

A clinical and neuroimaging approach to the evaluation of virtual reality efficacy for upper limb recovery after stroke.

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ABSTRACT

The treatment of upper limb motor function impairments and associated participation restrictions still represent a challenging therapy target in stroke neurorehabilitation. Recent evidence showed that virtual reality (VR) is better than conventional physiotherapy for the treatment of upper limb, after stroke. Both genetics and neurophysiological factors drive functional recovery and carrying the Val66Met single nucleotide polymorphism (SNP) of the brain derived neurotrophic factor (BDNF) was argued to be a potential determinant of poor motor recovery. Motor control theories postulate that the motor system pools groups of muscles in functional units called muscle synergies, to control voluntary movements. A determined number of muscle synergies, which is stable across subjects, but affected by stroke, allows the description of natural motor behaviour. Evidence from animals proposed a subcortical and spinal substrate for muscle synergies. In this thesis, a virtual reality environment commonly applied in real clinical settings for the treatment of upper limb after stroke, was used as a reference framework to test hypotheses on both the genetics and neurophysiological factors described above. The first part of the thesis explores whether carrying the Val66Met SNP BDNF determines a bad recovery of upper limb motor function and whether different brain morphologies are associated with each genotype, in stroke survivors. The second part of the thesis explores whether muscle synergies are represented in the human brain and whether their representation is affected by stroke. The third part of the thesis explores whether muscle synergies might represent a robust neurophysiological outcome to test differences in efficacy between VR-based treatments and conventional therapy. With regard to genetics, the key findings were that polymorphisms of the BDNF do not determine clinically detectable differences, but brain morphological differences exist, because of the genotypes, with bigger brain areas in carriers of the Val66Met SNP BDNF. Neurophysiological findings showed that muscle synergies are represented in the brain structures of the pyramidal motor system, but their representation extends to brain areas devoted to higher order cognitive functions, after stroke. Finally, it was found that VR-based therapy determines a better functional brain reorganisation around muscle synergies brain seeds, than conventional physiotherapy. More research is needed to determine whether these findings represent reliable modules which can be incorporated within a computational model of neurorehabilitation.

1. IMPACT OF STROKE AND ASSOCIATED REHABILITATION NEEDS

1.1. Stroke and cerebrovascular disease (CVD)

Stroke is defined by the National Library of Medicine (NLM) of the United States as: “a group of pathological conditions characterized by sudden, non-convulsive loss of neurological function due to brain ischemia or intracranial haemorrhage. Stroke is classified by the type of tissue necrosis, such as the anatomical location, vasculature involved, aetiology, age of the affected individual, and haemorrhagic vs. non-haemorrhagic nature.” (Adams et al., 1997). The term was introduced in the controlled vocabulary thesaurus of the Medical Subject Heading (MeSH) in 2000 and lastly defined in 2008. Previous MeSH indexing for stroke were “Cerebrovascular Disorders” (1964-1999), “Intracranial Arteriosclerosis” (1965-1999), “Intracranial Embolism and Thrombosis” (1965-1999), generally including the whole spectrum of pathological conditions affecting blood flow in the brain and leading to related syndromes associated with brain damage (e.g. headache, confusion, transient blindness, speech impairment, hemiparesis, swallowing difficulties).

Worldwide, stroke is either the leading cause of death or of disability for men and women of all ages, classes, and ethnic origins. Every year almost 16 million first-ever strokes occur in the world (Di Carlo, 2009) making this the second cause of death (Mathers et al., 2009) and the leading cause of disability (WHO, 2011). The epidemiology of stroke has been extensively studied, but differences between countries where data are collected (e.g. risk factors in the population, comorbidities, prevalence of cardiovascular disease, quality of medical care) make a precise estimation of prevalence and mortality difficult (Zhang et al., 2012). Indeed, the related social costs associated with stroke are difficult to calculate, as mortality is dependent on the length of stay in hospital and diagnosis-related group (DRG) and the rehabilitation costs are impossible to determine (Peltola, 2012). Moreover, the outcomes related to residual motor abilities are the main burden influencing stroke-related costs, as they are directly responsible for the loss of productive capacity in survivors.

Several epidemiological surveys have been conducted on cerebro-vascular disease in European countries. For example in the United Kingdom the Oxford Vascular Study (OXVASC) reported a standardised overall incidence of 1.62 (95% CI = 1.43–1.82), with a peak in people older than 85 years, to 16.36 (95% CI = 11.7–21.1) (Rothwell et al., 2004). More recently a review estimated incidence, prevalence and mortality by pooling the data coming from studies sampled in 6 different countries (i.e. France, Germany, Italy, Spain, UK and US) (Zhang et al., 2012).

The findings demonstrated that:

- comparison of epidemiological data between countries should be done with caution because of the heterogeneity of the studies (e.g. prevalence: Italy, 0.15%; UK, 1.7%; US, 2.6%);
- incidence of subarachnoid haemorrhagic strokes is not related to age, increasing up to 50 years in both genders, but losing any pattern for patients older than 50;
- incidence of stroke is higher in males than females, except for subarachnoid haemorrhagic stroke that is more incident in women;
- in the English population the prevalence of stroke seems to follow a decreasing trend;
- mortality depends on: incidence of stroke in the population; quality of medical care; risk factors in the population (e.g. prevalence of cardiovascular disease and comorbidities).

The estimates of the total costs of stroke are very variable from country to country, in relation to the difficulty of calculating the indirect costs resulting from disability and mortality. In detail it has been demonstrated that the variability is clearly explained by length of stay in hospital and diagnosis-related group (DRG), nonetheless it is still impossible to determine the rehabilitation course followed by the patients, as well as the treatment setting of each patient (e.g. stroke unit, intensive care unit) (Peltola, 2012). The main costs of stroke survivors are related to their residual motor disabilities that interfere with personal, social and productive activities.

1.1.1. Neuropathology and pathophysiology of stroke

The medical syndrome characterising stroke depends on a large variety of neuropathological and pathophysiological mechanisms. Ischemic stroke is defined as a syndrome caused by reduction or occlusion of blood flow in the arterial circulation of the brain (Rosamond et al., 2007). A broad classification of ischemic stroke pathophysiology recognises three main categories: primary vascular pathologies (e.g. atherosclerosis, arterial dissection), cardio-aortic pathologies (e.g. atrial fibrillation, patent foramen ovale, myocardial ischemia) and haematological pathologies (e.g. prothrombotic or hyperaggregable states). In the first case, the mechanism involved is an artery-to-artery embolism, in the second case a cerebral arterial occlusion occurs following embolism, in the third case venous or intracardiac thrombus formation is at the origin of the occlusion. Differently, the pathophysiology of haemorrhagic stroke involves vascular rupture followed by bleeding in the brain parenchyma. Therefore, growing of haematoma may lead to increased brain pressure in few hours, thus the first treatment line is frequently focused on arresting haematoma growth, by medical or surgical therapies. The high mortality rate associated with haemorrhagic stroke, ranging from 35% to 40% (Cohen et al., 2006), depends on direct destruction or compression of critical brain areas, in case of severe or untreated bleeding the uncontrolled improvement of intracranial pressure may lead to circulatory arrest. Overall, haemorrhagic stroke represents about 12% to 15% of stroke aetiology (Thom et al., 2006). The most common risks associated with brain haemorrhage are: long-standing hypertension; cerebral amyloid angiopathy (the main aetiology for lobar haemorrhage); vascular malformation (any location); impaired coagulation (e.g. haematological malignancies, liver disease); cerebral tumour; abuse of sympathomimetic drugs (e.g. cocaine, amphetamine). Haemorrhagic stroke can be classified according to aetiology (i.e. idiopathic, anticoagulation, vascular malformation, associated medical or neurological disease), or to brain location (i.e. lobar, deep hemispheric, brainstem, cerebellar). Overall, measures of primary prevention for stroke are mainly related to healthy lifestyle (e.g. good nutrition, no smoking, no alcohol or drug abuse and medical treatment of systemic diseases), but the main measure of prevention in case of hypertension is lowering of blood pressure.

Recently the development of tools for quantitative analysis of motor deficits has given the opportunity to increment the amount of data in clinical practice to better

study human motor behaviour with consequent important practical implications. In fact, it is now possible to study the anatomical structures involved by the lesion to characterize better the resulting motor deficits and, consequently, to plan individually modified therapeutic approaches.

From a physiopathological perspective, a large body of evidence has demonstrated that the location of the stroke lesion is strictly related to the severity of the consequent upper limb motor deficit. Specifically, it was argued that patients with cortical stroke have a better motor outcome than patients with subcortical stroke. Furthermore, patients with mixed cortical plus subcortical stroke tend to improve more than patients with pure subcortical stroke, despite the expected larger size of the mixed lesions. Consequently, even small subcortical lesions produce devastating motor effects. The probability of upper limb motor recovery after stroke is hence strictly linked to the anatomical lesion:

- 75% for patients with lesions restricted to the cortex (M1; pre-motor area, PMA; supplementary motor, SMA);
- 38.5% for those with subcortical or mixed cortical plus subcortical lesions not affecting the posterior limb of the internal capsule (PLIC);
- 3.6% of those with involvement of the PLIC plus the adjacent corona radiata, the basal ganglia or the thalamus (Piron et al., 2009b).

1.1.2. Diagnostic issues in stroke

In its very acute manifestation, stroke needs to be differentiated among different possible pathologies. Together with the confirmation of stroke diagnosis, hemorrhagic or ischaemic aetiology have to be defined, with the aim to plan the best form of treatment for each individual patient. In fact, time contingency is fundamental to determine the consequent outcome of recovery in the acute, sub-acute and chronic phases.

The main aim of differential diagnosis is to obtain evidence of focal brain damage, as most possibly explained by the concurrent neurological clinical picture. Thus, the major conditions to exclude are: transient ischaemic attack (whose symptoms resolve within 24 hours); hypertensive encephalopathy (distinguished by evidence of oedema on CT or MRI); hypoglycaemia (e.g. diabetes; low levels of serum glucose); complicated migraine (characterised by positive symptoms and negative MRI) (Alberts et al., 2011); seizures and postictal deficits (evidence at

EEG and no signs of lesions on MRI; typical eye deviation towards the hemiparetic side which might be due to stroke in the pons or the thalamus); conversion and somatisation disorders (characterised by absence of infarction on MRI); Wernicke's encephalopathy (commonly present history of alcohol abuse and decreased thiamine blood level); brain tumours (evidence on CT scan). The principal diagnostic evidence common to all the listed pathologies is the absence of infarction on cerebral imaging assessment (i.e. CT and/or MRI). In case of suspected neurological deficits due to either metabolic (e.g. diabetes) or neurophysiological (e.g. seizure, migraine) aetiology, additional tests (e.g. EEG, blood samples) will be needed, to confirm diagnosis.

From a clinical point of view, there are common signs and symptoms (retrievable from clinical history and anamnesis), that allow expert practitioners to make a diagnosis of stroke, possibly differentiating between an ischaemic or haemorrhagic origin. If clinical key, common and uncommon factors are correctly interpreted, then the most appropriate tests need to be prescribed to confirm the diagnosis of ischaemic (Table 1.1, 1.2) or haemorrhagic (Table 1.3) stroke.

Table 1.1. Key and common diagnostic factors of ischaemic stroke

FACTOR	DEFINITION	CLINICAL INTERPRETATION
Vision loss Or Visual field deficit*	Monocular vision (transient)	<ul style="list-style-type: none"> • Cervical carotid stenosis unilateral deficit • Vertebrobasilar ischemia (unilateral or bilateral deficit) • Posterior circulation ischaemia
Weakness*	Complete or partial loss of muscle strength in: <ol style="list-style-type: none"> 1. Face 2. Arm 3. Legs 	<ul style="list-style-type: none"> • Large hemispheric involvement if all 3 involved • Rarely bilateral • Hemiparesis associated with lacunar strokes
Aphasia*	Impairment in any language function, either producing or understanding	<ul style="list-style-type: none"> • Dominant hemispheric ischaemia
Ataxia*	Impaired motor coordination	<ul style="list-style-type: none"> • Involvement of the cerebellum or its fibres connections • Posterior circulation stroke associated with fine (hand) motor coordination and gait
History of TIA	Temporary disruption in the blood supply to part of the brain, whose effects last from	<ul style="list-style-type: none"> • Present in almost 50% of stroke related to a cervical artery atherosclerosis • Higher risk of subsequent stroke

	few minutes to 24 hours maximum.	<ul style="list-style-type: none"> • 5% of TIA patients have a stroke within 2 days
Sudden onset of symptoms	Starting over seconds to minutes and worsening step-wisely, fluctuating or stuttering	<ul style="list-style-type: none"> • Slow progression could be related to other aetiologies (e.g. intracerebral haemorrhage) • Differentiating between multiple step-wise worsening and gradual decline
Negative symptoms	Loss of functions (e.g. visual loss, numbness, weakness)	<ul style="list-style-type: none"> • Positive symptoms (e.g. hallucinations, athetosis) are mostly related to seizures or psychiatric disorders
Sensory loss	Inability to perceive sensorial stimulations on neurological examination	<ul style="list-style-type: none"> • Cortical lesion may induce fine sensory impairments (e.g. 2-point discrimination, graphaesthesia, stereognosis)
Altered sensation	Sensory loss and paraesthesia (numbness)	<ul style="list-style-type: none"> • Non dermatomeric distribution (peripheral nerve disease)
Headache	Pain anywhere in the region of the head or neck	<ul style="list-style-type: none"> • Insidious and gradually increasing (haemorrhage) • Sudden with gradual moderation (subarachnoid haemorrhage) • Intracranial hypertension (cerebral sinus thrombosis, space-occupying lesion) • Complicated migraine
Diplopia	Double vision of a single object in any of the space direction	<ul style="list-style-type: none"> • May occur in patients with posterior circulation ischaemia
Dysarthria	Motor speech disorders resulting in poor articulation of phonemes	<ul style="list-style-type: none"> • Associated with facial weakness or cerebellar dysfunction • Due to posterior circulation ischaemia or lacunar infarct
Gaze paresis	Altered coordination of eyes motion	<ul style="list-style-type: none"> • Commonly horizontal and unidirectional • Associated with anterior circulation ischaemia • Deviation towards hemiparetic side (controlateral to brain hemisphere) might depend on seizure or infarct of the pons or thalamus
Arrhythmias, murmurs, pulmonary oedema	Comorbidities of the cardiovascular systems predisposing patients to stroke	<ul style="list-style-type: none"> • Atrial fibrillation (i.e. higher risk of for cardioembolic ischaemic stroke) • Paroxysmal atrial fibrillation • Indication to anticoagulants treatment

The key diagnostic factors are marked with an asterisk (*). TIA: transient ischemic attack

Table 1.2. Uncommon diagnostic factors associated with ischaemic stroke

FACTOR	DEFINITION	CLINICAL INTERPRETATION
Vertigo Dizziness	Subjective spinning sensation, described as feeling like being on a ship in choppy seas	<ul style="list-style-type: none"> • Associated with nystagmus • Symptom of posterior circulation ischaemia
Nausea and/or vomiting	Starting over seconds to minutes and worsening step-wisely, fluctuating or stuttering	<ul style="list-style-type: none"> • Posterior circulation ischaemia • Increased intracranial pressure
Horner's syndrome	<p>Postganglionic lesions at the level of internal carotid artery (third-order neuron disorder) that releases epinephrine, with signs of:</p> <ul style="list-style-type: none"> • ipsilateral miosis, • ptosis, • enophthalmus, • with or without anhidrosis. 	<ul style="list-style-type: none"> • dissection of ipsilateral carotid artery • posterior circulation ischaemia
Altered level of consciousness / Coma	Reduced or absent level of alertness	<ul style="list-style-type: none"> • large anterior circulation, thalamic, bihemispheric, ischaemia • coma is more associated with brain stem ischaemia • higher levels of urgency (breathing support) • seizures and large haemorrhage to rule out
Confusion	Sensory loss and paraesthesia (numbness)	<ul style="list-style-type: none"> • common in older people with previous strokes or cognitive impairments • Wernicke's aphasia to rule out

Table 1.3. Key and common diagnostic factors associated with haemorrhagic stroke

COMMON	UNCOMMON
Neck stiffness	Haemathological disorder (e.g. bleeding diathesis, dengue haemorrhagic fever)
Medical history of atrial fibrillation or liver disease	Vertigo (cerebellar haemorrhage)
Visual changes (hemianopia: haemorrhage in visual pathway; diplopia: haemorrhage in brain stem)	Nausea / vomiting
Photophobia	Altered consciousness / coma (e.g. large hemispheric or posterior fossa haemorrhages)
Sudden onset	Gaze paresis (often horizontal and unidirectional)
Altered sensation	
Weakness	
Sensory loss	
Aphasia	
Ataxia	
Headache	

With the aims to result in a complete assessment of the clinical picture and to set up the most appropriate therapy, a set of indicated tests represents the first choice to confirm the diagnosis of ischaemic (Table 1.4, 1.5) or haemorrhagic (Table 1.6, 1.7) stroke.

Table 1.4. Indicated tests for first diagnosis of ischemic stroke.

TEST	RESULT	INTERPRETATION
CT head	<ul style="list-style-type: none"> • Hypoattenuation (darkness) of the brain parenchyma • Hyperattenuation (brightness) in an artery indicates clot • Loss of grey matter • White matter differentiation • Sulcal effacement 	<ul style="list-style-type: none"> • Most important test to differentiate haemorrhagic from ischaemic stroke (Kucinski, 2005) • Normal within the first few hours in ischaemic stroke
MRI brain	<ul style="list-style-type: none"> • Acute ischaemic infarct: brightness on DWI • Ischaemic territory: increased signal on T2 sequences 	<ul style="list-style-type: none"> • DWI and gradient-echo sequences are more accurate than CT • Equivalent for haemorrhage but more sensitive for

Serum glucose	<ul style="list-style-type: none"> • Exclusion of hypo/hyperglycaemia for focal neural signs 	<p>infarction detection, than CT (Chalela et al., 2007)</p> <ul style="list-style-type: none"> • Hyperglycaemia is associated with poor recovery and risk of haemorrhagic transformation of ischaemic stroke (Baird et al., 2003) • Every patient with TIA or stroke should be screened for diabetes mellitus.
Serum electrolytes	<ul style="list-style-type: none"> • Electrolyte disturbance 	
Serum urea and creatinine	<ul style="list-style-type: none"> • Exclude renal failure 	<ul style="list-style-type: none"> • Potential contraindication to some stroke interventions (e.g. specific hydration protocols)
Cardiac enzymes	<ul style="list-style-type: none"> • Exclude MI 	<ul style="list-style-type: none"> • Concurrent MI and stroke
ECG	<ul style="list-style-type: none"> • Exclude arrhythmia or myocardial ischaemia 	<ul style="list-style-type: none"> • 24h of ECG monitoring, to exclude atrial fibrillation. • Ambulatory ECG for 30 days to diagnose paroxysmal atrial fibrillation in cryptogenic stroke (Gladstone et al., 2014)
Full blood count	<ul style="list-style-type: none"> • Exclude anaemia and thrombocytopenia 	<ul style="list-style-type: none"> • Contraindication for thrombolytic, anticoagulants, antithrombotic treatments in acute stroke.
PT and PTT (with INR)	<ul style="list-style-type: none"> • Exclude coagulopathy 	<ul style="list-style-type: none"> • Thrombolysis as acute treatment, should not be delayed in case of history of anticoagulants use or coagulopathy.

CT: Computer Tomography; MRI: Magnetic Resonance Imaging; DWI: Diffusion Weighted Images; TIA: transient ischaemic attack; MI: myocardial infarct; ECG: electrocardiogram; PT: prothrombin time; PTT: partial thromboplastin time; INR: international normalised ratio

Among the variety of new techniques available in imaging, CT/MRI perfusion-weighted imaging is emerging as a test for patients presenting beyond 3 to 4.5 hours, to identify cerebral regions that may be at risk of subsequent infarction (Kane et al., 2007).

Table 1.5. Tests to consider for diagnosis of ischemic stroke.

TEST	RESULT	INTERPRETATION
Serum toxicology screen	<ul style="list-style-type: none"> Exclusion of alcohol or drug abuse 	
Stool guaiac test	<ul style="list-style-type: none"> Negative for occult blood 	<ul style="list-style-type: none"> Exclusion of gastrointestinal bleeding
Chest X-Ray	<ul style="list-style-type: none"> Exclusion cardiomegaly, aortic dissection, pneumonia 	<ul style="list-style-type: none"> Exclusion of other relevant cardio-pulmonary conditions.
CT / MR angiography	<ul style="list-style-type: none"> Exclude acute arterial occlusion, atherosclerotic stenosis, arterial dissection, reversible vasoconstriction syndrome, Moyamoya disease, fibromuscular dysplasia 	<ul style="list-style-type: none"> CT has better spatial resolution than MR angiography, to identify arterial occlusion or stenosis
Carotid ultrasound	<ul style="list-style-type: none"> To identify critical stenosis or cervical artery occlusion 	<ul style="list-style-type: none"> More frequent in sub-acute phase
Transcranial Doppler ultrasound	<ul style="list-style-type: none"> Arterial occlusion of major branches of the circle of Willis 	<ul style="list-style-type: none"> Limited spatial resolution compared with angiography

CT: Computer Tomography; MR: Magnetic Resonance

Table 1.6. Indicated tests for first diagnosis of haemorrhagic stroke.

TEST	RESULT	INTERPRETATION
Non-infused CT head	<ul style="list-style-type: none"> Hyperdense lesion 	<ul style="list-style-type: none"> Combined with CT angiography
Chemistry panel	<ul style="list-style-type: none"> Normal 	<ul style="list-style-type: none"> Abnormal results suggest other aetiologies
Full Blood Count (FBC)	<ul style="list-style-type: none"> Normal 	<ul style="list-style-type: none"> Low platelet count suggest secondary haemorrhage
Clotting test	<ul style="list-style-type: none"> Normal 	<ul style="list-style-type: none"> To rule out coagulopathy
ECG	<ul style="list-style-type: none"> Large inverted T waves (change of cerebral origin) 	<ul style="list-style-type: none"> To rule out angina
Platelet function test	<ul style="list-style-type: none"> Abnormal aggregation 	<ul style="list-style-type: none"> Risk of brain haemorrhage expansion
Urine drug screen	<ul style="list-style-type: none"> Positive/negative 	<ul style="list-style-type: none"> To rule out drugs abuse
Pregnancy test (women of childbearing age)	<ul style="list-style-type: none"> Positive/negative 	<ul style="list-style-type: none"> Specific medical management
Liver function test	<ul style="list-style-type: none"> Deranged 	<ul style="list-style-type: none"> Acute liver failure may lead to bleeding, cerebral oedema, intracranial hypertension

CT: Computer Tomography; ECG: electrocardiogram

Table 1.7. Tests to consider for diagnosis of haemorrhagic stroke.

TEST	RESULT	INTERPRETATION
CT/MR angiography and venography	Negative	<ul style="list-style-type: none"> To rule out aneurysm, AVM, thrombosis
Or		<ul style="list-style-type: none"> Recommended <45yy, lobar haemorrhage
Conventional angiography		<ul style="list-style-type: none"> MR lower spatial resolution, than CT Conventional is the reference for small AVM
MRI brain with DWI and GRE	Acute haemorrhage hypointense (dark) in GRE	<ul style="list-style-type: none"> Acute ischemic infarct bright on DWI 5%-20% GRE are asymptomatic bleeding
MRI brain with SWI	Lesions hypointense	<ul style="list-style-type: none"> More sensitive than GRE (Pettigrew et al., 2006)

CT: Computer Tomography; MR: Magnetic Resonance; AVM: artero-venous malformation; DWI: diffusion-weighted imaging; GRE: gradient-echo sequence; SWI: Susceptibility Weighted Imaging

1.1.3. Acute treatment of stroke for reducing the effect of brain damage

Treatment in the acute phase of stroke is strictly dependent on the time span between presentation of symptoms and access to a stroke unit, together with the presence of contraindications for thrombolysis in case of an ischemic aetiology. In fact, the possibility to undergo thrombolysis leads to significant better outcome of recovery, if successful in eligible patients. Different evidence has been produced by several scientific societies and national health systems, for the use of tissue plasminogen activator (tPA) 24 hours before aspirin. So far, across countries differences exist about the time window from stroke onset to consider administration of tPA to patients (within 3 hours for NIH, within 4.5 hours for the UK-NHS), but all agree on the necessity to exclude contraindications.

Following this critical clinical decision, standard acute care, regardless of aetiology, is based on: supporting vital parameters (i.e. breath, ventilation, oxygen saturation); assessing swallowing impairments (to avoid aspiration pneumonia); activating deep venous thrombosis prophylaxis (TVP) together with early mobilisation. Large trials are currently running to study the efficacy of very early mobilisation (within 24 hours) after stroke to improve long term outcomes (Askim et al., 2014, Bernhardt, 2012, Bernhardt et al., 2006, Bernhardt et al., 2008, Bernhardt et al., 2015b, Bernhardt et al., 2015c, Bernhardt et al., 2009, Cumming et al., 2008, Cumming et al., 2011), but reaching the vertical stance and adapting to gravity as early as possible is known to stabilise vital parameters and reduce complications due to immobilisation.

1.1.4. Evidence from brain imaging in stroke

The systematic introduction of magnetic resonance imaging (MRI) and functional MRI (fMRI) both in research and in clinical settings, allowed the redefinition of the role of well-known structures traditionally considered as the proper motor system (corticospinal tracts; cortico-cerebellar, cortico-striato-thalamo and cortico – cortical circuits). Moreover, it has also been possible to infer the existence of a so called “extended motor system”. The extended motor system could be considered as the whole set of networks linking classical motor areas with non-motor functions, including perception, emotion, language and music (Rowe and Siebner, 2012).

Motor learning deals with plasticity of the central nervous system (CNS) in healthy, as well as in stroke patients, implying that both the proper and the extended motor systems are instrumental to the development of new skills. Based on this classification, it could be argued that techniques such as MRI-based morphometry and voxel-based morphometry (VBM) allow the investigation of macroscopic structural changes (Granert et al., 2011), while fMRI allows the detection of microscopic changes at the level of synaptic weights. With fMRI the spatial resolution ranges from 1mm to 5mm, while the temporal resolution is measured in seconds (Cramer and Bastings, 2000). Several fMRI protocols have been proposed for the assessment of relevant changes in cortical plasticity, during motor recovery. Among them index finger tapping, reaching or pointing movements, button presses, joystick control and motor imagery, have been the most investigated in the available literature (Rowe and Siebner, 2012). The main problem affecting whatever paradigm is used deals with the difficulty of differentiating between changes due to real motor recovery and the ones reflecting differential performance. Possible corrections that should be considered when analysing data, are:

- normalization of performance matching it with perceived effort, as a measure of relative task difficulty (Ward et al., 2004),
- minimisation of performance confounds with resting state acquisition in the same subject

Some evidence from fMRI studies in stroke patients demonstrated that:

- bilateral activation of motor, premotor and parietal cortical areas is commonly reported during active movements of the paretic hand (Calautti et al., 2001)
- the persistence of a pattern of bilateral activation during voluntary movements is a predictor of poor motor recovery (Rehme et al., 2011). Nevertheless, it is still debated if this finding should be considered as maladaptive recovery or as evidence of ongoing compensatory strategies.
- reduced interhemispheric functional connectivity in the extended motor system at rest is associated with severe motor impairment (Carter et al., 2010),

- the preservation of resting-state connectivity between the ipsilesional motor cortex and the thalamus, SMA and middle frontal gyrus is a predictor of good motor recovery at 6 months after stroke.
- increased functional connectivity with the ipsilesional fronto-parietal networks, bilateral thalamus and cerebellum (Park et al., 2011) is present in subcortical motor stroke,

The body of knowledge on brain reorganization patterns post stroke is continuously increasing and changing, as a function of the development of new computational tools for data analysis. Therefore, not all the available evidence is coherent and caution should be taken when describing the dynamic changes occurring in the brain after a stroke.

Nonetheless, Richards and co-workers (2008) reported the weighted effect sizes of using fMRI as an outcome measure (referring to specific experimental paradigms) to map movement-dependent stroke recovery. The findings from the meta-analysis indicated that the overall effect size – including transcranial magnetic stimulation (TMS), fMRI, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) – was large ($ES = 0.84 \pm 0.15$, 95% CI = 0.76-0.93) and also robust, as at least 42 null studies are required to lower the effect size to a non-significant level. Moreover, specifically related to fMRI, it was found that active elbow or finger flexion/extension are the most informative paradigms when the following variables are considered as outcomes: volume of brain activity, laterality index or number of active voxels.

1.2. The effect of rehabilitation on recovery processes after stroke

1.2.1. Neurophysiology of stroke recovery and implications for rehabilitation

Motor recovery in stroke can be considered as a direct function of cortical plasticity and the adaptation ability of the entire motor system. Nevertheless it is still strongly debated how much of the recovery process after brain lesions relies on true or compensatory repairing mechanisms (Arya et al., 2011a). True motor recovery is defined as that occurring when undamaged, new or alternative pathways convey signals to the same muscles, whose cerebral areas were interested by the lesion. Conversely, compensatory motor recovery is considered as that occurring when different muscles are activated to accomplish the same functional task (Krakauer, 2006). In the case of true recovery, several mechanisms (e.g. unmasking, changes in synaptic weights and activation of redundant circuitries) are supposed to be involved in the re-mapping of the motor system (Teasell et al., 2005). In the case of compensative recovery, different areas enlarge their cortical representation in order to supply the damaged one. Both patterns occur after stroke and are equally fundamental to regain the best motor and functional recovery. Motor learning after stroke involves true, as well as compensatory recovery, nevertheless the first one is widely considered the main goal of the emerging neurophysiologically based techniques for motor rehabilitation.

1.2.2. Computational theories of rehabilitation for motor function after stroke

According to the Sherrington adage (1924): “to move things is all that mankind can do, for such the sole executant is muscle, whether in whispering a syllable or in felling a forest” (Kandel and Schwartz, 2000; p. 675), the motor system could be considered the principal output effector allowing the so called “life of external relation”.

Many components contribute to transform iteratively stimuli from the external world into neural signals, which are finally conveyed back to the body (Figure 1.1). Among them muscles, receptors (e.g. exteroceptors, proprioceptors), neural pathways (e.g. ventromedial, dorsolateral, corticobulbar tract, corticospinal tract, reticulospinal tract, segmental spinal networks) and cortical networks (e.g. Brodmann's areas 4 and 6) cooperate with the aim of producing a coherent motor behaviour. The aim of movement neuroscience is to investigate how such complex structures can be integrated into a unique functional system, able to transform sensory signals into motor commands and to clarify which mechanisms underpin their related control (Wolpert and Ghahramani, 2000).

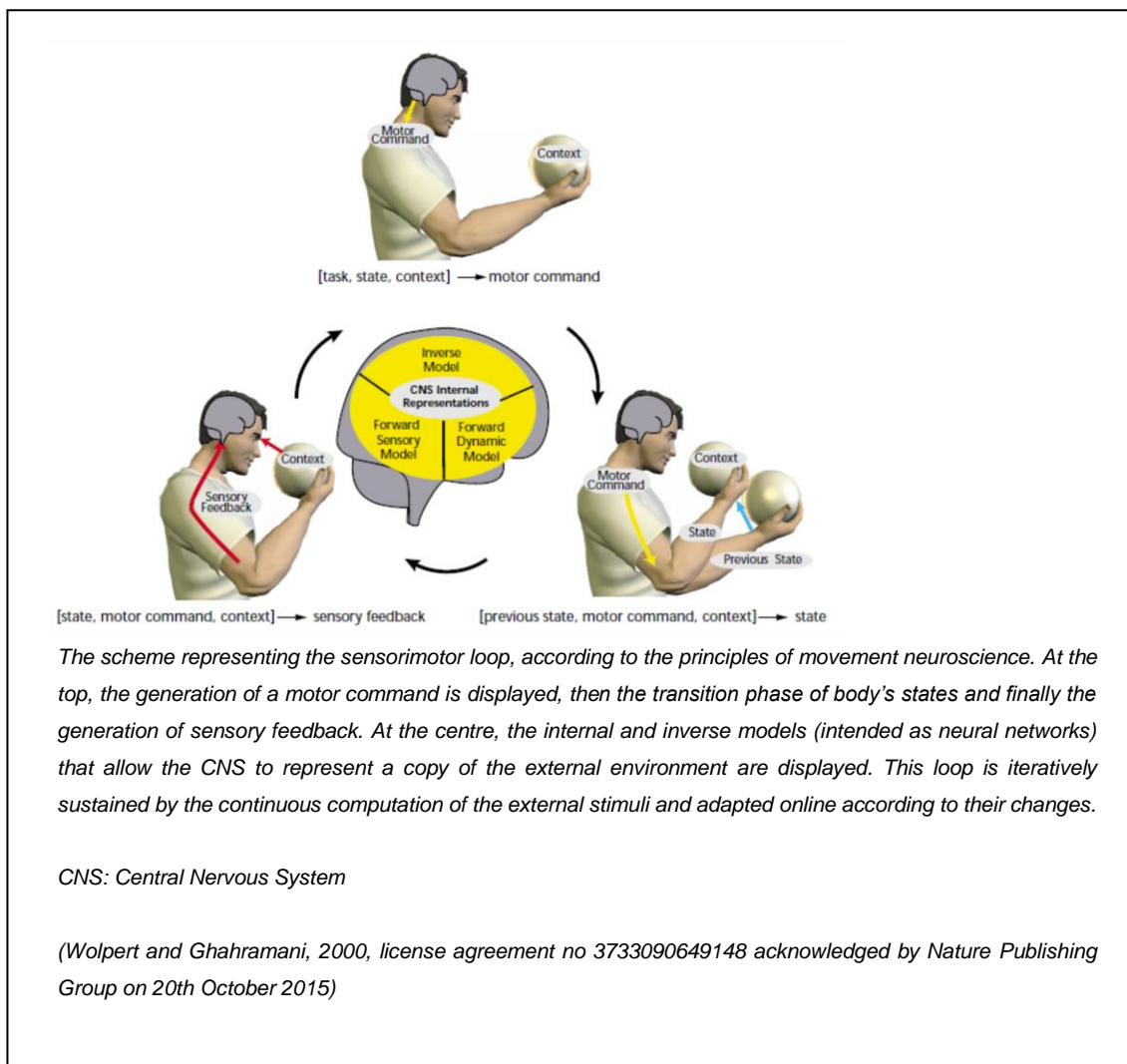


Figure 1.1. The sensorimotor loop.

The motor system is organized according to a hierarchical architecture allowing an efficient functionality within its physical constraints (e.g. muscles, bones and

joints). Four major components are recognized as being the motor system scaffolding, each one representing a different level, according to a periphery-central direction (Kandel and Schwartz, 2000):

- 1st level: spinal cord (reflex responses)
- 2nd level: brain stem and reticular formation (integration of ascending and descending pathways)
- 3rd level: motor cortex (Brodmann's area 4, representing 30% of corticospinal and corticobulbar fibres devoted to the activation of descending motor commands)
- 4th level: premotor cortical areas (Brodmann's area 6, representing 30% of corticospinal and corticobulbar fibres devoted to programming of movements).

Three common aspects characterize those levels:

1. they contain somatotopic maps;
2. each level receives information from the periphery;
3. higher levels control information that reaches them, allowing or suppressing the transmission of the afferent volley by means of a sensory relay.

The cerebellum and the basal ganglia complete the hierarchical architecture of the motor system (Doya, 2000a): the former modulating anticipatorily the activity of the brain stem and of the cortex, the latter intervening in movements' selection and underpinning reward mechanisms related to successful behaviours (Frey et al., 2011).

This top-down architecture is also regulated by means of parallel circuits (e.g. interneurons, descending fibres from supraspinal regions, neuronal gating) that, working independently, increase the modularity of the entire system and so its flexibility in accomplishing the demanding requirements coming from an unpredictable external environment.

From Bernstein's perspective (1967), the problems of degrees of freedom and degrees of constraint have been postulated taking into consideration three major points:

1. in the animal species the levels of control achieved for voluntary motion, with regard to intrinsic complexity, are so fluent, reliable, and widely

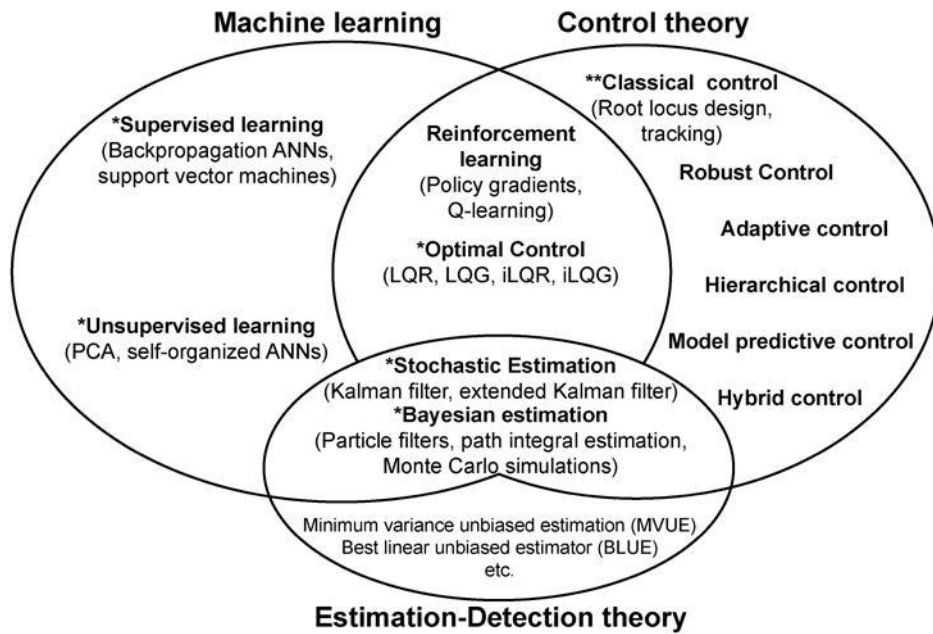
manifest, that they must be underwritten by principles of the most basic and general kind.

2. the level of sensory corrections (i.e. the mechano-receptive machinery embedded in the body's deformable tissues) that sustain the so called "haptic perceptual capabilities" is hugely integrated, underpinning motion fluency.
3. the systematic exploitation and modulation of the level by other functional levels must engage further abstract principles.

Several models have been proposed in the literature to describe how the motor system acts in its interaction with the external environment (Han et al., 2008, Schweighofer et al., 2009), and to explain intrinsic mechanisms underpinning neurophysiological functions (Kutch and Valero-Cuevas, 2012). The general purpose of a model is to formalize coherently all the possible relationships existing between anatomical structures and neurophysiological mechanisms composing a functional system; through their implementation it is easier to figure out weaknesses in a theoretical framework and formulate new hypotheses to be tested experimentally. Those models are commonly based on mathematical functions explaining, in whole or in part, human behaviour (Sejnowski et al., 1988).

Commonly, a model for motor function (i.e. neuro-musculo-skeletal model) needs a reference computational environment where problems of reduction of dimensionality and redundancy can be solved, preferably online, with fast computation. Among the large number of components available, the following are necessary for a reliable model: skeletal mechanics, musculo-tendon routing, forward models for muscle activations and inverse models for sensory optimisation. Finally, models of learning, solutions and functional relationships need to be coded.

Valero-Cuevas and collaborators (2009) reviewed the current state of the art on mathematical approaches so far implemented in applied research, to study neuromuscular function in humans (Figure 1.2).



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** most common used techniques; * upcoming techniques

Figure 1.2. Interaction among different mathematical approaches to study human movement (Valero-Cuevas et al., 2009).

Recently, Frey and co-workers (Frey et al., 2011) presented one of the most complete model considering, in a unique framework, the neuroanatomy, neurophysiology and computational aspects clinically useful for the interpretation of human motion.

The model relies on six principles of functional anatomy:

1. evidence of anatomical gradients and parallel circuits (Table 1.8):
 - a. in the parietal and premotor cortices, the level of activation smoothly changes according to the difficulty of motor planning and control, required by the task;
 - b. in the frontal lobe an anterior-to-posterior gradient is responsible for the transformation of an action's intended goal into effective motor commands;
 - c. parieto-frontal circuits code for a stimulus' location and estimation of body's state (sensory-to-motor transformation) (Wolpert and Ghahramani, 2000)
 - d. in the premotor areas medial to lateral gradients are involved in planning of internally guided actions (e.g. motor imagery).

Table 1.8. Brain circuitries and gradients involved in motor execution. (Adapted from, Frey et al., 2011).

Circuit / Gradient	Function	Structure/circuit involved
Anterior (abstract processes) to posterior (immediate requirements) gradient in the frontal lobe	Associative areas for goals and motivation	Amygdala Hypothalamus Ventral striatum
Parieto-frontal circuits	Comparison of sensory feedbacks Stimulus location in space / body state estimation Spatial aspects of movement Familiar movements	Parietal cortex Posterior parietal areas Right parietal cortex Left parietal cortex
SPL – PMd	Control of goal directed upper limb movements (reaching/grasping), based on visuo – proprioceptive feedbacks mediated by joints and skin stimuli mediated by peripheral vision (extrafoveal visual space)	SPL MIP, V6A
IPL – PMv	Feeding/avoiding objects approaching to face Transformation of visual feedback in grasping posture for manipulation Mirror neurons	VIP – PMvc (F4) IPS(AIP) – PMvr (F5) PFG – PMvr (F5) – Area 44
Medial – lateral gradient in PMA	Motor planning of internally guided actions Motor planning of response to stimuli actions	Medial PMA Lateral PMA

AIP: Anterior Part of IPS; IPL: Inferior Parietal Lobule; IPS: Intraparietal Sulcus; MIP: posterior half of the Medial wall of the IPS; PFG: Posterior Fusiform Gyrus; PMA: Premotor Areas; PMd: Dorsal Premotor Cortex; PMv: Ventral Premotor Cortex; PMvc (F4): Caudal Part of Ventral Premotor Cortex; PMvr (F5): Rostral Part of Ventral Premotor Cortex; SPL: Superior Parietal Lobule; VIP: Ventral Intraparietal Areas; V6A: Medial Component of Area 19

2. Overlapping synergies in the primary motor cortex. The activation of every single neuron induces cascades of events mediating excitatory and inhibitory effects at the same time. The consequent organization should be most probably based on overlapping networks that synergistically produce common effects targeted to set of muscles, instead of a one-to-one effectors' control (Figure 1.3).

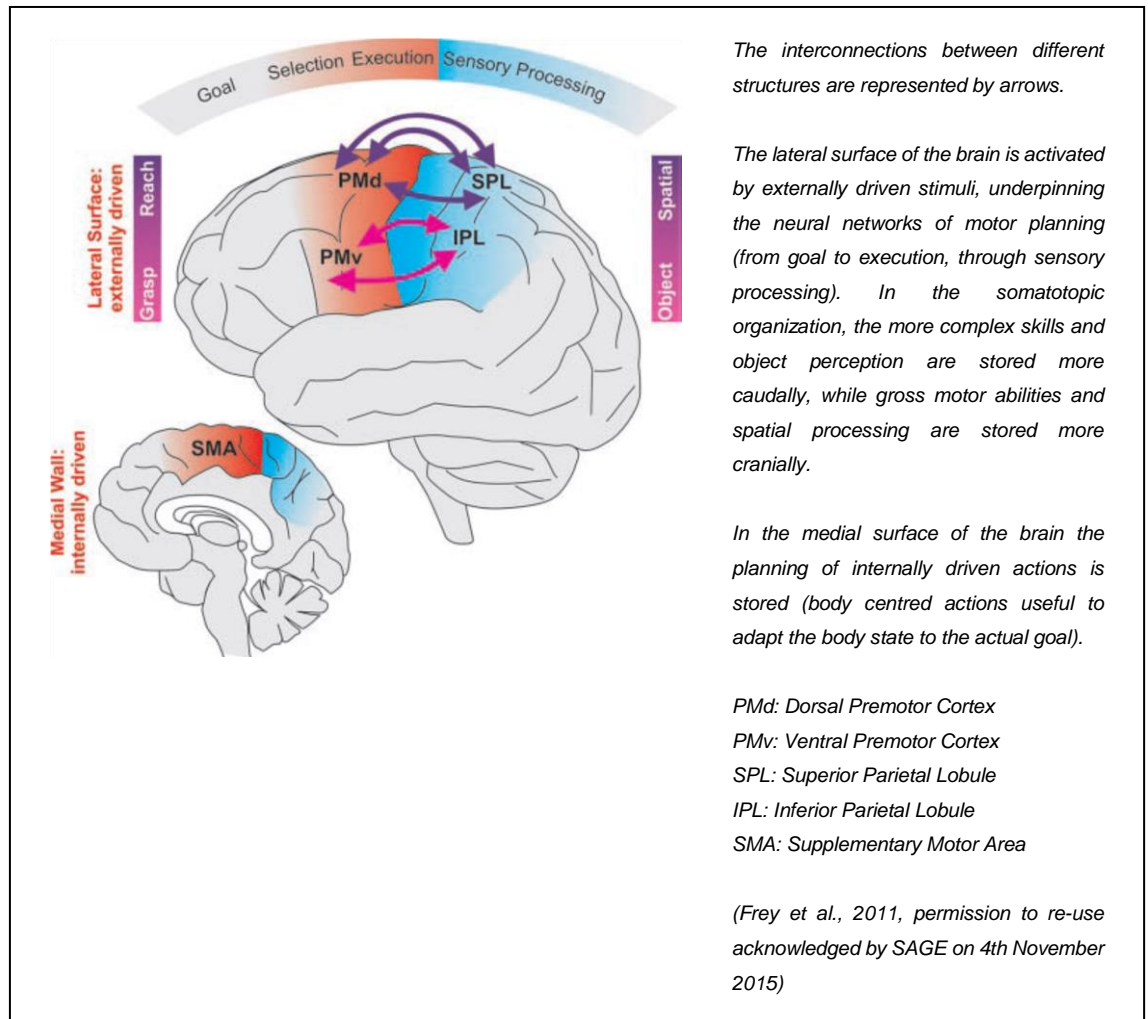


Figure 1.3. Overlapping synergies in motor cortical areas.

3. The cerebellum as a predictor of cognitive and motor tasks: it facilitates the learning of new kinematics and internal models, by means of cerebral cortex and brain stem forward modulation (Table 1.9).

Table 1.9. Functions and structures of the cerebellum involved in motor execution. (Adapted from, Frey et al., 2011).

Function	Structure/circuit involved
Carrying error signals in relation to movements execution; tuning of cerebellar output	Inferior olive
Comparing different signals from cerebral cortex	Cerebellar cortex
Integration of eye – head – body signals to control balance	Vestibulocerebellum
Hands movements coordination; cognitive functions	Neocerebellum
Verbal selection; working memory	Right cortex of neocerebellum
Spatial working memory; non-verbal reasoning	Left cortex of neocerebellum

4. Involvement of basal ganglia in movement selections and reward. The basal ganglia are designed to not mix information between different cortical areas. They intervene when automatic selections of motor programs are needed, to modulate the propagation of information and mediate the rewarded learning through dopaminergic circuits (Table 1.10).

Table 1.10. Functions and structures of basal ganglia involved in motor execution. (Adapted from, Frey et al., 2011).

Function	Structure/circuit involved
Scaling of force, amplitude, acceleration	BG – motor/premotor cortex
Selection of novel, not habitual actions	BG – prefrontal cortex

BG: Basal Ganglia

5. Parallel pathways from the cortex to the spinal cord. Several cortical areas project to the brain stem, all outputting to the spinal cord (Table 1.11).

Table 1.11. Functions and structures of cortex – spinal cord pathways involved in motor execution. (Adapted from, Frey et al., 2011).

Function	Structure/circuit involved
Activation of distal muscles	M1
Encoding of movements' kinematics	Superior Parietal Cortex; PMA
Mediating cortical outputs	Corona Radiata; Internal Capsule
Bilateral and ipsilateral projection to spinal cord	RSTs

M1: Area 4; PMA: Premotor area; RSTs: Reticulospinal Tracts

6. Modular organization of the spinal cord. The spinal cord is a complex architecture of computational circuitries integrating descending commands with sensory reflex. The reflex pathways are estimated to support from 30% to 40% of the output of voluntary contraction force.

The presence of both parallel and series circuitries provides a biological hardware, realistically able to sustain the high-performance computation required for online management of a “motor control” problem. In this sense, the computational, anatomical, physiological (CAP) model also provides information about the computational capabilities of the motor system.

Intended as a computational machine the motor system is affected by the so called “curse of dimensionality” (Bernstein, 1967), in other words the dilemma of controlling a system composed by a high number of elements. For example, estimating an approximate number of 200 muscles working like body actuators and assuming that they can only contract and relax, the possible combinations of different states correspond to $\sim 2^{200}$: a number too high to be managed online. Moreover, in a multi-joint system, the same purpose could be reached by means of different combinations of the same elements (redundancy), further increasing the degrees of freedom (DoF).

If we consider a feasible biological scenario, the picture is more complex than the one illustrated, for example the muscles can have potentially infinite states of contraction. For this reason, algorithms are needed to solve the matter of control, in order to reduce the DoF and increase the accuracy of motor performance.

The most studied computational principles, assumed to take place in the motor system, are reported in tables 1.12 and 1.13, with their hypothesised anatomical substrate.

Table 1.12. Computational processes for motor control and related neuroanatomical substrates

<i>Predictive feed-forward control (internal models)</i>		
Computational process	Description	Structure/circuit involved
Forward model	A neural simulator that predicts (in the causal and forward – direction) the sensory consequences of an action given the current state and efference copy of motor command (Wolpert et al., 2011)	Motor cortex, parietal cortex, interior olive, Cerebellum (Frolov and Dufossé, 2006)
Inverse model	A neural simulator that model the necessary motor commands, which would transform the current position into the desired one.	Motor cortex, parietal cortex, interior olive, Cerebellum (Frolov and Dufossé, 2006)
<i>Feedback control</i>		
Computational process	Description	Structure/circuit involved
Reactive control	Use of sensory inputs to update ongoing motor commands	1 st and 2 nd level of the motor system hierarchy
Biomechanical control	Modulation of limb compliance	Peripheral receptors (exteroceptors, proprioceptors)
Visuo – haptic integration	The process that combines visual information (e.g. the visual size of an object) and haptic information (e.g. the felt size of a grasped object) into a single percept (e.g. its size) (Wolpert et al., 2011).	Motor and visual cortex

Table 1.13. Computational processes for motor learning and related neuroanatomical substrates

Computational process	Description	Structure/circuit involved
Unsupervised (use – dependent learning)	Through a concise representation of sensory state, context, and action find appropriate modular architecture for a given task (Doya, 2000a).	Motor cortex (Doya, 2000a).
Reinforcement	Learning process based on the prediction of reward signal. Perform evaluation of current situation and selection of the appropriate action by a set of candidate (Doya, 2000a).	Basal ganglia: reward signal encoded in dopaminergic fibers from the substantia nigra (Doya, 2000a).
Knowledge of performance (kinematic feedback)	Sustain reinforcement learning. Information indicating the quality or patterning of a movement (e.g. displacement, velocity, joint motion) (Young and Schmidt, 1992).	Basal ganglia: reward signal encoded in dopaminergic fibers from the substantia nigra.
Knowledge of result	Sustain reinforcement learning. Extrinsic or augmented information indicating the success of an action with regard to an environmental goal (Schmidt and Young, 1991).	Basal ganglia: reward signal encoded in dopaminergic fibers from the substantia nigra.
Supervised (Error – based)	Learning adaptation process based on the comparison between the estimated outcome and the achieved one. It can reduce the average error to zero, but do not provide a mechanism for improvement.	Inferior olive, cerebellum, motor cortex
Observational	Learning process based on the observations of others (action observation) (Rizzolatti and Luppino, 2001).	Adjustment of kinematic error: left IPS, left PMd and right cerebellar cortex. Action prediction: dorsolateral prefrontal cortex Outcome prediction: ventromedial prefrontal cortex (Burke et al., 2010).

Based on those assumptions, the general principle of existence of “synergies” at different levels was postulated, whose aim was to downscale complexity to more manageable structures. The notion of synergy is cognate with notions of organization, cooperative activity, collective behavior, low-dimensionality and metastability. A synergy, therefore, can be considered like a stable organization whose components are always ready to participate in other stable organizations. In Bernstein’s functional hierarchy (1967), the level responsible for forming synergies of large muscle groups and different patterns of locomotion is referred to as the level of muscular–articular links or synergies. The synergistic activity of all the mechanism so far described allows the emergence of human voluntary motor behaviour. Nevertheless, the majority of the underlining relationships still remains unknown. Moreover, from an experimental point of view it is not yet feasible to control for all the interferences occurring while performing a paradigmatic task. Thus, solutions that permit the reduction of the DoFs are useful, not only to clarify control strategies stored in the motor system, but mostly to set up effective approaches for studying human motor behaviour, especially when affected by neurological disorders.

1.2.3. Assessment of functional recovery after stroke

The assessment process in rehabilitation represents the critical appraisal of the actual residual function of a patient. The aim is to provide them with the best treatment, according to current evidence based practice. For optimal clinical decision, it is fundamental to iterate assessment procedures, from admittance to discharge, taking into consideration three main domains:

1. patient’s values and beliefs (e.g, needs, current health status, wishes),
2. resources available in life-living environment of the patient (e.g. time, costs, laws, rules),
3. experience and clinical skills of the therapist.

Within this framework, measurement of a patient’s function represents the most reliable way to objectify prognosis of recovery and efficacy of therapies.

An outcome can be defined as any detectable event changing across (i.e. presence or not of a specific clinical condition) or along (i.e. modification of a defined clinical condition) time, in a single or group of patients, in the group of caregivers or community, because of a clinical intervention (Wade, 1992).

Likewise, it is considered an outcome measure a sequence of measurement procedures, structured in a standardized protocol, with the aim to score meaningful information on the health status of a person. Outcome measures can be classified according to different reference models, but the most comprehensive has been agreed and published by the World Health Organisation (WHO) in 2001 and it is known as the International Classification of Functioning, Disabilities and Health (ICF) (World Health, 2001). The first step to plan a reliable measure is the choice of ICF domains and constructs to quantify, then the outcome measure is chosen among many according to its best clinimetric properties (Figure 1.4).

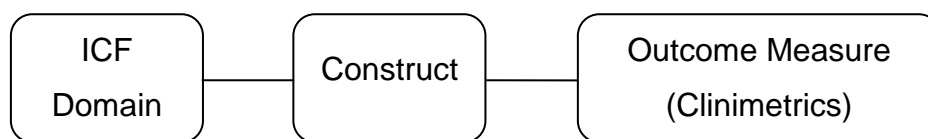


Figure 1.4. Flowchart delineating the process of choice of outcome measures.

Nevertheless, the score obtained from the administration of an outcome measure is not necessarily related to the subjective perception and satisfaction of a single patient, because every step in the decision flow introduces an uncertain source of variability, affecting the power of final clinical inference.

A large literature has been published on the use of stroke scales and their clinical interpretation. The scales reported in table 1.14 are known to be the most reliable to forecast prognosis in the acute phase, to plan rehabilitative strategies and to evaluate a patient's perspective.

Table 1.14. Summary of the most common scales for general assessment after stroke.

Scale	Acronym	Clinical validity
National Institute of Health stroke scale	NIHSS	Intraobserver reliability ICC=0.93 Interobserver reliability ICC=0.95 0.4<CC<0.8 with infarct volume (i.e. MRI, CT) Acute phase>3 excellent outcomes at 3 and 7 days Acute phase>15 poor outcomes at 3 months
modified Rankin Scale	mRS	Inter-rater reliability k=0.25, without structured interview Inter-rater reliability k=0.74, with structured interview 0.4<CC<0.5 with infarct volume (i.e. MRI, CT) Poor responsiveness from admission to discharge
Functional Independence Measure	FIM™	MCID = 22 p.ts (total), 17 p.ts (motor), 3 p.ts (cognitive) Adequate correlation with length of hospital stay (r = -0.39)

ICC: Intra-Class Correlation; CC: correlation coefficient;

The NIHSS is a 15-items scale developed for fast and reliable general assessment of stroke patients in emergency departments. The advantage of its implementation lies on the possibility to have a reliable measure of stroke in a critical environment, regardless of the background of any health professional present in that setting. In any case, the final and detailed clinical administration has to be done by a trained and certified neurologist. The main areas of measurement are: level of consciousness (3 items), gaze, visual fields, facial palsy, limbs strength (4 items), coordination (ataxia), sensory function, language, speech (dysarthria), hemi-inattention (extinction). A change of 2 points has been indicated as reference for a minimal clinically important difference (Tilley et al., 1996). Patients with a score between 6 and 13 are eligible for referral to acute inpatient rehabilitation (Kasner, 2006). Kwakkel and collaborators (2010) demonstrated that the 13-items NIHSS has an excellent accuracy (72%) in predicting recovery of ADLs at 6 months, when administered in the first 9 days after stroke. The main pitfall of the NIHSS is related to the complete absence of cranial nerves assessment, thus brainstem and cerebellum infarctions are hard to detect and the consequent score might not be coherent with the real clinical

picture. Moreover, the aetiology and pathophysiology of stroke is not considered, thus a complete history, imaging and neurological examinations are still needed. The mRS (Sulter et al., 1999) is commonly used to measure disability after stroke, by seven different grades ranging from no symptoms (0), to dead (7). Its usefulness relies on the possibility to detect broad problematic areas for patients, by structured interview, not detectable by impairment-based assessment usually administered during office visits. Another important aspect of mRS frequently exploited in clinical research is the dichotomisation of the scale to divide stroke patients in bad or good responder, with regard to specific outcomes. Its main pitfall is lack of specificity, since it is not possible to retrieve which specific ICF construct (e.g. cognitive function, visual loss, force, dexterity) is determining the current disability, thus the population of patients falling in the same rank is affected by large clinical variability.

The FIM™ includes 18 items (i.e. 13 motor tasks, 5 cognitive tasks), each ranging from 0 (not executable) to 7 (complete independence). Certification is required for scale administration. Despite the level of evidence is not yet satisfying for FIM™ properties (Chumney et al., 2010), its clinimetrics is still one of the most reliable for patients admission to rehabilitation hospitals. Particularly, both motor and cognitive scores at hospital admission are the most significant predictors of length of stay (Heinemann et al., 1995).

The worst effect of a stroke is the possibility that a large spectrum of body structures and functions can be disrupted because of the brain lesion. Nevertheless, motor recovery has direct implications for the overall restoration of autonomy, thus motor function represents one of the most measured constructs after stroke. An incredible number of outcome measures have been developed for the assessment of motor function and their clinimetrics have been studied extensively. Nowadays, several databases (e.g. www.rehabmeasure.org , www.strokingengine.ca) offer free access to updated reviews of the most common outcome measures available for motor impairments caused by stroke (Table 1.15).

Table 1.15. Summary of the most common scales used for the assessment of motor function, after stroke

Outcome measure	Acronym	ICF Domain
ABILHAND ¹		Activity, Participation
Action Research Arm Test ^{1,2}	ARAT	Activity
Box and Block Test ^{1,2}	BBT	Activity
Chedoke Arm and Hand Activity Inventory ^{1,2}	CAHAI	Activity
Chedoke-McMaster Stroke Assessment ^{1,2}	CMSA	Body function, Activity
Frenchay Arm Test ^{1,2}	FAT	Activity, Participation
Nine Hole Peg Test ^{1,2}	NHPT	Body function, Activity
Purdue Pegboard Test ^{1,2}	PPT	Body function
Stroke Arm Ladder ¹		Activity, Participation
Stroke Impact Scale ^{1,2}	SIS	Activity, Participation
Upper Extremity Function Test ¹	UEFT	Activity
Wolf Motor Function Test ^{1,2}	WMFT	Activity
Functional Gait Assessment ^{1,2}	FGA	Activity
6 Minute Walk Test ^{1,2}	6MWT	Activity
2 Minute Walk Test ^{1,2}	2MWT	Activity
10 Meter Walk Test ²	10MWT	Activity
Dynamic Gait Index ²	DGI	Activity
Modified Ashworth Scale ^{1,2}	AS / MAS	Body structure, Body function
Timed Up and Go ^{1,2}	TUG	Activity
Fugl-Meyer Assessment Scale ^{1,2}	FMA	Body function
Functional Ambulation Category ²	FAC	Activity
Motor Assessment Scale ^{1,2}	MAS	Activity
Rivermead Mobility Index ^{1,2}	RMI	Activity

¹: www.strokingengine.ca; ²: www.rehabmeasure.org

Recently, a predictive algorithm for recovery of upper limb motor function after stroke has been proposed by Stinear and co-workers (2007, 2010, 2012), the so called “predictive recovery potential (PREP)”. This algorithm (Figure 1.5) combines clinical (i.e. Medical Research Council score of shoulder abduction and fingers extension, 72 hours after stroke), neurophysiological (i.e. presence of motor evoked potentials elicited in affected upper limb by transcranial magnetic stimulation) and neuroimaging (i.e. asymmetry index of fractional anisotropy in the posterior limbs of the internal capsules measured with diffusion-weighted MRI) parameters to calculate a reference score able to predict the recovery of upper limb motor function soon after stroke. For neuroimaging parameter an index based on fractional anisotropy (FA) of both posterior limbs of the internal capsules defines the integrity of this structure in stroke affected hemisphere, as

compared with the contralesional hemisphere. This index has positive values when the posterior limb of the internal capsule is more impaired in stroke affected hemisphere, than non-affected hemisphere and negative values in the opposite case, whereas a value of 0 indicates the both structures have the same FA (i.e. the same integrity).

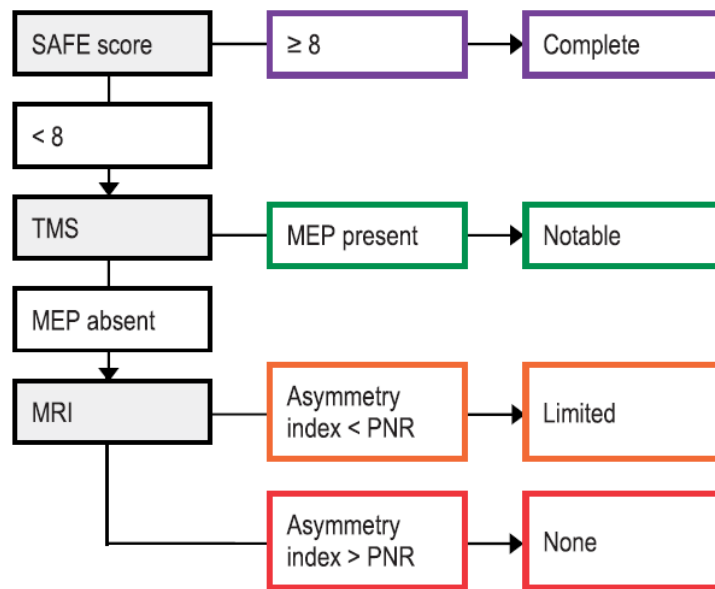


Figure 1.5. The PREP algorithm for recovery of upper limb motor function after stroke (Stinear et al., 2012)¹.

SAFE: sum of MRC from shoulder abduction (SA) and finger extension (FE); TMS: transcranial magnetic stimulation; MEP: motor evoked potential; MRI: magnetic resonance imaging; Asymmetry index: fractional anisotropy in posterior limbs of internal capsule measured with diffusion-weighted MRI; PNR: point of no return.

A point of no return (PNR) was calculated for this index, which is related to the baseline level of upper limb motor function measured by the action research arm test (ARAT). The boundary value for PNR of the fractional anisotropy asymmetry index has been suggested to be 0.15 in the acute phase and 0.25 in the chronic one. Overall, the score obtained from PREP allows the stratification of motor function recovery and the association to feasible rehabilitation goals (Table 1.16).

¹ The material from Stinear et al. (2012) is not published under any open access (OA) or creative commons (CC) license and permissions request must be requested from Journals Permissions directly

Table 1.16. Definitions of PREP recovery and related rehabilitation goals
(Adapted from Stinear et al., 2012).

Recovery	Definition	Rehabilitation Goal
Complete	Potential to return to normal or near-normal limb function within 12 weeks	Task specific therapies for fast recovery of ADLs.
Notable	Potential to using their affected limb in most of ADLs within 12 weeks, but normal function is unlikely.	Strength, coordination, fine motor control training, to maximize recovery and minimize compensation with the other hand.
Limited	Potential to have some movement in their affected limb within 12 weeks, but it is unlikely to be used functionally for ADLs	Reducing impairment, in order to promote adaptation and incorporation of the affected upper limb in ADLs.
None	Minimal movement in their affected limb, with little improvement at 12 weeks.	Prevention of secondary complications, such as spasticity, reducing disability by learning to complete ADLs with healthy side.

The PREP proposal represents a big step forward for rehabilitation-oriented prognosis, because it incorporates all the most evident tests available for motor function recovery, from simple clinical tests to advanced diagnostic techniques, in an easy and stepwise flowchart. Nevertheless, it has to be considered that not all the proposed techniques are easily available, nor were other paramount clinical factors like the level of cognitive functions, awareness and comprehension, considered. Thus, this algorithm has strong predictive values when referred to the primary motor system, but nothing is known when considering areas of the extended motor system as potential contributors to broad recovery.

1.3. Rehabilitation of the upper limb after stroke

1.3.1. Rehabilitation modalities

The rapid restoration of post-stroke deficits and the attainment of a lifestyle as close as possible to the pre-morbid state are among the main, partially unachieved purposes of neurological rehabilitation. Surprisingly, there are few therapeutic approaches to restore lost functions and currently researchers are working to develop treatments related as closely as possible to neurophysiological principles coming from basic science.

Recently, innovative treatments have considerably increased the repertoire of available therapeutic and rehabilitative strategies. Considering that almost half of the stroke survivors present reduced functionality of the upper limb (Dromerick et al., 2006), great efforts have been spent to develop the most effective strategies for the recovery of motor function of this motor district.

A large number of approaches (e.g. Kabat, Brunstrom) based on neurophysiology of the motor system have been proposed in past decades, by many authors (Paci, 2003). In motor rehabilitation the Bobath concept has been for a long time one of the reference points among the so called neurodevelopmental treatments (Kollen et al., 2009). The method, originally developed for the treatment of patients affected by cerebral palsy, was systematically coded for stroke patients and published for the last time by the original authors in 1990 (Bobath, 1990). Its main principles rely on the elicitation of the missing components of movements, as a consequence of a stroke, inhibiting abnormal movement patterns and promoting the physiological ones. The application of the method requires specific patient handling skills to guide them through effective execution of the requested tasks.

Recently, increasing evidence has sustained the effectiveness of feasible technology aided approaches aimed at augmenting mechanisms of physiological recovery involved in the production of voluntary movements after a brain lesion. Among them, the best studied approaches are: electromyography (EMG) biofeedback (Woodford and Price, 2007), robot-assisted therapy (Mehrholz et al., 2012), virtual reality (VR) based interventions (Laver et al., 2011), constrain induced movement therapy (CIMT) (Sirtori et al., 2009) and functional electrical stimulation (FES) (Pelton et al., 2012). However, despite advances in the

understanding of stroke aftermaths and development of innovative rehabilitation methods, there is no evidence that suggests superior efficacy of one method over others. Several gaps need filling to better understand the relationship between the neuroanatomical structures and the neurophysiological mechanisms involved in human voluntary movements and unravel possible pipelines leading from brain activations to natural behaviour.

Following a neurological disease, it is not possible to treat all movement disorders only by physical rehabilitation; in fact some individuals will benefit also from pharmacological, surgical or orthotic interventions and there is no unique consensus on how to deliver motor treatment. Nevertheless, certain general principles are always present in effective rehabilitation treatments, independently from the chosen technique.

According to Pomeroy and co-workers (2011) six principles should encompass every therapeutic program:

1. The establishment of a “contract” between patient and the therapy team;
2. Analysis of behavioural deficits according to the principles of brain reorganization previously described (see paragraph 1.2.2);
3. Reliable measurements of impairment, function and activity before, during and after therapy (see paragraph 1.2.3);
4. Planning of a rehabilitation program according to the prognosis of a patient (see paragraph 1.2.3);
5. Administration of an appropriate amount of therapy in terms of specificity, intensity (dose) and repetitiveness (Han et al., 2008);
6. The presence of an appropriate therapeutic environment shaped for motor learning (modular spaces, possibility to augment feedbacks, adequate to subjects’ comprehension and attention).

Within this framework it is possible to classify current rehabilitation techniques in three major groups according to the following descriptions (Figure 1.6):

- *Priming techniques*: interventions that may prepare the sensorimotor system for increased plasticity promptness through direct stimulation (physical or sensorial) of tissues;
- *Augmenting techniques*: interventions that enhance the effects of sensorimotor interaction during practice;

- *Task-specific practice*: interventions based on massive practice of specific tasks performed in real environment, with the aim of prompting the best generalization of learning in real life (Platz et al., 2001, Platz, 2004).

The concept of priming after stroke deals with the issue of promoting substitution or restitution strategies for recovery of lost functions, due to the lesion location in the brain. With restitution is meant the possibility to reinstate function in the ipsilateral hemisphere interested by the lesion, with the aim to maximise motor recovery. In this regard, the more is the residual excitability in the lesioned M1 the better the prognosis for motor recovery. When excitability is predominant in the unaffected hemisphere a substitution strategy is recommended, aimed at inhibiting over activation of the unaffected side potentially masking the affected one with maladaptive mechanisms.

Recent advances in neurophysiological techniques provide methods to condition temporarily neural networks by administration of electrical (tDCS) or magnetic (rTMS) fields to the brain, through the scalp. This brain stimulation is able to modulate synaptic balance between neurons promoting the so called metaplasticity (i.e. the plasticity of synaptic plasticity) (Cassidy et al., 2014). With a wider meaning and rehabilitation purposes all the modalities able to induce a temporary modification of any structure in the musculoskeletal (e.g. soft tissue of passive mobilisation, tactile stimulation) and neurological systems (e.g. motor and visual imagery, action observation) is considered as promoting priming of the structures involved in expressing voluntary motor behaviour.

The concept of augmented modalities deals with the evidence that enrichment of the external environment, where animals or subjects are requested to interact with, leads to significant modifications of their own functional systems, both at a central (e.g. CNS) and a peripheral level (e.g. muscles). Experiments in rats demonstrated that animals living in environments providing greater opportunities for physical activity, motivation and socialisation, after experimentally induced stroke, show augmented sprouting and brain plasticity, than those living in standard laboratory cages (Dobrossy and Dunnett, 2004, Nithianantharajah and Hannan, 2006). This evidence has been applied also to stroke rehabilitation and all the artificial environments (e.g. robot, virtual reality, biofeedback) augmenting specific features and providing feedback information on results and performance of tasks accomplished are considered as the clinical translation of enriched environments. So far, clinical evidence suggests that this approach is successful

particularly for impairment oriented treatment, but more insights on the mechanisms involved are needed (Takeuchi and Izumi, 2013).

The concept of task-specific practice comes from the movement and motor skill learning literature (Schmidt and Young, 1991, Carr and Shepherd, 2011) and has been defined by Teasell and collaborators (2008) as training or therapy where patients “practice context-specific motor tasks and receive some form of feedback”. This wide definition can be applied to almost all the therapeutic settings available in rehabilitation care, therefore all the modalities aimed to massive practice of everyday tasks with real-world objects are intended as task-specific practice in clinics. The aim is to achieve optimal function performance which can be replicated in everyday activities, thus to improve quality of life in ecological environments.

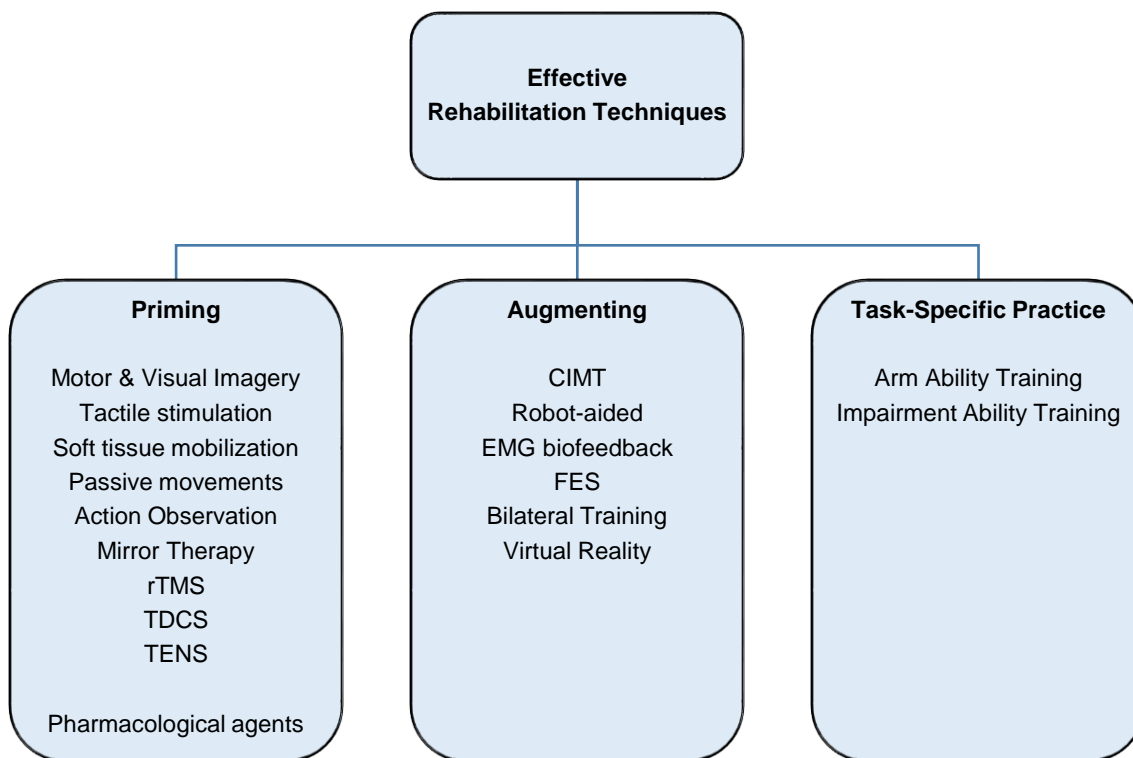


Figure 1.6. Classification of motor rehabilitation techniques.

rTMS: repetitive Transcranial Magnetic Stimulation; TDCS: Transcranial Direct-Current Stimulation; CIMT: Constraint Induced Movement Therapy; EMG: Electromyography; FES: Functional Electrical Stimulation; TENS: Transcutaneous Electrical Nerve Stimulation

1.3.2. Neural plasticity in patients undergoing rehabilitation of the upper limb after stroke

The wide availability of imaging (e.g. fMRI) and neurophysiological (e.g. PEM, TMS) techniques for the study of structural and functional modifications of the CNS after brain lesion have allowed scientists to explore whether rehabilitation modalities might act as key factors to induce neural plasticity. In neurorehabilitation, neural and cortical plasticity are mostly intended like a combination of spontaneous recovery and goal-directed reorganisation induced by motor therapy. Because of the criticisms in quantifying the dose of conventional rehabilitation therapies provided, the most recent motor therapies have been studied more extensively from a neurophysiological point of view. Arya and co-workers (2011a) have recently reviewed the evidence available on neural reorganisation induced by movement therapies targeted to the upper limb, after stroke (Table 1.17).

Table 1.17. Neural reorganisation induced by specific rehabilitation modalities for the upper limb, in stroke patients.

Motor therapy	Technique	Brain areas
Task-specific training	fMRI	Decreased activation: unaffected hemisphere Increased activation: primary sensorimotor cortex; both hemispheres
CIMT	fMRI, MRI	Increased cortical excitability, metabolic rate, blood flow Increased grey matter volume: sensory and motor cortical areas bilaterally
Mental imagery / practice	fMRI	Increased activation: cerebellum, premotor, primary motor cortex, striatal sensorimotor network
Robotics	fMRI	Increased activation: sensorimotor cortex
Virtual Reality	fMRI	Increased activation: ipsilesional SM1 Decreased activation: bilateral SM1s, contralesional premotor cortex

fMRI: functional magnetic resonance imaging; CIMT: constraint induced movement therapy SM1; primary sensorimotor cortex

Recently, some evidence has been provided on brain plasticity specifically induced by virtual reality based therapies, after stroke. August and collaborators (2006) made the first single healthy subject pilot fMRI to test whether the visualization of a hand virtual avatar or not-anthropomorphic avatar of the hand,

induced different brain activations when doing motor tasks. Findings indicated that visualising a virtual hand moving, while doing voluntary motor tasks with the hand, activated largest motor areas (primary motor cortex, dorsal premotor and supplementary motor areas, anterior cingulate cortex, anterior intraparietal cortex, superior temporal gyrus) than watching simple objects not related to hand shape. The same group of author also provided the first evidence on brain activation reshaping due to virtual reality training, after stroke. In their pilot study changes in task-related fMRI and in functional connectivity during resting state acquisition were analysed, in two stroke patients (Saleh et al., 2012). Results showed that the extent of activation decreased in both subjects after VR-based therapy at task related fMRI, while M1 and SMA increased their connectivity after training. These results were also supported by improvements at behavioural level as measured by the Wolf Motor Function Test and the Jebsen Test of Hand Function. A larger pilot trial on 8 consecutive stroke patients was run by Orihuela-Espina and collaborators (2013) using a motor imagery block paradigm, to test reorganisation of brain activation after gesture therapy mediated by interaction with VR environments. Their results confirmed the main involvement of prefrontal cortex and cerebellar activity for the recovery process of upper limb motor function, following a stroke.

1.3.3. Efficacy of virtual reality based therapy in stroke patients: clinical evidence

Virtual reality is an innovative technology consisting of a computer based environment that represents a 3D artificial world and has been already applied in many fields of human activities (Steuer, 1992). New computer platforms permit human-machine interactions in real time, therefore the possibility of using VR in medicine has become available, for example, for stroke survivors and can be used for rehabilitative therapeutic procedures (Piron et al., 2001a). Combining VR-based systems with motion tracking tools allows the study of the kinematics of arm movements in the restorative process after stroke. Furthermore, the possibility of modifying the artificial environment, in which the patients can interact, may be a method to promote motor learning, by means of enhancement of feedbacks about movement characteristics, such as knowledge of performance and knowledge of results (Piron et al., 2010, Piron et al., 2009a).

A recent Cochrane Library review (Laver et al., 2011) pooled the results from different studies of the effect of VR to improve motor, cognitive, gait and balance functions and activities of daily living (ADL), after stroke. The authors concluded that, despite encouraging significant results, there is still not sufficiently strong evidence that VR therapy has a better effect than conventional therapy, so more data are needed to determine the effectiveness of this approach on the different post stroke sequelae. Notably, taking into account only upper limb function, all studies included in the review, indicated that a VR approach yielded better motor and ADL outcomes, when compared with conventional therapy. Considering the reported meta-analysis it was estimated, that the difference in effect size (calculated as standardized mean difference: SMD) between motor VR therapies and standard ones was 0.53 (CI 95% = 0.25-0.81) for the recovery of motor function and 0.81 (CI 95% = 0.39-1.22) for independence, in favour of VR-based treatments. In addition, in the case of rehabilitation of upper limb motor function based on virtual reality, a therapy dose effect was observed. Specifically, when the intervention is delivered for less than 15 hours of training, this treatment becomes ineffective.

A subsequent Cochrane publication from Pollock and co-workers (2014) compared in an overview of reviews, the clinical effect of all the modalities for the treatment of upper limb after stroke (i.e. repetitive task training provided both for more or less of 20 hours, bilateral versus unilateral training, CIMT, virtual reality, mirror therapy, mental practice), with some level of evidence available. The results confirmed two fundamental points:

1. the need to provide motor treatments for a sufficient number of sessions (i.e. 15 to 20 hours overall), in order to change significantly clinical outcomes, regardless of therapeutic modality;
2. moderate evidence, based on the “grading of recommendations assessment, development and evaluation” criteria (GRADE) (Guyatt et al., 2008), that the best improvements are achieved by these modalities focused on the reinforcement of the visual-motor loop (i.e. virtual reality, mirror therapy, mental practice).

Going into details of these results, virtual reality turned out to have the most consistent clinical effect, as compared to the others included in the review. In fact its standardised effect is in the top rank of the modalities considered (SMD = 0.53), but its variability range is the smallest overall (CI 95% range = 0.56).

Soon after this piece of evidence, Laver and co-workers updated their original review on the effect of VR for stroke rehabilitation (2015b) and this time evidence was extracted from 37 trials involving 1019 participants overall, compared with the 19 trials involving 565 participants included in the previous review (Laver et al., 2011). The new results added some stronger evidence on the effect of VR based rehabilitation for the recovery of upper limb, thus a more precise overall effect was calculated for motor function both regardless of the outcome measure assessed (SMD = 0.29 [0.09, 0.49]) and with regard to the Fugl-Meyer upper extremity scale (MD = 3.30 [1.29, 5.32]). The dose effect was also confirmed, making VR-based therapy more effective than conventional physical therapy after 15 hours of training (SMD = 0.31 [0.07, 0.55]), together with transferability of achievements to activities of daily living (ADLs) (SMD = 0.42 [0.18, 1.29]). More interesting in the updated review was that the increased amount of literature available allowed a new comparison with a hard clinical meaning, namely the use of a combination of VR and conventional therapy versus the same amount of conventional physical therapy. This type of comparison is the closest available to what can be provided in a real clinical setting, where multimodal therapies are constantly administered to patients because of the need to exploit as much as possible the best time window available for recovery. The results gave new insights on the potential clinical transfer of VR-based therapies into real clinics of patients undergoing intensive rehabilitation.

Overall, when VR and conventional therapies are provided, the positive effect on global motor function is higher, than using only VR-based therapy (SMD = 0.44 [0.15, 0.73]). With regard to dose effect, a significant improvement is achievable with less than 15 hours of training, when VR and conventional therapies are combined (SMD = 0.40 [0.05, 0.75]). On the contrary, more than 15 hours are needed if only specialised VR systems are used (SMD = 0.31 [0.07, 0.55]). Finally, also the transferability to ADLs was confirmed (SMD = 0.40 [0.05, 0.75]) and was observed that positive results are achieved only when specialised VR systems designed for rehabilitation are used (SMD = 0.42 [0.07, 0.76]), rather than commercial gaming systems (e.g. Nintendo Wii®).

When looking at subgroups of patients in relation to time since stroke onset (figure 1.7), an interesting pattern appears. In the first 6 months after stroke, the gain is significantly better by using only specialised VR systems, with a surprising big effect size for the field of rehabilitation interventions (SMD = 0.78 [0.28, 1.29]).

Conversely, the combination of VR with conventional modalities in the first six months seems to be as effective as the same amount of conventional physical therapy (SMD = 0.29 [-0.11, 0.70]).

After 6 months the picture seems to flip, in fact using only VR systems is as effective as conventional physical therapy (SMD = 0.21 [-0.04, 0.46]), but the combination of both modalities turns to be more effective than the same amount of conventional rehabilitation intervention alone (SMD = 0.50 [0.00, 1.01]).

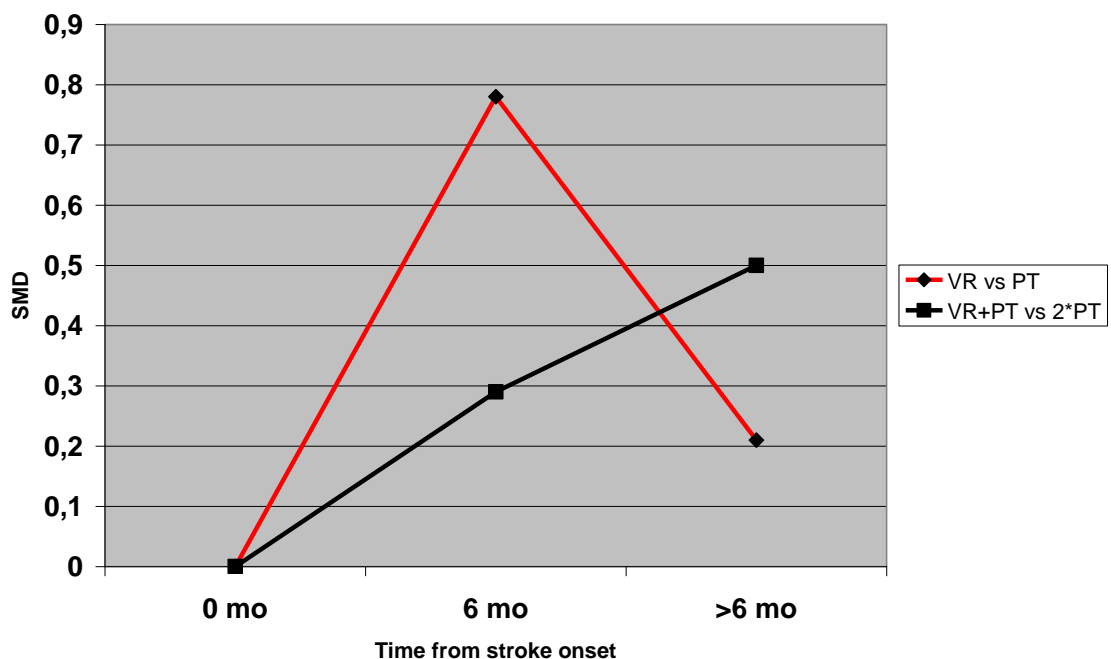


Figure 1.7. Standardised effects of virtual reality based and physical therapy modalities with regard to time since stroke, for the recovery of upper limb motor function.

SMD: standardised mean difference; VR: virtual reality; PT: physical therapy.

A potential interpretation might be related to the intensity and specificity of stimulations that it is possible to provide using VR-based treatments. In the first 6 months after stroke it is widely acknowledged that recovery is mainly driven by spontaneous mechanisms of CNS reorganisation, thus training patients in a well-controlled environment, where augmented information is available, might help to maximise the functional adaptive plasticity of the CNS. Whereas, longer than 6 months from stroke onset more compensatory strategies are supposed to be recruited for sustaining functional recovery, so combining high specific

impairment-oriented training with functional practice might provide better outcomes.

On these bases, it becomes paramount to provide evidence for a better understanding of the advantages and limits of VR therapy applications to the plurality of deficits consequent to a stroke.

For clarity and completeness, the evidence provided in the reviews reported above, even though being the highest methodologically available in the literature, is updated to November 2013, namely the last search run as claimed by Laver and co-workers (2015b).

To fill this gap, papers published from 1st November 2013 to 26th October 2015 were reviewed to detect potential new evidence on the topic.

The review was run on PubMed with the following string:

- (“Virtual Real”[Journal] OR (“virtual”[All Fields] AND “reality”[All Fields]) OR “virtual reality”[All Fields] OR “Int J Virtual Real”[Journal] OR (“virtual”[All Fields] AND “reality”[All Fields]) OR “virtual reality”[All Fields]) AND (“stroke”[MeSH Terms] OR “stroke”[All Fields]) AND (“rehabilitation”[Subheading] OR “rehabilitation”[All Fields] OR “rehabilitation”[MeSH Terms]) AND (“upper extremity”[MeSH Terms] OR (“upper”[All Fields] AND “extremity”[All Fields]) OR “upper extremity”[All Fields])”.

Thirty five records were retrieved, in accordance with the time filter applied (“2013/11/01”[PDAT]: “3000/12/31”[PDAT]). With the aim to update the current evidence on the topic, only studies comparing VR-based therapy with other treatments, for the recovery of upper limb motor function after stroke were considered.

From the screening of title and abstract, 16 studies were included and 19 excluded (i.e. 2 protocols; 5 guidelines or non-systematic reviews; 10 proof of concepts, feasibility or cohort studies; 2 non-controlled study). After full-text reading 6 more studies were excluded (2 healthy controls; 1 cross-sectional study from larger RCT; 1 treatment of fine finger dexterity; 1 full text not retrieved; 1 non-controlled study). In the following paragraphs, a short description of the main findings from every single study is reported.

Choi and collaborators (2014) studied the effectiveness of commercial gaming-based virtual reality (i.e. Nintendo Wii™), as compared to conventional occupational therapy (OT). Patients (N = 20) survived to a stroke in the last 3

months, with residual impairment of the upper limb (Fugl-Meyer Assessment upper limb, FMA-UL < 50 out of 66 points) and without severe cognitive impairments, were randomly allocated to Wii™ (N = 10) or OT treatments (N = 10). Both treatments lasted 20 sessions provided daily, 5 days a week for 4 weeks. Results indicated that both treatments are effective to promote recovery of the upper limb (except grip strength for Wii™ group), but none of the outcome measures (i.e. FMA-UL, manual function test, box and block test and grip strength) were significantly different between groups, after treatments. This result sustained the evidence that commercial VR game-based systems are potentially not useful to gain better results than conventional therapies.

Kiper and co-workers (2014) compared two groups of stroke patients in a hospital setting, one undergoing VR-based training with a specialised system, the other treated by standard physical therapy. Both treatments were provided for 20 sessions over 1 month, with daily session of 1 hour, 5 days a week for 4 weeks. The results were in favour of VR treatment both at the level of outcome measures (i.e. FMA-UL, FIM®) and kinematic parameters of standardised movements (i.e. time and smoothness of execution). Subgroup analysis in ischaemic or haemorrhagic stroke onset, found that only FIM® was different between subgroups, probably due to the normal difference in slope of recovery because of aetiology. Overall the available evidence suggested significant clinical difference due to the type of treatment in favour of VR-based training.

Kottink et al. (2014) compared patients with impairment of the upper limb due to a stroke treated by specialised VR system (i.e. FurballHunt) with conventional reaching exercises. Both treatments were provided, by single session lasting 30 minutes each, three times a week, for 6 weeks (18 sessions for 9 hours overall). None of the outcome measures assessed (FMA-UL and Action Research Arm Test) were significantly different between groups after treatment, but also in this study lack of treatment intensity might be argued.

In the study from Lee D and collaborators (2014) different concepts of VR training were compared for the first time to test whether different exercise parameters, both augmented in an artificial environment induce different treatment outcomes. Namely, these authors compared symmetric with asymmetric bilateral practice, both performed in a VR system mirroring movements performed with the unaffected arm. Results were in favour of asymmetric practice (i.e. moving one limb in one direction and the other in the opposite one, at 1 Hz frequency) that

resulted in better proximal gross motor function and range of movement, hand grasping and grip strength.

The study by Shin and co-workers (2014) tested the feasibility of using a specialised VR system (RehabMaster™, a device integrating 3D sensors with infrared projectors), for the treatment of upper limb motor impairment after stroke. Its clinical effect was tested alone and in combination with OT, as well as compared with the same amount of OT. Both treatments were provided for 10 sessions in comparable independent groups and the results were in agreement with evidence from the Cochrane reviews. In fact, 10 sessions were enough to induce a significant change in outcome measures such as the Fugl-Meyer and Barthel Index, but when compared with conventional OT no significant differences were observed. An interpretation based on the lack of intensity might be appropriate for these results.

A subsequent study from the same group (Shin et al., 2015) overcame the limit of intensity maintaining the same study design, but with treatments provided for 20 sessions, instead of 10. In this second trial the results turned to be in favour of VR-based training at the level of upper limb motor function, depression reduction and less emotional problems, thus confirming the positive multi-domain effects of long and intense VR-based treatment.

Yin and co-workers (2014) compared two groups of stroke patients in a hospital based setting in Singapore, undergoing VR treatment (N = 13) or conventional OT (N = 13). Both treatments were provided for 10 sessions of 30 minutes on a daily basis, 5 days a week for 2 weeks. The results showed no significant difference between treatments for all the outcome measures assessed (i.e. FMA-UL, Action Research Arm test, Motor activity Log and FIM®), neither soon after the end of treatment nor at one month follow up. Also in this case lack of intensity might be an appropriate interpretation of these findings. Interestingly, the authors, based on the effect size calculated from their results, indicated in 192 the sample size for future studies on training of the upper limb with virtual reality.

Three of the included studies explored the potential advantages arising from the possibility to combine priming (transcranial direct current stimulation, tDCS) with augmenting (VR) modalities. Lee and Chun (2014) compared the combination of tDCS and VR training, with tDCS stimulation or VR training alone, for the treatment of upper limb after stroke. The results clearly indicated that combining tDCS with VR induced significant better outcomes than providing the two

modalities by themselves. This study was an adjunctive piece of evidence on the possibility to ameliorate the effect of rehabilitation therapies when effective treatments are combined.

Viana and collaborators (2014) replicated a study similar to that by Lee and Chun (2014), finding that tDCS combined with Wii™-based treatment for upper limb, after stroke has a better effect than Wii™ alone, in reducing spasticity at wrist level as measured by the modified Ashworth scale. Nevertheless, Zheng and collaborators (2015) published the largest randomised double-blind clinical trial on the topic, which provided strong evidence that merging the two modalities led to significant better outcome in the domains of motor function and independence, than providing the same modalities independently.

To conclude and strengthen the evidence that using VR technologies is advantageous for the treatment of upper limb function after stroke, the cortical mechanisms implicated in the recovery process need to be better understood. In the following chapters of this thesis, experimental evidence aimed at filling this gap in knowledge will be reported.

2. AIMS OF THE STUDIES

The current evidence on the recovery of upper limb motor function after stroke is still inadequate to describe exhaustively all the possible implicated mechanisms. Similarly, the possibility to improve clinical outcomes after rehabilitation treatment is strictly dependent on a thorough understanding of the mechanisms ruling motor control.

With regard to the proposed models and premises reviewed in the introduction chapter, a reference framework has been postulated, with the aim to suggest hypotheses on the potential mechanisms involved in the recovery of upper limb motor function, following a stroke. The main objects of this framework (figure 2.1) are represented; on one side there is a schematisation of the motor system, where the relationship between planning and execution of voluntary movements of the upper limb can be linearly explicated, regardless of any pathological condition. On the other side, the schematisation of an artificial environment based on virtual reality (VR) in which the patient is requested to interact with is displayed. In this scheme, motor tasks displayed in VR can be controlled online by the patients' behaviour through data streaming coming from sensors placed on their own body. The real-time inter-dependency between motor behaviours and modification of the VR environment mediated by sensors, allows a closed-loop interaction that represents the clinical setting for rehabilitation of the upper limb motor function, which is the focus of study in this PhD project. The same closed-loop setting has been considered like the reference paradigm for the experiments reported in this thesis, with the aim to understand which mechanisms are involved at different levels (i.e. imaging, genetics, neurophysiology) and which are the relationships with outcome measures at the level of different International Classification for Functioning, Disability and Health (ICF) domains.

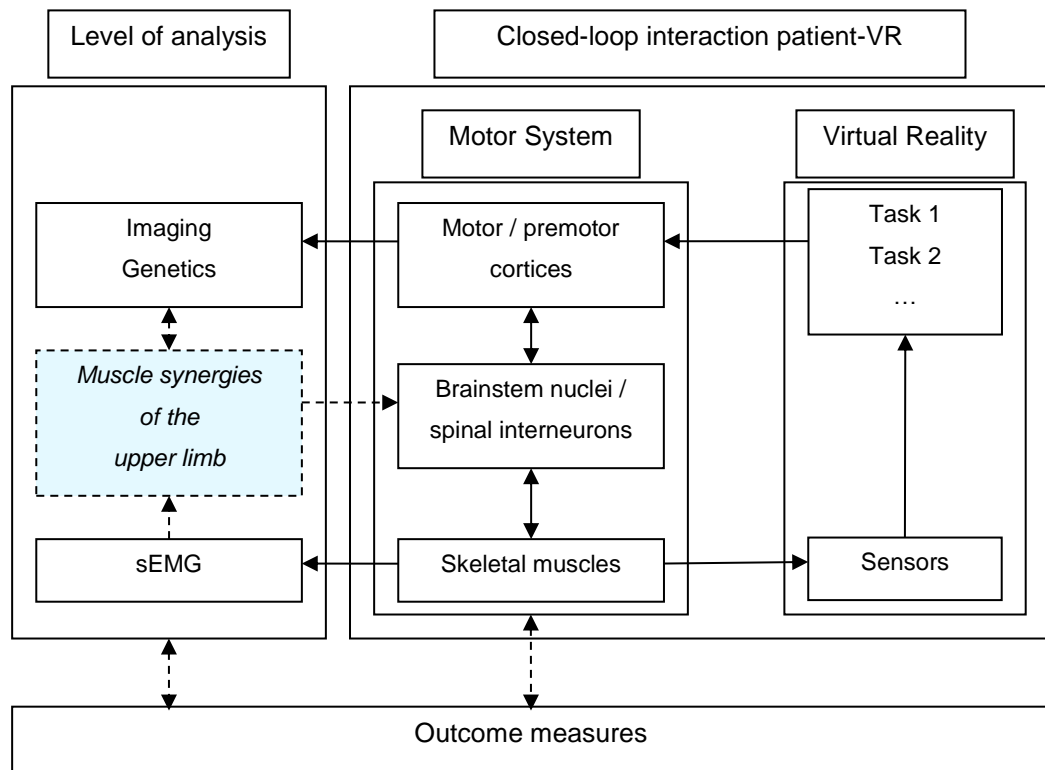


Figure 2.1. Scheme for closed-loop interaction between upper limb and VR

On the left, the computational techniques used in the experiments carried out for this thesis are shown as it is the level that each experimental hypothesis is meant to test. On the right, the reference framework for a closed-loop interaction between an artificial environment and a patient's behaviour is schematised. The analysis of muscle synergies represents the most recent and the less known of the computational techniques used, thus dashed lines represent the unknown relationships between imaging, genetics and electromyography which will be tested in the experiments carried out in this thesis.

This particular framework has been already tested in clinical settings, with successful results (Piron et al., 2005, Piron et al., 2007, Turolla et al., 2007, Piron et al., 2009a, Piron et al., 2009b, Piron et al., 2010, Kiper et al., 2011, Turolla et al., 2013) in the rehabilitation of upper limb motor function, after stroke. Nevertheless, all sets of evidence so far provided are at the level of clinical outcome measures, without any insight on the potential neurophysiological mechanisms implicated.

To fill this gap and try to unhook the black box from the motor system side, in this PhD project stroke patients who were treated with a VR-based rehabilitation programme have been studied using an embedded longitudinal assessment with genetics, imaging, neurophysiological and clinical outcome measures.

The aim of this project is to examine whether a specific therapy targeted to upper limb recovery induces a better reorganization of the central nervous system, compared with standard care and if any consolidation occurs over time, post stroke.

It is hypothesized that muscle synergies, to date interpreted like a mathematical reduction of the recorded sEMG activity, might have a neuroanatomical correlate in the brain, thus representing a biological substrate for motor control, potentially disrupted by stroke. Whether evidence will demonstrate that specific brain structures could act as repository for muscle synergies, it can be postulated, that a rehabilitation technique based on motor learning rules (i.e. VR-based rehabilitation) induces a better reorganization of functions in the brain, than unspecific techniques. In this case, a therapeutic modality improving motor control would result in a more focused activation and structural change of target brain areas, than any standard rehabilitation therapy.

The experimental hypotheses we intend to test through a series of planned studies are reported in the following list. For each question, a different set of studies have been carried out.

1. Can genetic factors be determinants of upper limb motor recovery after stroke?

Recent evidence argued that a specific polymorphism of the BDNF (i.e. Val66Met) might be implicated in promoting different magnitude of motor recovery due to different expression of free BDNF throughout body tissues. BDNF expression can be modulated by intensity of physical activity. Considering VR treatment for the upper limb as a controlled clinical setting where homogeneous treatment intensity is provided, the potential effects of being a carrier or a non-carrier of the Val66Met polymorphism were studied at a clinical and neuroanatomical level. The results of this investigation are reported in study 1 and 2 in chapter 3.

2. Is there a neuroanatomical substrate for muscle synergies of the upper limb in humans and what is the relationship between brain lesion and muscle synergy activation after stroke?

It has been postulated that motor control in humans might rely on functional coupling of many muscles that are activated as a single functional unit shared

across several motor tasks (muscle synergy). Experiments in high and low vertebrates have demonstrated that muscle synergies have a neural representation in the CNS. Whether a similar anatomical representation exists also in humans was investigated in experiments 3 and 4 reported in chapter 4.

3. Is the effectiveness of a VR-based treatment greater than that of a standard therapy approach for upper limb rehabilitation in stroke patients in terms of clinical and imaging outcomes? What is the added value of a virtual reality based treatment for upper limb rehabilitation in stroke patients? Is it possible to have some insight on its effectiveness by looking at possible biological changes using functional neuroimaging?

A large amount of evidence has proved that a VR based approach is superior to conventional physical therapy for the recovery of upper limb after stroke, both at the level of gross motor function and independence. Nevertheless, the mechanisms implicated in the longitudinal recovery of motor function after stroke are not yet fully understood, especially with reference to specific therapeutic modalities such as VR-based treatments.

With the aim to study the neurophysiological mechanisms underpinning this difference, a controlled clinical trial was designed to compare several validated outcome measures, mainly in the ICF domain of impairment and activities together with changes in the neurophysiology of muscle synergies in relation to functional neuroimaging. The treatment modality received (i.e. VR or conventional physical therapy) was considered as the factor determining potential different outcomes. The clinical and functional neuroimaging results from this study are reported as experiment 5 in chapter 5.

In the following paragraphs methodologies, materials and results from the experiments carried out during this PhD project are reported.

3. ROLE OF THE BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) ON THE RECOVERY OF UPPER LIMB AFTER STROKE

3.1. Background

3.1.1. Introducing the neurobiology of motor skills acquisition and learning

The possibility of learning new motor skills can be defined, from a kinematic point of view, like the ability to optimise sequences of smoothed and accurate movements with the aim to accomplish specific tasks (Monfils et al., 2005). For this purpose a large variety of different but mostly unknown mechanisms are implicated at several levels (e.g. genetics, neuroanatomy and neurophysiology) (Hammond, 2002). It has been hypothesized that the development of sequences of skilled movements involves the strengthening of the spatiotemporal relationship between specific networks while weakening others. This process may occur through changing the connectivity between specific sets of corticospinal neurons through changes in synaptic efficacy (Hess and Donoghue, 1994). This spatial and temporal reorganisation of synaptic weights is known as a connectivity map, the activation of which has been postulated as the biological substrate for all functional behaviours in animal and humans. Some specific principles characterise the organisation of connectivity maps in the motor system: (1) the representation of individual movements is distributed and highly interspersed with adjacent cortical regions (fractured somatotopy); (2) adjacent cortical areas are densely interconnected through the corticospinal tract bundles (interconnectivity); (3) the higher the dexterity of a movement, the larger the proportion of the map represented in the related cortical areas (area equals dexterity); (4) internal and external environmental stimuli are the drivers for dynamic changes of maps' topography (plasticity); (5) the disruption of motor maps causes inability to produce skilled limb movements but does not abolish movement.

In recent years a new fundamental property became evident related to plasticity of connectivity maps in the motor system. Since the studies by Nudo et al. (2011,

2007a, 2006, 2007b, 2000, 2003, 1996, 1996a, 2001, 1996b), it has been widely accepted that, following anatomical lesions affecting structures of the motor system, both motor maps and skilled movement can be restored by the administration of massed practice motor training and rehabilitation (Kleim et al., 2003b). These findings strengthened the idea that motor maps reflect a level of synaptic connectivity within the motor cortex that is required for the performance of skilled movement. At the biological level this evidence was confirmed by experiments on rats where acetylcholinergic inputs to the motor cortex were removed before skill training, thus preventing learning dependent map reorganization in their motor cortex, with consequent impairment of motor learning (Conner et al., 2003). On these bases skill training might induce changes in synaptic efficacy exploiting plasticity within the motor cortex, with consequent changes in map topography (figure 3.1). At the behavioural level, the main parameter driving this retrograde effect of training on brain plasticity is intensity, defined in the case of motor skill training as the amount of repetition executed for specific tasks. In order to induce an effective brain reorganisation a certain threshold (i.e. number of repetitions) needs to be crossed. This effect is known as experience-dependent neuroplasticity and in animal models it was estimated that a range between 1000 to 10000 repetitions of the same task (trials) is needed to observe a permanent change at synaptic level (Kleim and Jones, 2008).

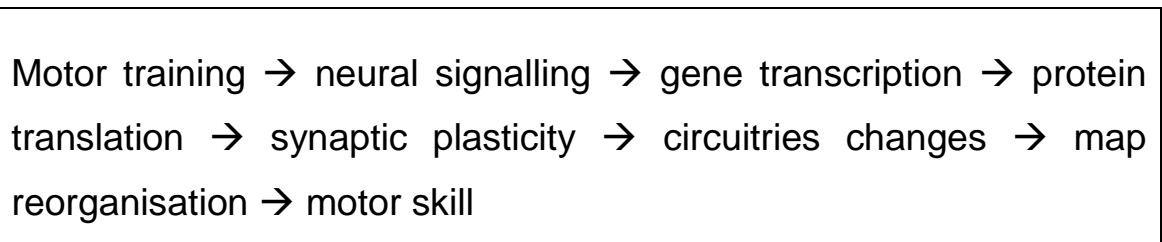


Figure 3.1. Cascade of neural events from behavioural training to motor skill acquisition.

In animal experiments, it has been observed that behavioural motor learning is mediated by promotion of long-term potentiation (LTP) plasticity and by inhibition of long-term depression (LTD). Moreover, none of these two occurs when only muscular activity (or motor activities) without learning is requested. At the level of the motor cortex the number of synapses and connections increases during the early stages of training (Kleim et al., 2003a, Kleim and Jones, 2008) and in experimental settings this phenomenon can be manipulated, promoting or

inhibiting skill learning, by injection of inhibitors of protein synthesis into the motor cortex (Luft et al., 2008). In the motor cortex of rats acetylcholine (Ach) and N-methyl-D-aspartate (NMDA) receptor antagonists block LTP, whereas gamma-aminobutyric acid (GABA) receptor antagonists facilitate LTP (Hess and Donoghue, 1994). Jacobs (1991) observed that injecting GABA antagonists induced an improvement of cortical representation of movements, whereas the injection of Ach antagonists abolished learning dependent map reorganisation in the motor cortex (Conner et al., 2003). Other effects observed in association with central inhibition of motor maps reorganisation were: decrease in number of synapses, weakened post synaptic response and impairments in motor skills. The possibility to induce active functional reorganisation in cortical circuitries following focal lesions of the central nervous system, by experience-dependent neuroplasticity, has opened new perspectives in rehabilitation medicine, with the final aim to restore functions fundamental for daily living.

3.1.2. Evidence on the role of “brain-derived neurotrophic factor” (BDNF) for the reorganisation of motor maps after stroke

Neuroplasticity of neuronal tissues is mediated by the action of many protein factors promoting or reducing survival, development and functioning of neurons. This family of proteins is known as neurotrophins and belongs to a class of growth factors which regulate signals capable of inducing cells' survival, differentiation or growth. Neurotrophins (e.g. nerve growth factor, NGF; brain-derived neurotrophic factor, BDNF; neurotrophin-3, NT-3; neurotrophin-4, NT-4) induce the remodelling of neuronal tissues underpinning the process of neuroplasticity for motor skill learning (Levi-Montalcini and Hamburger, 1951).

Candidate neural signals involved in long term potentiation of motor skill learning include BDNF, which is implicated in modulating dendritic morphology (Tolwani et al., 2002) and cortical map organisation (Rocamora et al., 1996a, Rocamora et al., 1996b) and its expression is modulated by aerobic exercises.

BDNF has been purified for the first time in the mammalian brain in 1982 (Barde et al., 1982) and represents the most expressed neurotrophin, both during development and in adult age. BDNF is found throughout all of the CNS such as in the hippocampus, cortex, basal forebrain, the amygdala and the cerebellum as well as in the retina, motor neurons, the kidneys, saliva and the prostate. This

evidence suggests that both areas implicated in high cognitive function (e.g. learning, memory) (Yamada and Nabeshima, 2003) and peripheral activation might be regulated by BDNF (Mandel et al., 2009).

In humans, the gene coding for the BDNF is mapped on chromosome 11 at the level of short limb 13 (11p13), it has four 5' exons (exons I-IV) and one 3' exon (exon V) encoding the mature BDNF protein (Binder and Scharfman, 2004). The BDNF mainly binds to tyrosine kinase receptors (e.g. tropomyosin-related kinase A and B, TrkA and TrkB). Ligand-induced receptor dimerization results in kinase activation, leading to a variety of intracellular signalling cascades. Trk receptors exist both in full length and truncated forms. Most of the known functions attributed to BDNF are associated with full length Trk receptors; nevertheless other important functions have been suggested for truncated forms, such as: growth and development (Luikart et al., 2003), negative modulation of trkB receptor expression and function (Haapasalo et al., 2002), upregulation of astrocytes with sequestration of BDNF following injuries (Saarelainen et al., 2000a, Saarelainen et al., 2000b), modulation of neuronal vulnerability and activation of glial calcium signalling (Rose et al., 2003).

Several stimuli have been described to alter BDNF gene expression in animals; as an example, light stimulation increases BDNF in the visual cortex (Castren et al., 1992), whisker stimulation has the same effect in the somatosensory cortex of rats (Rocamora et al., 1996b). Moreover, electrical stimulation can induce fixation of learning and memory by long term potentiation in the hippocampus, increasing BDNF expression (Castren et al., 1993). Recently, physical exercise has been described as the most important behavioural stimulation able to increase the expression of BDNF throughout body tissues (Neeper et al., 1995). In motor rehabilitation after injuries of the central nervous system (CNS), aerobic exercise has been proposed as the most important factor inducing upregulation of BDNF. In fact, the secretion of BDNF is modulated by activity dependent pathways, able to promote neuroplasticity because of practice, through bidirectional blood-brain barrier transport (Mang et al., 2013). Colcombe and co-workers (2003) have suggested that the appropriate dose of aerobic exercises to induce an effective activation of BDNF secretion should be a minimum practice of three times per week at a heart rate of at least 70% of the individual's maximum heart rate. The largest effects, for both healthy and stroke patients, have been observed for prolonged programmes, including a combination of resistance and

aerobic trainings practiced longer than 30 minutes, for 6 months (Kluding et al., 2011, Rand et al., 2010). The benefits observed in patients undergoing aerobic exercises include both cognitive (e.g. executive functions, planning, scheduling, working memory) and motor (e.g. mobility, balance, gross motor function) domains.

The BDNF is produced with different compositions of amino acid at specific positions (polymorphism) determining different individual genotypes. The most studied single nucleotide polymorphism (SNP) is the one where one or both valines (val) are changed with equivalent methionine (met) at position 66 (Val66Met) of the precursor peptide proBDNF. It has been estimated that this SNP results in a 25% reduction of activity-dependent secretion of BDNF in the CNS (Chen et al., 2004). The prevalence of the Val66Met BDNF SNP is different and varies according to the ethnic group, ranging from 25%-30% in Caucasians, to 70% in Asians (Shimizu et al., 2004). Evidence in animal models demonstrated that carrying the Val66Met BDNF SNP affects performance both in cognitive and motor tasks. Thus it has been postulated that the Val66Met SNP might have a potential detrimental role in the recovery process after injuries of the CNS, by affecting response to rehabilitation therapies.

In the following experiments a group of stroke patients undergoing the same rehabilitation treatment for the upper limb, were genotyped as carriers or non-carriers of the Val66Met BDNF SNP. The aim was to test whether genetics is a determinant for recovery of upper limb motor function and whether any anatomical mechanisms underpins recovery.

3.2. Experiment 1: Clinical effect of carrying the Val66Met single nucleotide polymorphism of the BDNF, for recovery of upper limb motor function after stroke

3.2.1. Introduction

The role of the Val66Met BDNF SNP in patients surviving a stroke has been extensively studied, because of its potential implication in determining different clinical outcomes when carried. Numerous studies have been carried out both in Asiatic (Kim et al., 2008, Kim et al., 2012b, Kim et al., 2012a) and western countries (Mang et al., 2013, Mirowska-Guzel et al., 2012, Mirowska-Guzel et al., 2014, Siironen et al., 2007, Vilkki et al., 2008, Westbroek et al., 2012, Zhou et al., 2011) where the expression of the Val66Met BDNF SNP is significantly different, with higher prevalence in the Asiatic countries.

Evidence has been provided that the Val66Met BDNF SNP is associated with poor recovery and autonomy in women experiencing ischemic stroke, but not in the case of stroke of haemorrhagic aetiology (Mirowska-Guzel et al., 2014). Kim and co-workers confirmed in a large East Asian cohort that carrying the Val66Met BDNF SNP is significantly associated with poor motor and cognitive recovery at 2 weeks and 1 year after stroke (2012b). Several other authors found this association between poor outcomes of recovery and carrying the Val66Met BDNF SNP also in other populations, such as: survivors of aneurysmal subarachnoid haemorrhage, brain arteriovenous malformation post-surgery (Siironen et al., 2007, Westbroek et al., 2012). In the domain of cognitive functions controversial results indicated that memory and learning performance are worse in healthy subjects carrying the Val66Met BDNF SNP, but the same difference is not observed after aneurysmal subarachnoid haemorrhage (Vilkki et al., 2008). Finally, post-stroke depression (PSD) has been observed to be significantly associated with carrying the met/met genotype, but not the val/met and val/val genotypes (Kim et al., 2008). Zhou and co-workers (2011) reported in a longitudinal study that carriers of the Val66Met BDNF SNP have a lower serum concentration of the BDNF than non-carriers, suggesting that the polymorphism might be involved in the pathogenesis of PSD.

The framework on the implications of carrying specific single nucleotide polymorphisms for recovery after brain lesions is still undefined and not thoroughly described. There is no current evidence on the potential role of carrying the Val66Met BDNF SNP for the recovery of upper limb motor function after stroke. The aim of this study was to test whether a difference exists in clinical outcome measures in patients undergoing a specific virtual reality-based treatment for the upper limb after stroke, according to their genotype.

3.2.2. Experimental hypotheses

The aim of this study was to test whether the Val66Met BDNF SNP is a determinant factor in the recovery of upper limb motor function after stroke. A controlled non randomised clinical trial was designed to test differences in the outcome measures between a group of carriers of the SNP of interest and a group of non-carriers. The following hypotheses were tested:

- H0: outcomes measures are not different after a rehabilitation treatment specifically focused on the upper limb, because of the Val66Met BDNF genotype.
- H1: non-carriers of the Val66Met SNP have better recovery than carriers of the Val66Met SNP, after a rehabilitation treatment specifically focused on the upper limb.

3.2.3. Methods

3.2.3.1 Participants

The cohort of post-stroke patients who took part in this study was selected from admissions to the Cerebrovascular Disease Unit of the San Camillo Hospital, Venice, Italy. Within this cohort of patients, those with hemiparesis as a result of a first stroke in the region of the middle cerebral artery (MCA) were screened. Occlusion of the MCA frequently accompanied by contralateral hemiparesis is the most common type of lesion, occurring in the majority (65%) of strokes resulting from cerebrovascular diseases (Sacco et al., 1984). This patient group accounts for approximately 2/5 of the overall admitted patients. CT/MRI scan demonstrated different combinations of brain lesions (i.e. large damage involving most of the

vascular territory of the MCA or more discrete lesions of the cortical and/or subcortical areas supplied by branches of the MCA). Moreover, the patients included in the study were those with a Motor Arm sub-score between 1 and 3 on the Italian version of the National Institutes of Health Stroke Scale (IT-NIHSS). The floor (i.e. 0) and ceiling (i.e. 4) scores of the Motor Arm sub-score clinically represent normal motor function and complete absence of voluntary movements; the score range 1 to 3 included, therefore, represents clinically the standardised definition of any maintenance of residual voluntary motor activation of the upper limb (Pezzella et al., 2009).

The following conditions were considered as exclusion criteria: the presence of moderate cognitive decline defined as a Mini Mental State Examination (Folstein et al., 1975) score < 20/30 points; the finding of severe verbal comprehension deficit defined as a number of errors > 13 (Tau Points < 58/78) on the Token Test (Huber et al., 1984); evidence of apraxia and visuospatial neglect interfering with upper arm movements and manipulation of simple objects in all the directions within the visual field, as assessed through neurological examination and report in the patient's clinical history and/or evidence from the neurological examination of the presence of behavioural disturbances (i.e. delusions, aggressiveness and severe apathy/depression) that could affect compliance with the rehabilitation programs. These criteria were decided for the feasible screening, within a defined population of stroke survivors, of those patients most likely capable of managing interaction with a challenging rehabilitation setting based on VR, independently of their outcomes at baseline.

All consecutive inpatients accepted to the unit (N = 118) were screened and 75 of those met the above criteria and were, therefore, referred for VR treatment of the upper limb (table 3.1).

Table 3.1. Demographic and clinical characteristics at baseline of patients sampled for genetic analysis.

Outcome	Carriers (n=31)	Non-carriers (n=44)	p
Age, years	58.29±16.76	63.88±9.88	0.454 ^a
Sex (M/F)	14/17	34/10	0.004 ^b *
Hemisphere (R/L)	16/15	22/22	1.000 ^b
F-M UE	41.29±18.38	40.98±18.38	0.825 ^a
FIM	95.25±17.87	92.54±21.40	0.294 ^a
RPS	24.79±11.61	24.80±10.74	0.946 ^a
NHPT	0.10±0.14	0.11±0.12	0.575 ^a

M: male; F: female; R: right; L: left; FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity; RPS: reaching performance scale; NHPT: nine hole pegboard test. Results are reported as mean and standard deviation, m (SD). Mann-Whitney U test (a) or Chi-Squared test (b) respectively, were used to test differences between carriers or non-carriers of the Val66Met polymorphism. Statistical threshold was set at $p < 0.05$ (*).

Detailed description of outcome measures considered are reported in the paragraph “Outcome measures” (paragraph 3.2.4.3) of the “Materials” section (paragraph 3.2.4). Their BDNF genotype was characterised from blood samples collected by the Laboratory of Microbiology and Molecular Biology at the IRCCS San Camillo Hospital Foundation (Venice, Italy) according to the protocol approved by the Institutional Review Board (IRB – Ethical Committee Ref.: Prot. 08/09 vers. 1) on 3rd November 2009. All the details of genotyping are reported in the “Analysis of the BDNF polymorphism” paragraph (3.2.4.2). From genotyping 41.3% of the patients were found to be carriers of the Val66Met BDNF SNP, which reflected a slightly higher prevalence for a Caucasian sample, than reported in the epidemiological literature. In the comparison between genotype subgroups all the variables with the exception of sex (more males in the non-carrier group) were comparable at baseline.

3.2.4. Materials

3.2.4.1 Virtual reality intervention for upper limb: Reinforced Feedback in a Virtual Environment (RFVE)

RFVE is currently provided to all patients admitted to the Neurorehabilitation Department of the San Camillo Foundation Hospital in Venice (Italy). The RFVE therapeutic modalities have already been published in previous papers (Piron et al., 2009b, Piron et al., 2010). During treatment, which is provided in a darkened room to minimize distracting stimuli (e.g. lights, sounds, environmental features), the patient is seated on a chair in front of a screen placed on the wall handling a sensorised object or wearing a sensorised glove, depending on whether they have preserved grasping ability. The sensorised object or glove is considered as the end-effector interacting with the virtual environment. The virtual environments are generated by the Virtual Reality Rehabilitation System (VRRS®. Khymeia Group Ltd. Noventa Padovana, Italy) including a:

- high definition LCD projector;
- wall screen
- desktop station (Intel Core 3.2 GHz, RAM 2GB, dedicated graphic card NVIDIA® GeForce® 8400 GS with 3D accelerator)
- 3D electromagnetic motion tracking system (Polhemus LIBERTY®, Colchester, VT, US)
- dedicated software.

The RFVE therapy involves performing different kinds of motor task with the patient holding a real manipulable object in their hands while interacting with a virtual scenario with movement monitored by means of the motion-tracking system. For instance, a simple reaching-aiming movement, such as putting a glass on a shelf, is represented in the virtual scenario and is represented by a virtual glass and shelf. The correct trajectory is displayed in the background of the virtual scene to facilitate the patient's perception and adjustment of his/her motion errors to target, by means of on line visual knowledge of their performance and results (Figure 3.2a). The therapist selects the characteristics and complexity of the motor tasks by changing the position or the orientation of virtual objects. The complexity of the motor tasks can be enhanced by complicating the required movements adding objects/barriers into the virtual scenario. As a consequence,

the patients are forced to activate different sets of upper arm muscles to meet the increasingly difficult task requirements (Figure 3.2b).

To edit the scenarios, the physical therapist holds in their hand the real object with a receiver positioned on it and performs the correct motor task. The virtual scenario displays the correct movement path and then the patient is required to emulate the correct movement performed earlier by the therapist.

The therapist was present at every session for the entire duration of the treatment, as in a standard one-to-one setting. The therapist role was to manage the virtual environment to adapt it to the current patient's physical condition and to guide the patient with verbal instructions in case of difficulties during the execution of the interactive exercise.

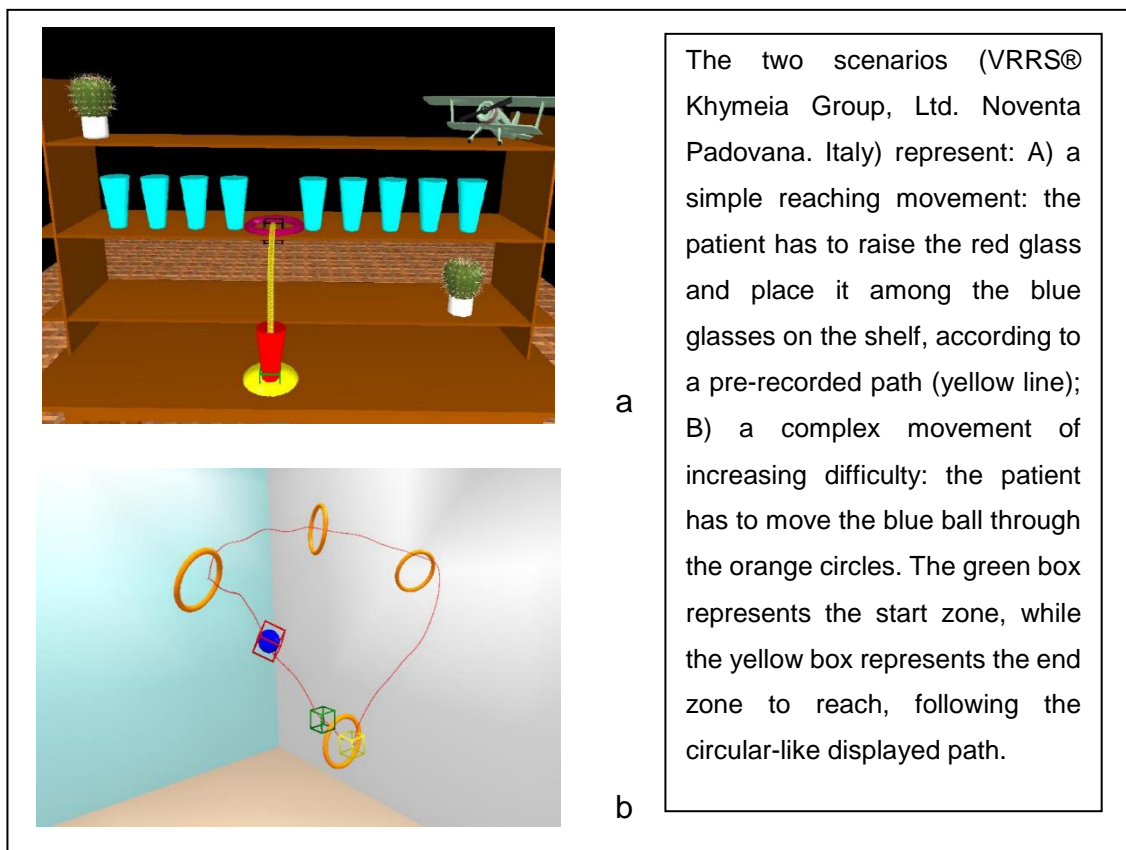


Figure 3.2. Motor exercises in the virtual environment.

3.2.4.2. Analysis of the BDNF polymorphism.

Genetic analysis was carried out in the Laboratory of Microbiology and Molecular Biology at the IRCCS San Camillo Foundation Hospital in Venice (Italy) using DNA extracted from blood samples. Specific written informed consent was obtained from every participant at time of hospital admittance (MOD. 182/FH/V06/13/11) as approved by the IRB (Prot. 08/09 vers. 1).

Genotypisation was performed using the high resolution melting (HMR) method (Reed et al., 2007). The DNA region of interest was amplified by a specific primer and the resulting melting curves were compared to those of reference samples previously sequenced, to obtain the family of polymorphism each patient was associated with (i.e. Val/Val, Val/Met, Met/Met). A blind control on random samples was also carried out by replicating the sequencing and genotypisation from the same samples in a laboratory external to the hospital.

3.2.4.3. Outcome measures

The Fugl-Meyer upper extremity (F-M UE) scale was considered as the primary outcome measure, since it is the most frequently used measure in stroke rehabilitation research. The F-M UE is an ordinal scale with an overall score ranging from 0 to a maximum of 66 for upper limb motor function. The upper limb section has 33 items, including reflexes tests, movement observation, testing of grasp abilities and assessment of motor co-ordination. The score for each item is: 0 Unable to perform; 1 Able to perform in part; 2 Able to perform (Deakin et al., 2003). Its clinimetric properties are known and the inter-rater reliability ($r=0.98$ to 0.99), the intra class correlation ($ICC=0.99$) (Croarkin et al., 2004) and the minimal clinically important difference ($MCID = 9$ pts.), have been estimated (Arya et al., 2011b).

The secondary outcome measures were:

1. The functional independence measure (FIM®) scale (Keith et al., 1987) was used to measure independence in activities of daily living (ADLs). The FIM® scale is a trademark of the Uniform Data System for Medical Rehabilitation, a division of UB Foundation Activities Inc. (Buffalo, NY, US) and is considered the worldwide reference rehabilitative outcome to measure independence in ADLs. It is composed by 18 items, (i.e. 13 measuring motor independence, 5 measuring cognitive tasks). Every item can be scored between 1 and 7 points on an ordinal scale, ranging from complete dependence to complete independence, respectively. Thus, the total score ranges from 18 to 126 points. The MCID has been calculated in stroke population (Beninato et al., 2006), and a change of 22 points is considered as clinically meaningful (i.e. 17 points for the motor subscale, 3 points for the cognitive subscale) and its test-retest reliability is

considered excellent in the elderly adult population for both the motor (ICC = 0.90) and the cognitive (ICC = 0.80) domains (Hobart et al., 2001).

2. The reaching performance scale (RPS) is an outcome measure that measures the ability to reach for a target with the upper limb while in a seated position (42 cm height). It has been developed by Levin and co-workers (2004) and the patient is requested to reach for and grasp a cone placed on a table (72 cm height) at close and far distances (i.e. 1 cm and 30 cm from the front edge of the table, respectively). The assessor has to score different components of the reaching gesture for 12 items (i.e. 6 items for close target, 6 items for far target) each one with a score ranging from 0 to 3 points. Some clinimetrics properties of the RPS have been studied revealing poor to excellent overall reliability of the scale ($0.14 < K \text{ score} < 0.85$) and good to excellent inter-rater reliability ($0.58 < ICC < 0.95$) (Levin et al., 2004).
3. A modified version of the nine hole pegboard test (mNHPT) was administered to test fine skills of hand motor function. In the NHPT the patient is requested to place 9 pegs in 9 holes squared in a 3x3 matrix, without any predisposed order (Wade, 1989). The time needed to place and remove the pegs is registered with a maximum time of 50 seconds allowed to complete the task. Because a high functional level of the hand is needed to complete the task, only the time to place and not to remove pegs was registered. Thus, the task was simplified to avoid potential floor effect because of the expected high percentage of patients not able to complete the placement, thus not able even to remove at least one peg from the holes. To compute a valid score to be registered for all the patients, regardless of their ability to perform the mNHPT, a peg/time index was calculated. This index is a continuous variable indicating the amount of pegs placed every second and ranges from 0 (no pegs placed at all) to infinite (0 seconds needed to place 9 pegs). The higher the index, the better the performance.

3.2.4.4. Statistical analysis for this controlled clinical trial

The demographic and clinical characteristics of enrolled patients are reported as mean and standard deviation. The patients were divided in two sub-groups according to their genotype, respectively: carrier (i.e. Val/Met, Met/Met) and non-

carrier (i.e. Val/Val). A two way analysis of variance (ANOVA) was run to test for differences between groups of carriers and non-carriers of the Val66Met BDNF SNP on: F-M UE, FIM, RPS, and NHPT. The change between time of assessment of these outcome measures was considered as the within factor. Once explored the potential interactions within and between factors influencing the final outcomes, direct comparisons of average scores were carried out using parametric (i.e. paired t-test, t-test for independent samples) or non-parametric (i.e. Wilcoxon test, Mann-Whitney U test) statistics depending on data distribution.

3.2.5. Results

All patients completed treatment without reporting any side effects. The ANOVA revealed that time, i.e. before/after treatment, had a significant impact on inducing improvements on all of the outcome measures (F-M UE: $F = 54.36$, $p = 0.000$; FIM®: $F = 38.69$, $p = 0.000$; RPS: $F = 10.04$, $p = 0.002$; mNHPT: $F = 17.02$, $p = 0.000$). Genotype polymorphism, i.e. being a carrier or non-carrier of the Val66Met BDNF SNP, had no significant impact on all of the outcome measures (F-M UE: $F = 0.01$, $p = 0.922$; FIM®: $F = 0.62$, $p = 0.433$; RPS: $F = 0.17$, $p = 0.678$; mNHPT: $F = 0.04$, $p = 0.850$). The interaction between time and polymorphism, however, was not significant (F-M UE: $F = 0.56$, $p = 0.456$; FIM®: $F = 0.00$, $p = 0.987$; RPS: $F = 0.10$, $p = 0.754$; mNHPT: $F = 0.27$, $p = 0.609$). The same results were confirmed by post-hoc direct comparisons within the groups of carriers and between them (table 3.2). All the outcomes improved significantly after treatment, but none of them was significantly different between genotype sub-groups (FIM®: $p = 0.706$; F-M UE: $p = 0.630$; RPS: $p = 0.940$; mNHPT: $p = 0.829$).

Table 3.2. Effect of the RFVE therapy in Val66Met SNP carriers and non-carriers.

Outcome	Carrier (N = 31)		Non-carrier (N = 44)	
	Before	After	Before	After
FIM®	95.25±17.87	102.65±16.30*	92.54±21.40	98.93±19.85*
F-M UE	41.29±18.38	46.65±19.44*	40.98±18.38	46.73±18.39*
RPS	24.79±11.61	28.15±9.88*	24.80±10.74	27.29±11.34*
mNHPT	0.10±0.14	0.16±0.20*	0.11±0.12	0.15±0.15*

Data are reported as means and standard deviations. FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity; RPS: Reaching Performance Scale; mNHPT: modified Nine Hole Pegboard Test. (*) Wilcoxon test, (#) Mann-Whitney U test, statistical threshold was set at $p < 0.05$.

3.2.6. Discussion

According to the current evidence in the literature, this is the largest study exploring the effect of a specific single nucleotide polymorphism (i.e. Val66Met) on the recovery of upper limb motor function, after stroke. The results contribute to confirm that carrying the Val66Met BDNF SNP does not influence recovery of upper limb motor function after stroke at the clinical level. The outcomes considered for measuring upper limb change in function covered the whole domain of motor function (i.e. reaching ability, gross motor function, hand dexterity), thus it can be argued that carrying or not carrying this polymorphism does not affect recovery for this specific district, after brain lesion. These findings are similar to the ones achieved by Vilkki and collaborators (Vilkki et al., 2008) for verbal memory performance, thus it can be argued that specific performance abilities are not affected by specific genetic profiles of the BDNF. Furthermore, this negative finding might be justified with the large variability in clinical picture resulting from the variety of brain lesions observed in patients, which could mask the action of a single nucleotide polymorphism.

No between group difference was found on the variable measuring level of independence (FIM). This finding is apparently not in line with evidence from Kim and collaborators (2013) who in a study of stroke patients reported a worse level of disability (measured by the modified Rankin Scale) in carriers of the Val66Met BDNF SNP than in non-carriers. Nevertheless, the reported difference was detected at 3 months after discharge (chronic phase), but not at 1 month soon after hospital discharge. This result is in favour of the hypothesis that the effect of the polymorphism might emerge and consolidate at the later point after the rehabilitation intervention has been discontinued. From an overall perspective all

the evidence seems to converge toward a protective role of rehabilitation, which, by minimising the effect of individual genetic profiles, mitigates the acknowledged detrimental effect of physical and cognitive inactivity, after diseases of the central nervous system. Given these premises, in the following experiment the potential neuroanatomical mechanisms implicated in the recovery of upper limb motor function, due to specific rehabilitation modalities will be studied combining clinical and imaging outcomes.

3.3. Experiment 2. Effect of carrying the Val66Met polymorphism on structural anatomy of the brain in stroke patients

3.3.1. Introduction

Qin and co-workers (2014) have recently studied the effect at the behavioural and anatomical levels of carrying the Val66Met BDNF SNP in rat models. Their findings suggested a new hypothesis on the role played by the Val66Met BDNF SNP in the recovery of motor function after stroke. Two samples of rats carrying or not carrying the SNP were exposed to sham and real occlusion of the middle cerebral artery, inducing a stroke with main involvement of the striatum. At the behavioural level, the outcomes analysed were: kinematics of gait and walk, while volumes of brain structures, perimeter and number of neuronal connections were chosen as anatomical outcomes. All the outcome measures were assessed after stroke in the very acute phase (i.e. 3 and 5 days), subacute phase (i.e. 1 and 2 weeks) and monitored every month up to the chronic phase (i.e. 6 months). The results indicated that the striatum contralateral to the stroke side was bigger in the Val66Met BDNF SNP carriers than in the non-carriers, due to a bigger cell body perimeter and area, but not to larger dendritic arborisation. This histological difference conveyed a better motor recovery of gait performance, although no significant between group differences in its kinematics were observed. Moreover, using pharmacological agents, it was demonstrated that the recovery of gait relied on the hypertrophied striatum. In fact, the administration of muscimol, a GABA receptor agonist, severely affected gait performance in both groups, but had worse effect on the Val66Met BDNF SNP carriers than the non-carriers. The non-carrier sub-group was also quicker than carriers in regaining baseline

performance after muscimol interruption. This evidence demonstrated that better behavioural performance in carriers was heavily sustained by the contralateral striatum. Overall, the interpretation of Qin's results revealed a potential positive effect of carrying the Val66Met BDNF SNP in the recovery process of motor function after stroke, but rigidly dependent on the integrity of contralateral brain structures (particularly the striatum), underlying a potential maladaptive role in the long term. On the basis of this evidence from an animal model, it has been hypothesised that also in humans carrying or not carrying the Val66Met BDNF SNP might have an impact on structural reorganisation of the brain after stroke, potentially related to different patterns of motor function recovery. In our experiment an innovative virtual reality based treatment, specially focused on the upper limb, was considered as a reference setting to test whether expected improvement of motor function might be affected by patients' genotype.

3.3.2. Experimental hypotheses

The aim of this study was to test both, at the behavioural and anatomical levels, whether a similar pattern exists even in humans which have suffered a stroke. The following hypotheses were tested, using Voxel Based Morphometry regression analyses:

- H0: brain structures' volume is the same both in carriers and non-carriers of the Val66Met SNP.
- H1: brain structures' volume is bigger in carriers than non-carriers of the Val66Met SNP.

3.3.3. Methods

3.3.3.1. Participants

VBM correlation analysis was carried out on a sample of 19 consecutive patients (Carrier = 10, Non-carrier = 9), among the 75 enrolled in study 1, whose descriptive characteristics are reported in table 3.3.

Table 3.3. Demographic and clinical characteristics of the patients sampled for VBM analysis.

Outcome	Overall (n=19)	R hemisphere lesion (n=10)	L hemisphere lesion (n=9)	p
Age, years	65.13±11.62	61.88±11.40	68.38±11.62	0.780 ^a
Sex, M/F	15/4	8/2	7/2	0.906 ^b
Time since stroke onset (months)	95.38±81.26	4.51±3.10	1.85±1.43	0.095 ^a
Val66Met (carrier/non-carrier)	10/9	6/4	4/5	0.498 ^b
FIM	92.56±19.36	91.63±24.07	93.50±14.90	0.604 ^a
F-M UE	39.81±15.32	37.38±16.96	42.25±14.19	0.720 ^a
RPS	24.44±9.72	24.63±12.06	26.25±7.46	0.888 ^a
NHPT	0.09±0.11	0.07±0.09	0.11±0.13	0.721 ^a

M: male; F: female; R: right; L: left; FIM: Functional Independence Measure; RPS: reaching performance scale; NHPT: nine hole pegboard test; F-M UE: Fugl-Meyer Upper Extremity. Results are reported as means and standard deviations, m (SD). Mann-Whitney U test (a) or Chi-Squared test (b) were used to test differences between patients with lesions in the right or left hemisphere. Statistical threshold was set at $p < 0.05$ (*).

The two groups were comparable at baseline for all the variables both demographically and clinically.

3.3.4. Materials

3.3.4.1. Magnetic resonance image (MRI) acquisition

Each patient underwent a standard structural MRI acquisition protocol which included a fluid-attenuated inversion recovery (FLAIR) scan and a three dimensional T1-weighted structural MRI scan. These scans were acquired on a 1.5T Philips Achieva MRI system. Coronal FLAIR acquisitions were obtained for a detailed assessment and characterization of cerebral infarctions (TR/TE/inversion time [T1]/NEX = 11000/140/2600/2; matrix size = 256 x 256; FOV = 240 mm; slice thickness = 7.0 mm with no gap between slices, in-plane spatial resolution of 0.898 x 0.898 mm/pixel). The three dimensional T1-weighted scan was acquired with a Turbo Field Echo sequence. The Voxel dimensions were 1.1 X 1.1 X 0.6 mm and field of view was 250 mm with a matrix size of 256 X 256 X 124. For this latter MRI sequence, total acquisition time was 4 min 27 s (repetition time, RT: 7.4 ms, echo delay time, TE: 3.4 ms and flip angle 8 degrees).

3.3.4.2. Statistical analysis for clinical outcome measures

The aim was to test also in the subsample of patients studied by VBM regression whether differences existed in the clinical outcomes after treatment. To this end the same statistical analyses described in study 1 were used.

3.3.4.3. Voxel Based Morphometry (VBM) analysis.

A number of pre-processing steps were carried out to isolate grey and white matter from the 3D T1-weighted structural scans before performing the statistical analysis using SPM8 (Wellcome Trust Centre for Neuroimaging). In detail, to correct for global differences in brain shape, structural images were warped to standard stereotactic space and segmented to extract grey matter, white matter and cerebrospinal fluid. The grey and white matter segments were then modulated to correct for changes in volume induced by nonlinear normalization and smoothed using a Gaussian filter set at 8 mm to reduce possible error from between-subjects variability in local anatomy and render the data more normally distributed. After every step, for every subject, the resulting image was visually checked to assure that the output was still intact and suitable for further processing. The overall group was divided according to the hemisphere involved by the stroke lesion (i.e. right, left). This was necessary because in VBM analysis, when images are not flipped by applying rotation matrixes, the voxels significantly associated in one hemisphere would be subtracted by the voxels in the contralateral hemisphere, when running the statistical tests. Thus, to minimise biases coming from the application of algebraic transformations (with potential distortions in the resulting anatomy), the two groups of patients with right and left damaged hemispheres were analysed separately.

Finally, smoothed grey and white matter segments were modelled using the presence of the Val66Met polymorphism, age, gender and F-M UE score at baseline as factors. Differences between carriers and non-carriers, for both right and left hemisphere damaged sub-groups were tested using t-test with age, gender and F-M UE at baseline as covariates. Height of statistical threshold level was set at $p < 0.05$, Family Wise Error (FWE) corrected. When no significant clusters emerged from FWE correction, the statistical threshold was set at $p < 0.001$ uncorrected and an extent threshold of 10 voxels was applied as spatial filter, to maintain only the biggest clusters as final outcome.

The results from the VBM analyses were standardized with the stereotactic grid of Talairach and Tournoux (Talairach and Tournoux, 1988), with a cube range of 1mm for labelling brain areas.

3.3.5. Results

All the patients completed treatment without reporting any side effects. From a clinical perspective, the results in the sub-sample of patients studied with MRI in this experiment confirmed the findings observed in experiment 1. All clinical outcome measures were comparable at baseline and ANOVA revealed that time was a significant factor inducing improvements of all outcome measures (F-M UE: $F = 10.78$, $p = 0.005$; FIM: $F = 12.43$, $p = 0.003$; RPS: $F = 10.35$, $p = 0.006$; NHPT: $F = 16.31$, $p = 0.001$). In addition, genotype, i.e. being a carrier or non-carrier of the Val66Met BDNF SNP, had no significant effect and no significant between group differences were found for any of the outcome measures (F-M UE: $F = 1.15$, $p = 0.302$; FIM: $F = 0.24$, $p = 0.631$; RPS: $F = 0.92$, $p = 0.355$; NHPT: $F = 3.46$, $p = 0.084$). The interaction between time and polymorphism, however, was significant but only for the RPS ($F = 5.03$, $p = 0.042$) and NHPT ($F = 10.72$, $p = 0.006$) outcome measures.

VR treatment for the upper limb induced a significant improvement of F-M UE ($Z = -2.38$, $p = 0.017$), FIM ($Z = -2.20$, $p = 0.028$) and NHPT ($Z = -2.26$, $p = 0.024$) within the group of carriers, while F-M UE ($Z = -2.52$, $p = 0.012$) and NHPT ($Z = -2.02$, $p = 0.043$), but not FIM, improved significantly within the group of non-carriers. In the post-hoc comparisons between groups only the NHPT score ($Z = -2.33$, $p = 0.022$) was significantly higher in the non-carriers than in carriers of the Val66Met BDNF SNP (table 3.4).

Table 3.4. Effect of the RFVE therapy in Val66Met SNP carriers and non-carriers, sampled for VBM analysis.

Outcome	Carrier (N = 10)		Non-carrier (N = 9)	
	Before	After	Before	After
FIM	90.40±18.57	97.50±19.40*	96.17±21.89	101.67±20.90
F-M UE	37.00±15.95	42.00±19.60*	44.50±14.27	52.17±12.43*
RPS	24.60±11.29	25.70±11.59	26.83±7.11	33.00±5.14
NHPT	0.07±0.10	0.08±0.11*	0.12±0.13	0.23±0.10*#

Data are reported as means and standard deviations. FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity; RPS: Reaching Performance Scale; NHPT: Nine Hole Pegboard Test. (*) Wilcoxon test, (#) Mann-Whitney U test, statistical threshold was set at $p < 0.05$.

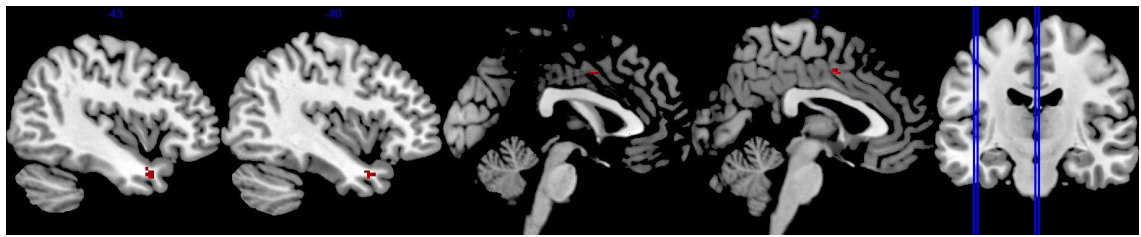
At the neuroanatomical level, VBM analysis found no significant clusters in which non-carriers of the polymorphism had bigger volumetric values than the Val66Met carriers, thus indicating that all the brain structures in non-carriers were smaller than in the Val66Met carriers. Conversely, there were many significant clusters both in grey and white matter in which Val66Met carriers had bigger volumetric values than non-carriers. This pattern of result was found in both the right and the left hemisphere damaged subgroups.

In the subgroups with lesions caused by stroke in the right hemisphere (tables 3.5 and 3.6) the cingulate and superior temporal gyri (white matter), BA38 and the thalamus (grey matter) were found to be significantly bigger in Val66Met carriers than in non-carriers (figures 3.3, 3.4, 3.5).

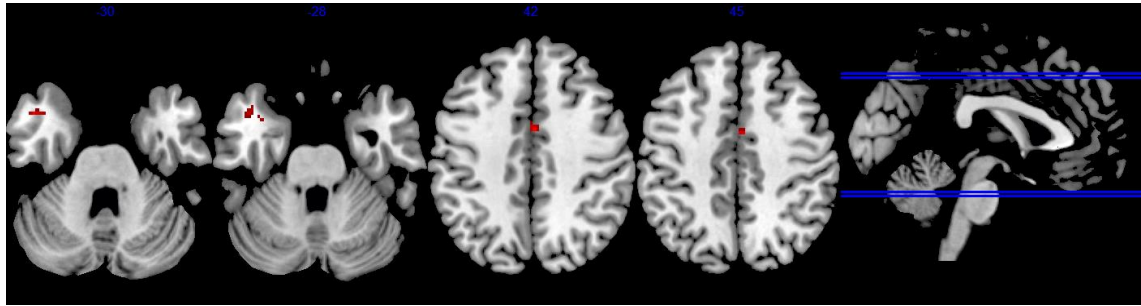
Table 3.5. Brain areas associated with carrying the Val66Met polymorphism in right hemisphere stroke patients (grey matter).

Brain Area	L/R	Brain Area	no of voxels	Talairach coordinates		
				X	Y	Z
Contralateral to lesion						
WM	L	Cingulate Gyrus	18	0.33	-5.43	41.60
WM	L	Superior Temporal Gyrus	27	-41.36	10.37	-22.47
GM	L	BA38	21	-32.14	6.42	-20.88

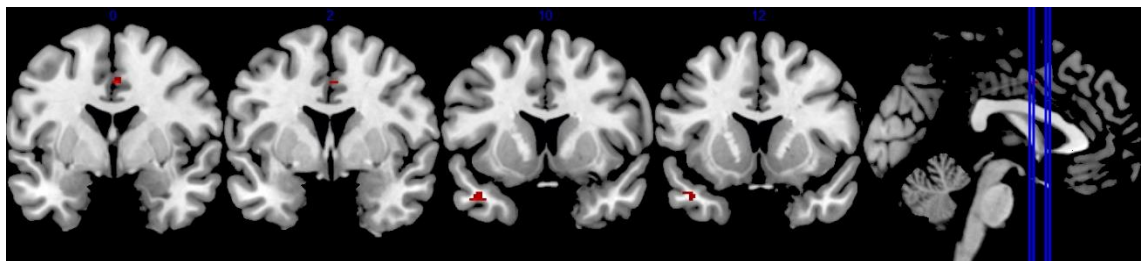
GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. Hits are the number of voxels for the indicated label included in the cube range of 1mm.



(a)



(b)



(c)

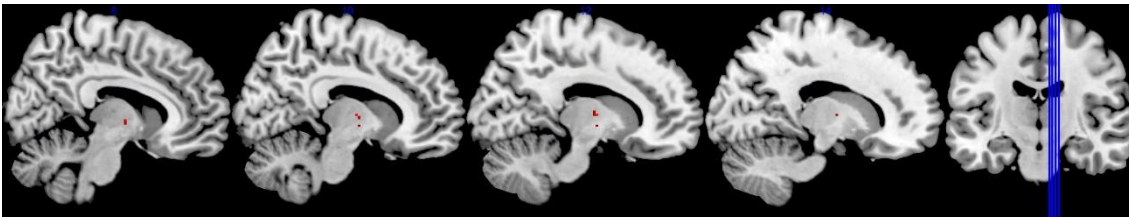
Figure 3.3. Graphical display of bigger brain volumes in right hemisphere stroke carriers of the Val66Met polymorphism (grey matter).

The cluster of significant voxels (red) is shown superimposed on a standard anatomical template. (a) sagittal plane; (b) transversal plane; (c) coronal plane.

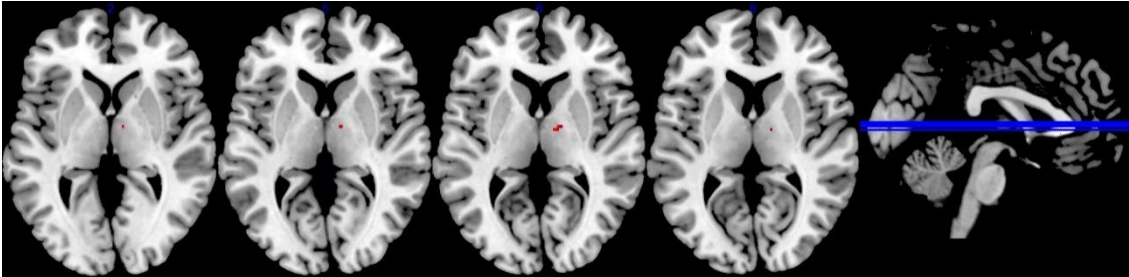
Table 3.6. Brain areas associated with carrying the Val66Met polymorphism in right hemisphere stroke patients (white matter).

Brain Area	L/R	Brain Area	no of voxels	Talairach coordinates		
				X	Y	Z
Ipsilateral to lesion						
GM	R	Thalamus (Ventral Anterior Nucleus)	12	10.00	-7.94	9.10
GM	R	Thalamus	12	8.24	-7.23	1.93

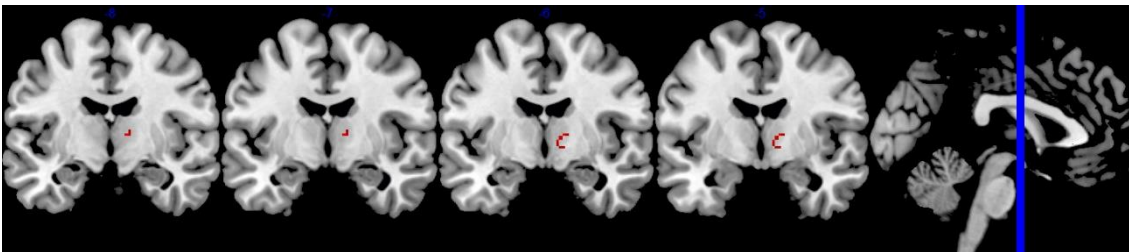
GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. Hits are the number of voxels for the indicated label included in the cube range of 1mm.



(a)



(b)



(c)

Figure 3.4. Graphical display of bigger brain volumes in right hemisphere stroke carriers of the Val66Met polymorphism (white matter).

The cluster of significant voxels (red) is shown superimposed on a standard anatomical template.

(a) sagittal plane; (b) transversal plane; (c) coronal plane.

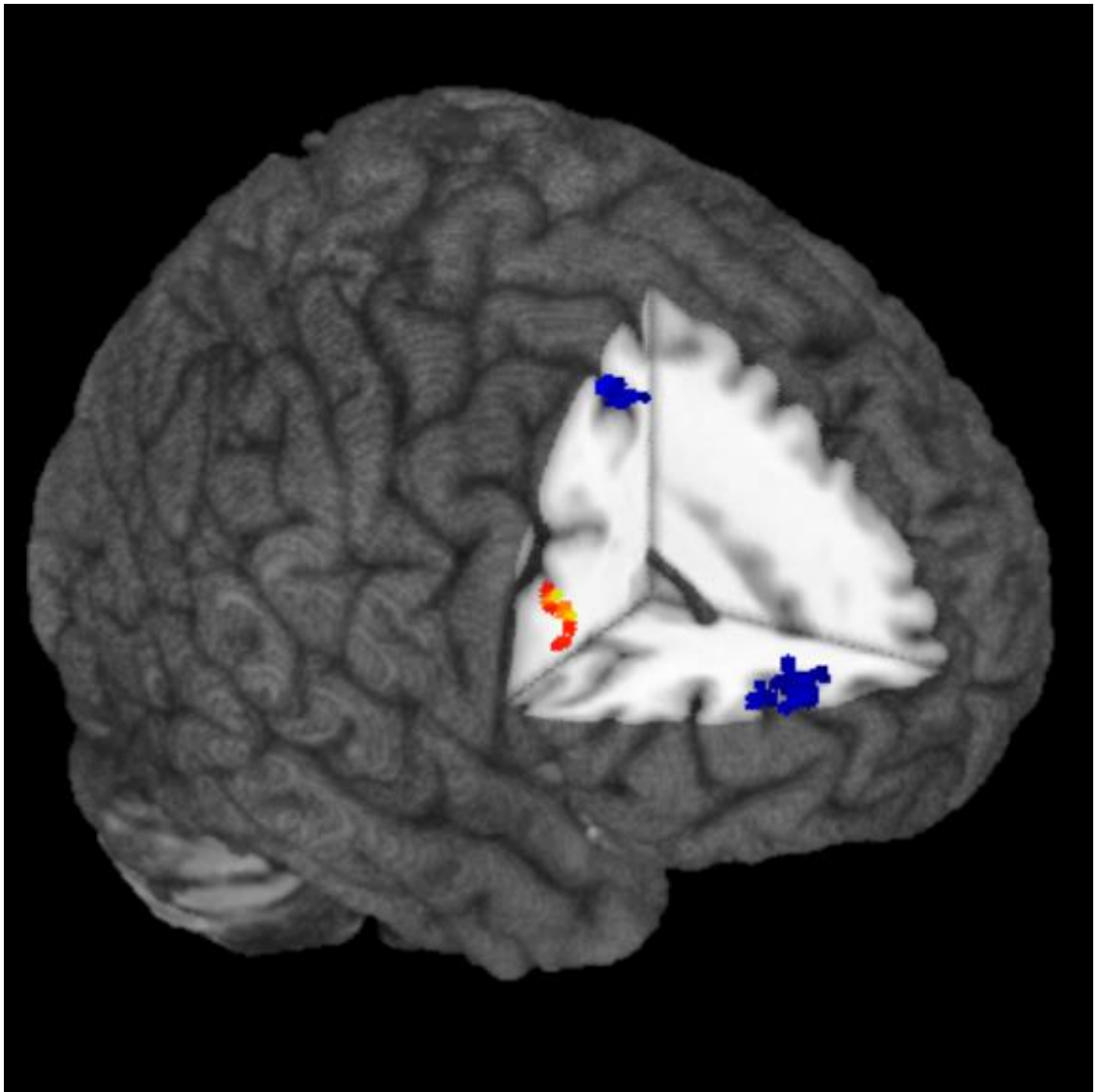


Figure 3.5. 3D rendering of bigger brain volumes in right hemisphere stroke carriers of the Val66Met polymorphism.

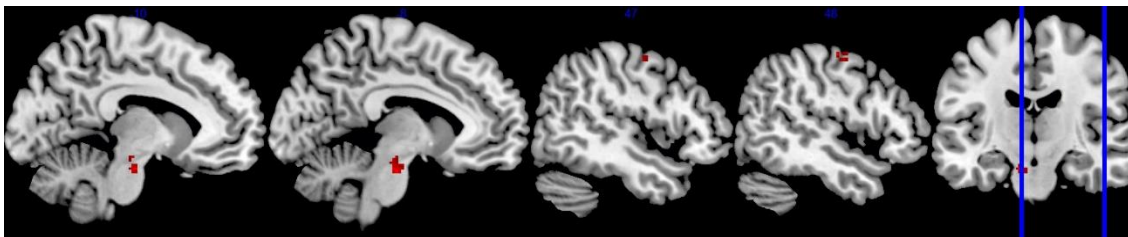
The clusters of significant voxels ($p = 0.001$ uncorrected; threshold = 10 voxels) for both white matter (red) and grey matter (blue) are shown superimposed on a standard 3D rendering of the brain.

In the left hemisphere lesioned group (tables 3.7 and 3.8), the precentral, supramarginal and sub gyri (white matter), BA6, BA3 and the substantia nigra in the midbrain were significantly bigger in Val66Met carriers than in non-carriers. (figures 3.6, 3.7, 3.8).

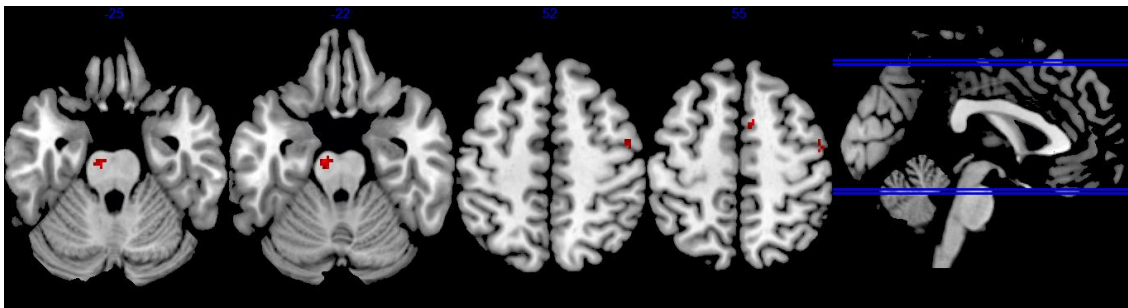
Table 3.7. Brain areas associated with carrying the Val66Met polymorphism in left hemisphere stroke patients (grey matter).

Brain Area	L/R	Brain Area	no of voxels	Talairach coordinates		
				X	Y	Z
Ipsilateral to lesion						
WM	L	Midbrain	27	-10.05	-14.71	-17.11
GM	L	Substantia Nigra	18	-10.15	-17.27	-10.14
Contralateral to lesion						
GM	R	BA6	24	5.74	0.77	54.90
WM	R	Precentral Gyrus	13	42.71	-16.19	53.91
GM	R	BA3	5	42.71	-16.19	53.91

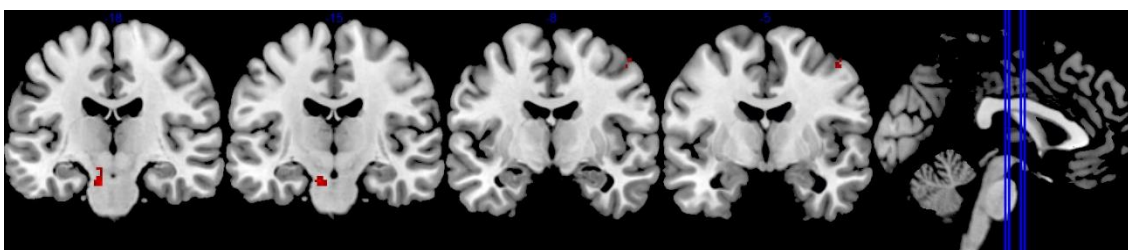
GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. Hits are the number of voxels for the indicated label included in the cube range of 1mm.



(a)



(b)



(c)

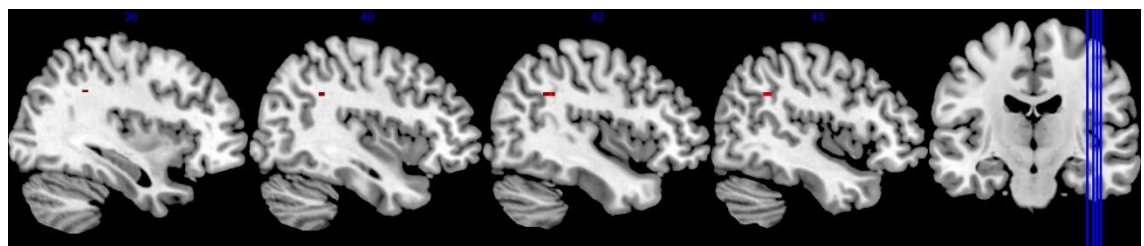
Figure 3.6. Graphical display of bigger brain volumes in left hemisphere stroke carriers of the Val66Met polymorphism (grey matter).

The cluster of significant voxels (red) is shown superimposed on a standard anatomical template. (a) sagittal plane; (b) transversal plane; (c) coronal plane.

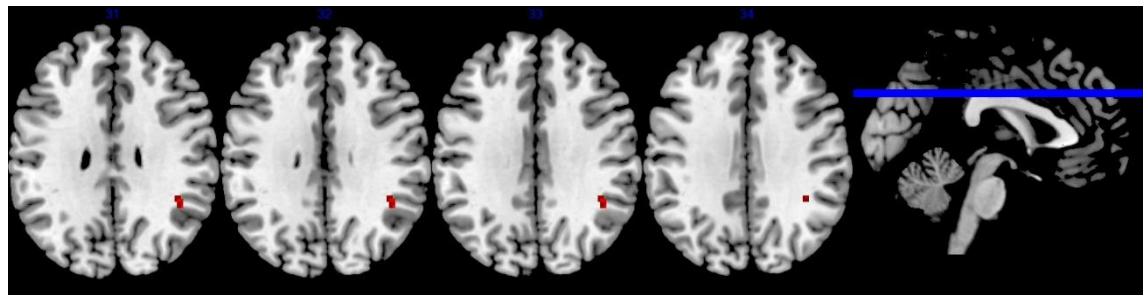
Table 3.8. Brain areas associated with carrying the Val66Met polymorphism in left hemisphere stroke patients (white matter).

Brain Area	L/R	Brain Area	no of voxels	Talairach coordinates		
				X	Y	Z
Contralateral to lesion						
WM	R	Supramarginal Gyrus	27	39.20	-51.35	28.90
WM	R	Sub-Gyral	27	33.64	-45.90	31.13

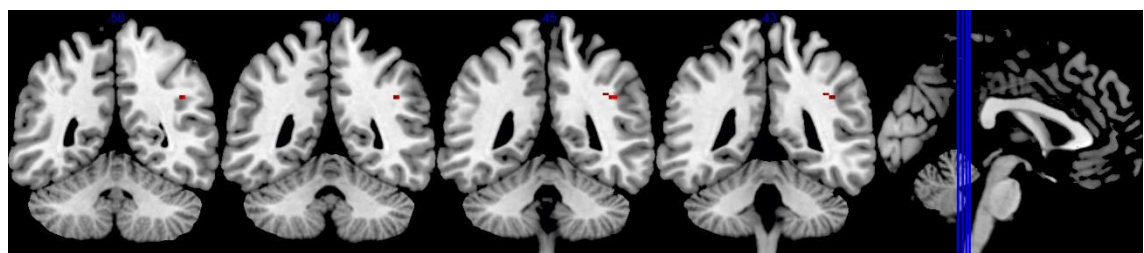
GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. Hits are the number of voxels for the indicated label included in the cube range of 1mm.



(a)



(b)



(c)

Figure 3.7. Graphical display of bigger brain volumes in left hemisphere stroke carriers of the Val66Met polymorphism (white matter).

The cluster of significant voxels (red) is shown superimposed on a standard anatomical template. (a) sagittal plane; (b) transversal plane; (c) coronal plane.

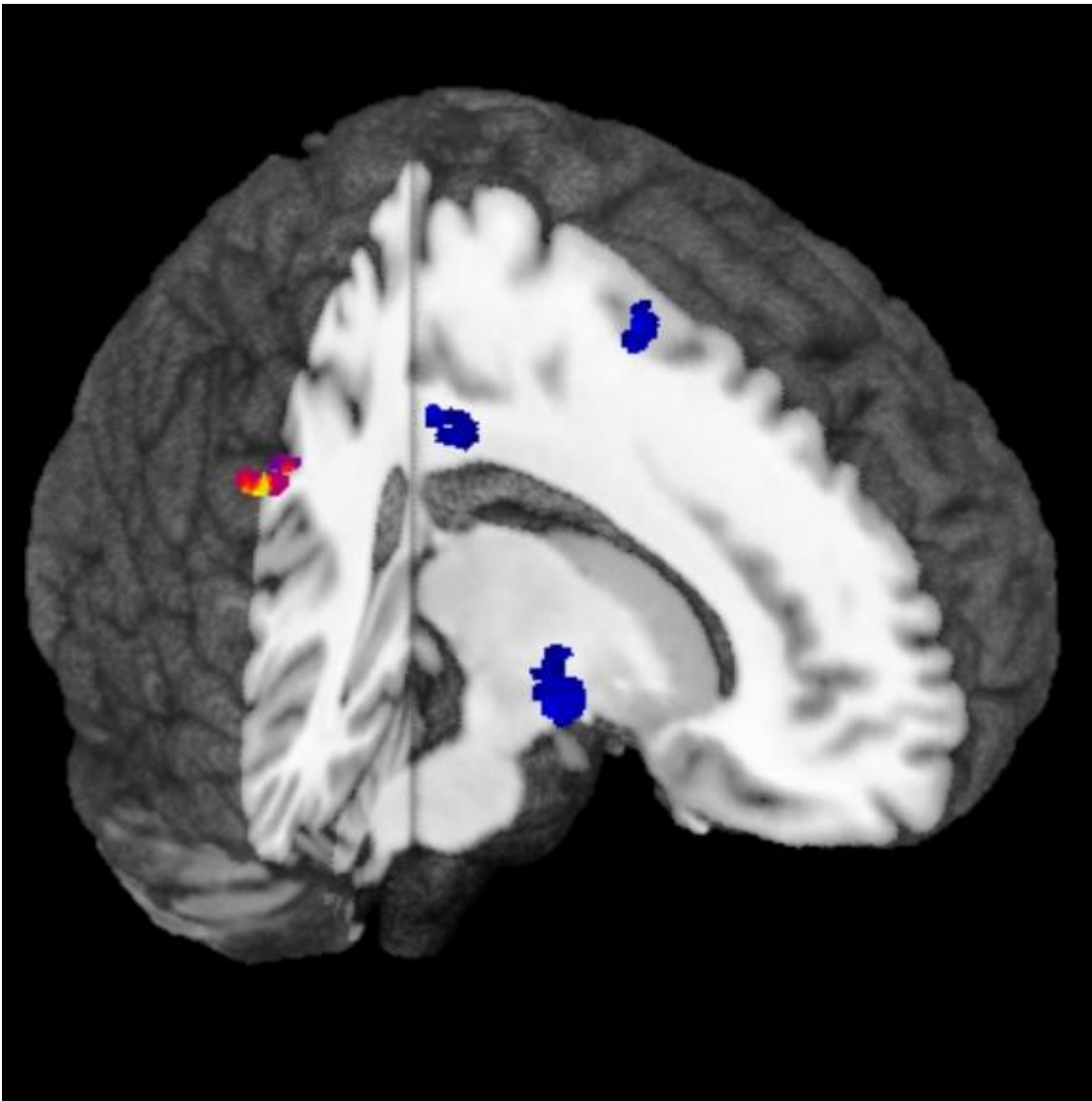


Figure 3.8. 3D rendering of bigger brain volumes in left hemisphere stroke carriers of the Val66Met polymorphism.

The cluster of significant voxels ($p = 0.001$ uncorrected; threshold = 10 voxels) for both white matter (red) and grey matter (blue) are shown superimposed on a standard 3D rendering of the brain.

3.3.6. Discussion

The results reported above come from a cross-sectional study including a sample of 19 patients, whose upper limb motor function was impaired at different levels, due to stroke occurred at variable distance in time. This sample was studied by imaging with the aim to compare brain structural volumes between two groups of patients carrying different genetic profiles of the BDNF. The specific polymorphism studied was the Val66MET BDNF SNP, which has been argued to be responsible for different patterns of recovery in animal models. The main

finding was that the differences observed in animals were observed also in our sample of human patients. All the brain structures in the non-carriers group were smaller than in the carriers' ones, as in rats. In details, the brain areas found to have a bigger volume in the carriers group were:

- Contralateral
 - Cingulate Gyrus
 - Precentral Gyrus
 - Superior Temporal Gyrus
 - Supramarginal Gyrus
 - Sub-Gyral
 - BA3
 - BA6
 - BA38
- Ipsilateral
 - Thalamus (Ventral Anterior Nucleus)
 - Midbrain
 - Substantia Nigra

In the hemisphere contralateral to the brain lesion, all the clusters bigger in carriers than non-carriers were in cholinergic motor areas mainly devoted to recognition of motor gestures (supramarginal and superior temporal gyri), integration and association of somatosensory stimuli (BA3) and voluntary motor activation (BA6, precentral gyrus). Conversely, in the hemisphere ipsilateral to the site of the stroke the dopaminergic system of the basal ganglia (i.e. thalamus, midbrain) was bigger in non-carriers, than carriers. This evidence is coherent with the one found by Qin and co-workers (2014) in animals, but flipped in terms of hemisphere laterality. In fact, the contralateral and not the ipsilateral thalamus was bigger, moreover no cortical or subcortical areas were found bigger in the comparison between the affected and the non-affected hemisphere in rats. A potential explanation for this controversial finding might be found in the district and type of training studied in this sample of patients. In fact, studies on animals were targeted to explore the recovery of gait after experimental induced stroke. Differently, the results reported in this experiment are referred to a sample of patients undergoing upper limb rehabilitation by a treatment specifically tailored to amplify augmented motor learning. The reinforcement learning paradigm is sustained by the reward pathways which are mediated by dopaminergic

circuitries (Doya, 2000a, Doya, 2000b); thus the intensive training based on augmented feedbacks, such as knowledge of performance (KP) and knowledge of results (KR), may have facilitated an augmented activation of the structures included in the cortico – basal ganglia – thalamic loop. This finding is anatomically coherent with the rationale of the treatment proposed, in fact the bigger volumes found in carriers of the polymorphism potentially represent the extrapyramidal tracts (i.e. vestibulospinal tract, reticulospinal tract) with ipsilateral innervation. In this regard, these results are fully coherent with canonical anatomy of the extrapyramidal motor system.

As for clinical outcome measures exclusively, the findings were slightly different, but coherent with the results of experiment 1. The RPS did not improved significantly after treatment in both groups and the FIM changed significantly after treatment only in the experimental group. These results might be interpreted as due to lack of statistical power for outcome measures whose minimal detectable change has not been established, like for FIM and RPS, thus the detection of a significant improvement can be biased by the variability of a small sample.

Outstandingly, a significant difference between the two groups was observed after treatment on the NHPT. It is not possible to infer any relationship between genotype profile and recovery of hand dexterity with current data, but it might be postulated that different availability of free BDNF could sustain different recovery in those districts which are phylogenetically newer such as the hand as compared to the whole upper limb. Specific studies need to be designed in the future to explore this hypothesis.

The result achieved in this experiment showed that hypertrophy of specific brain areas mostly overlaps between humans and animal. Moreover, the results from stroke patients are strongly coherent with the rationale of the virtual-reality based treatment proposed for the recovery of upper limb motor function. This evidence allows to close the computational – anatomical – physiological loop, and to speculate about the possible neurophysiological mechanisms occurring in patients undergoing rehabilitation treatment, in a real clinical setting. This evidence is encouraging to postulate that the mechanisms of recovery after stroke observed in animals at histological and anatomical level might happen also in humans.

As a consequence, a possibility for genetic-to-rehabilitation translation exists to start interpreting clinical observations from a combined genetic-imaging

perspective, for each individual patient. The genetic-to-rehabilitation translation represents a feasible path to tailor personalised rehabilitation therapies for people surviving a stroke. As an example, knowing the genetic profile of a patient with regard to the Val66Met BDNF SNP might be predictive of fundamental clinical information, such as:

- No difference exists in terms of recovery gain between genotypes, thus both carriers and non-carriers of the Val66Met BDNF SNP are good candidates for intensive rehabilitation care directed to the upper limb.
- Carriers of the polymorphism are more likely, than non-carriers to rely on preserved functionality of the non-lesioned hemisphere, thus rehabilitation modalities facilitating (e.g. transcranial direct current stimulation, tDCS), or inhibiting (e.g. constraint induced movement therapy, bilateral arm training) the activation of the non-affected hemisphere should be considered (Cheeran et al., 2008, Fritsch et al., 2010).
- When the upper limb is the target of motor rehabilitation, feedbacks such as KP and KR are useful in both genotypes, but more likely to promote a change also at the anatomical level in carriers of the Val66Met BDNF SNP.

3.4. Limitations

Some limitations need to be acknowledged for both experiments reported in this chapter. In general terms, the statistical power needed for a reliable study comparing different genotypes in a population of patients is not commonly agreed on. Nevertheless, a sample of 100 subjects is considered sufficiently powerful for external validity, when prevalence is not below 1 in 1500 cases, as for rare diseases. The prevalence of the Val66Met BDNF SNP is ethnicity dependent but its frequency is never below 30% as it is the case for Caucasians where is least frequent, and can reach a frequency of around 70% in Asians. In this regard, the sample size of 75 patients included in experiment 1 is very close to reach reliable external validity, while the sample size of 19 patients included in experiment 2 is far from the appropriate target. Nevertheless, to overcome this issue in experiment 2 very specific outcomes, such as brain volumes, were explored with very powerful statistics, corrected for repeated measures and very low threshold of statistical significance accepted ($p < 0.001$). Another sample issue is the one related to ethnicity. As reported in the introduction paragraphs, prevalence is

significantly different because of the ethnicity, thus the current external validity of these results is limited to Caucasian patients, but not extendable to Asians.

Another limitation is related to the absence of long term follow up in the patients enrolled. Kim and collaborators (2013) reported differences in groups of carriers and non-carrier at 3 months after hospital discharge; moreover evidence from Qin and co-workers in animals (2014) demonstrated that carriers of the polymorphism have a drastic drop in their performance once compensatory mechanisms of the non-lesioned hemisphere are inactivated. Inactivity due to interruption of rehabilitation care might be interpreted as the ecological equivalent of inactivation of compensatory mechanisms; thus it is feasible to expect a detriment of outcome measures at certain time distance from discharge. Due to these patients being treated in hospital facilities, it was not possible to collect long term follow up measures after hospital discharge and all patients were treated intensively according to very specific physical therapy protocols based on virtual reality. Considering the available knowledge, it is most likely that possible differences between groups due to the polymorphism were compensated by the treatment provided, thus confirming that specific rehabilitation treatments might be a protective factor against the possible detrimental effects of different genetic profiles.

Another limitation common to the two experiments is the set of outcome measures chosen. In fact, referring to the International Classification for Functioning Disability and Health (ICF) the domains of body structures, functions (F-M UE, RPS, NHPT) and activities (FIM) were exhaustively covered, but no outcomes were collected with respect to the level of participation of patients. In consideration that the World Health Organisation (WHO) defines rehabilitation as “[...] a process aimed at enabling them [patients] to reach and maintain their optimal physical, sensory, intellectual, psychological and social functional levels. [...]” the domain of individual participation in society should be considered like the reference aim to pursue. In this regard, the present data do not allow any inference to be made on any potential translation of findings into final quality of life of any individual subject, thus future studies should include also outcome measures in the domain of participation, with the aim to explore what is finally translated into real daily life.

A final limitation specific for experiment 2 is the lack of histological examinations, to test whether the larger volumes detected were somehow related to

hypertrophic cells or bigger number of connections. This issue is technically still hard to address in humans, since direct histological exam of brain tissue is not yet systematically feasible in patients and imaging techniques available for humans are not sufficiently spatially defined to explore in these details the target tissues. It is not yet possible, therefore, to demonstrate in humans which are mechanisms that play at the histological level during brain reorganisation after stroke.

4. MUSCLE SYNERGIES' CHARACTERISTICS IN PATIENTS UNDERGOING VIRTUAL REALITY-BASED TREATMENT FOR THE UPPER LIMB, AFTER STROKE

4.1. Background

4.1.1. Introducing the neurophysiology of motor control and motor learning of the upper limb in humans: “the curse of dimensionality”.

Successful execution of voluntary movements relies on the functional integration of several parts of the central nervous system (CNS), such as M1, dorsal and ventral premotor areas, the supplementary motor area. Motor brain areas are able to control peripheral structures by descending neural signals destined to the spinal interneurons, motoneurons, and skeletal muscles to produce voluntary movements. How the motor cortical areas are able to control such a large complexity is still a matter of debate. Over several decades it has been implicitly postulated that activities of neurons in the primary motor areas are somehow correlated with some parameters of movement. Starting from earlier investigations by Evarts (1968), neural correlates have been found virtually for every parameter examined such as force, direction and speed of movement, end point position, joint motion, and muscle activation (Fetz, 1993, Churchland and Shenoy, 2007). More recently, the focus shifted from kinematics of movement to changes in synaptic balance because of the need to express different motor behaviours. Kurtzer and co-workers (2005) found that many neurons changed their load sensitivity in the transition from movement to posture and vice versa. At present, a growing number of investigators have become aware that this plethora of correlations makes it hard to understand how the spinal circuitries can manage the interpretation of these mixed descending cortical signals. An additional factor contributing to the difficulty of deciphering the nature of descending motor activities is the fact that the CNS needs to coordinate the actions of a large number of muscles with thousands of motor units in the limbs, even for the simplest movements. These muscles represent a large number of degrees of freedom (DoFs) in the motor system to be controlled and specified.

Given the complexity of the dynamic relationship between joint torques and their resulting joint motions, as well as the considerable redundancy in joint actions across all muscles, how the motor system coordinates the activations of the muscles has remained obscure. Presumably, the CNS copes with this apparent difficulty of controlling movements by using a certain simplified control strategy. The elucidation of the nature of such a strategy and the understanding of how it can be implemented by the motor cortex and spinal cord represents two highly important questions in motor neuroscience. Evidence from physiology studies has provided experimental support that the CNS may solve the computational complexities of motor control through a modular architecture (Bizzi et al., 1991). Physiological evidence for the existence of such a modular organization was obtained by direct stimulations of the interneuronal system of the lumbar spinal cord of the frog, rat and cat (Saltiel et al., 2001). Electrical or chemical stimulation of different loci of the spinal premotor circuitry imposes a different balance of muscle activations (muscle synergies), generating different patterns of force fields which can be recorded at the limb end point across the workspace. The link between the force field evoked by microstimulation and muscle synergies was provided by Loeb and co-workers (Loeb et al., 2000). But, most importantly, Mussa-Ivaldi and co-workers (1994) showed that co-stimulation of two different spinal loci in a spinalized frog produced a force field similar to the vectorial sum of the force fields evoked by stimulation of the individual loci. This finding provided the experimental basis for suggesting that movement generation occurs through a linear combination of muscle synergies organized within the spinal cord. On the basis of these results Bizzi and collaborators (Overduin et al., 2008) have put forward the hypothesis that descending cortical signals represent neuronal drives which select, activate and combine, in a flexible way, spinal cord modules which express robust muscle synergies. Different motor behaviours then emerge as different synergies are recruited to different degrees of activation. Recent publications provided support for this hypothesis with evidence obtained also from humans. More precisely it was observed that in stroke patients, the muscle synergies for both the affected and unaffected upper limbs were similar, despite differences in motor performance and superficial electromyography (sEMG) between the arms. This evidence supports the idea that muscle synergies are robust and resistant to brain lesion (Cheung et al., 2009b). More recently, it has also been observed that muscle synergies of the impaired limb (upper and lower)

are characterized by different patterns, like merging (Clark et al., 2010) and fragmentation (Cheung et al., 2012b). These patterns were found to be significantly different in patients, according to the severity of motor impairment and distance from stroke. Namely, severely impaired patients showed an increased number of merged synergies, while patients distant from stroke showed fragmentation of synergies (Cheung et al., 2012b). These findings suggest that muscle synergies could be considered as physiological markers of motor cortical damage.

4.1.2. Evidence of a neural association of limb muscle synergies in animals.

In general terms, studies on muscle synergies are conducted collecting large amounts of EMG data from animals or humans while they are performing motor tasks, whose variability is explained by basic vectors extracted through the application of mathematical algorithms. A wide range of algorithms have been proposed for analyses, such as factor analysis (FA) with varimax rotations (Basilevsky, 2009), non-negative matrix factorisation (NMF) (Lee and Seung, 1999), principal component analysis (PCA) (Krishnamoorthy et al., 2003), independent component analysis (ICA) (Bell and Sejnowski, 1995), ICA applied to subspace of PCA (ICAPCA) (Makeig et al., 1997), probabilistic version of ICA (pICA) with non-negativity constraints (Højen-Sørensen et al., 2002). Tresch and co-workers (2006) demonstrated that performance of each algorithm is comparable, provided that specific assumptions are respected. One of the main issues with this computational analysis deals with demonstrating that muscle synergies are not just a mathematical explanation of data collected within the experimental paradigms, but that a neurophysiological substrate exists, justifying the biological plausibility of the existence of muscle synergies. An option to solve this issue from an experimental perspective relies on the possibility to manipulate the biological tissues supposed to store muscle synergies, predicting new muscle synergies' structures to be tested with the application of mathematical algorithm. Several experiments have been reported, with the aim to demonstrate that experimental dismantling of specific structures in the motor system results in coherent outputs from the mathematical models applied to EMG datasets. Nevertheless, few models have demonstrated that the results obtained with this

approach were sufficiently robust in an experimental paradigm context and valid for natural motor behaviour as well (Cheung et al., 2005).

The following experiments were aimed at testing the hypothesis that reduction in number of muscle synergies' modules depends on the disruption of specific brain areas (i.e. cortical and subcortical) and of the corticospinal tract. Three different methodologies were used. First, a pilot study using region of interest (ROI) analysis was carried out to test whether the lesional weight (defined as the frequency with which a brain structure was involved in the lesion caused by the stroke) was significantly different between group of patients with high or low volumes of brain damage (defined as lower or higher than 10 cm³) and whether the number of muscle synergies was different between those groups. A second study using voxel based morphometry (VBM) and diffusion tensor imaging (DTI) analyses was run to test which brain areas and white matter microstructure respectively were correlated with modification of muscle synergies' structure.

4.2. Experiment 3: Effect of brain lesion on the number of upper limb muscle synergies: a region of interest (ROI) analysis.

4.2.1. Introduction

The matter of whether size, side or sites of brain damage have implications in the clinical sequelae of stroke has been debated for a long time. Page and collaborators (2013) have studied the association between stroke lesion volume and motor impairment of the upper extremity in a large sample of 139 ischemic stroke patients, in both the mild and chronic phase. Lesion volume was calculated by manual tracing of the ischemic lesion on each MRI or CT slice from diagnostic scans, while upper limb impairment of motor function was measured by the upper limb section of the Fugl-Meyer (F-M UE) scale, the Arm Motor Ability Test (AMAT) and Functional Ability (FA) scale. Their results indicated that the F-M UE score was not related with brain lesion volume and that only a small variability in the residual functional level can be predicted by this imaging parameter, which, however, had no clinical meaning. In this study no labelling of cortical or

subcortical structures involved by the lesion was done, thus the volume of the lesion as a whole was only considered, regardless of the anatomical structures disrupted by the brain infarct.

Darling and collaborators (2011), using quantitative histology in primates have studied the motor consequences of lesions in the white or grey matter, both at cortical and subcortical level. Their animal model confirmed that greater impairment of upper limb motor function was related to bigger lesions in the white matter. These authors, therefore, suggested that also in humans, motor deficits following a stroke might be the consequence of major involvement of subcortical white and grey matter structures.

Evidence suggests that the volume of brain lesion does not predict the level of upper limb motor impairment, after stroke. Nevertheless, no data are available on the potential relation between brain lesion volume and alterations of motor control mechanisms, such as muscle synergies. As described in previous paragraphs it has been argued that muscle synergies might work as a filter at spinal level, to reduce the complexity of controlling peripheral muscles, when activated by supraspinal signals. Thus, the possibility that the amount of neuronal loss, defined as brain volume, could affect the structure of the motor system deputed to peripheral control needs to be explored.

4.2.2. Experimental hypothesis

The aim of this study was to test whether brain lesions in the main areas of the motor system (i.e. BA 3, 1, 2; BA 4; BA 6, the corona radiata, the corticospinal tract) were somehow related with changes in the number of muscle synergies.

The following hypotheses were tested:

- H_0 : patients with overall low volume of brain lesion ($\leq 10 \text{ cm}^3$) have the same number of muscle synergies as patients with high volume of brain lesion ($> 10 \text{ cm}^3$).
- H_1 : patients with overall low volume of brain lesion ($\leq 10 \text{ cm}^3$) have a significant higher number of muscle synergies than patients with high volume of brain lesion ($> 10 \text{ cm}^3$).

4.2.3. Methods

4.2.3.1. Participants

The cohort of post-stroke patients who took part in this study was selected from admissions to the Cerebrovascular Disease Unit of the San Camillo Hospital, Venice, Italy. Within this cohort of patients, those with hemiparesis due to a first stroke in the region of the middle cerebral artery (MCA) were screened. Occlusion of the MCA frequently accompanied by contralateral hemiparesis is the most common type of impairment, occurring in the majority (65%) of strokes due to cerebrovascular diseases (Sacco et al., 1984). This patient group accounts for approximately 2/5 of the overall admitted patients. CT/MRI scan demonstrated different combinations of brain lesions (i.e. large damage involving most of the vascular territory of the MCA or more discrete lesions of the cortical and/or subcortical areas supplied by branches of the MCA). Moreover, the patients included in the study were those with a Motor Arm sub-score between 1 and 3 on the Italian version of the National Institutes of Health Stroke Scale (IT-NIHSS) (Pezzella et al., 2009). This score was considered as a reliable criterion for assessing the maintenance of residual voluntary motor activation.

The following conditions were considered as exclusion criteria: the presence of a moderate cognitive decline defined as a Mini Mental State Examination (Folstein et al., 1975) score < 20/30 points; the finding of severe verbal comprehension deficit defined as a number of errors > 13 (Tau Points < 58/78) on the Token Test (Huber et al., 1984); evidence of apraxia and visuospatial neglect interfering with upper arm movements and manipulation of simple objects in all the directions within the visual field, as assessed through neurological examination and report in the patient's clinical history or if there were evidence in the neurological examination of behavioural disturbances (i.e. delusions, aggressiveness and severe apathy/depression) that could affect compliance with the rehabilitation programs. These criteria were decided for the feasible screening, within a defined population of stroke survivors, of those patients most likely capable of managing interaction with a challenging rehabilitation setting based on VR, independently of their outcomes at baseline.

According to the above criteria, 19 patients were screened and 8 of them excluded because of major brain dysmorphia (e.g. cortical atrophy, midline shift, aneurism) after visual inspection of MRI scans. The final sample included 11 patients (7 males and 4 females) all right-handed with a mean age of 60.0 ± 13.8

years and a mean distance from stroke onset of 17.4 ± 17.6 months. The mean F-M UE was 60.0 ± 13.8 points (4 patients with F-M UE < 35 points, severe motor impairment; 7 patients with F-M UE ≥ 35 points, mild motor impairment) and a mean lesion volume of 12.2 ± 13.8 cm³ (8 patients had a lesion of the left hemisphere and 3 of the right hemisphere). The detailed characteristics for every patient are reported in table 4.1.

Table 4.1. Clinical and neurophysiological characteristics of the patients sampled for ROI analysis.

Sub	Age	Sex	Time since stroke onset (months)	Affected hemisphere	No synergies unaffected arm	No synergies affected arm	Lesion volume (cm ³)	F-M UE
BV	76	M	10.6	L	9	7	16.78	34
BG	58	M	0.9	L	7	8	0.73	66
CA	83	M	2.7	L	8	9	0.72	10
ME	42	M	2.1	L	8	8	1.17	64
BA	45	F	1.6	L	7	8	20.63	66
RC	52	F	6.3	L	7	7	0.22	14
SP	45	M	12.8	R	8	9	1.70	66
FA	61	F	43.6	L	7	9	2.02	61
SG	64	M	45.3	R	6	7	20.97	66
RA	76	F	35.6	L	7	6	37.31	65
TB	58	M	29.9	R	6	7	31.46	23

M: male; F: female; L: left; R: right; F-M UE: Fugl-Meyer Upper Extremity. All patients were right handed.

4.2.4. Materials

4.2.4.1. Analysis of the upper limb muscle synergies

To extract the upper limb motor synergies, surface electromyographic activity (sEMG) was recorded from 16 muscles from both the normal and the stroke-affected arm while the patient performed a variety of standardised motor tasks (figure 4.1). The recorded muscles included all major muscles of the shoulder and upper-arm and the tasks for both arms were identical, except that their trajectories were mirror images of each other, depending on limb side. All motor tested tasks were performed in a virtual reality setting built within the Virtual Reality Rehabilitation System (VRRS®. Khymeia Group, Noventa Padovana, Italy). The equipment included a computer workstation connected to a 6 degrees

of freedom (DOF) motion-tracking system (Polhemus G4®, Vermont, US), a high-resolution LCD displaying the virtual scenarios on a large screen and a flexible software processing the motion data coming from the receiver of the end-effector placed on the dorsal face of the hand. The VRRS allowed the participants to perform the requested motor tasks, while the movement of the entire biomechanical arm system's end-effector was simultaneously represented in a virtual scenario.

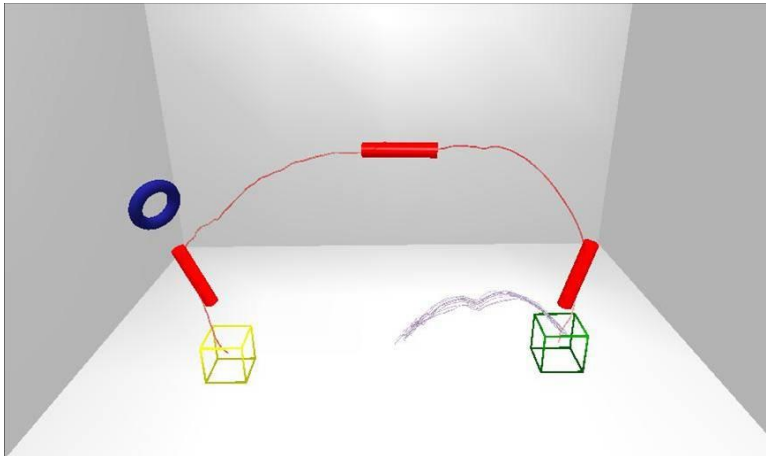


Figure 4.1. Example of a task a patient was requested to perform while interacting with VR

The blue donut is the virtual avatar of the sensorised end-effector; the green and the yellow boxes represent the start and end point of the task, respectively; the red solid line represents the spatial task to be accomplished, while red cylinders are visual constraints to increase task difficulty. The black solid lines on the right are examples of kinematics performed by a patient while trying to perform the requested task with the affected arm.

The task procedure for every sensor placement followed the SENIAM's (Surface ElectroMyoGraphy for the Non-Invasive assessment of Muscles-European Community project <http://www.seniam.org/>) recommendations for skin preparation of the patient, placement, fixation and testing of the sensor and its connection.

We followed the same recommendations also for the sensor locations in the following muscles: Triceps brachii (electrode 1: medial head; electrode 2: lateral head); Biceps brachii (electrode 3: short head; electrode 4: long head); Deltoideus anterior (electrode 5); Deltoideus medius (electrode 6); Deltoideus posterior (electrode 7); Trapezius superior (electrode 8). In order to ensure consistency of the placement position of the EMG electrodes across

assessments, the anatomical landmarks listed in table 4.2 were used as reference.

Table 4.2. Landmarks and numbered channels for each of the sampled muscle.

Channel	Muscle name	Surface anatomy landmarks
1	Infraspinatus	Posterior angle of acromion
2	Teres major	Posterior angle of acromion
3	Superior trapezius	Posterior angle of acromion
4	Rhomboid major (or Medial trapezius)	Posterior angle of acromion; Medial side of the spine of the scapula
5	Pectoralis major, clavicular head	Coracoid process; Sternoclavicular joint
6	Deltoid, anterior part	Anterior angle of acromion
7	Deltoid, medial part	Anterior angle of acromion
8	Deltoid, posterior part	Posterior angle of acromion
9	Triceps brachii, lateral head	Posterior angle of acromion
10	Biceps brachii, short head	Anterior angle of acromion; Intertubercular sulcus
11	Biceps brachii, long head	Anterior angle of acromion; Intertubercular sulcus
12	Brachialis	Anterior angle of acromion; Intertubercular sulcus
13	Brachioradialis	Lateral epycondile
14	Pronator teres	Medial epycondile
15	Triceps brachii, medial head	Posterior angle of acromion
16	Supinator	Lateral epycondile

For all the other muscles (Figure 4.2): Rhomboid Major (electrode 9); Brachioradialis (electrode 10); Supinator (electrode 11); Brachialis (electrode 12); Pronator Teres (electrode 13); Pectoralis Major (electrode 14: clavicular head); Infraspinatus (electrode 15); Teres Major (electrode 16) standard clinical procedures were followed (Perotto and Delagi, 2005).

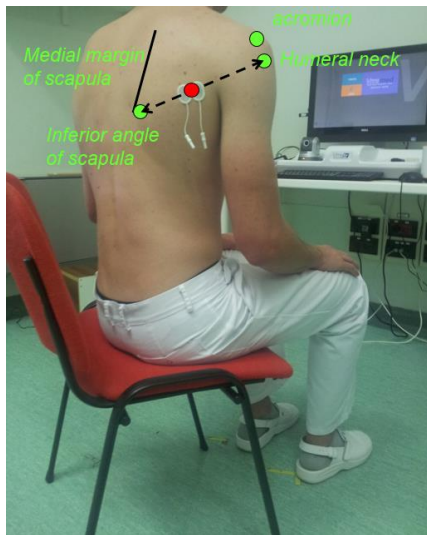


Figure 4.2. Example of bipolar electrodes placement (i.e. Teres Major) following standard clinical procedures.

The green spots and the black solid line are the surface anatomical landmarks for retrieving the reference point (red spot) to be included between the two electrodes. In the background the VRRS® system used for VR tasks administration and motion tracking.

The instrumental setup for signal acquisition was composed by:

- EMG-USB2+ amplifier (OTBioelettronica, Torino, Italy)
- 72001-K/12 electrodes (AMBU Neuroline, Ballerup, Denmark).

The non-negative matrix factorization algorithm (Lee and Seung, 1999) was applied to extract from the sEMG the minimal number of synergies necessary for a reconstruction of at least 80% of the source signal ($R^2 \geq 0.80$).

4.2.4.2. Magnetic resonance image (MRI) acquisition

Each patient underwent a standard structural MRI acquisition protocol which included a fluid-attenuated inversion recovery (FLAIR) scan, a three dimensional T1-weighted (3DT1W) structural MRI scan and a 32 directions diffusion weighted imaging (DWI) scan. These scans were acquired on a 1.5T Philips Achieva MRI system. Coronal FLAIR acquisitions were obtained for a detailed assessment and characterization of cerebral infarctions (TR/TE/inversion time [T1]/NEX = 11000/140/2600/2; matrix size = 256 x 256; FOV = 240 mm; slice thickness = 7.0 mm with no gap between slices, in-plane spatial resolution of 0.898 x 0.898 mm/pixel). The 3DT1W scan was acquired with a Turbo Field Echo sequence. The Voxel dimensions were 1.1 X 1.1 X 0.6 mm and field of view (FOV) was 250 mm with a matrix size of 256 X 256 X 124. Total acquisition time was 4 min 27 s (repetition time, RT: 7.4 ms, echo delay time, TE: 3.4 ms and flip angle 8 degrees,

number of sagittal slices 280). The DWI 32 directions scan was acquired to analyse the bundle's integrity in the white matter tracts. The voxels size was 2.5 X 2.5 X 3.0 reconstructed as 1.67 X 1.67 X 3.0, with a FOV of 240 X 240 X 135 (RL, AP, FH). The total acquisition time was 27'11" for 45 parallel slices acquired interleaved with no gap and a thickness of 3mm (TR: 8280, TE: 70ms, flip angle 90 degrees, b factor: 600).

4.2.4.3. Region of Interest (ROI) analyses of lesion location.

The coronal FLAIR acquisitions were used for delineation and quantification of lesions. Lesion mapping was carried out using a digital adaptation of the method devised by Damasio and Damasio (1989, Feinberg et al., 2010). For each patient, lesions were drawn manually using MRIcro (Rorden and Brett, 2000) in their own native space (MRI scan) and superimposed onto the specific template (i.e. Damasio or Talairach template) that best matched the orientation of the MRI image. A digital dimensional scaling procedure was carried out to fit digitally each lesion of each individual patient onto the template in order to convert lesions from native space into a standard template space. Lesion size in cm³ was determined from the native space by multiplying the two dimensional lesion size by slice thickness (7mm). The lesioned brain areas were identified using the labelling of the cytoarchitectonic (i.e. Brodmann areas, BAs), vascular and anatomical areas marked on each template. All lesion drawings were independently verified by a second person (AV) who was blind to the behavioural results at that time. For every patient, the number of times that a brain area was involved by the lesion was computed in all the slices (area's absolute frequency), then its lesional weight (i.e area's relative frequency) was calculated as follows:

$$lesional_weight = \frac{\text{no of label per area}}{\text{no of total labels}}$$

4.2.4.4. Statistical analysis for ROI characterisation of lesion location.

The following statistical procedures were applied to the ROI analyses, with the aim to test whether the anatomical site of brain lesion was related to the amount of motor impairment in the upper limb affected by stroke. Potential associations between clinical (i.e. time from stroke onset; Fugl Meyer upper extremity scale, F-M UE) and neurophysiologic variables (i.e. No of synergies in the affected and

unaffected arm, volume lesion) were studied using Spearman's rank correlation test (ρ). Moreover, the Spearman rank correlation test (ρ) was used for testing the reliability of the ROI analysis, considering the finding of a significant positive correlation between total volume of brain lesions and number of the labelled brain areas as a proof of internal consistency of the lesional weight as described above. BAs (i.e. 3,1,2; 4; 6) including the primary motor cortex (M1) and associative areas (pre-motor area and supplementary motor area), the corona radiata (CR) and the internal capsule (IC), were considered as representatives of the corticospinal tract in the brain. To explore whether the stroke lesion had disrupted the structures composing the corticospinal tract differently, a log-linear model was used to study any difference in the relative lesional weight. The Wilcoxon test was used to test whether the disruption of cortical (i.e. BAs 3,1,2; 4; 6) and subcortical (i.e. CR and IC) areas, as well as of the IC and other structures of the corticospinal tract, was different within group and subgroups. Chi-square test (χ^2) was used to study whether cortical (i.e. BAs 3,1,2; 4; 6) and subcortical (i.e. CR and IC) areas, as well as the IC and other structures of the corticospinal tract, were differently disrupted between patients with mild (F-M UE \geq 35 points) and severe (F-M UE $<$ 35 points) motor impairment.

4.2.5 Results

No significant correlations were found among variables, which indicates the absence of strong associations and potential dependence through them. A strong positive correlation was found between the total volume of brain lesions and the number of labelled brain areas involved in the brain lesions ($\rho=0.936$, $p<0.000$), demonstrating internal consistency of the lesional weight as computed with the ROI analysis.

The contingency table exploring the log-linear model assumptions revealed that only for the IC the expected frequency was over the critical value of 5, as a consequence no further analysis was carried out since any analysis would have been underpowered (Fig 4.3).

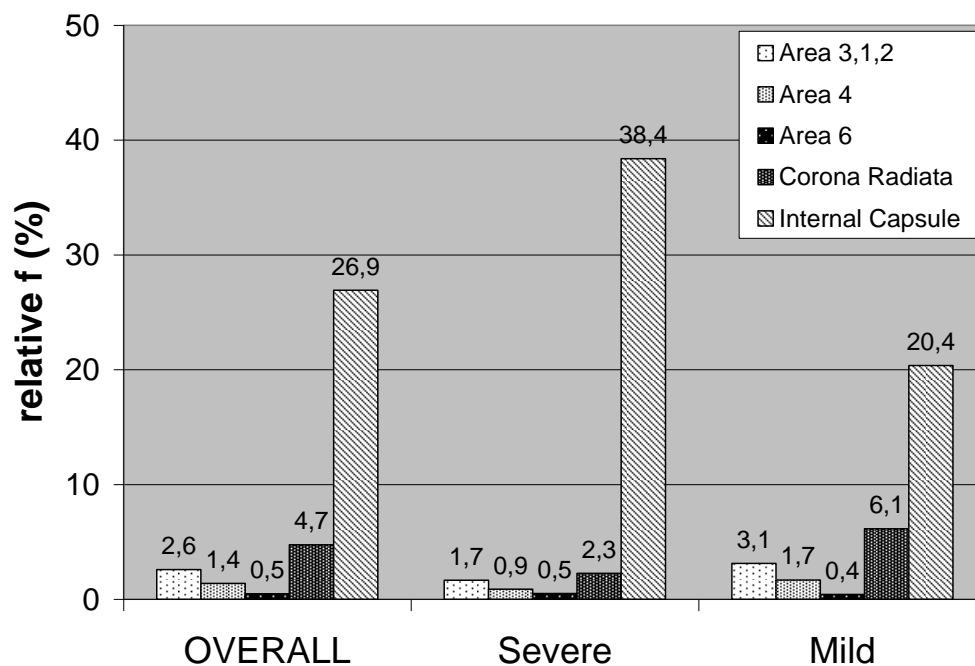


Figure 4.3. Lesional weight for corticospinal tract structures.

The first comparison was run between patients with a total volume of brain lesion equal or lower than 10 cm^3 , versus those with a total volume of brain lesion higher than 10 cm^3 . The threshold of 10 cm^3 was set arbitrarily as an ideal one by looking at single patient data from our sample, but no clinical meaning is further associated with this value. The dependent variables for this comparison were the number of muscle synergies extracted in the affected arm and the Fugl-Meyer Upper Extremity score, between groups (table 4.3).

Table 4.3. Characteristics of low volume and high volume brain lesion groups.

	Low Volume group ($\leq 10 \text{ cm}^3$) (N=6)	High Volume group ($> 10 \text{ cm}^3$) (N=5)	p value
Volume (cm^3)	1.09 (0.67)	25.43 (8.59)	0.006*
No muscle synergies	8.33 (0.82)	7.00 (0.71)	0.028*
F-M UE	46.83 (27.07)	50.80 (20.73)	0.575

F-M UE: Fugl-Meyer Upper Extremity. Results are reported as mean and standard deviation, m (SD). Mann-Whitney U test was used to test differences between patients with low ($\leq 10 \text{ cm}^3$) or high ($> 10 \text{ cm}^3$) volume brain lesion, statistical threshold was set at $p < 0.05$ (*).

Results indicated that the number of synergies extracted from the affected arm was significantly different when considering the whole volume of brain lesion as

criteria, but not the F-M UE assessment scale, as expected from published evidence. When looking at differences in lesional weight considered as involvement of white matter fibres or motor cortical areas (i.e. BA 3, 1, 2; BA 4; BA 6), it was observed that fibres were relatively more involved than cortical areas overall ($z = -2.091$, $p = 0.037$), but the differences in subsamples of patients with high and low volume of whole brain lesion were not significantly different ($\chi^2=3.733(1)$, $p=0.053$). The probability value was very close but not significant, probably because of the low expected frequency (i.e. 1.2) of cortical lesions in the high volume group (Figure 4.4).

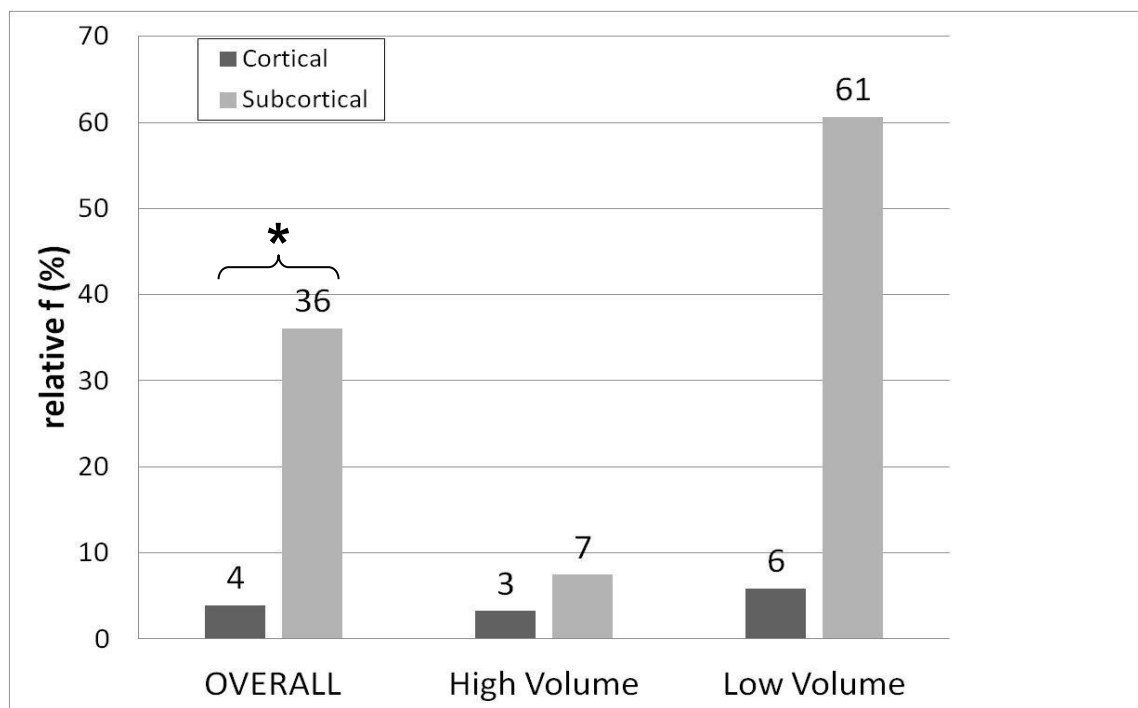


Figure 4.4. Lesional weight of the cortical and subcortical structures of the corticospinal tract, with regard to the volume of brain lesion. High Volume > 10 cm³; Low Volume ≤ 10 cm³.

The same analysis was carried out according to levels of motor impairment, as measured by the Fugl-Meyer Assessment scale for upper extremity (F-M UE). The patients were defined as severe when the F-M UE was lower or equal to 35 out of 66 points and mild when the score was higher than 35 out of 66 points (Table 4.4).

For these subsamples neither volume of brain lesion nor number of muscle synergies were significantly different between groups.

Table 4.4. Characteristics of mild and severe groups.

	Severe (F-M UE \leq 35) (N=4)	Mild (F-M UE $>$ 35) (N=7)	p value
Volume (cm ³)	12.30 (14.91)	12.08 (14.41)	0.527
No muscle synergies	7.50 (1.00)	7.86 (1.07)	0.490
F-M UE	20.25 (10.66)	64.86 (1.87)	0.006*

F-M UE: Fugl-Meyer Upper Extremity. Results are reported as mean and standard deviation, m (SD). Mann-Whitney U test was used to test differences between patients with severe (F-M UE \leq 35) or mild (F-M UE $>$ 35) motor impairment, statistical threshold was set at $p < 0.05$ (*).

In the overall group the lesional weight of the subcortical structures was significantly higher than in the cortical structures ($z=-2.135$, $p=0.033$). However, in the two subgroups of motor impairment no differences between the lesional weight of cortical and subcortical structures were found ($\chi^2=1.526(1)$, $p=0.217$) probably due to the low expected frequency of cortical lesions (i.e. 3.4 mild group, 4.6 severe group) (Fig. 4.5). Additional analyses, however showed that the IC (Fig. 4.6) was significantly more affected by the stroke lesion ($\chi^2=5.609(1)$, $p=0.018$), when the patient presented with a moderate to severe impairment of the upper limb motor function than when the motor upper limb impairment was mild.

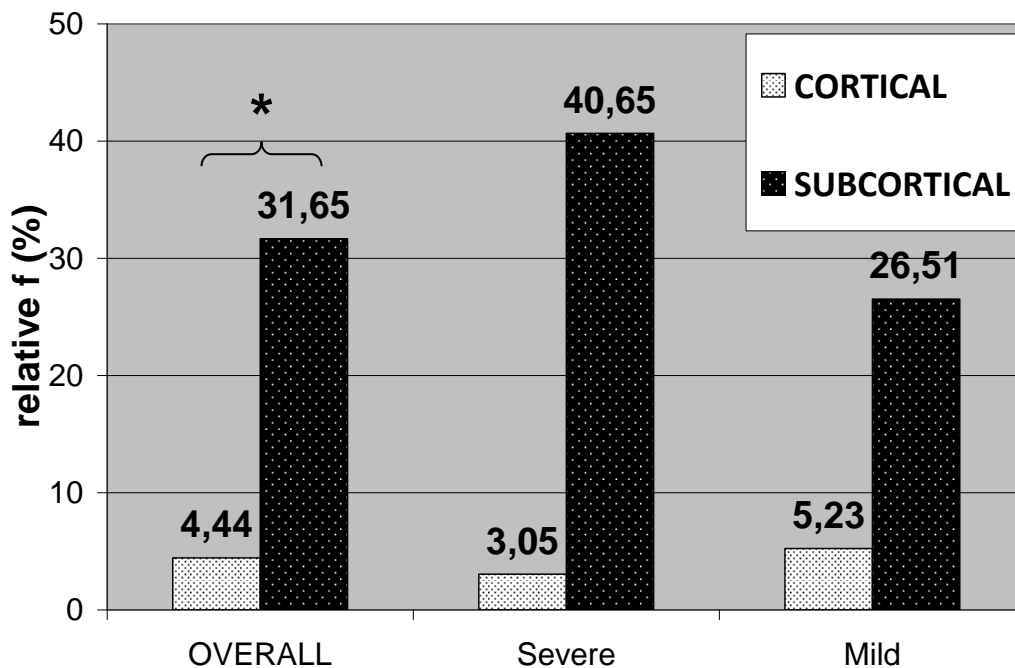


Figure 4.5. Lesional weight of the cortical and subcortical structures of the corticospinal tract.

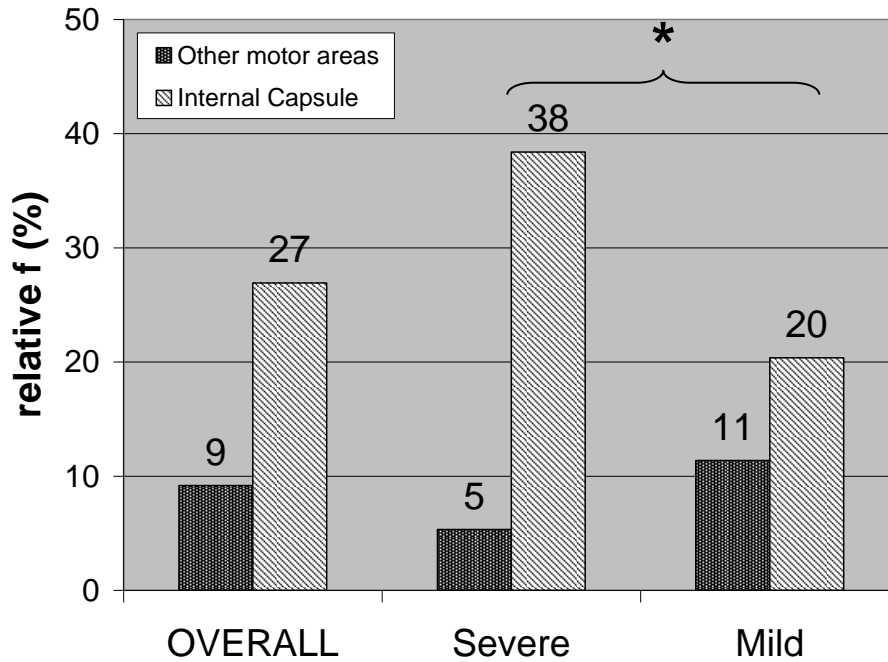


Figure 4.6. Lesional weight of the internal capsule and other motor areas of the corticospinal tract.

4.2.6. Discussion

The first evidence on the effect deriving from the lesion of the corticospinal tracts' fibres was provided by the ROI analysis. In fact, the reconstruction of brain lesion allowed the comparison between normalised weights computed for every area. The results clearly indicated that in our sample, representing typical stroke patients with motor impairments, the involvement of subcortical structures and of the internal capsule was significantly higher compared with other representative areas playing a role in the motor system. Unfortunately, the small number of patients currently enrolled did not allow a powerful statistical comparison in subgroups of patients with high and low volume of infarction, as well as in subgroups of patients with severe and mild motor impairments, as defined by the F-M UE scale score. Furthermore, patients were recruited in the study regardless of the distance from stroke onset; thus, the time window varied extensively (i.e. from 0.9 to 45.3 months). This characteristic should be considered as a significant bias affecting the current sample, thus these findings need to be confirmed with similar methodologies in a bigger group of more homogeneous patients. Nevertheless, these pilot data indicated a trend that will be tested more powerfully

by VBM analyses in experiment 4 in this chapter, in which the inhomogeneity of distance from stroke of this experiment will be also addressed by enrolling patients in whom distance from stroke was more homogeneous. The findings of the effect of the IC lesion on motor outcomes has been already reported (Qiu et al., 2011). Lesional involvement of this structure is the best predictor associated with poor motor outcomes (sensitivity: 73.7%; specificity: 100%; positive predictive value: 100%, negative predictive value: 89.1%) (Puig et al., 2011). All predictor values, however, were studied in the very acute phase (within 12 hours after stroke onset) to 90 days after the event: nothing is known about the effects of these predictors for good or poor recovery of upper limb motor function due to rehabilitation, after stroke.

4.3. Experiment 4: Effect of brain lesion for the production of complex voluntary movements with upper limb, after stroke: Voxel Based Morphometry (VBM) and Diffusion Tensor Imaging (DTI) analyses.

4.3.1 Introduction

Recently, two experiments have provided direct plausible explanations for this issue. Roh and collaborators (2011) collected EMG data from the limbs of bullfrogs while they were performing a large variety of natural motor behaviours (i.e. jump, swim, kick and step) and from elicited cutaneous reflexes. Data were collected and muscle synergies extracted by NMF procedure before and after surgical transection of the CNS at different levels for comparison, within the same animal (Figure 4.7). The findings indicated that all natural motor behaviours were still partially present since the medulla oblongata was preserved after transection, but with spinal preparation (i.e. transection performed caudally to the medulla oblongata) only cutaneous reflexes could be elicited. The analysis of EMG data showed the existence of two types of muscle synergies: (1) muscle synergies that were shared before and after transection and (2) muscle synergies that were specific after transection. All the shared types survived until medullary preparation, while in spinalised bullfrogs only specific synergies were present, after transection. These authors concluded that muscle synergies of the limbs in bullfrogs are plausibly hard-wire plastic circuitries, organised and represented at the level of the brain stem and the spinal cord, activated by supraspinal areas to express natural motor behaviour.

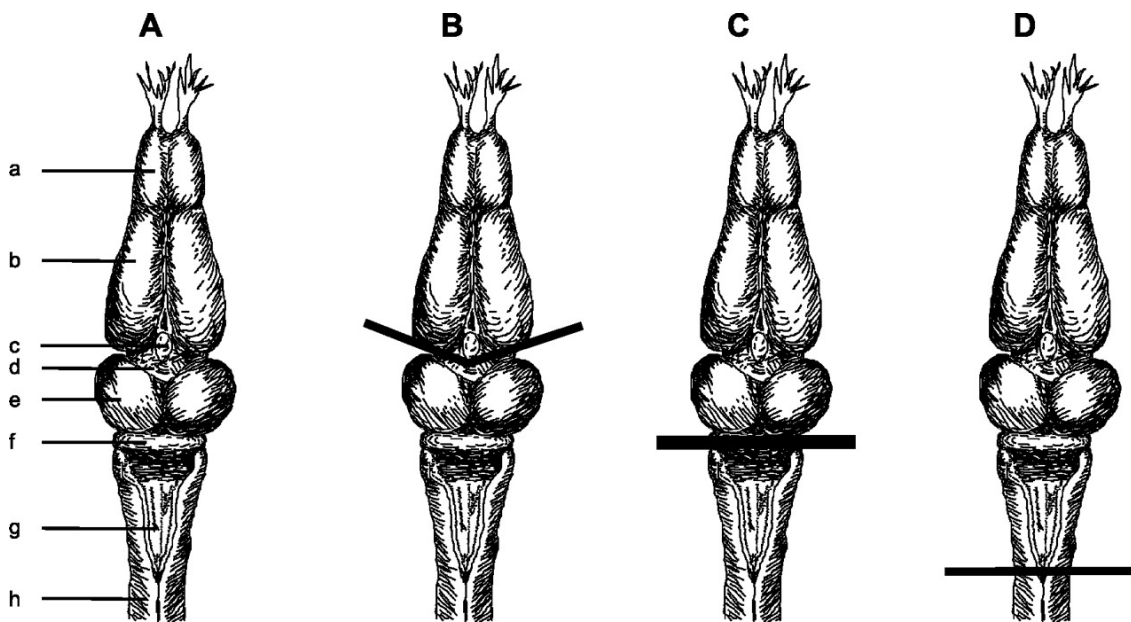


Figure 4.7. Schematic diagram of the bullfrog central nervous system (CNS) and transection at 3 different levels of the neuraxis (Roh et al., 2011).

The bullfrog CNS (A): olfactory lobes, a; cerebral hemispheres, b; pineal gland, c; thalamus, d; optic lobes, e; cerebellum, f; medulla, g; spinal cord, h. From left to right: (B) transection (solid line) performed at the level of the caudal end of the 3rd ventricle (brain stem preparation), (C) at the level of the caudal end of the pons (medullary preparation), (D) at the level of caudal end of the 4th ventricle (spinal preparation).

Rathelot and Strick (2009) contributed to demonstrate that the topography of the corticomotoneurons for different muscles overlaps extensively in the primary motor cortex (M1). They exploited the retrograde transneuronal transport properties of the rabies virus to map the histological organisation of different cell layers in M1, in the upper limb of rhesus monkeys. Shoulder, elbow and fingers muscles were injected with rabies virus in different samples of monkeys, then the distribution of viruses was reconstructed in normalised anatomical maps of M1 using histological tests after autopsy, for comparison. Their findings demonstrated that proximal muscles (i.e. shoulder, elbow) are represented in the rostral layer of M1, an old structure common to many mammals. Neurons controlling these muscles are commonly indicated as neurons of the corticospinal tract (CST), thus keeping connections with inter-neurons in the spinal cord, to generate voluntary motor activity. Elsewhere, fingers muscles, devoted to fine motor skills, are represented in a caudal region of M1, which is a new layer of cells keeping direct connections with second-order motor neurons in the ventral horn of the spinal cord. This new layer is present only in some higher primates and humans and has been considered the essential substrate to learn new highly

skilled movements, such as those of the hand (Figure 4.8), based on the synergistic combination of different muscles.

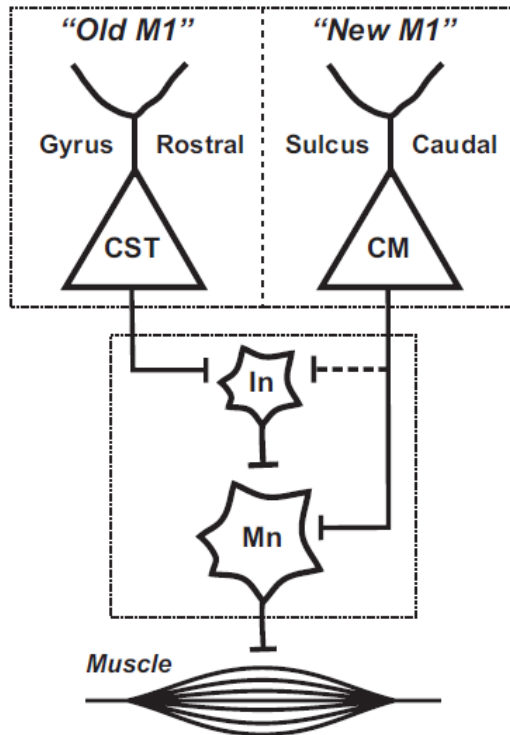


Figure 4.8. Scheme of the histological organisation of M1 for voluntary control of the upper limb, in higher primates (Rathelot and Strick, 2009, reproduced with written permission granted by PNAS on the 26th of October 2015).

New M1 is located caudally and has CM cells that make direct connections with motoneurons. Old M1 is located rostrally and has CST neurons that influence motoneurons indirectly through their connections with spinal interneurons. CM, cortico-motoneuronal; CST, corticospinal; In, interneurons; Mn, motoneurons.

Although starting from different types of experimental evidence, both groups proposed the idea that voluntary motor control of the upper limb relies on a modular architecture of the motor system, based on the concept of muscle synergies. Muscle synergies of the upper limb are intended as interneurons circuitries represented presumably in the brainstem, aimed at reducing the dimensionality of peripheral structures, for control of proximal movements. Conversely, execution and learning of fine motor skills of the hand rely on direct cortico-motoneuronal control bypassing these interneurons, thus requiring a larger and newer cortical representation. Nevertheless, experimental evidence that muscle synergies have representations in biological substrates in humans like it has been observed in other animals has yet to be provided.

4.3.2. Experimental hypotheses

The first aim of this study was to explore which brain areas were anatomically related with the maintenance of movement complexity (i.e. high number of muscle synergies' modules). The following hypotheses were tested, using Voxel Based Morphometry regression analyses:

- H_0 : the maintenance of a high number of muscle synergies' modules is not influenced by lesions in canonical brain areas (i.e. BA 3,1,2; BA 4; BA 6; corona radiata, internal capsule) within the motor system.
- H_1 : the maintenance of a high number of muscle synergies' modules is influenced by lesions in canonical brain areas (i.e. BA 3,1,2; BA 4; BA 6; corona radiata, internal capsule) within the motor system.

The second aim of this study was to test whether lesions of white matter tracts (e.g. the corticospinal tract, the pons, the brainstem) were associated with the reduction of the number of muscle synergies' modules. The following hypothesis was tested, by DTI regression analysis:

- H_0 : the number of muscle synergies' modules in the affected upper limb does not change when fibres in white matter tracts are disrupted (i.e. the fractional anisotropy index is higher).
- H_1 : the number of muscle synergies' modules in the affected upper limb is lower when fibres in white matter tracts are disrupted (i.e. the fractional anisotropy index is higher).

4.3.3. Methods

The methods of acquisition of sEMG signals and the extraction of the number of muscle synergies from upper limb movements, as well as the brain MRI sequences acquired were the same as in experiment 1.

4.3.3.1. Participants

The cohort of post-stroke patients who took part in this study and criteria for inclusion were the same as in experiment 1. Among the first 61 patients screened for enrolment, VBM correlation analysis was carried out on 43 patients who had good quality T1W3D scans and met inclusion criteria. The descriptive characteristics of the sample are reported in table 4.5.

Table 4.5. Clinical and neurophysiological characteristics of the patients sampled for VBM analysis.

Outcome	Overall (n=43)	Right hemisphere lesion (n=18)	Left hemisphere lesion (n=25)
Age, years	60.81±14.41	64.17±9.98	58.40±16.69
Sex, M/F	32/11	15/3	17/8
Time since stroke onset, months	8.09±12.90	12.22±18.56	5.11±5.02*
Handedness, R/L	42/1	17/1	25/0
FIM	95.33±28.41	86.31±31.26	101.61±25.05
F-M UE	38.67±21.16	34.67±21.61	41.56±20.78

M: male; F: female; R: right; L: left; FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity. Results are reported as mean and standard deviation, m (SD). Mann-Whitney U test was used to test differences in demographic data between patients with lesions in the right or left hemisphere, statistical threshold was set at $p < 0.05$ (*).

The overall group was divided according to the hemisphere involved by the stroke lesion (i.e. right, left) because of the need to analyse separately the 3DT1W structural images acquired. In VBM analysis, if anatomical lesions are not flipped by applying rotation matrixes, the voxels representing the loss of brain tissue in the right hemisphere would be subtracted by the ones in the left hemisphere, when running statistical tests. Thus, to minimise biases coming from the application of algebraic transformations (with potential distortions in the resulting anatomy), the two groups of patients with right and left injured hemispheres were analysed separately. The group with damage in the left hemisphere was significantly closer to stroke onset, than the one with damage in the right hemisphere (Mann-Whitney test: $U = 140.50$, $z = -2.08$, $p=0.037$), but all other outcomes were comparable.

Among the 43 patients enrolled for VBM studies it was possible to collect diffusion weighted images (DWI) to carry out DTI analysis in a subsample of 13 patients (7 male, 6 female), with a mean age of 61.69 ± 13.26 years.

4.3.3.2. Voxel Based Morphometry (VBM) analysis.

A number of pre-processing steps were carried out to isolate grey and white matter from the 3DT1W structural scans before performing the statistical analysis using SPM8 (Wellcome Trust Centre for Neuroimaging). In detail, to correct for global differences in brain shape, structural images were warped to standard stereotactic space and segmented to extract grey matter, white matter and

cerebrospinal fluid. The grey and white matter segments were then modulated to correct for changes in volume induced by nonlinear normalization and smoothed using a Gaussian filter set at 8 mm to reduce possible error from between-subjects variability in local anatomy and render the data more normally distributed. After every step, for every subject, the resulting image was visually checked to ensure that the output was of good quality and suitable for the following pre-processing steps.

Finally, smoothed grey and white matter segments for both brain lesions in the right and left hemisphere were entered into independent correlation analyses with:

1. the number of muscle synergies extracted from the upper limb affected by stroke (positive and negative correlation);
2. the number of muscle synergies extracted from the not affected upper limb (positive correlation);
3. the number of muscle synergies shared between the two upper limbs (positive correlation).

Age and gender were also included in the model as covariates. Height threshold was set at $p < 0.05$, Family Wise Error (FWE) corrected. When no significant clusters emerged at this stringent level of threshold, a more liberal threshold level was set at $p < 0.001$, or $p < 0.005$ uncorrected accordingly. When needed an extent threshold of 10 voxels was applied as a spatial filter, to maintain only the bigger clusters as final outcome.

The results from VBM analyses were standardized with the stereotactic grid of Talairach and Tournoux (Talairach and Tournoux, 1988), with a cube range of 1mm for labelling brain areas.

4.3.3.3. Diffusion tensor imaging (DTI) analysis

Among the large availability of computational approaches for diffusion tensor imaging (DTI) analysis the deterministic track termination technique was chosen (Tournier et al., 2011), by the definition of a threshold for fractional anisotropy (FA). The aim of this technique is to determine automatically the termination of a white matter tract, thus to compare alteration of hard-wired connectivity between the brain areas with regard to a reference skeleton. Under the conventional assumption that FA is highest in white matter tracts (i.e. FA = 1) and lowest in

grey matter and cerebrospinal fluid (i.e. FA = 0) a threshold was imposed to determine the end of tract propagation (i.e. FA < 0.2).

Diffusion weighted imaging (DWI) scans underwent a number of pre-processing steps before being entered into statistical analyses to test for white matter tracts integrity. Every DWI scan for every patient was corrected for eddy currents, then all the non-brain tissues were removed and the binary brain mask extracted, by the brain extraction tool (BET), to exclude non brain voxels from further consideration. Finally, the diffusion tensors were reconstructed manually uploading the diffusion weighted data, the BET binary brain mask extracted previously, the gradient directions and the b values. Then, a tract-based spatial statistics (TBSS) voxelwise analysis of multi-subject diffusion data (Smith et al., 2006) was carried out. Starting from the DWI images previously pre-processed, all the FA data were extracted and adapted to standard space, thus FA images and skeletons were created and all subjects data projected onto the mean FA skeleton. Finally, a GLM model corrected by randomisation method (number of permutations = 500) for multiple comparisons, was run with number of muscle synergies' modules as regressor and age and gender as covariates. The aim of this analysis was to find which voxels were correlated with the reduction of muscle synergies in the white matter tracts. Height threshold level was set at $p < 0.05$. All the analyses were run using the FMRIB (Functional Magnetic Resonance Imaging of the Brain) Software Library (FSL) 5.0 (Smith et al., 2004, Woolrich et al., 2009, Jenkinson et al., 2012), released by the FMRIB Analysis Group at Oxford University (UK).

4.3.4. Results

4.3.4.1. Correlation between brain areas and number of muscle synergies in the non-affected upper limb

The VBM analysis revealed that significant clusters were detectable only in white matter areas of patients with lesions in the right hemisphere (i.e. Sub-Gyral ipsilaterally and Brainstem contralaterally) (Table 4.6; Figure 4.9).

Table 4.6. Brain areas associated with numbers of unaffected muscle synergies' modules in patients with right hemisphere lesions.

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Ipsilateral to lesion						
WM	R	Sub-Gyral	20	20.66	-9.44	43.37
WM	R	Sub-Gyral	27	24.56	2.94	32.00
Contralateral to lesion						
WM	L	Brainstem	27	-2.73	-17.13	-11.81

GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. Hits are the number of voxels for the indicated label included in the cube range of 1mm.



Figure 4.9. 3D rendering of brain volumes correlated with muscle synergies in the non-affected limb (white matter).

The cluster of significant voxels ($p = 0.001$ uncorrected; threshold = 10 voxels) is shown superimposed (red) on a standard 3D anatomical template.

4.3.4.2. Correlation between brain areas and number of muscle synergies shared by both upper limbs

The VBM analysis revealed that only in patients with lesions in the right hemisphere the number of muscle synergies shared between the two arms was associated with the Sub-Gyral region, ipsilaterally to the brain lesion in white matter areas (Table 4.7).

Table 4.7. Brain areas associated with numbers of shared muscle synergies' modules in patients with right hemisphere lesions.

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Ipsilateral to lesion						
WM	R	Sub-Gyral	15	20.66	-2.16	45.86

GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of voxels = number of voxels for the indicated label included in the cube range of 1mm.

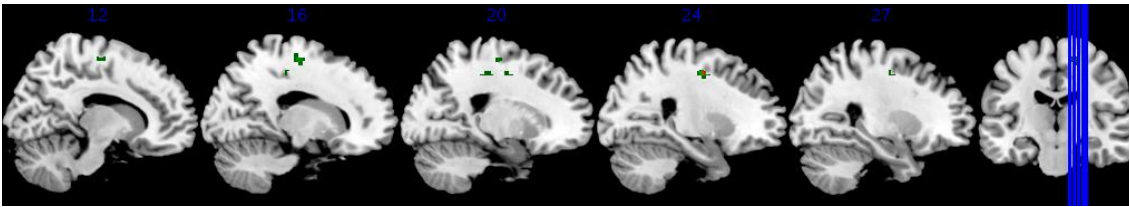
4.3.4.3. Correlation between brain areas and number of muscle synergies in the affected upper limb

VBM analysis revealed that in patients with lesions in the right hemisphere the number of muscle synergies was positively associated with the following white matter areas: the Sub Gyral region and the Medial Frontal Gyrus, in both cases ipsilaterally to the stroke lesion (Table 4.8; Figure 4.10, 4.11).

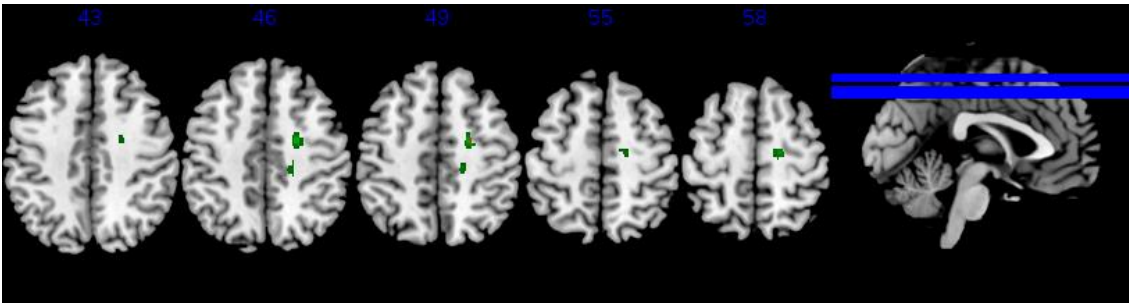
Table 4.8. Brain areas associated with number of affected muscle synergies' modules, in patients with right hemisphere lesions (positive correlation).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Ipsilateral to lesion						
WM	R	Sub-Gyral*	27	20.60	-13.52	46.59
WM	R	Sub-Gyral	27	15.00	-32.12	44.73
WM	R	Medial Frontal Gyrus	24	14.91	-21.81	54.71

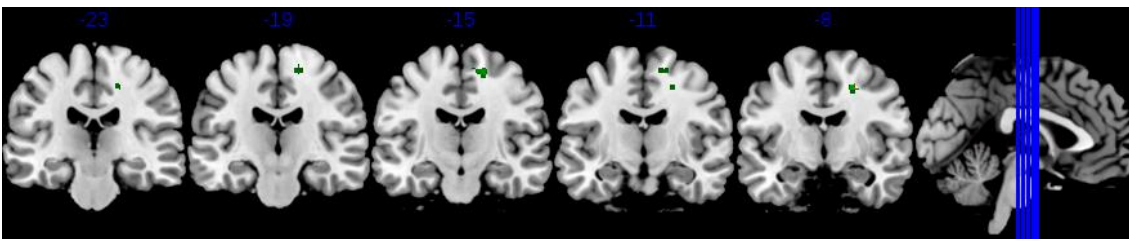
*GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of voxels = number of voxels for the indicated label included in the cube range of 1mm. * indicates clusters survived to FWE correction.*



(a)



(b)



(c)

Figure 4.10. Graphical display of brain volumes correlated with muscle synergies in the affected limb (white matter).

The cluster of significant ($p = 0.001$ uncorrected; threshold = 10 voxels, blue; $p = 0.05$ FWE corrected, red) voxels is shown superimposed on a standard anatomical template. (a) sagittal plane; (b) transversal plane; (c) coronal plane.

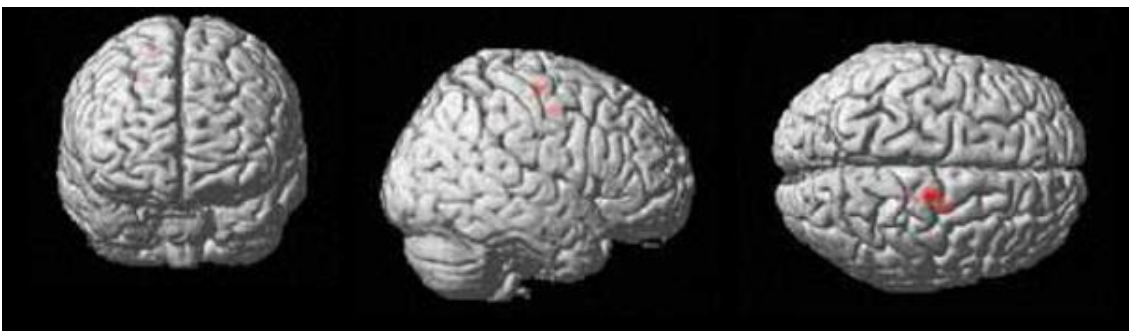


Figure 4.11. 3D rendering of brain volumes correlated with muscle synergies in the affected limb.

The cluster of significant voxels ($p = 0.001$ uncorrected; threshold = 10 voxels, blue; $p = 0.05$ FWE corrected, red) in the white matter are shown superimposed on a standard 3D anatomical template.

In patients with lesions in the left hemisphere white matter areas in the Cingulate Gyrus region were negatively associated with the number of muscle synergies, whereas no significant clusters were detected in patients with lesions in the right hemisphere (Table 4.9, Figure 4.12).

Table 4.9. Brain areas associated with reduction of numbers of affected muscle synergies' modules, in patients with left hemisphere lesions (negative correlation).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Ipsilateral to lesion						
WM	L	Cingulate Gyrus	27	-8.8	-20.94	27.37

WM: white matter; L = left. No of voxels = number of voxels for the indicated label included in the cube range of 1mm.



Figure 4.12. 3D rendering of brain volumes negatively correlated with muscle synergies in the affected limb.

The cluster of significant voxels ($p = 0.001$ uncorrected; threshold = 10 voxels, red) in the white matter are showed superimposed on a standard 3D anatomical template.

4.3.4.4. Correlation between fibres of the white matter tracts and number of muscle synergies in the affected upper limb

The voxels in the white matter tracts whose FA index was below the threshold of 0.2, thus representing discontinuity of the same tract, are displayed in red superimposed on the mean white matter tracts skeleton (green) obtained from pre-processing (Figure 4.13, 4.14, 4.15).

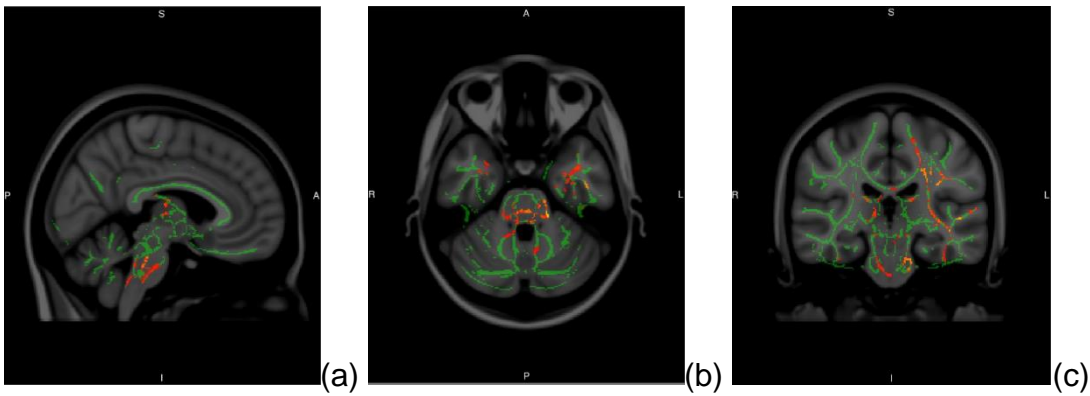


Figure 4.13. Graphical display of lesions in the Brain Stem correlated with reduction of muscle synergies in the affected limb.

The voxels significantly correlated with reduction of muscle synergies' modules (red) are displayed superimposed onto the mean skeleton (green). (a) sagittal plane; (b) transversal plane; (c) coronal plane.

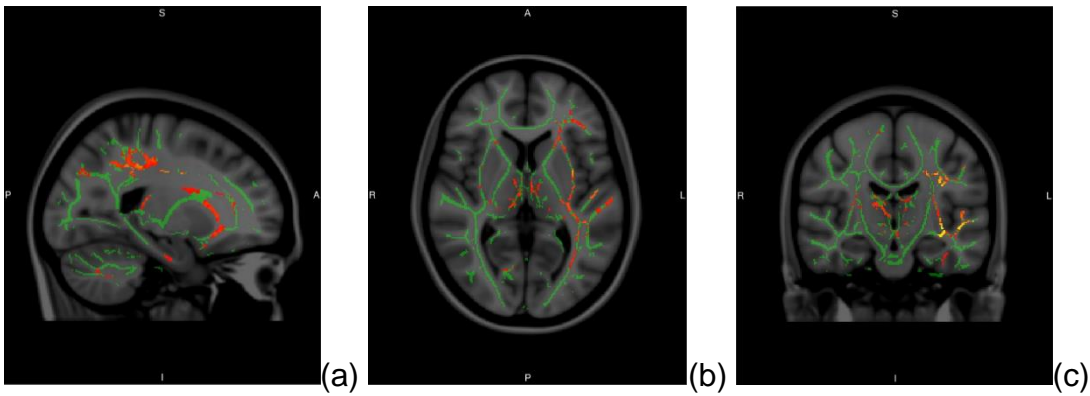


Figure 4.14. Graphical display of lesions in the corticospinal tract correlated with reduction of muscle synergies in the affected limb.

The voxels significantly correlated with reduction of muscle synergies' modules (red) are displayed superimposed onto the mean skeleton (green). (a) sagittal plane; (b) transversal plane; (c) coronal plane.

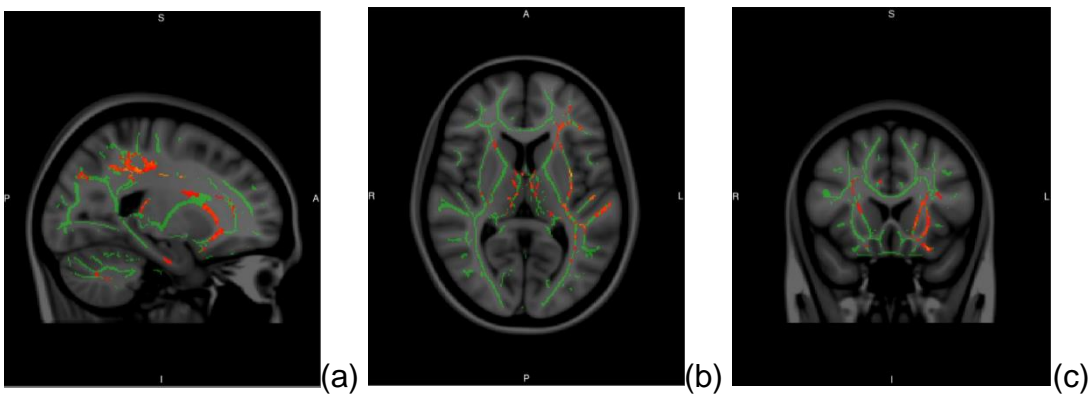


Figure 4.15. Graphical display of lesions in the Putamen Caudate correlated with reduction of muscle synergies in the affected limb.

The voxels significantly correlated with reduction of muscle synergies' modules (red) are displayed superimposed onto the mean skeleton (green). (a) sagittal plane; (b) transversal plane; (c) coronal plane.

The regions of interest from the resulting images were automatically labelled using the Harvard-Oxford cortical and subcortical structural atlas (Desikan et al., 2006) and the Jülich histological (cyto- and myelo-architectonic) atlas (Eickhoff et al., 2006). Visual exploration of statistical maps indicated that disruption of white matter tracts in the brainstem, the corticospinal tract and in the Putamen and Caudate areas were associated with a reduction in the number of muscle synergies' modules in the stroke-affected upper limb.

4.3.5. Discussion

From VBM analyses only 4 white matter (Table 4.10) structures were found to be anatomically related with the number of muscle synergies' modules. In our theoretical framework muscle synergies' modules represent the primitive architecture that is voluntarily activated by descending neural signals from motor cortical areas, to produce finalised motor behaviour with the upper limb. More in details the number of modules might represent an index of the complexity of the movement the motor system is able to express, once activated. Thus, the higher the number of modules observed, the richer the motor repertoire the individual may be capable of producing.

Table 4.10. White matter structures anatomically related to the number of muscle synergies' modules

Area	Function
Medial Frontal Gyrus	Executive mechanisms
Cingulate Gyrus	Limbic system, emotion, learning, memory.
Sub-Gyral	Memory, motor planning, motor imagery, learning complex motor skills, direct control of fingers movements.
Brainstem	Conduction of ascending and descending pathways signalling, origin of the cranial nerves from 3 rd to 12 th , integration of vital functions (e.g. respiratory, cardiac, pain, consciousness)

Overall, our findings showed that the number of muscle synergies' modules has a positive association with the Sub-Gyral region in the affected hemisphere, the

significance of which is consistent regardless of the arm considered (i.e. affected, unaffected). Remarkably, the Sub-Gyral cluster was the only one surviving family wise correction in the impaired arm, targeting the Sub-Gyral region like a robust anatomical substrate, whose integrity might be considered fundamental for the maintenance of muscle synergies, after stroke. Moreover, two other clusters were positively associated with the number of muscle synergies' modules: the brainstem contralateral to hemispheric lesion and the Medial Frontal Gyrus (executive functions), ipsilaterally. On the contrary, the reduction of muscle synergies' modules seems to be associated with areas devoted to action observation (Cingulate Gyrus) (Calvo-Merino et al., 2005) ipsilaterally. These results, coherently with animal models, allow researchers to argue that integrity of the corticospinal tract, to the spinal cord, might play a key role in the activation of muscle synergies' modules for the production of voluntary fine movements (Caggiano et al., 2016) also in humans. This provides new evidence that muscle synergies should be considered like sub-entities of the motor system and not just computational explanations of the sEMG signals collected.

It is interesting to outline that the reported results came only from patients with lesions in the right hemisphere for positive associations, and only from patients with lesions in the left hemisphere for negative associations. These two groups were significantly different only for distance from stroke onset. On average, patients with right hemisphere lesions were enrolled at 1 year, while patients with left hemisphere lesions at 5 months after stroke. Previous evidence demonstrated that reduction in the number of muscle synergies depends on a phenomenon called "merging" of modules (Cheung et al., 2009b), which is related to the level of motor function preserved after stroke. Thus, the higher the motor function is preserved after stroke, the more likely the number and shape of muscle synergies' modules are the same in both the affected and unaffected arms. As well established, motor function is suddenly abolished soon after stroke, but improves constantly along the recovery process; thus, patients closer to stroke onset are expected to represent the ones with less capability of actuating voluntary movements than patients more distant from stroke. Moreover, it is largely acknowledged that at 1 year after stroke all the spontaneous recovery mechanisms have been fully exploited and the ability to produce voluntary behaviours is expressed at its maximum in stroke survivors. On these bases, it might be argued that in our sample the left hemisphere injured patients, being the

closer to stroke onset, were more informative on the brain structures needed to maintain the ability to produce voluntary movements, conversely the right hemisphere injured patients provided more information on the brain structures actually responsible for the production of voluntary motor behaviour. Nevertheless, since the differences between stroke in the left or right hemispheres cannot be explained in terms of muscle synergies (i.e. no differences in terms of numbers of modules) in our sample, it can be argued that the recovery of motor function after stroke is a multi-factorial process involving many unknown variables. More powerful analyses (e.g. larger sample of patients, optimised algorithms for sEMG factorisation), therefore, will be needed in future studies to test the hypothesis that muscle synergies are robust biomarkers to target the recovery of motor function, with a clinical meaning. With reference to computational frameworks recently proposed for neurorehabilitation (Frey et al., 2011, Pomeroy et al., 2011, Reinkensmeyer et al., 2016), this finding gives indications that a much larger and more distributed neural network is involved in the activation of muscle synergies' modules in the affected arm, than in the unaffected arm.

The reported results come from a VBM correlational study including a sample of 43 patients, whose upper limb motor function was impaired at different levels, as a consequence of a stroke with variable time distance onset. To understand which mechanisms of motor control were disrupted by the stroke and what type of correlation there was between the characteristics of the brain lesions and their clinical manifestation, all patients were studied in detail using MRI imaging, neurophysiological and clinical measures. The findings provide encouraging evidence in support of the hypothesis that it is possible to observe a relationship between anatomical lesions and the behavioural manifestation of motor impairment, based on the concept of muscle synergies. This insight comes from the evidence that a neural substrate for muscle synergies exists also in humans as previously demonstrated for more phylogenetically primitive animals. This analysis was targeted to study the disruption of muscle synergies for the whole brain. The results obtained confirmed the need for high integration between several brain areas to produce voluntary movements, not only at a functional, but also at a structural level. As a conclusion, this study provides adjunctive evidence that the production of complex voluntary movements in humans emerges from the cooperation of different brain areas deputed to musculoskeletal activation, as

well as to higher order cognitive functions (e.g. executive functions, motor planning and imagery), thus confirming that both the proper and a more extended motor system are needed to close the sensory-motor loop, as difficulty of motor tasks increases. Moreover, the result of this integration is linearly transferred to the periphery through inter-neuronal circuitries represented by muscle synergies. As current limitations of results reported in this chapter, it has to be acknowledged that findings come from a cross-sectional study with patients not treated with either innovative or conventional rehabilitation therapies. Thus, at this stage it is arguable that our findings represent the effect of stroke lesion per se, indeed what should be expected in terms of consequences when anatomy, functional activation and connections are disrupted. None is yet referable to potential positive effect of rehabilitation modalities for the restoration of motor function.

On this basis, it is also difficult to infer whether the reduction in the number of muscle synergies we observed can be interpreted as the homologous of the so called “merging” phenomenon (i.e. loss of independent activation of a single muscle synergy) described for the lower limb by Clark and co-workers (2010) and observed also in the upper limb by Cheung and co-workers (2012b). However, none of the cited studies considered any link between merging of muscle synergies and loss of tissues in associated brain structures. This is the first time that such an association is confirmed by experimental data, providing support for the hypothesis already tested in other vertebrates (Saltiel et al., 2001) that a biological representation of muscle synergies might be structurally encoded in the motor system.

The results from the DTI analysis were the most detailed to study the implications of specific anatomical lesions affecting motor control, in terms of integrity of white matter bundles. Our findings confirmed the hypothesis, stemming from animal models, which considers muscle synergies as hard-wired networks biologically represented in the brainstem. In fact, together with the corticospinal tract, a reduction in the number of muscle synergies’ modules was found to correlate positively with disruption of white matter tracts in the brainstem and in the Putamen and Caudate areas.

This finding suggests that muscle synergies might have a distributed anatomical representation converging in the corticospinal tract to activate populations of muscles as a single functional unit. This result represents an outstanding step forward in the interpretation of alterations of motor behaviour after stroke, since

muscle synergies are emerging as a potential biomarker for monitoring the recovery process in stroke survivors.

4.4. Conclusion

The described results come from three correlational studies including a sample of 67 patients in all (i.e. 11 patients for the ROI study, 43 patients for the VBM study, and 13 patients for the DTI study), whose upper limb motor function was impaired at different levels, as a consequence of a stroke occurred at variable distance in time. To understand which mechanisms of motor control were disrupted by the stroke and what type of correlation there was between the characteristics of the brain lesions and their clinical manifestation, all the patients were studied in detail using MRI imaging, neurophysiological and clinical measures. The findings provided encouraging evidence in support of the hypothesis that it might be possible to observe relationships from anatomical lesions to behavioural manifestation of motor impairment, based on the concept of muscle synergies. This insight comes from the evidence that a neural substrate for muscle synergies exists also in humans as previously demonstrated for more phylogenetically primitive animals. In particular, the results suggested that when the brain lesion involves deep structures in the white matter, mostly represented by fibres of the corticospinal tract, muscle synergies' modules in the affected upper limb are more likely to be reduced in number.

This analysis was targeted to study the disruption of muscle synergies for the whole brain. The results obtained confirmed the need for high integration between several brain areas to produce voluntary movements, not only at functional, but also at structural level. As a conclusion, these studies provide adjunctive evidence that the production of voluntary movements in humans emerges from the cooperation of different brain areas deputed to musculoskeletal activation, as well as to higher cognitive functions (e.g. attention, vision, semantic recognition), thus confirming that both the proper and a more extended motor system are needed to close the sensory-motor loop, as much as the difficulty of motor task increases. Moreover, the result of this integration is linearly transferred to the periphery through inter-neuronal circuitries represented by muscle synergies. The concept of muscle synergies currently represents one of the most promising methodologies to interpret the dynamic of voluntary motor behaviour, based on

biological data (i.e. sEMG). This approach has several implications for rehabilitation, as it allows a computational interpretation of clinical pictures, up to now described only by measures of impairment somehow related to functional activities in real environment. Moreover, the so called “muscle synergies analysis” (Safavynia et al., 2011) allows clinicians to provide patients with therapies that are tailored on their own personal impairment, ideally closing the sensorimotor loop in a more physiological manner and providing them with personalised programmes of recovery. In addition, considering the need to understand better the neurophysiological mechanisms involved in the recovery process after stroke, muscle synergies represent a potential biological marker for monitoring whether effective rehabilitation therapies might induce tuning of muscle synergies’ activation and shaping of their structure. Finally, characterizing particular patterns of muscle synergies as predictors of functional outcomes represents a challenging opportunity for tailoring rehabilitation therapy so that it is targeted to the specific needs of a single patient.

4.5. Limitations

Some limitations affecting the robustness of the current findings should be acknowledged. First, the small sample size for each single study (i.e. 11 patients for the ROI study, 26 patients for the VBM study and 13 for the DTI study) didn’t allow strong inferences on the major part of those relevant questions which are of interest to neuroscientists as well as clinicians. In fact, despite the evidence from a previous study demonstrating that “muscle synergies analysis” works efficiently also in small groups (Cheung et al., 2009b), the possibility to explore subgroups characteristics is heavily affected by the sample size dimension. On the other hand, the characteristics of the enrolled patients are not yet representative of the wide range of potential clinical pictures induced by a stroke. Second, the cross-sectional design did not allow the observation of any change of muscle synergies when patients are rehabilitated and did not permit the observation of over time changes in their correlation with structures involved by the brain lesion. As a consequence, the characterisation of muscle synergies during the recovery process needs to be investigated.

Third, the lesion wasn't masked when 3DT1W sequences were processed, thus some distortion might have occurred in the normalisation processing, potentially biasing the anatomical interpretation of the results.

Overall, there is no final agreement among researchers about what would be the gold standard when using automated computational methods to target anatomical structures in the brain and this agreement is still far from being in place. All the chosen methods have intrinsic limitations in identifying the correct anatomical structures, because of major issues in data acquisition and a priori statistical assumptions leading to potential misalignment in anatomy reconstruction.

A first point of strength in the reported experiments comes from the large amount of evidence provided by research on animal models that have been referred to, for the development of the current experimental hypotheses. Secondly, the theoretical reference framework we used was tested by three different techniques and all the results were coherent with the possibility that the findings were not outputs from computational biases, but feasible biological phenomena.

In addition, as a further limitation it needs to be acknowledged that variability in distance from stroke of recruited patients still represents a major issue for reaching any final conclusions which might be of clinical relevance. In fact, large part of the knowledge about plasticity mechanisms comes from animal models developed to study functional recovery from immediately after stroke up a bigger distance in time from onset in that which can be referable as the sub-acute phase in humans surviving a stroke. A significant percentage of patients recruited for studies reported in Chapter 4, however, were in their chronic phase (i.e. over one year after stroke) of the disease. Thus, it remains difficult to clarify and speculate on which part of the findings of these studies might be the outcome of spontaneous recovery mechanisms, and which can be, instead, the result of rehabilitation induced neural plasticity.

5. EFFICACY OF VIRTUAL REALITY-BASED TREATMENT FOR RECOVERY OF UPPER LIMB, AFTER STROKE: NEUROIMAGING EVIDENCE

5.1. Background

5.1.1. The recovery of upper limb motor function after stroke

Impairment of the motor function is commonly the most disabling consequence occurring after stroke. The clinical manifestation is characterised by reduction, if not complete abolishment, of gross and fine motor dexterity in the entire side of the body contralateral to the affected hemisphere. Especially in the acute phase, functional recovery is supported by the neural tissue residual capacity for plasticity, which is aimed at promoting functional cortical reorganisation for the restoration of lost abilities.

The aim of neurorehabilitation is to sustain and maximise the process of brain reorganisation, contemporarily avoiding potential maladaptive neuroplasticity, to ensure the best possible outcome for each individual patient. As a whole, neurorehabilitation is devoted to the development of specific therapeutic modalities, able to induce the best neuroplastic modification leading to desirable permanent effects. In recent years, innovative therapeutic methods, the rationale of which is based on motor learning principles, have been shown to provide better clinical effects than traditional methods. The best evidence in this regard has been produced for task-specific training (Rensink et al., 2009, Timmermans et al., 2010), constraint-induced movement therapy (Corbetta et al., 2010), robotic training (Mehrholz et al., 2012, Mehrholz et al., 2007), motor imagery (Pollock et al., 2014) and virtual reality training (Laver et al., 2015b).

After a stroke most brain lesions in the cortical and subcortical motor areas result in severe impairment of functioning of the upper limb, the recovery of which still remains unsatisfactory in 33% to 66% of patients, at 6 months from onset (Nijland et al., 2010). This evidence is argued to be responsible for long term lack of autonomy in ADLs, in stroke survivors. Determining which impaired functions are responsible for poor recovery is fundamental for tailoring motor rehabilitation to each individual's own characteristics. Based on the International Classification of

Functioning, Disability and Health (ICF) several functions are implicated for the production of successful voluntary movements, including: joint mobility, muscle power and tone, or deviation of musculoskeletal districts like shoulder or hand. All of them are potentially affected following a stroke, but the weight of their involvement vary within each individual clinical picture. A sequential timeline of motor function restoration after stroke has been proposed since the late sixties by Brunnstrom (1966) and till the most recent “computation, anatomy and physiology” model of Frey (2011), the most accredited view was that an extended knowledge of the brain mechanisms underpinning motor control are needed for the development of innovative rehabilitation modalities.

The upper limb is the most complex musculoskeletal district the brain needs to control voluntarily to realise an effective interaction with the external environment. Its intriguing biomechanical architecture allows coverage of a large number of complex tasks, by real-time smooth coordination of a huge number of degrees of freedom. Moreover, all tasks can be performed either with only one limb or both. The recovery of bilateral upper limb functioning after stroke has been modelled by Han and co-workers (2008), combining three different computational models: the first for the motor cortex, the second simulating a reward-based decision making paradigm and the third emulating a physical therapy intervention based on repetition of reaching movements. In their experiment the authors simulated a cortical lesion in the motor cortex model that was subsequently trained by the physical therapy model. The results demonstrated that the presence of supervised learning rules controlled by the reward model led to a better repopulation of the lesioned motor cortex and to better behavioural results (i.e. lower errors in reaching a target), than their absence. These findings support the hypothesis that the specific contents of physical exercises are the main drivers for optimal regaining of the best motor function. Namely, stimulating the reward-based circuits via specific augmented feedbacks, such as knowledge of performance and knowledge of results, is a successful path to follow to improve motor function after stroke. Another important finding was the identification of a threshold of number of task repetitions to practice (about 420 trials), with lower numbers leading to no meaningful rehabilitation effect. The authors postulated that the absence of a sufficient dose of task repetitions after stroke might promote maladaptive plasticity inducing the “learned non-use” phenomenon. The “learned non-use phenomenon” was described in monkeys like the learning process of

suppressing voluntary movements in the deafferented limbs (Taub et al., 2006). This event is partially reversible by forcing the limb to be moved using shaped stimuli. As a whole, all the augmenting techniques like the “constraint induced movement therapy” (Taub et al., 1998, Wolf et al., 2008, Wolf et al., 2010), or the virtual reality approach (Piron et al., 2001b, Piron et al., 2010, Piron et al., 2005) are based on this rationale, given the brain lesion as the central onset of peripheral musculoskeletal districts deafferentation and rehabilitation aimed to revert the “learned non-used” phenomenon.

In this chapter the results from a clinical and imaging experiment comparing two groups of stroke patients undergoing virtual reality or conventional rehabilitation of the upper limb are presented. The aim was to test whether the exposure to different therapeutic modalities providing different amount of augmented feedbacks for motor learning, induced a different functional reorganisation of the brain after treatment.

5.1.2. Evidence on detection of functional MRI activation in the white matter

Vessels in the white matter are able to support detectable hemodynamic changes due to brain activation, but:

- blood oxygenation level dependent (BOLD) contrast is three to seven times lower in the white matter, than in the grey matter
- BOLD signal is meant to originate mainly from post-synaptic potential (grey matter), than from action potentials. Post-synaptic potential accounts for less than 1% of the energy consumption in the white matter thus unlikely to be the substrate for detectable BOLD signal. Most of the signalling is mediated by spiking activity in the white matter which represents action potentials, but evidence has been reported that changes in BOLD signal might be related also to spiking activity to localise fMRI activation (Rauch et al., 2008).

White matter represents almost 50% of the brain volume and there is emerging evidence that detection of BOLD contrast in the white matter is not an artefact. Nevertheless, there is still poor reporting in the literature as compared with ones related to grey matter activation.

Poor literature reporting of BOLD fMRI contrast in the white matter might depend on established practices that have considered historically this signal as, respectively:

- A nuisance regressor to be subtracted during pre-processing to improve detection of BOLD changes in the grey matter.
- A signal not eligible to be acquired by T2* or T2 standard sequences, thus not to be analysed.
- A potential artefact (e.g. motion, partial volume effects, physiological noise), to be ignored when present in the results.

Other potential biases coming from established methodologies, beliefs and other potential misinterpretation of BOLD fMRI signal in the white matter relies on neurophysiological assumptions, which not all have been demonstrated to be realistic. Vessels section is almost the same in both grey and white matter, but density is significantly lower in the white matter (50% less than in the grey matter). As a consequence, the whole blood flow circulating in the white matter has been estimated to be one-tenth to one-third smaller than in the grey matter. Thus, higher field scanners and specific T2* and T2 sequences might help to increase the sensitivity of detecting BOLD contrast in the white matter.

Recently, despite the limitations reported above, evidence has been provided using a 1.5T scanner (Fabri et al., 2011), that BOLD fMRI signals is reliably detectable in the corpus callosum. More specifically, different experiments with healthy subjects demonstrated that the corpus callosum is specifically activated anteriorly by taste, centrally by motor tasks (e.g. bimanual coordination), at the midpoint and posteriorly by tactile tasks, posteriorly by visual stimuli. Moreover, the genu of the corpus callosum is activated when administering visual tasks (Tettamanti et al., 2002) and the splenium when administering recognition tasks (Myers and Sperry, 1985). Also the internal capsule has been reported to be activated when performing fine motor tasks, such as finger tapping (Mazerolle et al., 2013).

Ding and co-workers (2013) have reported white matter functional connectivity with seed based resting state fMRI. Seed were extracted in the white matter from previous structural DTI analysis in the same subjects. Results showed greater correlation patterns of BOLD signal along the whole white matter tracts, than in other directions, demonstrating that integrity of fibres is associated with the presence of valid BOLD fMRI contrast.

In chapters 3 and 4 of this thesis the fundamental role of subcortical brain structures and of white matter fibre tracts in the interpretation of upper limb motor function impairment following a stroke has been highlighted. Given the evidence listed in this paragraph and considering that BOLD signal in the white matter is frequently reported as associated with motor tasks execution, in the following experiment the results will consider also white matter as a feasible target of analysis to explore functional changes in the related brain structures.

5.2. Experiment 5: Brain functional reorganisation in stroke patients undergoing virtual reality based treatment for rehabilitation of upper limb motor function

5.2.1. Introduction

Virtual reality is considered among the newest technologies available for medical purposes, together with robotics and devices for neuromodulation (e.g. transcranial magnetic stimulation, TMS; transcranial direct-current stimulation, tDCS). Its clinical effectiveness has been demonstrated very recently with an acceptable level of evidence (Laver et al., 2015a, Laver et al., 2015b), but consistent amount of research is still needed to understand which are the best modalities for treatment administration, which are the most suitable populations to be treated with this technique and primarily which are the basic mechanisms underpinning its efficacy. Very few studies have been published reporting outcomes of brain functional changes resulting from the interaction with a virtual environment.

August and collaborators (2006) reported the first pilot study on a single healthy subject. The experiment was carried out with a 3T MRI scanner using a block-design paradigm. The subject wore a sensorised glove controlling the representation of a virtual hand (condition 1) or an oval shape (condition 2) that was displayed through MRI compatible goggles. While lying in the scanner a subject was asked to observe first a finger flexion-extension movement, then to replicate it voluntarily at a predetermined frequency of 1 Hz. Conditions 1 and 2 were compared to study whether different representations of virtual objects might

activate different brain areas. The results indicated that secondary motor areas compatible with the hand cortical representation (e.g. dorsal premotor and supplementary motor areas, anterior cingulate cortex, anterior intraparietal cortex, superior temporal gyrus) were activated during condition 1, but not during condition 2. The authors concluded that cortical activation of movements' imitation is related to the features of the displayed virtual objects, thus this issue needs to be considered when using virtual reality for rehabilitation purposes, with the aim to promote the best cortical reorganisation.

The first pilot study carried out with stroke patients has been published by Saleh and co-workers (2012), investigating the effect of 2 weeks of robot-aided combined with virtual reality training for the upper limb. Two patients were studied collecting clinical outcome measures and functional connectivity analysis both in resting state and task based conditions. The chosen region of interest (ROI) for functional connectivity analysis was the primary motor area (M1), while the motor task was the same as described by August (2006). Both patients improved their clinical outcome measures, and functional connectivity related to resting state and task based conditions changed similarly after treatment, in each patient. Nevertheless, while for one subject connectivity between the affected M1 and its contralateral homologous decreased, for the other patient functional connectivity increased. The authors concluded that resting state analysis is a powerful tool to study functional brain reorganisation in stroke patients undergoing rehabilitation treatments. In fact, the results from resting state are similar to the ones coming from block design task based paradigms, thus overcoming the issue related to being unable to test patients who are not able to produce voluntary movements, but patterns of functional reorganisation are subject-dependent, however.

The first longitudinal study on stroke patients undergoing a virtual reality game-based therapy for the upper limb has been published recently by Orihuela-Espina and co-workers (2013). They enrolled 8 patients that were studied with both behavioural and block-design task based fMRI outcomes. The results indicated that behavioural improvements are matched with contralesional activation of the motor cortex, activation of cerebellar areas and compensatory activation of the prefrontal cortex.

Finally, Bao and collaborators (2013) compared 5 patients undergoing virtual reality game-based therapy with 18 healthy subjects exposed to the same training. The MRI outcomes were collected before and after treatment and 12

weeks after the end of training. Results indicated that soon after the end of virtual reality training, the contralateral sensorimotor cortex is mainly activated in stroke patients as compared to healthy subjects, targeting this area as the driver for recovery of upper limb motor function after stroke.

To date, the mechanisms involved in stroke patients undergoing virtual reality rehabilitation for the recovery of upper limb motor function have been explored with weak experimental designs and results were reported from very small cohorts of consecutive patients. The level of evidence is low, thus not allowing the inference of any definitive conclusion. To overcome this lack of current knowledge, a pilot randomised controlled trial (RCT) was planned to address the following experimental questions:

- Which are the functional reorganisation mechanisms occurring after virtual reality based rehabilitation of the upper limb, after stroke?
- Is effectiveness of a VR-based treatment higher than a standard therapy approach for upper limb rehabilitation in stroke patients?
- Are the brain mechanisms underpinning recovery of motor function different, because of the treatment provided?

5.2.2. Experimental hypotheses

The aim of the proposed clinical trial was the assessment of the efficacy of a virtual reality based intervention for treatment of upper limb motor impairment after stroke. Moreover, imaging and neurophysiological data were collected according to the protocol described in the following paragraphs, for the detection of the neural mechanisms supporting the recovery process as a consequence of rehabilitation.

The experimental hypotheses to address this aim were:

- H_0 : patients undergoing VR-based treatment for recovery of upper limb motor function show the same pattern of brain functional reorganisation (fMRI) as patients treated by conventional therapy.
- H_1 : patients undergoing VR-based treatment for recovery of upper limb motor function show greater brain functional reorganisation (fMRI) that is related to specific brain volumes representing motor control function, than patients treated by conventional therapy.

5.2.3. Methods

A single blind randomised clinical trial (RCT) has been designed with stroke patients recruited among those admitted to the Neurorehabilitation Department at the San Camillo Foundation Hospital in Venice (Italy) (Figure 5.1). The reporting in the following paragraphs accomplishes the “Consolidated Standards of Reporting Trials (CONSORT)” (Moher et al., 2001) acknowledged as the international standard for transparent reporting of RCTs. Ethical approval for this study was granted by the Ethics Committee of the Venice province (Prot. 2015.14).

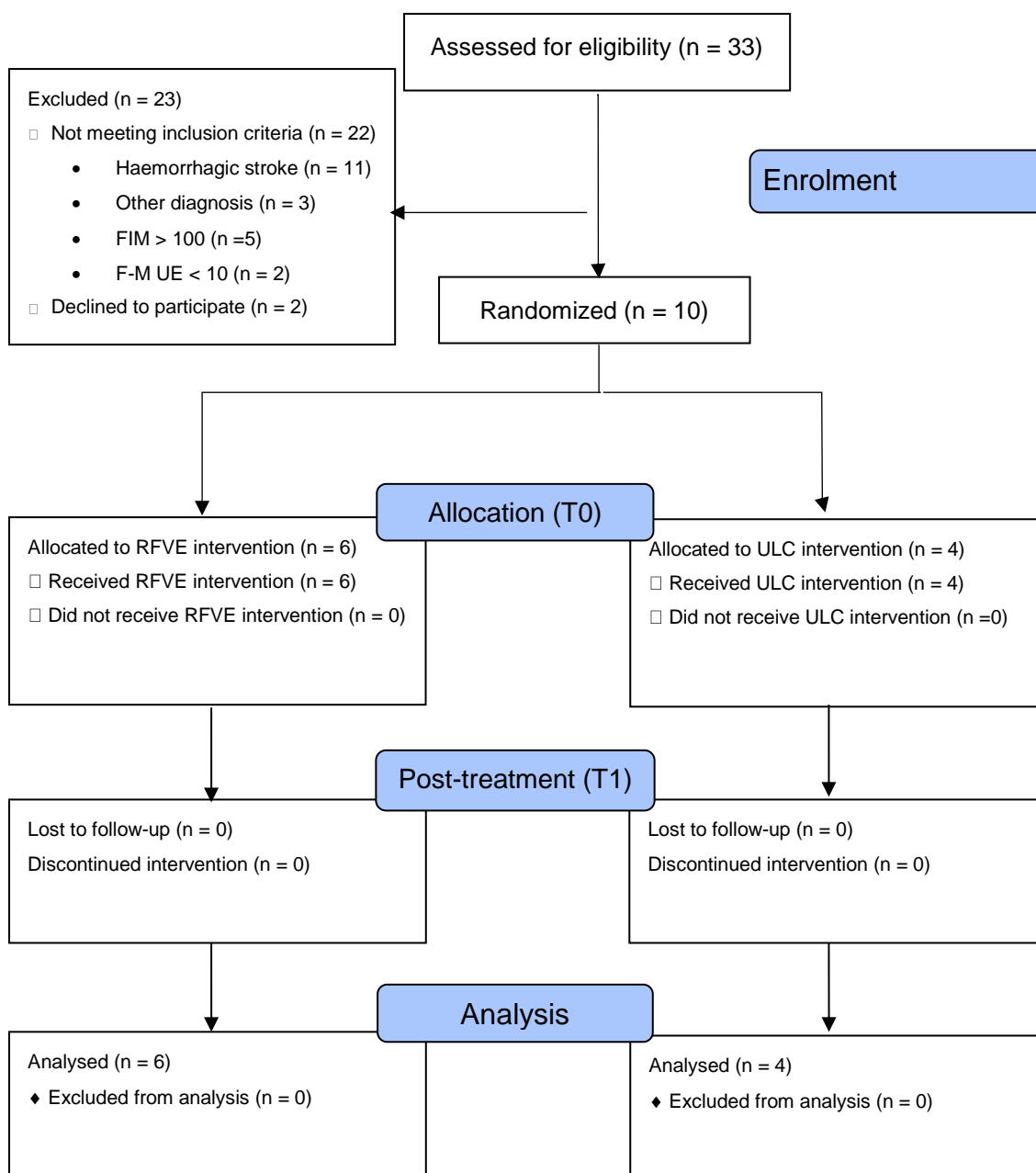


Figure 5.1. Consort like study flow diagram.

RFVE: Reinforced Feedback in Virtual Environment; UCL: upper limb conventional rehabilitation; FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity scale.

All those patients able to give consent to participate in the study and meeting the inclusion/exclusion criteria described in the “Population” paragraph were considered eligible for participation in the study. The enrolled patients were allocated to one of the following groups according to a simple randomization procedure:

1. Reinforced Feedback in Virtual Environment (RFVE) group (experimental group): the patients were asked to execute motor exercises with the upper limb while interacting with a virtual environment. During the execution of the motor task a virtual representation of the arm’s end effector was displayed on a screen placed on a wall, amplifying the results of the performed path.
2. Conventional rehabilitation treatment (control group): the patients were asked to follow with their arm simple or complex trajectories, to reach for different target positions, to grasp and manipulate objects.

The patients enrolled in both groups underwent clinical and instrumental assessments, before (T0) and after (T1) treatment.

A simple random number sequence was generated by a computer and the allocation concealment was guaranteed using sequentially numbered, opaque sealed envelopes.

5.2.3.1. Population

The stroke patients eligible for this trial were identified according to the 7th edition of the Italian guideline for prevention, care and rehabilitation of stroke (Stroke Prevention and Educational Awareness Diffusion - SPREAD) (Inzitari and Carlucci, 2006). Every patient was classified according to the Oxford Community Stroke Project (OCSP) criteria (Bamford et al., 1991) and the diagnosis confirmed by CT or MRI examinations. Once enrolled in the study every patient underwent MRI assessments. Each MRI session included a three dimensional T1-weighted structural scan, a T2-weighted axial scan, and a fluid attenuated inversion recovery (FLAIR) scan, all acquired on a 1.5 Tesla MRI Philips Achieva® system available at the I.R.C.C.S. San Camillo Foundation Hospital, Venice (Italy). Images have been analysed using the Statistical Parametric Mapping software (Wellcome Trust Centre for Neuroimaging).

All patients meeting the following criteria were considered eligible for enrolment in this study.

Inclusion criteria:

- diagnosis of stroke occurred at least 6 months before the enrolment;
- a score between 1 and 3 on the upper limb sub-item on the Italian version of the National Institute of Health stroke scale (IT – NIHSS) (Pezzella et al., 2009)
- a score lower than 100 points out of 126 on the Functional Independence Measure (FIM) scale (Keith et al., 1987);
- a score higher than 10 out of 66 on the Fugl – Meyer upper extremity (F-M UE) scale (Fugl-Meyer et al., 1975), indicating the presence of residual motor function sufficient to avoid frustration caused by inability to interact with an artificial environment. The F-M UE score was considered as the primary outcome measure for sample size calculation.

Exclusion criteria:

- non stabilised fractures;
- diagnosis of depression/delusion;
- associated traumatic brain injury;
- drug resistant epilepsy;
- evidence of ideomotor apraxia (medical history);
- evidence of visuospatial neglect (medical history);
- severe impairment of verbal comprehension defined as a score higher than 13 errors on the Token test (i.e. score < 58 out of 78 Tau points).

Such criteria guaranteed the enrolment of a representative population of stroke patients still needing an intensive rehabilitation program to achieve a level of functional recovery as close to the level of independence and autonomy they had before onset of their stroke.

5.2.3.2. Sample size calculation

In a recent Cochrane review (Laver et al., 2011) the authors provide estimates of the effect size of improvement achieved by virtual reality aided treatment in comparison to that achieved by the use of standard physical therapy, to regain

upper limb motor function, after a stroke; the Fugl-Meyer upper extremity (F-M UE) scale was used as the primary outcome measure. The F-M UE scale is the most frequently used measure in stroke rehabilitation research; it is an ordinal scale with scores ranging from 0 to a maximum of 66 for the upper limb motor performance. The upper limb section has 33 items, which include testing of reflexes, movement observation, testing of grasp abilities and assessment of motor co-ordination. The score for each item is: 0 Unable to perform; 1 Able to perform in part; 2 Able to perform (Deakin et al., 2003). Its clinimetric properties are known and the inter-rater reliability ($r=0.98$ to 0.99), the intra class correlation ($ICC=0.99$) (Croarkin et al., 2004) and the minimal clinically important difference (MCID = 9 pts.) have been estimated (Arya et al., 2011b). The experimental and control treatments proposed in this project are based on the same principles as the one reported by Laver and co-workers (2011) and this meta-analysis demonstrated that the patients treated with virtual reality obtained a mean improvement of 4.43 points [CI 95%: 1.98 – 6.88] on the F-M UE scale when compared with patients undergoing standard treatments. Based on these premises, the calculated effect size from data from the meta-analysis is $d = 0.54$. Arya and co-workers (2011b) have reported a MCID of 9 points on the F-M UE scale. However, the one calculated by Laver and co-workers (2011) should be considered as more precise and large enough to detect a MCID due to VR based rehabilitation compared with conventional rehabilitation of the upper limb, after stroke. Power calculation indicated that 55 patients per group have to be enrolled for $\alpha=0.05$ and $1-\beta=0.8$. Nonetheless, considering an expected drop-out rate of 20% (i.e. 11 patients per group) the sample size should be increased to 66 patients per group (i.e. 132 overall).

5.2.3.3. Participants

For this pilot study 10 right-handed participants meeting the inclusion criteria were enrolled in the clinical trial (figure 5.1). Three of them had a lesion in the right hemisphere and the other seven a lesion in the left hemisphere. According to the randomisation sequence, four of them (all left hemisphere lesioned) were referred for control treatment, while the remaining six were allocated to the experimental treatment (3 with right hemisphere lesion and 3 with left hemisphere lesion). The demographic and clinical characteristics of the enrolled patients are reported in table 5.1.

Table 5.1. Demographic and clinical characteristics of the patients sampled for resting state analysis.

Outcome	RFVE (n= 6)	CTRL (n= 4)	p
Age, years	60.00±12.62	57.00±13.06	0.592
Sex, M/F	5/1	4/0	0.389
Hemisphere affected (R/L)	3/3	0/4	0.091
Time since stroke onset, months	9.68±5.86	4.94±3.28	0.171
Synergies affected limb (n)	8.67±1.21	8.00±1.41	0.352
F-M UE	36.50±16.33	45.00±25.18	0.476
FIM	91.00±33.79	119.00±6.48	0.038*
RPS	24.33±10.31	24.75±15.65	0.762
NHPT	0.05±0.08	0.22±0.27	0.476

M: male; F: female; R: right; L: left; FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity. Results are reported as mean and standard deviation, m (SD). Mann-Whitney U test was used to test differences in demographic data between patients with lesions in the right or left hemisphere, statistical threshold was set at $p < 0.05$ (*).

5.2.4. Materials

5.2.4.1. Interventions

The experimental intervention was the reinforced feedback in virtual environment (RFVE), already described in chapter 3 (see paragraph 3.2.4.1), while the control treatment was based on upper limb conventional (ULC) rehabilitation.

The ULC program was based on traditional rehabilitation techniques aimed at restoring upper limb motor functions and based on the Bobath principles (Bobath, 1990). The patients were asked to perform a wide range of exercises, including: shoulder flexion-extension, abduction-adduction, internal-external rotation, circumduction, elbow flexion-extension, forearm pronation-supination, hand-digit motion. Standardized instructions and modalities were followed when providing exercises to the patients in order to control for any variability in leading the therapy session due to the therapist. For example, the patients were asked to perform different reaching exercises (e.g. frontally, diagonally, from a low to a high surface or to different targets on a plane, while seated or standing); abducting the shoulder in sitting position using free weights; sliding arms and hands on a table; taking objects to the face or mouth; pouring liquids or objects between containers; pronating and supinating the forearm to a target. The programme was designed using the free access “PhysioTherapy eXercises” web tool (<https://www.physiotherapyexercises.com/>), developed by the Australian

Physiotherapy Association in partnership with the World Confederation for Physical Therapy (WCPT).

To facilitate motor skill relearning, patients underwent a sequence of motor tasks of increasing difficulty. Firstly, the patients were asked to control isolated movements without postural control and subsequently postural control was included. Later, complex movements were practiced. Based on residual motor capacities, the therapist could be placed next to the hemiparetic side of the patient, while seated on the edge of the therapy bed, to support the trunk as well as the arm and to assist them during the execution of the motor task. Conversely, when a patient could control its trunk the therapist was seated in front of him controlling the correct execution of the task and providing verbal instructions to improve motor performance.

The usage of “PhysioTherapy eXercises” allowed the improvement of homogeneity in the delivery of conventional treatment. Detailed descriptions of the exercises’ modalities have been reported in the Appendix 1.

5.2.4.2. Dose of intervention

In both intervention groups a physical therapist was constantly present during the session and modified the rehabilitation program in accordance with the patient’s current motor capacity and needs. At the end of every session, the therapist discussed with the patients the results obtained during that therapy session.

As demonstrated by Laver and co-workers (2011), VR based therapies are effective as conventional motor therapies, when provided for more than 15 hours. Based on this evidence, in both groups the interventions lasted 1 h/day, 5 days/week, for 4 weeks for a total of 20 sessions (i.e. 20 treatment hours). In the case that one or more sessions were missed, the same were recovered within the admission period to the hospital, up to the completion of the planned protocol.

5.2.4.3. Outcome measures

The following outcomes were recorded along the entire duration of the trial (reported divided for time of registration).

Enrolment (inclusion / exclusion criteria):

- IT – NIHSS
- Functional Independence Measure (FIM)

- Fugl-Meyer Upper Extremity (primary outcome)

Allocation (T0):

- Reaching Performance Scale (Levin et al., 2004),
- Modified Ashworth Scale (Bohannon and Smith, 1987),
- Nine Hole Pegboard Test (Wade, 1989),

Follow – up (T1):

- Fugl-Meyer Upper Extremity
- Functional Independence Measure (FIM)
- Reaching Performance Scale,
- Modified Ashworth Scale,
- Nine Hole Pegboard Test,

All the adverse events, from enrolment to the end of the study, were registered and considered for safety analysis.

5.2.4.4. Statistical analysis

The demographic and clinical characteristics of enrolled patients are reported as mean and standard deviation. A two way analysis of variance (ANOVA) was used to test the interaction of time (within factor) and treatment (between factor) influencing the final clinical outcomes. The distribution of the clinical variables were studied using the Kolmogorov – Smirnov test, according to these results parametric or non-parametric test were used to study differences within and between groups.

5.2.4.5. Magnetic resonance image (MRI) acquisition

Each patient underwent a standard structural MRI acquisition protocol which included a fluid-attenuated inversion recovery (FLAIR) scan, a three dimensional T1-weighted (3DT1W) structural MRI scan and a 32 directions diffusion weighted imaging (DWI). These scans were acquired on a 1.5T Philips Achieva MRI system. Coronal FLAIR acquisitions were obtained for a detailed assessment and characterization of cerebral infarctions (TR/TE/inversion time [T1]/NEX = 11000/140/2600/2; matrix size = 256 x 256; FOV = 240 mm; slice thickness = 7.0 mm with no gap between slices, in-plane spatial resolution of 0.898 x 0.898

mm/pixel). The 3DT1W scan was acquired with a Turbo Field Echo sequence. The Voxel dimensions were 1.1 X 1.1 X 0.6 mm and field of view (FOV) was 250 mm with a matrix size of 256 X 256 X 124. Total acquisition time was 4 min 27 s (repetition time, RT: 7.4 ms, echo delay time, TE: 3.4 ms and flip angle 8 degrees, number of sagittal slices 280). The DWI 32 directions scan was acquired to analyse the bundle's integrity in the white matter tracts. The voxels size was 2.5 X 2.5 X 3.0 reconstructed as 1.67 X 1.67 X 3.0, with a FOV of 240 X 240 X 135 (RL, AP, FH). The total acquisition time was 27'11" for 45 parallel slices acquired interleaved with no gap and a thickness of 3mm (TR: 8280, TE: 70ms, flip angle 90 degrees, b factor: 600).

The echo planar imaging (EPI) parameters for resting state acquisition were:

- Scan Duration [sec]: 264
- Max. number of slices/locations: 20
- Max. number of dynamics: 120
- Repetition time [ms]: 2000
- FOV (ap,fh,rl) [mm]: 230; 120; 230
- Repetition time = 2 s
- Echo delay time = 50 ms
- Flip angle: 90°
- Voxel dimensions: 3.28 x 3.28 x 6.00 mm³
- Field of view: 230 mm

Two EPI scans were acquired. Total scanning time was 63 minutes and 30 seconds.

5.2.4.6. Resting state analysis.

All the EPI functional scans were pre-processed before being included in statistical models for groups' comparison. In details all the EPI scans were sliced timed for timing correction, realigned for 3D motion correction and normalized to a standard template for group analyses. A band pass filter (cut-off frequencies: 0.008Hz, 0.1Hz) and a Gaussian spatial smoothing with a kernel of 6mm width, were applied. Then a seed-based analyses was run on the basis of regions of interest (ROIs) obtained from VBM analyses reported in chapter 4. The coordinates of the seeds included for signal extractions were the centres of clusters surviving a threshold of $p < 0.001$ uncorrected, with an extent threshold of 10 voxels, for the anatomical correlates of muscle synergies in the affected

arm (see Chapter 4). Then, every ROI was selected with a radius of 10mm around the centre of these clusters. For inferential analysis a general linear model (GLM) was applied in two steps. At first level analysis, the two EPI runs were connected in one series and regressed to the signals extracted for every seed, for each subject. The models estimated at first level were used as inputs for second level analysis. At the second level analysis a series of two-sample t-tests were applied to compare differences in BOLD signal within and between the two groups. For within group analysis, the activation before RFVE and ULC treatments were compared with respective activation collected at the end of the rehabilitation sessions. For differences between RFVE and ULC treatments the activations collected after treatment were compared. Family wise correction (FWE) was applied in the first instance. If there were no clusters surviving correction, the statistical threshold was set at $p < 0.001$ uncorrected, with an extent threshold of 10 voxels. All the MRI analyses were run using SPM8 (Wellcome Trust Centre for Neuroimaging).

5.2.5. Results

All the patients finished their planned treatment without reporting any side effect. Analysis of variance revealed that time was a significant factor within each group only for the F-M UE ($F = 16.72$, $p = 0.003$). Type of treatment received, however, was not a significant factor for any of the outcome measures (F-M UE: $F = 0.24$, $p = 0.639$; FIM: $F = 3.36$, $p = 0.104$; RPS: $F = 0.06$, $p = 0.806$; NHPT: $F = 1.44$, $p = 0.264$).

As reported in table 5.2 only the F-M UE improved significantly after RFVE treatment ($Z = -2.20$, $p = 0.028$), but none of the outcomes were significantly different between the two groups (FIM: $p = 0.257$; F-M UE: $p = 0.610$; RPS: $p = 0.352$; NHPT: $p = 0.610$).

Table 5.2. Clinical effect of RFVE and ULC treatments.

Outcome	RFVE (N = 6)		CTRL (N = 4)	
	Before	After	Before	After
F-M UE	36.50±16.33	44.83±13.82*	45.00±25.18	48.25±23.39
FIM	91.00±33.79	85.50±38.02	119.00±6.48	119.25±6.99
RPS	24.33±10.31	24.83±10.76	24.75±15.65	28.25±11.33
NHPT	0.05±0.08	0.11±0.20	0.22±0.27	0.23±0.25

Data are reported as means and standard deviations. FIM: Functional Independence Measure; F-M UE: Fugl-Meyer Upper Extremity; RPS: Reaching Performance Scale; NHPT: Nine Hole Pegboard Test. (*) Wilcoxon test, (#) Mann-Whitney U test, statistical threshold was set at $p < 0.05$.

5.2.5.1. Brain functional reorganisation after RFVE and ULC treatment of the upper limb.

The first comparison to be carried out was that between the brain activation detected before and after RFVE and ULC treatments, within each group. Functional brain activation did not change in the grey matter of the ULC groups. In patients undergoing RFVE treatment who had lesions in the right hemisphere the related BOLD signal showed significant changes in one region of interest (ROI) (Table 5.3).

Table 5.3. Brain areas activated after RFVE treatment (grey matter ROIs, right hemisphere).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Affected hemisphere (R)						
ROI MNI: -48, 6, -8. Talairach: -45.33, 4.75, -3.25 (BA22)						
GM	R	BA24	18	9.60	-10.90	39.44

ROI: region of interest; GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of Voxels = number of voxels for the indicated label included in the cube range of 1mm.

The results from ROIs in the white matter were more consistent than those observed in grey matter.

In the RFVE group the brain activation was increased after treatment both for patients with lesion in the right and left hemispheres (Table 5.4).

Table 5.4. Brain areas activated after RFVE treatment (white matter ROIs).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Affected hemisphere (R)						
ROI MNI: -22, -6, 46. Talairach: -21.94, -11.26, 44.28 (Sub-Gyral)						
WM	L	Sub-Gyral	27	-27.19	5.59	25.97
WM	L	Sub-Gyral	27	-32.69	9.70	22.66
ROI MNI: 18, -18, 58. Talairach: 14.91, -23.67, 54.54 (Medial Frontal Gyrus)						
WM	L	Sub-Gyral	12	-18.08	-30.20	26.33
WM	R	Cingulate Gyrus	27	17.11	-22.93	27.62
WM	R	Superior Frontal Gyrus	27	11.30	11.75	57.83
ROI MNI: 18, -24, 50. Talairach: 14.99, -28.57, 46.87 (Sub-Gyral)						
GM	R	Amygdala (BA28)	26	17.66	-6.06	-10.41
Affected hemisphere (L)						
ROI MNI: 20, 2, 46. Talairach: 16.95, -4.01, 45.62 (Cingulate Gyrus – BA24)						
WM	L	Fusiform Gyrus	27	-32.49	-49.18	-9.94
GM	L	BA19	21	-27.00	-47.86	-4.32
ROI MNI: 18, -18, 58. Talairach: 14.91, -23.67, 54.54 (Medial Frontal Gyrus)						
GM	L	Cerebellar Culmen	23	-12.24	-55.57	-3.0
GM	R	BA37	12	30.46	-47.46	-10.51
GM	R	BA37	18	23.04	-47.60	-8.85
ROI MNI: 18, -24, 50. Talairach: 14.99, -28.57, 46.87 (Sub-Gyral)						
GM	R	BA47	24	30.83	13.73	-20.93
GM	R	BA34	17	23.31	3.75	-14.79
WM	L	Corpus Callosum	27	-4.77	23.47	15.43
GM	R	Cerebellar Culmen	27	30.52	-52.53	-16.40
GM	R	Cerebellar Culmen	27	24.94	-47.26	-12.39

ROI: region of interest; GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of Voxels = number of voxels for the indicated label included in the cube range of 1mm.

Also in the ULC group a significant change in brain activation was observed after treatment, in patients with lesions in the left hemisphere (Table 5.5).

Table 5.5. Brain areas activated after ULC treatment (white matter ROIs).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Affected hemisphere (L)						
ROI MNI: -22, -6, 46. Talairach: -21.94, -11.26, 44.28 (Sub-Gyral)						
WM	R	Middle Temporal Gyrus	27	52.56	-37.45	1.62
GM	R	BA6	21	54.45	-4.27	8.40
ROI MNI: 18, -18, 58. Talairach: 14.91, -23.67, 54.54 (Medial Frontal Gyrus)						
GM	L	Cerebellar Uvula	27	-30.52	-66.60	-24.17
GM	L	BA6	12	-10.59	26.99	37.28
GM	L	BA8	12	-51.16	22.84	23.59
ROI MNI: 18, -24, 50. Talairach: 14.99, -28.57, 46.87 (Sub-Gyral)						
GM	L	BA9	16	-45.68	11.28	26.19
ROI MNI: 14, -10, 62. Talairach: 11.18, -16.55, 58.75 (Medial Frontal Gyrus)						
GM	R	Cerebellar Vermis	19	4.52	-69.51	-14.84

ROI: region of interest; GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of Voxels = number of voxels for the indicated label included in the cube range of 1mm.

5.2.5.2. Differences in brain functional reorganisation between RFVE and ULC treatments.

The resting state comparison between RFVE and ULC group after treatment was run only for patients with stroke in the left hemisphere, because all the patients in the ULC groups had stroke lesions in the left hemisphere.

The results revealed that patients treated by RFVE had significant larger functional brain activations after treatment, than patients treated by the UCL approach (Table 5.6; Figure 5.2), for ROIs in the grey matter.

Table 5.6. Brain areas activated after RFVE, but not ULC treatment (grey matter ROIs).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Affected hemisphere (L)						
ROI MNI: -48, 6, -8. Talairach: -45.33, 4.75, -3.25 (BA22)						
GM	L	BA6	18	-56.95	-4.44	33.53
WM	L	Anterior Cingulate	18	-13.77	25.43	-4.36
Affected hemisphere (L)						
ROI MNI: 4, 48, 30. Talairach: 2.44, 40.32, 35.16 (BA6)						
WM	L	Extra-Nuclear	27	-25.41	- 40.65	18.01
WM	L	Lateral Ventricle	27	-27.22	- 51.29	11.57
WM	L	Lingual Gyrus	27	-17.95	- 73.18	4.25
WM	L	Extra-Nuclear	27	-16.20	- 46.46	19.42
WM	L	Precentral gyrus	18	-51.09	6.94	13.08
WM	L	Sub-Gyral	21	-34.49	- 70.36	-4.77

ROI: region of interest; GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of Voxels = number of voxels for the indicated label included in the cube range of 1mm.

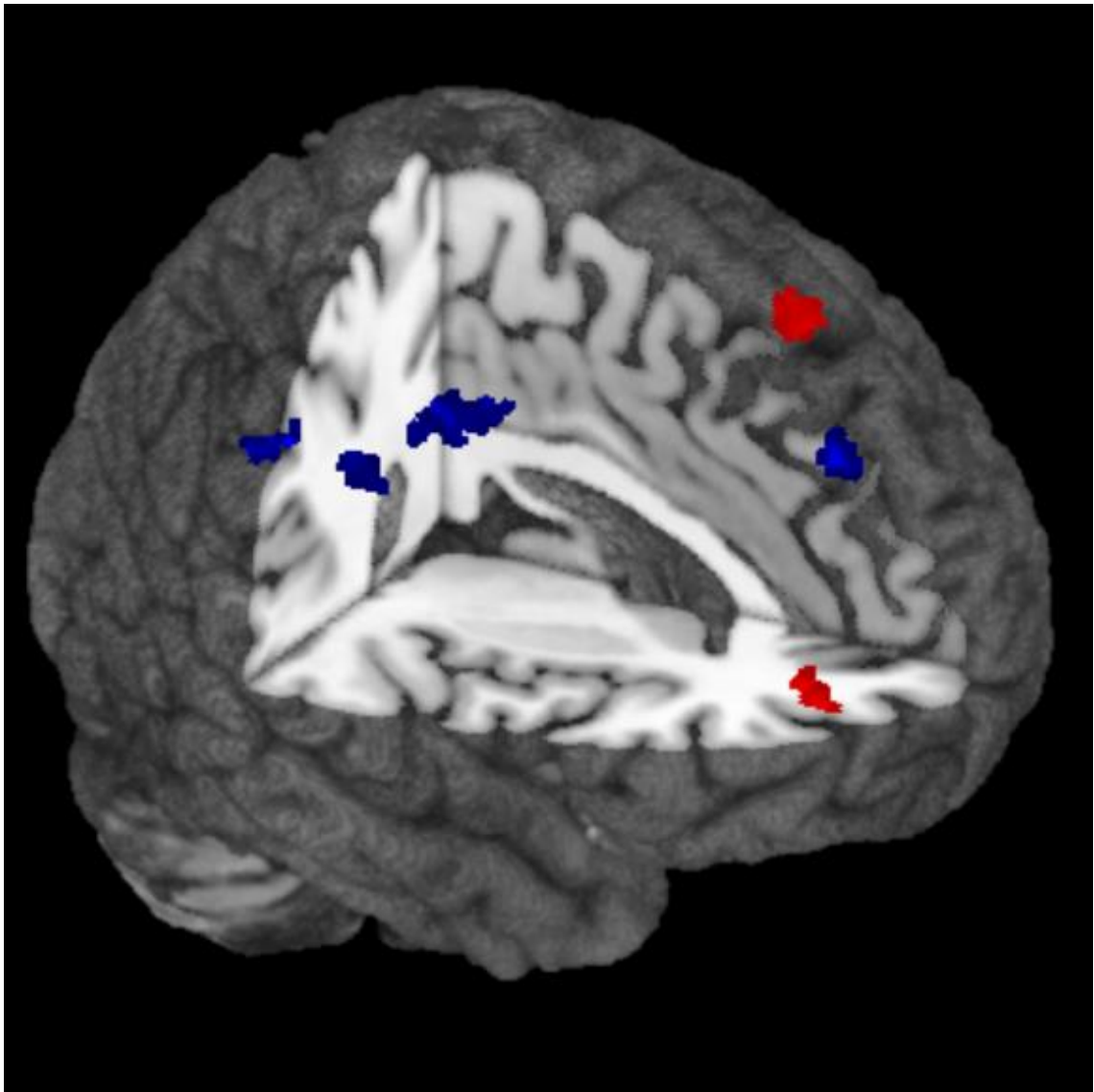


Figure 5.2. 3D rendering of differences in resting state BOLD signal after RFVE treatment, but not conventional treatment (grey matter, left hemisphere stroke).

The cluster of significant voxels ($p = 0.001$ uncorrected; threshold = 10 voxels) is showed superimposed on a standard 3D anatomical template. Red: -48, 6, -8 ROI; Blue: 4, 48, 30 ROI.

Only a small cluster of 27 voxels was found more activated in the ULC than the RFVE group, in the left grey matter of the declive of the cerebellar vermis (Talairach coordinates: -30.63x, -14.72y, -14.72z).

As for white matter activation the picture was more distributed, than in grey matter (Table 5.7, 5.8), when comparing the neuro-functional effects of RFVE and ULC treatments.

Table 5.7. Brain areas activated after RFVE, but not ULC treatment (white matter ROIs).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Affected hemisphere (L)						
ROI MNI: -22, -6, 46. Talairach: -21.94, -11.26, 44.28 (Sub-Gyral)						
WM	L	Fusiform Gyrus (BA20)	16	-43.46	-33.52	-15.85
WM	L	Fusiform Gyrus	18	-52.89	-45.69	-6.35
GM	L	BA7	7	-13.10	-58.29	63.39
				-15.00	-47.62	69.78
ROI MNI: 20, 2, 46. Talairach: 16.95, -4.01, 45.62 (Cingulate Gyrus – BA24)						
GM	R	BA5	18	7.33	-45.00	61.40
ROI MNI: 18, -18, 58. Talairach: 14.91, -23.67, 54.54 (Medial Frontal Gyrus)						
GM	L	BA38	15	-41.49	5.78	-13.89
WM	L	Superior Temporal Gyrus	27	-50.75	11.24	-11.73
GM	R	Amygdala	27	23.30	-7.25	-17.64
WM	R	Parahippocampal Gyrus	21	30.75	-6.94	-21.08
ROI MNI: 18, -24, 50. Talairach: 14.99, -28.57, 46.87 (Sub-Gyral)						
GM	L	BA38	15	-47.02	13.26	-13.28
WM	R	Sub-Gyral	24	22.19	-53.87	55.40
				16.26	-46.59	-57.52
WM	R	Middle Frontal Gyrus	25	48.71	19.93	30.41
WM	L	Inferior Frontal Gyrus	18	-22.92	18.90	-14.14
ROI MNI: 14, -10, 62. Talairach: 11.18, -16.55, 58.75 (Medial Frontal Gyrus)						
WM	R	Sub-Gyral	27	24.41	-7.25	40.04
GM	R	BA7	24	24.16	-60.29	44.02

ROI: region of interest; GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of Voxels = number of voxels for the indicated label included in the cube range of 1mm.

Table 5.8. Brain areas activated after ULC, but not RFVE treatment (white matter ROIs).

Brain Area	L/R	Brain Area	No of voxels	Talairach coordinates		
				X	Y	Z
Affected hemisphere (L)						
ROI MNI: -22, -6, 46. Talairach: -21.94, -11.26, 44.28 (Sub-Gyral)						
WM	R	Extra-Nuclear	27	24.69	-7.02	18.44
GM	R	BA41	21	39.52	-25.21	11.57
WM	R	Precentral Gyrus	24	50.56	-7.50	22.44
WM	R	Middle Temporal Gyrus	27	52.56	-37.45	1.62
WM	L	Sub-Gyral	27	-23.61	-4.44	32.28
WM	L	Sub-Gyral	27	-23.68	-14.10	34.97
WM	L	Sub-Gyral	27	-27.29	-13.39	27.77
WM	R	Sub-Gyral	18	31.94	-22.84	26.08
WM	R	Extra-Nuclear	18	28.34	-20.26	19.05
WM	R	Sub-Gyral	18	24.54	-20.93	26.13
WM	R	Cingulate Gyrus	27	11.57	-10.04	30.55
ROI MNI: 18, -18, 58. Talairach: 14.91, -23.67, 54.54 (Medial Frontal Gyrus)						
GM	L	BA44	27	-51.00	13.05	8.25
WM	L	Inferior Parietal Lobule	27	-49.55	-35.63	25.29
WM	L	Extra-Nuclear	12	-23.52	-44.04	14.12
ROI MNI: 18, -24, 50. Talairach: 14.99, -28.57, 46.87 (Sub-Gyral)						
WM	L	Inferior Frontal Gyrus	18	-47.49	2.50	19.93
ROI MNI: 14, -10, 62. Talairach: 11.18, -16.55, 58.75 (Medial Frontal Gyrus)						
WM	R	Precentral Gyrus	27	50.48	-9.89	27.62
GM	R	BA40	15	48.32	-30.43	45.45
WM	R	Corpus Callosum	18	7.87	-24.57	25.50
WM	L	Corpus Callosum	18	-6.87	-16.70	22.40

GM: grey matter; WM: white matter; L = left; R = right; BA = Brodmann Area. No of Voxels = number of voxels for the indicated label included in the cube range of 1mm.

5.2.5. Discussion and conclusion

In paragraphs above preliminary results from a randomised clinical trial, comparing two treatments for the upper limb (i.e. RFVE, ULC) in stroke survivors, have been reported. As expected, at the level of clinical outcome measures no differences between the two treatments were detected. In fact, given an effect size of $d = 0.54$ the number needed to treat is about 3.362, thus every 3 to 4 patients treated in the experimental group, only one does not improve significantly

in the experimental group. In the current sample of 10 patients the chance to observe a significant difference was very small. Nevertheless, the high spatial resolution of fMRI is a powerful tool to explore whether differences exist in the functional activation of the brain, due to the treatment received. Moreover, this kind of assessment of these forms of treatment has not been reported in the literature yet.

As for brain functional changes related to regions of interest (ROIs) in the grey matter (Table 5.9), only the RFVE group showed an improvement after treatment. Specifically, BA24 was more activated than BA22. On the contrary, no differences at the level of the grey matter were detected in the ULC group after treatment.

Table 5.9. Grey matter structures functionally activated by RFVE or ULC treatment.

Brodmann Area	General functions	Motor function
5 – Somatosensory Association Cortex	Involved in somatosensory processing and association	Imagery, tool use-gesture (left hemisphere) execution, mirror neurons, bimanual manipulation, saccadic eye movement
6 – Premotor cortex and Supplementary Motor Cortex (Secondary Motor Cortex)	Planning of complex, coordinated movements	Sequencing, planning, learning (SMA), preparation, initiation, imagery, interlimb coordination, smiling
7 – Somatosensory Association Cortex	Involved in locating objects in space. Point of convergence between vision and proprioception to determine where objects are in relation to parts of the body	Imagery, tool use-gesture (left hemisphere) execution, mirror neurons, bimanual manipulation, saccadic eye movement
8 – Supplementary motor area	Initiating, maintaining, coordinating and planning complex motor sequences	Motor learning, motor imagery, motor control, planning, executive functions, memory, attention
9 – Dorsolateral prefrontal cortex	Short term memory, evaluating recency, overriding automatic responses, verbal fluency, error detection, auditory verbal attention, inferring the intention of others, inferring deduction from spatial imagery, inductive reasoning,	Executive control of behaviour

	attributing intention, sustained attention involved in counting a series of auditory stimuli	
19 – Secondary visual cortex	Visual processing	Visual mental imagery
24 – Anterior cingulate gyrus	Emotion	Motor preparation/planning, motor imagery, response to vestibular-oculo-motor stimuli
34 – Dorsal entorhinal cortex	Olfactory processing	Navigational skills, landmark retrieval
37 – Temporal, fusiform gyrus	Lexico-semantic association	Semantic of visual perception
38 – temporal pole	Language process, executive functions	
40 – Supramarginal gyrus part of Wernicke's area	Reading (i.e. meaning, phonology)	Executive control, visually guided grasping, gesture imitation, visuomotor transformation, planning, repetitive passive movements, sensory feedback
41 – Primary auditory cortex, Heschl's gyrus	Auditory processing	Auditory priming
44 – Broca's Area	Speech production	Mirror neurons for expressive movements, motor speech programming, motor response inhibition.
47 – Inferior frontal gyrus	Semantic and phonological processing	Motor inhibition.
Amygdala	Processing of memory, decision-making and emotional reactions.	
Cerebellar Vermis (culmen, uvula)	Computation of body position in the space	Body posture, locomotion

SMA: Supplementary Motor Area

As for the ROIs in the white matter (Table 5.10), RFVE treatment increased functional activation in the Superior Frontal Gyrus and in the Anterior Cingulate Gyrus in patients with brain lesions in the right hemisphere. In patients with lesions in the left hemisphere, the Inferior Frontal Gyrus, the Superior Temporal Gyrus, the Lingual and Fusiform Gyri were more activated, after RFVE treatment.

In the ULC group only results for patients with lesions in the left hemisphere were available. As for ROIs in white matter, all the areas subtending executive functions (i.e. BA6, BA8, BA9) and motor coordination were found to be more activated after the ULC treatment.

Table 5.10. White matter structures functionally activated by RFVE or ULC treatment.

Area	Function
Superior Frontal Gyrus	Working memory
Middle Frontal Gyrus	Executive functions, decision-related processing
Inferior Frontal Gyrus	Language processing, speech production, inhibition of actions
Superior Temporal Gyrus	Auditory processing, language comprehension, emotions perceptions
Middle Temporal Gyrus	Contemplating distance, recognition of known faces, accessing word meaning while reading
Anterior Cingulate Gyrus	Reward, decision-making, empathy, impulse control, emotion
Cingulate Gyrus	Limbic system, emotion, learning, memory.
Precentral Gyrus	Primary Motor Cortex (BA4)
Lingual Gyrus	Processing vision
Fusiform Gyrus	Processing of colour, face and body recognition, word recognition
Parahippocampal Gyrus	Memory encoding and retrieval
Inferior Parietal Lobule	Perception of emotion in facial stimuli, interpretation of sensory information, language, mathematical operations, body image
Corpus Callosum	Transferring motor, sensory and cognitive information between hemispheres

Similar results, were found when the RFVE and ULC groups were compared, in patients with lesions in the left hemisphere. For ROIs in grey matter, patients receiving the RFVE treatment showed a larger activation of the primary motor cortex (BA4), supplementary motor cortex (BA6) and of the reward system than patients treated by ULC. For ROIs in the white matter, the results showed a comparable activation of areas devoted to language processing, memory processing and emotional control, for both RFVE and ULC treatments. Nevertheless, the somatosensory association cortex was activated after RFVE, but not after ULC treatment.

Overall, this first controlled study of patients undergoing different rehabilitation modalities for the recovery of upper limb motor function after stroke, showed that the specificity of stimuli provided within the therapeutic session can act like primers of specific areas in the brain. Cortical reorganisation is bigger in patients asked to interact with multi-sensory stimuli provided by reinforced feedback in

augmented environments, while rehabilitation modalities provided in standard environments seem to exploit better executive functions mechanisms and motor control strategies (left hemisphere lesion, only).

Moreover, different mechanisms are activated according to the hemisphere affected by the stroke, namely patients with lesions in the right hemisphere seem to rely more on working-memory processing, while patients with lesions in the left hemisphere appear to rely more on phonological processing and integration of sensory (visual-auditory) stimuli coming from the external world.

This is the first time that functional brain reorganisation of patients undergoing virtual reality based treatment is compared with the one of patients receiving conventional physical therapy. In this regard, these results represent the first validation of the neuro-functional effect of different rehabilitation modalities, for the recovery of upper limb after stroke. The aim of this comparison was to explore whether the different contents of the physical exercise administered to patients might result in differences in functional brain reorganisation.

The findings are in favour of the hypothesis that the brain follows different paths of functional reorganisation, according to the contents of the administered stimuli. Indeed, the bigger is the complexity of motor interaction required (e.g. number of coherent stimuli needed to be controlled simultaneously), the larger is the activation of higher functions which are integrated with the successful execution of the requested motor action.

5.3. Limitations

The current preliminary results are only from a preliminary pilot trial leading to a much larger randomised clinical trial (RCT). On this basis, several limitations are currently present. First the estimated sample size required could not be met within the timeframe of this PhD, thus the findings are thoroughly underpowered, but particularly with regard to the primary outcome chosen (i.e. Fugl-Meyer Upper Extremity scale). A secondary bias due to the current sampling was that all the patients in the control (ULC) group had a lesion in the left hemisphere, thus only comparisons with matched patients for this variable in the experimental (RFVE) group were possible for resting state fMRI.

Due to the small sample size no significant difference between groups at clinical level were found and family wise correction in the resting state fMRI analysis was

not feasible. The former limitation was not considered because testing clinical differences was not an aim at this stage of the RCT, while the latter issue was overtaken by setting the threshold for statistical significance at $p = 0.001$ uncorrected and applying an extent spatial filter of 10 voxels, thus to consider only the biggest clusters for final conclusions.

The results are however encouraging and a fully powered study should yield informative meaningful findings.

6. FINAL DISCUSSION

The general aim of this PhD project was to explore the neuronal mechanisms implicated in the recovery of upper limb motor function after stroke in humans, when technology-aided modalities are used for rehabilitation therapies. The need for specific investigations in the population of stroke survivors relies on several reasons. Stroke is a complex syndrome recognised as the first cause of disability and the second cause of mortality worldwide (Mathers et al., 2009). Moreover, the social costs associated with stroke care are the highest in the medical rehabilitation field because of the years people have to live with disability and loss of productivity experienced by survivors. More specifically, both direct and indirect costs are challenging to estimate due to the difficulties in predicting mortality, the use of different diagnostic-related group (DRG) at emergency admittance, the variability in length of stay in the hospital and the multiplicity of treatment settings (e.g. stroke unit, intensive care unit, rehabilitation care unit) exploited along the rehabilitation course, for each patient (Peltola, 2012). From a clinical perspective, the definition of stroke is vague and different classifications exist for type of tissue necrosis, lesion location or aetiology, thus widening the heterogeneity of its clinical description. The multiple sequelae of stroke impair a large number of functions in different domains (e.g. cognitive, motor, speech, memory, problem solving, mood), heavily compromising autonomy in activities of daily living of survivors. Upper limb motor function represents the hardest to recover amongst all the ones impaired by brain lesion, especially after a long delay from stroke onset (Dromerick et al., 2006). The probability to regain good motor function of the upper limb is influenced by the brain areas (site) affected by stroke. Evidence in humans revealed that a cortical stroke (preferably in the primary motor areas) results in milder motor impairment than subcortical lesions, whereas mixed cortical-subcortical lesions have a better chance to recover, than pure subcortical stroke lesions (Piron et al., 2009b).

After brain insult both true and compensatory recovery mechanisms spontaneously drive the restoration of normal functionality, as best as possible to approximate function before stroke. It is widely accepted that the recovery process along the rehabilitation course is a combination of both true and compensatory mechanisms mediated by neuroplasticity (Teasell et al., 2006). The reference goal for neurorehabilitation, particularly for therapeutic modalities for which the rationale is based on neurophysiological principles (e.g. technology-

aided therapies) is the facilitation of true recovery, against the instauration of compensatory adaptive behaviours, which are generally considered as maladaptive. Most of the strategies adopted in neurorehabilitation to vicariate true recovery exploit the experience-dependent neuroplasticity phenomenon, which occurs when a large amount of repetitions (between 1000 and 10000 trials) is practised for a sufficient time length (15 days at least) (Kleim and Jones, 2008). In this regard, the role of neurotrophins has been reconsidered recently like a determinant factor influencing recovery after stroke. In particular, the brain-derived neurotrophic factor (BDNF), which represents the most studied neuronal growth factors purified in mammals, has been largely investigated because of its primary action in modulating dendritic morphology when new learning, both at the level of motor and high cognitive functions, occurs (Tolwani et al., 2002). Moreover, its expression and activation can be increased by different agents like aerobic exercises, making BDNF an intriguing biological target to study, with the aim to increase functional recovery after brain lesion. The BDNF can be synthesised with different compositions of amino acid sequences (single nucleotide polymorphism, SNP), which determines different genotypes. The most prevalent SNP of the BDNF is the Val66Met, whose effect is to reduce the amount of BDNF freely available throughout body tissues. A worse recovery after lesions of the CNS has been associated with the Val66Met BDNF SNP, because of the learning impairment due to the lack of available BDNF (Kim et al., 2012b, Mirowska-Guzel et al., 2014). Evidence on the role of Val66Met BDNF SNP for functional recovery is currently controversial in stroke patients, due to its different prevalence between Asiatic (70%) and Caucasian (30%) ethnicities. Thus, external validity of results is very poor due to the high heterogeneity of sampled subjects, making interpretations difficult to generalise. Moreover, no evidence exists at all on the specific effect of Val66Met BDNF SNP for the recovery of upper limb motor function, after stroke. Given these premises, it was suggested that carrying or not carrying the Val66Met BDNF SNP might result in different clinical outcomes after stroke, when receiving specific upper virtual reality (VR) based rehabilitation for the treatment of upper limb motor function.

The results from experiment 1, which enrolled 75 consecutive stroke patients rehabilitated by non-immersive VR-based treatment for upper limb, indicated that there is no difference at the level of clinical outcome measures, between carriers and non-carriers of the Val66Met BDNF SNP. VR treatment improved

significantly gross motor function of the upper limb, autonomy and hand dexterity in both carriers and non-carriers of the polymorphism. This finding has strong clinical implications demonstrating that genotype is not a determinant for clinical recovery, when the same doses of controlled rehabilitation therapy are provided. This is paramount in terms of access to rehabilitation services, which should not be referred on the basis of predetermined individual factors, such as genetics, but tailored on individual characteristics of each patient. As detailed before, it is not possible to extend these results to the worldwide population of strokes patients, because of the differences in prevalence of SNP expression across ethnicities. Nevertheless, it is the first time that groups of patients receiving the same amount of controlled rehabilitation therapy are compared because of their genetic profile; in the future, studies should consider strictly this indication when discussing trials' results which compare the effects of controlled rehabilitation therapies. Another interpretation for the current clinical results is that it might be possible that different functions and body districts have specific recovery pathways, depending on their distributed representation within the CNS. In this regard, it is arguable that the upper limb, due to its complex architecture and cognitive demands needed to be controlled actively, might exploit a larger number of distributed connections with other brain areas, than other districts such as the lower limbs. For gait function, recovery mechanisms might rely more on anatomical integrity of subcortical spinal tracts and less on compensatory neuroplasticity mediated by the motor cortex, thus genetic factors influencing learning (e.g. BDNF) might be less determinant for the final rehabilitation outcome. Conversely, in the case of upper limb motor function a larger motor reserve might be activated after brain lesion and several learning mechanisms might drive neuroplasticity for cortical reorganisation, and functional recovery. Moving to in-depth exploration of mechanisms influencing motor recovery, Qin and co-workers (2014) used animal models (rats) to demonstrate that regaining of gait performance after stroke relies on hypertrophy of the thalamus contralateral to the affected hemisphere, in carriers of the Val66Met BDNF SNP. To test this hypothesis, the authors used pharmacological agents (i.e. muscimol) to inactivate the thalamus, with the result to impair more severely walking function in carriers of the Val66Met BDNF SNP, than in non-carriers. These findings demonstrated that external agents, such as drugs, are able to induce selective anatomical and behavioural changes with functional meaning and rehabilitative

implications, because of the genotype. On this basis, it has been postulated that a similar phenomenon might operate also in humans after stroke and that other stimuli, like physical exercises for the rehabilitation of upper limb motor function, might also be effective in inducing structural changes in the brain.

In experiment 2 we tested the hypothesis that also in human stroke survivors carrying the Val66Met BDNF SNP might lead to similar structural changes in certain brain areas. A voxel based morphometry (VBM) analysis was run in a subsample of 26 patients among the ones enrolled in experiment 1. At the level of clinical outcome measures, the results confirmed the same finding as in experiment 1, except for hand function that was better regained in non-carriers of the Val66Met BDNF SNP than in carriers. Theoretically speaking, this clinical evidence from experiment 2 allows the speculation that hand function might be the most responsive outcome to measure, for the detection of genotypes' effect on the recovery of motor function after brain injuries. A similar genetic predisposition has been proposed for the development of other neurological diseases, such as the so called work-related focal dystonia (Altenmuller et al., 2012). Work-related focal dystonia is a neurological disease affecting elite professionals, such as musicians (e.g. pianists, violinists, flautists, trombone players, guitarists, drummers) or athletes (e.g. golf, dart, cricket, baseball, snooker), its prognosis is typically good, with complete restoration of previous function (Furuya et al., 2014a, Furuya et al., 2014b, Furuya et al., 2014c), after resting from professional practice for a certain amount of time. Nevertheless, in the worst cases its treatment might require significant changes in performing technical gestures, or even complete cessation of professional practice. A clear physiopathological mechanism has not been recognised yet, but it is widely acknowledged that this disease is an extreme case of cortical maladaptive plasticity, due to super-training in healthy subjects. Curiously, also genetic predisposition has been proposed as a trigger of this disease (Altenmuller et al., 2009, van der Steen et al., 2014, van Vugt et al., 2014), potentially associated with carrying the autosomal dominant gene DYT-7 (Jankovic and Ashoori, 2008). Clinically speaking, manifestation of focal dystonia usually impairs active motor control of hands and lips, which are the most represented body districts in both the motor and sensory homunculus (Penfield and Boldrey, 1937). On this basis, it might be speculated that the hand might represent the likely body district in the

upper limb where impairments of fine motor function can be detected in relation to genetic factors.

At the level of structural imaging, only carriers of the polymorphism showed larger volumes of specific brain areas than non-carriers. In the hemisphere contralateral to stroke lesion areas devoted to motor processing (BA 6 and gyri), sensory integration (BA 3 and gyri) and verbal comprehension (BA 38) were larger, whereas in the hemisphere ipsilateral to the stroke lesion the thalamus and substantia nigra were larger. These results are coherent with the animal model suggesting that after stroke hypertrophy of specific brain areas exists in carriers of the Val66Met BDNF SNP, but not in non-carriers. Nevertheless, the body district (i.e. upper limb) and the associated functions (e.g. reaching) studied in this experiment were different from the ones studied in animals (i.e. lower limb, gait). This discrepancy does not allow a perfect superposition of expected anatomical landmarks from animals to humans. In fact, differently from results in rats the anatomical structures found as having larger volumes in the VBM analysis in experiment 2 were in both hemispheres (i.e. ipsilateral and contralateral to the brain lesion). In the contralateral hemisphere, the brain areas in which larger volumes were detected were the ones involved in comprehension, planning and sensory integration functions for the production of voluntary movements. As a whole, the picture emerging from experiment 2 seems to confirm that genetic polymorphisms might have a role in neuroplasticity processes after stroke. Specifically, for stroke patients undergoing VR-based rehabilitation for upper limb motor function and carrying the Val66Met BDNF SNP the brain structures which have as a main role the direct excitation of the motor cortex are bigger in the ipsilateral hemisphere, whereas the brain areas which have as a main role that of optimising voluntary motor execution are bigger in the contralateral hemisphere. To determine whether these structural differences exist from birth or represent a different reorganisation strategy after stroke, both a large cohort study on healthy volunteers and a longitudinal VBM analysis on patients undergoing rehabilitation of the upper limb need to be carried out in the future.

The motor system is characterised by a huge number of elements combinable in different modalities, to reach the same target. The control of such a high-dimensional system represents a considerable computational problem to be solved quickly and efficiently by the CNS, with the purpose of guaranteeing optimal motor performance in every environmental context. A theoretical

approach to solve this problem has been based on the concept of synergies. A synergy has been conceptualised as the pooling of different components of the system in stable functional units, ready to be activated in larger stable organisations, thus reducing the degrees of freedom that need to be controlled, therefore simplifying the computational demanding needed to achieve a successful result. In the field of motor neuroscience, it has been proposed that the CNS might operate exploiting similar strategies to cope with the high dimensionality of the motor system, with the goal to produce smooth, well-coordinated, low energy consumption and targeted voluntary movements. At the muscular level, the pooling of several muscles in one single functional group is called muscle synergy and represents the instantaneous activation of all the muscles, each one activated with its own specific amplitude. The same functional unit has been considered also like a task dependent primitive module, shared across subjects. Muscle synergies have been proposed as a powerful tool to explain the large variability of the whole set of free natural movements, with a small number of components (Tresch et al., 2006). Based on this approach it has been suggested that the CNS might control primitive modules directly, instead of each single muscle both in animals and healthy humans (Bizzi et al., 2002, d'Avella et al., 2006). Several studies have demonstrated that the number of muscle synergies' modules is somehow affected after stroke (Cheung et al., 2009a, Gizzi et al., 2011). Namely, the reduction of modules' number is proportional to the severity of motor function impairment: the higher the impairment, the lower the number of modules needed to explain the residual movements (Cheung et al., 2012a, Clark et al., 2010). Reciprocally, the number of muscle synergies' modules can be considered as an index of the ability to express complex motor behaviours with the following statement: the higher the number of modules, the higher the complexity of natural movements can be expressed voluntarily. A current limitation of this approach is that neurophysiological evidence on the existence of muscle synergies is still missing, since it has not been demonstrated that modules are represented in a specific neuronal substrate, somewhere in the CNS. Experiments 3 and 4 aimed at providing evidence to fill this gap.

In experiment 3 the relationships between site of brain lesion, impairment of upper limb motor function and number of muscle synergies' modules, were explored with a region of interest (ROI) analysis, in a sample of 19 patients.

Specific targeted brain areas (i.e. BA 3,1,2; BA 4; BA 6; corona radiate; internal capsule) were considered as the primary structures composing the pyramidal motor system and their contribution to the whole brain lesion, in terms of lesional weight, was computed as the relative frequency a structure was involved by the lesion. Therefore, this index was regressed against severity of motor impairment and number of muscle synergies' modules to test any causal relationship. The results indicated that stroke lesion was more frequently observed in subcortical structures (i.e. corona radiate; internal capsule), than cortical areas (i.e. BA 3,1,2; BA 4; BA 6). Unluckily, the ROI analysis was underpowered to detect a significant difference between the involvement of single brain areas which could explain a reduction of both motor function and number of muscle synergies' modules. The internal capsule was the only structure for which it was possible to conclude that patients with severe impairment of upper limb motor function had a larger involvement of this structure, than mild ones. At the level of muscle synergies, the only significant difference was observed between subgroups of patients with different brain lesion volumes: patients with a volume bigger than 10cm³ had a smaller number of residual muscle synergies' modules.

These results are coherent with evidence from animal models (Darling et al., 2011) and imaging studies in humans (Page et al., 2013), claiming that the brain site affected by stroke and not the volume of the whole lesion is the main predictor of final motor function impairment. However, at the imaging level quantitative indexes able to describe individual motor impairments due to stroke are still complex to compute and provide information only about average recovery measured by general outcomes long term (Stinear et al., 2012, Stinear, 2010). Experiment 3 exploited the number of muscle synergies' modules as an index which was matched with imaging data for the first time. It was, therefore, possible to test the hypothesis that some linear relation between anatomy of the brain lesion and motor outcome of a single subject might exist. This opportunity enlightened the possibility to create an individual profile of the motor consequences of a stroke, linking brain anatomy with voluntary motor behaviour, at a neurophysiological basis.

Because of the low power of the ROI analysis, in experiment 4 the causal relationships between upper limb motor function, muscle synergies and brain morphology were explored with VBM and diffusion tensor imaging (DTI) analyses, in a sample of 26 and 13 participants, respectively. Data from both the

healthy and stroke affected upper limb were analysed, to have a matched control of the direct effect of stroke lesion anatomy on motor function. The findings of this analyses indicated that the number of muscle synergies' modules in the healthy upper limb correlated with brain structures of the pyramidal system (figure 6.1 a), whereas the number of muscle synergies' modules in the stroke affected upper limb was related to a larger number of brain areas, beyond the pyramidal tract. In fact, brain areas devoted to executive, comprehension and speech functions were also significantly correlated with reduction of the number of muscle synergies' modules in the stroke-affected upper limb (figure 6.1 b).

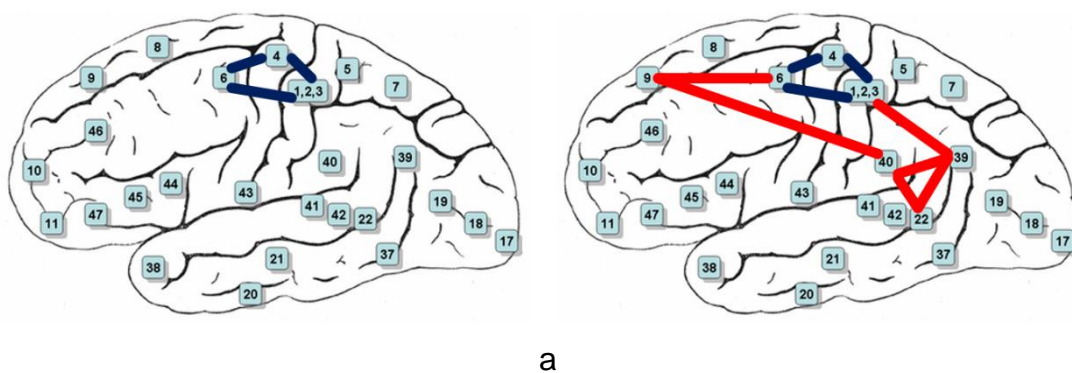


Figure 6.1. Schematised representation of the Brodmann Areas correlating with synergies' modules, in the healthy and stroke-affected upper limb.

The Brodmann Areas (i.e. 4; 6; 1,2,3) correlating with synergies' modules in healthy arm (blue) represent the sensory integration and projection cortical areas of the pyramidal tract (a). The Brodmann Areas correlating with synergies' modules in stroke affected arm (b) include over the cortical areas of the pyramidal tract (blue), a larger set of brain areas (red) involved in executive, speech and comprehension functions (BA9, BA39, BA22, BA40).

This is the first time that analysis of muscle synergies is used in combination with VBM and DTI techniques. The main innovation of this combined approach relies on the possibility to explore whether any anatomical correlates exist for a computational strategy demonstrated to be biologically plausible in animals (Bizzi et al., 2000, Overduin et al., 2008, Saltiel et al., 2001, Tresch et al., 2002), as well as feasible for neuro-musculoskeletal modelling (Sartori et al., 2012, Gonzalez-Vargas et al., 2015) and rehabilitation engineering applications for body machine interfaces (e.g. prosthetics and robotics control) (Dosen et al., 2015). The robustness of the non-negative matrix factorisation (NMF) method for data mining (Lee and Seung, 1999) has allowed its application in a wide range of biomedical

fields for classification of electromyography (Tresch et al., 2006), electroencephalography (Delis et al., 2016) and imaging (Anderson et al., 2014) signals. The possibility that a continuity of findings across domains exists because of the application of the same classification methods was to be expected, but had never been tested before. The results from these experiments are not the final biological demonstration that muscle synergies modules are hardwired in the CNS. In fact, only direct stimulation, ablation or biochemical manipulation of neural tissue will be the highest experimental demonstration of their ontology, as it has done in animal studies (Bizzi et al., 1995). Nevertheless, the coherence of results obtained across experiments, together with their plausibility within a general framework of functioning of the motor system (Frey et al., 2011) offer a general interpretation of the mechanisms involved in determining the impairment of upper limb motor function after stroke. As acknowledged, the production of complex voluntary movements by the upper limb is underpinned by the correct functional activation of the primary motor and sensory cortices and propagated through the intact corticospinal tract. The more the pyramidal system is disrupted, such as after stroke, the more other brain areas, usually devoted to cognitive-related tasks (e.g. procedural, speech, comprehension), are recruited structurally as part of the proper motor system. The structural enlargement of the motor system after a brain lesion might be interpreted as the need for larger computational reserves, aimed at handling the undiminished dimensionality of the musculoskeletal system at the periphery, with the purpose to solve the unchanged complexity when interacting with the external physical environment (figure 6.2).

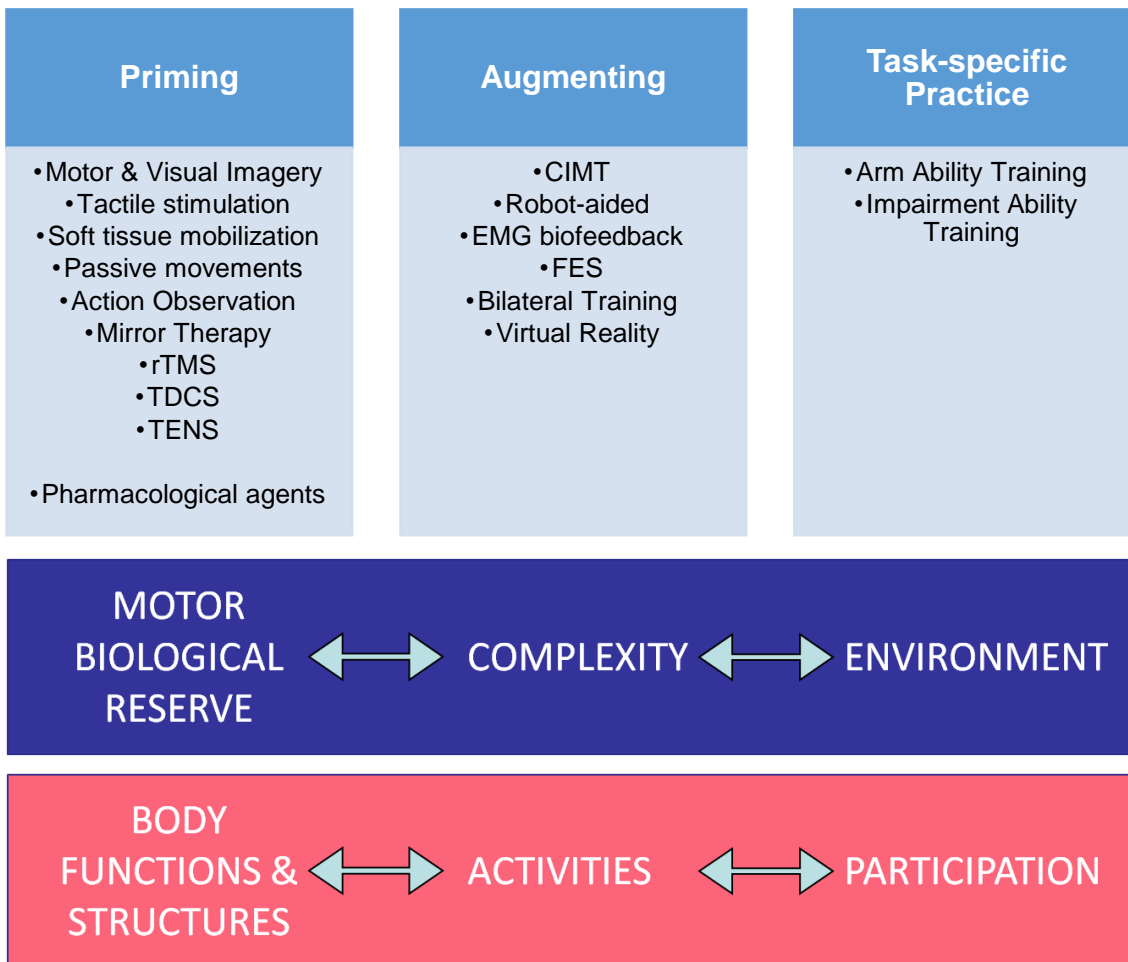


Figure 6.2. Biological, computational and functional purposes of rehabilitation modalities. with ICF related domains.

Each rehabilitation modality group, as defined in the paragraph 1.3.1, is represented overlying its own alleged purpose and international classification of functioning (ICF) domain, so that a bio-computational balance can be achieved for the production of effective voluntary movements.

The results from experiment 4 suggest that the concept of muscle synergies might represent a concrete argument to explain how the CNS can handle the balance between the high dimensionality of its musculoskeletal system and unpredictability of the external world. Indeed, muscle synergies might operate as a linear transducer of the motor system intention, from internal states of the body to the external environmental context. This controller has the advantage to be easier to control, but without losing contents on behavioural complexity.

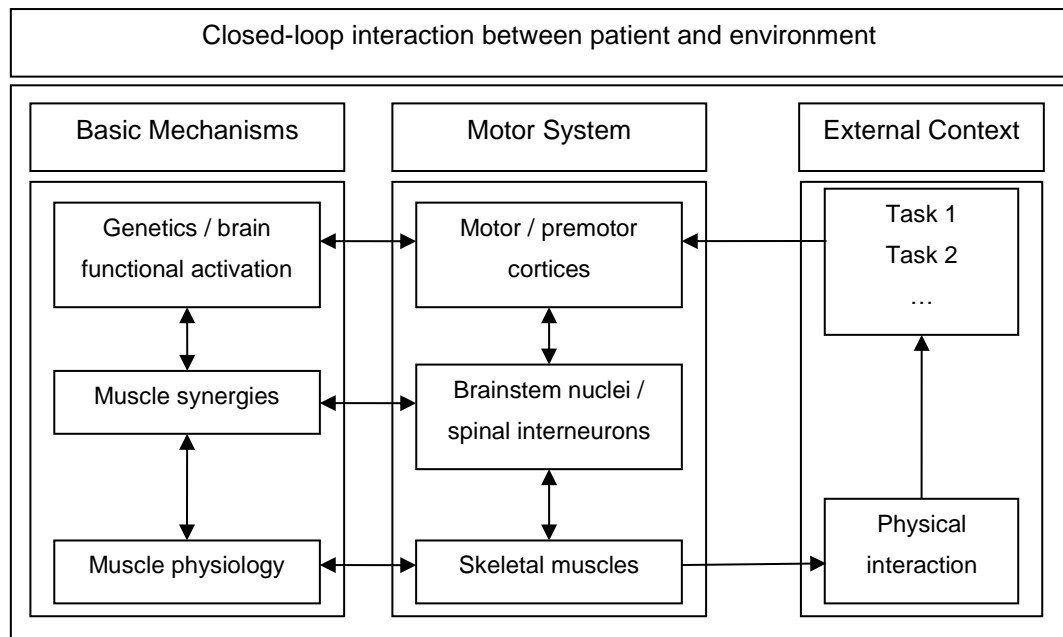


Figure 6.3. Scheme for mechanisms ruling the closed-loop interaction between the motor system and the external environment.

On the left, the basic mechanisms ruling the closed-loop interaction between human motor behaviour and external environment are represented. On the right, the reference framework of the closed-loop subject-environment interaction is schematised.

The results of the ROI and DTI analyses confirmed that this motor biological reserve is affected when the corticospinal tract is anatomically disrupted; moreover, VBM analysis suggested that the CNS tries to compensate for this lack of capability, by compelling extended brain components to cover the unchanged demands of intentional actions (figure 6.3). True recovery might be postulated, therefore, as the ability of the CNS to restore its biological motor reserve, as close as possible to the one available before the injury. Conversely, compensation recovery might be postulated as the inability to recover throughout this path, thus building newer reserves, by exploiting alternative brain areas and control strategies. Within this framework, muscle synergies' modules can be intended as an index to quantify this ability, by comparing their presence and activation between the healthy and stroke-affected arms and by monitoring their expression along the recovery path. Due to the computational flexibility and robustness of the mathematical methods applied for muscle synergies' extraction, this application represents a feasible approach both for group and single subject analyses. In experiment 5 the clusters of voxels representing muscle synergies in the brain were used for seed-based resting state fMRI.

As previously described, the motor system has multiple connections with many non-motor areas throughout the whole brain, thus stroke sequelae usually affect multi-domain functions. Currently, it is still difficult to assess exhaustively all the impairments following a stroke and even harder to predict their effect on rehabilitation outcomes. The literature review carried out in the first chapter of this thesis, provided an update of the recent evidence on motor rehabilitation after stroke, and together with the results from first four experiments, allowed the classification of rehabilitation modalities (i.e. priming, augmenting, task-oriented), the identification of the specific target each one is referred to and the respective ICF domain are supposed to tackle (figure 6.3). In the field of stroke rehabilitation time contingency, specificity, intensity, exercise parameters and therapy doses are acknowledged to be critical aspects to consider for the planning of effective rehabilitation programmes, regardless of the modality chosen. Several pieces of evidence have been provided on the prominence of exercise features and, as an example, the effect of very early mobilisation (i.e. within 24 hours from stroke onset) represents the current edge of experimental investigations (Bernhardt, 2012, Bernhardt et al., 2015a, Bernhardt et al., 2006, Bernhardt et al., 2008), with reference to time contingency. To date, there is still no consensus on which is the best treatment form for regaining upper limb motor function after stroke (Loureiro et al., 2011), but a recent overview of reviews from Pollock et al. (2014) provided evidence that the best treatments are the ones focused on the reinforcement of the visual-motor loop, such as virtual reality (VR), mirror therapy and mental practice. Clinically speaking, augmenting techniques (e.g. virtual reality, robotics) might be considered like the reference standard for impairment-oriented treatment of the upper limb (Takeuchi and Izumi, 2013). For VR-based treatments, efficacy has been demonstrated by Laver et al. (2015b), whose meta-analyses showed that in stroke patients this modality is better than conventional rehabilitation therapies to improve upper limb motor function (SMD = 0.29 [0.09, 0.49]), with some transferability to regained autonomy by patients (SMD = 0.42 [0.18, 1.29]). Moreover, it was demonstrated that a dose effect exists with a minimum of 15 hours of therapy needed for detecting a clinical effect (SMD = 0.31 [0.07, 0.55]) and that specialised VR systems are better than commercial gaming systems (SMD = 0.42 [0.07, 0.76]). Nevertheless, the mechanisms underpinning this clinical evidence are still unknown and matter for debate. To tackle this issue a feasible approach would be the comparison of functional brain

reorganisation between patients undergoing VR-based and those having conventional therapy as treatment of upper limb motor impairment.

In general terms, imaging studies carried out with stroke patients have suggested that bilateral hemispheric activation is common after brain lesion, but its persistence when voluntary movements are performed is a predictor of poor motor recovery (Rehme et al., 2011). Complimentary, preservation of activation in the hemisphere ipsilateral to the lesion at resting state analysis is a good predictor of motor recovery (Park et al., 2011). Still it remains to be clarified whether the presence of bilateral hemisphere activation should be considered a maladaptive or compensatory phenomenon in motor recovery. Despite uncertainty coming from fMRI studies, this technique remains amongst the most robust available to study the brain changes occurring during the recovery process after stroke. The variability of experimental results might be due to its sensitivity, but also depending on the heterogeneity of paradigms (e.g. sequences, tasks, scanners, processing procedures) used in each study. Specific technical limitations affecting the power of fMRI in detecting brain reorganisation related to motor tasks are: poor temporal resolution, given that 2 to 4 seconds is the average time necessary to detect changes in BOLD signal; the paucity of motor-tasks doable in the scanner (e.g. finger tapping, joystick control), not representative of the whole set of human motor behaviours, neither executable by the largest part of stroke patients. Resting state fMRI allows the overcoming of the limitations involved in executing motor-tasks in the scanner, since patients are not required to engage in any voluntary activation.

Some imaging evidence is available for each of the rehabilitation modalities reported above. Indeed, priming approaches seem to increase the activation of both the primary and the extended motor system; augmenting techniques, such as robotics, seem to increase the activation of sensorimotor cortex, while virtual reality seems to decrease the activation of premotor cortex contralateral to the lesioned hemisphere; task-oriented practice seems to decrease the activation of the unaffected hemisphere. Unluckily, very few studies have used imaging to explore the specific effect of VR-based treatments for upper limb rehabilitation after stroke and none of them compared groups of patients doing VR-based therapy with those undergoing conventional rehabilitation therapies.

Experiment 5 was designed to fill this gap with a large randomised controlled trial (RCT), whose sample size was calculated in reference to the expected difference

at the Fugl-Meyer upper extremity scale, between groups of patients doing VR-based treatment or conventional physical therapy for the upper limb. The estimated difference was inferred from meta-analyses by Laver et al. (2011), thus the statistical power, guaranteed at the level of clinical outcome measures, was overestimated for imaging outcomes. The results presented in this thesis are preliminary and not clinically meaningful, but still represent the first evidence available at the level of brain functional reorganisation on the effect of different rehabilitation treatments for the upper limb, after stroke. This experiment was carried out with only 10 patients allocated randomly to VR-based treatment or conventional therapy, but both treatments were provided for the same amount of sessions of the same time length and over the same period of time. The exercises provided in both treatments were matched (see Appendix 1), thus the same exercise toolbox was available regardless of the settings. In this way intensity, exercise contents and therapy doses were matched as better as possible between treatments, whereas the main difference relied on the specificity of the feedback provided by the augmented environment exploited for practice: the virtual reality system in the experimental group and conventional physiotherapy in the control group. With the aim to collect fMRI from all the enrolled patients, thus minimising the bias of collecting fMRI only in mild patients able to perform active motor tasks in the scanner, a resting state fMRI acquisition was chosen. Finally, to base the resting state analysis on the most suitable available neurophysiological rationale, a seed-oriented approach was preferred, using the clusters of voxels resulting from the VBM analyses in experiment 4 as the seeds of interest. So strictly constrained, the resting state fMRI findings revealed that functional reorganisation around the seeds, presumably representing the neuronal substrate of muscle synergies' modules in the brain, occurred in patients treated by virtual reality, but not by conventional physiotherapy. This result should not be interpreted just as favourable to VR, against traditional physiotherapy methods. Widening the interpretation, it might be concluded that when the aim of an intervention is to reduce the impairment of a specific function (e.g. upper limb motor function), a specific treatment based on augmented environments, providing specific information and feedback on performance and results of the tasks accomplished, thus useful to improve learning, is more likely to induce reorganisation of brain activation around foci specific for that function, than a less specific intervention. In fact, functional reorganisation was observed within both

groups after therapy, but in the comparison between groups the reorganisation around seeds was more focused in the experimental group. As for the ICF framework, VR settings should not be intended just as a treatment which is better than standard ones, but rather a useful tool enabling the therapist to be more specific for impairment-oriented treatment of specific body structures and functions, affected by some pathological accident.

6.1 Studies limitations

The whole set of studies reported in this PhD thesis allowed the achievement of new knowledge about the current state of the art on rehabilitation of upper limb motor function, after stroke. The results, however, should not be considered as definitive because of some technological constraints and methodological limitations affecting the power of inferences.

Firstly, the set of outcome measures considered was mostly in the ICF domains of “body structures and functions” and “activities”, thus very little can be said on how VR-based modalities improve participation of stroke survivors. This is actually a major limit not in line with the final aim of rehabilitation defined by the world health organisation (WHO) as: “[...] a process aimed at enabling” patients “to reach and maintain their optimal physical, sensory, intellectual, psychological and social functional levels. Rehabilitation provides disabled people with the tools they need to attain independence and self-determination”. In fact, starting from the inventory of impairments the gold purpose of rehabilitation should be improvement of patients’ quality of life. So far, it can be claimed that because of the effect obtained at functional and activity levels some amelioration is clinically detectable in patients’ real life, but which are the causes of this gain still remains unknown and chance findings or placebo effects cannot be excluded. The same limit is at the basis of criticisms on the current deployment of VR-systems within national health systems, with the aim of delivering special services based on this modality. The Cumberland consensus working group (Cheeran et al., 2009) proposed a circular model to translate research findings from basic science to service delivery for stroke patients. The translational pipeline is driven from organisational catalysts from basic science to health services and by biological catalysts on the way back to basic research. This model has been taken into consideration for the design of all the experiments reported in this thesis. In fact,

all the hypotheses postulated and experimental designs set tried to link together, as best as possible, the different levels of analyses from biology to behaviour. Obviously, the whole picture cannot be controlled in all the degrees of freedom and due to the current available knowledge it was not possible to achieve robust conclusions on the impact of VR-aided rehabilitation in patients' real life. Nevertheless, it should be considered that nowadays technology is dramatically pervading daily life of people, not only in western society, and changing social behaviours and habits drastically. Integration of technology in health service delivery can no longer be excluded from the agenda, thus also new background knowledge on how technology-aided therapies work at the level of basic mechanisms is needed, to guide translational research from bench, to bedside, to real life for each patient.

Secondly, the multi-modality of stimuli provided by the VR artificial environment used in the experiments did not allow any speculation on whether the determinants of the observed differences were the specific features of the exercises, or the complexity of VR as a whole. Evidence from the fields of general psychology and sport sciences indicates that motor learning is influenced by both internal and external focuses, which the CNS estimates from internal state of the body and external environmental characteristics, respectively. For instance, for the effect of internal focuses on learning, studies on runners showed that giving feedback on the main end-effector is better than correcting secondary errors of the run pattern to improve performance (Corte et al., 2015), while for the role of extrinsic feedback (i.e. external focus) on learning, studies on elite golf players showed that amplification of error is a better learning strategy, than direct verbal instruction to correct technical errors in performing swing movements (Milanese et al., 2016). This level of details, aimed at understanding the best learning strategies to be adopted in a real clinical setting, is not yet achievable in experimental studies on stroke patients. First of all, it still needs to be thoroughly explored whether learning is preserved or disrupted after stroke, then it has to be clarified which are the determinants of recovery mediated by the rehabilitation path. The background needed to move forward in this direction in stroke rehabilitation research is still defective. There are, however, active trials, currently recruiting participants, investigating these issues (Zucconi et al., 2014). Another important aspect to consider in VR settings is the strong visual component guiding interaction. As it is known, upper limb and hand movements are mainly

performed under visual control, especially when new learning of fine skills is needed (Archambault et al., 2015, Filimon, 2010). The role of visual feedback for restoration of motor function has been completely reconceptualised with the discovery of mirror neurons (Rizzolatti and Luppino, 2001). As a consequence, innovative rehabilitation approaches have been proposed (Buccino et al., 2006), becoming very popular and vastly studied, based on the rationale that imagining to perform real motor tasks (motor imagery), looking at others while performing transitional gestures (action observation), or watching visual illusions of one's own movements (mirror therapy) might improve motor performance, and consequently motor recovery in the case of brain lesions. Similarly to the case of VR-based therapies, current evidence on the effectiveness of these approaches for stroke rehabilitation is not definitive (Barclay-Goddard et al., 2011, Thieme et al., 2012, Pollock et al., 2014), and therefore it is not possible to discriminate which components of the visual-motor loop are more involved and in which phase after stroke onset, during the recovery process. There is, however, a major limitation of these studies which has to be acknowledged. The patients enrolled for the studies reported in this thesis did not have a complete neuropsychological assessment. In fact, only the Mini Mental State Examination was administered to exclude patients with severe cognitive impairments. It is widely acknowledged, however, that a wide range of higher cognitive functions are involved in the visual-motor loop. Thus, future studies should consider the administration of a comprehensive neuropsychological assessment to all patients included in the studies, with the aim of characterising subgroups of patients who might be good or bad responders, to ascertain whether their response to treatment depends on their residual cognitive abilities.

Some methodological issues represent current limits of the results reported. In experiment 1 the allocation was done just on the basis of genotype, given that it was not possible to know whether the BDNF polymorphism was present or not, before DNA sequencing. This is a non-random criterion, thus a sampling bias cannot be excluded and future studies might consider the prevalence of the polymorphism according to the ethnicity studied, to implement a balanced sampling procedure. In the same experiment the absence of follow-up did not allow any inference to be made on whether the observed differences result from a neuroplastic recovery mechanism after stroke, or whether these were predetermined during development because of genotype. On the same basis all

the cross-sectional designs in study 2, 3 and 4 did not allow conclusions to be reached on the effect of VR treatment with regard to brain morphometry and connectivity of muscle synergies' modules. Conversely, these limitations were overcome in experiment 5 by designing a powerful RCT, but the sample size calculated was not achieved, thus also for the last experiment the conclusions are not definitive. Finally, for all the experiments the treatments provided (i.e. VR, conventional) were carried out with the continuous presence of the physiotherapist. This issue still represents nowadays the major criticism of running RCTs in neurorehabilitation. In fact, in this field of research it is still difficult to disentangle how much of the measured effects are operator dependent, placebo, or real efficacy due to treatments' rationale. To date, this compromise is still hard to avoid, but the introduction of technological devices, embedding part of these principles will give the opportunity to improve the methodological quality of RCTs, by making the treatment partly independent of the single operator. This scenario should not be intended as a risk for the professional role of therapists in their daily practice, but on the contrary should be seen as an opportunity to empower, by acquiring deeper knowledge and insights about treatments, their interventions in terms of specificity, effectiveness and deployment.

6.2 Suggestions for future research

The research field of innovative technologies in rehabilitation (e.g. virtual reality, telerehabilitation, robotics) has increased hugely in last decades (Brochard et al., 2010). This trend has been sustained by several recent phenomena like the spreading of low cost technologies able to monitor physical activities of each subject for 24 hours a day, the enlargement of connectivity bandwidth and easy access to cloud services making data sharing feasible and powerful, the easy manufacturing of experimental prototypes using 3D printers. Other fields of research such as neurophysiology have demonstrated that cortical plasticity and learning can be modulated by other priming techniques like repetitive transcranial magnetic stimulation (rTMS) (Avenanti et al., 2012) or transcranial direct-current stimulation (TDCS) (Bastani and Jaberzadeh, 2012, Bolognini et al., 2009). A direction to pursue for future studies should consider the effect of combining priming with augmenting techniques, with the aim to augment the effects

achievable from the administration of each one independently. Some studies have been recently carried out with this purpose (Lee and Chun, 2014, Bolognini et al., 2011). Several questions remain, however, unanswered about the possible outcome/contribution of using combinations of different treatment modalities: which is the contribution of each technique? Does the combination empower or interfere with basic mechanisms underpinning recovery and operating at central level? Does the combination increase the risks of side effects? Future research might discuss whether the combination of different known treatment modalities should be considered as a specific modality in itself, thus experiments exploring whether the same or new basic mechanisms operate when different techniques are merged, will be needed. The influence of genetics on the recovery process after lesion of the CNS represents the most challenging edge for future research. As an example, recent evidence on animal models offered evidence, at histological level, that brain morphology is affected by different genotype profiles, after stroke. Recently, a revolutionary genetic technique (i.e. optogenetics) allowed the exploration of neuronal functioning *in vivo* during natural behaviours both in animals and humans. Optogenetics is a biological technique exploiting light to activate genetically-modified neurons, whose ion-channels are made light-sensitive by specific key-reagents. The first method for the application of this technology in mammalian neurons was published by Zemelman and co-workers (2002), after that several applications for neuroscience research have been reported and recently this technique was proposed also as a therapeutic tool for the treatment of retinitis pigmentosa in humans (Francis et al., 2013). The deployment of such techniques in rehabilitation research will open outstanding possibilities to explore which are the mechanisms occurring *in vivo* during recovery, with spatial and time resolutions not yet achievable nowadays.

Finally, future research should take into consideration the inclusion of reliable outcome measures for the detection of patients' quality of life and participation changes, due to each rehabilitation modality. In fact, according to the Cumberland consensus model (Cheeran et al., 2009), findings coming from all the phases of the research pipeline need to be translated in the delivery of health services only when results have a significant impact on patients' real life.

6.3 Conclusion

Over the three years of data collection for the whole set of experiments reported in this thesis, 215 patients were screened and 122 (56,7%) of them were enrolled and analysed. A first consideration is on the generalisability of the results obtained, still not representing the complete profile of the population of stroke patients who can be seen in clinics.

Very recently Reinkensmeyer and co-workers (2016) have proposed the first computational model for neurorehabilitation. This model is characterised by three main elements:

1. Quantitative indexes of patient's practice.
2. Computational models of plasticity-driven recovery, based on the phenomenon of experience-dependent plasticity.
3. Quantitative predictive regression models of patient's expected outcomes.

The model represents the first quantitative attempt to provide tools for interpreting motor behaviours after lesion of the CNS, planning effective rehabilitation therapies and predicting successful outcomes, with the final aim of improving translation of effective therapeutic modalities in real clinical practice. Overall, the model is composed by basic modules representing the hypothesised functions involved in neurorehabilitation, such as: action choice modules, right/left brain cortices/hemispheres modules, reward function models, reward-based learning modules, error-based learning modules.

The experiments reported in this thesis postulated that a similar model exists, with a main focus on the spinal mechanisms underpinning motor control and their representation in the brain. The hypotheses postulated started from a complimentary background as the one used for the computational model for neurorehabilitation, but in the absence of a reference framework, only published for the first time in April 2016. The results obtained fit with the model proposed by Reinkensmeyer and co-workers, thus muscle synergies represent an adjunctive module which can be implemented in the computational framework for neurorehabilitation with different meanings: a module to transduce neuronal firing from different brain areas into linear activation of peripheral muscles, a module providing a neurophysiological bio-

marker to detect true or compensatory recovery in motor function, a module with drivers for the detection of voluntary motor activation in body-machine interface applications, a module to predict neurophysiological outcomes for testing the efficacy of rehabilitation techniques. Most of the knowledge on the topic still needs to be explored and more evidence needs to be gathered to claim that these concepts might represent a concrete step ahead to base neurorehabilitation on neuroscientific evidence, with the best still to come in the field of rehabilitation neuroscience.

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Appendix 1. Description of the upper limb exercises provided in the interventions (Paragraph 5.2.4.1)

(Adapted from www.physiotherapyexercises.com)

Reaching while seated

Therapist's aim: To improve the ability to sit unsupported.

Client's aim: To improve your ability to sit unsupported.

Therapist's instructions: Position the patient in short sitting on a plinth with an object placed on a stool obliquely in front of them. Instruct the patient to reach for the object without using their other hand for support.

Client's instructions: Position yourself sitting over the edge of a plinth with an object placed on a stool in front and to the side of you. Practice reaching for the object without using your other hand for support.

Progressions and variations: Less advanced: 1. Perform similar activities sitting in a wheelchair. 2. Ensure thighs and feet are well supported. 3. Decrease reaching distance. More advanced: 1. Decrease thigh support. 2. Sit on stools of different height. 3. Increase reaching distance. 4. Instruct patient to carry out different tasks. 5. Instruct patient to move objects between their two hands.

Precautions: 1. Ensure that the patient does not fall forwards.

Reaching diagonally in sitting

Therapist's aim: To improve the ability to reach.

Client's aim: To improve your ability to reach.

Therapist's instructions: Position the patient in a sitting position with an object obliquely in front of them. Instruct the patient to reach and pick up the object with their hand. Ensure that the shoulder does not elevate or internally rotate.

Client's instructions: Position yourself sitting with an object obliquely in front of you. Practice reaching forwards to pick up the object with your hand. Ensure that you do not hitch your shoulder or move your elbow out to the side.

Progressions and variations: Less advanced: 1. Position the object closer and at a less oblique angle. 2. Use an object that is easy to manipulate. More advanced: 1. Position the object further away and at a more oblique angle. 2. Use an object that is difficult to manipulate.

Reaching from a low surface to a high surface

Therapist's aim: To improve the ability to reach.

Client's aim: To improve your ability to reach.

Therapist's instructions: Position the patient in a sitting position with a block on a table in front of them. Instruct the patient to lift a cup from the table to the top of the block. Ensure that the arm flexes forward rather than abducts and the shoulder does not elevate.

Client's instructions: Position yourself sitting with a block on a table in front of you. Practice lifting a cup from the table to the top of the block. Ensure that your elbow stays tucked in and your shoulder does not hitch.

Progressions and variations: Less advanced: 1. Lift the hand only. 2. Use a lower block. More advanced: 1. Add water to the cup. 2. Use a cup that can deform. 3. Use a higher block.

Reaching to different targets

Therapist's aim: To improve the ability to reach.

Client's aim: To improve your ability to reach.

Therapist's instructions: Position the patient in a sitting position in front of a table that has markers on it. Instruct the patient to move an object from one marker to another with their hand.

Client's instructions: Position yourself sitting in front of a table with markers on it. Practice moving an object from one marker to another with your hand.

Progressions and variations: Less advanced: 1. Use an object that is easy to pick up. 2. Move the object through a smaller distance. More advanced: 1. Use an object that is difficult to pick up. 2. Move the object through a larger distance.

Shoulder abductor strengthening in a sitting position using free weights

Therapist's aim: To strengthen the shoulder abductors.

Client's aim: To strengthen the muscles at the side and top of your shoulder.

Therapist's instructions: Position the patient in a sitting position with their shoulder adducted. Instruct the patient to abduct their shoulder with their elbow extended.

Client's instructions: Position yourself sitting in a chair. Start with your arm down beside your body. Finish with your arm above your head. Ensure that you keep your elbow straight.

Progressions and variations: More advanced: 1. Progress using strength training principles.

Precautions: 1. Ensure that the chair does not tip backwards.

Sliding the arm forwards on a table

Therapist's aim: To improve the ability to protract the shoulder.

Client's aim: To improve your ability to reach forward.

Therapist's instructions: Position the patient in a sitting position with their arm extended on a table at shoulder height in front of them. Place a slide sheet under their arm and an object a small distance beyond their fingertips. Instruct the patient to slide their arm forwards to touch the object. Ensure that the elbow remains straight and the trunk remains against the chair.

Client's instructions: Position yourself sitting with your arm resting straight out on a table in front of you. Place a slide sheet under your arm and an object a small distance beyond your fingertips. Practice sliding your arm forwards to touch the object. Ensure that you keep your elbow straight and your back against the chair.

Progressions and variations: Less advanced: 1. Position the object closer to the fingertips. 2. Place the arm in an airsplint. More advanced: 1. Position the object so that maximum protraction is required. 2. Remove the slide sheet. 3. Remove the table.

Sliding the hand forwards on a table

Therapist's aim: To improve the ability to forward flex the shoulder.

Client's aim: To improve your ability to move your arm forward.

Therapist's instructions: Position the patient in a sitting position with their hand resting on a table in front of them and an object on the table. Instruct the patient to slide their hand forward to touch the object. Ensure that the patient does not elevate or internally rotate their shoulder.

Client's instructions: Position yourself sitting with your hand resting on a table in front of you and an object on the table. Practice sliding your hand forward to touch the object. Ensure that you do not hitch your shoulder or move your elbow out to the side.

Progressions and variations: Less advanced: 1. Sprinkle talcum powder on the table to reduce friction. 2. Decrease the distance to the object. More advanced:

1. Increase the distance to the object. 2. Lift the hand forwards rather than sliding it on the table.

Standing and reaching

Therapist's aim: To improve the ability to reach forward when standing.

Client's aim: To improve your ability to reach forward when standing.

Therapist's instructions: Position the patient in a standing position with a table at hip level a small distance in front of them and an object at shoulder height a little further than an arm-length away. Instruct the patient to reach forward and pick up the object. Ensure that their hips make contact with the table.

Client's instructions: Position yourself standing with a table at hip level a small distance in front of you and an object at shoulder height a little further than an arm-length away. Practice reaching forward to pick up the object. Ensure that your hips make contact with the table.

Progressions and variations: Less advanced: 1. Position the table and object closer. More advanced: 1. Position the table and object further away.

Taking a cup to the mouth

Therapist's aim: To improve the ability to bring a cup to the mouth.

Client's aim: To improve your ability to bring a cup to your mouth.

Therapist's instructions: Position the patient in a sitting position with a cup on a table in front of them. Instruct the patient to lift the cup up to their mouth. Ensure that their head remains erect.

Client's instructions: Position yourself sitting with a cup on a table in front of you. Practice lifting the cup up to your mouth. Ensure that you keep your head up straight.

Progressions and variations: Less advanced: 1. Lift the hand to the mouth without holding a cup. More advanced: 1. Add water to the cup.

Precautions: 1. Ensure appropriate for patients with swallowing difficulties.

Reaching forward to an object

Therapist's aim: To improve the ability to grasp an object.

Client's aim: To improve your ability to pick up an object.

Therapist's instructions: Position the patient in a sitting position with three objects placed slightly apart on a table in front of them. Instruct the patient to reach

forward and pick up the middle object without touching the objects on either side. Ensure that pre-shaping of the hand occurs at the start of the reaching movement. *Client's instructions:* Position yourself sitting with three objects placed slightly apart on a table in front of you. Practice reaching forward to pick up the middle object without touching the objects on either side. Ensure that you shape your hand to match the middle object as you start reaching.

Progressions and variations: Less advanced: 1. Place the objects further apart. 2. Use a middle object with a smaller diameter. 3. Use a middle object that cannot deform. More advanced: 1. Place the objects closer together. 2. Use a middle object with a larger diameter. 3. Use a middle object that can deform. 4. Pick up a middle object that is filled with water.

Pouring water between cups

Therapist's aim: To improve the ability to pronate and supinate the forearm.

Client's aim: To improve your ability to rotate your forearm.

Therapist's instructions: Position the patient in a sitting position with two cups on a table and holding a third cup filled with water. Instruct the patient to pour water into each cup on the table by pronating and supinating their forearm. Ensure that the elbow remains bent at about 90 degrees to minimise shoulder rotation.

Client's instructions: Position yourself sitting with two cups on a table and holding a third cup filled with water. Practice pouring water into each cup on the table by rotating your forearm one way and then the other way. Ensure that you keep your elbow bent.

Progressions and variations: Less advanced: 1. Perform the exercise without water in the cup. 2. Use a cup that does not deform with pressure. 3. Support the forearm with a sandbag. More advanced: 1. Perform the exercise without any support under the forearm. 2. Use smaller diameter cups or a bottle.

Extending the arm to a target in lying

Therapist's aim: To improve the ability to reach forward and protract the shoulder.

Client's aim: To improve your ability to reach forward.

Therapist's instructions: Position the patient in a supine position with their arm and fingers extended vertically and a target object a small distance above their fingertips. Instruct the patient to reach up and touch the object with their fingertips. Ensure that the trunk stays on the bed and the elbow remains straight.

Client's instructions: Position yourself lying on your back with your arm out straight and pointing vertically. Practice reaching up to touch a target object a small distance above your fingertips. Ensure that your body remains in contact with the bed and your elbow is held straight.

Progressions and variations: Less advanced: 1. Position the object closer to the fingertips. 2. Place the arm in an airsplint. 3. Support the arm (e.g. against a wall). More advanced: 1. Position the object so that maximum protraction is required.

Forearm supination and pronation to a wall target

Therapist's aim: To improve the ability to reach for objects.

Client's aim: To improve your ability to reach for objects.

Therapist's instructions: Position the patient in a sitting position in front of a table and wall with their elbow bent and a ruler taped to their hand. Draw two target lines on some paper on the wall. Instruct the patient to supinate and pronate their forearm until the ruler reaches the target lines. Ensure that internal rotation at the shoulder level does not occur.

Client's instructions: Position yourself sitting in front of a table and wall with your elbow bent and a ruler taped to your hand. Draw two target lines on some paper on the wall. Practice rotating your forearm back and forth until the ruler reaches the target lines. Ensure that the hand does not move sideways.

Progressions and variations: Less advanced: 1. Decrease distance between the target lines. More advanced: 1. Increase distance between the target lines to maximum range. 2. Tape a small weight to the ruler.

Cupping the hand while picking up a plate

Therapist's aim: To improve the ability to manipulate objects with the hand.

Client's aim: To improve your ability to manipulate objects with your hand.

Therapist's instructions: Position the patient in a sitting position with a plate on a table in front of them. Instruct the patient to pick up the plate by placing their fingers underneath and thumb on top of the plate. Ensure that cupping of the hand occurs.

Client's instructions: Position yourself sitting with a plate on a table in front of you. Practice picking up the plate by placing your fingers underneath and thumb on top of the plate. Ensure that your palm forms a cup shape when lifting the plate.

Progressions and variations: Less advanced: 1. Pick up a lighter plate. 2. Position the plate closer. More advanced: 1. Pick up a heavier plate. 2. Position the plate further away. 3. Place small objects (eg. small balls) on the plate.

Elbow flexor strengthening in supine using free weights

Therapist's aim: To strengthen the elbow flexors.

Client's aim: To strengthen your biceps.

Therapist's instructions: Position the patient in a supine position. Instruct the patient to flex their elbow.

Client's instructions: Position yourself lying on your back. Start with your arm down beside your body. Finish with your hand up near your shoulder. Ensure that your elbow is held beside your body.

Progressions and variations: More advanced: 1. Progress using strength training principles.

Elbow extensor strengthening in lying without weights

Therapist's aim: To strengthen the elbow extensors.

Client's aim: To strengthen the muscles that straighten your elbow.

Therapist's instructions: Position the patient in a supine position with their arm extended vertically. Instruct the patient to bend their elbow to bring their palm to touch their forehead and then straighten it again. Ensure that the upper arm remains vertical and only the forearm moves.

Client's instructions: Position yourself lying on your back with your arm held vertically. Start with your elbow straight. Finish with your elbow bent and your palm touching your forehead. Ensure that your upper arm doesn't move and only your forearm moves.

Progressions and variations: Less advanced: 1. Support the proximal part of the arm. More advanced: 1. Progress using strength training principles.

Elbow flexor strengthening in sitting using free weights

Therapist's aim: To strengthen the elbow flexors.

Client's aim: To strengthen your biceps.

Therapist's instructions: Position the patient in a sitting position with their elbow and shoulder extended. Instruct the patient to flex their elbow.

Client's instructions: Position yourself sitting with your arm down beside your body. Start with your elbow straight. Finish with your elbow bent. Ensure that you keep your elbow tucked in beside your body.

Progressions and variations: More advanced: 1. Progress using strength training principles.

Lifting up the arm in sitting.

Therapist's aim: To improve the ability to reach.

Client's aim: To improve your ability to reach.

Therapist's instructions: Position the patient in a sitting position with their arm supported on a table and extended in front of them. Instruct the patient to lift their arm off the table to the target. Ensure that the shoulder does not elevate and the elbow remains extended.

Client's instructions: Position yourself sitting with your arm supported on a table and extended in front of you. Practice lifting your arm off the table to reach the target. Ensure that your shoulder does not raise and your elbow remains straight.

Progressions and variations: Less advanced: 1. Apply an elbow splint to keep the elbow extended. More advanced: 1. Increase lifting height and weight.

Pouring beans from a cup

Therapist's aim: To improve the ability to supinate and pronate the forearm.

Client's aim: To improve your ability to rotate your forearm.

Therapist's instructions: Position the patient in a sitting position while holding a foam cup filled with beans. Instruct the patient to supinate their hand and empty the beans onto the table. Ensure that the elbow is bent at about 90 degrees to minimise shoulder rotation.

Client's instructions: Position yourself sitting while holding a foam cup filled with beans. Practice rotating your hand outwards to empty the beans onto the table. Ensure that you keep your elbow bent.

Progressions and variations: Less advanced: 1. Use a cup that does not deform with pressure. 2. Support the forearm with a sandbag. More advanced: 1. Hold onto a heavier object.

Wrist extensor strengthening in sitting using free weights

Therapist's aim: To strengthen the wrist extensors.

Client's aim: To strengthen your wrist muscles.

Therapist's instructions: Position the patient in a sitting position with their arm supported on a table, their forearm pronated and their hand over the edge of the table. Instruct the patient to extend their wrist.

Client's instructions: Position yourself sitting with your arm supported on a table, your palm facing downwards and your hand over the edge of the table. Start with your wrist dropped downwards. Finish with your wrist pulled upwards.

Progressions and variations: More advanced: 1. Progress using strength training principles.

Wrist extensor strengthening in supine using free weights

Therapist's aim: To strengthen the wrist extensors.

Client's aim: To strengthen your wrist muscles.

Therapist's instructions: Position the patient in a supine position with their elbow semi-flexed and wrist fully flexed and a weight attached around their hand. Instruct the patient to extend their wrist.

Client's instructions: Position yourself lying on your back with a weight attached around your hand. Start with your wrist dropped downwards. Finish with your wrist pulled upwards.

Progressions and variations: More advanced: 1. Progress using strength training principles.

Wrist flexor strengthening in supine using free weights

Therapist's aim: To strengthen the wrist flexors.

Client's aim: To strengthen your wrist muscles.

Therapist's instructions: Position the patient in a supine position. Instruct the patient to flex their wrist.

Client's instructions: Position yourself lying on your back. Start with your wrist dropped downwards. Finish with your wrist pulled upwards.

Progressions and variations: More advanced: 1. Progress using strength training principles.