjects affected basal ganglia ($n = 8$), centrum semiovale and temporal lobes ($n = 7$) corona radiata ($n = 5$), insula ($n = 5$), and the frontal lobe ($n = 5$). Comparing the two groups of cognitive recovery, no statistical difference was found in lesion volume (good cognitive recovery: 12.90 cm^3 [1.13 – 48.23]; poor cognitive recovery: 17.99 cm^3 [9.80 – 36.00]) ($Z = -0.446$; $P = 0.656$), affected hemisphere or stroke severity at baseline measured by the NIHSS scale (good cognitive recovery: 9.50 ± 6.437; poor cognitive recovery: 10.70 ± 7.027; $t = -0.7373$ (16), $P = 0.714$].

Neuropsychological Characteristics

Stroke group in general demonstrated a significant acute-to-subacute improvement in the following cognitive tests: MMSE, SFT (naming animals in one minute), Boston Naming Test, TMTA, and the GPT (Table III). We have to emphasize that improvement means increase (score) in the first three and decrease (time to complete) in the last two tests.

There was no significant difference between the two stroke groups in any cognitive test evaluated in the acute phase. In the subacute phase however, patients with good cognitive recovery performed significantly better in the TMTA ($P = 0.053$) and the number of omissions in the attention subtest ($P = 0.052$) than patients with poor cognitive recovery (data not shown).

fMRI Analysis

Compared with the healthy control group, the stroke group showed significant alteration in the following six RSNs: increased brain activity in (1) frontal network; (2) fronto-temporal network; (3) DMN, (4) secondary network,

TABLE II. Clinical and neuroimaging characteristics of the stroke patients

Patients	Baseline severity (NIHSS)	Infarct side and location	Infarct volume (cm^3)	Vascular distribution
Stroke patients with good cognitive recovery				
1	1	R. frontal cortex	0.1	MCA_ACA (M2-M3)
2	2	L. precentral cortex + CR	0.3	MCA (M2-M3)
3	17	R. basal ganglia	8.2	MCA (M1)
4	9	R. basal ganglia + CR	17.6	MCA (M1)
5	14	R. basal ganglia + insula + CR	36.0	MCA (M1)
6	4	R. occipital cortex + centrum semiovale	53.2	PCA (P2)
7	13	R. insula + temporal and frontal cortex	124.0	MCA-ACA (M2)
8	16	R. basal ganglia	3.6	MCA
Stroke patients with poor cognitive recovery				
9	5	R. frontal and parietal cortex + premotor cortex + IC	4.6	MCA (M2)
10	22	L. basal ganglia	9.2	MCA (M1)
11	3	L. centrum semiovale	10.0	MCA (M1)
12	7	R. insula + inferior frontal cortex	14.5	MCA (M2)
13	5	R. temporo-parietal cortex	15.0	MCA (M2-M3)
14	7	R. temporo-occipital cortex	20.9	PCA
15	21	R. frontal cortex + lenticulate	24.0	MCA-ACA (M1)
16	13	R. temporo-parietal cortex and IC	34.0	MCA (M1)
17	7	R. basal ganglia + CR	42.0	MCA-ACA (M1)
18	17	R. temporo-parietal + basal ganglia	175.0	MCA (M1)

Abbreviations: CR: corona radiata; IC: intern capsule; L: left; M1: first segment of the MCA; M2: second segment of the MCA; M3: third segment of the MCA; MCA: middle cerebral artery; NIHSS: National Institute of Health Stroke Scale; P2: second segment of the PCA; PCA: posterior cerebral artery; R: right.

and decreased brain activity in (5) Basal Ganglia network, and (6) parietal network (Fig. 2, first column)

All abovementioned alterations could be detected when comparing patients with poor cognitive recovery separately to healthy control group (Fig. 3, second column). However, patients with good cognitive recovery demonstrated significant increase of activity only in the Fronto-Temporal and the DMN, as well as significant decrease of activity in the Basal Ganglia network when compared to healthy control group (Fig. 3, third column).

Relationship Between RSNs Activity and Performance on Cognitive Tests

Whole-brain ANCOVA Comparing the activity of the significant RSNs in the group of patients with poor cognitive recovery and the healthy control group we found: (a) a lower correlation between Basal Ganglia activity change and SFT score and a higher correlation between Basal Ganglia activity and TMTA time (Table IV), (b) a higher correlation between Frontal activity and SFT score, and (c) lower correlation between Frontal activity and TMTA time (Table V).

DISCUSSION

This study aims to identify resting-state functional connectivity patterns characterizing ischemic stroke in subacute phase and their relations with cognitive recovery. Our pICA analysis identified eighteen relevant components matching the standard RSN reported in healthy subjects [Beckmann et al., 2005]. From these eighteen RSNs, only six showed significant between-group differences. In comparison with the healthy control group, the stroke group showed increased activity in the Frontal, Fronto-Parietal, DMN and Secondary Visual networks, and decreased activity in the Parietal and Basal Ganglia networks. These alterations suggest that stroke event affected not only the lesioned hemisphere but the contralesional hemisphere too. Alterations were stronger in stroke patients with poor cognitive recovery, whereas stroke patients with good recovery only showed minimal alterations in three networks (DMN, Fronto-Temporal and Basal Ganglia networks).

RSN Connectivity and Motor Recovery in Stroke Populations

Resting-state studies have already been carried out in stroke populations in relation to motor recovery investigating interhemispheric resting activity as a measure of normal function [Carter et al., 2010; Golestani et al., 2012] and the activity of the ipsilesional primary motor cortex [Park et al., 2011]. They have found that reduced interhemispheric activity at rest is associated with motor deficits [Golestani et al., 2012], recovery of a normal interhemispheric coherence is important for a normal function, and motor impairments are not related to interhemispheric

TABLE III. Neuropsychological tests scores at acute and subacute phase for the stroke group

	ACUTEPHASE (within 72 h; $n = 18$)	SUBACUTEPHASE (at 3 months; $n = 18$)	t (df)	P	r
General cognitive function					
MMSE	25.72 ± 3.23	27.22 ± 2.57	−3.040 (17)	**0.007**	**0.35**
Sustained attention					
MoCA subtest	10.11 ± 1.27	10.33 ± 1.28	−0.776(17)	0.449	–
Digit span forward (WAIS-III)	4.61 ± 1.09	4.72 ± 1.28	−0.46 (17)	0.651	–
Working memory					
Digit span backwards (WAIS-III)	3.22 ± 1.35	3.44 ± 1.04	−0.940(17)	0.361	–
Premotor functions					
Luria' sequences (/5)	3.61 ± 2.30	4.22 ± 1.66	−1.77(17)	0.094	–
Rhythms subtest (/10)	6.00 ± 2.91	6.83 ± 3.07	−1.567(17)	0.135	–
Interference and inhibitory control (/3)	2.22 ± 1.00	2.50 ± 0.85	−1.426(17)	0.172	–
Verbal fluency					
Letter (P)	7.33 ± 4.25	8.83 ± 4.69	−1.775(17)	0.941	–
Semantic (animals)	10.06 ± 5.23	13.83 ± 4.54	−3.688(17)	**0.002**	**0.44**
Language					
Boston naming test	9.11 ± 3.06	10.83 ± 2.41	−3.511(17)	**0.003**	**0.42**
Understanding (/6)	5.83 ± 0.38	5.94 ± 0.23	−1.458(17)	0.163	–
Psychomotor speed (s)					
Trail making test A (s)	203.89 ± 101.15	107.67 ± 85.48	5.024(17)	**<0.001**	**0.60**
Grooved pegboard test (preferred hand; s)	274.27 ± 74.01	108.39 ± 72.06	7.214(17)	**<0.001**	**0.75**
Visuospatial skills					
Line cancellation test (/36)	30.78 ± 10.38	31.94 ± 7.91	−0.557(17)	0.585	–

Score values are reported as means ± standard deviations for each test (Paired Samples T Test).
Abbreviations: df: degrees of freedom; FBI: frontal behavioral inventory; GDS: geriatric depression scale; MMSE: mini mental state examination; MoCA: montreal cognitive assessment; S-IQCODE: short informant questionnaire on cognitive decline in the elderly.
$r = 0.10$ (small effect: effect explains 1% of total variance).
$r = 0.30$ (medium effect: effect accounts for 9% of the total variance).
$r = 0.50$ (large effect = effect accounts for 25% of the total variance).

connectivity among attentional-related areas [Carter et al., 2010]. Furthermore, an increased asymmetry of brain activity at rest is attributed to rearrangements of activation over the bihemispheric sensoriomotor cortex [Park et al., 2011]. These studies were restricted to an a priori selection of specific regions of interest such us the somato-motor and the attentional network. To supplement these findings, our study focuses on recovery of cognitive functions during the first three months after stroke, which depend on the integration and segregation of several distinct brain networks requiring the study of the brain as a whole.

RSN Changes in Stroke

There is an on-going debate in the literature regarding the role of the contralesional hemisphere activity in stroke recovery. Considering the motor function, stroke patients typically show pathologically enhanced neural activity in a number of areas both in the lesioned and in the contralesional hemisphere [Grefkes et al., 2008]. It is pointed out that, early after stroke, the lesioned hemisphere cannot provide transcallosal inhibition, so the other hemisphere becomes hyperactive. These points the research efforts towards two hypotheses: first, that stroke recovery might encompass both degenerative phenomena and mechanisms of plasticity, [Cramer et al., 2008]; and second, that early after stroke contralesional recruitment may be a compensatory adaptation. The second hypothesis explains the multiplicity of deficits following a focal lesion, and the complexity of the neuroplasticity processes that underlie functional brain organization. According to it, in our study, stroke patients with poor cognitive recovery showed increased neural activity at rest in the left (contralesional) hemisphere for the frontal, fronto-temporal, secondary visual, and the anterior part of DMN.

The brain areas where we found increased activity at rest are related to cognitive functions impaired in our stroke patients, such as executive, attentional, and motor functions (GPT, TMTA, and SFT): the paracingulate cortex involved in top-down and bottom-up control to other areas [Allman et al., 2012]; the operculum performs task control [Dosenbach et al., 2008] and switches between the executive control network and the DMN [Seeley et al., 2007]; the anterior insula has been implicated in the salience network, which plays a role in initiation, maintenance and adjustment of attention, and the integrating information [Nelson et al., 2010]; and finally, the frontal pole contributes to inductive, analogical or relational reasoning, as well as prospective memory [Ramnani et al.,

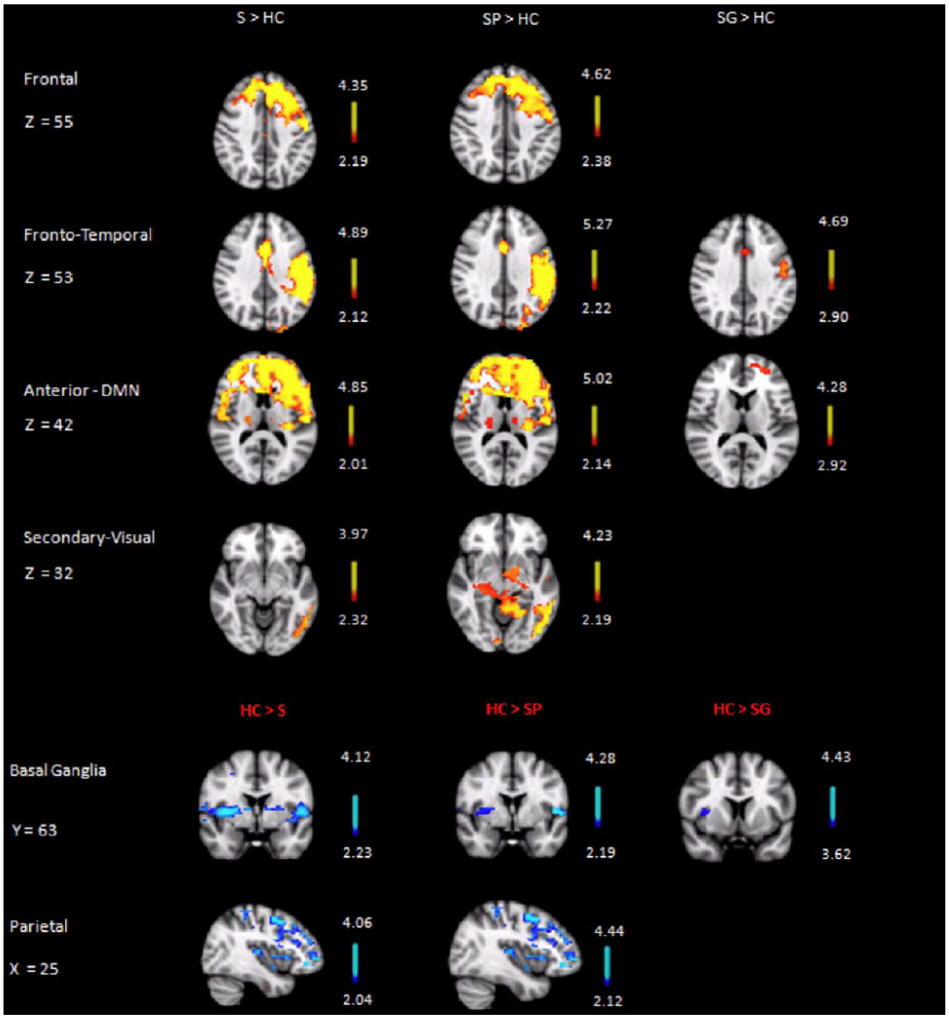

Figure 2.

Axial(Frontal, Fronto-Temporal, DMN and Secondary Visual), coronal (Basal Ganglia), and sagittal (Parietal) slices (MNI template) showing significant between-group differences in resting activity. HC: healthy control group; S: whole stroke group; SP: stroke patients with poor cognitive recovery; SG: stroke patients with good cognitive recovery. Images are depicted in radiological convention (R-L).

2004]. Interestingly, right precuneous cortex in stroke patients with poor cognitive recovery showed both a decreased activity in the Parietal network and an increased activity in the DMN. These findings support the hypothesis that the lesion does not only modify the activity of individual regions but it also affects functional networks as a whole, involving even regions located further from the lesion. Finally, stroke patients showed higher activity at rest in several areas of the secondary visual network. These areas are responsible for visuospatial processing and their lesion may induce neglect [Saalmann et al., 2007]. Most patients with poor cognitive

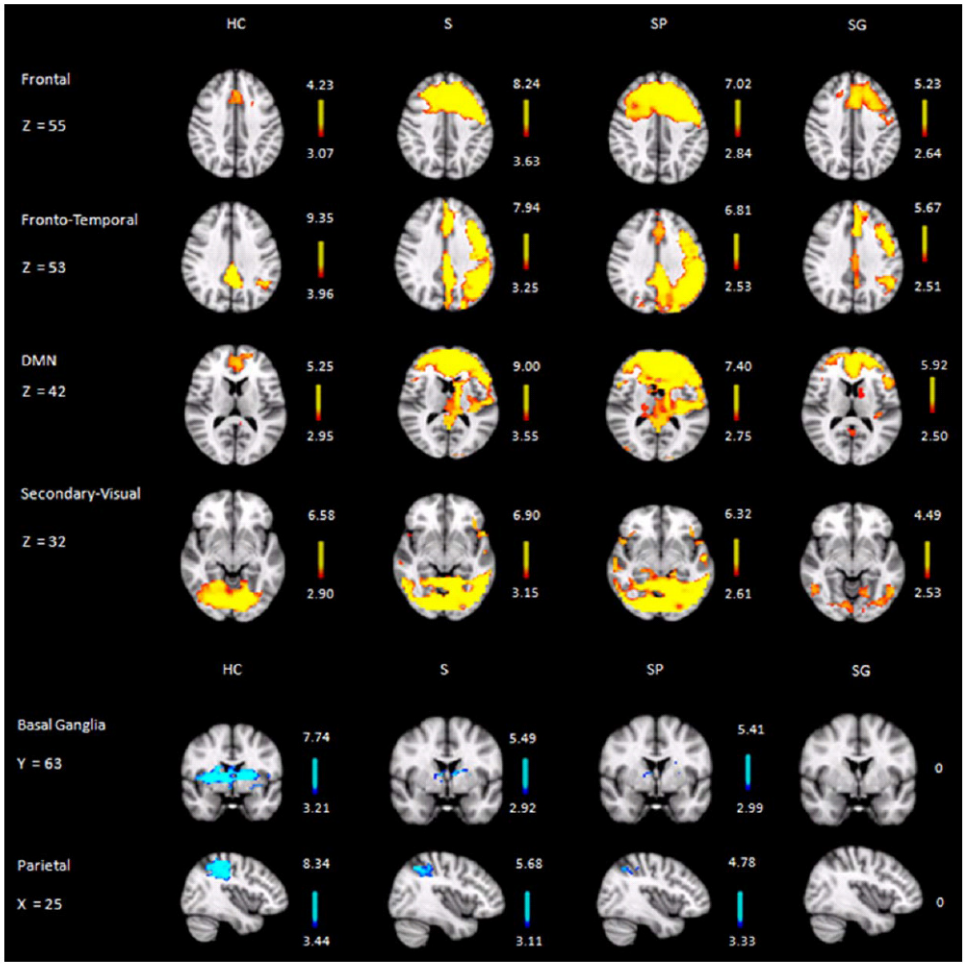

Figure 3.
Axial(Frontal, Fronto-Temporal, DMN and Secondary Visual), coronal (Basal Ganglia), and sagittal (Parietal) slices (MNI template) showing significant resting activity. HC: healthy control group; S: whole stroke group; SP: stroke patients with poor cognitive recovery; SG: stroke patients with good cognitive recovery. Images are depicted in radiological convention (R-L).

recovery presented neglect in the acute phase so that we hypothesize that recovery during the first three months is related to a compensative over-activity in contralesional areas. Stroke patients also showed decreased activity in the Basal Ganglia and in the Parietal networks. The former is related to psychomotor speed and attention, while the latter has already been described above.

RSN Changes as Compensatory Mechanisms

RSN changes by themselves could be interpreted as brain disturbances due to stroke. The fact that they were stronger in stroke patients with poor cognitive recovery also supports this hypothesis. However, a larger portion of brain activity at rest was in the left hemisphere (contralesional in

TABLE IV. Clusters of the basal ganglia network showing significant group-difference in correlations with the scores of the semantic fluency test and the trail making test, part A

	MNI coordinates					
Anatomical region	X	Y	Z	Voxels mm^3	$P \leq 0.05$ corrected	
Semantic fluency test						
Healthy control > poor cognitive recovery group						
					Z	P
R. paracingulate gyrus	21	43	18	3032	4.29	0.002
R. angular gyrus	10	18	30	83	3.61	0.021
L. frontal orbital cortex	28	35	14	20	3.46	0.032
L frontal orbital and frontal operculum cortex	33	38	17	12	3.35	0.043
L. cingulate gyrus, posterior division and precuneus cortex	23	20	21	6	3.33	0.046
Trail making test, part A						
Poor cognitive recovery group > healthy control						
					Z	P
L. postcentral gyrus	10	24	33	257	3.95	0.007

Note: Correlations between-group contrasts are cluster corrected for multiple comparison using randomize method ($P < 0.05$TFCE corrected; z-threshold of 2.3; Critical z for design efficiency calculation set fmri = 5.3).
Abbreviations: L: left; R: right.
Reported z values are two-sided.

the majority of the patients). This pattern of activity is in agreement with results obtained from stroke recovery research both in animal models and clinical patients showing that widespread changes in activity patterns can even extend to the unaffected hemisphere [Carmichael and Chesselet, 2002; Nelles et al., 1999; Schaechter and Perdue, 2008]. These altered circuits work within the intact contralesional (opposite to stroke) hemisphere [Biernaskie et al., 2005], leading to less lateralized (less crossed) activation.

Most importantly, the magnitude of these changes correlated well with cognitive performance: increased Frontal activity having a positive correlation with cognitive tests, and decreased Basal Ganglia activity having a negative correlation with cognitive tests. The (not significantly) weaker correlations in patients with good cognitive recovery, and the significantly weaker correlation or reverse correlation in healthy controls also support their compensatory nature. In stroke patients with poor cognitive recovery, they seem to have a negative effect on performance probably due to disruption of the interplay between the brain areas. When some of those brain areas are damaged in stroke patients, they compensate these damages via shifting the functional connectivity to favor unaffected brain areas. Therefore, patients demonstrating a larger shift in functional connectivity (i.e., better plasticity) provide a better cognitive performance.

However, these changes seem to play no role in recovery; they actually diminish to allow coming back to "normal" brain activity. That explains their weaker presence in patients with good cognitive recovery. This is in agreement with other studies linking improved recovery with regaining the"normal" brain activity [Dijkhuizen et al., 2012; Ramos-Cabrer, 2010; van Meer, 2010, 2011].

Methodological Considerations

Rs-fMRI is becoming an excellent tool for clinical studies, because it does not impose attentional demands or cognitive burdens on the patient. Although rs-fMRI has already been employed in other stroke studies, they do not take into account the whole range of brain networks as this study. Moreover, we not only employed a detailed neuropsychological evaluation covering the whole cognitive spectrum in acute stroke; but also investigated how they are associated with recovery. Finally, the study design allows examining how resting-state brain activity relates to recovery, and whether rs-fMRI has any predictive value regarding clinically relevant outcome. However, our sample is small due to our strict criteria. This may also decrease the sensitivity, and restrict the generalizability of our preliminary results.

Finally, although we did not reported statistical significant differences regarding the volume of ischemic lesions, their size was heterogeneous. This is a limitation because whereas recovery after a small ischemic lesion may involve preserved peri-infarct tissue with function similar to the infarcted tissue [Brown et al., 2009; Murphy and Corbett, 2009], for recovery after a large ischemic lesion, tissue with similar function may only be found at more distant sites, such as the premotor cortex (for motor cortex stroke) [Dancause et al., 2005; Frost et al., 2003] or regions in the unaffected contralateral hemisphere [Biernaskie et al., 2005] where structural remodeling has been observed [Takatsuru et al., 2009].

Summarizing, our results confirm our hypotheses and may expand our understanding of brain changes occurring after stroke, as well as stimulate new researches on lesion-induced network plasticity changes and fMRI biomarkers of recovery/progression not only in stroke but also in vascular cognitive impairment and vascular dementia.

TABLE V. Clusters of the frontal network showing significant group-difference in correlations with the scores of the semantic fluency test and the trail making test, part A

Anatomical region	MNI coordinates X	Y	Z	Voxels (mm^3)	$P \leq 0.05$ corrected Z	P
Semantic fluency test						
Poor cognitive recovery group > healthy control						
L. angular gyrus	34	18	28	284	3.95	0.007
R. posterior cingulate gyrus and precuneous cortex	20	19	25	178	3.78	0.012
R. angular gyrus	12	16	28	24	3.69	0.016
Trail making test, part A						
Healthy control > poor cognitive recovery group						
L. angular gyrus	35	17	26	1023	3.68	0.017
L. lateral occipital cortex, posterior division	14	11	22	41	3.38	0.04

Note: Correlations between-group contrasts are cluster corrected for multiple comparisons using randomize method ($P < 0.05$ TFCE corrected; z-threshold of 2.3; Critical z for design efficiency calculation set fmri = 5.3).
Abbreviations: L: Left; R: Right.
Reported z values are two-sided.

CONCLUSION

Brain connectivity changes is stroke patients have been already described in task related fMRI studies and in a few resting-state functional connectivity studies focusing on specific networks. Our less restricted study also demonstrated that these changes affect several brain networks, which not only explains the multiplicity of the deficits following a focal lesion but may also indicate compensatory brain plasticity. As a consequence, they are more pronounced in patients with poor cognitive recovery, whereas patients with good cognitive recovery show "normalization" of these compensatory changes. More importantly, there are strong correlations between functional connectivity changes and cognitive recovery further supporting the relevance of the study of resting-state functional data.

Our results suggest that resting-state fMRI provides information for cognitive recovery prognosis and could be a potential biomarker in stroke patients detecting early neural dysfunction and compensatory mechanisms prior to brain atrophy.

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3.2. Study II

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PLOS ONE

Structural Integrity of the Contralesional Hemisphere Predicts Cognitive Impairment in Ischemic Stroke at Three Months

Rosalia Dacosta-Aguayo[2], Manuel Graña[2], Marina Fernández-Andújar[1,4], Elena López-Cancio[3], Cynthia Cáceres[3], Núria Bargalló[5,6], Maite Barrios[7], Immaculada Clemente[1,4], Pere Toran Monserrat[9], Maite Alzamora Sas[9], Antoni Dávalos[3], Tibor Auer[8☯], Maria Mataró[1,4*☯]

1 Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain, **2** Group of Computational Intelligence, University of the Basque Country UPV/EHU, San Sebastian, Spain, **3** Department of Neurosciences, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Barcelona, Spain, **4** Institute for Brain, Cognition and Behaviour (IR3C), Barcelona, Spain, **5** Diagnostic Center for Image, Clinic Hospital, Barcelona, Spain, **6** Imatge Platform of IDIBAPS, Barcelona, Spain, **7** Department of Methodology of Behavioral Sciences, University of Barcelona, Spain, **8** MRC Cognition and Brain Sciences Unit, Cambridge, England, **9** Primary Healthcare Research Support Unit Metropolitana Nord, Institut Universitari d'Investigació en Atenció Primària (—IDIAP) Jordi Gol, Santa Coloma de Gramenet, Spain

Abstract

After stroke, white matter integrity can be affected both locally and distally to the primary lesion location. It has been shown that tract disruption in mirror's regions of the contralateral hemisphere is associated with degree of functional impairment. Fourteen patients suffering right hemispheric focal stroke (S) and eighteen healthy controls (HC) underwent Diffusion Weighted Imaging (DWI) and neuropsychological assessment. The stroke patient group was divided into poor (SP; n = 8) and good (SG; n = 6) cognitive recovery groups according to their cognitive improvement from the acute phase (72 hours after stroke) to the subacute phase (3 months post-stroke). Whole-brain DWI data analysis was performed by computing Diffusion Tensor Imaging (DTI) followed by Tract Based Spatial Statistics (TBSS). Assessment of effects was obtained computing the correlation of the projections on TBSS skeleton of Fractional Anisotropy (FA) and Radial Diffusivity (RD) with cognitive test results. Significant decrease of FA was found only in right brain anatomical areas for the S group when compared to the HC group. Analyzed separately, stroke patients with poor cognitive recovery showed additional significant FA decrease in several left hemisphere regions; whereas SG patients showed significant decrease only in the left genu of corpus callosum when compared to the HC. For the SG group, whole brain analysis revealed significant correlation between the performance in the Semantic Fluency test and the FA in the right hemisphere as well as between the performance in the Grooved Pegboard Test (GPT) and theTrail Making Test-part A and the FA in the left hemisphere. For the SP group, correlation analysis revealed significant correlation between the performance in the GPT and the FA in the right hemisphere.

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* E-mail: mmataro@ub.edu

☯ These authors contributed equally to this work.

Introduction

Stroke and cerebrovascular disease is a major cause of mortality and disability worldwide [1]. Approximately 64% of patients who have experienced stroke have some degree of cognitive impairment and up to a third of them develop dementia [2].

With cerebral ischemia, reductions in cerebral blood flow disrupt energy metabolism resulting in perturbation of ionic pumps and disruption of ionic homeostasis [3]. Cytotoxic edema occurrence produces both a decrease in the extracellular volume fraction and changes in membrane permeability [4]. These events reduce white matter (WM) integrity locally, at the primary lesion location, due to tissue damage or remotely as a consequence of anterograde Wallerian (WD) and/or retrograde axonal degeneration [5]. Furthermore, reduced WM integrity has been found to be associated with cognitive impairment [6,7,8].

The role of the non-injured hemisphere in stroke recovery is still controversial. Some imaging studies suggest that contralesional functional networks are significantly involved in post-stroke functional recovery [33,71]; although the interpretation of the results regarding to their positive or negative implication in patient's recovery is disputed (for a review of the literature see 71). At a structural level, some diffusion tensor imaging (DTI) studies report increased anisotropy in the contralesional hemisphere (e.g. thalamus) after stroke [24,25], structural remodeling in ipsilateral and contralesional corticospinal tracts [25] and changes in the number of neural pathways in areas both ipsilateral and contralateral to the stroke [34]. Studies of tract's fractional

anisotropy (FA) asymmetries have concluded that the contralesional corticospinal tract may play a role in motor recovery after unilateral stroke [72,73,74,23].

Animal studies of ischemic stroke often use FA to study the temporal evolution of WM changes [9,10,11,12]. From these studies it has been established that disruption of the brain tissue microstructure results in a significant reduction in the FA during the subacute and chronic phases of cerebral ischemia. Human studies usually use FA [13,14,15,16] and Mean Diffusivity (MD) [17,18,19] to study the recovery of the injured brain over time. Regarding FA values, microstructural integrity of normal-appearing WM improves during 1 and 2 years following ischemic stroke, achieving subsequent stabilization [14].

Animal studies suggest that structural remodeling of WM in both the ipsilesional and contralesional hemispheres plays a role in motor recovery [20,21,22,23]. On the other hand, DTI studies of stroke patients have demonstrated that reduced contralesional WM integrity of the corticospinal tract in chronic stroke [24] is associated with poorer motor skill recovery [25] in stroke patients compared to patients with better motor recovery and healthy controls (HC) [25]. In these studies, larger interhemispheric asymmetries in FA for this anatomical region have been associated with reduced motor recovery [26,27,28,29], reduced skill improvement in response to training [30] as well as motor dysfunction after stroke [27,31]. Although plastic changes in the contralesional hemisphere have already proved to play a role in stroke recovery [32,33,25,34], this role remains still unclear from a cognitive point of view.

Prior work suggests that DTI may provide information about different pathophysiological processes and may be one of the most sensitive neuroimaging biomarkers of vascular damage [35,36,37,8]. Although, FA is the most widely studied diffusion metric, there is a growing interest in investigating WM microstructural mechanism underlying the FA change by analyzing other – more direct – diffusion metrics, such as axial (AD) and radial (RD) diffusivity. A recent study identified RD and AD decrease accompanying FA increase in areas surrounding the anterior cingulate cortex after 4-week integrative body-mind training, a form of mindfulness meditation [38]. Reduction in RD is usually interpreted as improved myelination, whereas reduction in AD is associated with axon morphological changes, such as changes in axonal density or caliber [39,40].

The objectives of the study in this paper were: 1) to evaluate the effects of right hemispheric stroke on left hemispheric WM; 2) to investigate the microstructural mechanism underlying the eventual WM change 3) to assess their effect on cognitive recovery of stroke patients at three months after stroke. We test the following hypothesis: a) Stroke patients with poor cognitive recovery will show more widespread WM disruption in the contralesional (left) hemisphere; b) Stroke patients with good cognitive recovery will have better contralesional FA integrity; c) The better contralesional WM integrity will be related to the cognitive performance of stroke patients with good cognitive recovery. To address these questions we used a hypothesis-driven TBSS approach.

Materials and Methods

1.1. Subjects

This is a prospective and longitudinal study that included a group of consecutive ischemic stroke patients (S) admitted at the Stroke Unit at the Germans Trias i Pujol Hospital from August 2008 to May 2012 and a group of healthy controls (HC). Stroke patients underwent a complete neuropsychological assessment in the acute phase (first 72 hours), and another at three months when the DTI study was performed. In the control group, cognitive assessment and DTI were performed in the same day.

Potential participants were included in the study if they had 1) First territorial ischemic stroke in the territory of middle, anterior or posterior cerebral arteries (MCA, ACA, PCA); 2) modified Rankin Scale (mRS) score of 0 and Barthel score of 100 before admission; 3) Age in the range between 40 and 75 years; 4) Absence of severe aphasia (fourteenth scoring item of National Institute of Health Stroke Scale (NIHSS) $\leq$1); 5) Absence of alcohol or drug abuse, psychiatric comorbidities, or severe visual or hearing loss; 6) Absence of contraindications to undergo MRI or severe claustrophobia.

From the 30 patients included in the study, we chose patients with right hemisphere stroke (n = 17), from which 3 were discarded due to acquisition problems (n = 1), and because the lesion volume fell outside 1.5 times the interquartile range (n = 2). The final study sample comprised 14 patients.

Table 1. Demographical and Clinical data.

	HC (n = 18)	SG (n = 6)	SP (n = 8)	HC - SG	HC - SP	SG - SP
Sociodemographic Factors				p	p	p
Age (years)	63 (60.75–67)	58.67±11.021	66.50±6.370	0.867	0.155	0.172
Women	7 (38.9%)	1 (16.7%)	2 (25%)	0.319	0.413	0.615
Education (years)	7.33±4.087	9.50±3.728	8±5.904	0.264	0.741	0.597
Vocabulary subtest	37.78±7.856	36.83±12.024	33.86±10.976	0.825	0.326	0.650
Edinburgh Test	95.56±13.492	96.67±5.164	99.38±1.768	0.452	0.723	0.280
Vascular Risk Factors						
Hypertension	8 (44.4%)	3 (50%)	5 (62.5%)	0.590	0.223	0.413
Dyslipidemia	9 (50%)	3 (50%)	4 (50%)	0.680	0.664	0.704
Diabetes Mellitus	1 (5.6%)	2 (33.3%)	4 (50%)	0.143	**0.020**	0.471
Smoking	6 (33.3%)	1 (16.7%)	2 (25%)	0.414	0.607	0.563
Alcohol intake	9 (50%)	1 (16.7%)	4 (50%)	0.171	0.664	0.238

Values are means ± standard deviations in Student's t-test or medians (interquartile range) in Mann-Whitney test for continuous variables. Values are n (%) for categorical variables in Chi-square test and Fisher's exact test. p shows statistical comparison between groups.
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Table 2. Stroke characteristics and neurological impairment at baseline.

Patients	Baseline NIHSS	Hemisphere	Infarct location	Lesion Volume (cm^3)	Arterial Territory
STROKE GROUP WITH GOOD COGNITIVE RECOVERY					
1	1	R	Frontal	0.1	MCA_ACA
2	17	R	Basal Ganglia+Insula	8.2	MCA
3	4	R	Occipital Cortex+Centrum Semiovale	52.3	PCA
4	16	R	Lenticular	3.6	MCA
5	5	R	Fronto-Parietal+Premotor+Insula	4.6	MCA
6	7	R	Insula+Frontal inferior	14.5	MCA
STROKE GROUP WITH POOR COGNITIVE RECOVERY					
7	8	R	Temporo-Parietal posterior	15	MCA
8	7	R	Temporo-Occipital	20.9	PCA
9	7	R	Basal Ganglia+Corona Radiata	42	MCA_ACA
10	17	R	Lenticular+Fronto-Parietal	7.3	MCA
11	13	R	Temporo-Parietal+Intern Capsule	34	MCA
12	9	R	Fronto-Parietal+Insula	32.5	MCA
13	14	R	Basal Ganglia+Insula+Corona Radiata	36	MCA
14	9	R	Basal Ganglia+Corona Radiata	17.6	MCA

Abbreviations: NIHSS = National institute of Health Stroke Scale; R = right; MCA = Middle Cerebral Artery; PCA = Posterior Cerebral Artery; ACA = Anterior Cerebral Artery;
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Table 3. Neuropsychological performance.

	HC (n = 18)	SG (n = 6)	SP (n = 8)	HC - SG	HC - SP	SG - SP
General Cognitive Function				p	p	p
MMSE	29.11±1.28	27.50±1.38	27.25±2.49	**0.016**	**0.022**	0.250
Sustained Attention						
MoCA subtest (/11)	11	10.83±0.41	9.50±1.77	0.083	**0.000**	0.072
Digit Span Forward (WAIS-III)	5±1.14	4.67±1.37	4.75±1.49	0.560	0.642	0.462
Working memory						
Digit Span Backwards (WAIS-III)	3.83±1.04	3.83±0.75	3.38±1.30	0.747	0.274	0.643
Premotor functions						
Luria' sequences (/5)	5	4.67±0.82	3.87±1.81	0.083	**0.007**	0.227
Rhythms subtest (/10)	8.78±1.35	7±2.45	6.75±3.11	0.054	0.060	0.713
Interference and Inhibitory control (/3)	2.89±0.32	2.83±0.41	2.38±0.74	0.727	**0.028**	0.762
Verbal fluency						
Letter (P)	11.67±3.11	12.17±4.67	7.50±4.81	0.814	**0.014**	0.522
Semantic (Animals)	16.39±3.74	16.67±6.62	13.50±4.69	0.898	0.106	0.467
Language						
Boston Naming Test (/15)	11±2	13 (10–14)	9±3.02	0.152	0.056	**0.037**
Psychomotor speed *(seconds)*						
Trail Making Test A (seconds)	59.94±23.45	54.50±22.189	127.50±84.34	0.968	**0.046**	0.064
Grooved Pegboard Test (preferred hand; seconds)	72.33±11.87	68.83±14.26	146.25±97.16	0.557	**0.009**	0.109
Visuospatial Skills						
Right Line cancellation test (/18)	18	18	18	–	–	–
Left Line cancellation test (/18)	18	18	18	–	–	–

Values are means ± standard deviations in Student's t-test or medians (interquartile range) in Mann-Whitney test for continuous variables. Values are n (%) for categorical variables in Chi-square test and Fisher's exact test. p shows statistical comparison between groups. Abbreviations: MMSE = Mini Mental State Examination; HC = Healthy Control; SG = Stroke Group with good recovery; SP = Stroke group with poor recovery.
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Table 4. Clusters of significant FA, RD and AD differences between Stroke and Healthy Controls group.

Brain Lobe	Anatomical Region	Size (mm^3)	MNI coordinates			Z-max	Z	p	d
			x	y	z				
	Fractional Anisotropy								
	HC>S								
BG	Thalamic Radiation (R)	755	77	119	65	1.00	3.09	0.001	1.299
BG	Corticospinal Tract (R)	483	72	118	75	1.00	3.09	0.001	1.299
T	Inferior Fronto_Occipital Fasciculus (R)	704	68	143	59	1.00	3.09	0.001	1.299
F	Uncinate Fasciculus (R)	138	56	129	58	1.00	3.09	0.001	1.299
BG	Anterior Limb of Internal Capsule (R)	202	77	121	68	1.00	3.09	0.001	1.299
BG	Retrolenticular part of Internal Capsule (R)	393	66	148	67	1.00	309	0.001	1.299
	Radial Diffusivity								
	S>HC								
BG	Anterior Limb of Internal Capsule (L)	493	105	142	68	0.99	2.88	0.002	1.21
BG	Anterior Limb of Internal Capsule (R)	521	77	121	69	1.00	2.88	0.002	1.21
BG	Retrolenticular part of Internal Capsule (L)	265	126	101	69	0.99	2.88	0.002	1.21
BG	Retrolenticular part of Internal Capsule (R)	1441	72	147	60	1.00	2.88	0.002	1.21
BG	Posterior Thalamic Radiation (L)	393	123	64	82	1.00	2.88	0.002	1.21
BG	Posterior Thalamic Radiation (L)	393	51	117	53	1.00	2.88	0.002	1.21
BG	Anterior Thalamic Radiation (L)	1345	116	66	90	1.00	2.88	0.002	1.21
BG	Anterior Thalamic Radiation (R)	2125	71	142	75	1.00	2.88	0.002	1.21
BG	Corticospinal Tract (L)	1218	116	91	100	1.00	2.88	0.002	1.21
BG	Corticospinal Tract (R)	2052	63	107	93	1.00	2.88	0.002	1.21
T	Inferior Fronto-Occipital Fasciculus (L)	1059	123	65	83	1.00	2.88	0.002	1.21
T	Inferior Fronto-Occipital Fasciculus (R)	4340	69	141	59	1.00	2.88	0.002	1.21
F	Uncinate Fasciculus (L)	144	109	144	100	1.00	2.88	0.002	1.21
F	Uncinate Fasciculus (R)	752	56	127	59	1.00	2.88	0.002	1.21
	Axial Diffusivity								
	S>HC								
BG	Anterior thalamic radiation (R)	322	66	117	106	0.99	2.05	0.02	0.88
BG	Corticospinal Tract (R)	433	65	113	105	0.99	2.05	0.02	0.88
T	Inferior fronto occipital fasciculus (R)	342	55	75	83	0.99	2.05	0.02	0.88

Abbreviations: FA = Fractional Anisotropy; RD = Radial Diffusivity; AD = Axial Diffusivity; S = Stroke group; HC = Healthy Control group; BG = Basal Ganglia; T = Temporal; F = Frontal; L = Left; R = Right.

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Eighteen healthy volunteers from the Barcelona-Asymptomatic Intracranial Atherosclerosis (AsIA) study [41,42] matched by age, sex, education, and handedness with the stroke patients were recruited as the control group. None of them had a previous history of neurological or psychiatric diseases and brain scans were reported as normal. Information on demographical characteristics and vascular risk factors were collected in each patient based on their medical history.

The study was approved by the institutional ethics committee (Comissió de Bioètica de la Universitat de Barcelona (CBUB); Institutional Review Board (IRB) 00003099 Assurance number: FWA00004225; http://www.edu/recerca/comissiobioetica.htm and the research was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from each participant prior to taking part in the study.

1.2. Measurement of Cognitive Function and Grouping Criteria

Information about previous cognitive impairment was collected by a trained neuropsychologist with the short version of the Spanish Informant Questionnaire on Cognitive Decline in the Elderly (S-IQCODE) [43] and the Frontal Behavioral Inventory (FBI) [44] on admission day. Premorbid Intelligence was estimated using the vocabulary subtest of Wechsler Adults Intelligence Scale (WAIS-III-R) [45] at three months post-stroke.

Patients underwent two neuropsychological examinations at different times. First examination was performed within the first 72 hours after the stroke. We selected a test battery that covered a variety of possible cognitive manifestations of vascular brain injury. Attentional abilities were explored by the Digit Span Forward Test (WAIS-III-R) [45], the subtest of attention extracted from de Montreal Cognitive Test (MOCA) [46], and the Line Cancellation Test (LCT) [47]. Executive abilities were assessed with the Digit Span Backwards from WAIS-III-R) [48], part B of the Trail Making Test [47], Phonological fluency (letter P) [47],

Table 5. Clusters of significant FA and RD differences between Stroke groups with good and poor recovery and Healthy Controls group.

Brain Lobe	Anatomical Region	Size (mm^3)	MNI coordinates			Z-max	Z	p	d
			x	y	z				
	Fractional Anisotropy								
	HC>SG								
F	Genu of Corpus Callosum (L)	231	91	110	97	0.99	2.05	0.02	0.88
	HC>SP								
BG	Pontine Crossing Tract (L)	194	94	149	85	0.99	2.05	0.02	0.88
F	Genu of Corpus Callosum (L)	1034	96	144	90	0.99	2.05	0.02	0.88
	Body of Corpus Callosum (L)	555	99	94	94	0.99	2.05	0.02	0.88
BG	Corona Radiata (L)	1180	109	140	101	1.00	2.05	0.02	0.88
BG	Posterior Thalamic Radiation (L)	215	116	68	90	0.99	2.05	0.02	0.88
BG	External Capsule (L)	661	123	119	74	0.99	2.05	0.02	0.88
BG	Anterior Thalamic Radiation (L)	347	113	158	90	0.99	2.05	0.02	0.88
BG	Corticospinal Tract (L)	726	117	107	97	0.99	2.05	0.02	0.88
P	Cingulum (cingulate gyrus) (L)	284	100	148	90	0.99	2.05	0.02	0.88
P	Forceps major (L)	382	116	68	90	0.99	2.05	0.02	0.88
P	Forceps minor (L)	326	94	149	85	0.99	2.05	0.02	0.88
F	Inferior fronto occipital fasciculus (L)	414	110	148	98	1.00	2.05	0.02	0.88
T	Inferior longitudinal fasciculus (L)	133	117	68	91	0.99	2.05	0.02	0.88
F	Superior longitudinal fasciculus (L)	663	111	141	101	1.00	2.05	0.02	0.88
F	Uncinate fasciculus (L)	145	112	160	97	0.99	2.05	0.02	0.88
	Radial Diffusivity								
	SP>HC								
BG	Pontine crossing tract (L)	176	94	149	85	1.00	2.33	0.01	0.98
F	Genu of corpus callosum (L)	768	96	145	90	1.00	2.33	0.01	0.98
	Body of corpus callosum (L)	306	115	71	90	0.99	2.33	0.01	0.98
BG	Anterior limb of Internal Capsule (L)	376	100	132	70	0.99	2.33	0.01	0.98
BG	Corona radiata (L)	1551	109	140	101	1.00	2.33	0.01	0.98
BG	External capsule (L)	417	123	130	69	1.00	2.33	0.01	0.98
BG	Anterior thalamic radiation (L)	890	111	158	88	1.00	2.33	0.01	0.98
BG	Corticospinal tract (L)	460	118	110	91	1.00	2.33	0.01	0.98
P	Cingulum (cingulate gyrus) (L)	174	99	148	90	1.00	2.33	0.01	0.98
P	Forceps major (L)	165	116	68	90	0.99	2.33	0.01	0.98
P	Forceps minor (L)	247	94	149	85	1.00	2.33	0.01	0.98
F	Inferior fronto-occipital fasciculus (L)	274	110	147	98	1.00	2.33	0.01	0.98
F	Superior longitudinal fasciculus (L)	375	112	140	101	1.00	2.33	0.01	0.98

Abbreviations: SG = Stroke group with good recovery; SP = Stroke group with poor recovery; HC = Healthy Control group; BG = Basal Ganglia; T = Temporal; F = Frontal; P = Parietal; L = Left.

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and Semantic fluency (animals) [47]. Language abilities were assessed with spontaneous speech (talking briefly about his/her health problems), repetition, understanding items extracted from The Mental Status Examination in Neurology [48], writing of one sentence, item extracted from the Mini Mental State Examination Test (MMSE) [49], and naming with the short version (15-items) of the Boston Naming Test [50]. Premotor abilities were assessed with Luria's sequences test, Rhythms subtest extracted from the MOCA test [46], and interference and inhibitory control subtest extracted from the Frontal Assessment Battery [51]. Speed and visuomotor coordination were assessed with the part A of the Trail Making Test [47] and the Grooved Pegboard Test (GPT) [52]. Neuropsychological examinations also included the MMSE [49], as a global cognitive test and the Geriatric Depression Scale (GDS) [53]. The acute neuropsychological examination was performed in a fixed order that took approximately 60 minutes to complete. If the patient was fatigued, the testing was split between two sessions carried out in the same day.

Second cognitive examination took place at three months after stroke lasting at most 2 hours. It should be mentioned that for this study we only considered the tests that had been used in both examinations. HC received a similar neuropsychological assessment to S at the acute phase. Stroke patients were dichotomized into two subgroups according to their level of cognitive recovery

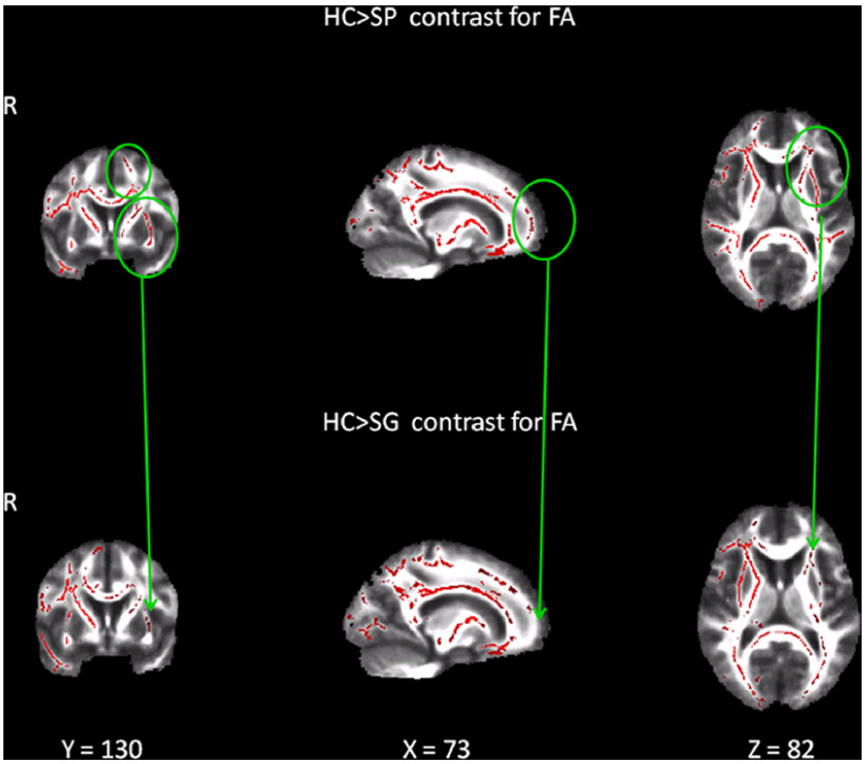

Figure 1. Significant changes in FA for the stroke group with poor recovery (SP) and the stroke group with good recovery (SG) at 3 months when compared with healthy controls (HC). The red color identifies clusters with significant decrease of FA. Statistical maps are represented in radiological convention (right corresponds to left hemisphere), superimposed on an MNI152 template. The threshold for significance was set at $p \leq 0.02$ corrected for multiple comparisons. Circles highlight the locus of clusters in the HC>SP contrasts not found in the HC> SG contrast.
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between acute and subacute phase. First, a paired t-test was conducted to determine the cognitive tests in which patients had significantly improved. Second, subjects were considered to demonstrate a good cognitive recovery (SG group) if they had normalized or improved 1.5 SD in at least three of these tests.

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA), version 17.0 for Windows. Baseline characteristics were summarized as mean ± standard deviation (SD) for continuous variables and proportions (n, %) for categorical variables. The threshold for statistical significance was set at $P<0.05$.

1.3. MRI Acquisition and Lesion Analysis

All images were acquired at a 3T Siemens Magneto TIM Trio (Siemens Diagnostics Healthcare, Erlangen, Germany) at the Image Platform of IDIBAPS, Centre de diagnostic per la Imatge from Hospital Clínic (CDIC), Barcelona. We used a 32-channel phased-array head coil with foam padding and head phones to restrict head motion and suppress scanner noise. The MRI protocol included a set of magnetization prepared rapid gradient echo (MP-RAGE) T1-weighted images (repetition time [TR]: 2300 ms; echo time [TE]: 3 ms; flip angle: 15°; field of view: 245 mm; and voxel size: $1\times1\times1$ mm^3) and two runs of DWI. DWI was acquired in 30 non collinear diffusion directions, with a b-value of 1.000 s/mm^2 and one with a value of 0 s/mm^2, with the following echo planar acquisition protocol: [TR]: 9300 mm; [TE]: 94 ms; flip angle, 15°; field of view: 240 mm; no gap; and voxel size: $2\times2\times2$ mm^3.

Infarct depth (cortical, subcortical or both), laterality (left/right) and vascular territory involved were determined in the first 24 hours employing Computed Tomography (CT) and/or Magnetic Resonance (MRI). Lesion volume was calculated in the subacute phase. T2-weighted images (TR: 5520 ms; echo time [TE]: 94 ms) and fluid attenuated inversion recovery images (FLAIR; [TR]: 9040 ms; [TE]: 85 ms; inversion time [TI]: 2500 ms; and voxel size: $0.86\times0.86\times6.5$ mm^3) were collected and analyzed by a trained neurologist (M.M). Lesion volume was determined using the three largest diameters along the three orthogonal axes divided with 2 (AxBxC/2) [54].

1.4. Image Pre-processing

DWI pre-processing included motion and eddy current correction using FSL's Eddy Correct Tool using the FMRIB Diffusion Toolbox (FDT) (Analysis Group, FMRIB, Oxford, UK). In order to eliminate spurious voxels, skull stripping of the T2 weighted $b=0$ volume was achieved using FSL's Brain Extraction

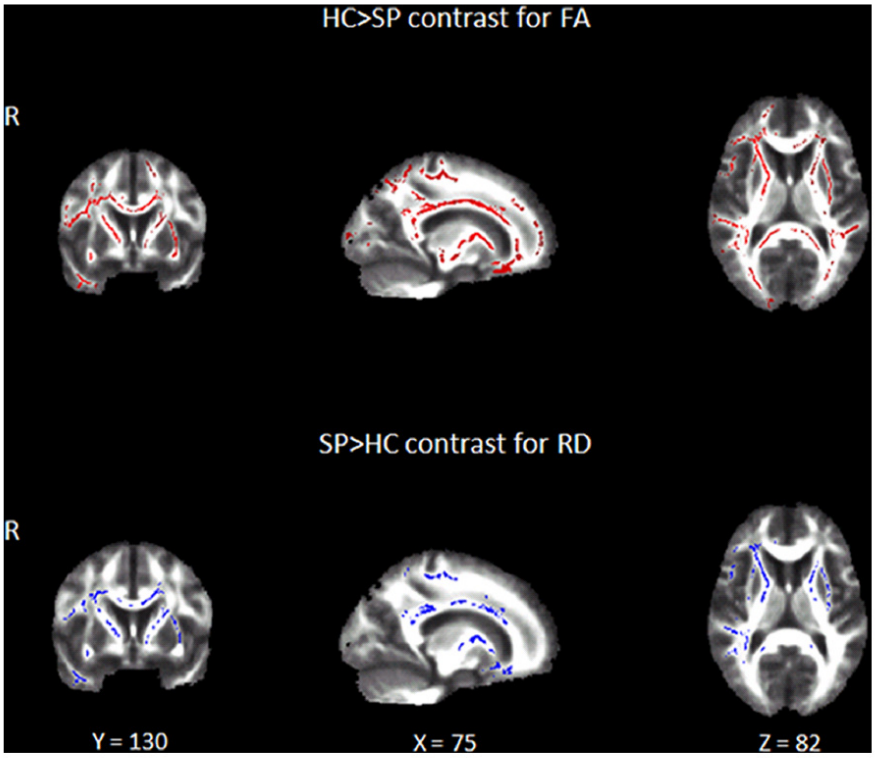

Figure 2. Significant changes in FA and RD for the stroke group with poor recovery at 3 months when compared with healthy controls. The red and blue colors show clusters of significant decrease of FA and increase of RD. Statistical maps are represented in radiological convention (right corresponds to left hemisphere) and are displayed superimposed on an MNI152 template. The threshold for significance was set at $p \leq 0.02$ corrected for multiple comparisons.
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Tool (BET) [55], and was used as a brain-mask for all other diffusion maps. The second DWI run was linearly co-registered (FLIRT) to the first, and the two runs have been averaged. FMRIB's Diffusion Toolbox - FDT v2.0 was used for the tensor modeling of the diffusion parameters to produce DTI data. Microstructural maps of fractional anisotropy (FA) and mean diffusivity (MD) were entered into group analysis using Tract Based Spatial Statistics - TBSS v1.2 [56] which is part of FSL data processing suite [57].

1.5. Diffusion Tensor Image Group Analysis

Tract-Based Spatial Statistics (TBSS). All subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT [58,59], which uses a b-spline representation of the registration warp field [60,61], resulting in all images transformed into 1 mm isotropic, MNI152 standard space. Next, all participants' FA volumes were averaged and a mean FA skeleton was created from all voxels with a FA threshold = 0.2 to reduce inclusion of voxels that are likely composed of multiple tissue types or fiber orientations. Each participants' aligned, standard space, FA maps were then projected onto this skeleton to create a 4D skeletonized volume (3D skeletal volume × number of subjects) which was then fed into voxelwise group statistics.

1.6. Statistical Analysis of the DTI Data

Randomize tool (v2.1; www.fmrib.ox.ac.uk/fsl/randomise/index.html) from the FMRIB software library with a number of permutation tests set to 5000, was applied on the FA maps [62. 56], to identify clusters of voxels that were significantly different between the HC and the S, SP, and SG groups. Significant clusters were identified using the Threshold-Free Cluster Enhancement (TFCE) choosing a more restrictive threshold at a p-value ≤ 0.02 corrected for Family Wise Error (FWE) via Gaussian Random Field theory [63].

Other diffusion-derived data (RD and AD) projections on the TBSS skeleton were also calculated for each subject. The spatial normalization transformations computed for the FA maps were applied on the RD and AD maps to achieve their nonlinear registration, which was projected on the TBSS skeleton. The resulting 4D volumes were also used for voxelwise cross-subject statistics.

The following statistical comparisons were made for each TBSS diffusion map: 1) Whole-brain analysis between HC and S subgroups; 2) Whole-brain ANCOVA analysis with the selected cognitive tests as covariates of interest to study differences between HC, SG, and SP; 3) Spearman's correlations analysis between the selected cognitive tests as covariates of interest and the TBSS diffusion maps.

Table 6. Clusters of significant negative (−) and positive (+) FA correlations between the different stroke groups and performance in the Semantic, Trail Making part-A and Grooved Pegboard Test.

Brain Lobe	Anatomical Region	Size (mm^3)	MNI coordinates			Z	p
			x	y	z		
	Semantic Fluency Test						
	+SG group						
Basal Ganglia	Retrolenticular part of IC (R)	3256	52	91	74	2.014	0.022
	Trail Making Test (part A)						
	−SG group						
F	Superior Corona Radiata (L)	15788	108	126	116	2.409	0.008
F	Inferior Fronto-Occipital Fasciculus (R)	7663	64	77	93	2.075	0.019
F	Inferior Fronto-Occipital Fasciculus (L)	4035	120	161	75	2.033	0.021
F	Superior Longitudinal Fasciculus (L)	264	124	121	93	1.995	0.023
P	Cingulum (L)	149	98	126	112	1.685	0.046
P	Forceps Major (L)	120	101	45	90	1.655	0.049
	Grooved Pegboard Test						
	−SG group						
F	Superior Corona Radiata (L)	27662	108	123	112	2.170	0.015
Basal Ganglia	Anterior Thalamic Radiation (L)	7188	108	126	114	2.054	0.02
P	Splenium of Corpus Callosum (L)	116	111	76	94	1.695	0.045
Basal Ganglia	Posterior Thalamic Radiation (L)	390	120	57	73	1.685	0.046
	−SP group						
T	Inferior Longitudinal Fasciculus (R)	917	46	101	74	1.825	0.034
F	Posterior Corona Radiata (R)	136	62	87	94	1.695	0.045

Abbreviations: F = Frontal, P = Parietal; T = Temporal; R = Right; L = Left.
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Results

3.1. Sample Characteristics

Demographic and clinical data are shown in Table 1. All subjects were right handed (mean = 96.91±10.08) except for one ambidextrous subject. There were no significant between-groups differences regarding to premorbid IQ, sex, gender and elapsed time between stroke onset and neuropsychological assessment in the acute phase (data not shown). Only a higher frequency of diabetes mellitus was found in the stroke group with poor cognitive recovery compared to the HC group. Table 2 shows stroke severity at baseline of the National Institute of Health Stroke Scale (NIHSS) and characteristics of the ischemic lesions (location, brain hemisphere, volume and vascular territory). All infarcts were in the territory supplied by the right MCA with the exception of 2 infarcts located in the right PCA territory. There were no significant between-group differences either regarding to the volume of the lesion ($t_{12} = -0.524$; $p = 0.610$), neurological severity, measured with the NIHSS ($t_{6.284} = 1.272$; $p = 0.248$) at baseline and at three months ($Z = 0.000$; $p = 1.000$), functional status, measured with the Barthel Scale at 3 months ($Z = -0.091$; $p = 0.928$) and the treatment received (all patients received mechanical Thrombectomy, with the exception of two patients, who received fibrinolytic treatment with rt-PA) (data not shown).

3.2. Neuropsychological Testing

Stroke groups in general demonstrated a significant acute-to-subacute improvement in the following cognitive tests: Mini-Mental State Examination (MMSE), Semantic Fluency (SF) (naming animals in one minute), Boston Naming Test (BNT), TMTA and the GPT (data not shown). The reader has to keep in mind that cognitive improvement corresponds to increasing score in the first three and decreased time to complete the last two tests. This fact is important for the interpretation of the correlation between FA and the test scores.

At the subacute phase, the SG group showed significant differences from the HC group only in the MMSE ($Z = -2.417$; $p = 0.016$). The SP group showed significant differences from the HC group in the following cognitive tests: MMSE ($Z = -2.294$; $p = 0.022$); Luria' sequences test ($Z = -2.704$; $p = 0.007$); Interference and Inhibitory Control test ($Z = -2.196$; $p = 0.028$); Phonetic Fluency test ($t_{24} = -2.661$; $p = 0.014$); Trail Making Test part-A ($t_{7.485} = -2.392$; $p = 0.046$); Grooved Pegboard Test ($Z = -2.613$; $p = 0.009$) and the Attentional subtest (MoCA) ($Z = -3.634$; $p = 0.000$).

Regarding to the two stroke subgroups, we only found significant differences between SG and SP in the BNT ($Z = -2.089$; $p = 0.037$) (Table 3).

3.3. Fractional Anisotropy

For the whole stroke group (S), significant decrease of FA was found only in right hemisphere when compared to the HC ($p = 0.001$, $d = 1.29$) (Table 4). When analyzed separately, both SP and SG groups continue to show significant FA disruption in the right hemisphere (data not shown). SP group showed significant decrease of FA also in several anatomical areas of the left hemisphere ($p = 0.02$, $d = 0.88$); whereas SG group showed significant FA disruption only in one anatomical area of the left hemisphere ($P = 0.02$, $d = 0.88$) (Table 5, Figure 1).

3.4. Axial and Radial Diffusivity

To investigate potential mechanisms underlying WM changes in stroke patients, both AD ($\lambda||=\lambda1$) and RD [$\lambda\perp=(\lambda2+\lambda3)/2$] maps were also analyzed. We found significant increase in both AD and RD for the S group ($p=0.002$, $d=1.21$ for RD; $p=0.02$, $d=0.88$ for AD) relative to HC group (Table 4). When analyzed separately, SP group showed significant increase of RD in the same anatomical areas where this group had shown significant FA decrease ($p=0.01$, $d=0.98$ for all the regions). The SG group did not show any significant change in either RD or AD in any region (Figure 2, Table 5).

3.5. Relationship between White Matter Integrity and Neuropsychological Function for the Stroke Subgroups

To assess if cognitive performance was associated with WM disruption, a whole-brain ANCOVA was performed using the WM skeleton given by the FA as dependent variable and the scores of the relevant cognitive tests as predictors, with diabetes mellitus as covariate of no interest. We regressed out the effect of diabetes because previous studies have shown that directly affects white and gray matter structures [64–67]. Clusters showing a significant correlation between cognitive test scores and FA are summarized in table 6. The SG group showed significant positive correlation between FA values located in the right retrolenticular part of the internal capsule (rIC) and scores in the Semantic Fluency test. Negative correlations were found between FA values located in the left hemisphere and the time spent to complete the GPT and the TMT part –A. For the GPT, the most significant areas were the left superior corona radiata (SCR) ($p=0.015$) and the left anterior thalamic radiation (ATR) ($p=0.02$). For the TMT part –A, the most significant areas were the left SCR ($p=0.008$), the right inferior fronto-occipital fasciculus (IFOF) ($p=0.019$) and the left IFOF ($p=0.021$). The SP group showed negative correlations between the time spent to complete GPT and FA values in the right inferior longitudinal fasciculus (ILF) ($p=0.034$) and the right posterior corona radiata (PCR) ($p=0.045$).

Between-group comparisons showed that correlation with semantic fluency scores was significantly stronger for the SP than for the SG in the right rIC ($p<0.001$). It was also stronger for the SP than for the HC in the right posterior thalamic radiation (pTR).

Discussion

One of the most important clinical questions after stroke is patient's potential for recovery from stroke-induced deficits. This question is of considerable interest given the impact of WM abnormalities for cognitive decline and the development of dementia after stroke [68]. WM changes after stroke are important determinants for presentation and severity of the neurological deficits as well as for prospects of recovery or secondary cognitive decline.

The present study aims to identify the effects of right hemispheric stroke on patient's cognitive recovery at three months after stroke. As an extension of previous studies, we were focusing on the left (contralesional) hemispheric WM. It has been reported that focal cerebral infarcts can lead to tissue alterations in remote connected regions, which are related to wallerian degeneration and cortical deafferentation. These secondary degenerative processes, usually detected within the ipsilateral hemisphere [69], can lead to a progressive atrophy [70], and they have been related to stroke recovery.

The role of the non-injured hemisphere in stroke recovery, however, is still controversial. In the contralesional hemisphere, only functional abnormalities have been identified in humans. Some imaging studies suggest that contralesional functional networks are significantly involved in post-stroke functional recovery [33,71]; although the interpretation of the results regarding to their positive or negative implication in patient's recovery is disputed (for a review of the literature see [71]). From a structural point of view, some DTI studies reported increased anisotropy in the contralesional hemisphere (e.g. thalamus) after stroke [24,25], structural remodeling in ipsilesional and contralesional corticospinal tracts [25], and changes in the number of neural pathways in both ipsilateral and contralateral areas [34]. Studies of tract FA asymmetries have concluded that the contralesional corticospinal tract may play a role in motor recovery after unilateral stroke [72,73,74,23].

In agreement with previous studies, we demonstrated that WM integrity (i.e. FA) was affected in the contralesional as well as in the ipsilesional hemisphere. Our findings also indicate that WM disruption is caused by demyelination rather than by axonal degeneration, as shown by the fact that the RD increase is more widespread than the AD increase. Axial and Radial components of the DTI tensor have been proposed as biomarkers of the type of neuronal damage [75,76]: AD measures diffusivity in the principal diffusion direction, and it is proposed as a biomarker of axonal damage [77,78], while RD is the average of diffusivities perpendicular to the principal direction of the tensor, and it is assumed to give information on the degree of demyelination [79,80,81].

In studies of small-vessel-disease both ischaemic demyelination and axonal loss have been found [82,83]. Recently [84], RD was found to be the strongest predictor of executive dysfunction. This finding was interpreted in the sense that the ischaemic demyelination has greater influence than axonal degeneration on the presence of cognitive impairment, therefore it was proposed as a more reliable biomarker than AD. Our study not only provides some support for the role of demyelination in stroke patients at three months after suffering a stroke, but also provides a relationship between this event and the presence of a poorer cognitive performance.

The relevance of these changes is demonstrated by the fact that patients with poor cognitive recovery showed stronger WM disruption in the left hemisphere. The correlation of the WM changes with cognitive performance – especially in the contralesional hemisphere – further supports their functional importance. Notice that a higher score in SF and a lower score in TMTA and GPT means better cognitive performance. Therefore, the combination of a positive correlation with SF scores and a negative correlation with TMTA and GPT scores means a positive correlation with cognitive performance in general. It is important to mention that this correlation was stronger in SG patients than in SP patients. This finding can be explained by the more severe damage of WM in the SP group: comparing Table 5 and 6, it is obvious that most of the brain areas showing correlation in SG group are affected in the SP group. Although, changes of the contralesional hemisphere can be due both to the degenerative and protective processes (i.e. compensation), our findings correspond to the WM degeneration as confirmed by their disruptive nature (decreased WM integrity mostly due to demyelination), and their correlation with cognitive performance (i.e. lower WM integrity co-occur with worse performance).

Our findings are in agreement with other studies with stroke patients [25,34] and extend our previous research with resting state [85] providing structural ground to the difficulty of SP patients to compensate their cognitive deficits after stroke.

SP patients showed significant deficits in attentional, motor, executive and processing speed functions when compared to HC. This profile has been related to vascular lesions in brain structures harboring frontal-subcortical circuits [86], something which is frequent in strokes that affect the vascular territory supplied by the MCA. Moreover, SP patients showed lower FA values in major left and right WM tracts that run along the anterior-posterior axis of the brain, supporting fronto-posterior and fronto-subcortical network interactions. These networks have been associated with executive functions [87]. Furthermore, WM disruption in the Body of Corpus Callosum for the SP group supports the suggestion made by Meguro et al (2000) who counted structural disruption of the corpus callosum as a sign of existing changes in the non-injured hemisphere. The relationships between structural changes reported here along with our previous findings imply their importance in clinical recovery and emphasize that not only lesion volume or lesion localization but WM integrity of the non-lesioned hemisphere are also important determinants of post-stroke recovery.

The generalizability of our findings is restricted by our relatively low sample size. On the other hand, our sample was quite homogenous regarding the lesion (all right-sided, first-time infarct) and demographic characteristics (e.g. vascular risk factors).

Conclusion

According to our knowledge, our study is the first characterizing WM changes in relation to cognitive recovery in patients at three months post stroke and matched healthy participants.

We have demonstrated not only the involvement of the contralesional hemisphere but also that its involvement correlates with cognitive recovery. The results reported in this paper broaden our view of the factors that may play a role in patient cognitive recovery after stroke. Future longitudinal studies may further improve our understanding of the evolution of poststroke changes in the contralesional WM microstructure and the relevance of the WM changes observed in this study.

Moreover, our results demonstrate that DTI provides information about the mechanism of WM pathology, and may help to explain apparent severity and cognitive outcomes. In the future, DTI may serve as a biomarker of cerebral plasticity and help evaluating a patient's response to rehabilitation. Predicting which patients will have worse outcomes in the chronic phase is a pivotal question for restorative neurology and can help us to adjust rehabilitation therapies more efficiently to each patient's needs.

Finally, taking into account that we assessed only stroke patients at 3 months following ischemic stroke, our results should be interpreted carefully. Inter-individual differences in brain structure might be the result of variations in life experience or of different genetic predispositions that should be take into account in future studies with large samples [88].

Author Contributions

Conceived and designed the experiments: MM AD RDA MG TA. Performed the experiments: RDA MG TA. Analyzed the data: RDA TA. Contributed reagents/materials/analysis tools: RDA TA MG. Wrote the paper: RDA. Revised manuscript: MM MG TA IC MB NB AD CC ELC MFA PTM MAS. Recruitment and exploration of patients: RDA MFA.

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3.3. Study III

Metabolic Imbalance, Default Mode Network Activity and White Matter Integrity in the healthy contralesional hemisphere related to Cognitive Outcome in Stroke Patients

Dacosta-Aguayo R*[a, b], Graña M[b], Falcón C, Fernández-Andújar M[a, d], López-Cancio E[c], Cáceres C[c], Bargalló N[e, f], Barrios M[g], Clemente IC[a, d], Guillem Pere[h], Fores-Raurell Rosa[h], Dávalos A[c], and Mataró M[a*]

[a] *Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain*

[b] *Group of Computer Science and Artificial Intelligence, University of the Basque Country, San Sebastian, Spain.*

[c] *Department of Neurosciences, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Barcelona, Spain*

[d] *Institute for Brain, Cognition and Behaviour (IR3C), Barcelona, Spain*

[e] *Diagnostic Center for Image, Clinic Hospital, Barcelona, Spain*

[f] *Imatge Platform of IDIBAPS, Barcelona, Spain*

[g] *Department of Methodology of Behavioral Sciences, University of Barcelona, Spain*

[h] *Primary Healthcare Research Support Unit Metropolitana Nord, Institut Universitari d'Investigació en Atenció Primària (-IDIAP) Jordi Gol, Santa Coloma de Gramenet, Spain*

*Corresponding authors M. Mataró and M. Graña at Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Passeig de la Vall d'Hebron, 171, 08035 Barcelona, Spain. Fax: +34 93 402 15 84. E-mail address: mmataro@ub.edu; Group of Computational Intelligence, University of the Basque Country (UPV/EHU), Paseo Manuel de Lardizábal, 1, 20018 Donostia, Gipuzkoa, Spain. Fax: +34 943 01 5590, E-mail address: manuel.grana@ehu.es

ABSTRACT

After acute stroke, diaschisis leads to changes in cerebral blood flow, white matter integrity and metabolism in areas connected to the ischemic lesion. Our goal was to investigate whether certain metabolites in the contralesional hemisphere are altered and if these alterations are correlated with resting-state activity, white matter integrity and cognitive outcome. 11 survivors of a single right ischemic stroke and 17 healthy controls were included. At three months after stroke, principal metabolites, fractional anisotropy and resting-state activity values were extracted in the left frontal WM region. Stroke group showed lower levels of Myoinositol (mI), Glycerolphosphocholine + Phosphocholine (Cho compounds), and Glutamate + Glutamine (Glx) when compared to controls. Levels of mI and Glx were associated with the Rhythms subtest; Cho levels were related with right line cancelation. Functional activity at rest was greater for the stroke group when compared to controls. This resting state activity was associated with levels of mI, N-Acetylaspartate (NAAG) and Glx and with the MMSE, TMT-part A and Groove Pegboard Test (GPT). Stroke group showed decreased fractional anisotropy when compared to controls and it was negatively associated with the GPT. Our preliminary data demonstrated persistent neurometabolic, resting-state activity and white matter alterations in the contralesional hemisphere of stroke survivors three months following stroke that were related with worse cognitive function. Understanding metabolic, functional and structural changes in the contralesional hemisphere might improve our knowledge of mechanisms involved in patient's cognitive and functional recovery.

Keywords: N-Acetylaspartate; Magnetic resonance imaging; Spectroscopy; Stroke; Recovery; Contralesional Hemisphere; Anterior Cingulate.

INTRODUCTION

Stroke is a leading cause of death and long-lasting disability with a high socioeconomic burden; among 30-day survivors of first-ever stroke, about half survive for 5 years; of survivors, one-third remain disabled, and 1 in 7 are in permanent institutional care (Hankey et al., 2002). Although it seems clear that a spontaneous and gradual return of some abilities mainly depends on brain plasticity in both ipsilesional and contralesional hemispheres (Lee and van Donkelaar, 1995; Seil, 1997; Steinberg and Augustine, 1997; Johansson, 2000; Hallett, 2001; Nelles, 2004; Hurtado et al., 2007), the role of transient or persistent contralesional changes is still a matter of active research (Grefkes et al., 2008; Schaechter et al., 2009; Wang et al., 2011).

After acute stroke, the normal flow of information between the ischemic region and areas connected to it via fiber tracts is disrupted causing functional deafferentation (Haberg et al., 2009). This phenomenon, known as diaschisis (von Monakow 1914), leads to changes in electrical activity, cerebral blood flow and metabolism in non-ischemic areas highly connected to the ischemic lesion (Gold and Lauritzen 2002; Enager et al. 2004). Those changes have been shown to cause clinical deficits exceeding the functional impairments expected from the location and size of the focal lesion (Seitz et al. 1999; Whishaw 2000).

Following a stroke the brain tries to compensate for loss of function through the reorganization of neuronal networks (van Meer et al., 2010; van Meer et al., 2012; Bury and Jones 2002). This reorganization involves the bilateral recruitment of the lesional and the contralesional hemispheres (Gerloff et al., 2006; Lotze et al., 2006) in which metabolic (Demougeot et al., 2001; Demougeot et al., 2003; Kang et al., 2009; Kobayashi et al., 2001; Munoz Maniega et al., 2008 et al., 2005; Craciunas et al., 2013; Takatsuru et al., 2009; Takatsuru et al., 2013), functional (Cramer, 2008; Cramer and Crafton, 2006; Wahl and Schwab, 2014; Ward, 2004; Ward et al., 2003; Weiller et al, 1993; Seitz et al, 1998; Dacosta-Aguayo et al., 2013a) and structural changes (Liu et al., 2008; Buffon et al., 2005; Gerloff et al., 2006; Brus-Ramer et al., 2007; Dancause et al., 2005; Dacosta-Aguayo et al., 2013b) take place.

Proton MR Spectroscopy (^{1}H-MRS) is considered in stroke research as a sensitive tool for the assessment of metabolic abnormalities detected even in the absence of structural MRI changes (Kobayashi et al., 2001). Regarding gray matter tissue, the metabolites most frequently assessed with the ^{1}H-MRS technique are N-acetyl-aspartyl-glutamate (NAAG), Creatine (Cr), Choline compounds (Cho), Myo-inositol (mI), and Lactate (Lac). Among them, NAAG contributes to the most prominent signal observed in the ^{1}H-MRS spectrum (Baslow, 2003). Therefore, it has been used as a biochemical marker for assessing neuronal viability/integrity after cerebral ischemia (neural loss) (Demougeot et al., 2001; Demougeot et al., 2003). In the ischemic area, two drastic spectral changes are observed First, within 24 hours after occlusion, levels of NAAG, Creatine, Choline compounds and Myoinositol decrease rapidly and severely (Zhang and Chopp., 2009; Yang et al., 2012; Parsons et al., 2000) whereas Lac, Glutamate and GABA increase and reach high levels (Houkin et al., 1993; Takagi et al., 1993; Hertz et al., 2008; Khang et al., 2000; Kobayashi et al., 2001; Munoz Maniega et al., 2008). Second, in the subacute phase (more than one month after onset), Lac remains high, Glutamate and GABA normalize, and NAAG, Choline compounds and Cr remain decreased (Muñoz-Maniega et al., 2008; Yang et al., 2012).

Although it is known that white matter is as sensitive as gray matter to ischemia (Hertz et al., 2008), remarkably little information is available about white matter tissue metabolic parameters during and after ischemic insults (Hertz et al., 2008; Rosenzbeig and Carmichael, 2013). However, following severe incomplete global ischemia a large decrease in Phosphocreatine has been demonstrated in white matter, without any change in gray matter, and the increase in Lac appeared to be larger in white matter than in gray (Lesnick et al., 1986; Veriac et al., 1992).

Still, the impact of focal ischemia on metabolism in remote non-ischemic tissue has not been investigated and results are unconclusive. In an experiment of middle cerebral artery occlusion in rats conducted by Haberg et al (2009), it was found that during the acute phase, Glutamate and GABA concentrations were decreased in the contralesional hemisphere. The significant reduction in both Glutamate and GABA content was interpreted as a consequence of the acute ischemic depolarization and subsequent functional deafferentation of a significant number of both GABAergic and glutamatergic transhemispheric connections. This reduction would contribute to neuronal reshaping

which may facilitate recovery by means of keeping metabolism activity low to allow hemispheric reorganization (Haberg et al., 2009). In another study on ischemic stroke patients conducted by Cirstea et al (2011), they found lower levels of NAAG and higher levels of mI in the contralesional hemisphere at 6 months after stroke. Levels of NAAG were correlated with time after stroke.

The study of the spatiotemporal patterns of blood-oxygen level-dependent (BOLD) fluctuations at rest stands for a novel approach in the understanding of intrinsic brain activity (Fox and Raichle, 2007). Several spatially distinct cortical regions exhibit similar temporal properties of the spontaneous ongoing low-frequency BOLD fluctuations which constitute the so-called resting state networks (RSNs) (Beckmann et al., 2005). Among them, the Default Mode Network (DMN) is a robust RSN whose dysfunction have been related to major psychiatric, neurodegenerative diseases and ischemic stroke (Greicius et al., 2008; Broyd et al., 2008; Seeley et al., 2009; Tuladhar et al., 2013; Dacosta-Aguayo et al., 2013a). Studies of motor recovery using resting state functional activity have paid attention mainly to changes in the resting-state functional connectivity (rsFC) of the ipsilesional primary sensorimotor cortex after stroke (Carter et al., 2010; Golestani et al., 2013; Park et al., 2011; Wang et al., 2010). In these studies it has been reported the following process of reorganization: (1) in the acute phase, rsFC is initially decreased and there is a reduced inter-hemispheric rsFC (Carter et al., 2010; Golestani et al., 2013; Park et al., 2011; Wang et al., 2010; Carter et al., 2012); (2) rsFC is gradually increased during recovery; (3) rsFC is finally restored to near normal or above normal levels at 10 weeks after stroke onset and there is a reinstatement of the inter-hemispheric rsFC (van Meer et al., 2012).

Prior work suggests that DTI may provide information about different pathophysiological processes and may be one of the most sensitive neuroimaging biomarkers of vascular damage and stroke (Crofts et al., 2011; Bucur et al., 2008; Burgmans et al., 2010). DTI studies of stroke patients have demonstrated that reduced contralesional WM integrity of the corticospinal tract in chronic stroke is associated with poorer motor skill recovery in stroke patients compared to patients with better motor recovery and healthy controls (Buffon et al., 2005; Liu et al., 2008). In these studies, larger interhemispheric asymmetries in FA for this anatomical region have been associated with reduced motor recovery (Schaechter et al., 2009; Jang et al., 2005; Lindenberg et al., 2010; Watanabe et al., 2001), reduced skill improvement in response to training (Thomalla et al., 2004) as well as motor

dysfunction after stroke (Jang et al., 2005; Stinear et al., 2007). Therefore, plastic changes in the contralesional hemisphere play an important role in stroke recovery (Qiu et al., 2011; Carmichael 2003; Gerloff et al., 2006; Crofts et al., 2011; Dacosta-Aguayo et al., 2013b).

We consider that the combined study of metabolic, functional and structural changes in the contralesional hemisphere through the combination of different neuroimaging techniques might bring new light on what changes occur, which are the mechanisms involved and how those changes affect cognitive performance.

In the present study we examine metabolic, functional and microstructural status of a single frontal region of white matter located in the healthy hemisphere in relation to cognitive outcome in first ever right hemispheric ischemic stroke patients at three months following stroke. We hypothesized that: 1) patients will show metabolic, functional and structural alterations when compared to healthy controls; 2) metabolite's concentrations will be related with functional, structural and cognitive measures in stroke patients.

To meet this aim we employed single ^{1}H-MRS, resting state fMRI and DTI. No prior study has combined those techniques to study plastic changes in white matter in the contralesional hemisphere at three months following an ischemic stroke.

2. MATERIALS AND METHODS

2.1. Study Participants

The stroke group comprised 29 consecutive patients admitted in the stroke unit of the Germans Trias i Pujol Hospital affiliated with the University Autonomous of Barcelona. Participants were aged 42 to 72 years, did not have a diagnosis of dementia or other neurologic disorder prior to the stroke, did not have severe aphasia (a score of < 2 on National Institutes of Health Scale; NIHSS) (Brott et al., 1989), and were well enough to consent to participate. Participants rating on the 16-item-Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) (Morales-González et al., 1992) as well as in the Frontal Behavioral Inventory (FBI) (Kertesz et al., 1997) indicated an absence of pre-

stroke cognitive impairment. Exclusion criteria included hemorrhagic stroke, persistent impairment of consciousness following stroke, concomitant central nervous system disease known to affect cognition, actual medical disease that is judged to possibly affect cognition secondarily, alcohol dependence and contraindications to MRI (pacemaker, metallic foreign bodies, and severe claustrophobia). Taking into account that the selected region for the spectroscopy was located in the left hemisphere, from these patients we only selected those that had an ischemic stroke in the right hemisphere (n = 17). From these 17 patients, 6 patients were discarded due to quality problems with their spectra data. The final sample comprised 11 patients with right stroke.

The comparison group comprised unpaid volunteers from the Barcelona AsIA Neuropsychological study (Miralbell et al., 2012; López-Cancio et al., 2011; Soriano-Raya et al., 2012). They were screened for absence of stroke, cognitive impairment and psychiatric disorder, with the same exclusion criteria as the stroke patients. Prior ethical approval was obtained from the University of Barcelona ethic committee (Comissió de Bioètica de la Universitat de Barcelona (CBUB); Institutional Review Board (IRB) 00003099 Assurance number: FWA00004225; http://www.edu/recerca/comissiobioetica.htm), and informed consent was obtained from each subject to participate in this study, which was conducted according to the provisions of the Helsinki declaration.

2.2. Neuropsychological data

Patients underwent neuropsychological examinations both within 72 hours after the stroke (acute phase) and after three months (subacute phase). For this study, we took into account neuropsychological data collected at three months after stroke. The neuropsychological battery comprised the following tests pertaining to various cognitive domains: Attentional abilities were explored by the Digit Span Forward Test (WAIS-III-R) (Wechsler, 1999), the subtest of attention extracted from de Montreal Cognitive Test (MoCA) (Nasreddine et al., 2005), and the Line Cancellation Test (LCT) (Strauss, 2006). Executive abilities were assessed with the Digit Span Backwards from WAIS-III-R) (Wechsler, 1999), part B of the Trail Making Test (Strauss, 2006), Phonological fluency (letter P) (Strauss, 2006), and Semantic fluency (animals) (Strauss, 2006). Language abilities were assessed with spontaneous speech (talking briefly about his/her health problems), repetition,

understanding items extracted from The Mental Status Examination in Neurology (Strub and Black, 2000), writing of one sentence, item extracted from the Mini Mental State Examination Test (MMSE) (Folstein, 1975), and naming with the short version (15-items) of the Boston Naming Test (Kaplan et al., 1983). Premotor abilities were assessed with Luria's sequences test, Rhythms subtest extracted from the MoCA test (Nasreddine et al., 2005), and interference and inhibitory control subtest extracted from the Frontal Assessment Battery (Dubois et al., 2000). Psychomotor speed and visual coordination were assessed with Grooved Pegboard Test (Ruff and Parker, 1993) and the part A of the Trail Making Test (Strauss 2006). Neuropsychological examinations also included the Mini Mental State Examination (MMSE) (Folstein, 1975), as a global cognitive test, the vocabulary subtest (Wechsler, 1999) as an estimation of the premorbid intelligence quotient (IQ) , and the Geriatric Depression Scale (GDS) (Yesavage et al. 1982). Trained psychologists (RDA and MFA) performed assessments. Participants were allowed breaks where appropriate to minimize the effects of fatigue on performance. Raw cognitive test scores and standard deviations are reported (Table 2).

2.3. Lesion analysis

Infarct depth (cortical, subcortical or both), laterality (left/right) and vascular territory involved were determined within the first 24 hours employing Computed Tomography (CT) and/or Magnetic Resonance (MRI). Lesion volume was calculated within the first days after stroke as the product of the three largest lesion diameters, along the three orthogonal axes, divided by 2 (Sims et al., 2009).

2.4. *Image acquisition*

MRI and ^{1}H-MRS were performed at three months after stroke on a whole-body 3T Siemens Magneto Trio (Siemens Diagnostics Healthcare, Erlangen, Germany) at the Image Platform of IDIBAPS, Centre de diagnostic per la Imatge from the Hospital Clínic (CDIC), Barcelona. We used a 32-channel phased-array head coil with foam padding and head phones to restrict head motion and scanner noise.

MRI protocol included a set of: 1) Magnetization Prepared Rapid Gradient Echo (MPRAGE) T1-weighted images (repetition time [TR]: 2300 ms; echo time [TE]: 3 ms; flip angle: 15°; field of view:

245 mm; voxel size: 1x1x1 mm^3; 2) Resting-state Blood Oxygen Level-Dependent data using an echo-planar imaging sequence (repetition time [TR] = 2s; echo time [TE] = 29ms; flip angle = 80°; in plane spatial resolution = 3×3 mm^2; field of view = 240×240mm^2; slice thickness = 4 mm; number of slices = 32; number of volumes = 240; acquisition time = 8 minutes); 3) two Diffusion Weighted Images (DWI) in 30 non collinear diffusion directions, with a b-value of 1.000 s/mm^2 and one with a value of 0 s/mm^2 ([TR]: 9300 mm; [TE]: 94 ms; flip angle, 15°; field of view: 240 mm; no gap; and voxel size: 2x2x2 mm^3) and 4) Spectra with the use of a double-spin echo point-resolved spectroscopy sequence (PRESS) with TR=1500 ms and TE=35 ms, data points 2048, with automatic shimming and water suppression. Water suppression spectra consisted of 128 acquisitions and spectra without water suppression consisted on 16 acquisitions. A single voxel technique was used to assay the metabolites of interest. The voxel was localized using the axial plane, within the left frontal white matter. The size of VOI as determined on MRI was 2x2x2 mm^3 in all cases. The VOI was placed in the same location for every subject guided by anatomical landmarks in the images. This position was chosen because the function in this region relies on the integrity of frontal–subcortical circuits whose disturbance is the hypothesized basis of the nature of the neuropsychological deficits seen in stroke patients (Almkvist, 1994). All acquisitions were performed three months after stroke to exclude transient changes in MRI parameters due to acute ischaemia (Figure 1).

2.5. Image processing and analysis

2.5.1. ^{1}H-MRS processing

Spectral raw data were processed by fully automated spectral analysis using LCModel, version 6.3-01, (Provencher, 1993; Provencher, 2001). The LCModel method analyzes an in vivo spectrum as a linear combination of concentration calibrated model in vitro spectra correcting for residual eddy current effects.

The following metabolites were collected: *N*-acetyl-aspartate plus *N*-acetyl-aspartyl-glutamate (NAAG), 2.02 ppm; total Creatine (Cr; composed of creatine and phosphocreatine), 3.03 ppm; Phosphocholine plus Glycerolphosphocholine (Cho compounds), 3.23 ppm; Myo-inositol (mI), 3.26 ppm; Glutamate plus Glutamine (Glu + Gln = Glx), 2.1 to 2.55 ppm (Figure 2). This method allows for

distinction between metabolites with overlapping signals to yield individual concentrations as well as providing summation data for overlapping resonances.

All spectra were visually inspected to ensure correct identification of the metabolites by LCModel. Since each concentration is reported with a confidence measurement (SD %; Cramer-Rao bounds (CRBs)) reflecting maximum-likelihood estimates and their uncertainties (Provencher, 2001), and in order to achieve a comparable quality of the spectroscopic data over the entire course of our study, only metabolite concentrations with a fitting error SD% of 15 or less were included in the final analysis of this paper.

Calculation of variations in voxel composition (Cerebrospinal Fluid (CSF), Gray Matter (GM) and White Matter (WM)) was performed with a semiautomatic interactive algorithm developed at the University of Barcelona based on SPM functions (Wellcome Department of Cognitive Neurology, Institute of Neurology, London). We chose to use absolute metabolite units without any internal reference due to the fact that it has been shown that the use of a metabolite as an internal reference in stroke might introduce potential bias (Parsons et al., 2000). As it is known that GM concentrations contribute to differences in metabolites (Doyle et al., 1995), we regressed out for GM proportions when comparing both groups and when performing partial correlations.

2.5.2. fMRI processing and analysis

The analysis was conducted using probabilistic Independent Component Analysis (pICA) as implemented in FSL 4.1.9 (FMRIB Centre, Department of Clinical Neurology, University of Oxford, www.fmrib.ox.ac.uk/fsl). Data preprocessing consisted of the removal of the first 6 volumes to ensure saturation and adaptation of the participants to the environment leaving 234 volumes for further analysis, removal of non-brain structures using Brain Extraction Tool (BET), motion correction using MCFLIRT, high-pass filtering with a frequency cut-off at 160s, low-pass temporal filtering (5.6s), spatial smoothing using a Gaussian kernel of full-width half-maximum of 5mm, intensity normalization, and affine linear registration to the MNI152 standard template. Absolute head movement was below 1.5mm for all participants. We selected the independent component map of the DMN. The procedure for

selecting the DMN within the whole set of components was based on the computation of spatial cross-correlation between each independent component and free available standard templates of RSNs (http://www.nitrc.org/projects/fcon_1000/) (Biswal et al., 2010). We then, made a co-registration of the VOI used in the ^{1}H-MRS analysis in every subject's DMN map created in the second stage with FEAT (FMRI Expert Analysis Tool, v.5.90). We used the dual-regression procedure (Filippini et al., 2009) to investigate between-groups differences in this VOI related to the DMN. The threshold of the voxel-wise differences was set at $p < .05$ (family wise error corrected).

2.5.3. Diffusion Tensor imaging processing

DWI images were analyzed with FDT (FMRIB's Diffusion Toolbox) software from FSL (v5.0.6). After extracting individual fractional anisotropy (FA) maps, we co-registered the FA maps to the WM masks obtained from FAST (FMRIB's Automated Segmentation Tool) bundled in FSL (Zhang et al., 2001) in order to ensure that GM contamination of FA characteristics in the spectroscopic VOI were minimal. After this, we extracted individual FA values from this mask and made partial correlations with SPSS software between those values and metabolite concentrations after controlling for age and GM partial volume effects.

2.6. Statistical Analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago), version 17.0 for Windows. Distributions of demographic variables were tested for normality by the Kolmogorov-Smirnov test and we examined group differences using parametric (*t* test) and nonparametric (Mann-Whitney test) independent sample tests for continuous variables and Chi-Square or Fisher's exact test for categorical variables. The threshold for statistical significance was set at 2-sided alpha error of .05. Analysis of covariance with univariate general linear model was used to test for between-group differences regarding metabolites controlling for voxel partial GM volume and age. Pearson's test was used for testing partial correlations between ^{1}H-MRS, fMRI and FA measures and neuropsychological scores for the entire sample after accounting for age as regressor of no interest. No correction for multiple comparisons was performed given the a priori main hypotheses listed in the introduction.

3. RESULTS

3.1. Demographical and clinical data

The demographic characteristics and clinical data of the study groups are described in Table 1. There were not statistical significant differences between stroke group and the control group regarding age, educational level, handedness (all participants were right-handed), premorbid IQ, gender or vascular risk factors. All patients had right hemispheric ischemic stroke affecting the Middle Cerebral Artery (MCA) with exception of two patients which had the right Posterior Cerebral Artery (PCA) affected.

3.2. Neuropsychological data

Stroke patients showed statistically significant differences at three months following stroke in the general cognitive function measured by the MMSE, sustained attention measured by the MoCA subtest, premotor functions measured by the Rhythms subtests, and psychomotor and speed measured by the time to complete the TMTA at three months after stroke (see Table 2).

3.3. ^{1}H-MRS findings and correlations with cognitive outcome

We found statistical significant differences regarding metabolite levels of mI (ANCOVA: F=17.629, 28, p<0.001), Cho compounds (ANCOVA: F=9.173, 28, p=0.005) and Glx (ANCOVA: F=4.945, 28, p=0.036) between the stroke group and the healthy control group after controlling for age and voxel partial GM volume (Table 4). mI was positively associated with Glx (r_s=0.482; p = 0.013); Cho compounds were associated with NAAG (r_s= 0.541; p = 0.004) and with Cr (r_s= 0.659; p <0.001); and Glx was associated with NAAG (r_s= 0.541; p = 0.004) taking into account the entire study sample (Table 3).

Regarding cognitive performance, we found statistical significant associations between the levels of those significant metabolites and cognitive performance for the entire study sample: 1) levels of mI and Glx were associated with the Rhythms subtest (MoCA) (r_s = 0.541; p = 0.004; r_s = 0.458; p =

0.019, respectively); 2) levels of Cho compounds were associated with right line cancelation (r_s = 0.461; p = 0.018) (Figure 3)

3.3. Functional findings and correlations with metabolites and cognitive outcome

The dual-regression results yielded significant differences regarding rs-fMRI in the VOI region located in the right frontal hemisphere between the stroke group (mean = 25.00 ±16.10) and the healthy control group (mean = 8.62 ± 5.04) (Z = -3.175; p = 0.001). Higher rs-fMRI activity was significantly associated with metabolite's levels of mI (r_s = -0.485; p = 0.012) and Glx (r_s = -0.398; p = 0.044) (Figure 4). Finally, this activity was significantly associated with the following cognitive tests: MMSE (r_s = -0.472; p = 0.011); TMT-part A (r_s = 0.507; p = 0.006); and GPT (r_s = 0.486; p = 0.009) (Figure 3).

3.5. Structural findings and correlations with metabolites and cognitive outcome

Stroke group showed significant decreased FA values in the selected VOI when compared with controls (t_{26} = 2.733; p = 0.011). We found a significant statistical association between those values and the GPT (r_s = -0.494; p = 0.009) for the entire study sample. No significant results were found between the levels of metabolites and FA values (Figure 3).

4. DISCUSSION

We introduce a novel approach to study poststroke plasticity in the contralesional hemisphere by measuring metabolites, specific to neurons (NAAG), glia (mI, Choline compounds) or to neuronal-glial (Glx) neurotransmission system, relating their concentration with DMN activity as well as with WM integrity, and linking such changes with cognitive outcome. Overall, we found the following results in the WM frontal VOI located in the contralesional hemisphere: 1) reduced levels of mI, Choline compounds and Glx for the stroke group when compared with healthy controls; 2) increased DMN activity in the group of stroke survivors when compared with healthy controls; 3) altered WM integrity

for stroke group when compared with the healthy controls; 4) Metabolite's concentrations, DMN activity and WM integrity were associated with cognitive performance.

The main finding of this study was that concentrations of metabolites related to glia and neuro-glia transmission were decreased in the contralesional hemisphere of the stroke patients and that those concentrations were correlated with DMN activity and cognitive performance. The reduction of Glx is in agreement with the study conducted by Haberg et al (2009) with middle cerebral artery occlusion in rats in which it was found a decrease in Glx and GABA concentrations in the contralesional hemisphere during the acute phase. This reduction was interpreted as a consequence of the acute ischemic depolarization and subsequent functional deafferentation of a significant number of glutamatergic transhemispheric connections. This decrease it is thought that would contribute to the reshaping of the neuronal receptive fields which may facilitate recovery by means of keeping metabolism activity low to allow hemispheric reorganization (Haberg et al., 2009). Our stroke patients were, however, at three months post stroke and lower levels of Glx were associated with higher levels of DMN activity which were, in turn, associated with worse cognitive performance. By the other hand, we found lower levels of mI and Cho compounds also related to worse cognitive scores. Lower levels of mI were correlated with higher levels of DMN activity. The presence of lower levels of mI and Cho compounds at the subacute phase in a frontal WM area of the contralesional hemisphere has not been reported in stroke patients. Cirstea et al (2011) and Craciunas et al. (2013) reported, in gray matter motor and premotor cortices, lower levels of NAAG and higher levels of mI in the contralesional in the contralesional hemisphere of stroke patients at 6 months after stroke and those levels were related with motor recovery at 6 months after stroke. Our preliminary results might be suggestive of that changes in metabolites related to glia and neuro-glial transmission system could be influencing resting activity and cognitive recovery of stroke patients at three months following a stroke.

Stroke patients showed higher DMN activity in the same VOI located in the contralesional hemisphere. This activity was negatively correlated not only with metabolite's levels of mI and Glx but with overall cognitive function (MMSE) and with processing speed (TMT-A, GPT). Relationships between functional activity in the DMN and Glx have been described recently (Hu et al., 2013; Kapogiannis et al., 2013). At a cellular level, neuronal activity is regulated by multiple neurochemical

processes including cycling of Glx and GABA, the major excitatory and inhibitory neurotransmitters in the Central Nervous System. The coordination between glutamatergic neurons and GABAergic interneurons is believed to have a direct impact on BOLD contrast (Logothetis et al., 2001; Buzsáki et al., 2007). Studies showing negative correlations between GABA concentration and BOLD signals (Northoff et al., 2007; Stagg et al., 2011) and positive correlation between Glx concentration and BOLD signal change has been recently reported (Enzi et al., 2012). In rs-fMRI, glutamatergic and GABAergic activities in the DMN may reach equilibrium to facilitate endogenous processes and when turning from the resting to a task state, the DMN suppression might be achieved by enhancing GABAergic activities to regulate glutamatergic activities (Hu et al., 2013). This is expected to have serious implications in neurological diseases that directly affect metabolite balance, such stroke. The relation between lower levels of an excitatory neurotransmitter with higher levels of DMN activity might be indicative of breakage of the equilibrium between neurotransmitter's and rs-fMRI activity and would be in agreement with our previous findings (Dacosta-Aguayo et al., 2013a) in which we found overall increase in the DMN in patients with worse cognitive recovery at three months after stroke. The association between DMN activity and cognitive tests is in agreement with several studies in which it has been found that DMN activity is correlated with behavioral performance (Anticevic et al., 2010; Hu et al., 2013).

Finally, WM structural integrity in the contralesional hemisphere was affected in the group of stroke patients and this alteration was negatively associated with processing speed. This finding of WM alteration and its relation with processing speed is in agreement with our previous study (Dacosta-Aguayo et al., 2013b) conducted with stroke patients at three months following a stroke by means of whole brain Tract Based Spatial Statistics (TBSS). In this study, we reported not only WM alterations in the tracts of the lesioned hemisphere but in the contralesional hemisphere too and the level of impairment of the contralesional hemisphere was predictive of the cognitive impairment of stroke patients.

We did not found any relation between metabolites and WM integrity nor between DMN activity and WM integrity. In healthy subjects, it is thought that there is a close relationship between functional and structural connectivity measures (Damoiseaux et al., 2009). In particular, when

structural connectivity is present, its integrity is related to the strength of functional connectivity (Honey et al., 2009). However, the presence of functional connectivity does not always reflect the presence of structural connectivity (Greicius et al., 2009). To our knowledge, it is not known how functional and structural connectivity measures relate in stroke patients.

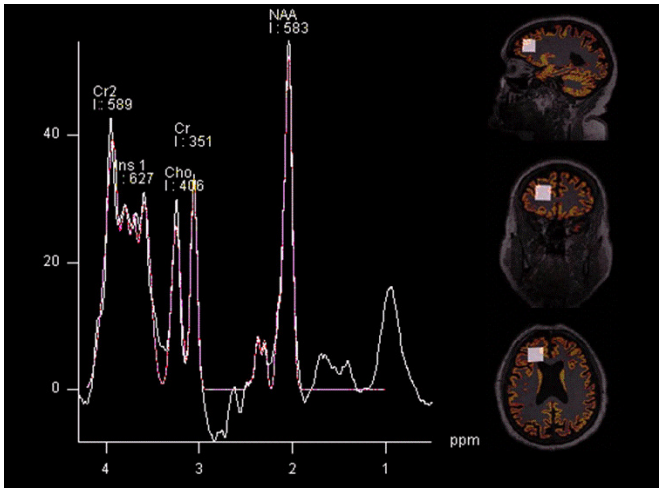
Spontaneous recovery is seen in stroke survivors weeks to months after the incident. Due to variability across subjects and across neurological domains and across within the same patient for different functions (Cramer, 2008), efforts of summarizing this process with precision have been frustrating. Although no power analysis was performed in this exploratory study, our data provide important information for formulating hypothesis in future confirmatory studies. In summary, stroke patients demonstrated significantly decreased mI, Glx and Choline compounds in the frontal WM region located in the contralesional hemisphere 3 months after stroke compared with normal control subjects. Levels of mI and Glx were related to DMN activity and with cognitive performance. Finally, WM integrity was impaired in stroke patients. No prior studies have reported those changes in the contralesional WM hemisphere of stroke patients at three months.

CONCLUSIONS

The current study provides further evidence that multimodal MRI approach may have a greater value broadening our understanding of the cellular, structural and functional processes underlying brain plasticity in vivo, promoting the development of future new therapeutic strategies addressed to the rehabilitation of stroke survivors.

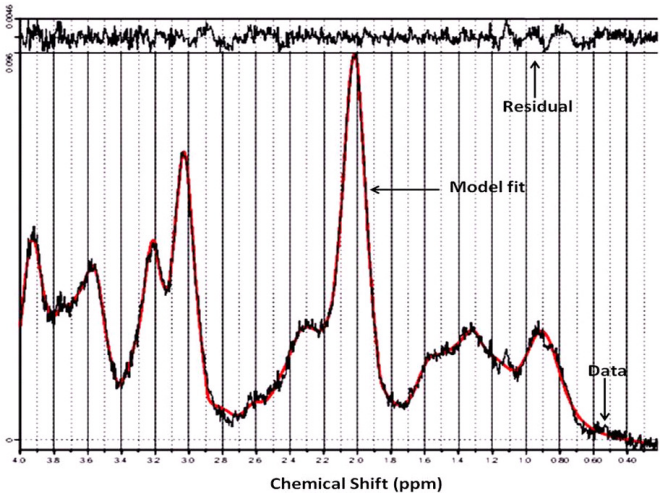
FIGURE LEGENDS

Figure 1.-



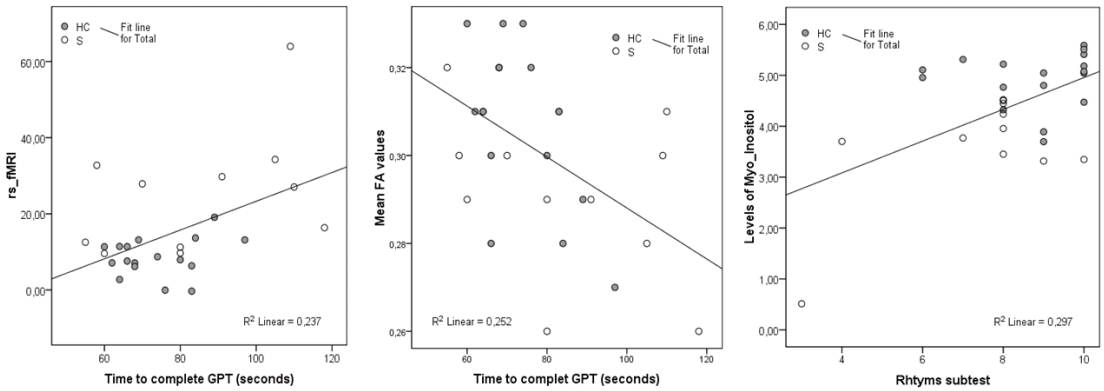
Anatomic image of the left white matter VOI with voxels borders for MRS. First right image, sagittal plane, second right image coronal plane, third right last image, middle transaxial plane. Left image shows ^{1}H-MRS spectra.

Figure 2.-



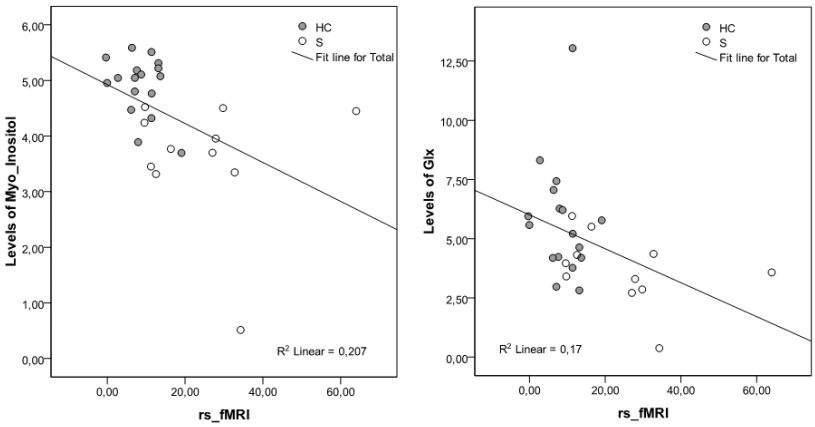
Representative left white matter VOI; the narrow line width indicates excellent data quality and the uniform residual represents excellent model fit.

Figure 3



Scatter plots showing partial correlations between rs-fMRI and GPT, Mean FA values and GPT and levels of mI and Rhythms subtest ($p < 0.05$).

Figure 4



Scatter plots showing partial correlations between rs-fMRI and mI, and rs-fMRI and Glx ($p < 0.05$).

Table1. Demographic and Clinical Characteristics of Controls and Patients Participants

	Control Participants (n = 17)	Patients with Stroke (n = 11)
Characteristics		
Age, mean (SD), years [a]	63.8 (3.6)	61.9 (7.9)
Educational level, mean (SD), years [b]	6.8 (3.6)	8.1 (6.1)
Edinburgh Test, mean (SD) [c]	95.3 (13.9)	97.7 (4.1)
Premorbid IQ, mean (SD) [d]	37.9 (8.1)	36.5 (10.4)
Gender, No.		
Male (%)	11 (64.7)	8 (72.7)
GDS, mean (SD)	2.3 (3.5)	4.6 (3.5)
Vascular Risk Factors, No (%)		
Alcohol intake	9 (52.9)	2 (18.2)
Smoking	6 (35.3)	3 (27.3)
Hypertension	8 (47.1)	5 (45.5)
Diabetes Mellitus	1 (5.9)	4 (36.4)
Dyslipidemia	9 (52.2)	6 (54.5)
S-IQCODE at 3 months, mean (SD)	51.8 (1.8)	53.6 (4.65)
NIHSS at 3 months, median (interquartile range)	…	1.50 (3.5-0)
Barthel Scale at 3 months, mean (SD)	…	89.55 (17.9)
Ranking Scale at 3 months, median (interquartile range)	…	2 (2-0.5)
Lesion volume (cm^3), mean (SD)	…	31.9 (36.2)
Anatomical regions affected	…	
Insular cortex	…	4
Lentiform nucleus	…	2
Basal Ganglia	…	2
Corona Radiata	…	2
Occipital lobes	…	2
Temporal lobes	…	3
Parietal lobes	…	4
Frontal lobes	…	3
Arterial distribution	…	
MCA	…	9
PCA	…	2

Abbreviations: IQ, Intelligence Quotient measured with the vocabulary subtest (Wechsler Adults Intelligence Scale (WAIS-III-R); GDS, Geriatric Depression Scale; NIHSS, National institute of Health Stroke Scale; S-IQCODE, Short version of the Informant Questionnaire on Cognitive Decline in the Elderly.

[a] t = 0.734; P = .476

[b] t = -0.695; P = .493

[c] z = 0.304; P = 1.00

[d] t = 0.409 ; P = .686

Table2. (Supplementary)- Neuropsychological tests scores at three months for the healthy control and stroke subgroups.

	HC (n = 17)	Stroke (n = 11)	p
General Cognitive Function			
MMSE	30 [30-28]	28 [29-26]	**<0.01**
Sustained Attention			
MoCA subtest (/11)	11 [11-10.5]	11 [11-10]	**0.03**
Digit Span Forward (WAIS-III)	4.9 ± 1.1	5.0 ± 1.5	0.91
Working memory			
Digit Span Backwards (WAIS-III)	3.0 [4-3]	4.0 [4-3]	0.92
Premotor functions			
Luria' sequences (/5)	5 [5-5]	5 [5-0]	0.07
Rhythms subtest (/10)	9 [10-8]	8 [8-7]	**0.04**
Interference and Inhibitory control (/3)	3 [3-2]	3 [2-1]	0.27
Verbal fluency			
Letter (P)	11.4 ± 2.9	9.3 ± 4.6	0.15
Semantic (Animals)	18 [18.5-13]	13 [17-12]	0.28
Language			
Boston Naming Test (/15)	11.1 ± 2.1	10.8 ± 2.6	0.79
Psychomotor speed *(seconds)*			
Trail Making Test A (seconds)	56.2 ± 23.6	80.6 ± 29.8	**0.02**
Grooved Pegboard Test (preferred hand; seconds)	73.7 ± 10.7	85.1 ± 22.9	0.15
Visuospatial Skills			
Right Line cancellation test (/18)	18 [18-18]	18 [18-18]	0.07
Left Line cancellation test (/18)	18 [18-18]	18 [18-17]	0.07

Values are means ± standard deviations in Student's t-test or medians (percentile 25-75) in Mann-Whitney test for continuous variables.

Table3. Between-group comparisons of metabolite absolute concentrations after correcting for CSF and adjusting for GM.

	HC (n = 17)	Stroke (n = 11)	Uncertainty (SD %)	p
NAA + NAAG	6.21 ± 1.17	5.43 ± 0.86	6	0.15
mI	5.10 [4.67 – 5.32]	3.81 [3.38 – 4.50]	6	**<0.001**
Glu + Gln	5.64 [4.24 – 6.73]	3.62 [2.89 – 4.40]	15	**0.04**
GPC + PCh	1.64 ± 0.30	1.26 ± 0.27	5	**0.01**
Cr + PCr	4.20 ± 1.67	3.21 ± 1.47	3	0.19

Abbreviations: CSF = Cerebrospinal Fluid; GM = Grey Matter; NAA = N-Acetylaspartate; NAAG = N-Acetylaspartylglutamate; mI = Myoinositol; Glu = Glutamate; Gln = Glutamine; GPC = Glycerophosphorylcholine; PCh = Phosphocholine; Cr = Creatine; PCr = Phosphocreatine. Values are expressed as mean ± SD except for mI and Glu+Gln, which are expressed as median and percentile [25-75].

Table4. - Tissue proportions from the VOI selected for doing Spectroscopy analysis

	HC (n = 17)	Stroke (n = 11)	p
White Matter	62.54 ± 8.97	69.08 ± 7.00	**0.05**
Grey Matter	30.79 ± 5.88	26.44 ± 5.48	0.06
CSF	6.66 ± 3.87	4.48 ± 2.67	0.1

Tissue composition was calculated with a homemade script based on SPM functions using MATLAB. Values are expressed as mean ± SD (Carles Falcó IDIBAPS Center, Barcelona).
CSF: Cerebrospinal fluid.

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3.4. Study IV

Impairment of Functional Integration of the Default Mode Network correlates with Cognitive Outcome at three months after Stroke

Rosalia Dacosta-Aguayo [a, b], Manuel Graña [b], Yasser Iturria-Medina [h], Marina Fernández-Andújar [a, d], Elena López-Cancio [c], Cynthia Cáceres [c], Núria Bargalló [e, f], Maite Barrios [g], Immaculada Clemente [a, d], Pera Toran [i], Rosa Forés[i], Antoni Dávalos [c], Tibor Auer*[j] Maria Mataró* [a]

[a] *Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain*

[b] *Group of Computational Intelligence, Dept. CCIA, University of the Basque Country UPV/EHU, San Sebastian, Spain.*

[c] *Department of Neurosciences, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Barcelona, Spain*

[d] *Institute for Brain, Cognition and Behaviour (IR3C), Barcelona, Spain*

[e] *Diagnostic Center for Image, Clinic Hospital, Barcelona, Spain*

[f] *Imatge Platform of IDIBAPS, Barcelona, Spain*

[g] *Department of Methodology of Behavioral Sciences, University of Barcelona, Spain*

[h] *Montreal Neurological Institute, Montreal, QC, Canada*

[i] *Primary Healthcare Research Support Unit Metropolitana Nord, Institut Universitari d'Investigació en Atenció Primària-IDIAP Jordi Gol, Santa Coloma de Gramenet, Barcelona, Spain*

[j] *MRC Cognition and Brain Sciences Unit, Cambridge, England*

**Shared latest authorship*

Corresponding author at Department of Psychiatry and Clinical Psychobiology,

University of Barcelona, Passeig de la Vall d'Hebron, 171, 08035 Barcelona, Spain.

Fax:+34934021584.E-mail address:mmataro@ub.edu (M. Mataró)

Rosalia Dacosta-Aguayo (MSc) rdacostaa@gmail.com

Manuel Graña (PhD) manuel.grana@ehu.es

Yasser Iturria-Medina (PhD) Iturria.medina@gmail.com

Marina Fernández-Andújar (MSc) m.fernandez@ub.edu

Elena López-Cancio (PhD) elenacancio@gmail.com

Cynthia Cáceres (PhD) caceresa@hotmail.com

Nuria Bargalló (PhD) bargallo@clinic.ub.es

Maite Barrios (PhD) maitebarrios@gmail.com

Imma Clemente (PhD) iclemente@ub.edu

Guillem Pera (PhD) gpera.bnm.ics@gencat.cat

Rosa Fores (PhD) rfores.bnm.ics@gencat.cat

Antoni Dávalos (PhD) adavalos.germanstrias@gencat.cat

Tibor Auer (PhD) Tibor.Auer@mrc-cbu.cam.ac.uk

Maria Mataró (PhD) mmataro@ub.edu

ABSTRACT

Resting-state studies conducted with stroke patients are scarce. The study of brain activity and connectivity at rest provides a unique opportunity for the investigation of brain rewiring after stroke and plasticity changes. This study sought to identify dynamic changes in the functional organization of the Default Mode Network of stroke patients at three months after stroke. Eleven patients (eight male and 3 female; age range: 48-72) with right cortical and subcortical ischemic infarctions and seventeen controls (eleven males and six females; age range: 57-69) were assessed by neurological and neuropsychological examinations and scanned with resting- state functional magnetic ressonance imaging. First we explored group differences in functional activity within the Default Mode Network by means of probabilistic Independent Component Analysis followed by a dual regression approach. Second, we estimated functional connectivity between eleven Default Mode Network nodes by means of partial correlation interaction weighted matrices (r_{ij}), and Network Based Statistics, followed, finally, by the study of graph analysis properties using graph theoretical approach. We found that patients had greater Default Mode Network activity in the left precuneus and the left anterior cingulate gyrus when compared with healthy controls ($p < .05$ Family Wise Error corrected). Network Based Statistics showed that stroke patients had significant impairment ($p = 0.014$; threshold = 2.00) in the connectivity between the following five Default Mode Network nodes: left superior frontal gyrus and posterior cingulate cortex ($t = 2.01$); left parahippocampal gyrus and right superior frontal gyrus ($t = 2.11$); left parahippocampal gyrus and left superior frontal gyrus ($t = 2.39$); right parietal and left superior frontal gyrus ($t = 2.29$). Finally, mean path length showed positive correlations with semantic fluency test ($r_s = 0.454$; $p = 0.023$), phonetic fluency test ($r_s = 0.523$; $p = 0.007$) and the Mini Mental State Examination ($r_s = 0.528$; $p = 0.007$). In conclusion, the ability to regulate activity of the Default Mode Network appears to be a central part of normal brain function in stroke patients. Our study expands the understanding of the changes occurring in the brain after stroke providing a new avenue for investigating lesion-induced network plasticity.

Keywords: Default Mode Network, Independent Component Analysis, Network Based Statistics, Graph Computational Analysis,

Abbreviations

amPFC = Anterior Medial Prefrontal Cortex

FBI = Frontal Behavioral Inventory

FWE = Family Wise Error

GDS = Geriatric Depression Scale

GPT = Grooved Pegboard Test

IQ = Intelligence Quotient

IQCODE = Informant Questionnaire on Cognitive Decline in the Elderly

iTL = Inferior Temporal Lobe

lITG = left Inferior Temporal Gyrus

LP = left Parietal

lPH = left Parahippocampal

lSFG = left Superior Frontal Gyrus

MFPC = Medial Prefrontal Cortex

MMSE = Mini Mental State Examination

MoCA = Montreal Cognitive Test

MRI = Magnetic Ressonance Imaging

mTL = medial Temporal Lobe

NIHSS = National Institutes of Health Scale National Institutes of Health Scale

PCC = Posterior Cingulate Cortex

piPL = posterior inferior Parietal Lobule

rITG = right Inferior Temporal Gyrus

ROIs = Regions of Interest

RP = Right Parietal

rPH = right Parahippocampal

rSFG = right Superior Frontal Gyrus

TMTA = Trail Making Test part-A

vmPFC = ventro medial Prefrontal Cortex

1. INTRODUCTION

Nearly one in four stroke patients has cognitive impairment severe enough to be diagnosed as dementia (Desmond et al., 2002; Sachdev et al., 2004; Sachdev et al., 2014) and another one in three has milder levels of cognitive impairment (Sachdev et al., 2006). While some patients may show recovery over an extended period (Desmond et al., 1996), an overall decline in cognitive function is to be expected (Aharon-Peretz, et al., 2002; del Ser et al., 2005; Nyenhuis et al., 2002; Srikanth et al., 2004; Sachdev et al., 2014) Post-stroke cognitive symptoms cannot be explained only by the location of the infarction, rather they seem to be attributable to impairment of cortical regions which are remote from the ischemic lesion. A possible explanation for these remote effects can be the disruption of neuronal function at remote cerebral regions belonging to certain functional networks (Kaiser et al., 2007; Crofts et al., 2011; Dacosta-Aguayo et al., 2014a).

Spontaneous restitution of lost cognitive function has been associated with brain plasticity, which is the brain ability to compensate for functional loss through reorganization of neuronal networks (van Meer et al., 2010; van Meer et al., 2012).

The Default Mode Network (DMN) is one of the most widely studied and precisely identified functional brain networks at rest. This network comprises a set of functionally highly connected regions including the Medial Prefrontal Cortex (MPFC), the Posterior Cingulate Cortex (PCC), the lateral Inferior Temporal Lobe (ITL) and the medial Temporal Lobe (mTL) and the posterior inferior Parietal Lobule (piPL) (Shulman et al., 1997; Raichle et al., 2001; Greicius et al., 2003). During rest periods, the DMN presents higher levels of activity than other networks (Fox et al., 2005; Buckner et al., 2008). It is hypothesized that the DMN is active while a person is not performing a goal oriented task, and that during this period the activity in the DMN reflects undirected, spontaneous, conscious mentation or monitoring of the external environment (Shulman et al., 1997; Raichle et al., 2001; Gusnard and Raichle, 2001) Consistently with this hypothesis, this network deactivates during cognitive task (Dasellar et al., 2004; Miller et al., 2008; Wang et al., 2010). The term ‘deactivate’ refers to the fact that there is a decrease of activity in the DMN during cognitive tasks compared to baseline. DMN disruption

has been commonly observed by resting state functional studies in patients with Mild Cognitive Impairment (Wang et al., 2006; Sorg et al., 2007), Vascular Cognitive Impairment with subcortical lesions (Sun et al., 2011), patients with carotid stenosis (Lin et al., 2014; Cheng et al., 2012) and patients with stroke (Tuladhar et al., 2013). Despite the considerable interest of this network, little is known about its possible alterations in ischemic stroke patients. Probabilistic Independent Component Analysis (pICA) can identify networks of co-activating brain areas based on a linear decomposition of the data and, therefore, it may miss nonlinear relationships in the data (Friston et al., 2000). Graph theory is a mathematical tool that has been recently applied to rs-fMRI data with the goal of studying the organization of network nodes (i.e. connections) at both the local and whole-brain levels (Bullmore and Sporns, 2007; Buckner et al., 2009). In both, structural and functional MRI data, brain networks have been found to be so consistently organized (Friston 2000; Buckner et al., 2009) that disruptions can be used as a biomarker (Nomura et al., 2010). Graph computational analysis allows detection of small-scale changes in regional organization that may underlie more global network changes.

We therefore investigated, as an extension of our previous study (Dacosta-Aguayo et al., 2014a), the alterations in the functional connectivity of the DMN in ischemic stroke patients during resting state condition, in order to assess more accurately DMN integration and segregation properties three months after ischemic stroke. For this purpose we used pICA, seed-based partial correlation analysis, NBS, and a computational graph analysis to determine the functional connectivity of the DMN in a group of subacute stroke patients after a right hemispheric stroke. We computed connectivity estimates between 11 specific ROIs belonging to the DMN (Watanabe et al., 2013) to study measures of connectivity between those ROIs, testing changes in local and global network structure that may occur following stroke. We chose different methods because they investigate the networks at various levels and therefore they complement each other: pICA detects nodes of the networks (voxelwise), seed-based connectivity analysis test local correlation between the different nodes and, finally, graph analysis investigates the global connectivity of the network. We hypothesized that functional connectivity measures at any level would reveal changes characteristic to the stroke patients. Previous studies indicate disruption in the inter-hemispheric connectivity (Chang et al., 2012; Tuladhar et al., 2013). There is also a growing evidence that some of the most affected regions are the medial parietal areas (posterior cingulate cortex) (Sun et al., 2011; Chang et al., 2012; Tuladhar et al., 2013), the medial prefrontal cortex (Lin et al., 2014; Tuladhar et al., 2013) and the hippocampus (Sun et al., 2011; Chang et al., 2012; Tuladhar et al., 2013). Finally, we expected to find a strong negative correlation between the size of the lesion and the functional connectivity, that is: the larger the injury, the worst the connectivity.

2. MATERIALS AND METHODS

2.1. Study Participants

The stroke group comprised 29 consecutive patients admitted to the stroke unit Germans Trias i Pujol Hospital affiliated with the University Autonomous of Barcelona. Participants were aged 42 to 72 years, they did not have a diagnosis of dementia or other neurologic disorder prior to the stroke, they did not have severe aphasia (a score of < 2 on NIHSS) (Brott et al., 1989), and they were well enough to consent to participate. Participants rating on the 16-item IQCODE (Morales-González, 1992) as well as in the FBI (Kertesz et al., 1997) indicated an absence of pre-stroke cognitive impairment. Exclusion criteria included hemorrhagic stroke, persistent impairment of consciousness following stroke, concomitant central nervous system disease known to affect cognition, actual medical disease that is judged to possibly affect cognition secondarily, alcohol dependence and contraindications to MRI (pacemaker, metallic foreign bodies, and severe claustrophobia). From these patients we only selected those that had a stroke in the right hemisphere (n = 17). From these 17 patients, 4 patients were discarded due to absence of apparent injury at three months according to the neuroradiologist report (based on T1-weighted image), and 2 more due to excessive motion during the functional measurement. The final sample comprised 11 patients with right hemispheric stroke. We chose to include only right hemispheric stroke patients because adding a few more (n = 12) subjects with very different lesion locations (i.e. lesion on the other hemisphere), would have added a lot of unimportant variance (i.e. noise). To control it, we would have to add extra regressors to the model, which, in turn, would have decreased the degree-of-freedom (i.e. sensitivity).

The control group comprised 17 stroke-free participants from the Barcelona AsIA Neuropsychology study (López-Cancio et al., 2011; Miralbell et al., 2012).They were screened for absence of cognitive impairment and psychiatric disorder on history and examination, with the same exclusion criteria as the stroke patients. Approval was obtained from the University of Barcelona ethics committee (Comissió de Bioètica de la Universitat de Barcelona (CBUB); Institutional Review Board (IRB) 00003099 Assurance number: FWA00004225; http://www.edu/recerca/comissiobioetica.htm), and informed consent was obtained from each participant, and study was conducted according to the provisions of the Helsinki declaration.

2.2. Neuropsychological data

Patients underwent neuropsychological examinations both within 72 hours after the stroke (acute phase) and three months later (subacute phase). The neuropsychological battery comprised the following tests pertaining to various cognitive domains: Attentional abilities were explored by the Digit Span Forward Test (WAIS-III-R) (Wechsler, 1999), the subtest of attention extracted from de Montreal Cognitive Test (MoCA) (Nasreddine et al., 2005), and the Line Cancellation Test (Strauss et al., 2006). Executive abilities were assessed with the Digit Span Backwards from WAIS-III-R) (Wechsler et al, 1999), Phonological fluency (letter P) (Strauss et al., 2006), and Semantic fluency (animals) (Strauss et al., 2006). Language abilities were assessed with spontaneous speech (talking briefly about his/her health problems), repetition, understanding items extracted from The Mental Status Examination in Neurology (Strub and Black, 2000), writing of one sentence, item extracted from the MMSE (Folstein et al., 1983) and naming with the short version (15-items) of the Boston Naming Test (Kaplan et al., 1983). Premotor abilities were assessed with Luria's sequences test (Folstein et al., 1983), Rhythms subtest extracted from the MoCA test (Nasreddine et al., 2005), and interference and inhibitory control subtest extracted from the Frontal Assessment Battery (Dubois et al., 2000). Psychomotor speed was assessed with the TMTA (Strauss et al., 2006) and the GPT (Ruff and Parker, 1999). Neuropsychological examinations also included the MMSE (Folstein et al., 1983), as a global cognitive test and the GDS (Yesavage et al., 1982). Assessments were conducted by trained psychologists. Participants were given breaks where appropriate to minimize the effects of fatigue on performance. Raw cognitive test scores and standard deviations are reported (Table 2).

2.3. Lesion analysis

Infarct depth (cortical, subcortical or both), laterality (left/right) and vascular territory involved were determined within the first 24 hours employing Computed Tomography and/or Magnetic Resonance. Lesion volume was calculated in the subacute phase as the product of the three largest lesion diameters, along the three orthogonal axes, divided by 2 (Sims et al., 2009).

2.3. Image Acquisition

Data were acquired at the subacute phase (3 months) using a Siemens Magnetom Trio operating at 3 Tesla at the Image Platform of IDIBAPS, Centre de diagnostic per la Image from Hospital Clínic (CDIC), Barcelona. We used a 32-channel phased-array head coil with foam padding and headphones to restrict head motion and scanner noise. Resting-state fMRI data were acquired

using an echo-planar imaging sequence (repetition time [TR] = 2s; echo time [TE] = 29 ms; flip angle = 80°; in plane spatial resolution = 3×3 mm^2; field of view [FOV] = 240×240 mm^2; slice thickness = 4mm; number of slices = 32; number of volumes = 240; acquisition time = 8 minutes). Participants were instructed to lie still with their eyes closed but remaining awake. All images were visually inspected to ensure that they did not contain MRI artifacts or excessive movement before analysis.

2.3.1. FMRI: Image Processing and Analysis

2.3.1.1. Independent Component Analysis of Resting-State fMRI Data

The analysis was carried out using pICA as implemented in MELODIC 3.10 bundled in FSL 4.1.9 (FMRIB Centre, Department of Clinical Neurology, University of Oxford, www.fmrib.ox.ac.uk/fsl). The fMRI data preprocessing consisted of the removal of the first 6 volumes to ensure saturation and adaptation of the participants to the imaging environment, leaving 234 volumes for further analysis, removal of non-brain structures using Brain Extraction Tool (BET), motion correction using MCFLIRT, high-pass filtering with a frequency cut-off at 150s, low-pass temporal filtering (5.6s), spatial smoothing using a Gaussian kernel with full-width half-maximum (FWHM) of 5mm, intensity in homogeneity normalization, and affine linear registration to the subject's anatomical T1-weighted image, and to the MNI 152 2mm resolution standard template. Absolute head movement was below 1.5mm for all participants included. pICA is a data-driven approach that decomposes the data into a set of Independent Components (ICs). Each IC consists of a triplet of a) time courses, b) spatial maps and c) subject modes, which characterize the signal variation across the temporal, spatial and subject domains. Subject modes are the quantification of the mean BOLD response for each subject within an IC. We selected the independent component map of the DMN from the whole set of ICs. The selection procedure was performed by visual inspection and spatial cross-correlation between the ICs and template available online (Biswal et al., 2010; Smith and Nichols, 2009). The ICs of the DMN was then introduced into a dual regression analysis (Filippini et al., 2009; Sharp et al., 2011). In this analysis the preprocessed functional data of each subject were first regressed against the spatial IC maps, yielding individual time series associated with the DMN. These time series were then used to regress again the individual preprocessed fMRI data and to obtain individual spatial maps. Spatial maps were finally tested for voxel-wise differences between groups using nonparametric testing with 5000 random permutations (Nichols and Holmes, 2002). We applied a mask to ensure that only voxels involved in the DMN were evaluated. After family-wise error (FWE) correction, differences with $p<0.05$ were considered significant. Anatomical labeling of activations was performed with reference to the Harvard-Oxford cortical and subcortical structural atlases (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases).

2.3.1.2. Seed-Based Functional Connectivity Analysis of the DMN

According to previous reports (Watanabe et al., 2013), we selected eleven spherical ROIs as representatives of the main nodes of the DMN (see Table 3). First level fMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, bundled in FSL. For each subject, the mean timeseries of all those ROIs was stored into a matrix of 11 columns (regions) by 234 rows (time points). In order to reduce the influence of head movement, six motion parameters were regressed out from each mean timeseries: the three sets of translations (in the x, y, and z directions) estimated in the image realignment phase with MCFLIRT after running MELODIC and the three first order differences of these translations.

On the regional functional representatives contained in these matrices, region-to-region partial correlation weighted matrices (r_{ij}) were calculated for each subject. Then, to normalize the correlation coefficients we applied Fisher's transformation (Raichle et al., 2001; Fransson et al., 2005; Long et al., 2008). Furthermore, we compute separate mean correlation weighted matrices for the patient and control groups.

2.3.1.3. Network Based Statistics analysis

We used an NBS approach (v1.2) (Zalesky et al., 2010) (http://www.nitrc.org/projects/nbs/) to isolate the components of the 11 x 11 connectivity weighted matrices that differ significantly between the two groups. A component is a set of interconnected edges in the connectivity matrix. The NBS analysis first performed a two-sample t-test at each edge independently to test the null hypothesis (H_0) that the values of connectivity between the two populations come from distributions with equal means. After that, a preselected T-value (T-threshold = 2) is used to threshold the statistical value calculated at each edge of the connectivity matrix to identify the set of supra-threshold edges. All interconnected components present in the set of supra-threshold edges are identified and their size (number of edges that the components comprise) is stored. Thus, a component is formed by interconnected supra-threshold edges at which the H_0 was rejected. To estimate the significance of each component, the NBS performed a nonparametric permutation test (K = 5000 permutations). A total of K random permutations are generated independently; for each permutation the group to which each subject belongs is randomly exchanged, and then the statistical test is recalculated in each permutation. After that, the same threshold is applied to create the set of suprathreshold links for each K permutation. Then, the size of the largest component in the set of supra-threshold links derived from each K permutation is stored, thus providing an empirical estimation of the null distribution of the maximal component size. Finally, the p-value of each observed connected component was corrected, calculating the proportion of the 5000 permutations for which the largest component size was greater

than the observed connected component size and then normalized by K. This allowed us to control the family-wise error (FWE)

2.3.1.4. Graph Network properties

Firstly, we calculated the following measures of functional segregation for every subject: clustering, defined as the inherent tendency to cluster nodes into tightly connected neighborhoods (Onnela et al., 2005; Watts and Strogatz, 1998), and local efficiency, defined as the average of the global efficiency of the level of the local subnetworks (Latora and Marchiori, 2001); secondly, we calculated measures of functional integration for every subject: mean path length, defined as the average distance (in terms of node-node connection) that must be traversed to connect one mode with another (Watts and Strogatz, 1998), and global efficiency , defined as a measure of how much parallel information can potentially be exchanged (Latora and Marchiori, 2001). Finally, we compared each patient's network measures with the corresponding values obtained in control participants, using Crawford's Z test as a more appropriate an established approach for small samples size (Crawford et al., 2004; Crawford et al., 2003a; Crawford et al., 2003b). We correlated those measures with patient's cognitive scores. Threshold of significance for those correlations was set at p = 0.05 FWE corrected by multiple permutation testing.

2.3.1.5. Statistical Analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago), version 17.0 for Windows. Distributions of demographic variables were tested for normality by the Shapiro-Wilk test and we examined group differences using parametric (*t* test) and nonparametric (Mann-Whitney test) independent sample tests for continuous variables and Chi-Square or Fisher's exact test for categorical variables. The threshold for statistical significance was set at 2-sided $p < 0.05$.

3. RESULTS

3.1. Demographical and clinical data

Anatomical regions and arterial distribution affected by the stroke are described in Table 1. In brief: patients showed lesions that affected the right hemisphere of the following regions: insular cortex

(n =4), parietal lobe (n =4), temporal lobe (n =3), frontal lobe (n =3), lentiform nucleus (n =2); basal ganglia (n = 2), corona radiata (n =2), and the occipital lobes (n =2). Lesion overlap is represented in Figure 1. There were no statistically significant differences between stroke group and control group regarding age, gender, years of education, premorbid IQ, handedness or vascular risk factors (Table 1).

3.2. Neuropsychological data

Stroke patients showed statistically significant impairment at three months following stroke in the general cognitive function measured by the MMSE, sustained attention measured by the MoCA subtest, premotor functions measured by the Rhythms subtests, and psychomotor and speed measured by the time to complete the TMTA at three months after stroke (Table 2).

3.3. DMN activity: Independent Component Analysis

Using pICA with temporal concatenation, a set of 55 independent components was estimated using the Laplace approximation to the Bayesian evidence of the model order (Minka, 2000; Beckmann and Smith, 2004). Within all ICs obtained we identified twelve common resting-state functional networks (van den Heuvel et al., 2010; Smith and Nichols, 2009; Biswal et al., 2010) (Figure 2) particularly the Default Mode Network (DMN) (Figure 2). Stroke patients had greater functional activity than controls within the DMN in the left cingulate gyrus and the left precuneus cortex (Figure 3). We extracted the DMN scores from those significant areas associated with each individual (patients and controls). These scores were further used to study the relationship between connectivity and cognition. We did not found any relationship between cognitive tests and DMN activity.

3.4. Network Based Statistics

Based in the network based statistics (NBS), we found affected connectivity between the following pair of ROIs for the stroke patients: left Superior Frontal Gyrus (lSFG) and Posterior Cingulate Cortex (PCC) (t = 2.01); left Parahippocampal (lPH) gyrus and right Superior Frontal Gyrus (rSFG) (t = 2.11); left Parahippocampal (lPH) gyrus and left Superior Frontal Gyrus (lSFG) (t = 2.39) and between right Parietal (RP) and lSFG (t = 2.29). All these connections were identified as part of an across-patients affected DMN sub network, altered with regard controls with a statistical significance of

p = 0.014 (see Table 3 for coordinates and abbreviations) (Figure 5). Results were corrected for multiple comparisons (5000 random permutation testing, FWE).

3.5. Graph Computational Analysis

We found statistical significant correlations between the mean path length and the following tests: scoring in the Semantic Fluency test (r_s = 0.454; p = 0.023), scoring in the Phonetic Fluency test (r_s = 0.523; p = 0.007), and scoring in the MMSE (r_s = 0.528; p = 0.007). Additionally, we found a non-significant correlation trend to the significance between the path length and the GDS (r_s = -0.380; p = 0.055), and between the global efficiency measure and the Semantic Fluency test (r_s = -0.378; p = 0.063). Results were corrected for multiple comparisons (5000 random permutation testing; FWE).

4. DISCUSSION

The purpose of this study was to provide further insight into the role of the different regions of the DMN in the cognitive outcome of patients with first ever ischemic stroke, as well as a better understanding of the underlying pathophysiology.

Previous works showed that localized brain lesions can cause connectivity-based changes in regions that are structurally intact and far from the lesion site (Crofts et al., 2011; Dacosta-Aguayo et al., 2014a; Dacosta-Aguayo et al., 2014b; He et al., 2007; Grefkes et al., 2008; Park et al., 2011; Ovadia-Caro et al., 2012; Carter et al., 2010; Carter et al., 2012; Golestani et al., 2013; Warren et al., 2009; Nomura et al., 2010; Tuladhar et al., 2013). The relationship between alterations in functional connectivity after stroke and behavioral outcome/performance has been previously reported. For example, He et al (2007) reported a breakdown of interhemispheric functional connectivity within the attention network in stroke patients with neglect symptoms. Symptom severity correlated with decreased connectivity, and recovery from symptoms correlated with the recovery of normal connectivity patterns. Similar results have been reported regarding the sensorimotor (Wang et al., 2010; Grefkes et al., 2008; Park et al., 2011; Ovadia-Caro et al., 2013; Carter et al., 2010; Carter et al., 2012; Golestani et al., 2013) and language networks (Warren et al., 2009). DMN disruption has been commonly observed by resting state functional studies in patients with Mild Cognitive Impairment (Wang et al., 2006; Sorg et al., 2007), Vascular Cognitive Impairment with subcortical lesions (Sun et al., 2011), patients with carotid stenosis (Lin et al., 2014; Cheng et al., 2012). Regarding the role of the DMN on cognitive performance in stroke patients, we know only of one recent study (Tuladhar et al., 2013) carried out. This study investigated the pathophysiology of memory dysfunction in first-ever right

and left stroke patients scanned 9-12 weeks after stroke onset. By means of group pICA, they found reduced DMN activity in stroke patients when compared with controls. We found, however, increased DMN activity in our stroke patients, which confirms our previous finding (Dacosta-Aguayo et al., 2014a). The increase of activity in the left anterior cingulate gyrus was related to line cancellation test ($r = -0.747$; $p = 0.000$) what is in agreement with models of attention that postulate the involvement of the anterior cingulate cortex in the attentional network (Thiel et al., 2004).

By means of graph computational algorithms we found that 1) impairment of the functional integration of the DMN was negatively associated with the size of the lesion in stroke patients, that is, as the size of the lesion is greater, the connectivity between the nodes is poorer; 2) there were more impaired nodes in the right and left (contralesional) frontal gyrus, the posterior cingulate cortex, the right parietal cortex and the left parahippocampal gyrus; 3) the disrupted mean path length between those nodes was associated to the cognitive performance in the Semantic Fluency Test, Phonetic Fluency Test, and MMSE. The size of the lesion has been, and still remains, a subject of discussion in the stroke literature along with the importance of the site of the lesion. These findings might contribute to clarify the reasons by which both size and localization are equally important in cognitive impairment after stroke: if the stroke lesion is big, the connectivity disruption between the different nodes of a network (or even nodes belonging to more than one network) will be greater enlarging the mean path length from one node to the other. On the other hand, if the lesion is situated in a critical node, such as the posterior cingulate cortex, or disrupts the connectivity of this node, the consequences of the lesion would be of significant relevance at short and long term. The impaired nodes were not only located in the lesioned hemisphere but in the contralesional hemisphere too. This finding reflects the general influence of localized lesions on distant functionally connected regions. Our findings are consistent with the concept of diaschisis after stroke, which was originally formulated to describe temporary clinical deficits related to areas remote from the area of damage (Achard et al., 2006) and has been expanded to include neurophysiological observations of depression of activity in remote, undamaged brain sites that are functionally connected to lesion areas (Carter et al., 2010; Crofts et al., 2011). However, the mean path length between the DMN nodes in stroke patients was positively associated with the MMSE as well as with the semantic and verbal fluency scores. Taking into account that lesions directly affect the mean path length, one would have expected that this association was negative; showing that mean path length disruption or elongation would explain poor cognitive performance in stroke patients. However, no such result was reported so far according to our knowledge. We can only speculate that those positive associations could perhaps reflect adaptive changes, i.e. decreased connectivity can be interpreted not as a consequence of the damage but rather a compensation by means of isolating damaged areas.

The association with the MMSE, a screening test that have been always related to cortical neurodegenerative disease, such as Alzheimer Disease, is an important finding. Whereas vascular

cognitive impairment and Vascular Dementia have been always related to a pattern of executive, motor, processing speed and attentional disabilities; Alzheimer Disease has been related mainly to episodic memory and visuospatial (parietal) related skills. Our finding is not only in agreement with other recent study (Tuladhar et al., 2013) regarding the involvement of the DMN in episodic memory but also is based on the fact that our patients showed impairment in the left parahippocampal gyrus.

The negative trend to significance correlation between the mean path length and the GDS (r_s = -0.38; p = 0.055) would be in agreement with other studies over patients diagnosed with major depressive disorder. Those patients presented a number of abnormal psychological and psychiatric symptoms characterized by multiple *self*-abnormalities (Grimm et al., 2009; Northoff, 2007; Sumner et al., 2010). The DMN has recently been shown to be important in *self*-referential activities (Fox et al., 2005; Fransson 2005). According to previous studies, there are striking differences between the DMNs of patients with major depressive disorder and healthy control participants (Johnson et al., 2009; Sheline et al., 2009; Zhu et al., 2012).

Finally, the association between the DMN activity and the semantic retrieval (fluency semantic test) is in agreement with a study of Grecius and colleagues (2003), who suggested that the retrieval and manipulation of episodic and semantic knowledge were likely elucidated by the DMN.

Our ischemic stroke patients showed more alteration in a measure of functional integration in the brain networks: the mean path length, computed as the global average of the graph's distance matrix (Watts and Strogatz, 1998). Cognitive functions depend on the integrated operation of large-scale distributed brain networks. The interaction between brain regions within a network and the interactions between networks are both important for efficient cognitive function. Numerous hubs (important nodes highly connected to the network that play global integrative processes, or play a critical compensatory role when the network is damaged) are mainly located in heteromodal association cortices which largely overlap the DMN (van den Heuvel and Sporns, 2011). Disturbances of the functional connectivity of those hubs are linked to neuropathology. Diseases that affect the rich-hub core of the brain may have more global effects on brain communication and thereby affect multiple cognitive domains. Whether these deficits can lead to increased susceptibility to develop vascular cognitive impairment and ensuing vascular dementia remains unclear. Applying graph theory to individual nodes of a network provides a potential explanation for disruptions of small-worldness organization of this network. A network perspective is fundamental to appreciate the pathophysiology of brain injury at the systems level, and the underlying mechanisms of recovery, as well as to develop novel strategies of rehabilitation.

5. CONCLUSIONS

The ability to regulate activity of the DMN appears to be a central part of normal brain function in stroke patients.

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FIGURES AND TABLES LEGENDS

Figure1. – Frequency distribution of the lesions for patients. Images are depicted in radiological convention (R-L)

Figure 2a and b: Twelve common resting state networks identified with pICA for the two groups.

Figure 3. - Orthogonal view. Increased activity within the Default Mode Network in patients with stroke relative to healthy controls. Red regions represent areas where the activity differed significantly between patients and controls. Significant at $p < .05$ (family-wise error corrected).

Figure 4. – Mean correlation coefficient weighted matrices (Fisher transformed) for every pair-wise region for the group of controls (A) and for the group of patients (B). (1) amPFC; (2) vmPFC; (3) lITG; (4) rITG; (5) LP; (6) RP; (7) lPH; (8) rPH; (9) lSFG; (10) rSFG; (11) PCC.

Figure 5. – Graph visualization of the correlation coefficient weighted matrices thresholded at $p < 0.05$, FWE corrected (5000 permutations). Mean correlations for the group of controls; mean correlations for the group of patients; significant differences between patients and controls. amPFC: anterior middle prefrontal cortex; vmPFC: ventro medial prefrontal cortex; rSFG: right superior frontal gyrus; lSFG: left superior frontal gryrus; rITG: right inferior temporal gyrus; lITG: left inferior temporal gyrus; lPH: left parahippocampal; rPH: right

parahippocampal; LP: left parietal; RP: right parietal; PCC: posterior cingulate cortex.

Figure 1.-

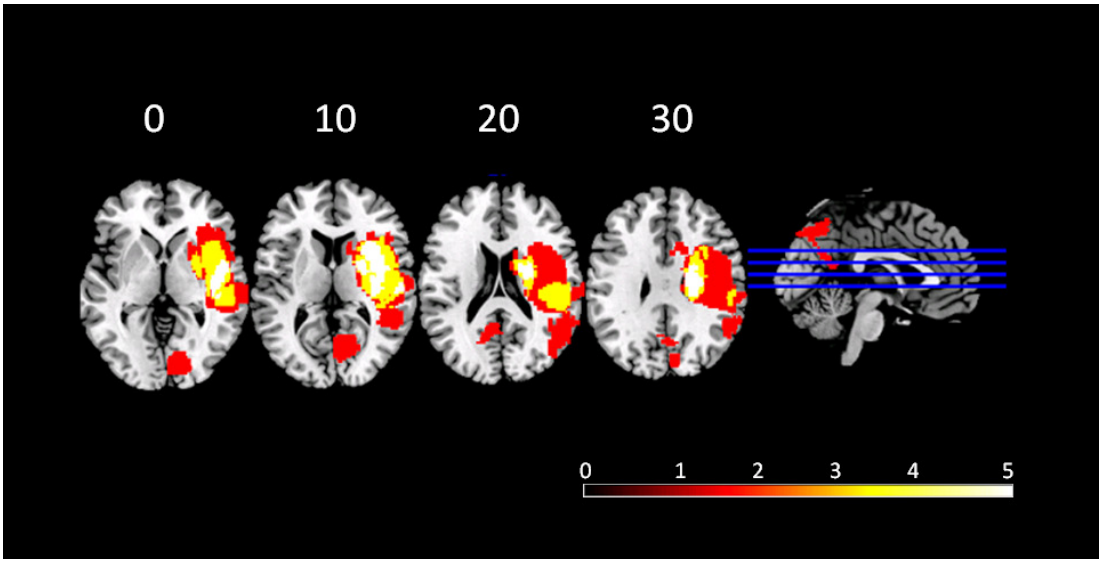
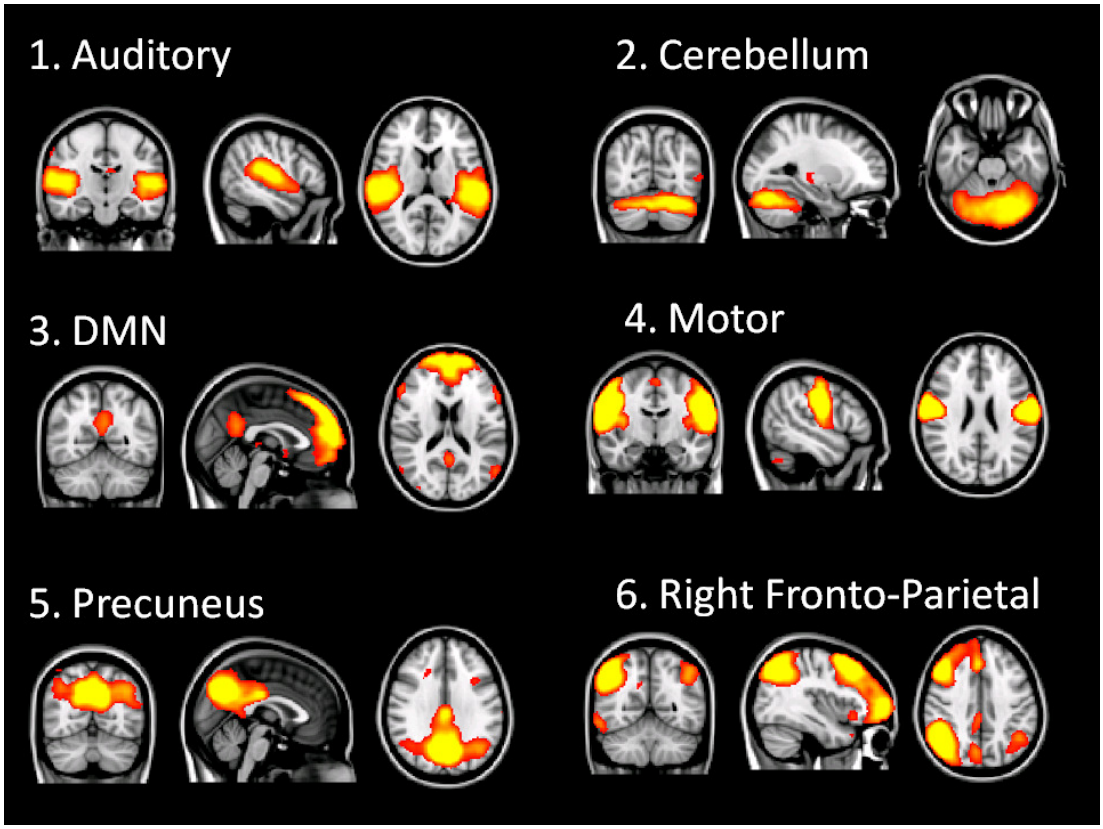


Figure 2.-



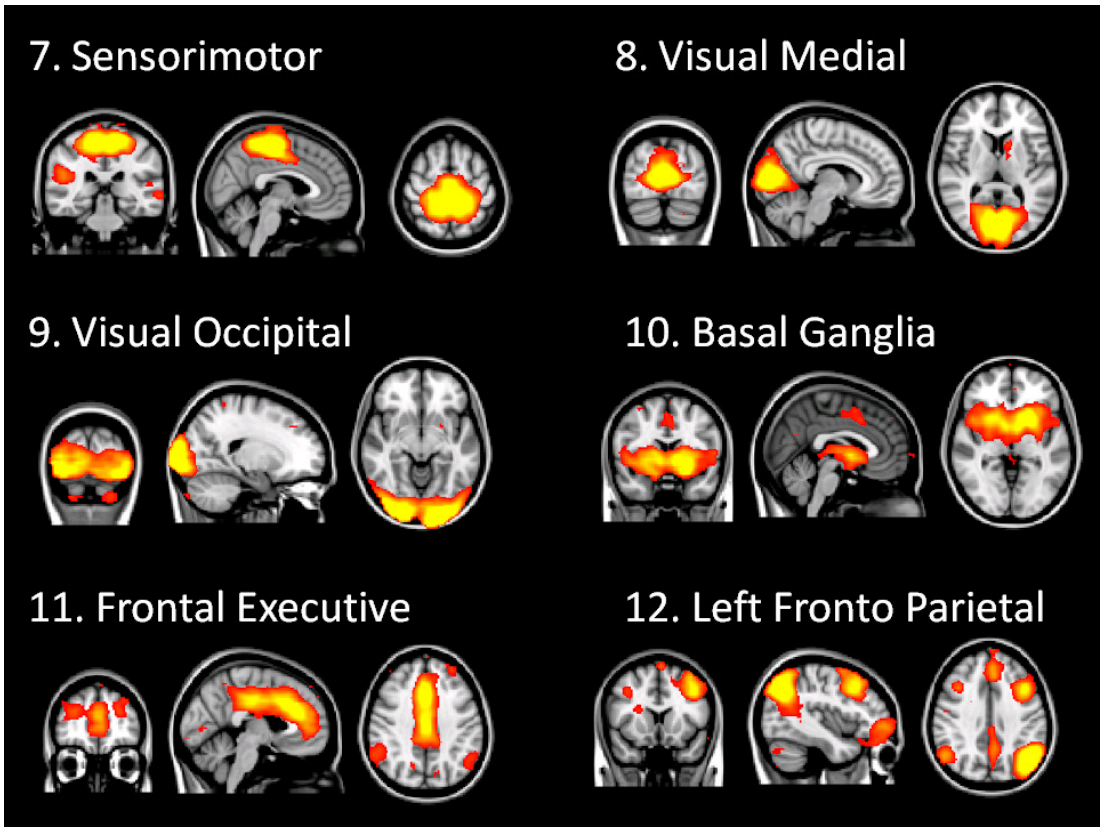

7. Sensorimotor
8. Visual Medial
9. Visual Occipital
10. Basal Ganglia
11. Frontal Executive
12. Left Fronto Parietal

Figure 3.-

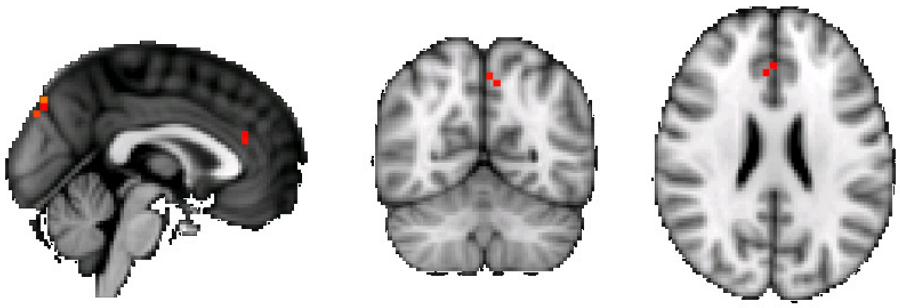
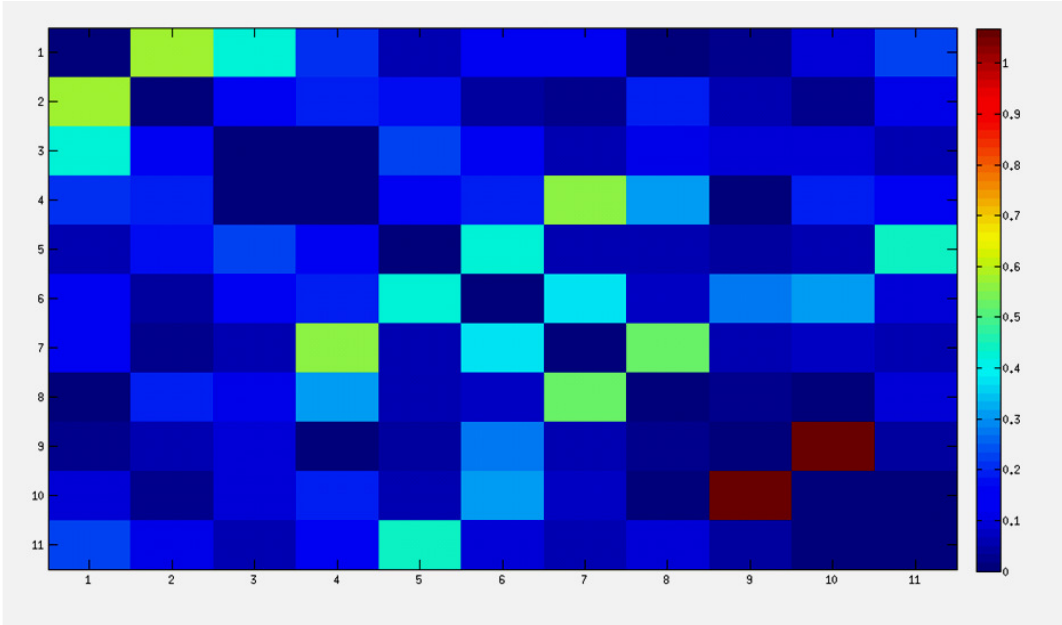


Figure 4.-

Controls



Patients

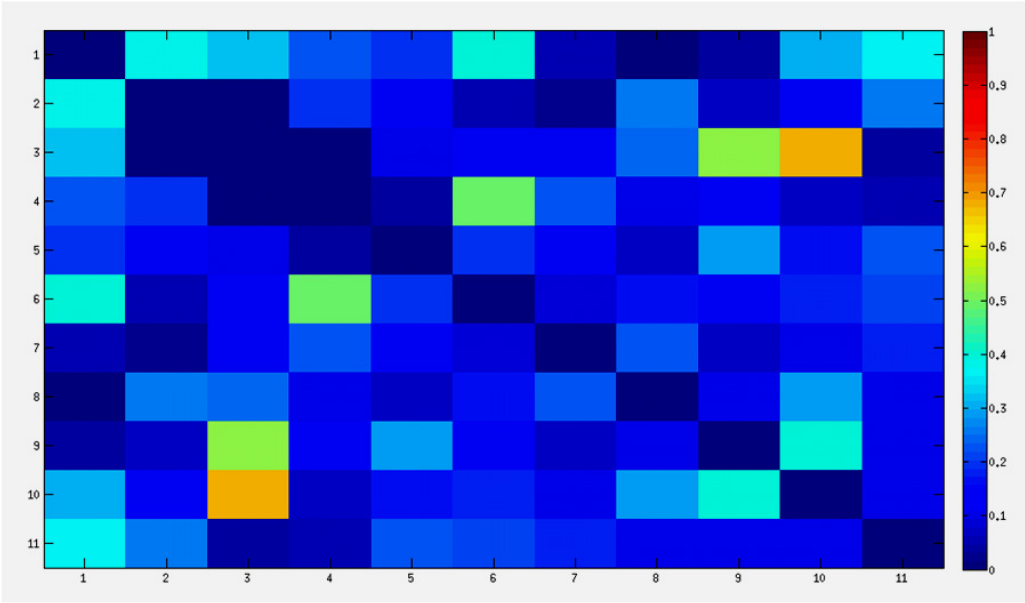


Figure 5.-

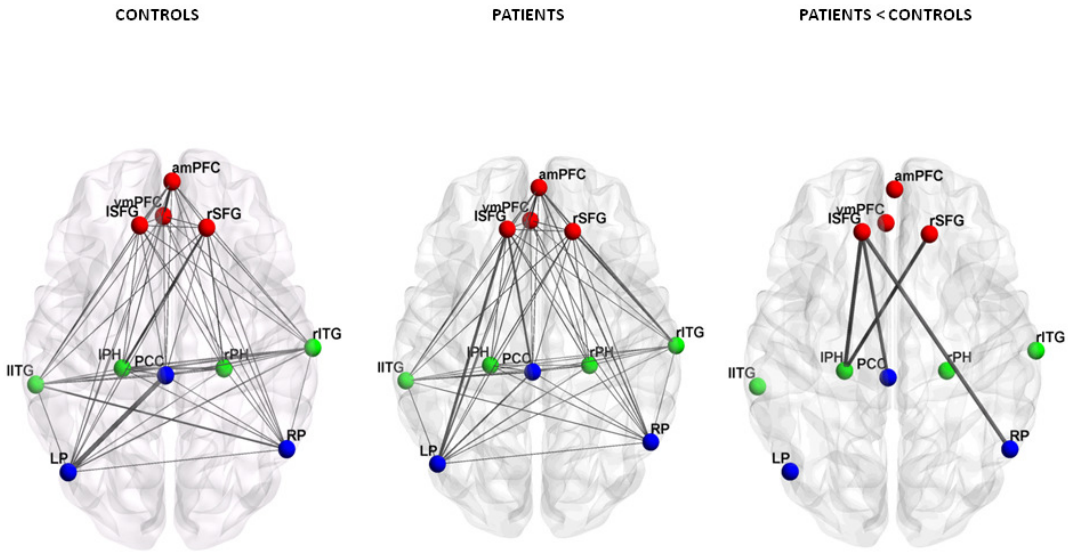


Table1. Demographic and Clinical Characteristics of Patients and Control Participants

Characteristics	Control Participants (n = 17)	Patients with Stroke (n = 11)
Age, mean (SD), years [a]	63.8 (3.6)	61.9 (7.9)
Educational level, mean (SD), years [b]	6.8 (3.6)	8.1 (6.1)
Edinburgh Test, mean (SD) [c]	95.3 (13.9)	97.7 (4.1)
Premorbid IQ, mean (SD) [d]	37.9 (8.1)	36.5 (10.4)
Gender, No.		
Male (%)	11 (64.7)	8 (72.7)
GDS	2.3 (3.5)	1 (2-0)
Vascular Risk Factors, No (%)		
Alcohol intake	9 (52.9)	2 (18.2)
Smoking	6 (35.3)	3 (27.3)

Hypertension	8 (47.1)	5 (45.5)
Diabetes Mellitus	1 (5.9)	4 (36.4)
Dyslipidemia	9 (52.2)	6 (54.5)
NIHSS at 3 months, median (interquartile range)	…	1.50 (3.5-0)
Barthel Scale at 3 months, mean (SD)	…	89.55 (17.9)
Ranking Scale at 3 months, median (interquartile range)	…	2 (2-0.5)
S-IQCODE at 3 months, mean (SD)	51.8 (1.8)	53.6 (4.65)
Lesion volume (cm^3), median (interquartile range)	…	17.60 (42-7.30)
Anatomical regions affected	…	
Insular cortex	…	4
Lentiform nucleus	…	2
Basal Ganglia	…	2
Corona Radiata	…	2
Occipital lobes	…	2
Temporal lobes	…	3
Parietal lobes	…	4
Frontal lobes	…	3
Arterial distribution	…	
MCA	…	8
ACA	…	2
PCA	…	2

Abbreviations: IQ, Intelligence Quotient measured with the vocabulary subtest (Wechsler Adults Intelligence Scale (WAIS-III-R); GDS, Geriatric Depression Scale; NIHSS, National institute of Health Stroke Scale; S-IQCODE, Short version of the Informant Questionnaire on Cognitive Decline in the Elderly.

[a] t = 0.734; p = .476

[b] t = -0.695; p = .493

[c] z = 0.304; p = 1.00

[d] t = 0.409 ; P = .686

Table2. - Neuropsychological tests scores at three months for the healthy control and stroke subgroups.

	HC (n = 17)	Stroke (n = 11)	p
General Cognitive Function			
MMSE	30 [30-28]	28 [29-26]	**<0.01**
Sustained Attention			
MoCA subtest (/11)	11 [11-10.5]	11 [11-10]	**0.03**
Digit Span Forward (WAIS-III)	4.9 ± 1.1	5.0 ± 1.5	0.91
Working memory			
Digit Span Backwards (WAIS-III)	3.0 [4-3]	4.0 [4-3]	0.92
Premotor functions			
Luria' sequences (/5)	5 [5-5]	5 [5-0]	0.07
Rhythms subtest (/10)	9 [10-8]	8 [8-7]	**0.04**
Interference and Inhibitory control (/3)	3 [3-2]	3 [2-1]	0.27
Verbal fluency			
Letter (P)	11.4 ± 2.9	9.3 ± 4.6	0.15
Semantic (Animals)	18 [18.5-13]	13 [17-12]	0.28
Language			
Boston Naming Test (/15)	11.1 ± 2.1	10.8 ± 2.6	0.79
Psychomotor speed *(seconds)*			
Trail Making Test A (seconds)	56.2 ± 23.6	80.6 ± 29.8	**0.02**
Grooved Pegboard Test (preferred hand; seconds)	73.7 ± 10.7	85.1 ± 22.9	0.15

Visuospatial Skills			
Right Line cancellation test (/18)	18 [18-18]	18 [18-18]	0.07
Left Line cancellation test (/18)	18 [18-18]	18 [18-17]	0.07

Values are means ± standard deviations in Student's t-test or medians (percentile 25-75) in Mann-Whitney test for continuous variables.

Table 3.- Coordinates of brain ROIs in the DMN

Region	**MNI Coordinates**		
	X	Y	Z
Anterior medial PFC (amPFC)	1	55	26
Ventro-medial PFC (vmPFC)	-3	40	0
Left SFG (lSFG)	-14	36	59
Right SFG (rSFG)	17	35	58
Left ITG (lITG)	-62	-33	-20
Right ITG (rITG)	66	-17	-19
Left parahippocampal gyrus (lPH)	-22	-26	-21
Right parahippocampal gyrus (rPH)	25	-26	-18
PCC	-2	-29	39
Left lateral parietal (LP)	-47	-71	35
Right lateral parietal (RP)	54	-61	36

PFC, prefrontal cortex; SFG, superior frontal gyrus; ITG, inferior temporal gyrus; PCC, posterior cingulate cortex.

An Active Learning approach for Stroke lesion segmentation on multimodal MRI data

Darya Chyzhyk[1], Rosalía Dacosta-Aguayo[1,3], Maria Mataró[2,3], Manuel Graña[1]

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[1]Grupo de Inteligencia Computacional, University of the Basque Country, Spain;
[2]Institute for Brain, Cognition and Behaviour (IR3C), [3]Dept. Psychiatry and Clinical Psychobiology, Faculty of Psychology. University of Barcelona, Spain

Abstract

The segmentation of lesion tissue in brain images of stroke patients serves to identify the extent of the affected tissues, to perform prognosis on its recovery, and to measure its evolution in longitudinal studies. The different regions of the lesion may have different imaging contrast properties in different image modalities, making difficult the automation of the segmentation process. In this paper we consider an Active Learning selective sampling approach to build image data classifiers from multimodal MRI data to perform voxel based lesion segmentation. We report encouraging results over a dataset combining functional, anatomical and diffusion data.

1 Introduction

Stroke is the third leading cause of death in industrialized countries [18]. Patients surviving stroke suffer an strong economic and personal impact, and it is highly desirable to provide accurate prognosis and motorization of the evolution of the patient. The localization of the stroke lesion by medical imaging means is a powerful non-invasive tool to support the clinical attention to the stroke patient. The kind of imaging used encompasses several modalities of Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI), which have specific sensitivities to the diverse parts of the lesion [18]. The automated localization of the brain areas affected by the stroke lesion can be stated as a classification problem, which predicts the lesion/non-lesion character of one voxel on the basis of the imaging data. This paper is devoted to explore the predictive capacity of Random Forests (RF) trained with an Active Learning strategy, to the automated segmentation of stroke lesion from several MRI modalities.

Active Learning by selective sampling: Given a labeled training set, supervised classifier learning consists in building a map of data features into a set

of classes possessing good generalization to predict the class of unseen data instances. Hence, validation processes involve simulating the existence of these future data events by cross-validation processes withholding some of the available data samples for test after training. The Active Learning approach consists in the progressive increase of the sampled data used for classifier training by some exploratory strategy. The idea of selective sampling [6] is based on the existence of an oracle that answers queries about the data which arise from an active exploration of the data domain. The active exploration takes the form of the computation of an uncertainty measure on the classification of the available data samples. Most uncertain samples are assumed to be more informative to build an accurate classifier, thus the oracle is queried about their ground truth labels and they are added to the training dataset. Testing is always reported on the data not used for train.

In general terms, Active Learning [22] soughs the simultaneous two-fold goal of maximizing the classifier accuracy generalization and using the minimal number of training samples whose ground truth label is required of the oracle, which is a human operator when dealing with image segmentation issues. Often, Active Learning starts from a small labeled data sample. The iterative process then performs the following steps (a) train a classifier, (b) apply the classifier to unlabeled data samples computing their uncertainty, (c) select the most uncertain, (d) ask the oracle about their labels, (e) add them to the training sample. The process convergence is often measured on the accuracy of the test data. The uncertainty measure definition derives from the classifier characteristics [22]. In ensemble classifiers, it often consists in some measure of the agreement of the individual classifiers.

Image segmentation by classification: Image segmentation can be realized as a classification process, each pixel receives a class label according to the associated image features which can be computed from the pixel neighborhood. For instance, the RF classifier [3] has been applied to delineate the myocardium in 3D ultrasounds of adult hearts [14], brain tissue segmentation [9, 24, 23], detection of several organs in computed tomography volumes [7, 8].

Active Learning has been successfully applied to classification of remote sensing images [17, 22, 21], medical image segmentation such as Abdominal Aortic Aneurysm computed tomography angiography images [5, 16], image retrieval based on semi-supervised Support Vector Machines [10], and the selection of a minimal collection of training images for the development of combined generative and discriminative models in the segmentation of CT scans [11].

Stroke lesion segmentation: In patients suffering a stroke, it is common practice to perform lesion volume estimation on Diffusion Weighted Imaging (DWI) data. However, it is argued [1] that while DWI is sensitive in the acute phase, it becomes less accurate during the subacute phase (i.e. 3 months after stroke insult). Nevertheless, lesion identification is also challenging for other modalities, such as Fluid Attenuated Inversion Recovery (FLAIR), because of large variations in shape, location signal intensity, and the existence of image artifacts and pre-stroke lesions. For this reason we have considered several manual delineations as the gold standard for classifier training, although the

feature vectors are always the same.

In the past, some attempts have been made on anatomical T1 weighted MRI data [19] to perform lesion identification using standard segmentation processes followed by a fuzzy clustering approach to detect outlier signal values in the segmented volume. The interactive segmentation of stroke and tumor lesions on FLAIR images is reported in [1]. The process includes the clustering of FLAIR signal into foreground and background classes. Lesions are extracted as hyperintense outlier voxels on the segmented foreground regions. The segmentation applying a Markov Random Field model on the fusion of multi-sequence MRI images is reported in [12]. The work is closely related to the elaboration of an atlas of brain territories which provides the topological background for the segmentation process. A z-score based test is applied to diffusion images to produce lesion identifications which are then used to build a map of lesion incidence in the occipital lobe [15] for use in clinical studies. A comprehensive review containing a critical appraisal of computational methods applied on MRI and CT image data to identify lesions in brain tissues and perform prognosis of its recovery is given in [18].

Paper contributions: The work reported in this paper follows a classification approach to automated stroke lesion segmentation, where the classifier is trained by an Active Learning selective sampling strategy. The classifier model chosen is the Random Forest (RF). A trained RF classifier is applied to each voxel independently to predict the class of the pixel, lesion versus no lesion. The voxel features which are the input of the classifier are the values of the scalar MRI data volumes and the scalar measures extracted from multidimensional data, described in section 2. Compared with other algorithms reported in the literature, the main advance of our approach is that it combines heterogenous information from diverse MRI modalities, while other algorithms work on a single image modality. Further contribution to the state of the art, is the Active Learning approach to sample selection, which allows an enhanced interactive work in clinical environments. Different from other reported works is the consideration of the construction of the gold standard for classification validation, given by manual delineations of the lesion ground truth. We explore the effect of the source data used by the expert neuroradiologist to carry out the manual delineation, finding quite different gold standards in some cases. The computational experiments carried out explore the effect of the variability in the provided the gold standard. To this end, we consider four different gold standards obtained from different image modalities.

Structure of the paper: Section 2 provides the explanation of the multimodal MRI data acquisition and pre-processing. Section 3 gives a description of the classification learning methods, including a detailed Active Learning algorithm. Section 4 presents the experimental design. Section 5 presents the experimental results. Section 6 contains a discussion of the results. Finally, section 7 gives the conclusions of our work.

2 Multimodal MRI data and its preprocessing

The details of MRI signal acquisition for each modality are given elsewhere. The pre-processing pipeline is specific for each kind of image modality. Hence a careful process has been carried out for each of them to ensure anatomical alignment and to remove noise sources. Most pre-processes have been carried out using FSL software library (FMRIB Centre, Department of Clinical Neurology, University of Oxford, www.fmrib.ox.ac.uk/fsl) [20]. Besides, the multidimensional signal, such as fMRI and DWI are subjected to some feature extraction processes that produce scalar measures for each voxel. These pipelines are as follows:

T1 weighted MRI preprocessing consisted on the removal of non-brain structures, linear registration of the skull stripped image to the 2mm resolution MNI152 template, nonlinear registration of the volume created from the previous linear registration to compensate the local changes around ventricles and sulci caused by atrophy, and, finally, we applied the estimated warps to the skull striped volume.

T2 and Flair image preprocessing consisted on the removal of non-brain structures, rigid linear co-registration of image to the subject's T1 skull striped volume (6DOF) and affine (12DOF) linear registration to the 2mm resolution MNI152 brain template.

fMRI Functional magnetic resonance imaging (fMRI) preprocessing consisted on the removal of the first 6 volumes to ensure saturation and adaptation of the subjects to the environment leaving 234 volumes for further analysis, removal of non-brain structures, motion correction, low-pass and high-pass temporal filtering, spatial smoothing using a Gaussian kernel of full-width half-maximum of 5mm, intensity normalization, rigid linear co-registration to the main structural image with 6 Degrees of Freedom (DOF), and posterior affine linear registration algorithm to the MNI152 standard template (12 DOF). Absolute head movement was below 1.5mm

- Amplitude of Low Frequency Fluctuations (ALFF) [25] and fractional Amplitude of Low Frequency Fluctuations (fALFF) [27] are low frequency oscillation measures of amplitude of the BOLD signal. ALFF is defined as the total power within the frequency range between 0.01 and 0.1 Hz. fALFF is defined as the power within the low-frequency range (0.01-0.1 Hz) split by the total power in the entire detectable frequency range [28].
- Regional Homogeneity (ReHo) estimates the similarity between the time series of a given voxel and its (27) nearest neighbors [26], computed as the Kendall's coefficient of concordance (KCC) [13]. The KCC values are standardized and smoothed (4mm FWHM) to build a voxel-based map for each subject.

DWI preprocessing data is as follows: After eddy current correction, non-brain voxels were extracted reducing inclusion of voxels that are likely composed of multiple tissue types or fiber orientations. In Diffusion Tensor Imaging (DTI), a voxel diffusion tensor is a 3×3 positive-definite symmetric matrix D, which can be represented by its decomposition as $D = \lambda_1\mathbf{g}_1\mathbf{g}_1^T + \lambda_2\mathbf{g}_2\mathbf{g}_2^T + \lambda_3\mathbf{g}_3\mathbf{g}_3^T$, where $\lambda_1 \geq \lambda_2 \geq \lambda_3$ and $\mathbf{g}_1$, $\mathbf{g}_2$, $\mathbf{g}_3$ are the eigenvalues and eigenvectors of D, respectively. Two scalar measures were extracted [2] from the voxel diffusion tensors: the mean diffusivity (MD) and the fractional anisotropy (FA). The first corresponds to the average eigenvalue:

$$MD = \frac{\mathrm{Tr}\,(D)}{3} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}. \tag{1}$$

The FA measures the fraction of the magnitude of D that can be related to anisotropic diffusion in a mean-squared sense (i.e. the extent of deviation from isotropic diffusivity in all direction). Its magnitude is also rotationally invariant, and independent from sorting of the eigenvalues. The FA is calculated as follows:

$$FA = \sqrt{\frac{1}{2}}\frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}. \tag{2}$$

Thus, isotropic diffusion is imaged as zero value and FA maximum value is one.

3 Classification Methods

This section provides a brief review of the classifier construction methods applied in the experiments. These methods refer to the actual classifier training, as well as the strategy used to increase the training data sample by an Active Learning approach.

3.1 Random Forest Classifiers

The random forests (RF) [3] is a collection of of decorrelated decision trees $\{h_t(\mathbf{x};\psi_t)\}_{t=1}^T$, where $\mathbf{x}$ is a d-dimensional feature vector, ψ_t are independent identically distributed random vectors that determine the tree construction, i.e. they characterize which variables are chosen to perform split at each branch of the decision tree, and the bootstrapping of the training data from the data sample. Given a dataset of N samples, a bootstrapped training dataset is used to grow tree $h_t(\mathbf{x};\psi_t)$ by recursively selecting a random subset of data dimensions $\hat{d}$ such that $\hat{d} \ll d$ and picking the best split of each node based on these variables. Unlike conventional decision trees, pruning is not required. The independent identically distributed random vectors ψ_t determine the random dimension selection.The output of each decision tree is a class prediction for the feature vector. The overall decision is taken by majority vote over the

outputs of the collection of decision trees. The RF classifier has shown to overcome over-fitting of individual trees, improving generalization. The RF classifier parameters were set after an exploration of several combinations of values by cross-validation experiments on a small training set extracted from the gold standard. Finally, we set the number of trees to 101, the number of variables randomly sampled at each split node to $\hat{d} = 5$, and the maximum depth of each individual decision tree to 5.

3.2 Active learning

Active Learning Selective Sampling [6, 21] performs an active exploration of the data domain, creating a map of the uncertainty associated with each data point. The uncertainty is an unsupervised measure, because we do not need to know the data point label, but the confidence of the classifier in the class prediction produced for it. For instance if the classifiers rely on some estimation of the class boundaries, the uncertainty would be inversely proportional to the distance of the point to the estimated boundary. Adding these points to the training set is expected to improve accuracy and generalization in the next iteration of classifier training.

Let $X = \{\mathbf{x}_i, y_i\}_{i=1}^{l}$ be a set of labeled samples for training, where $\mathbf{x}_i \in \mathbb{R}^d$ and $y_i \in \{1, \ldots, N\}$. Let be $U = \{\mathbf{x}_i\}_{i=l+1}^{l+u} \in \mathbb{R}^d$ the set of test data, with $u \gg l$, corresponding to the set of unlabeled pixels to be classified for test. At iteration k, the Active Learning algorithm selects from U^k the q candidates with maximal uncertainty in their class prediction with the current classifier trained on the current training set X^k. The selected samples $S^k = \{\mathbf{x}_i\}_{i=1}^{q} \subset U$ are labeled with labels $\{y_i\}_{i=1}^{q}$ by an oracle, often a human operator in interactive segmentation or the given ground truth. Finally, the set S^k is added to the current training set $X^{k+1} = X^k \cup S^k$ and removed from the pool of candidates ($U^{k+1} = U^k \backslash S^k$). The process is iterated until a stopping criterion on the accuracy obtained over the test set is met. Algorithm 1 summarizes the Active Learning process.

The estimation of each unlabeled sample uncertainty follows the committee approach [21], because we will be using RF as the classifier model. Assume that we have built a committee classifiers, i.e. a RF with T trees, so that classification is provided by the majority voting. The committee provides T labels for each candidate sample $\mathbf{x}_i \in U$, so that the uncertainty of its classification may be measured by the standard deviation σ^{RF} of the distribution of the class predictions provided by the individual decision trees. Formally:

$$\hat{\mathrm{x}} = \arg\max_{\mathrm{x}_i \in U} \{\sigma^{RF}(\mathrm{x}_i)\}$$

The standard deviation is a natural multi-class heuristic measure of classification uncertainty. If all classifiers in the committee agree on the class of a sample it has a zero prediction standard deviation, thus its inclusion in the training set does not bring additional information. On the other hand, a sample with maximum disagreement between the classifiers results in maximum standard deviation, introducing new information in the classification process.

Algorithm 1 Active learning general algorithm

Algorithm Inputs
–Initial training set $X^k = \{\mathrm{x}_i,\ y_i\}_{i=1}^{l} (X \in \mathcal{X},\ k = 1)$.
–Unlabeled test data $U^k = \{\mathrm{x}_i\}_{i=l+1}^{l+u} (U \in \mathcal{X},\ k = 1)$.
–Number of pixels q to add at each iteration (selected pixels S).

repeat
  Train a model with current training set X^k
  for *each* test sample in U^k **do**
    Evaluate uncertainty
  end for
  Rank the candidates in U^k according to uncertainty
  Select the q most uncertain pixels $S^k = \{\mathrm{x}_i\}_{i=1}^{q}$
  The oracle assigns a label to the selected pixels $S^k = \{\mathrm{x}_i,\ y_i\}_{i=1}^{q}$
  Increase the training set $X^{k+1} = X^k \cup S^k$
  Remove the batch from the unlabeled test data $U^{k+1} = U^k \backslash S^k$
  $k = k + 1$
until Test Accuracy $> \theta$

4 Experimental design

The experimental data: The experimental data considered was composed of ten volumes of scalar values, some of them corresponding to MRI data (i.e. T1, T2, FLAIR), others corresponding to scalar measures computed voxelwise from fMRI data (ReHo, ALFF, fALFF), and DWI data (AD, MD, RD, FA). Figure 1 illustrates the variety of the imaging data by showing an axial slice of each of them.

The classification process: The scalar valued volumes considered, i.e. T1, T2, FLAIR, ReHo, ALFF, fALFF, AD, MD, RD, and FA, provide the feature vectors for voxel classification: for each voxel site, the corresponding feature vector is composed of the values of these volumes a this voxel site. The RF classifier output is the class of the voxel site, lesion versus non-lesion. The RF classifier is trained by an Active Learning approach, starting from a small training set which enriched by informative samples in several steps. Each step implies retraining the RF classifier and testing on the remaining data to obtain the evolution of the classification accuracy.

Experimental design: The experimental design is illustrated in figure 2. From left to right, the first column of oval pink boxes shows the different image modalities considered. The second column of green boxes contain the process performed on the data, parenthesis contains the name of the software used. The third column of oval pink boxes displays the diverse scalar volumes obtained from each process. The red arrows indicate which scalar measure has been used by the Neuroradiologist to create the ground truth Lesion mask. The green arrows indicate which scalar measures has been entered in the Active Learning process in diverse combinations. The fMRI data is pre-processed

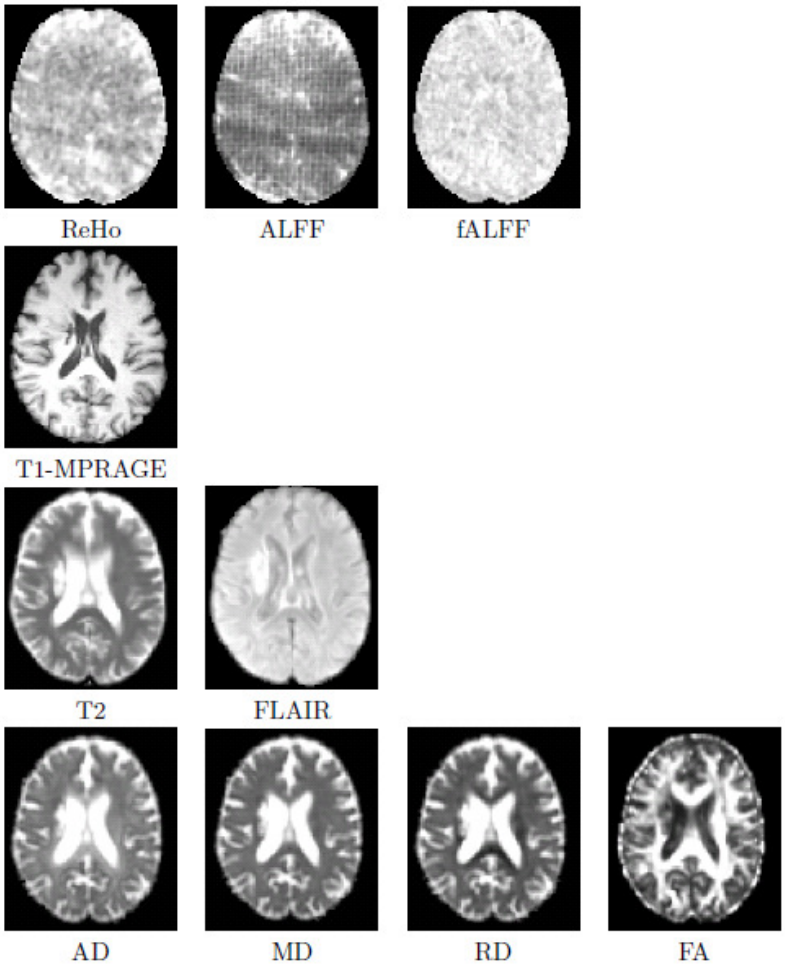


Figure 1: Brain axial slices of each of the image modalities considered in the paper.

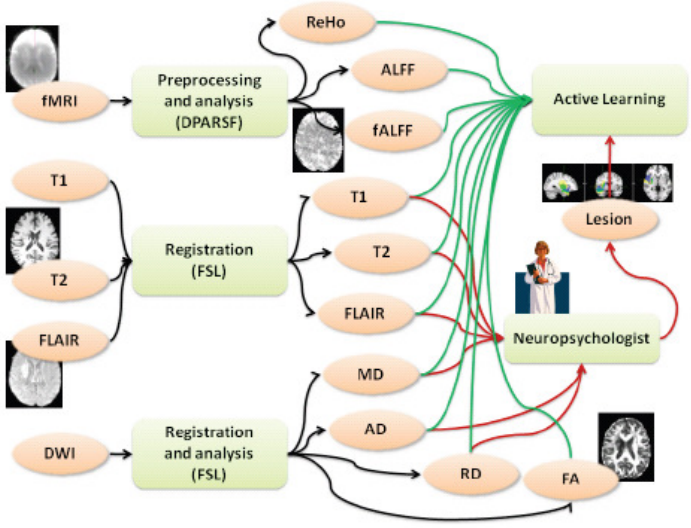
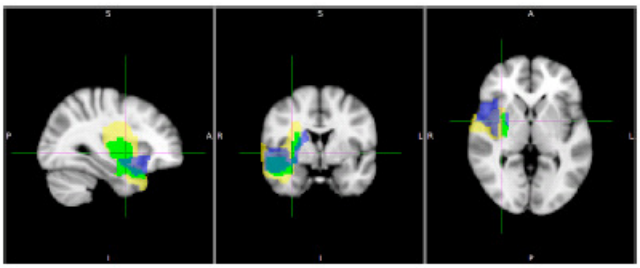


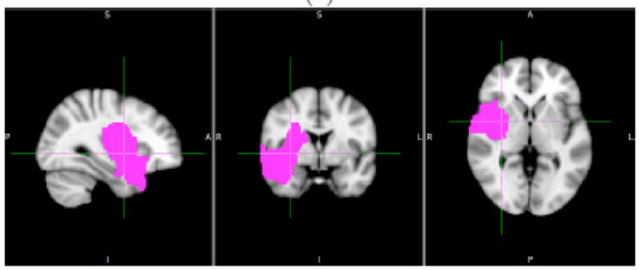
Figure 2: Pipeline description of the overall experimental design.

and scalar measures of brain activity are obtained using the DPARSF software (`http://rfmri.org/DPARSF`) [4]. The T1, T2 and FLAIR data are both taken as image features for classification and used by the Neuropsychologist to perform manual delineation of the lesion to provide the ground truth for active learning. The DWI data is preprocessed by FSL [20] and the tensor based scalar features are both taken as image features for classification and as the basis for lesion delineation. The four experiments reported below are separated by the actual gold standard for the classification, which depends on the image modalities used by expert neuroradiologist to perform the manual delineation of the lesion.

Lesion delineation: Lesion delineation is a delicate issue, because different results will be obtained from different image modalities. Figure 3(a) shows the overlapping of the manual lesion delineation obtained from the different modalities, yellow corresponding to T1, blue to T2 and FLAIR, and green to the DWI derived modalities. In fact, each manual delineation and its union give way to a different computational experiment where we test active learning construction of voxel prediction of lesion on the basis of the ten features obtained from the multi-modal MRI. Detailed results are given in the next section. The mask of the lesion was drawn at three months post-stroke using manual tracing on the T2-weighted, FLAIR, MPRAGE and Diffusion registered images (MD, AD, RD,FA) images by a trained clinical neuropsychologist. Lesion was drawn on each slice showing the infarct. The mask of the lesion was thresholded and binarized (lesion =1; brain = 0) avoiding partial volume effects (PVEs).



(a)



(b)

Figure 3: Manual delineation of the lesion by the neuropsychologist, (a) individual lesion delineations over different image data shown in different colors, (b) the union of the manual delineations for each image modality.

5 Experimental lesion detection results

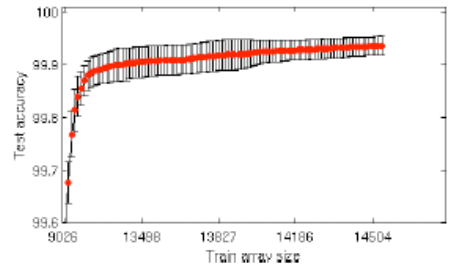
We have carried out four different segmentation experiments, each one assuming a different lesion delineation performed by the expert neuropsychologist over different MRI modalities. The segmentation by Active Learning Selective Sampling has been repeated ten times in each experiment. We report the evolution of the average and standard deviation of the accuracy, sensitivity and specificity of the classification at each iteration of the test data (disjoint from the training data being enriched at each iteration). The abscissa of the plots is the size of the training set. Besides, we show the overlap of the final lesion segmentation and the gold standard provided by the expert for each experiment.

Experiment 1: The first experiment considers the collection of all the individual manual delineations into the union lesion delineation of figure 3(b). That way, we consider that the expert is trying to extract information from all the available data. Figure 4 shows the plots of the average accuracy, sensitivity, and specificity of 10 repetitions of the active learning process. The accuracy and specificity reach very high values, close to 100%. The sensitivity, which is the true measure of the lesion detection, is close to 90% after the initial iterations, which is a very good result compared even with interater error. The evolution of the active learning is very fast, reaching values close to the final ones after a few iterations. Figure 5 gives a visualization of the overlap of the lesion identified by the Active Learning approach (red) and the gold standard given by the manual delineation (blue). Notice that there are no false positives, so all lesion detection is inside the actual gold standard.

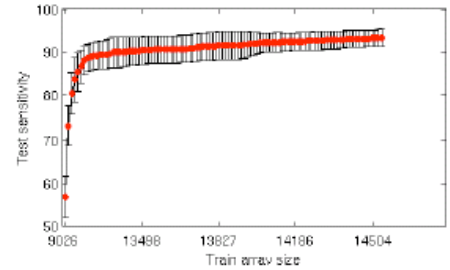
Experiment 2: The second experiment refers to the identification of the lesion taking as gold standard the manual delineation performed over the T1 data. Figure 6 gives the plots of the accuracy, sensitivity, and specificity. Sensitivity reaches above 80% in this experiment only after many iterations, which could confirm that T1 is not convenient source of information for lesion detection, even in subacute phase, 3 months after the stroke insult. Accuracy and specificity are very high, which is due to the absence of false positives. Figure 7 shows the overlap of the lesion identified by Active Learning (green) and the gold standard (red). We can appreciate some missing parts, and the lack of lesion detections outside the delineated gold standard.

*Experiment 3:*In the third experiment, the gold standard has been delineate over T2 weighted and FLAIR signals. Figure 8 gives the plots of accuracy, sensitivity, and specificity. Sensitivity reaches above 90%, while accuracy and specificity approach 100%. As in experiment 1, the lesion identification error is quite acceptable, close to interater errors. Our interpretation is that T2 and FLAIR contain most of the information that can be useful for lesion identification. Figure 9 shows the overlap of the lesion identified by Active Learning (blue) and the gold standard (yellow), which is very good.

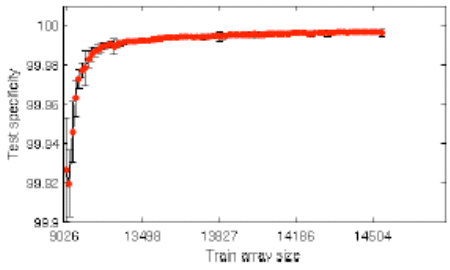
Experiment 4: The last experiment is performed on the gold standard delineated from the DWI data, specifically AD, MD and RD scalar measures extracted from the tensor information. Figure 10 gives the plots of the average accuracy, sensitivity, and specificity. We find that the classifier sensitivity is



(a)



(b)



(c)

Figure 4: First experiment: Average and standard deviations of the (a) accuracy, (b) sensitivity, and (c) specificity of the classification of the test data at each iteration.

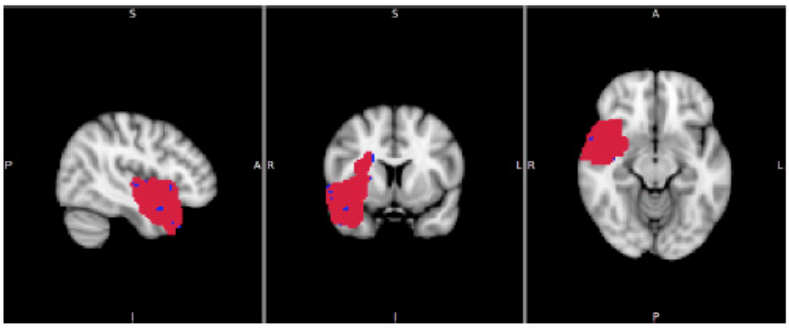


Figure 5: First experiment: overlap of the lesion identified by active learning classifier training (red) and expert delineation (blue).

the worse case, barely reaching 80% at the end of the process. This result may confirm the loss of information of the diffusivity measures after some time, more even after 3 months. Figure 11 shows the overlap between the lesion identified by Active Learning (yellow) and the gold standard (blue). Big portions of the lesion remain undiscovered by the learned classifiers, but the false positive ratio remains low as in previous experiments.

6 Discussion

The Active Learning approach means that the segmentation process will be basically interactive. That is, the human operator will act as the oracle, providing the class label for the most informative voxels detected by the classification uncertainty measure. The evolution of the accuracy, sensitivity and specificity measures in the summary plots of figure 12 shows that there are only a few iterations needed to reach a quality of the segmentation that can be accepted for practical clinical applications. Hence, the effort of the human operator will always be less than the effort required to perform the manual delineation. Moreover, attending to the variance bars, it is evident that the variability of the segmentation results decreases at each iteration of the Active Learning process, so that good results are to be expected in any situation, the learning process will correct any deviation from optimality. The proposed approach may lead to accurate and sound interactive lesion identification with clinical applicability. Overall, the classification results are encouraging, sensitivity reaches over 90% in some cases, which is above expected interrater agreement in many medical image analysis domains.

An important issue is the determination of the relation between image modalities and the quality of the gold standard lesion delineation. Our work seeks an answer to this question by the following experimental design: we have considered four different gold standards to be used as ground truth for Active Learning

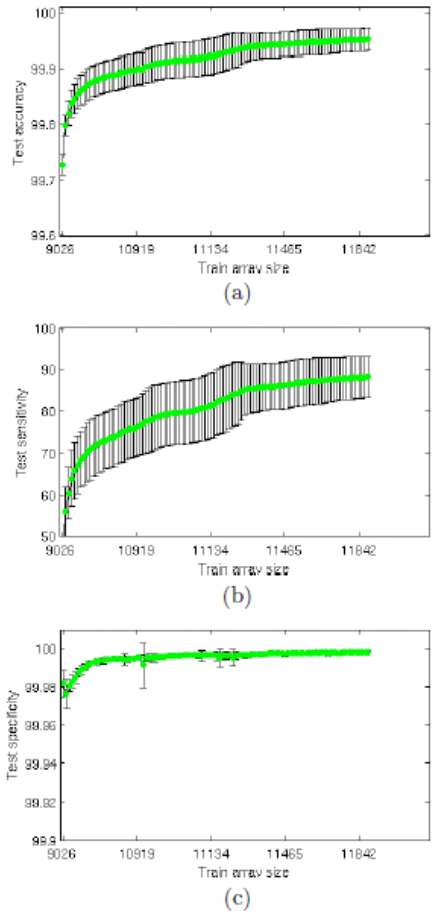


Figure 6: Second experiment: Average and standard deviations of the (a) accuracy, (b) sensitivity, and (c) specificity of the classification of the test data at each iteration.

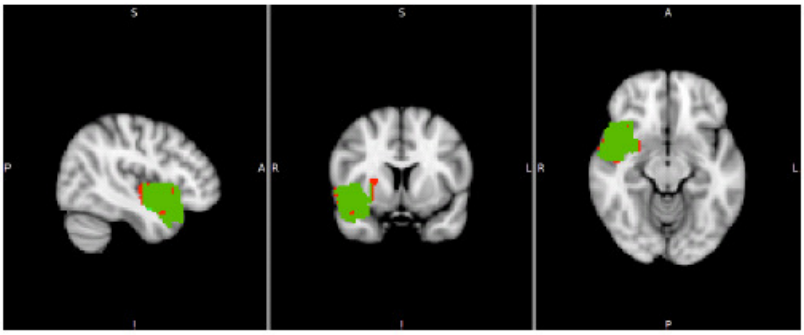


Figure 7: Second experiment: overlap of the lesion identified by active learning classifier training (green) and expert delineation (red).

construction of the RF classifiers carrying the lesion segmentation. The results are summarized in figure 12, overlapping the accuracy, sensitivity and specificity of the four experiments. The results show that the four gold standards are undistinguishable regarding accuracy and specificity, which are almost 100% in all cases. However, sensitivity is extremely different in some cases. The best agreement is between T2, FLAIR (experiment 3) and the whole union of gold standards (experiment 1), reaching 90% after few iterations. The use of the other data modalities to delineate the gold standard provides poor sensitivities, which can be interpreted as their lack of consistent information towards characterizing the lesion, despite the efforts of the expert.

7 Conclusions

The paper presents experimental results on the application of an Active Learning strategy to build classifier based stroke lesion segmentation over multi-modal MRI information. We tested RF classifiers on anatomical, diffusion and functional MRI data. The experimental results are encouraging of further research. An specific issue considered is the dependency of the segmentation results on the gold standard, which is highly dependent on the actual image modalities used for the manual delineation of the lesion. Future work will be extending this approach to more available datasets. Special attention will be given to the scattering of lesions, which is not present in the study reported in this paper. The fusion of CT and MRI data will be also considered.

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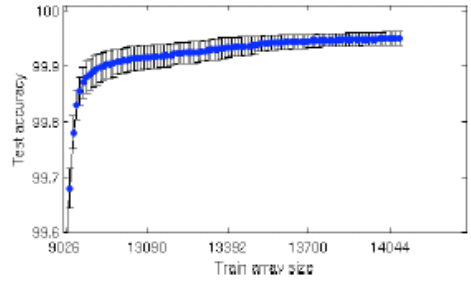
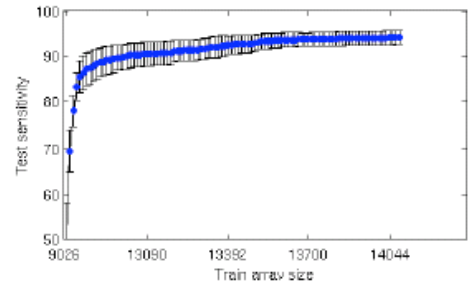
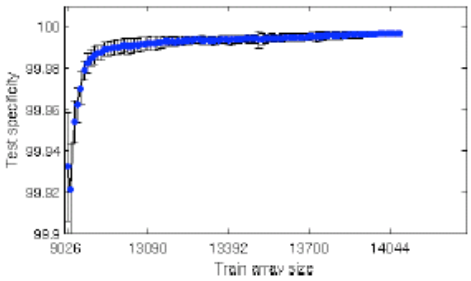


(a)



(b)



(c)

Figure 8: Third experiment: Average and standard deviations of the (a) accuracy, (b) sensitivity, and (c) specificity of the classification of the test data at each iteration.

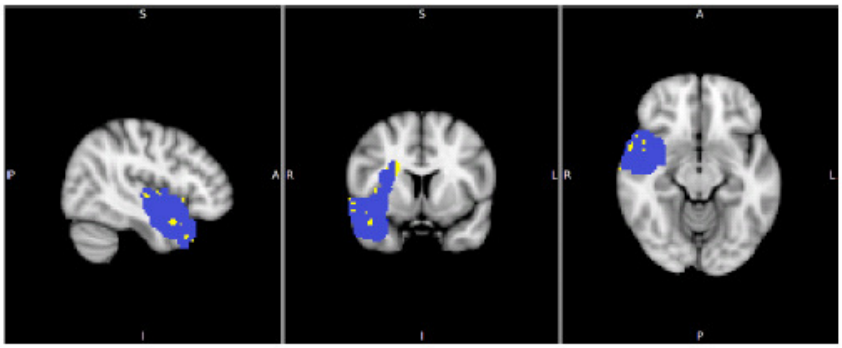


Figure 9: Third experiment: overlap of the lesion identified by active learning classifier training (blue) and expert delineation (yellow).

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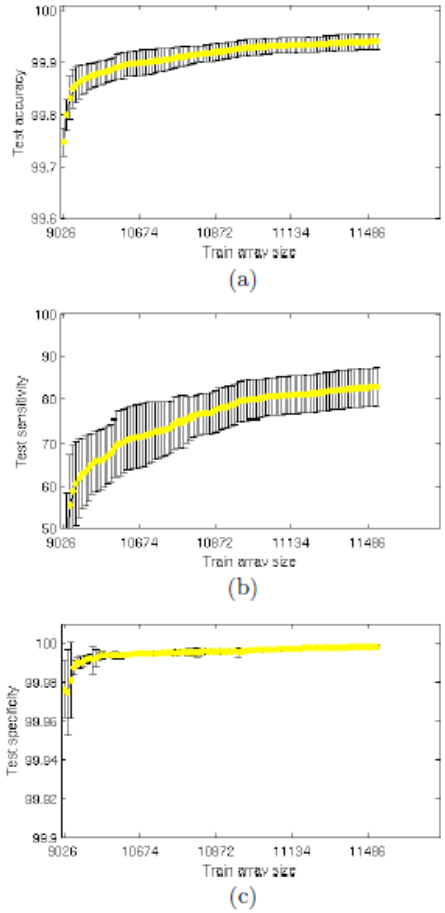

Figure 10: Fourth experiment: Average and standard deviations of the (a) accuracy, (b) sensitivity, and (c) specificity of the classification of the test data at each iteration.

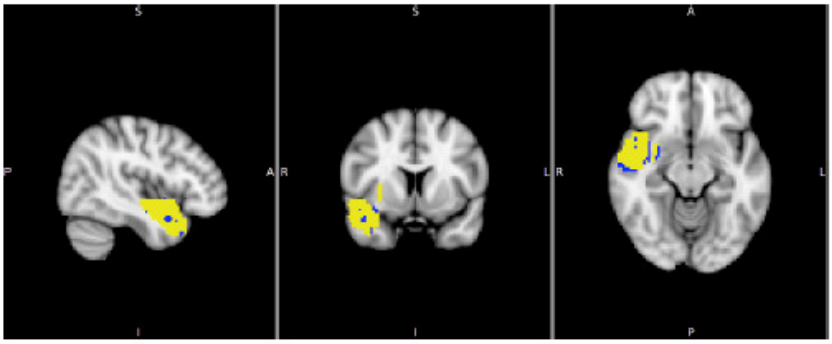

Figure 11: Fourth experiment: overlap of the lesion identified by active learning classifier training (yellow) and expert delineation (blue).

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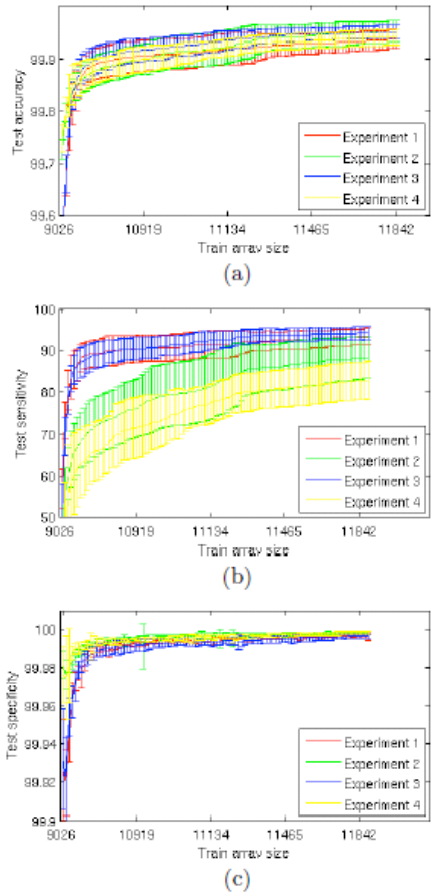

Figure 12: Overlapped experimental results of the four experiments: Average and standard deviations of the (a) accuracy, (b) sensitivity, and (c) specificity of the classification of the test data at each iteration.

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4. SUMMARY OF THE RESULTS

Study I

Compared to the healthy control group, the stroke group showed significant alteration in the following six RSNs: increased brain activity in (1) Frontal network; (2) Fronto-Temporal network; (3) Default Mode Network (DMN), (4) Secondary network, and decreased brain activity in (5) Basal Ganglia network and (6) Parietal network. Patients with good cognitive recovery demonstrated significant increase of activity only in the Fronto-Temporal and the DMN, as well as significant decrease of activity in the Basal Ganglia network when compared to healthy control group. Whole-brain ANCOVA comparing the activity of the significant RSNs in the group of patients with poor cognitive recovery and the healthy control group showed: (a) a lower correlation between Basal Ganglia activity change and SFT score and a higher correlation between Basal Ganglia activity and TMTA time, (b) a higher correlation between Frontal activity and SFT score, and (c) lower correlation between Frontal activity and TMTA time.

Study II

For the whole stroke group, significant decrease of FA was found only in right hemisphere when compared to the healthy control ($p = 0.001$, $d = 1.29$). When analyzed separately, both stroke group with poor cognitive recovery and stroke group with good cognitive recovery continue to show significant FA disruption in the right hemisphere. Stroke group with poor cognitive recovery showed significant decrease of FA also in several anatomical areas of the left hemisphere ($p = 0.02$, $d = 0.88$); whereas stroke group with good cognitive recovery showed significant FA disruption only in one anatomical area of the left hemisphere ($P = 0.02$, $d = 0.88$). To investigate potential mechanisms underlying WM changes in stroke patients, both AD ($\lambda_{||}=\lambda_1$) and RD [$\lambda_{\perp} = (\lambda_2 +\lambda_3)/2$] maps were also analyzed. We found significant increase in both AD and RD for the stroke group ($p = 0.002$, $d = 1.21$ for RD;

p = 0.02, d = 0.88 for AD) relative to healthy control group. When analyzed separately, stroke group with poor cognitive recovery showed significant increase of RD in the same anatomical areas where this group had shown significant FA decrease (p = 0.01, d = 0.98 for all the regions). The stroke group with good cognitive recovery did not show any significant change in either RD or AD in any region. To assess if cognitive performance was associated with WM disruption, a whole-brain ANCOVA was performed using the WM skeleton given by the FA as dependent variable and the scores of the relevant cognitive tests as predictors, with diabetes mellitus as covariate of no interest. The stroke group with good cognitive recovery showed significant positive correlation between FA values located in the right retrolenticular part of the internal capsule and scores in the Semantic Fluency test. Negative correlations were found between FA values located in the left hemisphere and the time spent to complete the GPT and the TMT part –A. For the GPT, the most significant areas were the left superior corona radiata (p = 0.015) and the left anterior thalamic radiation (p = 0.02). For the TMT part –A, the most significant areas were the left superior corona radiata (p = 0.008), the right inferior fronto-occipital fasciculus (p = 0.019) and the left inferior fronto-occipital fasciculus (p = 0.021). The stroke group with poor cognitive recovery showed negative correlations between the time spent to complete GPT and FA values in the right inferior longitudinal fasciculus (p = 0.034) and the right posterior corona radiata (p = 0.045). Between-group comparisons showed that correlation with semantic fluency scores was significantly stronger for the stroke group with poor cognitive recovery than for the stroke group with good cognitive recovery in the right retrolenticular part of the internal capsule (p < 0.001). It was also stronger for the stroke group with poor cognitive recovery than for the healthy control in the right posterior thalamic radiation.

Study III

We found statistical significant differences regarding metabolite levels of mI (ANCOVA: F=17.629, 28, p<0.001), Cho compounds (ANCOVA: F=9.173, 28, p=0.005) and Glx (ANCOVA: F=4.945, 28, p=0.036) between the stroke group and the healthy control group after controlling for age and voxel partial GM volume. Myoinositol (mI) was positively associated with Glutamato + Glutamine (Glx) (r_s=0.482; p = 0.013); Choline (Cho) compounds were associated with N-Acetyl-aspartyl-Glutamate NAAG (r_s= 0.541; p = 0.004) and with Creatine (Cr) (r_s= 0.659; p <0.001); and Glx was associated with NAAG (r_s= 0.541; p = 0.004) taking into account the entire study sample. Regarding cognitive performance, we found statistical significant associations between the levels of those significant metabolites and cognitive performance: 1) levels of mI and Glx were associated with the Rhythms subtest (MoCA) (r_s = 0.541; p = 0.004; r_s = 0.458; p = 0.019, respectively); 2) levels of Cho compounds were associated with right line cancelation (r_s = 0.461; p = 0.018).

The dual-regression results yielded significant differences regarding rs-fMRI in the VOI region located in the right frontal hemisphere between the stroke group (mean = 25.00 ±16.10) and the healthy control group (mean = 8.62 ± 5.04) (Z = -3.175; p = 0.001). Higher resting state functional magnetic resonance imaging (rs-fMRI) activity was significantly associated with metabolite's levels of mI (r_s = -0.485; p = 0.012) and Glx (r_s = -0.398; p = 0.044). Finally, this activity was significantly associated with the following cognitive tests: MMSE (r_s = -0.472; p = 0.011); TMT-part A (r_s = 0.507; p = 0.006); and GPT (r_s = 0.486; p = 0.009).

Stroke group showed significant decreased FA values in the selected VOI when compared with controls (t_{26} = 2.733; p = 0.011). We found a significant statistical association between those values and the GPT (r_s = -0.494; p = 0.009) for the entire study sample. No significant results were found between the levels of metabolites and FA values.

Study IV

Using the dual regression approach (Filippini et al., 2009), stroke patients had greater functional activity than controls within the DMN in the left precuneus and the left anterior cingulate gyrus. There were significant negative associations between those measures and the line cancellation subtest in stroke patients (r_s= - 0.747; p = 0.000). Based in the network based statistics (NBS), we found affected connectivity between the following pair of ROIs for the stroke patients: left superior frontal gyrus and posterior cingulate cortex (t = 2.01); left parahippocampal gyrus and right superior frontal gyrus (t = 2.11); left parahippocampal gyrus and left superior frontal gyrus (t = 2.39) and between right parietal and left superior frontal gyrus (t = 2.29). All these connections were identified as part of an across-patients affected DMN sub network, altered with regard controls with a statistical significance of p = 0.014. We found statistical significant correlations between the mean path length and the following tests: scoring in the Semantic Fluency test (r_s = 0.454; p = 0.023), scoring in the Phonetic Fluency test (r_s = 0.523; p = 0.007), and scoring in the MMSE (r_s = 0.528; p = 0.007).

Study V

Random Forest is a good classifier with high sensitivity and specificity to lesion segmentation at three months after stroke. FLAIR and T2_weighted images were the most appropriate images for lesion segmentation.

5. GENERAL DISCUSSION

This thesis comprises five studies developed to contribute to a better understanding of the functional, structural and metabolic status of stroke patients at three months following stroke. By means of different functional, structural and metabolic neuroimaging approaches we attempt to shed some light into the study of plastic mechanisms involved in ischemic stroke patients and how those changes are related to cognitive recovery.

In our first study we focused on resting-state functional connectivity (rs-FC) patterns of the whole brain on functional MRI captured three months after focal stroke using pICA approach (Beckmann et al., 2005). Previous works regarding motor recovery using rs-FC reported the following process of reorganization: (1) in the acute phase, rs-FC is initially decreased and there is a reduced inter-hemispheric rsFC (Carter et al., 2010; Golestani et al., 2013; Park et al., 2011; Wang et al., 2010; Carter et al., 2012); (2) rs-FC is gradually increased during recovery; (3) rs-FC is finally restored to near normal or above normal levels at 10 weeks after stroke onset and there is a reinstatement of the inter-hemispheric rs-FC (van Meer et al., 2012). In our study, performed three months following stroke, we found that stroke patients with worse cognitive recovery showed increased rs-FC in the lesioned as well as in the non lesioned hemisphere in four RSN (Frontal, Fronto-temporal, DMN and Secondary visual) and decreased rs-FC in two RSN (Parietal, Basal Ganglia). Stroke patients with better cognitive recovery, however, only showed increased rs-FC in two RSN (Fronto-Temporal and DMN) and decreased rs-FC in one RSN (Basal Ganglia) when compared with healthy controls. In agreement with our expectations, patients with worse cognitive recovery showed more rs-FC alteration not only in the lesioned hemisphere but, interestingly in the contralesional hemisphere. This pattern of activity is in agreement with results obtained from stroke recovery research both in animal models and clinical patients showing that widespread changes in activity patterns can even extend to the unaffected hemisphere (Carmichael and Chesselet, 2000; Nelles et al., 1999; Schaechter and Perdue, 2008). In the case of stroke

patients with worse cognitive recovery, the lesioned hemisphere could present more problems to provide transcallosal inhibition and the contralesional hemisphere became more hyperactive. Those changes might explain the multiplicity of the deficits following a focal lesion.

The DTI data supported and complemented the information obtained from the fMRI. We studied the effects of right hemispheric stroke on left hemispheric WM regarding cognitive recovery. DTI studies of stroke patients have demonstrated that reduced contralesional WM integrity of the corticospinal tract in chronic stroke is associated with poorer motor skill recovery in stroke patients compared to patients with better motor recovery and healthy controls (Buffon et al., 2005; Liu et al., 2008). In these studies, larger interhemispheric asymmetries in FA for this anatomical region have been associated with reduced motor recovery (Schaechter et al., 2009; Jang et al., 2005; Lindenberg et al., 2010; Watanabe et al., 2001), reduced skill improvement in response to training (Thomalla et al., 2004) as well as motor dysfunction after stroke (Jang et al., 2005; Stinear et al., 2007). Therefore, plastic changes in the contralesional hemisphere play an important role in stroke recovery (Qiu et al., 2011; Carmichael 2003; Gerloff et al., 2006; Crofts et al., 2011). In agreement with previous studies, we demonstrated that WM integrity (i.e. FA) was affected in the contralesional as well as in the ipsilesional hemisphere. Our findings are suggestive that WM disruption is caused by demyelination rather than by axonal degeneration, as shown by the fact that the RD increase is more widespread than the AD increase. Axial and Radial components of the DTI tensor have been proposed as biomarkers of the type of neuronal damage (Burzynska et al., 2010; Song et al., 2003): AD measures diffusivity in the principal diffusion direction and it is proposed as a biomarker of axonal damage (Kumar et al., 2010; Kumar et al., 2012), while RD is the average of diffusivities perpendicular to the principal direction of the tensor, and it is assumed to give information on the degree of demyelination (Hu et al., 2011; Keller et al.,

2009; Bennett et al., 2010). In studies of small-vessel-disease both ischaemic demyelination and axonal loss have been found (Pantoni, 2010; Englund, 2002). Recently (Lawrence et al., 2013), RD was found to be the strongest predictor of executive dysfunction. This finding was interpreted in the sense that the ischaemic demyelination has greater influence than axonal degeneration on the presence of cognitive impairment, therefore it was proposed as a more reliable biomarker than AD. Our study not only provides some support for the role of demyelination in stroke patients at three months after suffering a stroke, but also provides a relationship between this event and the presence of a poorer cognitive performance. The relevance of these changes is demonstrated by the fact that patients with poor cognitive recovery (SP) showed stronger WM disruption in the left hemisphere. The correlation of the WM changes with cognitive performance – especially in the contralesional hemisphere – further supports their functional importance. Notice that a higher score in Semantic Fluency test (SF) and a lower score in Trail Making Test part –A (TMTA) and Grooved Pegboard Test (GPT) means better cognitive performance. Therefore, the combination of a positive correlation with SF scores and a negative correlation with TMTA and GPT scores means a positive correlation with cognitive performance in general. It is important to mention that this correlation was stronger in stroke patients with good cognitive recovery (SG) than in SP patients. This finding can be explained by the more severe damage of WM in the SP group. Most of the brain areas showing correlation in SG group are affected in the SP group. Although changes of the contralesional hemisphere can be due both to the degenerative and protective processes (i.e. compensation), our findings give support to the WM degeneration as confirmed by their disruptive nature (decreased WM integrity mostly due to demyelination), and their correlation with cognitive performance (i.e. lower WM integrity co-occur with worse performance). Our findings are in agreement with other studies with stroke patients (Schaechter et al., 2009; Gerloff et al., 2006) and extend our previous research with resting

state (Dacosta-Aguayo et al., 2014a) providing structural ground to the difficulty of SP patients to compensate their cognitive deficits after stroke.

Moreover, SP patients showed lower FA values in major left and right WM tracts that run along the anterior-posterior axis of the brain, supporting fronto-posterior and fronto-subcortical network interactions. These networks have been associated with executive functions (Salthouse 2011). Furthermore, WM disruption in the Body of Corpus Callosum for the SP group supports the suggestion made by Meguro et al (2000) who counted structural disruption of the corpus callosum as a sign of existing changes in the non-injured hemisphere. The relationships between structural changes reported here along with our previous findings imply their importance in clinical recovery.

Functional and structural findings related to the contralesional hemisphere were complemented with our third study regarding metabolic abnormalities in a WM region located in the contralesional hemisphere. In this study we combined functional, structural and metabolic neuroimaging techniques in order to shed light to the plastic changes occurring in the contralesional hemisphere at different levels of specificity. The main finding of this study was that concentrations of metabolites related to glia and neuro-glia transmission were decreased in the contralesional hemisphere of the stroke patients and that those concentrations were correlated with DMN activity and cognitive performance. The reduction of Glx is in agreement with the study conducted by Haberg et al. (2009) with middle cerebral artery occlusion in rats in which it was found a decrease in Glx and GABA concentrations in the contralesional hemisphere during the acute phase. This reduction was interpreted as a consequence of the acute ischemic depolarization and subsequent functional deafferentation of a significant number of glutamatergic transhemispheric connections. This decrease it is thought that would contribute to the reshaping of the neuronal receptive fields which may facilitate recovery by means of keeping metabolism activity low to allow hemispheric

reorganization (Haberg et al., 2009). Our stroke patients were, however, at three months post stroke and lower levels of Glx were associated with higher levels of DMN activity which were, in turn, associated with worse cognitive performance. By the other hand, we found lower levels of mI and Cho compounds also related to worse cognitive scores.

Lower levels of mI were correlated with higher levels of DMN activity. The presence of lower levels of mI and Cho compounds at the subacute phase in a frontal WM area of the contralesional hemisphere has not been reported in stroke patients. Our preliminary results might suggest that changes in metabolites related to glia and neuro-glial transmission system could be influencing resting activity and cognitive recovery of stroke patients at three months following a stroke.

Stroke patients showed higher DMN activity in the same VOI located in the contralesional hemisphere. This activity was negatively correlated not only with metabolite's levels of mI and Glx but with overall cognitive function (MMSE) and with processing speed (TMT-A, GPT). Relationships between functional activity in the DMN and Glx have been described recently (Hu et al., 2013; Kapogiannis et al., 2013). At a cellular level, neuronal activity is regulated by multiple neurochemical processes including cycling of Glx and GABA, the major excitatory and inhibitory neurotransmitters in the Central Nervous System. The coordination between glutamatergic neurons and GABAergic interneurons is believed to have a direct impact on BOLD contrast (Logothetis et al., 2001; Buzsáki et al., 2007). Studies showing negative correlations between GABA concentration and BOLD signals (Northoff et al., 2007; Stagg et al., 2011) and positive correlation between Glx concentration and BOLD signal change has been recently reported (Enzi et al., 2012). In rs-fMRI, glutamatergic and GABAergic activities in the DMN may reach equilibrium to facilitate endogenous processes and when turning from the resting to a task state. The DMN suppression might be achieved by enhancing GABAergic activities to regulate glutamatergic activities (Hu et al., 2013). This is

expected to have serious implications in neurological diseases that directly affect metabolite balance, such stroke. The relation between lower levels of an excitatory neurotransmitter with higher levels of DMN activity might be indicative of breakage of the equilibrium between neurotransmitter's and rs-fMRI activity and would be in agreement with our first study (Dacosta-Aguayo et al., 2014a) in which we found overall increase in the DMN in patients with worse cognitive recovery at three months after stroke. The association between DMN activity and cognitive tests is in agreement with several studies in which it has been found that DMN activity is correlated with behavioral performance (Anticevic et al., 2010; Hu et al., 2013).

Finally, WM structural integrity in the same voi located in the contralesional hemisphere was affected in the group of stroke patients and this alteration was negatively associated with processing speed. This finding of WM alteration in this located region and its relation with processing speed is in agreement with our second study conducted with stroke patients at three months following a stroke by means of whole brain Tract Based Spatial Statistics (TBSS).

We did not found any relation between metabolites and WM integrity nor between DMN activity and WM integrity.

In our fourth study we investigated the alterations in the functional connectivity of the DMN in our ischemic stroke patients. DMN disruption has been commonly observed by rs-FC studies in patients with Mild Cognitive Impairment (Wang et al., 2006; Sorg et al., 2007), Vascular Cognitive Impairment no dementia with subcortical vascular lesions (Sun et al., 2011), and patients with carotid stenosis (Lin et al., 2014; Cheng et al., 2012), however, only a few studies have revealed that stroke patients also show functional connectivity changes in this network (Tuladhar et al., 2013; Li et al., 2014; Lasalle-Lagadec et al., 2012).

In this study we firstly found that stroke patients had greater functional activity than controls within the DMN in the left precuneus cortex and the left anterior cingulate gyrus.

Secondly, we found a significant negative correlation between connectivity for every pair of ROIs considered in our analysis and the size of the lesion. Thirdly, we found affected connectivity between the following pairs of ROIs for the stroke patients: left superior frontal gyrus and posterior cingulate cortex; left parahippocampal gyrus and right superior frontal gyrus; left parahippocampal gyrus and left superior frontal gyrus and between right parietal and left superior frontal gyrus. Finally, we found statistical significant correlations between the mean path length and the following tests: scoring in the Semantic Fluency test, scoring in the Phonetic Fluency test, and scoring in the MMSE.

The size of the lesion has been, and still remains, a subject of discussion in the stroke literature along with the importance of the site of the lesion. These findings might contribute to clarify the reasons by which both size and localization are equally important in cognitive impairment after stroke: if the stroke lesion is big, the connectivity disruption between the different nodes of a network (or even nodes belonging to more than one network) will be greater enlarging the mean path length from one node to the other. On the other hand, if the lesion is situated in a critical node, such as the posterior cingulate cortex, or disrupts the connectivity of this node, the consequences of the lesion would be of significant relevance at short and long term. The impaired nodes were not only located in the lesioned hemisphere but in the contralesional hemisphere too. This finding reflects the general influence of localized lesions on distant functionally connected regions.

Finally, we addressed the problem of lesion segmentation with stroke patients by means of Random Forest classifier. Accurate identification and quantification of lesion area is important for diagnosis, follow-up and therapy response assessments in stroke (Farr et al., 2010; Schiemanck et al., 2006). In patients suffering a stroke, it is common practice to perform lesion volume estimation on Diffusion Weighted Imaging (DWI) data. However, it is argued (Artzi et al., 2013) that while DWI is sensitive in the acute phase, it becomes less accurate

during the subacute phase (i.e. 3 months after stroke insult). Nevertheless, lesion identification is also challenging for other modalities, such as Fluid Attenuated Inversion Recovery (FLAIR), because of large variations in shape, location signal intensity, and the existence of image artifacts and pre-stroke lesions. After testing Random Forest classifiers in different MRI modalities (functional, anatomical and diffusion data), we found that FLAIR and T2_weighted data were the most sensitive in lesion detection at three months after stroke.

There are a number of caveats related to the studies that led to this thesis that should be mentioned: our sample is small due to our strict criteria. This may also decrease the sensitivity, and restrict the generalizability of our preliminary results. Although we only considered right stroke patients, in order to avoid heterogeneity of lesion location, the size of the lesion and the localization was still heterogeneous. This is a limitation because whereas recovery after a small ischemic lesion may involve preserved peri-infarct tissue with function similar to the infarcted tissue (Murphy and Corbett, 2009; Brown et al., 2009), for recovery after a large ischemic lesion, tissue with similar function may only be found at more distant sites, such as the premotor cortex (for motor cortex stroke) (Frost et al., 2003; Dancause et al., 2005) or regions in the unaffected contralateral hemisphere (Biernaskie et al., 2005) where structural remodeling has been observed (Takatsuru et al., 2009). In this sense, our results, although promising, should be taken as preliminary.

Future work would benefit from the development of multicenter studies that allow the recruitment of larger cohorts. Such studies would allow the study of the generalizability of the results reported in this thesis. One important field that should be further explored is the potential of the combination of different structural and functional modalities, along with

behavioral, physic and genetic assessments in the study of recovery and prognosis of ischemic stroke patients.

6. CONCLUSIONS

The main conclusions of this thesis, derived from the five studies developed can be summarized as follows:

1. Stroke patients show brain activity changes that affect several brain networks. These changes might explain the multiplicity of the deficits following a focal lesion. These changes are more pronounced in patients with poor cognitive recovery.

2. Stroke patients show microstructural changes not only in the lesioned hemisphere but in the contralesional hemisphere too. These changes were more pronounced in stroke patients with worse cognitive recovery.

3. Patients with ischemic stroke have metabolites' abnormalities related to glia and neuro-glia transmission in the contralesional hemisphere and those abnormalities are correlated with DMN activity and cognitive performance.

4. Dysfunction in the functional integration of the DMN has a role in explaining cognitive outcome in stroke patients.

5. Random Forest classifiers demonstrate that FLAIR and T2_weighted images are the most appropriate to perform automatic lesion segmentation at three months after stroke.

6. A focal ischemic injury has consequences at functional, structural and biochemical level not only in the injured hemisphere but around the brain. This global involvement explains the multiple deficits presented by those patients and it is related to the outcome.

7. Our findings are consistent with the concept of diaschisis after stroke, which was originally formulated to describe temporary clinical deficits related to areas remote from the area of damage

8. Functional and structural neuroimaging techniques provide information for cognitive recovery prognosis and can be a potential biomarker in stroke patients detecting early neural dysfunction and compensatory mechanisms prior to brain atrophy.

7. SUMMARY OF THE THESIS

Cambios funcionales, estructurales y metabólicos relacionados con recuperación cognitive en pacients con ictus isquémico

Glosario de abreviaciones

AD: Difusividad Axial

BI: Índice de Barthel

Cr: Creatina

FA: Anisotropía Fraccional

fMRI: Imagen de Resonancia Magnética Funcional

FSL: Free Software Library

Glx: Glutamato + Glutamina

mI: Mioinositol

mRS: Escala Rankin modificada

NAAG: N-Acetyl-Aspartyl-Glutamato

NBS: Network Based Statistics

NIHSS: National Institute Stroke Scale

pICA: probabilistic Independent Component Analysis

RD: Difusividad Radial

rs-fMRI: Resting state functional magnetic resonance

SPSS: Statistical Package for Social Sciences

Introduction

El accidente cerebrovascular isquémico es la segunda causa más común de muerte en el mundo y una causa importante de discapacidad de larga duración en la población de ancianos (Murphy y Corbett, 2009; Gorelick et al, 2011; Carmichael, 2012). Después de un derrame cerebral, el cerebro trata de compensar la pérdida de la función a través de la reorganización de las redes neuronales (van Meer et al, 2010; Van Meer et al, 2012; Bury y Jones 2002). Esta reorganización implica el reclutamiento bilateral tanto del hemisferio lesionado como del hemisferio contralesional (Gerloff et al, 2006; Lotze et al, 2006). Durante esta reorganización tienen lugar cambios a nivel funcional (Cramer, 2008; Cramer y Crafton, 2006; Wahl y Schwab, 2014, Ward, 2004; Ward et al, 2003; Weiller et al, 1993; Seitz et al, 1998; Dacosta - Aguayo et al, 2014a), estructural (Liu et al, 2008; Buffon et al, 2005; Gerloff et al., 2006; Brus - Ramer et al, 2007; Dancause et al, 2005; Dacosta - Aguayo et al, 2014b) y metabólico (Demougeot et al, 2001; Demougeot et al, 2003; Khang et al, 2009; Kobayashi

et al, 2001; Muñoz- Maniega et al, 2008; Craciunas et al, 2013; Takatsuru et al, 2009; Takatsuru et al, 2013).

Objetivos Generales e Hipótesis

El objetivo principal de esta tesis es estudiar los cambios plásticos que tienen lugar después de un ictus y, en particular, el papel del hemisferio contralesional en la recuperación cognitiva después del accidente cerebrovascular, desde un punto de vista funcional, estructural y metabólico. Para ello, utilizamos resonancia magnética funcional (fMRI), resonancia magnética estructural (DWI), imagen por espectroscopía y teoría de grafos aplicada a datos funcionales, además de pruebas neuropsicológicas para evaluar la recuperación de los pacientes tras un accidente cerebrovascular a los tres meses.

En primer lugar nos centramos en los patrones de estado de reposo cerebral y en los correlatos estructurales relacionados con la mejor y peor recuperación cognitiva. Este problema se ha investigado en los estudios I y II. En segundo lugar, nos centramos en la actividad DMN y en el desequilibrio metabólico relacionado con el rendimiento cognitivo. Este problema se ha investigado en los estudios III y IV. Finalmente, hicimos una aproximación técnica para el algoritmo de segmentación automática de la lesión. Este problema se ha investigado en estudio V.

Estudio I

Objetivos

1. Explorar si los pacientes con buena recuperación cognitiva muestran diferencias en los patrones funcionales en estado de reposo en comparación con los pacientes con mala recuperación cognitiva.
2. Determinar si estos patrones correlacionan con el rendimiento cognitivo.

3. Evaluar la existencia de factores pronósticos de la recuperación cognitiva

Hipótesis

1. Los pacientes con ictus mostrarán cambios en distintas redes de reposo tanto en el hemisferio lesionado como en el hemisferio contralesional.

2. Una de las redes de reposo dañadas en los pacientes con baja recuperación cognitiva será la DMN, dado que esta red está asociada con la mejor realización en tareas cognitivas.

3. Los pacientes con menor y mejor recuperación cognitiva mostrarán patrones de conectividad funcional distintos a los tres meses después del accidente cerebrovascular.

Estudio II

Objetivos

1. Evaluar los efectos microestructurales del ictus de hemisferio derecho en el hemisferio izquierdo.

2. Investigar el mecanismo subyacente a dichos cambios.

3. Evaluar los efectos de dichos cambios estructurales en la recuperación cognitiva a los tres meses después del ictus.

Hipótesis

1. Los pacientes con peor recuperación cognitive mostrarán mayor alteración microstructural en el hemisferio contralesional.

2. Los pacientes con mejor recuperación cognitiva tendrán mejor integridad de sustancia blanca en el hemisferio contralesional.

3. La mejor integridad de sustancia blanca en el hemisferio contralesional estará relacionada con un mejor rendimiento en los tests cognitivos en los pacientes con mejor recuperación cognitiva.

Estudio III

Objetivo

Examinar cambios metabólicos en una región frontal de sustancia blanca localizada en el hemisferio contralesional y asociar dichos cambios con el estado funcional, estructural y con el rendimiento cognitivo de pacientes con ictus.

Hipótesis

1. Los pacientes mostrarán alteraciones metabólicas, funcionales y estructurales comparadas con los controles.
2. Las concentraciones de los metabolitos estarán relacionadas con las medidas funcionales, estructurales y cognitivas de los pacientes.

Estudio IV

Objetivos

1. Estudiar la conectividad funcional de pacientes con ictus a los 3 meses.
2. Relacionar la conectividad funcional con ejecución cognitiva.
3. Estudiar diferencias entre pacientes y controles en relación a la DMN debido a su gran sensibilidad como marcador de deterioro cognitivo y demencia.

Hipótesis

1. Los pacientes con ictus mostrarán alteraciones en la conectividad de los distintos nodos de la DMN.
2. Estas alteraciones estarán relacionadas con el rendimiento cognitivo.

Estudio V

Objetivo

Realizar segmentación de lesiones en diferentes tipos de adquisiciones mediante clasificadores de Random Forest.

Resultados

Estudio I

En comparación con el grupo control sano, el grupo de pacientes mostró alteración significativa en las siguientes seis redes de reposo: aumento de la actividad cerebral en (1) la red frontal, (2) la red fronto-temporal, (3) Default Mode Network (DMN), (4) red visual secundaria, y disminución de la actividad cerebral en (5) la red de los ganglios de la base y

(6) la red parietal. Los pacientes con buena recuperación cognitiva demostraron un aumento significativo de actividad sólo en las redes fronto - temporal y en la DMN, así como una disminución significativa de la actividad en la red de los ganglios basales en comparación con grupo de control sano. Se llevó a cabo un análisis de la covarianza (ANCOVA) para comparar la actividad de las redes significativas entre el grupo de pacientes con mala recuperación cognitiva y el grupo de controles y encontramos: (a) una menor correlación entre los ganglios basales y la puntuación en el test de fluencia semántica y una mayor correlación entre la actividad de los ganglios basales y el TMTA, (b) una mayor correlación entre la actividad frontal y la puntuación obtenida en el test de fluencia semántica, y (c) una menor correlación entre la actividad frontal y el TMTA .

Estudio II

Para todo el grupo de pacientes, se encontró una disminución significativa de FA sólo en el hemisferio derecho, en comparación con el grupo de controles sanos ($p = 0.001$, $d = 1,29$). Cuando los grupos se analizaron por separado, en función de su recuperación cognitiva, el grupo de pacientes con peor recuperación cognitiva mostró disminución significativa de la FA en varias áreas anatómicas del hemisferio izquierdo ($p = 0,02$, $d = 0,88$), mientras que el grupo de pacientes con una buena recuperación cognitiva mostró alteraciones en la FA sólo en un área anatómica del hemisferio izquierdo ($p = 0,02$, $d = 0,88$). Para investigar los posibles mecanismos subyacentes a los cambios de sustancia blanca en los pacientes con ictus, se analizaron los mapas de difusividad axial (AD) ($\lambda \mid \mid = \lambda 1$) y de difusividad radial (RD) [$\perp = (\lambda 2 + \lambda 3) / 2\ \lambda$]. Se encontró un aumento significativo de AD y de RD para el grupo de pacientes ($p = 0.002$, $D = 1,21$ para RD, $p = 0,02$, $d = 0,88$ para AD) en relación al grupo de controles sanos. Cuando se analizaron los dos grupos de pacientes por separado, el grupo de pacientes con peor recuperación cognitiva mostró un aumento

significativo de RD en las mismas áreas anatómicas donde este grupo había mostrado disminución significativa FA ($p = 0,01$, $d = 0,98$ para todas las regiones). El grupo de pacientes con una buena recuperación cognitiva no mostraron ningún cambio significativo en ninguno de los índices de RD o AD. Para evaluar si el rendimiento cognitivo se asoció con una interrupción de las fibras de sustancia blanca, se realizó una ANCOVA de todo el cerebro usando el esqueleto de sustancia blanca como variable dependiente y las puntuaciones de las pruebas cognitivas relevantes como predictoras, con diabetes mellitus como covariable de interés. El grupo de pacientes con una buena recuperación cognitiva mostró correlación positiva significativa entre los valores de FA situados en la parte retrolenticular derecha de la cápsula interna y las puntuaciones en la prueba de fluidez semántica. Se encontraron correlaciones negativas entre los valores de FA ubicados en el hemisferio izquierdo y el tiempo para completar el GPT y el TMT parte -A. Para el GPT, las áreas más significativas fueron la corona radiata superior izquierda ($p = 0,015$) y la radiación talámica anterior izquierda ($p = 0,02$). Para el TMT –A, las áreas más significativas fueron la corona radiata superior izquierda ($p = 0,008$), el fascículo fronto-occipital inferior derecho ($p = 0,019$) y el fascículo fronto-occipital inferior izquierdo ($p = 0,021$). El grupo de pacientes con una mala recuperación cognitiva mostró correlaciones negativas entre el tiempo empleado para completar el GPT y la FA en el fascículo longitudinal inferior derecho ($p = 0,034$) y la corona radiata posterior derecha ($p = 0,045$). La comparación entre grupos mostró que la correlación con las puntuaciones de fluidez semántica fue significativamente más fuerte para el grupo de pacientes con mala recuperación cognitiva que para el grupo de pacientes con buena recuperación cognitiva en la parte retrolenticular de la cápsula interna derecha ($p < 0,001$). También fue mayor para el grupo de pacientes con una mala recuperación cognitiva que para el grupo de controles sanos en la radiación talámica posterior derecha.

Estudio III

Se encontraron diferencias estadísticamente significativas con respecto a los niveles de metabolitos de Mioinositol (mI) (ANCOVA: F = 17,629, 28, p < 0,001), Colina (Cho) (ANCOVA: F = 9.173, 28, p = 0,005) y Glutamato + Glutamina (Glx) (ANCOVA: F = 4.945, 28, p = 0,036) entre el grupo de pacientes y el grupo control sano después de controlar por edad composición parcial de sustancia gris en la región de interés. El Mioinositol (mI) se asoció positivamente con Glx (r_s = 0,482, p = 0,013); la Cho se asoció con el N-acetil-aspartil-glutamato (NAAG) (r_s = 0,541, p = 0,004) y con la Creatina (Cr) (r_s = 0,659, p < 0,001), y el Glx se asoció con NAAG (r_s = 0,541, p = 0,004), teniendo en cuenta la totalidad de la muestra del estudio. Con respecto al rendimiento cognitivo, encontramos asociaciones estadísticas significativas entre los niveles de dichos metabolitos significativos y el rendimiento cognitivo de toda la muestra de estudio: 1) niveles de mI y Glx se asociaron con el subtest de ritmos (MoCA) (r_s = 0,541, p = 0,004; r_s = 0,458, p = 0,019, respectivamente), 2) los niveles de Cho se asociaron con el test de cancelación de líneas derecha (r_s = 0,461, p = 0,018).

Se encontraron diferencias significativas en cuanto a la actividad de reposo, en el volumen de interés escogido para la extracción de metabolitos situado en el hemisferio frontal derecho, entre el grupo de pacientes (media = 25,00 ± 16,10) y el grupo controles sanos (media = 8,62 ± 5,04) (Z = -3,175, p = 0,001). La mayor actividad en reposo se asoció significativamente con los niveles de mI (r_s = -0,485, p = 0,012) y Glx (r_s = -0,398, p = 0,044). Finalmente, esta actividad se asoció significativamente con las siguientes pruebas cognitivas: MMSE (r_s = -0,472, p = 0,011); TMT- parte A (r_s = 0,507, p = 0,006), y GPT (r_s = 0,486, p = 0,009).

Finalmente, el grupo de pacientes mostró una disminución significativa de los valores de FA en el volumen de interés seleccionado en comparación con los controles (T_{26}= 2,733, p = 0,011). Se encontró una asociación estadística significativa entre estos valores y el GPT (r_s

= -0,494, p = 0,009) para la totalidad de la muestra del estudio. No se encontraron resultados significativos entre los niveles de los metabolitos y los valores de FA.

Estudio IV

Los pacientes mostraron una mayor actividad funcional que los controles en la DMN en el precuneus izquierdo y en la circunvolución anterior izquierda del cíngulo. Se encontraron asociaciones negativas estadísticamente significativas entre esta mayor actividad y el test de cancelación de líneas para los pacientes (r_s = - 0.747, p = 0.000). Se encontró una correlación negativa estadísticamente significativa entre la conectividad para cada par de ROIs considerados en nuestro análisis y el tamaño de la lesión (r_s = -0,752, p = 0,008). Encontramos conectividad afectada entre los siguientes pares de regiones de interés para los pacientes: circunvolución frontal superior y la corteza del cingulado posterior (t = 2,01); circunvolución del hipocampo izquierdo y circunvolución frontal superior derecha (t = 2,11); circunvolución del hipocampo izquierdo y circunvolución frontal superior izquierda (t = 2,39) y entre el parietal derecho y el giro frontal superior izquierdo (t = 2,29) con una significación estadística de p = 0,014. Se encontraron correlaciones estadísticamente significativas entre la longitud de camino y las siguientes pruebas: puntuación en la prueba de fluidez semántica (r_s = 0,454, p = 0,023), puntuación en la prueba de fluidez fonética (r_s = 0,523, p = 0,007) y la puntuación en el MMSE (r_s = 0,528, p = 0,007).

Estudio V

Los clasificadores de Random Forest mostraron una alta sensibilidad y especificidad en la detección de lesiones en comparación con el gold standard (delineación manual de la lesión) para distintos tipos de adquisiones. Las imágenes en FLAIR y potenciada en T2 fueron

las que mejores resultados obtuvieron, presentándose como las más adecuadas para la segmentación.

Discusión General

Esta tesis consta de cinco estudios desarrollados para contribuir a una mejor comprensión del estado funcional, estructural y metabólico de los pacientes con ictus a los tres meses después del accidente cerebrovascular. A través de diferentes enfoques de neuroimagen funcional, estructural y metabólica se intenta arrojar algo de luz en el estudio de los mecanismos plásticos involucrados en los pacientes con accidente cerebrovascular isquémico y cómo esos cambios se relacionan con la recuperación cognitiva.

En nuestro primer estudio nos hemos centrado en el estudio de los patrones de actividad funcional en estado de reposo (rs -FC) a los tres meses después del accidente utilizando el enfoque pICA (Beckmann et al., 2005). Hemos encontrado que los pacientes con accidente cerebrovascular con peor recuperación cognitiva mostraron aumento de actividad de reposo tanto en el hemisferio lesionado como en el hemisferio no lesionado en cuatro redes de reposo (RSN) (Frontal, Fronto -temporal, DMN y Secundaria visual) y disminución de actividad en dos RSN (parietal, ganglios basales). Los pacientes con una mejor recuperación cognitiva, sin embargo, sólo mostraron un aumento de actividad de reposo en dos RSNs (fronto-temporal y DMN) y disminución en una RSN (ganglios basales) en comparación con los controles sanos. De acuerdo con nuestras expectativas, los pacientes con peor recuperación cognitiva mostraron más alteraciones no sólo en el hemisferio lesionado sino en el hemisferio contralesional. Los datos DTI apoyaron y complementaron la información obtenida con el estudio de funcional. Se estudiaron los efectos del ictus cerebral hemisférico derecho sobre la integridad de sustancia blanca del hemisferio izquierdo y se relacionó con la recuperación cognitiva. De acuerdo con estudios anteriores, hemos

demostrado que la integridad de sustancia blanca (es decir, FA) se vio afectada tanto en el hemisferio lesionado como en el contralesional. Nuestros resultados también indican que dicha alteración está posiblemente causada por la desmielinización en lugar de por la degeneración axonal, como se muestra por el hecho de que el aumento de la difusividad radial es más elevado que el aumento de difusividad axial. La mayor parte de las áreas del cerebro que muestran la correlación en el grupo de pacientes con una buena recuperación cognitiva se ven afectadas en el grupo de pacientes con una mala recuperación cognitiva. Nuestros resultados están de acuerdo con otros estudios con pacientes con accidente cerebrovascular (Schaechter et al, 2009; Gerloff et al, 2006) y extienden nuestra investigación previa con estado de reposo (Dacosta - Aguayo et al, 2014a).

Los resultados funcionales y estructurales relacionados con el hemisferio contralesional se complementaron con nuestro tercer estudio sobre las anormalidades metabólicas en una región de sustancia blanca situada en el hemisferio contralesional. El principal hallazgo de este estudio fue que las concentraciones de metabolitos relacionados con la glía y la transmisión neuro - glial se redujeron en el hemisferio contralesional de los pacientes con accidente cerebrovascular y que esas concentraciones se correlacionaron con la actividad DMN y el rendimiento cognitivo. Se encontraron niveles más bajos de Glx. Estos niveles se asociaron con niveles más altos de actividad DMN que estuvieron, a su vez, asociados con un peor rendimiento cognitivo. También encontramos niveles más bajos de mI y Cho que se relacionaron con peores puntuaciones cognitivas. Niveles más bajos de mI se correlacionaron con los niveles más altos de actividad DMN. La presencia de niveles bajos de mI y Cho en la fase subaguda en una zona frontal del hemisferio contralesional no ha sido reportada en pacientes con accidente cerebrovascular isquémico. Nuestros resultados preliminares podrían ser indicativos de que los cambios en los metabolitos relacionados con la glia y sistema de transmisión neuro- glial podrían estar influyendo en la actividad de reposo

y en la recuperación cognitiva de los pacientes con ictus a los tres meses después de un accidente cerebrovascular.

Los pacientes con ictus mostraron mayor actividad en la DMN en el mismo volumen de interés utilizado para la extracción de los metabolitos. Esta actividad se correlacionó negativamente no sólo con los niveles de mI y Glx sino con la función cognitiva general (MMSE) y con la velocidad de procesamiento (TMT-A, GPT). La asociación entre la actividad DMN y pruebas cognitivas está de acuerdo con varios estudios en los que se ha encontrado que la actividad de DMN se correlaciona con el rendimiento cognitivo (Anticevic et al, 2010; Hu et al, 2013).

La integridad estructural en el hemisferio contralesional se vio afectada en el grupo de pacientes con accidente cerebrovascular y esta alteración se asoció negativamente con la velocidad de procesamiento. Este hallazgo y su relación con la velocidad de procesamiento está de acuerdo con nuestro segundo estudio. No se encontró ninguna relación entre los metabolitos y la integridad sustancia blanca ni entre la actividad de la DMN y la integridad de sustancia blanca.

El cuarto estudio se centró en las alteraciones en la conectividad funcional de la DMN en nuestros pacientes. En este estudio, en primer lugar encontramos que los pacientes con ictus tenían una mayor actividad funcional que los controles en la DMN en el precuneus izquierdo y en la circunvolución anterior del cíngulo. En segundo lugar, se encontró una correlación negativa significativa entre las puntuaciones de conectividad entre pares de ROIs considerados en nuestro análisis y el tamaño de la lesión. En tercer lugar, encontramos conectividad afectada entre el siguiente par de regiones de interés para los pacientes con accidente cerebrovascular: circunvolución frontal superior izquierda y corteza cingulada posterior; circunvolución del hipocampo izquierdo y circunvolución frontal superior derecha;

circunvolución del hipocampo izquierdo y circunvolución frontal superior izquierda y entre parietal derecho y la circunvolución frontal superior izquierda.

Estos hallazgos podrían contribuir a aclarar las razones por las cuales tanto el tamaño como la localización son igualmente importantes en el deterioro cognitivo después del accidente cerebrovascular: si la lesión es grande, la interrupción de conectividad entre los diferentes nodos de una red (o incluso nodos que pertenecen a más de una red) será mayor, ampliando la longitud media de la trayectoria de un nodo a otro. Por otro lado, si la lesión se encuentra en un nodo crítico, tales como la corteza cingulada posterior, las consecuencias de la lesión serían de relevancia significativa a corto y largo plazo. Los nodos alterados no sólo se encuentran en el hemisferio lesionado, sino también en el hemisferio contralesional. Este resultado refleja la influencia general de lesiones localizadas en regiones distantes pero conectadas funcionalmente.

Nuestros pacientes con accidente cerebrovascular isquémico mostraron una mayor alteración en una medida de la integración funcional en las redes del cerebro: la longitud de media de camino.

Por último, se abordó el problema de la segmentación de la lesión en pacientes con ictus a través de clasificadores de Random Forest. La identificación y cuantificación del área de lesión es importante para el diagnóstico, evaluación y seguimiento en el accidente cerebrovascular (Farr et al, 2010; Schiemanck et al, 2006). En los pacientes que sufren un accidente cerebrovascular, es una práctica común utilizar imágenes de difusión para llevar a cabo la estimación del volumen de lesión. Sin embargo, se argumenta (Artzi et al., 2013) que, si bien estas imágenes son sensibles en la fase aguda, se vuelven menos precisas durante la fase subaguda (es decir, 3 meses después del accidente cerebrovascular).

Por otra parte, la identificación de la lesión en otras modalidades de imagen, también es un reto debido a las grandes variaciones en la forma, la intensidad de la señal, así como la existencia de artefactos de la imagen y de las posibles lesiones previas al accidente cerebrovascular. Después de probar los clasificadores aleatorios de Random Forest en diferentes modalidades de resonancia magnética (datos funcionales, anatómicos y de difusión), se encontró que la imagen de FLAIR y los datos potenciados en T2 son los más sensibles en la detección de lesiones a los tres meses después del accidente cerebrovascular y que los clasificadores de Random Forest son sensibles para la clasificación de tejido lesionado.

Hay una serie de consideraciones metodológicas relacionadas con los estudios que llevaron a esta tesis que debe mencionarse: la muestra es pequeña debido a nuestros criterios estrictos. Esto puede disminuir la sensibilidad y restringir la generalización de nuestros resultados preliminares. El tamaño de la lesión y la localización es heterogéneo. Esta es una limitación porque mientras que la recuperación después de una pequeña lesión isquémica puede implicar tejido peri- infarto conservado con función similar al tejido infartado (Murphy y Corbett, 2009; Brown et al, 2009), la recuperación después de un gran lesión isquémica, el tejido con función similar sólo se puede encontrar en los sitios más lejanos, como la corteza premotora (para el accidente cerebrovascular corteza motora) (Frost et al, 2003; Dancause et al, 2005) o de las regiones en el hemisferio contralateral no afectado (Biernaskie et al., 2005), donde la remodelación estructural se ha observado (Takatsuru et al., 2009). En este sentido, nuestros resultados, aunque prometedores, deben tomarse como preliminares.

El trabajo futuro se beneficiaría del desarrollo de estudios multicéntricos que permiten el reclutamiento de cohortes más grandes. Tales estudios permitirán la generalización de los resultados presentados en esta tesis. Un campo importante que debe estudiarse más a fondo

es el potencial de la combinación de diferentes modalidades estructurales y funcionales, así como test neuropsicológicos, actividad física y evaluaciones genéticas en el estudio de la recuperación y el pronóstico de los pacientes con ictus isquémico.

Conclusiones

1. Los pacientes con ictus presentan cambios en la conectividad del cerebro que afectan varias redes cerebrales. Estos cambios son más pronunciados en los pacientes con mala recuperación cognitiva, mientras que los pacientes con buena recuperación cognitiva presentan un patrón similar a los pacientes sanos.

2. Los pacientes con ictus presentan cambios microestructurales no sólo en el hemisferio lesionado, sino en el hemisferio contralesional también. Esos cambios fueron más pronunciados en los pacientes con peor recuperación cognitiva.

3. Los pacientes con ictus isquémico tienen anormalidades metabólicas relacionadas con la glía y la transmisión neuro-glial en el hemisferio contralesional y esas anomalías se correlacionan con la actividad de la DMN y el rendimiento cognitivo.

4. La disfunción de la integración funcional de la DMN tiene un papel importante en la explicación del rendimiento cognitivo en pacientes con ictus.

5. Las imágenes de FLAIR y potenciadas en T2 son las más adecuadas para la realización de segmentación automática de la lesión en pacientes tres meses después del ictus.

6. La lesión isquémica tiene consecuencias a nivel funcional, estructural y metabólico no sólo en el hemisferio lesionado sino también en el contralesional. Esta afectación global explica los múltiples déficits que presentan estos pacientes y está relacionado con su recuperación cognitiva.

7. Nuestros resultados son consistentes con el concepto de diásquisis después del accidente cerebrovascular, el cual fue formulado originalmente para describir los déficits clínicos temporales relacionadas con las áreas alejadas de la zona de daño y se ha ampliado para incluir la actividad en sitios remotos del cerebro que están funcionalmente conectadas al área de la lesión.

8. Las técnicas de neuroimagen funcional y estructural proporcionan información para el pronóstico de recuperación cognitiva y puede ser un biomarcador potencial en pacientes con accidente cerebrovascular para la detección temprana de disfunción neuronal y mecanismos de compensación antes de la atrofia cerebral.

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