Motor skill learning after stroke: exploration of neurophysiological mechanisms with functional neuroimaging and therapeutic modulation by non-invasive brain stimulation

by

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List of abbreviations

ADL:	activities of daily living
AMPA:	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid
BA:	Brodmann area
BBT:	box and block test
BDNF:	brain derived neurotrophic factor
BG:	basal ganglia
C:	cortical
CBLM:	cerebellum
CNS:	central nervous system
CST:	corticospinal tract
CT:	computed tomography
DLPFC:	dorsolateral prefrontal cortex
PMd:	dorsal premotor cortex
EEG:	electroencephalogram
F:	female
FA:	flip angle
FAT:	Frenchay Arm Test
fMRI:	functional magnetic resonance imaging
FOV:	field of view
GABA:	γ-aminobutyric acid
GEE:	generalized estimating equations
GF:	grip force
GLM:	general linear model
DAMH:	damaged hemisphere
UNDAMH:	undamaged hemisphere
Hz:	hertz
JTT:	Jebsen Taylor Test
KG:	kilograms
L:	left
LD:	loading phase duration
LF:	load force
LI:	learning index
LK:	leukoaraiosis
LNU:	learned non-use
LTD:	long-term depression
LTP:	long-term potentiation

M:	male
M1:	primary motor cortex
MAS:	modified Ashworth scale
MaxHF:	maximal hand grip force
MCA:	middle cerebral artery
MEP:	motor evoked potential
mRS:	modified Rankin scale
MS:	millisecond
MT:	movement time
NIBS:	non-invasive brain stimulation
NIHSS:	national institute of health stroke score
NJ:	normalized jerk
NMDA:	N-methyl-D-aspartate
PET:	positron emission tomography
PI:	performance index
PLD:	preloading phase
PM:	premotor cortex
PPT:	Purdue pegboard test
PRT:	pursuit rotor task
R:	right
RCT:	randomized control trial
RT:	reaction time
rTMS:	repetitive transcranial magnetic stimulation
S:	second
SAT:	speed/accuracy trade-off
SC:	subcortical
SIS:	stroke impact scale
SLI:	subcortical lacunar infarctions
SMA:	supplementary motor area
SRTT:	serial reaction task
SSI:	small subcortical infarctions
TBS:	theta burst stimulation
tDCS:	transcranial direct current stimulation
TE:	echo time
TR:	repetition time
TS:	time shift
UDP:	use-dependent plasticity

ULD: unloading phase duration
ULES: upper limb electrical stimulation
VMT: visuomotor tracking
PMv: ventral premotor cortex
WHO: World Health Organization
WMFT: Wolf motor function test
WSO: World Stroke Organization

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CHAPTER 1: INTRODUCTION

1.1. Clinical, functional and socio-economic consequences of stroke

Stroke is defined as the neurological deficits that occur when blood flow supply to a part of the brain is suddenly interrupted. Eighty-seven percent of the strokes are secondary to the occlusion of a cerebral vessel (ischemic stroke), the remaining 13% to an intra-cerebral haemorrhage (haemorrhagic stroke) (Lloyd-Jones et al. 2009). Stroke induces sudden neurologic deficits and could result in death. Worldwide, stroke leads to death in 19.4% of stroke victims (Roger et al. 2012). According to the World Stroke Organization (WSO), one out of six persons in the world will suffer from a stroke; in other words, 795 000 new people are victims of a stroke every year, making stroke the first cause of adult disability in Western countries (Greenwood et al. 2009).

A majority of stroke patients encounters enduring physical and neuropsychological disabilities such as motor, speech, visual, cognitive and perceptual disorders. According to the World Health Organization (WHO) classification (International Classification of Functioning, Disability and Health), the consequences of stroke on patients can be classified as body (structural/functional) impairments, activity limitations and participation restrictions (Figure 1.1). Functional impairments refer to deficits in sensorimotor skills or to sensorimotor troubles (e.g. hemiparesis) and constitute one of the main problems for many stroke patients. Actually, functional impairments have been reported in up to 80% of stroke patients; only 12% of stroke survivors achieve complete motor recovery after six months (Kwakkel et al. 2003) and 30% are dependent on others during their lifetime (Laloux 2003). Hemiparetic stroke patients suffer from impairments in voluntary movements such as reaching and grasping due to a decrease in arm velocity, abnormalities in the initial movement direction and abnormal co-contractions of agonist and antagonist muscles (Hermsdorfer et al. 1999; Hermsdorfer et al. 2003; Halsband and Lange 2006; Lang et al. 2006; McDonnell et al. 2006). Stroke patients also present altered patterns of multi-joint coordination (Levin 1996; Zackowski et al. 2004), as well as a

general decrease in force and accuracy in arm movements, control of hand trajectories and fine hand control (Li et al. 2003). In addition, stroke patients may suffer from spasticity, an abnormal increase in muscular tone associated with exaggerated stretch reflexes (Watkins et al. 2002; Pizzi et al. 2005; Biering-Sorensen et al. 2006). Spasticity may further deteriorate voluntary movements' performance and lead to the development of abnormal movement's patterns and postures (Mirbagheri et al. 2007). Neglect (Pedersen et al. 1997) and aphasia (Berthier 2005) are also major disabilities which perturb stroke recovery and limit independence. Depression and anxiety are affective impairments often observed in stroke patients with severe motor deficits (Robinson and Merrill 2003). These disabilities could result in a lack of motivation and willingness which negatively influence the recovery process.

From an economic perspective, stroke is a burden both for the patients and for the society. At the personal level, stroke may lead to devastating individual limitations and dramatic social restrictions which may be associated with incapacity to work and important emotional troubles, leading to a financial disaster: need of nursing or other paramedical cares, supervision in daily life, expenses to accommodate the house, car, etc...(Laloux 2003; Di Carlo 2009). At the community level, the cost of stroke is distributed among different aspects, such as hospitalization, drugs, medical complications, rehabilitation process, loss of productivity etc…(Laloux 2003).

1.2. Natural recovery process after stroke

After stroke, recovery occurs spontaneously and has been documented in man and in animal models. This spontaneous recovery relies on several processes including the resolution of the metabolic dysfunctions, the intrinsic property of the central nervous system (CNS) to reorganized itself, a phenomenon called plasticity and behavioural compensations (Kwakkel et al. 2004; Pascual-Leone et al. 2005).

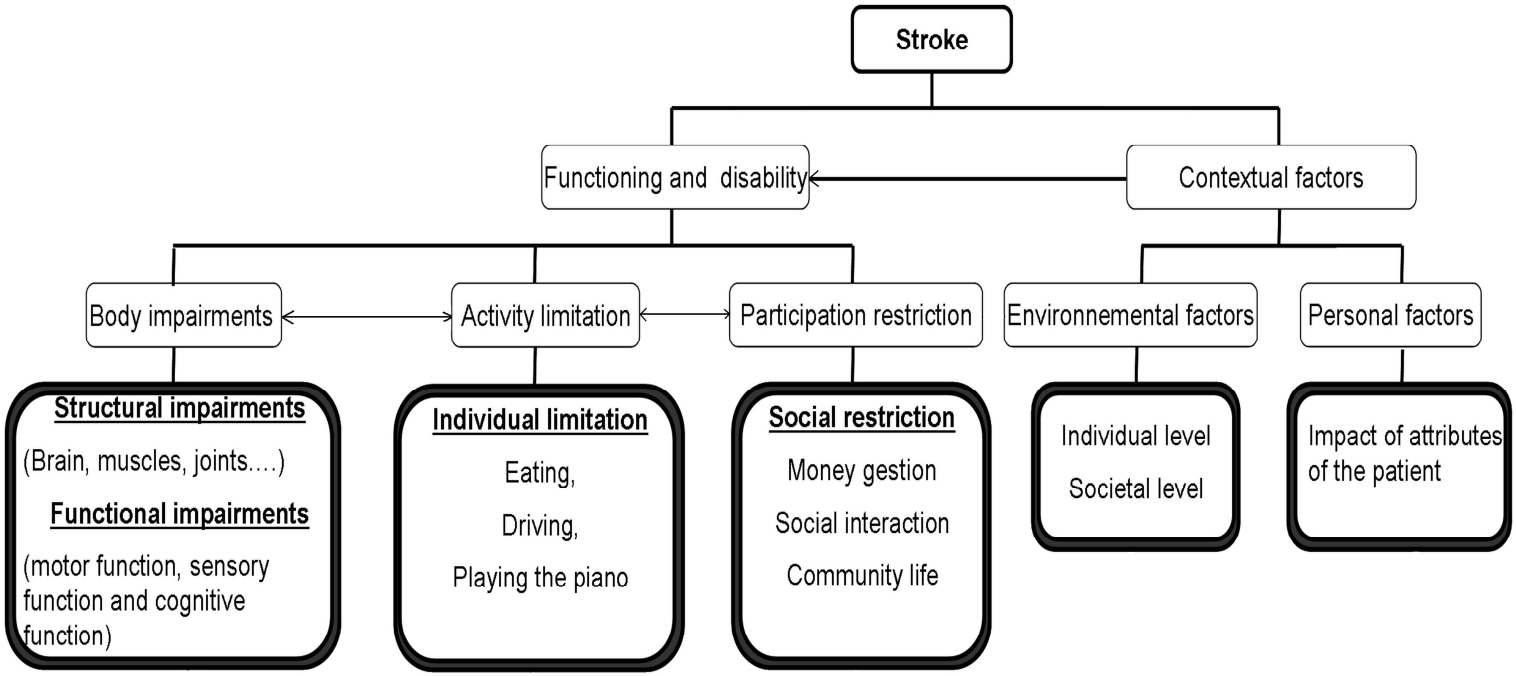

Figure 1.1: Impact of stroke on patients (modified from WHO 2001: *http://www.disabilitaincifre.it/documenti/ICF_18.pdf)*

1.2.1. Functional recovery and the resolution of metabolic dysfunctions

Longitudinal studies suggest that most of the recovery from hemiparesis takes place during the first six months after stroke, especially during the very first weeks (Buma et al. 2010; Langhorne et al. 2011). Post-stroke recovery can be divided in several phases (Kreisel et al. 2006), each phase being characterized by different metabolic events (for an extensive description of these events see (Kreisel et al. 2006; Wieloch and Nikolich 2006; Carey and Seitz 2007). In the hyperacute phase (up to few hours after stroke) important metabolic events such as cell death, oedema, metabolic depression, inflammation and axonal growth inhibition occur in the damaged hemisphere. These metabolic events start rapidly, and intensify in the following days. In the acute phase (up to few days after stroke), an increase in excitability of the intact hemisphere occurs (Delvaux et al. 2003). The subacute phase (up to few weeks afters stroke) is characterized by the resolution of some deleterious metabolic events which begun during the hyperacute phase such as oedema resolution and inflammatory processes. This is also the most dynamical period as changes in excitatory and inhibitory synapses, angiogenesis, gliogenesis and neurogenesis occur. In these early stages, the stroke patients experience either hemiplegia or hemiparesis and will hopefully start to recover voluntary mobility. They may also start developing compensatory strategies, before or at the same time as neurorehabilitation is initiated. During the consolidation stage, the neurofunctional alterations wane. The patients will adapt their daily activities to their new medical condition and slowly reach their theoretical maximal level of recovery, with a hypothetical plateau after 6 months. During the chronic phase, motor performances and deficits are supposed to remain stable. However, task-specific training and intensive neurorehabilitation programs can improve motor function, even years after the stroke (Carey et al. 2002). The chronic phase is associated with a restored metabolic activity in the damaged hemisphere.

1.2.2. Functional recovery and plasticity of the motor system

Plasticity refers to the ability of CNS to modify its activity or structure as an adaptive response to many challenging situations such as development, continuous adjustments when facing changing environmental conditions, training and learning, and in response to brain damages (Gomez-Fernandez 2000; Pascual-Leone et al. 2005). Several mechanisms play a role in brain plasticity; some of these mechanisms are also involved in memory formation. This Introduction focuses on plasticity associated with motor function recovery after stroke. Based on several imaging studies in humans (Ward et al. 2003; Lotze et al. 2006b) and on neurophysiological investigations in animals models (Nudo 2007; Kleim and Jones 2008; Xerri 2012), the motor function recovery process after brain damaged seems to be associated with plasticity in sensorimotor and premotor areas spared by stroke.

1.2.2.1. Molecular mechanisms of brain plasticity after stroke

The cellular mechanisms responsible for brain plasticity have been brought out in animals models (Hallett 2001) and consist in several processes such as remapping the cortical motor map by unmasking of silent connexions (Jacobs and Donoghue 1991), modifying the activity-dependent synaptic connectivity (i.e. strengthening or weakening existing synapse) by long-term potentiation (LTP) and long-term depression (LTD) (Trachtenberg et al. 2002; Malenka 2003), increasing the number of synapses and growth of new axon terminals (Toni et al. 1999), or modifying the membrane excitability (the cholinergic, noradrenergic and serotoninergic receptors are the most important receptors for cortical plasticity (Gu 2002)). These mechanisms of plasticity have been observed in animal subjected to experimental stroke and seem to sustain the recovery of motor function (Nudo et al. 1996; Plautz et al. 2003; Nudo 2006).

1.2.2.2. Evidence for post-stroke plasticity

In animal models, lesions of the primary motor cortex (M1) may induce peri-lesional reorganization, i.e. the remapping of motor function in

spared portions of M1 or in adjacent cortical areas. The first experimental evidence of functional brain reorganization after brain injury came from a study in macaque monkeys in 1950 (Glees and Cole 1950), which demonstrated that after a focal lesion of M1 in the thumb representation area, a new representation of the thumb appeared near the lesion. Since then, many other studies in non-human primates confirmed the existence of plasticity after brain injury (Jenkins et al. 1990; Nudo and Milliken 1996; Darling et al. 2011; Xerri 2012). In humans, stroke can also induce reorganization within the peri-lesional cortex. In stroke patients, the surface occupied by the hand motor representation in the damaged hemisphere ($M1_{damH}$), measured by transcranial magnetic stimulation (TMS) mapping, is reduced when compared to the hand representation in the undamaged M1 ($M1_{undamH}$) or compared to healthy controls. However, after neurorehabilitation, the hand motor representation in $M1_{damH}$ enlarged; this was associated with motor function recovery (Traversa et al. 1997). In addition, during movements with the paretic hand, the functional activation in $M1_{damH,}$ measured by functional magnetic resonance imaging (fMRI), may be initially dampened and may coincide with a compensatory recruitment of adjacent cortical areas (Nhan et al. 2004; Jaillard et al. 2005; Cramer 2008). However, this compensatory activation tends to decrease with motor function recovery, i.e. back towards a recovery of $M1_{damH}$ activation and a reduction of additional adjacent areas recruitment (Nhan et al. 2004; Jaillard et al. 2005; Cramer 2008).

Plasticity of the motor maps is not restricted to peri-lesional areas. In monkeys, a lesion of the M1 hand representation conducts to an enlargement of the hand representation in the ipsilateral ventral premotor cortex (PMv) (Frost et al. 2003). The recruitment of remote areas in the damaged hemisphere (especially the dorsal premotor (PMd) and sensorimotor areas) during movements with the paretic hand has been documented in human stroke patients (Carey et al. 2005; Jaillard et al. 2005). In addition, the recruitment of remote areas in the damaged hemisphere is compensatory, as demonstrated by the impairment in paretic

hand function observed when the function of PMd is disrupted by a single TMS pulse (Johansen-Berg et al. 2002b; Fridman et al. 2004).

Finally, even the undamaged hemisphere might be recruited after stroke and may play a role in functional recovery. fMRI and positron emission tomography (PET) studies demonstrated post-stroke cortical reorganisation associated with motor recovery and showed that abnormal activation patterns in the undamaged hemisphere can be observed during the performance of simple task after stroke, compared to healthy individuals. Exempli gratia (E.g.), after stroke, simple motor tasks involved contralesional brain activation, which tends to diminish in parallel with motor recovery, back towards predominantly ipsilesional activation (Chollet et al. 1991; Marshall et al. 2000; Nelles et al. 2001; Johansen-Berg et al. 2002a; Calautti and Baron 2003).

Recent and sophisticated methods of fMRI and electroencephalography (EEG) analysis permit to explore functional connectivity in the brain. The functional connectivity allows mapping brain networks by computing the strength of the correlation between spontaneous activities of areas belonging to a common network. In others words, this permits to detect the functional connection between spatially remote areas based on their amount of synchronous activity (Friston 2011). Exploring functional connectivity demonstrated that the network controlling motor execution is reorganised after stroke (Wang et al. 2010).

In addition, the use of diffusion tensor imaging (DTI) permits to evaluate the physical characteristics of white matter fibres by determining the quality of tissue microstructure depending on the molecular diffusion of water DTI measurements. DTI demonstrated that increase in fractional anisotropy (FA) or decrease in apparent diffusion coefficient (ADC) (supposed to reflect structural modifications of the white matter tracts (Assaf and Pasternak 2008; Neil 2008)) in the corticospinal tract (CST) are associated with motor recovery (Jang 2011).

In summary, after focal brain injury such as stroke, brain plasticity plays a central role in the compensatory reorganisation (Cramer 2008).

Following any brain damage, the CNS undergoes a deep functional and structural reorganisation that supports recovery of impaired sensory-motor and cognitive skills (Pineiro et al. 2001; Cramer 2008). Beyond this central role in the recovery of motor function, plasticity is also involved in post-stroke recovery of other functions disorders such as dysphagia, aphasia, neglect, visual impairments, etc… (Seghier et al. 2005; Schlaug et al. 2009; Allendorfer et al. 2012). However, not all forms of plasticity are beneficial; stroke may also lead to dysfunctions of brain activity or abnormalities in cortical excitability or imbalance in interhemispheric interactions (Elbert and Rockstroh 2004; Murase et al. 2004; Allred and Jones 2008).

1.2.3. Deregulated inter-hemispheric interactions after stroke

In the healthy human brain, the homotopic areas of the two hemispheres are tightly coupled, mostly through the corpus callosum (Bloom and Hynd 2005), and showed balanced reciprocal inhibitory interactions (Nowak et al. 2009). Most of the interhemispheric connections are inhibitory and might be modulated by circumstances (Ferbert et al. 1992; Perez and Cohen 2009). E.g., depending on the motor task, the interhemispheric interactions may be inhibitory or excitatory. In healthy individuals, movement onset is associated with a reversal of interhemispheric interaction from an inhibitory drive to an excitatory drive from the non-active motor cortex towards the active one (i.e. the motor cortex contralateral to the active hand) (Takeuchi et al. 2012).

After stroke, imbalance in interhemispheric interactions has been observed; these deregulated interhemispheric interactions were associated with motor dysfunction (Murase et al. 2004). TMS studies in stroke patients demonstrated that an abnormal interhemispheric inhibition persists from the $M1_{undamH}$ to the $M1_{damH}$ around the onset of the paretic hand movement (Murase et al. 2004; Takeuchi et al. 2012). The concept of interhemispheric rivalry suggests that the undamaged hemisphere could exert an abnormal (increased) interhemispheric inhibition to the damaged hemisphere (Duque et al. 2005; Sauerbrei and Liepert 2012).

This imbalance in interhemispheric interactions after stroke was first suggested by clinical clues. E.g., after stroke, involuntary mirrors movements of the non-active upper limb can be observed (Kim et al. 2003; Etoh et al. 2010; Beaule et al. 2012), suggesting an impairment in the normal interhemispheric inhibition, although this could also reflect the abnormal/compensatory recruitment of the ipsilateral CST to perform the task with the paretic hand. Furthermore, functional brain imaging during paretic hand movement showed over-activation of the contralesional motor network, suggesting an abnormal interhemispheric balance (Calautti et al. 2007). Finally, as already mentioned, studies measuring brain excitability with TMS formally demonstrated the existence of an interhemispheric imbalance with abnormally increased inhibitory drive from the $M1_{undamH}$ towards the $M1_{damH}$ (Murase et al. 2004; Kirton et al. 2008; Sauerbrei and Liepert 2012). The importance of these abnormal interhemispheric interactions correlated with the magnitude of motor dysfunction in stroke patients (Murase et al. 2004; Kirton et al. 2008; Sauerbrei and Liepert 2012). Thus, after stroke, the excitability/function of $M1_{damH}$ is impaired not only by the lesion itself but also by an additional and abnormal interhemispheric inhibition from $M1_{undamH}$.

Since this imbalance in interhemispheric inhibition exerts a deleterious effect on $M1_{damH}$ and prevents the full expression of its potential for motor recovery, re-balancing interhemispheric interactions and/or restoring excitability in the $M1_{damH}$ may become a therapeutic goal. Such a goal may be achieved by the use of non-invasive brain stimulations (NIBS).

1.3. Non-invasive brain stimulations (NIBS)

In addition to brain tissue destruction, stroke also induces a deregulation of cortical excitability (Butefisch et al. 2008; Carter et al. 2010; Kirton et al. 2010). Some of the post-stroke (motor) deficits could thus be lessened by restoring brain excitability/activity, e.g. rebalancing deregulated interhemispheric interactions. Due to their ability to modulate cortical excitability, NIBS such as repetitive TMS (rTMS) and transcranial direct current stimulation (tDCS) have an interesting therapeutic potential (Hummel

et al. 2005; Nowak et al. 2009; Bolognini et al. 2011). Three strategies of modulation of the cortical excitability by NIBS could be used: **i)** up-regulating the excitability of the damaged hemisphere, **ii)** down-regulating the excitability of the undamaged hemisphere, or **iii)** cumulating both with bilateral NIBS. The mechanisms of action as well as the technical parameters of rTMS and tDCS are described in the next section.

1.3.1 Repetitive transcranial magnetic stimulation rTMS:

1.3.1.1 Definition and mechanisms of action of rTMS

TMS is a painless method of NIBS. Magnetic pulses are administered through a coil placed on the scalp. These magnetic pulses pass through the skull and generate a local electric field within the brain, sufficient to depolarize cortical neurons and induce their firing (Barker et al. 1985; Allen et al. 2007). A broad variety of TMS pulses schedules may be used depending on the goal. The main modes of stimulation could be summarized as follow. First, the single-pulse TMS mode is mainly used for clinical diagnostic and research protocol. For example, single-pulse TMS is used to determine the stimulation intensity required to induce a motor response (the motor threshold, MTh) (Tranulis et al. 2006). Single-pulse TMS also permits to explore the integrity of the CST (Pennisi et al. 1999; Hendricks et al. 2002) through the persistence/lack of motor evoked potentials (MEP). MEPs are recorded from muscles with surface electromyography (EMG) electrodes and evoked by the recruitment of the CST originating from M1. Second, the paired-pulses TMS mode consists of two pulses separated by a short inter-pulse interval. The paired-pulses TMS mode is usually used for studying the cortical excitability of M1 (Ziemann et al. 1998b). Third, the rTMS mode relies on trains of several TMS pulses delivered at a defined frequency. This mode is usually used in clinical or research protocols as treatment or as a tool to modulate brain excitability and behaviour. Depending on the delay interval between the TMS pulses, the effects of rTMS on cortical excitability are different.. First, rTMS could be used in an excitatory mode (high-frequency rTMS), which induces an overall increase in cortical excitability when the pulses are repeated at a frequency

superior to 5 Hertz (Hz). Second, it could be used in an inhibitory mode (low-frequency rTMS), which induces an overall decrease in cortical excitability when the TMS pulses are repeated at a frequency equal or inferior to 1 Hz (Pascual-Leone et al. 1994; Wassermann et al. 1996a; Wassermann et al. 1996b). Theta bursts stimulation (TBS), another specific rTMS protocol, is based on low-intensity rTMS bursts at high-frequency (50 Hz, theta bursts). Two basic patterns of TBS are usually used (Huang et al. 2005): continuous TBS (cTBS), with theta bursts administered in a continuous train, decreasing cortical excitability, and intermittent TBS (iTBS) composed of interleaved trains of theta-burst which increase cortical excitability.

The modulations of cortical excitability observed after rTMS application are transient and decrease shortly after the end of the stimulation (up to 30 min for 30 min of stimulation) (Tsuji and Rothwell 2002). The modulation of cortical excitability induced in M1 may be quantified by measuring change in MEPs amplitude. An increase in MEPs amplitude is observed after high-frequency rTMS whereas a decrease in MEPs amplitude is observed after low frequency rTMS (Di Lazzaro et al. 2008; Di Lazzaro et al. 2011; Goldsworthy et al. 2012). rTMS also modulates oscillatory activity as measured by EEG (Helfrich et al. 2012).

The electric current induced by TMS into the brain leads to a depolarisation of neurones (Hummel et al. 2006; Wagner et al. 2007; Yue et al. 2009). Long-term after-effects mediated by rTMS rely on both LTP and LTD induction (Wang et al. 2011a) and on the induction of brain derived neurotrophic factor (BDNF) secretion (Yukimasa et al. 2006; Wang et al. 2011a).

Due to its ability to transiently modify motor cortex excitability, rTMS is now accepted as a standard tool for modulating human brain excitability in various research and clinical fields.

1.3.1.2. Behavioural effects of rTMS

A rapid and selective overview of the behavioural effects induced by rTMS in humans will be described in order to illustrate the potential of rTMS in the neuroscience and clinical domains.

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1.3.1.2.1. Some behavioural effects induced by rTMS in healthy individuals

Low-frequency rTMS applied over M1 induces a transient slowing of simple movements performed with the contralateral hand (Jancke et al. 2004) and an acceleration of movements performed with the ipsilateral hand (Avanzino et al. 2009). High-frequency rTMS over M1 induces a transient reduction in movement time and in reaction time (RT) in movements performed with the contralateral hand (Di Lorenzo et al. 2013). In healthy individuals, high-frequency rTMS applied over the left dorsolateral prefrontal cortex (DLPFC) improves transiently the self-perceived mood evaluated by the Beck Depression Inventory (Schaller et al. 2011). High-frequency rTMS applied over M1 or DLPFC induces transient analgesic effects in healthy individuals (Nahmias et al. 2009). Thus, through the modulation of brain excitability/activity, rTMS can induce transient behavioural modifications in healthy individuals.

1.3.1.2.2. Some behavioural effects induced by rTMS in stroke patients

Both high-frequency rTMS applied to $M1_{damH}$ and low-frequency rTMS applied to $M1_{undamH}$ induce a transient improvement in paretic hand function (for a detailed impact of rTMS on the paretic upper limb function, see next section (1.3.3)). Low-frequency rTMS applied over the leg area $M1_{undamH}$ improves transiently motor control in the lower limb and walking ability in stroke patients (Wang et al. 2012). In patients with post-stroke dysphagia, the application of high-frequency rTMS over the pharyngeal motor cortex of $M1_{undamH}$ during a two weeks treatment improves swallowing for at least two weeks follow-up period (Park et al. 2012). In acute stroke patients, high-frequency rTMS applied during a two weeks treatment over the posterior parietal cortex (PPC) in the damaged hemisphere induces a diminution of the visuospatial neglect immediately after the end of the stimulation (the duration of the after effect is not described) (Kim et al. 2013). In patients who present a non-fluent aphasia after stroke, low-frequency

rTMS applied over the right inferior frontal gyrus during a two weeks treatment induces an improvement in discourse production for at least the two months follow-up period (Medina et al. 2012). Although these studies have been performed in relatively small cohorts of stroke patients, they clearly demonstrate the high therapeutic potential of rTMS to improve post-stroke function and/or recovery.

1.3.1.2.3. Some behavioural effects induced by rTMS in patients with other medical conditions

High-frequency rTMS applied over the leg motor area daily during 15 days improves gait performance in patients with incomplete spinal cord injury, during at least the two weeks of follow-up period (Kumru et al. 2013). In a 5 day treatment in patients with Parkinson's disease, high-frequency rTMS over the leg motor area also improves gait performance during at least the six weeks follow-up period (Yip et al. 2012). In Parkinson's disease, high-frequency rTMS applied over the supplementary motor area (SMA) during a treatment of several weeks improves bradykinesia at least during the two follow-up weeks (Hamada et al. 2009). In patients with phantom pain, 20 Hz rTMS applied over M1 during a 5-day treatment produces a diminution of the perceived pain evaluated by the patients with a visual analogue scale during at least the two months of follow-up period (Ahmed et al. 2011). In patients with multiple sclerosis, a two-week period of rTMS stimulation over M1 improves spasticity in the lower limb during at least the 7 days of follow-up period (Centonze et al. 2007). A single session of rTMS or of TBS applied over the left transverse temporal gyrus (Heschl's gyrus, Brodmann area (BA) 41) reduces transiently tinnitus loudness in patients with chronic tinnitus (Lorenz et al. 2010). In migraines patients, high-frequency rTMS applied during 3 days over the left frontal cortex reduces headache frequency and severity during at least the 4 weeks follow-up period (Misra et al. 2012). High-frequency rTMS applied over the left prefrontal cortex in patients suffering from treatment-resistant depression induces an antidepressant effect as measured by a reduction in the score at the Hamilton Depression Rating Scale (Tarhan et al. 2012).

Thus, rTMS also has a very interesting therapeutic potential in a wide range of medical conditions characterised by abnormalities of brain excitability/activity.

1.3.1.3. Safety and advantages of rTMS.

The main risk with rTMS is to induce an epileptic seizure, especially in patients with brain damages and/or modified brain excitability (Nowak et al. 2006). Furthermore, rTMS application is uncomfortable for individuals as it produces unpleasant sensations on the scalp and as the TMS coil produces a loud clicking sound increased by stimulation intensity that can affect hearing after long exposure (Wassermann 1998). In contrast, the major advantages of rTMS compared to tDCS are **i)** a shorter time of stimulation, **ii)** a more focal stimulation. Indeed, during rTMS, the maximum magnitude of the induced electrical current density is spatially localized around the area targeted with the (focal) coil (Wassermann 1998).

Despite the risks carried with rTMS application, rTMS is an efficient tool with a high therapeutic potential to up- or down-regulate brain excitability/activity, and thus to influence behaviour in both healthy individuals and patients.

1.3.2. Transcranial direct current stimulation (tDCS):

1.3.2.1. Definition and mechanisms of action of tDCS

tDCS is a painless NIBS which consists in applying a low intensity direct current (between 0.5 to 2 mA) delivered between two soaked (with NaCl solution) electrodes (anode and cathode) placed on the scalp (Priori et al. 1998; Nitsche and Paulus 2000). For M1 stimulation, the classical electrode montage consists in two electrodes of the same size (up to 35cm²), one over M1 and the second electrode over the contralateral orbital region. Anodal stimulation of M1 increases cortical excitability as measured by an increase of MEPs amplitude in the contralateral hand or upper limb muscles whereas cathodal stimulation of M1 decreases cortical excitability as measured by a decrease of MEP amplitude in the contralateral hand or upper limb muscles (Nitsche and Paulus 2000; Nitsche and Paulus 2001;

Nitsche et al. 2003). The effects of tDCS on brain excitability are classically dichotomised in online effects (during tDCS application) and after-effects lasting well beyond the termination of tDCS.

Online tDCS effects on brain activity/excitability are supposed to be mediated through an action on sodium and calcium channels of the neuronal membrane (Gomez Palacio Schjetnan et al. 2013). tDCS induces a de-polarisation or an hyper-polarisation of cortical neurons thereby modulating their firing response (i.e. modulating the rate of action potential generation) (Terzuolo and Bullock 1956; Islam et al. 1995; Nitsche and Paulus 2000; Bikson et al. 2004). tDCS application also induces a modulation of the oscillatory activity as measured by EEG, (e.g. a significant and selective diminution of the power of theta band is observed after anodal stimulation (Jacobson et al. 2012)), or with fMRI as a modulation of functional connectivity between anatomically separated brain areas (Lindenberg et al. 2013; Sehm et al. 2013).

The duration of the modulation of cortical excitability is dependent on the intensity and the duration of tDCS. The after-effect of tDCS are longer when the intensity or/and duration are increased (Nitsche and Paulus 2000). For example, 30 min of tDCS applied over M1 induced a modulation of the MEP amplitudes up to 90 min (Nitsche et al. 2007; Lindenberg et al. 2010). Long-term after-effects of tDCS are mediated by the activation/insertion in the post-synaptic membrane of glutamate receptors [*N*-methyl-D-aspartate (NMDA)/2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA) and γ-Aminobutyric (GABA) (Nitsche et al. 2005; Stagg et al. 2009a; Brunoni et al. 2012; Gomez Palacio Schjetnan et al. 2013), which mediate LTP and LTD induction (Malenka and Nicoll 1999). The modulation of the glutamatergic system leads to the synthesis of key proteins such as BDNF (Fritsch et al. 2010; Clarkson et al. 2011), also involved in LTP formation.

As rTMS, tDCS is an efficient tool to modulate brain excitability and is already used in different research fields.

1.3.2.2. Behavioural effects of tDCS

A rapid and selective overview of the behavioural effects induced by tDCS in humans will be described in the following section, in order to illustrate the potential of tDCS as a tool in neuroscience and as a potentially very interesting clinical tool.

1.3.2.2.1. Some behavioural effects induced by tDCS in healthy individuals

In healthy individuals, the application of tDCS induces different transient effects depending on the polarity of tDCS: cathodal tDCS applied over M1 increases motor performance on a sequential finger tapping task with the ipsilateral hand and decreases motor performance with the contralateral hand (Vines et al. 2006). In contrast, anodal tDCS increases motor performance in the contralateral hand and decreases motor performance in the ipsilateral hand (Vines et al. 2006). Dual-tDCS (applying simultaneously anodal tDCS over M1 on one side and cathodal tDCS over the opposite M1) enhances motor function in the hand contralateral to the M1 under the anodal electrode (Vines et al. 2008). Anodal stimulation applied over the Wernicke's area shortens the naming latencies and improves the accuracy on a picture-naming task in healthy individuals (Fiori et al. 2011).

Thus, tDCS can modulate behaviour in healthy individuals, just as rTMS. So far, no direct comparison has demonstrated the superiority of tDCS over rTMS, or vice-versa.

1.3.2.2.2. Some behavioural effects induced by tDCS in stroke patients

A single session of anodal tDCS applied to $M1_{damH}$ or cathodal tDCS applied to $M1_{undamH}$ improves transiently motor performance of the paretic hand; a detailed description of the impact of tDCS on the paretic upper limb is provided in the next section (see 1.3.3). In addition, multiple sessions of anodal tDCS (Hesse et al. 2007), cathodal tDCS (Boggio et al. 2007), or dual-tDCS (Lindenberg et al. 2010) induce improvements of motor function in stroke patients lasting several weeks after the end of stimulation. Furthermore, a single session of anodal tDCS applied over the damaged

hemisphere improves transiently the voluntary control of the paretic ankle (Madhavan et al. 2011). Anodal tDCS applied over Wernicke's area during a five days treatment also shortens the naming latencies in aphasic patients during at least the 3 weeks of the follow-up period (Fiori et al. 2011). Anodal tDCS of the cortical pharyngeal motor representation in the damaged hemisphere applied during a 10 days treatment improves swallowing in acute stroke patients suffering from dysphagia during at least one month (the follow-up period) (Shigematsu et al. 2013).

As for rTMS, whereas these studies have been performed in relatively small cohorts of stroke patients, they unambiguously demonstrate the high therapeutic potential of tDCS to improve post-stroke function and/or recovery.

1.3.2.2.3. Some behavioural effects induced by tDCS in patients with other medical conditions

A single anodal tDCS session applied over the left temporoparietal area induces a significant transient reduction of tinnitus intensity and discomfort (Garin et al. 2011). Anodal tDCS applied over the left prefrontal cortex might be a good add-on tool in the treatment of anorexia (Hecht 2010). Cathodal tDCS, applied during a two weeks treatment, reduces the frequency and duration of epileptic seizure in a single epileptic patient during at least the two months follow-up period (Yook et al. 2011). There is a significant improvement in mood after anodal tDCS applied to the left prefrontal cortex applied during a three weeks treatment in patients with depression, which lasts at least three weeks (Loo et al. 2012). Anodal tDCS applied over M1 during 20 consecutive days in patients with episodic migraine diminishes migraine attack frequency as well as in the pain intensity during at least twelve weeks (Auvichayapat et al. 2012).

Thus, as for rTMS, tDCS has a very interesting therapeutic potential in a wide range of medical conditions characterised by abnormal brain excitability/activity.

1.3.2.3. Safety and advantages of tDCS

The low direct currents (1 to 2 mA) delivered by tDCS permit a safe use without any known risk of neural damages or epilepsy induction (Nitsche and Paulus 2001; Merrill et al. 2005). Nevertheless, special care is needed in patients with neurological disorders such as epilepsy (as tDCS alters regional excitability of the cortex and might theoretically activate the epileptic network) or in patients with skull damages (as those damages may distort the current flow and (theoretically) lead to electric burns). In addition to the limited side-effects (mild headache during stimulation, transient minor scalp burns) (Poreisz et al. 2007; Brunoni et al. 2012), tDCS is portable and does not need head restrain as rTMS, which makes tDCS easy to apply while the patient is receiving occupational or physical therapy. Furthermore, the large size of tDCS electrodes likely leads to the modulation of cortical excitability over a larger neural network than rTMS applied with a focal coil, which might potentially be interesting for neurorehabilitation. Finally, the sham tDCS mode permits better double-blind experiments and randomized controlled clinical trials than rTMS. Actually, tDCS induces less scalp sensation than rTMS and is generally not perceived by individuals at intensity below 1.5 mA (Dundas et al. 2007). In addition, sham tDCS provides a similar scalp sensation as real tDCS by the use of ineffective brief current pulses (around 100 µA, unable to modulate cortical excitability) delivered at regular interval. This allows a close control for placebo effects such as raised alertness or enhancement of attention (Gandiga et al. 2006).

Despite a lower spatial resolution than rTMS (wider current spread due to large electrode's size), tDCS is an efficient and secure NIBS which modulates efficiently brain excitability and could thus be used as a tool to modify brain excitability/activity, both in healthy individuals and patients.

1.3.3. NIBS improve post-stroke motor function of the paretic upper limb.

As detailed in the previous sections, rTMS and tDCS are NIBS techniques that can modulate the excitability/activity of the human (motor) cortex and enhance motor function. As previously mentioned, within the

framework of the interhemispheric rivalry model, three distinct strategies of NIBS could be applied (Figure 1.2).

The first strategy targets an up-regulation of the cortical excitability of the $M1_{damH}$. Both high-frequency rTMS and anodal tDCS up-regulate the excitability of the $M1_{damH}$ (Hummel et al. 2005; Kim et al. 2006). More importantly, both high-frequency rTMS (Yozbatiran et al. 2009) and anodal tDCS (Hummel et al. 2005; Hummel et al. 2006) applied over the $M1_{damH}$ during a single session of 20 min are able to enhance transiently motor performance on simple hand motor tasks such as Jebsen Taylor Test (JTT), nine Hole Peg Test (9-HPT) or maximum hand strength measurement in the paretic hand.

The second strategy targets a down-regulation of the cortical excitability/activity of the $M1_{undamH}$. Both low-frequency rTMS and cathodal tDCS decrease the excitability of the $M1_{undamH}$ (Takeuchi et al. 2008a; Zimerman et al. 2012) leading to a reduction of the interhemispheric inhibition from $M1_{undamH}$ to $M1_{damH}$, where cortical excitability is then released from pathological interhemispheric inhibition (Grefkes et al. 2010; Takeuchi et al. 2012). More importantly, both low-frequency rTMS (Mansur et al. 2005; Takeuchi et al. 2005; Nowak et al. 2008) and cathodal tDCS (Fregni et al. 2005; Takeuchi et al. 2005; Bradnam et al. 2011) applied during a single short session (from 10 to 25 min) enhance transiently motor performance of the paretic hand on simple tasks such as Purdue Pegboard Test (PTT) or simple reaction time (simple RT).

The third strategy aims to the bilateral modulation of cortical excitability. Both bi-hemispheric rTMS and dual-tDCS permit to simultaneously (or one just after the other for bi-hemispheric rTMS) increase the excitability of $M1_{damH}$ and decrease the excitability of $M1_{undamH}$. In addition, both bi-hemispheric rTMS applied in a single 30 min session (Takeuchi et al. 2009) and dual-tDCS applied in multiple sessions of 30 min (but the effect on motor task was already observed after the first session) (Lindenberg et al. 2010; Bolognini et al. 2011) improves motor performance on simple tasks such as JTT, Wolf Motor Function Test (WMFT), pinching rate and pinch force measurement in the paretic hand.

Depending on these different application strategies, a single session with a short (20-30 min) application of NIBS could modify motor performance in different tasks in both acute and chronic stroke patients. The Table 1.1 summarises the different studies in which this beneficial effect has been explored. Studies exploring the impact of tDCS on digital dexterity or temporal dynamic movement such as grip-lift task with the paretic hand are missing. In the current work, the attention has been focused on tDCS and especially on dual-tDCS.

Due to their ability to modify efficiently brain excitability after stroke and to induce beneficial (but transient) effects on motor performance even after a single session, and beneficial long-lasting effects on motor performance when applied during repeated sessions (Boggio et al. 2007; Hesse et al. 2007; Chang et al. 2010; Emara et al. 2010; Bolognini et al. 2011), NIBS could become efficient add-on tools in neurorehabilitation.

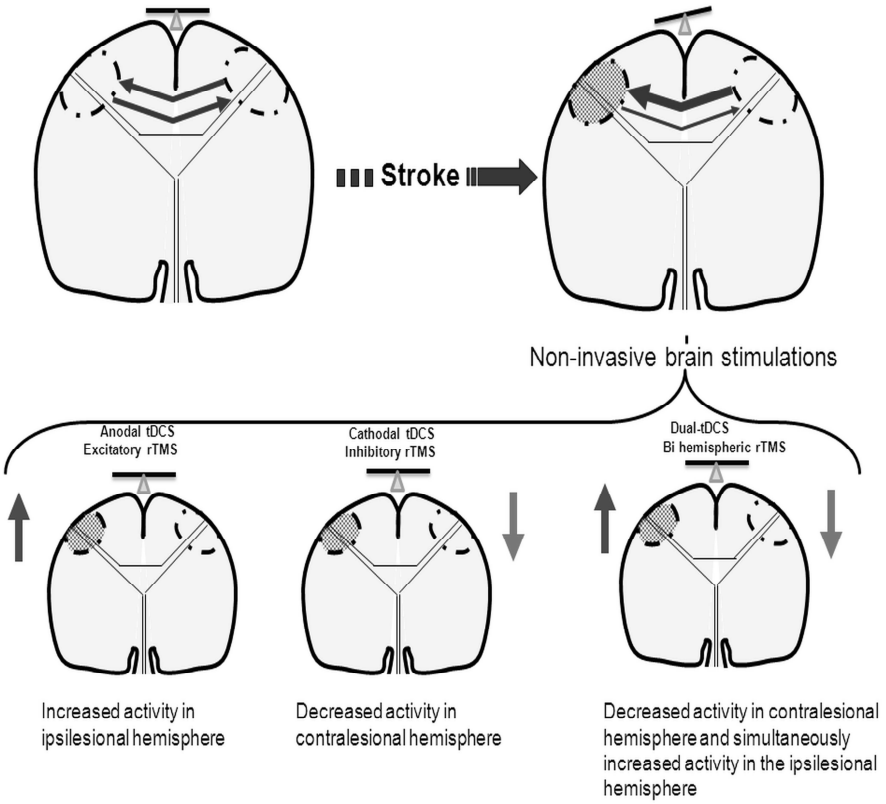

Figure 1.2: Impact of NIBS on interhemispheric interactions after stroke (based on the interhemispheric rivalry model). After stroke (right upper panel), unbalanced interhemispheric inhibition from $M1_{undamH}$ (large arrow) may further depress the excitability/activity of $M1_{damH}$, worsening residual motor function. These abnormal interhemispheric interactions might be re-balanced by three interventional approaches (lower panel) with NIBS targeting M1 (black circles). First, upregulation of excitability (up arrow) in $M1_{damH}$ (shaded area) with excitatory anodal tDCS or high-frequency rTMS.,. Second, down-regulation of excitability (down arrow) of $M1_{undamH}$ with inhibitory cathodal tDCS or low-frequency rTMS might induce a direct decrease of the interhemispheric inhibition from $M1_{undamH}$ to $M1_{damH}$, where cortical excitability is released from the additional pathological interhemispheric inhibition. Third, concomitant upregulation of excitability (up arrow) in $M1_{damH}$ and down-regulation of excitability (down arrow) of $M1_{undamH}$ by the use of dual-hemispheric tDCS (dual-tDCS) or successive rTMS on both hemispheres

1.4. Neurorehabilitation

This section focuses on the recovery from upper limb paresis after stroke. Whether the recovery processes supporting other post-stroke functional impairments (aphasia, neglect, dysphasia …) are identical to, share some common mechanisms with, or are radically different from post-stroke motor recovery will not be discussed here.

1.4.1. Predictors of motor function recovery after stroke

Predicting as accurately as possible the recovery potential of stroke patients is one of the main challenges for neurorehabilitation science, since this would allow to set realistic goals, to plan efficiently the neurorehabilitation program, to tailor it to individual patient's needs, to optimise the use of resources, to plan ahead patient's discharge, and to quantify the impact of new neurorehabilitation methods as a deviation from the expected recovery trajectory.

During the acute phase of stroke, several methods could be used to predict long-term functional recovery. Voluntary extension of fingers and shoulder abduction during the first 72 hours after stroke is associated with a complete recuperation of hand/upper limb function after six months in 60%

of the patients; the remaining 40% regain at least some motor function (Kwakkel et al. 2010; Nijland et al. 2010; Kwakkel et al. 2011).

The initial size and localisation of stroke MRI or computed tomography scanner (CT-scanner)) partly predicts motor recovery: the larger the lesion size, the poorer the long-term recovery (Chen et al. 2000). In addition, long-term motor recovery depends on the localisation of the lesion: deeper motor impairments are associated with stroke localized in the corona radiata, internal capsule or (primary) motor cortex i.e. the CST arises from M1, whereas extensive motor recovery is observed in patients with lesions localized in the putamen and the thalamus i.e. sparing the CST (Chen et al. 2000). The integrity of the CST, evaluated by MRI, is associated with functional recovery (Stinear et al. 2007). A significant ADC change in the cerebral peduncle seven days after stroke (which could represent early Wallerian degeneration) is associated with poor long-term recovery (DeVetten et al. 2010). Used early after stroke, TMS permits to explore the functional integrity of the CST through the persistence/lack of MEPs in the paretic limb (Pennisi et al. 1999; Hendricks et al. 2002). A lack of MEPs in the paretic upper limb during the first 72 hours after stroke is associated with a very poor long-term functional recovery in 40% of the patients and with no recovery at all in 60 % (Pennisi et al. 1999; Hendricks et al. 2002).

During the acute stroke phase, fMRI could also have a predictive value for functional motor recovery. Early recruitment of the SMA (BA 6) and of the inferior parietal cortex in the damaged hemisphere (BA 40_{damH}) during passive movements of the paretic hand predicts faster and larger recovery as early as two weeks after stroke and up to one year, whereas activation of the undamaged hemisphere is associated with a slower recovery at the same time period (Loubinoux et al. 2003).

Table 1. 1: Impact of NIBS on upper limb motor performance in chronic stroke patients

	Stroke	Design	Effects on paretic hand function	Study
rTMS	20 SC chronic	Single session 1Hz 25 min on $M1_{undamH}$	Acceleration of pinching rate immediately after the stimulation Return to baseline values 30 min after the stimulation No effect on Pinch force	(Takeuchi et al. 2005)
	10 SC patients in the first year after stroke	Single session 1 Hz on $M1_{undamH}$ and PMd_{undamH}	No significant effect on finger tapping with any stimulation Statistically significant improvement on reaction time, choice time and PTT after M1 stimulation compared to sham No statistically significant improvement on any parameters with PM stimulation compared to sham	(Mansur et al. 2005)
	15 SC acute	Single session 1Hz 25 min on $M1_{undamH}$	Frequency and velocity of index tapping, velocity of the wrist and peak grip aperture were improved immediately after the stimulation	(Nowak et al. 2008)
	15 SC chronic	Single session of stimulation 1Hz 25 min on $M1_{undamH}$	Acceleration of pinching rate and improvement of pinch force immediately after the stimulation	(Takeuchi et al. 2008b)
	12 SC acute	Single session 1Hz 10 min on $M1_{undamH}$	Improvement of both efficacy and coordination of grip lift immediately after the stimulation	(Dafotakis et al. 2008a)
	9 chronic	Single session 1Hz 20 min on $M1_{undamH}$	Compared to sham, inhibitory rTMS significantly decreased movement time, increased peak grasp aperture and induced a more coordinated movement.	(Tretriluxana et al. 2013)
	12 SC acute	Single session 1Hz 20 min on $M1_{undamH}$	Improvement in 9-HPT immediately after the stimulation No effect on maximal grip strength	(Liepert et al. 2007)
	12 patients minimum 12 weeks after stroke	Single session 20 Hz on $M1_{damH}$	No effect on FMT Improvement on maximal grip strength maintained at 1 week Improvement in 9-HPT 60 min after the stimulation	(Yozbatiran et al. 2009)

	30 SC chronic	Single session dual rTMS 10 Hz on $M1_{damH}$ 1 Hz on $M1_{undamH}$	Immediately after each stimulation: improvement on pinch force as well as on pinching rate These improvements are maintained at one week.	(Takeuchi et al. 2009)
	60 acute	Multiple sessions (10) 10 Hz on $M1_{damH}$ 1 Hz on $M1_{undamH}$	Finger tapping rate and activity index were improved after both stimulations These improvements were maintained 12 weeks after the end of the treatment.	(Emara et al. 2010)
	28 SC acute	Multiple sessions (10) 10 Hz on $M1_{damH}$	Real rTMS induced a greater effect than sham only for FMT and grip strength These improvements were conserved 3 months after the therapy.	(Chang et al. 2010)
	29 acute	Multiple sessions (5) 30 min 1Hz on $M1_{undamH}$ 30 min 10Hz on $M1_{damH}$	Improvement in grip strength and tapping frequency after both stimulations	(Sasaki et al. 2011)
	17 chronic	Multiple session (10) 10 Hz on $M1_{damH}$	Improvement in movement accuracy of sequential finger motor tasks after real rTMS compared to sham	(Chang et al. 2012)
	15 chronic 13 SC 2 C	Multiple sessions (5) 1Hz 20 min on $M1_{undamH}$	Improvements on JTT, PTT, simple and choice reaction time just after the 5 sessions of stimulation, maintained at two weeks.	(Fregni et al. 2006)
	10 SC chronic children	Multiple sessions (8) 1Hz 20 min on $M1_{undamH}$	Improvements on maximal grip strength as well as in upper limb function just after the 8 sessions of stimulation, maintained at one week.	(Kirton et al. 2008)
	52 acute 26 SC 26 C	Multiple sessions (10) 3Hz on $M1_{damH}$	Improvements on NIHSS, SSS and bartel index just after the 10 sessions of stimulation. Enhancement maintained 10 days after.	(Khedr et al. 2005)

	19 chronic 8 SC 11 C	Multiple sessions (10) rTMS 20Hz on $M1_{damH}$	real rTMS had no superior effect than sham on upper limb function (FMT) as on BBT	(Malcolm et al. 2007)
TBS	6 chronic 3 SC 3 C	Single session 20 min of cTBS on $M1_{undamH}$ 20 min of iTBS on $M1_{damH}$	Improvement of reaction time only with iTBS immediately after the stimulation, maintained to 30 min after the stimulation	(Talelli et al. 2007)
	10 SC chronic	Single session cTBS on $M1_{undamH}$ iTBS on $M1_{damH}$	Improvement of preload duration during grip lift task with both cTBS and iTBS. Opposite results on upper limb function (ARAT) : no effect with cTBS whereas degradation with iTBS	(Ackerley et al. 2010)
tDCS	6 chronic 3 SC 3 C	Single session 20 min of cathodal tDCS on $M1_{undamH}$ 20 min of anodal tDCS on $M1_{damH}$	Improvement on JTT with both cathodal and anodal tDCS just after the stimulation.	(Fregni et al. 2005)
	1 SC chronic	Single session 20 min of anodal tDCS on $M1_{damH}$	Improvement on JTT, pinch force and simple reaction time just after the stimulation	(Hummel and Cohen 2005)
	6 chronic 5 SC 1 C	Single session 20 min of anodal tDCS on $M1_{damH}$	Improvement on JTT immediately after the stimulation and maintained 20 min after	(Hummel et al. 2005)
	11 chronic	Single session 20 min of anodal tDCS on $M1_{damH}$	Improvement on both JTT and reaction time immediately after the stimulation	(Hummel et al. 2006)
	12 SC chronic	Single session 20 min of cathodal tDCS on $M1_{undamH}$	improvement of proximal upper limb function for mildly impaired patients and degradation for the more impaired patients	(Bradnam et al. 2011)

	8. acute 2 SC 7 C	Multiple sessions (6 weeks) 7 min of anodal tDCS on $M1_{damH}$	Improvement on JTT and upper limb function after the 6 weeks treatment	(Hesse et al. 2007)
	9 SC chronic	Multiple sessions (5) 20 min cathodal tDCS on $M1_{undamH}$	Improvement on JTT at the end of the 5 sessions treatment, maintained at 2 weeks	(Boggio et al. 2007)
	20 chronic 9 SC 11 C	Multiple sessions (5) 30 min dual tDCS on M1	Improvement in both FMT and WMFT after the session treatment, maintained one week after the end of the treatment	(Lindenberg et al. 2010)
	14 C chronic	Multiple sessions (14) 40 min dual tDCS on M1	Dual-tDCS lead to greater improvement compare to sham on JTT, FMT, maximum grip strength at the end of the therapy These improvements were maintained 4 weeks after the end of the	(Bolognini et al. 2011)
	13 chronic 7 SC 6 C	Single 10 min session Anodal tDCS on $M1_{damH}$ Cathodal tDCS on $M1_{undamH}$	Both anodal and cathodal tDCS induce a significant reduction of reaction time in chronic stroke patients	(Stagg et al. 2012)

Legend of table 1.1: SC: subcortical; C: Cortical; Hz: Hertz; tDCS transcranial direct current stimulation; rTMS repetitive transcranial magnetic stimulation; iTBS: intermittent Theta burst stimulation, cTBS: continuous Theta burst stimulation; M1: primary motor cortex; min: minutes; PM: premotor cortex; FMT: F*ugl-Meyer*; JTT: Jebsen Taylor Test; PPT: Purdue pegboard test; WMFT: Wolf Motor Function Test; ARAT: Action Research Arm Test. 9-HPT: 9 Hole Peg Test.

During the chronic phase of stroke, a better functional recovery of the paretic hand is associated with a switch of brain activation from a bilateral pattern towards a stronger activation in the damaged hemisphere during performing an action with the paretic hand (Nelles et al. 2001; Carey et al. 2002). A general principle emerges from experimental evidence: the more the reconfigured network looks like the original undamaged network, the better the recovery (Denny-Brown 1950; Ward et al. 2003; Krakauer 2004).

1.4.2. Neurorehabilitation programs

Currently, neurorehabilitation programs are initiated during the acute stroke phase, as soon as possible in the Stroke Unit and continue in neurorehabilitation centres, at home or in day-care centres (Figure 1.3). Neurorehabilitation programs should be tailored to the specific patient's needs and could consist in (a combination of) physiotherapy, occupational therapy, neuropsychological therapy and speech-language therapy.

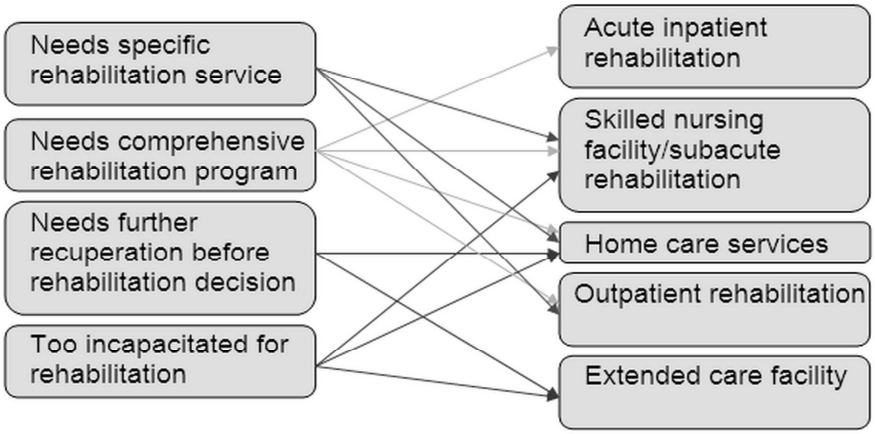

Figure 1.3: Neurorehabilitation process. (Modified from *The organisation of neurorehabilitation service* Textbook p522 Richard Zorowitz (Selzer et al. 2006)).

Regardless of the type used, neurorehabilitation programs attempt to achieve the (re-) integration of the patient in society. In the case of upper limb hemiparesis, the emphasis is put on increasing the amount of use of the paretic arm by enhancing spontaneous functional recovery (Taub 1999;

Taub et al. 2002; Teasell et al. 2010). To fight against motor deficits, the most common method consists in facilitating movements in the paretic extremity or teaching compensatory techniques to perform daily life activities (Taub 1999; Taub et al. 2002; Teasell et al. 2010). To improve motor performances of the paretic arm, neurorehabilitation methods could rely on a panel of different strategies, most of which are still experimental (Ward and Cohen 2004). Additional methods could be used to increase the benefit of neurorehabilitation such as applying electrical stimulation on the paretic hand (Wu et al. 2006) or pharmacological treatments modulating the adrenergic and dopaminergic pathways (Pariente et al. 2001; Chollet et al. 2011).

Recently, the idea that motor learning is a key factor in post-stroke recovery and neurorehabilitation has been explicitly formulated and started to be tested scientifically (Matthews et al. 2004; Krakauer 2006; Shmuelof and Krakauer 2011; Dipietro et al. 2012; Kantak et al. 2012). That is why, in addition to classical rehabilitation methods, specific therapies relying on motor learning theories could be used to enhance motor recovery (Krakauer 2006) such as constraint-induced movement therapy (Hakkennes and Keating 2005), virtual reality-based rehabilitation (da Silva Cameirao et al. 2011; Verschure 2011) as demonstrated also in the ENGAGE (Enhanced Neurorehabilitation: Guided Activity-based Gaming Exercise) study (Reinthal et al. 2012). Recently, specific bimanual therapies (hand–arm bimanual intensive therapy HABIT (Gordon et al. 2007)) based on motor learning have been developed in children. Even if HABIT has been developed in children, we can infer that these approaches could also be efficient in adult stroke patients. Actually, they are based on the rationale that i) most of the activities in daily life are bimanual and ii) after stroke, an efficient neurorehabilitation program has to take into account these parameters and to insist on bimanual coordination and not only on the revalidation of the paretic upper limb.

1.5. Different forms of motor learning and their neural substrates

Different forms of motor learning have been defined in an artificial - yet convenient - way based on experimental protocols: adaptation learning, and the broader motor skill learning category (Classen et al. 1998; Hallet 2005; Shadmehr and Wise 2005; Krakauer and Mazzoni 2011; Shmuelof and Krakauer 2011). It is yet not clear whether use-dependent plasticity (UDP) or experience-dependent plasticity may be considered as a specific form of motor learning. These different motor learning processes are not mutually exclusive and likely co-exist in everyday life; it is however convenient to break up experimentally these processes to study the building blocks of motor learning. Each of these forms of motor learning relies on the relative contribution of cortical areas (motor/premotor network (Figure 1.4), higher order cognitive areas…) and subcortical structures,

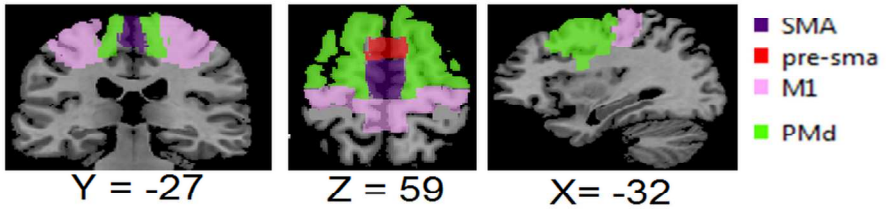

Figure 1.4: Localisation of the motor/premotor network based on the Talairach Daemon (*http://www.talairach.org* (Talairach and Tournoux 1988)) and on (Picard and Strick 2001; Nachev et al. 2008; Kim et al. 2010b). SMA (Brodmann areas, (BA) 6): supplementary motor areas,, M1 (BA 4) : primary motor cortex, PMd (BA 6) : dorsal pre-motor cortex.

1.5.1. Use-dependent plasticity (UDP)

UDP is a form of elementary motor memory encoded within M1 when simple ballistic movements are performed repeatedly (Butefisch et al. 2000). By modifying transiently the motor representations within M1, such training transitorily bias the direction of movements evoked by TMS towards the trained movement direction (Classen et al. 1998; Butefisch et al. 2000).

M1 is the neural substrate of early encoding of UDP, as demonstrated by the cancellation of UDP when inhibitory rTMS is applied over the contralateral M1 just after UDP training (Muellbacher et al. 2002). NMDA receptor activation in M1 is essential to induce motor performance improvement associated with UDP; the activation GABA receptors prevents UDP (Butefisch et al. 2000). NMDA/GABA receptors are known to be essential for the induction/blockade of LTP and may play a key role for encoding basic motor memories within M1 (Hess et al. 1996; Stefan et al. 2006). The LTP formation in M1 is also dependent on dopamine release (Molina-Luna et al. 2009); e.g. a PET study demonstrated the involvement of dopamine release in the basal ganglia (BG) during UDP formation (Floel et al. 2008). The BDNF, which facilitates LTP formation, is crucial for UDP (Cirillo et al. 2012). These observations reinforce the idea that a LTP-like mechanism sustains UDP encoding within M1. By extension, some authors have applied the term UDP to any form of training requiring the repetition of a set of movements or actions, as typically performed during neurorehabilitation. The term experience-dependent plasticity has been recently coined and better fits with these more elaborated forms of training (Kleim and Jones 2008). UDP should refer specifically to the paradigms involving simple ballistic movements and elementary motor memories biased by repeated training. Thus, UDP is a basic form of motor memory encoded early within M1, which could serve as a building block for more elaborated forms of motor learning, especially motor skill learning.

1.5.2. Adaptation learning

Adaption learning has been studied with ballistic movements such as reaching or throwing (Krakauer 2009; Pearson et al. 2010). Adaptation learning supports the rapid improvement of sensorimotor performances in response to altered environment such as rotated visual feedback or external perturbation (e.g. with a force field applied by a robot) (Krakauer and Mazzoni 2011; Shmuelof and Krakauer 2011). Through trial and errors, adaptation learning allows a quick return to the (quasi-) baseline performance, but with neither additional gain compared to baseline nor

enduring changes (Krakauer and Mazzoni 2011). Typically, when the perturbation is suddenly removed, normal individuals exhibit an after-effect characterised by a few overshoot trials in the direction opposite to the perturbation; this after-effect is corrected very quickly. It is supposed that adaptation learning relies on a switch between or an update of internal models leading to performance's modification through reducing sensory prediction errors. An internal model could be defined as a neuronal representation of the interaction between the body and environment (Wolpert and Ghahramani 2000; Rizzolatti and Wolpert 2005; Lonini et al. 2009).

On the one hand, adaptation learning may be supported by a bilateral network of (sub-)cortical structures (Scheidt et al. 2012) such as the cerebellum, preSMA, PPC, BG, M1 and PMd or PMv areas (Debas et al. 2010; Mutha et al. 2011b; Scheidt et al. 2012). On the other hand, fMRI studies in human individuals (Imamizu et al. 2000; Imamizu et al. 2003) and studies in monkeys (Kawato and Gomi 1992) demonstrated a key implication of the cerebellum in adaptation learning. Furthermore, disrupting M1 activity with rTMS does not perturb adaptation learning (Baraduc et al. 2004), whereas disrupting the cerebellum activity with rTMS induces intensity-dependent perturbation of adaptation learning (Jenkinson and Miall 2010). The cerebellum may thus be the critical structure devoted to adaptation learning (Shmuelof and Krakauer 2011).

1.5.3. Motor skill learning

Although motor skill learning may involve any part of the body, this section focuses on the upper limb and hand. Through training, motor skill learning leads to the acquisition and the lasting retention of (new) sensorimotor aptitudes superior to baseline (i.e. skills) involving enduring changes in the operating characteristics of motor patterns: shift of the SAT, some degree of automatisation and reduction of variability (Hallet 2005; Krakauer and Mazzoni 2011; Shmuelof et al. 2012). Skills range from the asymptotic optimisation of a simple ballistic movement to expert

performance of complex behaviours involving a coordinated sequence of movements across multiple parts of the body (e.g. elite athletic performance, playing a piano concerto…). Typically, motor skill learning proceeds through two stages: an early and fast stage characterised by large, online (within-session) gains and a late, slow stage during which smaller incremental improvements are gained online and offline across multiple training sessions; the respective proportion of fast/slow stages is highly variable and skill-specific (Dayan and Cohen 2011). The consolidation of motor skill learning involves off-line mechanisms and is improved by sleep (Siengsukon and Boyd 2009b).

Motor skill learning relies on a complex and flexible network involving subcortical structures, M1, PM and higher-order cortical areas such as DLPFC (Chen et al. 2000; Dayan and Cohen 2011; Hardwick et al. 2013). In healthy individuals, the M1 contralateral to the training hand is a key structure for the early stages of motor skill learning as demonstrated by fMRI (Karni et al. 1995) and by studies using inhibitory rTMS to interfere transiently with M1 activity (Hotermans et al. 2008). Interestingly, in healthy individuals, online motor skill learning is enhanced by high-frequency excitatory rTMS over M1 (Kim et al. 2004); and anodal excitatory tDCS over M1 enhances both online motor skill learning and offline motor skill consolidation (Reis et al. 2009; Zimerman and Hummel 2010). Motor skill memories might be split in two main components: the goal-based component i.e. the representation of the spatial goal of the skill, and the movement component i.e. the movements needed to achieve that goal (Robertson 2009). A recent theory suggests that if the goal-based component relies on higher-level cognitive areas such as the DLPFC, and that M1 is an essential locus for the movement component (Kantak et al. 2010; Shmuelof and Krakauer 2011). In addition, brain activation in non-primary motor areas is associated with motor skill learning: fMRI and PET activation changes have been observed in SMA (VanMier et al. 2004) as well as in PMv (Jenkins et al. 1994) during early sequential motor skill learning. The retention of the learned skills is improved by excitatory rTMS applied over the left PMd (Siengsukon and Boyd 2009b). A recent fMRI

meta-analysis confirms that the left PMd is a key area in the human motor skill learning network (Hardwick et al. 2013). Higher-order limbic and associative areas, such as the DLPFC (Tanji and Hoshi 2001); hippocampus (Gheysen et al. 2010) and parietal cortex (Hikosaka et al. 1996) are involved in motor skill learning, especially for complex tasks with an important cognitive load.

Subcortical structures are also involved in motor skill learning. The cerebellum may acquire the optimal internal model for sequence performance, contribute to on-going error correction, and provide comparisons with similar skills acquired previously, facilitating the acquisition of new skills, and play a role in inter-manual transfer (Ghilardi et al. 2000; Imamizu and Kawato 2009; Debas et al. 2010; Penhune and Steele 2012). The exact contribution of the BG in motor skill learning is still unclear. On one hand, both the striatal system and the putamen are involved in motor chunking during sequential motor skill learning (Orban et al. 2011; Penhune and Steele 2012);(Wymbs et al. 2012).Motor chunking is a process involved in sequential motor skill learning; motor chunking contributes to build motor sequences by combining motor elements into units of behavior that are later assembled as a sequence (Sakai et al. 2003). On the other hand, through the release of dopamine, the BG are involved in the reward process which plays an important role in motor learning (Wachter et al. 2010; Abe et al. 2011; Izawa and Shadmehr 2011). It is however not clear whether the BG contribute to the improvement of movement performance itself (Shmuelof and Krakauer 2011). Furthermore, the BG may be involved in decision making or action selection (Kurniawan et al. 2010), the cognitive processes leading to the choice of the most efficient (sequence of) actions in order to accomplish a goal. Action selection involves the frontal areas (Shadmehr and Wise 2005; Kurniawan et al. 2010; Krakauer and Mazzoni 2011), DLPFC (Brown and Sherrington 1912; Gu 2002; Philiastides et al. 2011), PM and SMA (Lowenstein et al. 1995; Cisek et al. 2003). Action selection and decision making might be considered as a higher-order component of motor skill learning which permits to optimise movements (sequences).

On the one hand, specific forms or aspects of motor learning seem to rely predominantly on specific brain structures: UDP on M1, adaptation learning on the cerebellum…. On the other hand, more complex forms of motor learning such as sequential motor skill learning may activate a distributed network involving several structures cooperating in a determined temporal sequence leading to action selection, chunking and sequence elaboration, and improvements in the quality of movement performance. In real life, these different aspects of motor learning are likely intermingled and the nodes of this motor skill learning network may be adaptively recruited depending on the circumstances and the task requirements. It is worth noting that the emphasis on M1 may reflect a bias due to the relative straightforward experimental accessibility to M1 (e.g. with TMS).

1.5.4. Molecular basis of motor learning.

Animals studies demonstrated that protein synthesis underlies LTP, LTD, synaptogenesis and cortical plasticity, which are all crucial phenomena necessary for the formation of the so-called motor memories within M1 (Luft et al. 2004). BDNF is essential for LTP, permitting the insertion of new glutamate receptors in the post-synaptic membrane (Carvalho et al. 2008). The *BDNF* gene shows a nucleotide polymorphism leading to an amino acid substitution at position 66 (*BDNF* Val66Met) that is associated with altered motor plasticity and fMRI patterns (McHughen et al. 2010), less efficient motor learning and reduced responsiveness to NIBS (Kleim et al. 2006; Fritsch et al. 2010; McHughen et al. 2010). Thus, protein synthesis in M1 is essential for motor skill memories formation through the regulation of synaptic plasticity and excitability. Ultimately, the genetic background may determine the ability to achieve fast and successful motor skill learning, and thus also condition the potential for functional recovery after stroke.

1.6. Motor learning and stroke

1.6.1. Is motor learning preserved after stroke?

Post-stroke neurorehabilitation programs are based on the implicit assumption that, beyond the resolution of acute metabolic events (ischemia, oedema ...) and the development of functional and structural plasticity, at least some motor learning abilities are retained (or recover) and also play a key role in recovery (Kwakkel et al. 2004). In order to achieve recovery, stroke patients have to learn how to optimise the functioning of their spared neural structures, by exploring different movement strategies and the corresponding states of brain activity, likely through a trial-and-error process. However, impairments of specific aspects of motor learning have been reported after injury to discrete parts of the brain.

1.6.1.1. Motor learning with the non-paretic upper limb (Table 1.2)

Subtle but unquestionable (sensori-) motor impairments have been demonstrated in the ipsilesional non-paretic upper limb of stroke patients (Sunderland 2000; Noskin et al. 2008). Therefore, the question arises to whether motor learning is also altered in the ipsilesional non-paretic upper limb. So far, UDP in the non-paretic upper limb has not been formally explored; it would however be surprising that UDP would be abolished in the non-paretic upper limb.

In a classical adaptation protocol involving a robot that imposed force-fields curving the arm trajectory during reaching movements, chronic hemiparetic patients with various stroke lesions exhibited a normal adaptation learning pattern with their non-paretic arm (Takahashi and Reinkensmeyer 2003). In a paradigm involving a rotated visual feedback during reaching movements, patients with a left parietal stroke showed impaired adaptation learning with the non-paretic upper limb, whereas patients with a right frontal stroke achieved adaption learning but had impaired online trajectory correction (Mutha et al. 2011b). Stroke of the inferior parietal lobule also impairs adaptation learning and the generalisation of reaching with the ipsilesional non-paretic hand when the

gain of the visual feedback is distorted compared to actual movement (Palluel-Germain et al. 2011). So far, adaptation learning with the ipsilesional non-paretic upper limb has not been studied in animals.

The capacity to learn new motor skills with the non-paretic upper limb is preserved since chronic stroke patients are able to achieve motor skill learning with visuomotor tracking (VMT) tasks (Winstein et al. 1999) or modified versions of the serial reaction time task (SRTT, a classical paradigm used to study motor sequence learning consisting in repeatedly respond to a fixed set of stimuli in which each cue signals that a particular response (i.e., button press) needs to be made, the RT is the mean outcome of this kind of task) (Pohl et al. 2001). Whereas apraxic patients with a left PMd stroke presented no trouble in learning SRTT with their left non-paretic upper limb, they showed impairment in intentional retrieval of the learned sequence (Dovern et al. 2011). Lesions of the prefrontal cortex impair but do not abolish motor skill learning during the SRTT with the ipsilesional hand (Gomez Beldarrain et al. 1999), and stroke involving the BG interferes with the capacity to benefit from explicit information when learning implicit motor sequences (Boyd and Winstein 2004b). Cerebellar strokes leading to ataxia and deficits in anticipatory adjustments do not abolish learning and retention of VMT skills with the non-ataxic upper limb (Boyd and Winstein 2004a). It is worth noting that in rats, experimental ischemic stroke of the sensorimotor cortex is associated with an *enhancement* of motor skill learning with the ipsilesional non-paretic forelimb (Hsu and Jones 2006).

Thus, just as motor function is subtly impaired in the ipsilesional non-paretic hand after stroke (Noskin et al. 2008), some forms or aspects of motor learning may be slightly impaired in the ipsilesional non-paretic upper limb of stroke patients, although the capacity to achieve different forms of motor learning seems to be preserved overall.

1.6.1.2. Motor learning with the paretic upper limb (Table 1.3)

In chronic stroke patients, the classical UDP paradigm has not been applied without additional therapeutic intervention such as Levodopa

administration (Floel et al. 2005). In this experiment, there was a slight trend in the placebo condition suggesting that UDP with the paretic hand was not abolished in all of the studied stroke patients (Floel et al. 2005). Along the same line, in experience-dependent paradigms, UDP-like plasticity is preserved in chronic hemiparetic patients (Butefisch et al. 1995).

In the paretic arm, the residual capacity to achieve adaptation learning / implement internal models correlates with impairment severity. When reaching movements with the paretic upper limb are perturbed by a force-field, severe deficits or lack of adaptation learning are observed only in the most severely impaired patients (Takahashi and Reinkensmeyer 2003). It is however worth mentioning that it may be difficult to disentangle motor *performance* deficits from motor *learning* deficits in severely impaired patients with high inter-trial variability.

Cerebellar strokes can impair visuomotor adaptation learning with the affected upper limb (Werner et al. 2010). To date, adaptation learning with the paretic upper limb has not been studied in animals.

There is currently no clear evidence that stroke or focal brain injury abolishes motor skill learning with the paretic upper limb since patients remain able to learn new motor skills such as 3-D motor skill learning tasks (Platz et al. 1994), self-paced maximal rate (Askim et al. 2009), modified versions of SRTT (Boyd et al. 2010), or sequential VMT tasks (Meehan et al. 2011b). However, impairments of specific aspects of motor skill learning have been reported. After damage to the M1 or S1 hand area of monkeys, paretic hand motor function partly recover through repetitive training of skilled movements, although learning is severely impaired (Nudo et al. 1996). Similarly, lesions of the prefrontal cortex do not abolish sequential motor skill learning but deteriorate both implicit and explicit component of motor skill learning when using a SRTT (Gomez Beldarrain et al. 1999) or sequential pursuit tracking task (Gomez Beldarrain et al. 2002), suggesting that the prefrontal cortex is involved in sequential aspects of motor skill learning and/or in the strategic processes identifying the goal of movements. Studies with focal BG damages led to conflicting results. In mixed groups of patients with left and right BG stroke using their paretic hand, SRTT was

impaired compared to control subjects, but not abolished (Vakil et al. 2000; Exner et al. 2001).

In contrast, the integrity of the striatum seems necessary to achieve motor sequence learning in monkeys (Miyachi et al. 1997) or to learn skilled reaching movements in rats (Wachter et al. 2010). These differential results could be explained by the large extend of the BG strokes in humans studies. In fact in the studies involving patients with BG injury (Vakil et al. 2000; Exner et al. 2001), the BG strokes did not systematically involve the same nuclei (striatum, putamen, globus palidus…). This is why further studies are warranted, especially to determine the impact of striatum lesion on motor skill learning in stroke patients. Finally, cerebellar stroke impairs but does not abolish learning VMT skills with the (ipsilesional ataxic) upper limb in humans (Hatakenaka et al. 2012).

To sum up, specific impairments of the different forms of motor learning have been reported after stroke or focal injury to different brain structures. However, even in severely impaired patients, at least some forms of motor learning seem to be preserved or recovered.

1.6.2. Involvement of motor learning in post-stroke recovery

In the (sub)acute stroke stage, fast spontaneous recovery relies on the resolution of acute metabolic events; in the subacute and chronic stages, slower motor recovery may rely on functional and structural reorganisation of the spared areas and their connexions (Loubinoux et al. 2003; Ward and Frackowiak 2006; Xerri 2012). Beyond the resolution of acute metabolic events (ischemia, oedema, …) and the concomitant re-activation of spared but stunned neural structures (i.e. peri-lesional cortex (Clarkson et al. 2010)), recovering motor function after stroke might be conceptualised as learning to use the remaining neural resources to improve motor planning, execution, feedback and control, i.e. a form of motor skill learning. It is still unclear whether post-stroke motor recovery relies on re-learning of damaged or lost motor engrams or on the *de novo* acquisition of new motor skills and

internal models (Krishnan 2006; Schubring-Giese et al. 2007; Hosp and Luft 2011).

Motor recovery can take two main different behavioural forms or strategies: **i)** compensation and **ii)** true recovery (Krakauer 2006; Nudo 2006). Compensation leads to the development of new behavioural strategies to achieve goals; e.g. using the non-paretic hand instead of the paretic hand to grab a glass of water. Compensation may rely on a mix of motor learning processes. It may involve UDP, the continuous improvement of sensorimotor abilities through training (i.e. motor skill learning), and decision making in the sense of selecting an alternative strategy to attain goals. If switching from an internal model (*using the paretic hand to grab the glass of water*) to another one (*using the non-paretic hand to grab the glass of water*) may prove useful, adaptation learning may be involved as well.

By contrast, true recovery is based on the recurrence of normal movement patterns supported by the plastic reconfiguration of brain networks providing functional vicariance. Undamaged brain regions may be adaptively recruited and generate the adequate commands to the same muscles that were used before stroke (Krakauer 2006). Again, true recovery likely relies on a combination of UDP, motor skill learning, decision making and/or adaptation learning. E.g., recovering a normal pattern of coordinated muscles activation may be considered as an extreme form of adaptation learning, in the sense that stroke is a perturbation. In this case, adaptation learning would be involved for progressively reducing sensory prediction errors in order to update the damaged internal models or to switch to undamaged internal models. However, it is not clear whether the persistence of stroke-induced impairment (i.e. the perturbation) would drive continuous adaptation learning) or whether, at some point, adaption would become permanent, leading to a transition between adaptation learning (which is by definition transient and reversible) and motor skill learning. UDP and motor skill learning are likely involved in recovery since recruiting efficiently the undamaged brain structures in order to generate the appropriate commands might be considered as learning new - and challenging – sensorimotor skills.

A recent study in subacute stroke patients suggests that recovery relies more on motor (skill) learning than on adaptation (Dipietro et al. 2012).

Strikingly, in rats, re-acquiring skilled reaching movements with the paretic forepaw learned before motor cortex damage is slower than learning the same skill *de novo* (Hosp and Luft 2011). Further studies are needed to clarify these issues, with the ultimate aim to improve neurorehabilitation techniques.

1.6.3. Enhancement of motor learning

Enhancement of motor learning is an attractive option for improving post-stroke motor recovery since it could speed up neurorehabilitation, increase its effects and translate into generalisation and long-term functional gains. Pioneering experimental studies have shown that there are different ways to enhance motor learning.

1.6.3.1. Enhancement of motor learning in healthy individuals

Several simple behavioural interventions enhance motor learning in healthy individuals; a selection of the most innovative/promising is presented in the following section, starting with behavioural/contextual approaches. UDP may be enhanced by external pacing (Ackerley et al. 2011), which might be a useful add-on in neurorehabilitation. Using random training schedules and adapting difficulty/intensity enhances motor learning (Choi et al. 2008). Feedback about task performance should be provided at low frequency since high frequency feedback impairs motor skill learning (Badets and Blandin 2011).

Sleep improves off-line motor skill learning by diminishing errors and improving speed (Walker et al. 2003), maybe through the restoration of the (synaptic) fatigue in the circuitry involved in learning (Walker et al. 2003). Motor skill learning is enhanced by reward, whereas punishment/neutral stimuli have no effect (Abe et al. 2011); this emphasizes the importance of reward and motivation likely acting through the dopaminergic system.

Table 1.2: Motor learning in the non-paretic upper limb

Motor learning forms	stroke patients	lesion localisation	learning task	motor learning	ref
Motor adaptation learning	13 chronic	7 SC, 6 C	force fields adaptation	not impaired	(Takahashi and Reinkensmeyer 2003)
	20 chronic	C	VMT	impaired after left parietal lesion, not impaired after right frontal lesion	(Mutha et al. 2011a)
	13 chronic	C	visuomotor gain adaptation	impairment with supramarginal gyrus lesion	(Palluel-Germain et al. 2011)
	10 chronic	C	VMT	impaired	(Schaefer et al. 2009)
Motor skill learning	40 chronic	C	Sequential VMT	not impaired	(Winstein et al. 1999)
	47 chronic	C	SRTT	not impaired	(Pohl et al. 2001)
	10 chronic	BG	Sequential VMT	impaired only when explicit information are given	(Boyd and Winstein 2004b)
	7 chronic	cblm	Sequential VMT	Deficit in temporal accuracy	(Boyd and Winstein 2004a)
	12 acute	cblm	PRT	Impaired with both paretic and non-paretic limb	(Hatakenaka et al. 2012)
	16 chronic	BG	SRTT	Impaired with both paretic and non-paretic limb	(Vakil et al. 2000)
	14 chronic	cblm	SRTT	not impaired	(Gomez-Beldarrain et al. 1998)

Legend of Table 1.2: C: cortical stroke, SC: subcortical stroke, cblm : cerebellar lesion, BG : basal ganglia lesion, SRTT: serial reaction time task, VMT: visuomotor tracking task, PRT: pursuit rotor task

Pharmacological agents have long been tested to enhance memory or motor learning. Whereas a dopamine agonist improves motor skill learning, dopamine antagonists depress it (Kumari et al. 1997). Other pharmacological agents (amphetamines, acetylcholinesterase inhibitors) improve UDP in healthy individuals (Ziemann et al. 2006), as well as D-amphetamine, maybe by enhancing dopamine release (Butefisch et al. 2002).

Recently, NIBS received increasing attention given their potential to improve motor learning (see sections 1.3 and 1.7). Two main techniques of NIBS have been successfully tested, rTMS and tDCS (Reis et al. 2008). High-frequency rTMS applied over the non-dominant M1 can improve motor skill learning with the non-dominant hand in healthy individuals (Kim et al. 2004). Furthermore, early expression of the *BDNF* gene (which is essential for LTP induction and for motor learning) is induced by rTMS (Muller et al. 2000). Similarly, anodal tDCS applied over M1 during (Reis et al. 2009) or just after training (Tecchio et al. 2010) improves online and off-line motor skill learning in healthy individuals.

In healthy individuals, there are numerous ways to efficiently improve motor learning, ranging from simple contextual/behavioural adjustments to sophisticated methods using NIBS. One of the great challenges of motor learning neuroscience and neurorehabilitation science is to implement and to test the efficiency of these new methods enhancing motor learning in patients with motor deficits.

Table 1.3 Motor learning in the paretic upper limb

Motor learning forms	stroke patients	lesion localisation	learning task	motor learning	ref
Experiment-dependent plasticity	10 acute	SC	task-oriented arm training	not impaired	(Nelles et al. 2001)
	27 acute	7 SC 20C	simple ADL or physiotherapy task	not impaired	(Butefisch et al. 1995)
Motor adaptation learning	15 chronic	cblm	VMT	impaired	(Werner et al. 2010)
	13 chronic	7 SC 6 C	force fields adaptation	impaired	(Takahashi and Reinkensmeyer 2003)
	10 chronic	4 SC 6 C	VMT	impaired only in the most severely affected patients	(Dancause et al. 2002)
Motor skill learning	10 chronic	SC	Sequential VMT	not impaired	(Bosnell et al. 2011)
	18 chronic	SC	Sequential VMT	not impaired	(Boyd et al. 2010)
	20 chronic	12 SC 8 C	Sequential VMT	not impaired	(Carey et al. 2007)
	26 chronic and 22 acute	C and SC	SRTT	Not impaired in non apraxic patients Impaired in apraxic patients	(Dovern et al. 2011)
	9 chronic	SC	Sequential VMT	not impaired	(Meehan et al. 2011b)
	13 chronic	BG	Sequential VMT	Impairment only in motor sequence chunking	(Boyd et al. 2009)

	37 chronic	22 BG and 15 thalamic	SRTT	not impaired with BG lesions but impaired with thalamic lesion	(Exner et al. 2001)
	16 chronic	BG	SRTT	Impaired with both paretic and non paretic limb	(Vakil et al. 2000)
	10 chronic	7 SC 3 C	Sequential VMT	not impaired	(Carey et al. 2002)
	10 chronic	C	SRTT	impaired	(Gomez Beldarrain et al. 2002)
	12 acute	cblm	PRT	Impaired with both paretic and non paretic limb	(Hatakenaka et al. 2012)
	14 chronic	cblm	SRTT	impaired	(Gomez-Beldarrain et al. 1998)
	20 stroke patients	12 SC 8 C	3-D motor skill learning task & Sequential VMT	not impaired	(Platz et al. 1994)

Legend of Table 1.3: C: cortical stroke, SC: subcortical stroke, cblm: cerebellar lesion; BG: basal ganglia, PRT: Pursuit rotor task ; VMT: visuomotor tracking task, SRTT: serial reaction time task; ADL: activities of daily living

1.6.3.2. Enhancement of motor learning in stroke patients (Table 1.4)

As in healthy individuals, numerous interventions (mental practice, sleep, reward, physical training …) enhance motor learning in stroke patients.

Theoretically, invasive brain stimulation of M1 could improve motor learning after stroke. However, the first clinical trial with M1 epidural stimulation involving stroke patients, the EVEREST study (Harvey and Winstein 2009) designed to assess the impact of combined direct cortical stimulation and experience-dependent plasticity, did not confirm positive pilot results (Brown et al. 2006), probably due to an inefficient (imprecise) M1 localisation.

In stroke patients, UDP in the paretic upper limb is enhanced by Levodopa intake (Floel et al. 2005) or by repetitive peripheral nerve stimulation (Sawaki et al. 2006). Selective serotonin reuptake inhibitors (SSRI, e.g. fluoxetine) improve motor recovery supported by standard physical and occupational therapy (i.e. experiment-dependent plasticity) in stroke patients (Chollet et al. 2011). Although not designed to explore specifically motor learning, several studies combining NIBS (rTMS or tDCS) with rehabilitative training or experience-dependent plasticity demonstrated enhanced recovery, supposedly through motor learning mechanisms (Edwards et al. 2009; Takeuchi et al. 2009; Koganemaru et al. 2010; Avenanti et al. 2012).

Sleep improves motor skill learning in stroke patients (Siengsukon and Boyd 2009a) and physical training such as aerobic exercise improves motor skill learning on a SRTT with the non-paretic hand (Quaney et al. 2009). Feedback about performance during training enhances motor skill learning in stroke patients (Boyd and Winstein 2001). Levodopa administered before training sessions improves SRTT (Rosser et al. 2008). Peripheral nerve stimulation enhances learning of a VMT skill with the paretic hand (Bhatt et al. 2007). Up-regulation of $M1_{damH}$ by high-frequency rTMS improves SRTT in chronic stroke patients (Kim et al. 2006) and down-regulation of the $M1/S1_{undamH}$ by cTBS or cathodal tDCS enhances motor

skill learning on a sequential VMT (Zimerman et al. 2012) or a modified version of SRTT (Meehan et al. 2011a). It has to be mentioned that with NIBS, the stimulated area is larger than with invasive epidural stimulation, this could partly explain the differential results between these two types of techniques.

Currently, several techniques could be used to improve motor skill learning in stroke patients. Among these different therapeutic options, NIBS are appealing for enhancing neurorehabilitation.

1.7. NIBS and motor skill learning improvement

As demonstrated by animal experimentation (Rioult-Pedotti et al. 1998; Ivanco and Greenough 2000), motor learning underlying motor recovery relies on brain plasticity mechanisms such as increment in synaptic efficacy, dendrites growth, increases in dendritic spines, and synaptogenesis. Plasticity within $M1_{damH}$ could be enhanced by NIBS (Ziemann et al. 1998a). tDCS and rTMS have both demonstrated their ability to improve motor skill learning and short-term retention (see specific NIBS references in Table 1.4). The ability of NIBS to improve motor skill learning could be explained by the similar molecular mechanisms between NIBS long-term effects and motor skill learning induction and storage. Recent studies demonstrated that NIBS long term-effects rely on brain plasticity induction and are especially related to BDNF secretion (Cheeran et al. 2008; Fritsch et al. 2010; Li Voti et al. 2011). This is why an appealing option to increase functional recovery in stroke patients relies on the combined use of motor learning strategies and NIBS to reinforce the acquisition and retention of the motor skill, and to enhance plasticity within the M1 by increasing BDNF production or LTP formation.

Table 1.4: Selected therapeutic interventions enhancing motor learning improvement in stroke patients with the paretic hand

motor learning forms	stroke patients & lesion location	motor learning task	intervention	Results	ref
Motor skill learning	11 SC 9 C chronic	Sequential VMT	ULES	Improvement of : Training alone : 20%* on BBT 22%*JTT ULES alone : 14% on BBT 6% JTT Combination: 20%* on BBT 11%* JTT No significant improvement on accuracy in tracking task Only combination session lead to an significant brain reorganisation related to learning.	(Bhatt et al. 2007)
	8 SC 7 C	complex sequential finger motor task	10 Hz rTMS M1damH	10% improvement on accuracy and MT after real rTMS compare to sham	(Kim et al. 2006)
	8 SC 4 C chronic	Sequential VMT	cTBS M1 undamH or S1 undamH	After M1 stimulation: improvement 14 % on MT; 18 % on RT, 22% on accuracy After S1 stimulation: improvement 14 % on MT; 10 % on RT, 16% on accuracy	(Meehan et al. 2011a)
	6 SC 6 C chronic	SRTT	explicit feedback on performance	50 ms diminution of RT after training with explicit feedback	(Boyd and Winstein 2001)
	19 C chronic	SRTT	sleep	There is an improvement of RT after a night of sleep : 14% in prefrontal lesions patients; 4% in parietal lesions patients and 7% in control individuals.	(Gomez Beldarrain et al. 2008)
	19 SC 21 C chronic	VMT	sleep	Patients improved them self in motor skill learning (10% in accuracy) (both explicit and implicit) after sleeping compare to no sleep between practice and retention test.	(Siengsukon and Boyd 2009c)

	7 SC 9 C chronic	VMT	sleep	Patients improved them self in motor skill learning (35% in time lag, and 16% in accuracy) after sleeping compare to no sleep between practice and retention test	(Siengsukon and Boyd 2009a)
	10 SC 8 C chronic	SRTT	Levodopa	Levodopa improved motor skill learning (diminution of reaction time by 150% between sham and Levodopa)	(Rosser et al. 2008)
	12 SC 25 C chronic	pointing task	explicit feedback on performance	Feedback on global performance improved more motor skill learning (50%) than no feedback (25%)	(Cirstea et al. 2006)
	12 SC chronic	SRTT	cathodal tDCS M1 undamH	cathodal tDCS improved motor skill learning compare to sham 20%	(Zimerman et al. 2012)
	11 C 7 SC chronic	Sequential VMT	dual-tDCS	Enhanced online motor skill learning, enhanced long-term retention (x10)	(Lefebvre et al. 2013a)
UDP	8 chronic	physical training	action observation	improvement in the magnitude of memory formation after action observation (10%)	(Celnik et al. 2008)
	7chonic	physical training	PNS	PNS enhance training effect of UDP (24%)	(Sawaki et al. 2006)
	9 SC chronic	physical training	Levodopa	Improvement in the magnitude of training effect with Levodopa (8%) compared to sham (2%)	(Floel et al. 2005)
experience-dependent plasticity	26 SC 4 C chronic	standard oriented task	1Hz rTMS M1 undamH	Improvement on JTT of 30% (compare to sham) after stimulation and at 3 months	(Avenanti et al. 2012)
	20 SC chronic	pinching task	1Hz rTMS M1 undamH	rTMS improved the acceleration of the affected hand of 20% up to one week	(Takeuchi et al. 2008b)

	30 SC chronic	pinching task	1Hz rTMS M1 undamH 10 Hz rTMS M1 damH cumulative	Improvement of 20% on acceleration of affected hand improved to 30% at one week after 1Hz rTMS Improvement of 15% on acceleration of affected hand up to one week after 10Hz rTMS	(Takeuchi et al. 2009)
	10 chronic	training on ADL task	mental practice	improvement on ARAT (18 %) and FM (10%) after therapy	(Page et al. 2009)
	5 SC 10 C chronic	training on ADL task	action observation	Improvement on trained task after action observation therapy : 14% on FAT and 6% on WMFT	(Ertelt et al. 2007)
	177 SC I 27 C 204	occupational therapy	rTMS	rTMS improved effect of occupational therapy 11% on FMA and 24% on WMFT at the end of the treatment and up to 6% on FMA and 28% on WMFT 8 weeks after the treatment.	(Kakuda et al. 2012)
	46 C chronic	physical therapy	Fluoxetine	Physical therapy associated with Fluoxetine was associated with good recovery. 35% extra of patients with good recovery after treatment compared to sham or maprotiline and 40% less patients with poor recovery after treatment compared to sham or maprotiline	(Dam et al. 1996)
	118 C acute	physical therapy	Fluoxetine	90 days after stroke, patients treated with Fluoxetine in addition to physical therapy presented a superior score on FMA (25%)	(Chollet et al. 2011)

Legend of Table 1.4: undamH: undamaged hemisphere; damH: damaged hemisphere; C: cortical stroke, SC: subcortical stroke; BG: basal ganglia, VMT: visuomotor tracking task, SRTT: serial reaction time task; rTMS : repetitive transcranial magnetic stimulation, cTBS continuous theta burst stimulation, tDCS: transcranial direct current stimulation, M1: primary motor area, S1: primary somatosensory cortex ULES: upper limb electrical stimulation, dual-tDCS (concomitantly anodal over ipsilesional M1 & cathodal over contralesional M1), UDP: use-dependent plasticity , FAT: Frenchay Arm Test, WMFT: Wolf Motor Function Test; SIS: Stroke Impact Scale, FMA: Fugl-Meyer Assessment, RT: Reaction time, JTT: Jebsen Taylor test, BBT: Box and Block Test, MT : movement time.

1.8. Specific methods and paradigm used in this thesis.

1.8.1. Motor skill learning task: circuit game

For this thesis, we developed a new motor skill learning paradigm **i)** involving a speed/accuracy trade-off (SAT) - according to the most recent definition, the SAT is one of the characteristic signature of motor skill learning (Krakauer and Mazzoni 2011) -, **ii)** requiring the use of the whole upper limb to perform complex sequences of movements and **iii)** having clearer ecological relevance to daily life activities. This motor skill learning paradigm task named “circuit game” requires moving a pointer across a circuit path using a computer mouse held in one hand. The instructions are to move the cursor as quickly and accurately as possible, accurately means keeping the cursor within the path of the circuit (i.e. keeping the centre of the cursor on the midline of the circuit).

This task has been designed as a repetitive sequential unimanual visuomotor task exploring upper limb function. In accordance to the ICF (see Figure 1.1), this task permits to explore functional limitation after stroke but is not clearly related to an activity limitation. So, it could not be used to assess daily life improvement after training.

We developed five circuits of equal length and difficulty, but requiring performing a different sequence of movements (Figure 1.5). For quantifying performance improvements and motor skill learning during training on the circuit game the error, velocity and normalized jerk were analysed. Error was

defined as the surface area generated by the difference between the real trajectory and the ideal trajectory in the midline of the track. Velocity was the first derivative of the position. Normalized jerk (NJ) was computed with the formula $NJ = \sqrt{1/2 * \int_{Tstart}^{Tend} jerk^2(t)dt * duration^5 / length^2}$ (Contreras-Vidal and Buch 2003b; Caimmi et al. 2008) where the jerk is the third derivative of the position. The NJ reflects the smoothness of the movements, with the underlying assumption that smoother movements (smaller NJ) are associated with a higher level of skill (another signature of motor skill learning (Nelson 1983; Shmuelof et al. 2012)). The error and velocity were expressed with arbitrary grid unit (u) as u/s for velocity and u² for error. One arbitrary grid unit (u) displayed on the computer screen is equivalent to a distance of 0.3 cm in straight line by the computer mouse.

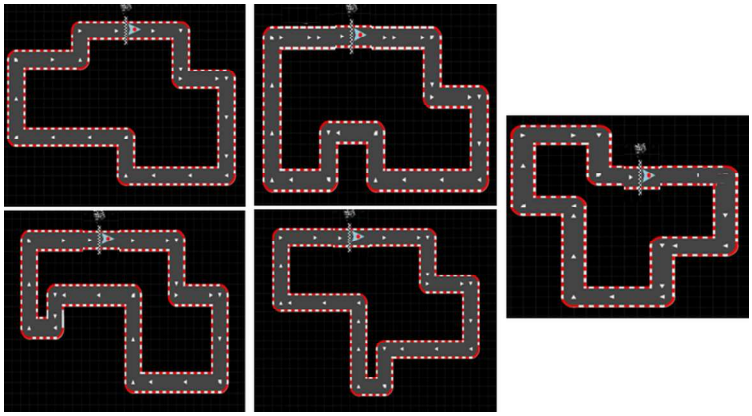

Figure 1.5: The five different versions of the circuit game. In each circuit is displayed the cursor (bleu arrow), its centre (red dot) and the midline of the path (white dots in the grey path).

Thus, in order to compute an index **i)** combining these two parameters expressed with different units and **ii)** not skewed by the much greater size of velocity compared to error, the error and velocity were normalised as Pe and Pv. Thus, Pe and Pv were calculated as

Pe = a / subject error

Pv = subject speed / b

This normalisation has been performed using a constant term for velocity (b) and for error (a) obtained on a group of seven stroke patients to compare the difficulty of the task depending on different versions or on a group of 18 healthy volunteers who have been trained on the circuit game to explore if the task induced on-line motor skill learning behaviour.

Next, Pe and Pv were combined to calculate the performance index (PI) PI = Pe x Pv. Finally, the evolution of performance across the training as a percentage from Baseline (i.e. the Learning Index, LI) was calculated with the formula: LI = [(PI - PI baseline)/PI baseline] x 100.

As mentioned, to verify that these circuits were of equal difficulty, seven chronic stroke patients performed each circuit during 5 min in a random order, after breaks of 5 min. The mean velocity, error, NJ and laps number were not statistically different between the five circuits of equal length.

In addition, to explore the ability of our task to induce on-line motor skill learning, 18 healthy volunteers were trained on the circuit game during 30 min (30 s on the circuit followed by 30 s of rest). During this training period, all the volunteers (except one) achieved motor skill learning (Figure 1.6). In addition, by the dissection of the SAT components (i.e. the respective evolutions of speed and accuracy) three distinct motor skill learning behaviours could be defined (Table 1.5).

Table 1.5: **Motor skill learning behaviour**

	Motor skill learning					**No learning**					
	Shift			**Fit**							
Pe	↗	↗	→	↗↗	↘	↘	→	↘	↗	↘↘	→
Pv	↗	→	↗	↘	↗↗	↘	↘	→	↘↘	↗	→
PI	↗↗	↗↗	↗↗	↗	↗	↘	↘	↘	↘	↘	→
LI	↗	↗	↗	↗	↗	↘	↘	↘	↘	↘	→

Pe: performance error, Pv: performance velocity, PI Performance index, LI learning index

First, the shift behaviour involves a global performance improvement (i.e. increase of the LI) with no concomitant degradation of one of the operating characteristic (speed or accuracy). Second, the fit behaviour involves a slight global performance improvement (i.e. mild LI increase) due to a strong improvement of one of the operating characteristic (e.g. speed) concomitant to a small degradation of the second one (e.g. accuracy). Finally, the non-learning behaviour involves a degradation of global performance over time (i.e. *decreasing* LI), with either a simultaneous degradation of the two operating characteristics, a stagnation of one parameter and a degradation of the second one or a strong degradation of one component concomitant to a small improvement of the second one. The non-learning behaviour also encompasses a stagnation of the global motor performance over time (i.e. unchanged LI). In this pilot experimentation, motor performance deteriorated in one healthy individual; half of the others adopted a shift pattern and the remaining the fit pattern.

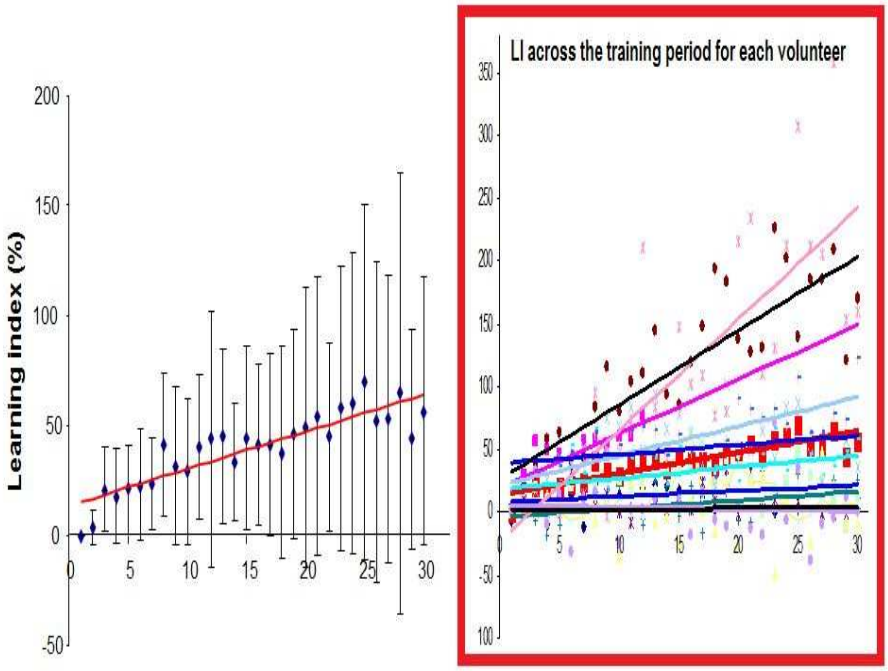

Figure 1.6. Motor skill learning in healthy volunteers (n=18). Left panel: At the whole-group level, the global LI across the 30 min training session. Right panel: Individual LI evolution of the 18 volunteers.

This paradigm, the circuit game, is used in the next chapters of this thesis to explore the neural substrates of motor skill learning in healthy volunteers (Chapter 4) and stroke patients (Chapter 5) and to explore the impact of dual-tDCS on motor skill learning in chronic stroke patients (Chapters 4 and 6).

1.8.2. Non-invasive stimulation method: dual-tDCS

As described in the sections 1.3 and 1.7., several methods of NIBS could be used to efficiently modulate brain excitability after stroke. In this thesis, we chose to apply dual-tDCS simultaneously over M1 of both hemispheres, damaged and undamaged. As dual-tDCS aims at a bilateral modulation of cortical excitability, it is an appealing option to rebalance the interhemispheric interaction after stroke. Previous studies in healthy individuals and chronic stroke patients suggest a greatest motor performance improvement with dual-tDCS than with uni-hemispheric tDCS (Vines et al. 2008;Lindenberg et al. 2010). Therefore, we chose to apply dual-tDCS despite recent results suggesting that cathodal tDCS over the $M1_{undamH}$ could induce a deterioration of the paretic arm motor function for the most impaired patients (Bolognini et al. 2011). In addition, it should be mentioned that cathodal tDCS over the $M1_{undamH}$ could possibly impair motor function of the non-paretic hand.

This is why we included additional measurement of the non-paretic hand performance to explore these potential deleterious effects (see Chapters 2, 3 and 6). We also explored whether dual-tDCS induced a different effect depending on the clinical characteristics of the patients.

In our experiments (Chapters 2, 3 and 6), the duration of dual-tDCS is either 20 or 30 min with either an efficient (real) continuous direct current of 1 mA or a sham stimulation, with only a transient stimulation followed by

inefficient small current pulses applied to elicit continuous sensation over the scalp and to permit continuous impedance level checking.

1.8.3. Randomized placebo-controlled double-blind cross-over design

The experiments (Chapters 2, 3 and 6) were designed as Randomized Controlled Trials (RCT) with a randomized, double-blind, placebo-controlled, cross-over design. All our patients participated in two sessions (real/sham dual-tDCS) in a placebo-controlled balanced order, which is based on an inclusion list; with a double-blind placebo-controlled application of dual-tDCS [neither the patients nor the experimenters knew the nature and the order of the stimulation (real or sham dual-tDCS)].

This specific design presents both advantages and disadvantages. The main disadvantages of this design are that the performance at the second session could be improved (carry-over effect) by the order of the intervention (real dual-tDCS during the first session), or by the practice/learning effect (repeated sessions).The performance at the second session could also be limited by a ceiling effect (unlikely with only one previous training session). Nevertheless, the randomized, double-blind order mitigates the impact of these interactions on our global analysis as half of the patients received the real dual-tDCS during the first session and the other ones received the sham. For the studies exploring motor skill learning (chapters 3 and 6), the patients were trained on the circuit game with different tracks, involving another sequence of movements and so reducing the learning/practice effect.

The main advantage of this design is to allow each patient enrolled to be his own control and ensure similar patients' characteristics for each condition (real and sham dual-tDCS)..

This is crucial from our point of view to limit the variability that can be expected in studies involving patients with various brain lesions etc… This is the main reason why we chose this design. Considering the confounding effects inherent to our design, we performed additional analyses only on the first session (as if we did a parallel group design study).

1.9. Purpose of the thesis

The main purposes of the present work are **i)** to explore the impact of dual-tDCS on motor function in chronic stroke patients, **ii)** to explore the capacity of dual-tDCS to improve motor skill learning and long-term retention in chronic stroke patients, **iii)** to explore the neural substrates underlying motor skill learning in both healthy individuals and chronic stroke patients, and **iv)** to unveil the neural substrates of tDCS-enhanced motor skill learning in chronic stroke patients. More specifically, the following questions will be addressed:

i) Does dual-tDCS improve post-stroke hand motor performance? This question is discussed in Chapter 2 by testing digital dexterity and precision grip before, during and after the application of 20 min of dual-tDCS in 19 chronic stroke patients.

ii) Are online motor skill learning and its long-term retention improved by dual-tDCS? This question is discussed in Chapter 3 by evaluating online motor skill learning improvement during 30 min application of dual-tDCS and the motor performance retention one week later, in 18 chronic stroke patients.

iii) What are the specific brain activation patterns induced by early motor skill learning in healthy individuals? This question is addressed in Chapter 4 by exploring, with fMRI, brain activation during a new motor skill learning task involving a speed-accuracy trade-off in 20 healthy volunteers.

iv) Does motor skill learning after stroke rely on a reorganised brain activation pattern? This question is addressed in Chapter 5 by exploring, with fMRI, the brain activation pattern during early training on a new motor skill learning task involving a speed-accuracy trade-off in 25 chronic stroke patients working with their paretic hand.

v) What are the neural substrates underlying the dual-tDCS-induced enhanced long-term retention of motor skill learning in chronic stroke patients? This question is currently explored in an on-going study in 19 chronic stroke patients; the preliminary results are presented in Chapter 6.

CHAPTER 2: Dual-tDCS improves precision grip and dexterity of the paretic hand after stroke: a RCT.**

***Chapter 2 is a modified version of an article submitted to Neurorehabilitation and Neural Repair similarly named by S. Lefebvre, J.L. Thonnard, P. Laloux, A. Peeters, J. Jamart, Y. Vandermeeren(Lefebvre et al. 2013b)*

Abstract:

Background After stroke, deregulated interhemispheric interactions influence residual paretic hand function. Anodal or cathodal transcranial direct current stimulation (tDCS) can rebalance these abnormal interhemispheric interactions and improve motor function. **Objective** We explored whether dual-hemisphere tDCS (dual-tDCS) in participants with chronic stroke can improved fine hand motor function in two important aspects: precision grip and dexterity. **Methods** Nineteen chronic hemiparetic subjects with mild to moderate impairment participated in a double-blind, randomized trial. During two separate cross-over sessions (real/sham), they performed 10 precision grip movements with a manipulandum and the Purdue Pegboard Test (PPT) before, during, immediately and 20min after dual-tDCS applied simultaneously over the ipsilesional (anodal) and contralateral (cathodal) primary motor cortices. **Results** 20min after dual-tDCS, the precision grip performed with the paretic hand improved significantly with reduction of the grip force/load force ratio by 7% (-0.29 after dual-tDCS vs 0.04 after sham dual-tDCS) and in preloading phase duration by 18% (-86 ms after dual-tDCS vs +27 ms after sham dual-tDCS) when compared to sham. The dexterity of the paretic hand started improving during dual-tDCS and culminated 20 min after the end of dual-tDCS (PPT score +2.1 pegs in 30 s : +38% versus +0.4 pegs in 30 s +5% after sham). The maximal improvements in precision grip and dexterity were observed 20min after dual-tDCS. These improvements correlated negatively with residual hand function quantified with ABILHAND. **Conclusions** One bout of dual-tDCS improved the motor control of precision grip and digital dexterity beyond the time of stimulation.. These results suggest that dual-tDCS should be tested in longer protocols for neurorehabilitation and with moderate to

severely impaired subjects. The precise timing of stimulation after stroke onset and associated training should be defined.

2.1. Introduction

Stroke is one of the leading causes of long-term disability. According to the World Stroke Organization, only approximately 12% of stroke survivors achieve complete motor recovery after 6 months (Kwakkel et al. 2003). The majority of stroke patients presents hemiparesis, characterized by abnormal muscle activation and coordination in the paretic arm (Lang et al. 2005), difficulties in strength control (Lodha et al. 2011), digital dexterity (Nowak et al. 2007), interjoint coordination (Cirstea et al. 2003), and precision grip (Hermsdorfer et al. 2003). After a stroke, poor upper-limb motor recovery is coupled with a relative imbalance in interhemispheric excitability, namely a down-regulation of excitability in the ipsilesional primary motor cortex (M1) and/or an enhanced excitability in the contralesional M1 (Murase et al. 2004). Therefore, rebalancing the deregulated interhemispheric interactions is an appealing therapeutic option to improve motor recovery after a stroke (Nowak et al. 2009). Indeed, non-invasive brain stimulations such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) have been shown to rebalance interhemispheric excitability and improve motor function in stroke patients (Takeuchi et al. 2005; Harvey and Stinear 2010; Bolognini et al. 2011; Kakuda et al. 2012). Anodal tDCS applied to the ipsilesional M1 or cathodal tDCS applied to the contralesional M1 improve motor performance of the paretic hand (Fregni et al. 2005). When compared to rTMS applied to both hemispheres (Takeuchi et al. 2009) or complex combinations of rTMS and tDCS on opposite hemispheres (Lindenberg et al. 2010; Bolognini et al. 2011), dual-hemisphere tDCS (dual-tDCS) is particularly attractive for treating stroke patients since tDCS is a simple, safe, and inexpensive method to rebalance disturbed interhemispheric interactions and improve paretic hand function. The pioneer studies which demonstrated that tDCS improves hand motor performance in stroke patients focused on relatively crude or basic measurements of speed and

errors in task execution or maximal force during voluntary contraction, (Fregni et al. 2005; Hummel et al. 2006; Bolognini et al. 2011; Nair et al. 2011). On the other hand, rTMS or theta burst stimulation (TBS) have been shown to improve the precision grip dynamics and dexterity of the paretic hand (Liepert et al. 2007; Dafotakis et al. 2008a; Ackerley et al. 2010) . Currently, the potential of tDCS to improve precision grip or dexterity has not yet been explored.

The goal of the present study was to test the hypothesis that, compared to baseline, two important aspects of fine hand motor function relevant from an ecological point of view, the motor control of precision grip and the digital dexterity, improved more after real than sham dual-tDCS in chronic stroke patients. We also explored the early time-course of functional changes induced by dual-tDCS.

2.2. Methods

The study was approved by the local Ethical Committee and conducted according to the recommendations of the Helsinki Declaration. Written informed consent was obtained at enrolment.

2.2.1. Patients

Nineteen chronic stroke patients were included in the study (Figure 2.1). The inclusion criteria were i) being a chronic (>6 months) stroke patient aged 18–80 years, ii) with an initial motor deficit in the upper limb clinically evident during at least one week, and iii) having a hemispheric vascular brain lesion demonstrated by cerebral imaging[1]. The exclusion criteria were the presence of i) intracranial metal, ii) epilepsy, iii) alcoholism, iv) pregnancy, v) cognitive impairment or psychiatric disorder, and vi) being unable to perform the task or understand the instructions. Seventeen patients had an ischemic stroke. Of these subjects, patients 12 and 16 had a secondary hemorrhagic transformation, and patients 4 and 8 had an intracerebral haemorrhage. Eleven patients presented subcortical stroke (Figure 2.2). The degree of

[1] *It has to be mentioned that some patients had lesion in the pons which is not an hemispheric lesion*

overall disability was quantified with the modified Rankin Scale (mRS) (Bonita and Beaglehole 1988) and manual ability was quantified with the ABILHAND scale (Penta et al. 2001; Wang et al. 2011b) (Table 2.1). ABILHAND is a measure of manual ability that has been defined as the capacity to manage daily activities requiring the use of upper limbs, whatever the strategies involved (Penta et al. 2001). During a structured interview, the patients scored the 23 ABILHAND items as impossible, difficult, or easy.. The ABILHAND items refer to bimanual tasks such as 'Fastening the zipper of a jacket', 'Tearing open a pack of chips', 'Cutting meat' or 'Hammering a nail'. Patients responses were classified according to the published calibration ranging approximately from -3.5 to 6 logits, where smaller logits (-3.5) are associated with a self-perceived greatest difficulty to perform the task (Penta et al. 2001).The digital dexterity impairment was also quantified by the mean score of three trials with each hand on the Purdue Pegboard Test (PPT) (Tiffin and Asher 1948; Costa et al. 1963) (Table 2.1). Unfortunately, the National Institutes of Health Stroke Scale (NIHSS) (Kasner et al. 1999) score was not available for a majority of patients. Nevertheless, all presented upper limb motor deficits, such as hand weakness and/or disorders in fine alternating movements or dysmetria, as explicitly reported in all the medical records. At the time of inclusion, all the stroke patients were able to perform a reaching movement and a precision grip between the thumb and index finger.

2.2.2. Design

The patients participated in a randomized control trial (RCT) consisting of two sessions (real/sham dual-tDCS) performed at least one week apart in a balanced order (inclusion list), with a double-blind, placebo-controlled experimental design.

During familiarization, each patient performed ten grip-lift movements with the paretic hand to allow for fast adaptation/learning effects (Johansson and Westling 1984). Each session was divided into four evaluation periods [prior to tDCS (Baseline), during tDCS (During), immediately after tDCS (After),

and 20 min after tDCS (After 20 min)], during which the patients performed ten grip-lifts with the paretic hand and the PPT three times with each hand.

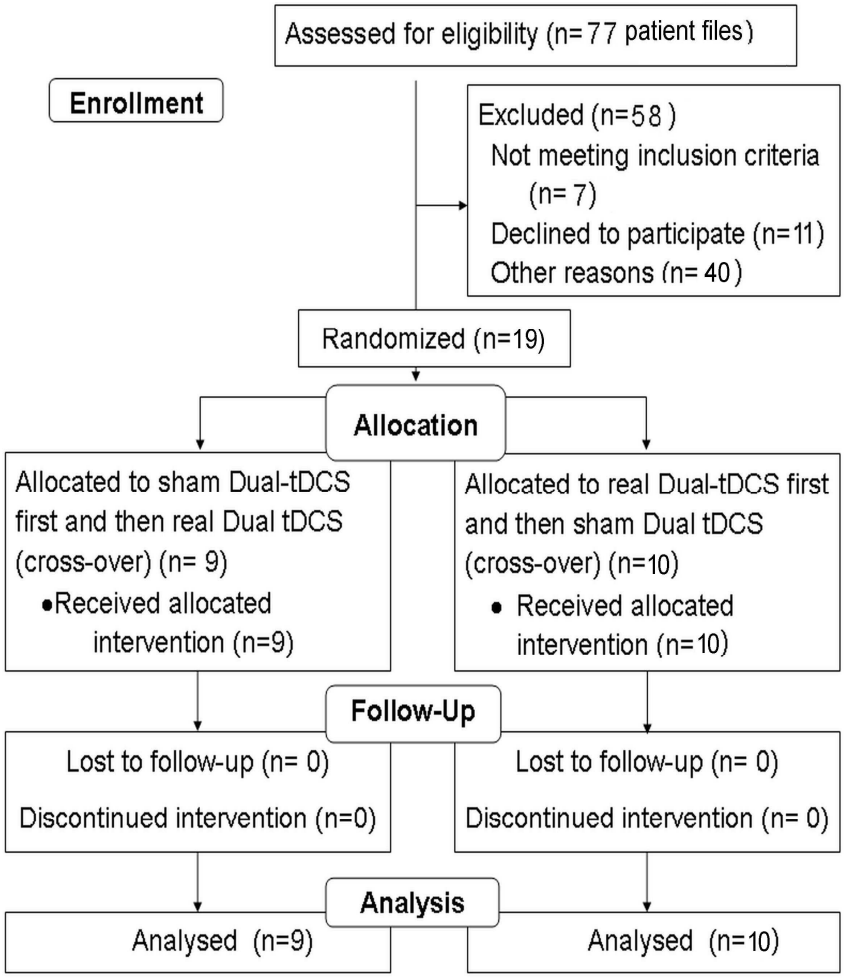

Figure 2.1: CONSORT Flow Diagram Allocation/Randomization method: A first experimenter established an inclusion list with the codes for real and sham dual-tDCS, in a pseudo-randomized and balanced order. These codes were used by a second experimenter to apply dual-tDCS in a double-blind fashion

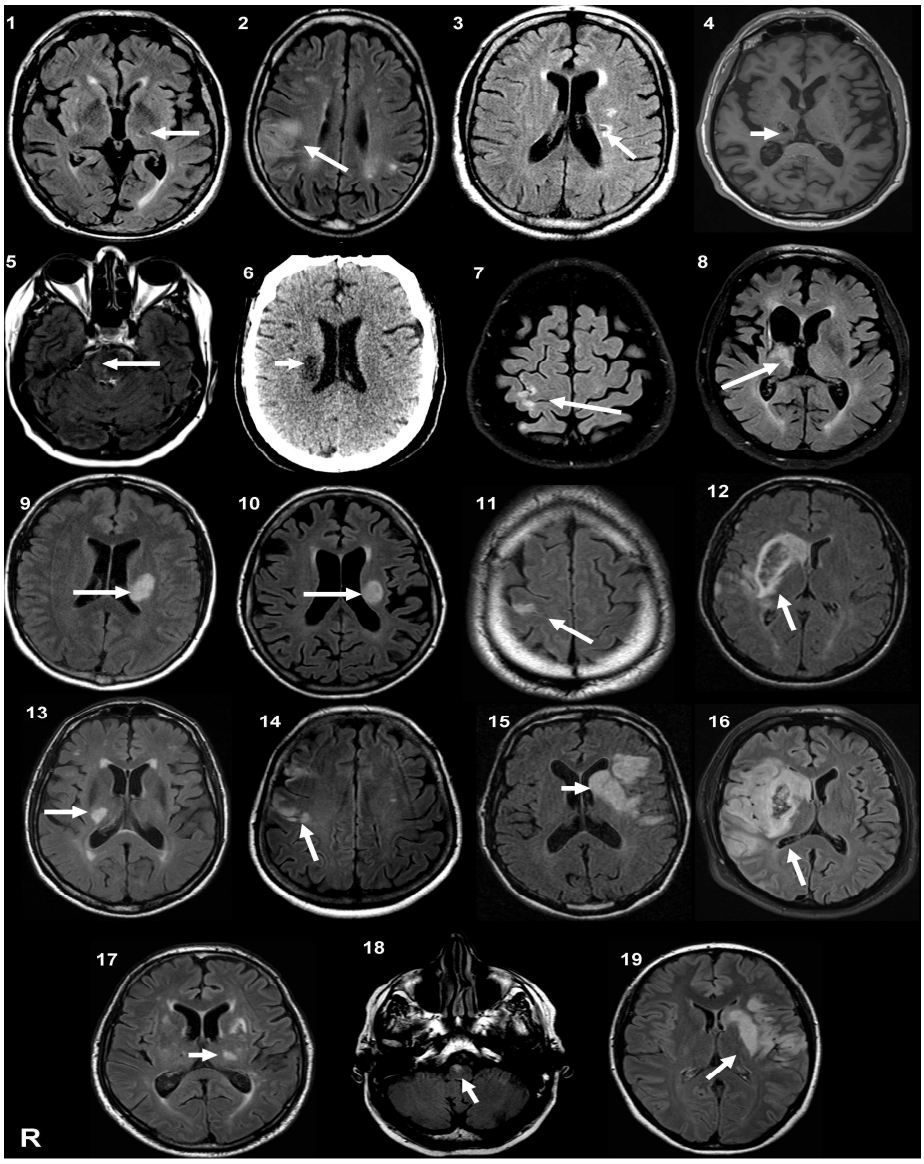

Figure 2.2: Brain imaging of the stroke patients Magnetic resonance imaging (MRI) or computed tomography (patient 6) at the level of the main stroke injury; T_2-weighted FLAIR except for patient 4 (T_1). Patients 4 and 8 had an intracerebral haemorrhage. There was a slight secondary hemorrhagic transformation in patients 12 and 16. Patients, 2, 14, and 19 had at least one other lesion compatible with a previous, minor stroke. Patients 8, 11, 13, and 17 had associated leukoaraiosis. Patients 1, 2, 11, 13, and 18 had some small chronic subcortical infarcts. Patients 3, 4, and 18 had some small chronic subcortical lacunar infarcts

Table 2.1. Baseline characteristics of the chronic stroke patients

	Gender	Age	Time since stroke (years)	Main stroke lesion	Additional vascular lesions	DH	PH	PH PPT (n)	PH PPT Z-score	N-PH PPT (n)	ABILHAND (logits)	mRS
1	M	65	9	SC	SSIs	R	R	8.3	-2.93	10.7	2.5	2
2	M	54	5	C	SSIs, C	R	L	3	-2.95	10.3	0.8	2
3	M	57	8	SC	SLIs	R	R	3.3	-5.33	7	0.4	3
4	M	61	6	SC (H+)	1 SLI	L	L	2.3	-6.93	12.3	0.3	3
5	F	56	3	SC	-	L	L	7.3	-1.87	9.3	1.8	2
6	M	55	4	SC	-	R	L	7.7	-1.39	12.7	1.2	1
7	M	63	2	C	-	L	R	8.3	-2.93	12.7	2.6	1
8	M	68	1	SC (H+)	LK	R	L	8	-3.13	9.3	2.8	2
9	M	64	4	SC	-	R	R	5	-5.13	16.3	1.5	2
10	F	57	3	SC	-	R	L	0	-6.80	11.3	-1.2	3
11	M	56	3	C	LK, SSIs	R	L	8.3	-1.19	11.3	3.9	2
12	M	49	3	C	-	R	L	6	-1.96	13.7	1.7	2
13	M	70	5	SC	LK, SSIs	L	L	10.3	-0.19	12.0	1.7	2
14	F	49	2	C	C, SS-C	R	L	10.3	-1.96	13.7	/	1
15	M	69	4	C	-	R	R	3.7	-6.0	9.7	1.5	2

16	M	63	1	C	-	R	L	1.3	-7.6	10.3	1.9	2
17	M	76	4	SC	LK, SLIs	R	R	12	0.4	10.7	6.0	0
18	M	70	3	SC	SSIs	R	L	8	-1.23	11.3	/	2
19	F	35	3	C	SS-C	R	R	16.6	0.58	17.3	4.4	2
		60 ± 1	**4 ± 2**					**6.9 ± 4.0**	**-3.08 ± 2.5**	**11.7 ± 2.4**	**2.1 ± 1.5**	**2 ± 1**

Legend Table 2.1[1] M: male, F: female, SC: subcortical stroke, C: cortical stroke, H+: intracerebral hemorrhage, R: right, L: left, PPT: baseline Purdue Pegboard Test score, n: number of pegs inserted in 30 s (mean of three trials), mRS: modified Rankin Scale, SSIs: small subcortical infarctions, SLI: subcortical lacunar infarctions, and LK: leukoaraiosis. In addition to the main stroke lesion, the majority of stroke patients presented additional vascular lesions: SSIs, SLI, and LK. PH: paretic hand; N-PH: non-paretic hand. Z-scores were calculated on paretic hand mean PPT score compared to normative data (Tiffin and Asher 1948; Desrosiers et al. 1995). Missing ABILHAND values were reported by backslashes

[1] *Patients who had a score of 0 at the PPT (unable to insert a single peg) or the maxHF were unable to perform the tasks despite repeated attempts. Patients with missing value (in ABILHAND scale) did not complete the questionnaire*

2.2.3. Intervention

Dual-tDCS was delivered by an Eldith DC-Stimulator® (NeuroConn, Ilmenau, Germany). The electrodes (35 cm²) were soaked in 0.9% NaCl. A Magstim 200² (Magstim Company, UK) with a figure-of-eight coil was used to determine the hot spot eliciting consistent movements in the contralateral hand. The anode was positioned over the ipsilesional M1, and the cathode was placed over the contralesional M1. During real dual-tDCS, the stimulator delivered 20 min of stimulation at 1 mA (fade in/out 8 s). During sham dual-tDCS, a short up-ramp (8 s fade-in) was followed by 40 s of direct current, and 8 s of fade-out, after which ineffective current pulses (110 µA over 15 ms, peak current 3 ms) were delivered every 550 ms. The first experimenter established an inclusion list with the Eldith codes (real/sham) for each session. These codes were used in a double-blind fashion by the second experimenter.

2.2.4. Hand function assessment

The primary outcome measures were the preloading phase duration (PLD) and grip force/load force ratio (GF_L/LF_L) for the precision grip, two variables which are typically impaired in chronic stroke patients (Hermsdorfer et al. 2003; Nowak et al. 2003; Dafotakis et al. 2008b), and the Purdue Pegboard Test (PPT) score for quantifying digital dexterity (Gallus and Mathiowetz 2003; Mansur et al. 2005).

To measure the forces [perpendicular: left and right grip forces, averaged as the global grip force (GF), and tangential: the load force (LF)] during the grip-lift task, a manufactured manipulandum fitted with three strain gauges (force transducers) and weighting (275 g) was used (GLM Arsalis®, Louvain-la-Neuve, Belgium). Analog signals were amplified, filtered with a Bessel 150-Hz cut-off low-pass 4^{th} order filter, and sampled at 2000 Hz. Data were analyzed offline.

During the grip-lift task, the patients were seated with their hand resting on a desk. The manipulandum was placed in front of them. The patients were asked to apply the minimal forces necessary to grasp the

manipulandum between the thumb and index finger, lift it 20 cm above the desk, hold it stationary for 3 s, and then replace it.

Temporal parameters of grip-lift movements were assessed by measuring the durations of three periods (Johansson and Westling 1984): i) the preloading phase (PL) duration (PLD), the delay between the onset of GF and the onset of LF; ii) the loading phase duration (LD), the delay during which both GF and LF increased until LF equaled the weight of the manipulandum (2,75 N); and iii) the unloading phase duration (ULD), when LF dropped below the manipulandum's weight until the end of the movement. Dynamical parameters were assessed by measuring the maximum downward force applied during the PL (PLF) and the efficiency of GF scaling relative to the load induced by raising the manipulandum. The latter was computed as the ratio between GF and LF (GF_L/LF_L) at the end of the lift. The coordination between LF and GF was quantified by a cross-correlation function between the first derivative of LF and GF [dLF/dt (LF rate) and dGF/dt (GF rate)] (Duque et al. 2003). This cross-correlation function was computed for the loading period. The cross-correlation was characterized by a time shift (TS1) quantifying the delay to obtain the best possible overlap between the dGF/dt and dLF/dt curves with 0.5 ms steps, and a correlation coefficient (R) that reflected the strength of this correlation.
To assess digital dexterity with the PPT, the patients had to pick up as many pegs as possible one by one and insert them into the holes of a board in 30 s (Tiffin and Asher 1948; Gallus and Mathiowetz 2003). The PPT score was the mean number of pegs placed in the holes during three trials for each hand (Tiffin and Asher 1948) (Table 2.1).

2.2.5. Statistical analysis

The analysis of grip-lift parameters was performed using regression of repeated measures with generalized estimating equations (GEE) to consider the multiplicity of intercorrelated values in each patient (Liang and Zeger 1986). This analysis was used to evaluate the impact of Stimulation (real/sham) and Time (Baseline, During, After, and After 20 min) for each grip-lift parameter. Repeated measures analysis of variance (RM-ANOVA)

was used to explore the effects of Stimulation and Time on the mean PPT scores. For pair-wise post-hoc comparisons, t-tests corrected for multiple comparisons (Bonferroni) were computed between each period and baseline; and separately between sham and real dual-tDCS for After 20 min period.

PPT score improvement at the period After 20min with real dual-tDCS was correlated with age, mRS, and ABILHAND by Pearson's coefficient. The PPT score improvement was compared according to the localization of lesion and whether the paretic hand was dominant or not using the Student's t-test. A p-value of 0.05 was considered statistically significant. All the statistical tests were two-tailed. Statistical analyses were performed using SPSS® 15.0 (SPSS Inc., Chicago, USA).

2.3. Results

2.3.1. Precision grip

A significant interaction between Time and Stimulation was observed for three parameters with the GEE analysis: the PLD ($p < 0.001$), the GF_L/LF_L ($p = 0.009$) and the ULD ($p < 0.001$) (Figure 2.3), suggesting that real dual-tDCS led to greater improvements than sham over time. At the baseline, there were no statistically significant differences between real and sham dual-tDCS for any of the precision grip parameters. For the After 20 min period, only real dual-tDCS significantly improved from Baseline the PLD (real: -86 ms $p = 0.023$, -18%; sham:+27 ms $p = 0.4$, +6%), and the GF_L/LF_L (real: -0.29 $p = 0.014$, -7%; sham:=0.04 $p = 0.9$, +1%). The ULD was significantly improved from Baseline only with real dual-tDCS both during the After period (real: -37 ms $p < 0.001$, -4%; sham:+72 ms $p = 0.5$, +7%) and the After 20 min period (real: -22 ms $p = 0.004$, -2%; sham: +10 ms $p = 0.8$, +1%). In addition, there was no Time x Stimulation interaction for TS1 but the TS1 showed significant improvement from Baseline to After 20 min with both real (+19 ms $p = 0.007$, +63%) and sham (+20 ms $p = 0.039$, +70%) dual-tDCS. Finally, for the After 20 min period, comparisons between sham and real dual-tDCS showed a significant improvement for PLD

(p = 0.036; effect size: 0.4) and only a non-statistically significant trend for ULD (p = 0.1) and GF_L/LF_L (p = 0.1).

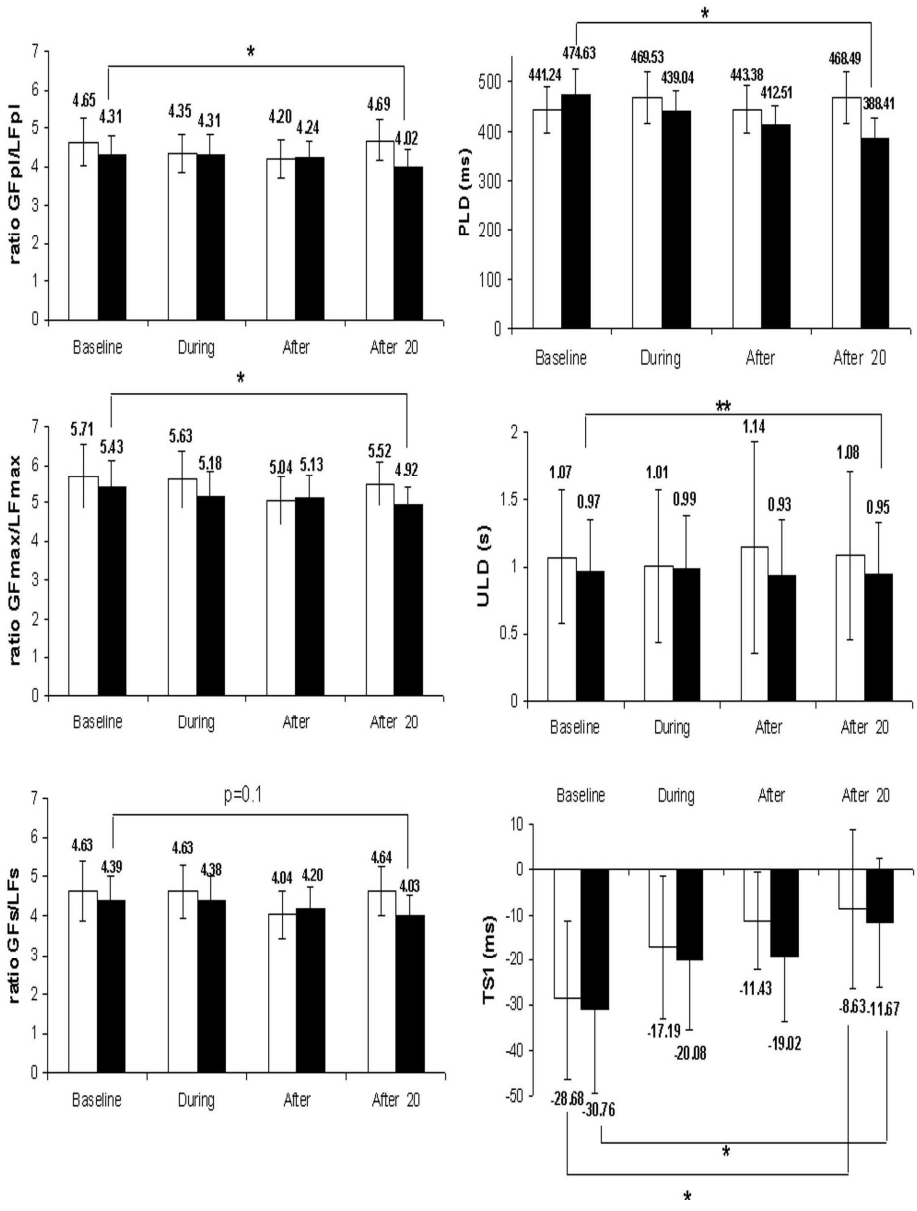

Figure 2.3: Precision grip parameters under sham and real dual-tDCS Changes at the group level (mean ± SEM) under real (black bars) and sham (white bars) dual-tDCS over the four evaluation periods (Baseline, During, After, and After 20 min). PLD: preloading phase duration, TS1: time shift 1, ULD: unloading phase duration, GF: grip force, LF: load force.

2.3.2. Digital dexterity

For the paretic hand, there was continuous improvement over time only with real dual-tDCS, and the maximal improvement was observed after 20 min (+2.1 pegs in 30 s, +38% after real dual-tDCS, vs +0.4 pegs in 30 s ,5% after sham Figure 2.4). The RM-ANOVA demonstrated a significant interaction between Time and Stimulation ($p < 0.001$), suggesting that real dual-tDCS led to greater improvements than sham over time. Post-hoc analyses confirmed that there was no significant performance improvement with sham dual-tDCS over time, whereas there were statistically significant performance improvements with real dual-tDCS between Baseline and During ($p = 0.003$), Baseline and After ($p < 0.001$), and Baseline and After 20 min (maximal improvement: +2.1 pegs in 30 s, +38%, $p < 0.001$). There was a statistically significant difference between real and sham dual-tDCS exclusively for the After 20 min period ($p < 0.001$; effect size: 0.3).

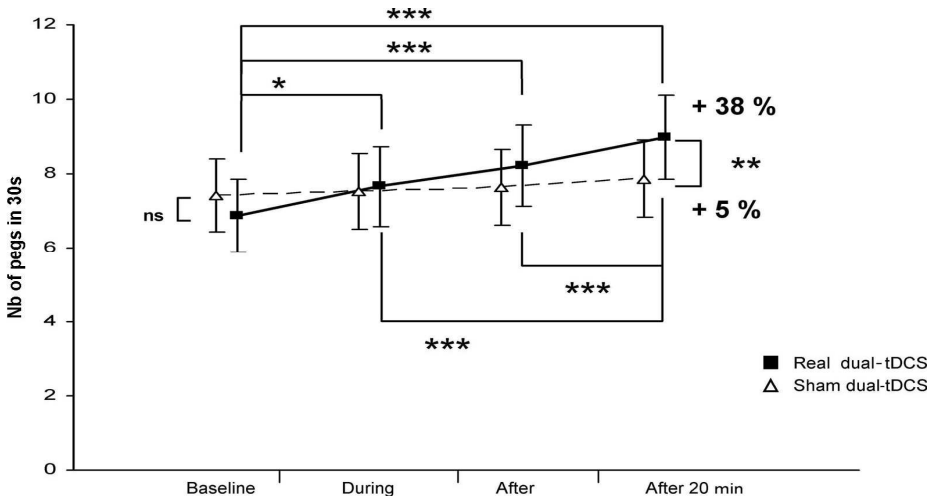

Figure 2.4[1]: Purdue Pegboard Test (PPT) scores of the paretic hand under sham and real dual-tDCS Changes in the PPT score (mean ± SEM) for the paretic hand under real (black squares) and sham (white triangles) dual-tDCS over the four evaluation periods (Baseline, During, After, and After 20 min).NS = not statistically significant, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$

[1]*The dexterity of the paretic hand started improved during dual-tDCS and culminated 20 min after the end of dual-tDCS (PPT score +2.1 pegs in 30 s : +38% versus +0.4 pegs in 30 s +5% after sham)*

For the non-paretic hand (Figure 2.5), RM-ANOVA showed that there was only a significant effect of the factor Time [$p < 0.001$; no effect of Stimulation ($p = 0.9$) and interaction between Time and Stimulation ($p = 0.3$)]. These results suggest a progressive performance improvement regardless of the stimulation type (real/sham), with a maximal improvement after 20 min (real: +0.8 pegs in 30 s ,+6%, sham +1.3 pegs in 30 s , +11%).

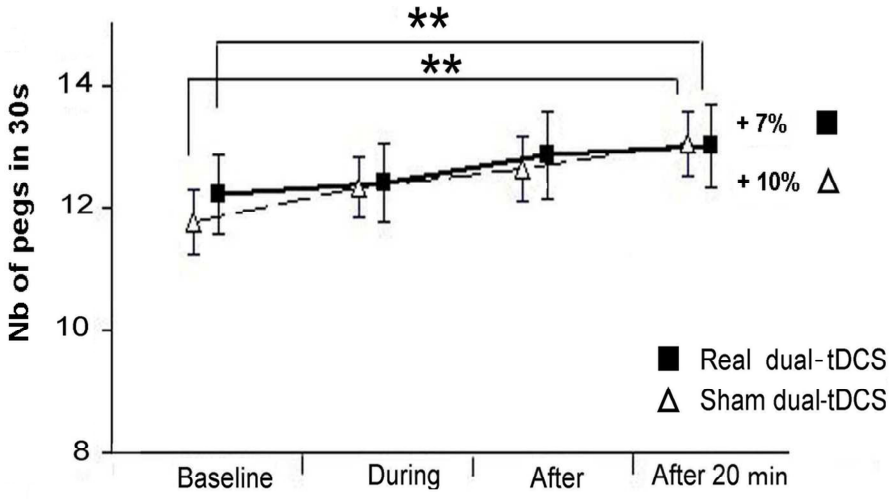

Figure 5: Purdue Pegboard Test (PPT) scores of the non-paretic hand under sham and real dual-tDCS Changes in the PPT score (mean ± SEM) for the non-paretic hand under real (black squares) and sham (white triangles) dual-tDCS over the four evaluation periods (Baseline, During, After, and After 20 min). * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$.

2.3.3. Correlation analyses

Correlations were performed to determine whether baseline clinical characteristics could predict the individual percentage of improvement at the period After 20min with dual-tDCS. The patient's age, localization of the lesion (cortical/subcortical), whether the paretic hand was dominant or not, and the mRS did not significantly correlate with the PPT score improvement ($p = 0.87$, $p = 0.60$, $p = 0.4$, and $p = 0.2$, respectively). In contrast, the ABILHAND score significantly correlated with the PPT improvement under dual-tDCS ($r=-0.54$; $p = 0.025$).

2.4. Discussion

The main finding of this study is that, in chronically, and mild to moderately impaired persons after stroke, 20 min of dual-tDCS induced a large, rapid and protracted improvement in performance with the paretic hand relative to baseline on a complex digital dexterity task (PTT) and a smaller, delayed improvement on the dynamics of precision grip after real dual-tDCS, compared to sham.

2.4.1. Dual-tDCS improves precision grip and dexterity with the paretic hand

Stroke has devastating effects on the precision grip ranging from eradication to typical impairments such as a prolonged preloading phase duration (PLD), an excessive grip force leading to an abnormal grip force/load force (GF/LF) ratio, an abnormal time shift between the GF and LF increases, or an excessive preload force (Hermsdorfer et al. 2003; Quaney et al. 2005; McDonnell et al. 2006; Raghavan et al. 2006; Nowak et al. 2007). The precision grip impairments we observed in stroke patients were in line with those reported in previous articles (Johansson and Westling 1984; Raghavan et al. 2006; Dafotakis et al. 2008a).

Real but not sham dual-tDCS improved the GF/LF ratio of the paretic hand compared to baseline (-7% (i.e. a decrement of excessive GF), versus sham dual-tDCS +1%, effect size: 0.3), that may reflect a finer control of the grip forces, a better processing of the somatosensory feedback, or a more accurate planning (Hermsdorfer et al. 2008). By comparison, inhibitory rTMS over the contralesional M1 led to a larger improvement in the GF/LF ratio (-30 %, versus sham rTMS -4%; effect size: 0.8, n=12) in patients with acute subcortical stroke (Dafotakis et al. 2008a). Compared to sham, real dual-tDCS also improved the rapidity of execution of the precision grip task by diminishing the PLD (-18%, versus sham dual-tDCS +6%, effect size: 0.4) compared to baseline; inhibitory TBS over the contralesional M1 led also to a similar significant diminution of the PLD (-20%, versus sham TBS + 12%, effect size: 0.5, n=16) (Ackerley et al. 2010). Whereas the unload period (Johansson and Westling 1984) has received little attention in previous

studies, dual-tDCS induced a small but statistically significant shortening of the unload period duration (-2%). Finally, the improvement of the time shift relative to baseline during both real and sham dual-tDCS may suggest a nonspecific training effect. The surprising finding was that, by contrast to rTMS studies (Dafotakis et al. 2008a; Nowak et al. 2008; Ackerley et al. 2010) or to other tDCS studies (Fregni et al. 2005; Hummel et al. 2006), the improvements were not observed during or just after dual-DCS but 20 min after the end of the stimulation period. This delayed improvement will be discussed below.

Dexterity is another aspect of fine sensorimotor function that may be severely impaired by stroke (Hermsdorfer et al. 2003; Quaney et al. 2005; Raghavan et al. 2006; Nowak et al. 2007; Calautti et al. 2010). In the current experiment, unlike sham, real dual-tDCS induced a strong and rapid online improvement in digital dexterity of the paretic hand relative to baseline (Tiffin and Asher 1948; Gallus and Mathiowetz 2003; Hummel et al. 2005). Strikingly, the maximal improvement was protracted and observed 20 min after the termination of dual-tDCS. The magnitude of this improvement was +38% (versus +5% after sham, effect size: 0.3). By comparison, classical tDCS in stroke patients led to 10% improvement on the Jebsen Taylor Test (versus 2% deterioration after sham, effect size: 1.6; n=6) (Hummel et al. 2005) and to 6 % improvement on simple reaction time (versus 5% deterioration after sham, effect size: 1.7; n=11) (Hummel et al. 2006). In another study with stroke patients, rTMS improved the PPT score by 33% (versus 5% after sham, effect size: 0.8; n=10) (Mansur et al. 2005).

In the current study, dual-tDCS induced various improvements in fine motor functions of the paretic hand, some improvements were equivalent (PLD) and other weaker (GF/LF ratio and PPT) compared to previous studies using classical tDCS or rTMS. It is worth noting that the differences may arise from the heterogeneity in stroke populations, stimulation paradigms, and outcome measures. In order to demonstrate a superiority of tDCS over rTMS for improving hand motor function in stroke patients or vice-versa, a formal comparison has to be carried out with a randomized trial.

2.4.2. Temporal dynamic of the improvements driven by dual-tDCS

In previous studies with rTMS (Dafotakis et al. 2008a; Nowak et al. 2008) and tDCS (Fregni et al. 2005; Hummel et al. 2005) the improvements of the paretic upper limb functions were measured during or just after non-invasive brain stimulation; the temporal dynamic of these improvements after the end of stimulation has received little attention. Strikingly, in the current experiment, the dexterity of the paretic hand improved continuously over time and culminated 20 min after the end of dual-tDCS, coincidentally with the delayed improvement of precision grip. This may reflect a protracted/delayed effect of dual-tDCS on fine functions of the paretic hand, previously overlooked by less sensitive measures and tests.

Alternatively, dual-tDCS may have strongly improved a training-dependent effect, i.e. motor skill learning, at least for the PPT. If this interpretation is correct, dual-tDCS could become an extremely efficient add-on therapy to boost neurorehabilitation since the amount of practice with the paretic hand, if any, was very small (50 precision grip trials, 12 PPT trials). According to the current results, the application of dual-tDCS during motor practice of complex tasks (precision grip and PPT) might place the motor system of chronic stroke patients in an optimal state for improving training-dependent performances, after a short break to avoid fatigue.

2.4.3. Differential impact of dual-tDCS on precision grip and dexterity

Why did dual-tDCS induce a greater performance improvement on PPT (+38%) than on precision grip parameters (-18% at best)? The precision grip was relatively well recovered in the majority of our chronic stroke patients, and their residual performance level may have been too high to be sensitive to a single 20-minute session of 1-mA dual-tDCS; the delayed improvement may represent a warm-up effect driven by the combination of practice and dual-tDCS. Alternatively, the precisions grip's dynamics may be less sensitive to dual-tDCS-driven performance improvement given the fact that the grasping movements may have been performed thousands of time by the stroke patients in everyday life, while the PPT was more novel and challenging, leaving more room for improvement.

Thus, performing the PPT may lead to a broader and stronger recruitment of the cortical areas devoted to attention, motor planning and control, and feedback processing than precision grip. In turn, this would both increase the natural afferent inputs towards M1 and lead to functional improvement under dual-tDCS. Indeed, one of the hypothesized mechanisms of action of tDCS is a modulation of the neuronal resting membrane potential, which tunes the receptiveness of the target cortical area to ongoing afferent inputs (Paulus 2011). In other words, if dual-tDCS modifies the receptivity of the target areas (M1) to ongoing afferent inputs, then the large improvements in the PPT may be due to an additive modulation through a potential increase of ongoing afferent inputs driven by the more challenging, novel PPT.

2.4.4. Relevance to neurorehabilitation

The improvements in paretic hand performance were not at the expense of non-paretic hand function, since the PPT scores with the non-paretic hand did not deteriorate, but rather improved slightly over time. Even though the precision grip of the non-paretic hand has not been assessed, it would be surprising to observe a deterioration since inhibitory rTMS applied over the contralesional hemisphere did not induce a negative effect (Dafotakis et al. 2008a). Thus, dual-tDCS does not seem to carry a risk of impairing the fine functions of the non-paretic hand, at least in stroke patients with characteristics similar to those involved in the current study.

As suggested by the correlation between the ABILHAND scores and the improvement on the PPT, dual-tDCS had a stronger impact in the more impaired stroke patients. Despite the fact that none of them had a very severe impairment of hand function, the present cohort closely matches the characteristics of stroke patients seen in real life (i.e., multiple vascular lesions, different types of vascular injuries, and old age). These results are thus encouraging in the perspective of implementing dual-tDCS as a new tool in the neurorehabilitation of a broad range of stroke patients, with different lesion locations, natures, and extents.

2.4.5. Limitations of the study

The current experiment has several limitations. First, the full temporal dynamic of dual-tDCS remains to be explored since the last measurements were performed 20 min after the end of stimulation. Second, the current experiment has been undertaken with the idea to modulate abnormal interhemispheric interactions in stroke patients, on the basis of previous studies using unilateral non-invasive brain stimulations (Murase et al. 2004; Fregni et al. 2005; Takeuchi et al. 2005; Nowak et al. 2009; Bolognini et al. 2011) '. Since no measure of cortical excitability with TMS has been performed in the current study, such experiments should be performed to explore the mechanisms of the improvements driven by dual-tDCS. Third, the sample of chronic stroke patients was heterogeneous. We think this is both a weakness and strength, since dual-tDCS seems have a beneficial effect on fine motor function in patients with different forms of stroke and with an extensive lesion burden[4].

2.5. Conclusions

The current study is the first to demonstrate that dual-tDCS applied in chronic stroke patients improves the dynamic of precision grip and the digital dexterity of the paretic hand, two important aspects of fine hand motor function. This improvement is independent of stroke type and does not cause deterioration of motor performance with the non-paretic hand.

Given the fact tDCS may be easily implemented in clinical settings, is able to enhance fine motor function of the paretic hand in stroke patients, is painless, easy and safe to use, tDCS is in the pole position for a successful bench-to-bedside translation. The full temporal dynamic of the improvements induced by dual-tDCS remains to be established to ensure an optimal implementation of dual-tDCS in the neurorehabilitation of the paretic hand in stroke patients.

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[4] *We acknowledge that the stroke patients involved in this experiment had only a slight to moderate impairment (since they were able to perform the grip lift task as an inclusion criterion); so this observation may not apply to patients with more severe impairment.*

CHAPTER 3: Dual-tDCS enhances online motor skill learning and long-term retention in chronic stroke patients.**

***Chapter 3 is a modified version of an article similarly named by S. Lefebvre, , P. Laloux, A. Peeters, P. Desfontaines, J. Jamart, Y.Vandermeeren, published in Frontiers in human neurosciences in 2013 (Lefebvre et al. 2013a).*

Abstract : Background Since motor learning is a key component for stroke recovery, enhancing motor skill learning is a crucial challenge for neurorehabilitation. Transcranial direct current stimulation (tDCS) is a promising approach for improving motor learning. The aim of this trial was to test the hypothesis that dual-tDCS applied bilaterally over the primary motor cortices (M1) improves online motor skill learning with the paretic hand and its long-term retention. **Methods** Eighteen chronic stroke patients participated in a randomised, cross-over, placebo-controlled, double bind trial. During separate sessions, dual-tDCS or sham dual-tDCS was applied over 30 min while stroke patients learned a complex visuomotor skill with the paretic hand: using a computer mouse to move a pointer along a complex circuit as quickly and accurately as possible. A learning index involving the evolution of the speed/accuracy trade-off (SAT) was calculated. Performance of the motor skill was measured at baseline, after intervention and one week later. **Results** After sham dual-tDCS, eight patients showed performance worsening. In contrast, dual-tDCS enhanced the amount and speed of online motor skill learning compared to sham ($p < 0.001$) in all patients; this superiority was maintained throughout the hour following. The SAT was shifted more consistently after dual-tDCS (n=10) than after sham (n=3). More importantly, one week later, online enhancement under dual-tDCS had translated into superior long-term retention (+44%) compared to sham (+4%). The improvement generalised to a new untrained circuit and to digital dexterity.

Conclusion A single session of dual-tDCS, applied while stroke patients trained with the paretic hand significantly enhanced online motor skill learning both quantitatively and qualitatively, leading to successful long-term

retention and generalisation. The combination of motor skill learning and dual-tDCS is promising for improving post-stroke neurorehabilitation.

3.1. Introduction

In the field of stroke neurorehabilitation, motor learning has recently become the focus of a great deal of attention. Motor skill learning is particularly attractive since practice-induced improvement of sensorimotor performance supports development of new aptitudes (skills), which provide the flexibility to adapt to changing conditions. Motor skill learning is defined as a training-induced improvement in motor performance characterised by a shift in the speed/accuracy trade-off (SAT) that persists over time (Reis et al. 2009; Dayan and Cohen 2011; Krakauer and Mazzoni 2011). In other words, motor skill learning requires long-term improvement of both speed and accuracy or improvement of one of these parameters without a simultaneous worsening of the other. Operationally, motor skill learning is demonstrated by improvement over baseline performance during a delayed retention test. Motor skill learning relies on neuroplasticity i.e. this aptitude of the brain to be durably modified by experience and to adapt to changing circumstances (Pascual-Leone et al. 2005). As showed by functional brain imaging (e.g. functional magnetic resonance imaging, fMRI), learning any complex task engages a coordinated motor learning network involving multiple brain areas (Krakauer 2006; Kantak et al. 2010; Dayan and Cohen 2011; Krakauer and Mazzoni 2011; Shmuelof and Krakauer 2011; Kantak et al. 2012; Penhune and Steele 2012). The cerebellum seems necessary for adaptation learning and the primary motor cortex (M1) for learning motor skills (Shmuelof and Krakauer 2011). For learning sequential motor actions, the striatal system is involved in chunking[1] (concatenating successive movements into chunks), the cerebellum acquires internal models optimising performances and contributing to error correction, and M1 stores the learned sequence (Shmuelof and Krakauer 2011; Penhune and Steele 2012). Long-lasting

[1] *Motor chunking does not only involve the striatal system but also other subcortical areas such as the putamen, and corticals areas such as the fronto-parietal network (Wymbs et al. 2012)or Broca's area (Clerget et al. 2012)*

changes in synaptic excitably such as long-term potentiation (LTP) and long-term depression (LTD), protein synthesis and synaptogenesis in the motor cortex are the neural substrates allowing motor learning (Luft et al. 2004; Monfils et al. 2005). Similarly, LTP-like plasticity of M1 seems to be involved in the formation of motor memory in healthy volunteers (Stefan et al. 2006). Ultimately, the genetic background could determine the potential to achieve successful motor skill learning. E.g., the brain-derived neurotrophic factor (BDNF) gene is one of the multiple genes that influence synaptic plasticity and repair (Bath and Lee 2006). The BDNF gene shows a common single nucleotide polymorphism leading to an amino acid substitution at position 66 (BDNF Val66Met) that is associated with altered motor plasticity and fMRI patterns (McHughen et al. 2010), less efficient motor learning and reduced responsiveness to non-invasive brain stimulations (Kleim et al. 2006; Reis et al. 2009; McHughen et al. 2010).

Several lines of evidence support the concept that motor learning is an essential component of motor recovery after stroke. First, recovery of motor function after stroke, whether spontaneous or driven by neurorehabilitation, shares common substrates with motor skill learning. Motor skill learning and functional plasticity leading to post-stroke motor recovery share striking similarities in terms of brain networks, fMRI activations, changes in cortical excitability revealed by transcranial magnetic stimulation (TMS) or underlying molecular and genetic substrates (Pascual-Leone et al. 2005; Kreisel et al. 2007; Dayan and Cohen 2011; Krakauer et al. 2012).

Second, the capacity to achieve at least some forms of motor learning is preserved in most, if not all, stroke patients[1]. For example, use-dependent plasticity, a basic form of motor memory relying on the repetition of a single movement, is conserved in stroke patients (Butefisch et al. 1995; Nelles et al. 2001). Adaptation learning, i.e. the rapid recovery of baseline performance levels under altered experimental conditions such as distorted

[1] *It has to be mentioned that the preservation of the motor learning with the paretic upper limb ability in the stroke patients population could only be explored in the in patients who conserved some residual voluntary mobility in the upper limb.*

visual feedback or force field perturbation of ballistic movement, is generally conserved in patients with hemispheric stroke (Takahashi and Reinkensmeyer 2003). In contrast, patients with damage to the cerebellum or posterior parietal cortex may present specific impairments in adaptation learning (Werner et al. 2010; Palluel-Germain et al. 2011). Motor skill learning appears to be conserved after stroke, as shown by studies using various tasks such as the serial reaction time task, finger sequence tapping, or visuomotor tracking (Carey et al. 2007; Boyd et al. 2010; Bosnell et al. 2011; Dovern et al. 2011; Meehan et al. 2011b). It is worth noting that impairments of specific aspects of motor skill learning may follow injury to the thalamus (Exner et al. 2001), cerebellum (Boyd and Winstein 2004a; Dirnberger et al. 2010; Hatakenaka et al. 2012) or prefrontal cortex (Gomez Beldarrain et al. 1999). Generally, motor skill learning appears to be preserved after stroke, though some aspects may be impaired after damage to specific brain areas.

Third, after a stroke, spontaneous recovery is mediated by a coordinated reorganisation of the undamaged cortical areas, their connections and corticospinal projections, subcortical structures (cerebellum, basal ganglia), and spinal cord circuitry (Byrnes et al. 1999; Johansen-Berg 2007; Xerri 2012). Recovering motor function after stroke might be conceptualised as learning to use the remaining neural resources to improve motor planning, execution, feedback and control. Thus, motor recovery after stroke could be a form of motor skill learning. It is still unclear whether post-stroke motor recovery requires the re-learning of damaged/lost motor engrams or the acquisition of new motor skills and internal models. Nevertheless, motor skill learning is undoubtedly one of the key mechanisms underlying the recovery of motor function after stroke.

This is why improving motor skill learning is a major target for neurorehabilitation. It is therefore not surprising that several neurorehabilitation methods have recently been developed on the premise of enhancing motor skill learning (Boyd and Winstein 2001; Bhatt et al. 2007; Rosser et al. 2008; Abe et al. 2011). Given their capacity to modulate cortical excitability and enhance behavioural performances, non-invasive

brain stimulations such as repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS) are particularly attractive as add-on interventions for enhancing post-stroke recovery (Reis et al. 2008). After stroke, deregulated interhemispheric interactions such as unbalanced interhemispheric inhibition from the contralesional M1 towards the ipsilesional M1 influence residual paretic hand function (Murase et al. 2004). Accordingly, rTMS and tDCS can improve residual motor function of the paretic upper limb, likely by rebalancing abnormal interhemispheric interactions (Nowak et al. 2009), enhancing ipsilesional M1 excitability (Hummel et al. 2005; Kim et al. 2006), reducing contralesional excitability (Takeuchi et al. 2008a; Zimerman et al. 2012) or doing both (Lindenberg et al. 2010; Bolognini et al. 2011).

Moreover, rTMS can enhance motor learning in stroke patients. High-frequency rTMS applied over the ipsilesional M1 of chronic stroke patients while they trained on a finger sequence tapping task with the paretic hand induced online improvement compared to sham rTMS (Kim et al. 2006). Continuous theta burst stimulation (cTBS, a specific form of rTMS) applied over the contralesional M1 or primary somatosensory cortex (S1) before training on a serial targeting task with the paretic hand improved performance and retention on the following day, as well as improvement on a novel task (Meehan et al. 2011a). However, broad use of rTMS in clinical settings is hindered by several factors: i) risk of inducing a seizure especially in patients with a brain lesion (Nowak et al. 2006; Lomarev et al. 2007), ii) relative difficulty of use, iii) uncomfortable sensations, iv) lack of convincing sham rTMS and v) price of rTMS devices. Given its safety (Nitsche and Paulus 2001; Merrill et al. 2005), portability, user-friendly and patient-friendly features, existence of convincing sham stimulations (Gandiga et al. 2006) and lower price, tDCS seems more likely than rTMS to rapidly become a therapeutic adjuvant in neurorehabilitation. In healthy volunteers, anodal tDCS over the contralateral M1 improves the SAT on a visuomotor task involving serial pinch contractions, enhances motor skill learning and long-term retention (Reis et al. 2009). In chronic stroke patients, a recent study showed that cathodal tDCS over the contralesional M1 improved motor skill

learning on a finger sequence tapping task, as well as overnight retention (Zimerman et al. 2012).

To date, evidence supporting motor skill learning improvements has been mostly based on online improvement or very specific tasks restricted to stroke patients with an excellent motor recovery (e.g., able to perform complex finger sequence task). It is only recently that studies have used a modern definition of motor skill learning (i.e. shift of the SAT) and/or investigated long-term retention (Reis et al. 2009; Meehan et al. 2011a). Therefore, before implementing non-invasive brain stimulations as therapeutic adjuvants in stroke neurorehabilitation, it is mandatory to test the impact of non-invasive brain stimulation on long-term retention of motor skills in stroke patients and to develop motor skill learning tasks **i)** involving a SAT, **ii)** requiring the activation of the whole upper limb in complex sequences of movements and/or **iii)** having clearer ecological relevance to daily life activities.

The present study tested the hypothesis that dual-tDCS applied in chronic stroke patients while they learned a new motor skill with the paretic upper limb enhances long-term retention of the motor skill (primary aim). Secondary aims were to test whether dual-tDCS **i)** improves online motor skill learning, **ii)** modifies the quality of motor skill learning by shifting the SAT, and **iii)** allows generalisation of improvement beyond the learned motor skill.

3.2. Patients and Methods

3.2.1 Population

The protocol was approved by the local Ethical Committee (Comité d'éthique médicale, CHU Mont-Godinne, UCL) and was conducted according to the recommendations of the Helsinki declaration. Eighteen patients with a chronic stroke provided written informed consent after reviewing the following inclusion criteria: i) being a chronic (>6 months) stroke patient aged 18-80 years, ii) presenting a chronic motor deficit in the upper limb, iii) having an hemispheric vascular brain lesion demonstrated by

cerebral imaging (Figure 3.1). Exclusion criteria were: being unable to perform the task or to understand instructions, presence of intracranial metal, epilepsy, alcoholism, pregnancy, cognitive impairment or psychiatric disorder. Ten patients had an ischemic cortical stroke, six a subcortical ischemic stroke, and two (#4 and 5) had an intracerebral haemorrhage (Figure 3.1, Table 3.1). Some patients had more than one type of stroke. Residual dexterity was quantified with the baseline Purdue Pegboard Test (PPT, see below), residual manual ability with the ABILHAND scale (Penta et al. 2001) (Table 3.1), and the overall degree of disability with the modified Rankin Scale (mRS) (Bonita and Beaglehole 1988).

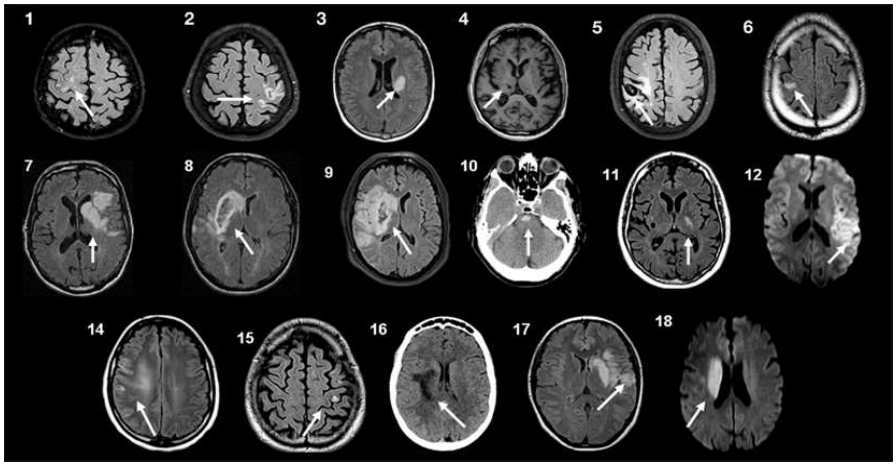

Figure 3.1. Brain imaging Magnetic resonance imaging (MRI) or computed tomography (CT) scans at the level of the stroke for each patient. Patients 10 and 16 had CT scans, patient 4 had a T_1-weighted MRI, patients 12 and 18 had Diffusion-Weighted Imaging (DWI), and all others had FLAIR T_2-weighted MRI. Patients 4 and 5 had an intracerebral haemorrhage. Patients 8 and 9 had a slight secondary haemorrhagic transformation. Patients 1, 15 and 17 had at least one other lesion compatible with a previous, minor stroke. Patient 6 had associated leukoaraiosis and small chronic subcortical infarcts. Patient 4 had small chronic subcortical lacunar infarcts. For patient 13, the MRI scans were not retained in the patient's medical folder, but a detailed neuroradiological report permitted localisation of the lesion (Table 3.1).

Table 3.1 Baseline patient characteristics

	Gender	Age	Time since stroke (years)	Main stroke lesion	Additional vascular lesions	DH	PH	PH PPT (n)	N-PH PPT (n)	PH MaxHF (Kg)	N-PH MaxHF (Kg)	ABILHAND (logits)	mRS
1	F	65	0.5	C	SC-C	R	L	1.3	10.7	16	28	1.5	3
2	M	67	2	C	-	R	R	10.3	12.7	36	45	2.3	2
3	M	64	4	SC	-	R	R	6.3	14.3	38	42	1.5	2
4	M	61	6	SC (H+)	1 SLI	L	L	1	13.7	34	41	0.3	3
5	M	61	4	C (H+)	-	R	L	6.3	11.3	36	51	0.3	2
6	M	56	3	C	LK, SSIs	R	L	7.7	12.3	30	41	3.9	2
7	M	69	4	C	-	R	R	7.3	10.7	53	38	1.5	2
8	M	49	3	C	-	R	L	7	16.3	28	47	1.7	2
9	M	63	1	C	-	R	L	3	13.7	26	35	1.9	2
10	M	65	0.8	SC	-	R	L	13	14.7	49	55	5.5	1
11	M	70	3	SC		R	R	9	11.3	35	47	4.3	1
12	M	55	1	C	-	R	R	9	11.7	55	50	1.9	2
13	F	68	3	SC	-	R	R	10.3	13	22	22	2.4	2
14	F	36	2	C	-	R	L	9.7	13.7	17	19	1.3	2
15	M	79	3	C	SC-C	R	R	2.3	8	28	40	1	3

16	F	61	3	SC	-	R	L	0	15.3	0	29	1.2	3
17	F	35	3	C	SC-C	R	R	17.3	17.3	33	31	4.4	2
18	F	61	1	SC	-	R	L	7	17.3	21	25	1.6	3
		61 ± 9	**2.6 ± 1.5**					**7.1 ± 4.5**	**13.2 ± 2.5**	**31 ± 14**	**38 ± 11**	**2.1 ± 1.5**	**2 ± 1**

Legend of Table 3.1. Baseline patient characteristics[1,2] M, male; F, female; SC, subcortical stroke; C, cortical stroke; H+, intracerebral haemorrhage; R, right; L, left; PPT, Baseline Purdue Pegboard Test score; n, number of pegs inserted in 30 s (mean of three trials); MaxHF, Maximal hand grip force; mRS, modified Rankin Scale; Kg, kilograms. In addition to their main stroke lesion, five stroke patients presented additional vascular lesions: SSIs, small subcortical infarctions; SLI, subcortical lacunar infarctions; LK, leukoaraiosis.

[1] *Patients who had a score of 0 at the PPT (unable to insert a single peg) or the maxHF were unable to perform the tasks despite repeated attempts.*

[2] *Patients # 3, 4, 6, 7, 8, 9, 17 participated in a previous study exploring the impact of dual-tDCs on precision grip and digital dexterity (Chapter 2, (Lefebvre et al. 2013b))*

3.2.2 Study design (Figure 3.2.A)

The study was a randomized, placebo-controlled (sham), double-blind, cross-over trial involving two blocks of two sessions each (Figure 3.2A and Supplementary Figure 3.1). Each block consisted of motor skill learning under dual-tDCS or sham dual-tDCS in the first session (Intervention) and a retention test one week later in a second session (Delayed Recall).The interval between Delayed Recall of session 1 and Intervention of session 2 was at least one week (1.4 ± 0.7 week).

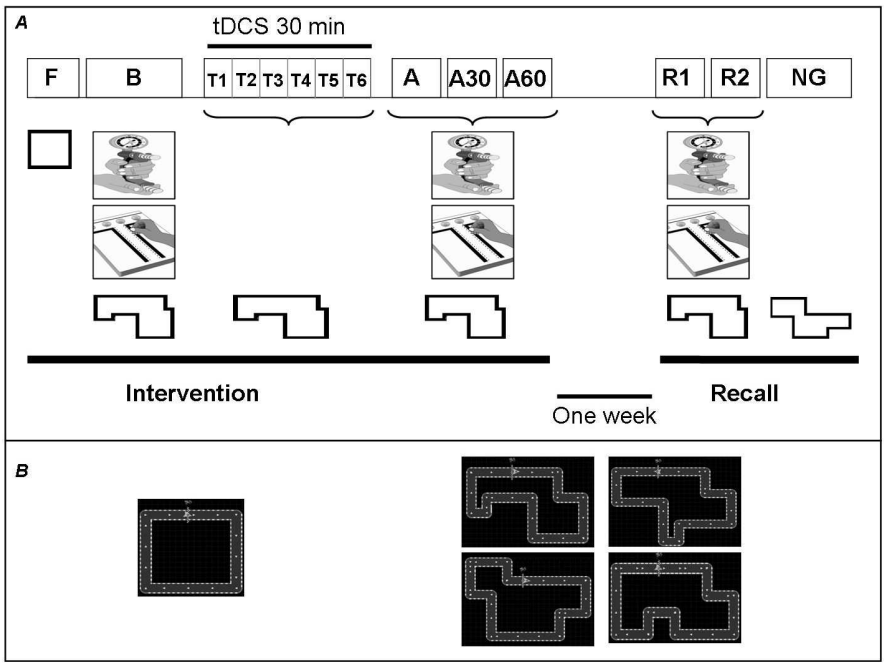

Figure 3.2. Study design Upper Panel (A). Study design: Patients participated in two intervention sessions, each of which was followed by a Delayed Recall session. Intervention sessions comprised 6 periods: Familiarisation (F), Baseline (B), Training (T), and Immediate (A1), 30 min (A2) and 60 min (A3) tests. Delayed Recall sessions comprised 3 periods: Recall 1 (R1), Recall 2 (R2) and New Circuit Game (NG) tests. During F, patients performed an easy circuit over one minute. During B, A1, A2, A3, R1 and R2, patients performed the Purdue pegboard test (PPT), Maximal hand grip force (MaxHF) and the circuit game with the specific circuit assigned to that session. During T, patients performed five blocks of six trials of the circuit game (with the specific circuit assigned to that session). During these Training, patients received 30

min of dual-tDCS or sham, based on their randomisation order. During NG, patients performed a New Circuit Game of the same length and difficulty. Lower Panel (B). Left, square circuit used for Familiarization; Right, the four circuits of identical length and complexity used for motor skill learning and New Circuit Game

The Intervention session comprised the six successive periods. (1) The Familiarisation involved performing one minute of habituation on a simple version of the motor skill learning task (a square circuit) with the paretic hand. (2) The Baseline included **i)** measuring the maximal grip force of each hand with a Jamar dynamometer over three trials to determine mean maximum hand grip force (MaxHF), **ii)** performing the Purdue Pegboard Test (PPT) three times with each hand to determine the mean number of pegs placed (Gallus and Mathiowetz 2003) and **iii)** performing the motor skill learning task (see below) with the paretic hand during two blocks of 30 s, with 30 s of REST between blocks. (3) The Training involved learning the task by performing the motor skill with the paretic hand over 30 min, alternating 30-s blocks of training and REST, while receiving dual-tDCS (Stagg et al. 2011). (4-6) The Early Recall tests were conducted immediately, 30 min and 60 min after completing training and involved measurement of **i)** MaxHF, **ii)** PTT, and **iii)** performance of the motor skill with the paretic hand over 5 min, alternating 30-s blocks of testing and REST.

The Delayed Recall session was performed one week later and comprised three periods: (1) Recall 1 and (2) Recall 2 which were identical to the Early Recall tests; and (3) New Circuit Game which involved performing an alternative version of the motor skill over 5 min, to test for a generalisation effect on a novel, untrained circuit.

3.2.3 Motor skill learning

Stroke patients trained on the circuit game, which induces motor skill learning and retention in healthy volunteers (Lefebvre et al. 2012). Patients were comfortably seated and held a computer mouse on a desk with their paretic hand. A complex circuit was displayed on a computer screen (Figure

3.2.B). The instructions were: Use the computer mouse to move the pointer as fast and accurately as possible over the circuit. Accurately means keeping the cursor within the track. Improvement during training is expected. To motivate the patients, a high score reflecting their error and velocity during the previous block was displayed on the screen during REST periods. Behavioural data (error and velocity) were stored and analysed off-line. Four different circuits of identical length and complexity (i.e. equal number of corners and segments arranged in a different order) were used during the two Intervention sessions and the two New Circuit Game tests (Figure 3.2.B). A pilot experiment in another group of stroke patients (n=7, age: 62 ± 5.5 years, four had a cortical lesion and three a subcortical lesion, all presented chronic upper limb paresis) demonstrated that these circuits were of equal difficulty. The seven chronic stroke patients of the pilot group performed each circuit during 5 min in a random order, after breaks of 5 min. The mean velocity, error and laps number were not statistically different between the four circuits ($p = 0.07$; $p = 0.37$; $p = 0.28$ respectively).

To quantify performance on the motor skill, error and velocity were extracted and combined in a performance index (PI). Error was defined as the surface area between the pointer's trajectory and the midline of the track. Velocity and error were averaged in bins of 3 s, resulting in 10 values for each 30-s Training block. Normalised mean error (Pe = a/subject mean error) and normalized mean velocity (Pv = subject mean velocity/b) were used to compute the PI, which is designed to increase when error diminishes and/or when velocity increases (PI= Pv*Pe). a and b are constant values of error and velocity derived from the pilot group of seven other stroke patients, (see Appendix 3.1). Increase in PI reflects enhanced motor skill performance, defined as an improvement of the SAT.

To quantify evolution of motor skill learning over time, a learning index (LI) was calculated for each block. The evolution of the PI from Baseline was expressed as LI = [(PI - PI baseline)/PI baseline] x 100. An increment of LI over time reflects an improvement in performing the motor skill relative to Baseline (i.e. motor skill learning). The LIs from five consecutive Training blocks were grouped and used for statistical analysis.

As reported previously (Lefebvre et al. 2012), three main patterns of evolution can be observed: 1) no learning (i.e. lack of change or worsening), 2) motor skill learning with a fit pattern (i.e. improvement of the LI limited by an opposite evolution of Pv and Pe), and 3) motor skill learning with a shift pattern (i.e. improvement of the LI due to improvement of both Pe and Pv or to an improvement of one parameter without a concomitant deterioration of the other). Since the shift pattern demonstrates a clear improvement of the SAT , it reflects more efficient motor skill learning than the fit pattern (Lefebvre et al. 2012).

For the New Circuit Game, PIs from five consecutive blocks were grouped and used to compare generalisation of motor performance on an untrained circuit (real dual-tDCS versus sham). Since the New Circuit Game consisted in only five blocks, no LI (reflecting changes) could be computed but only online performance (PI).

3.2.4 Dual-tDCS

An Eldith DC-Stimulator® (NeuroConn, Ilmenau, Germany) delivered dual-tDCS via two soaked (NaCl 0.9%) electrodes (35 cm²). The hot spot eliciting consistent movements in the contralateral hand was localized using a Magstim 200² (Magstim Company, UK) with a figure-of-eight coil to localize left and right primary motor cortices (M1). For patients 15 and 16, the Magstim 200² was not available, and M1 were localized using the international 10/20 EEG system where C3 and C4 correspond to M1. The anode electrode was positioned over the ipsilesional M1 and the cathode electrode over the contralesional M1. For dual-tDCS, stimulation at 1 mA (fade in/out 8 s) was applied over 30 min. For sham dual-tDCS, a short current up-ramp (8 s fade-in) was followed by 30 s of direct current to induce similar scalp sensations, then by 8 s of current fade-out. The Eldith® codes corresponding to tDCS and sham tDCS were selected by an experimenter to establish an inclusion list with a pseudo-randomised, balanced order (see the CONSORT flow diagram in Supplementary Figure 3.1). These codes were used in a double-blind fashion by a second experimenter. None of the patients reported adverse effects with tDCS.

3.2.5 Statistical analysis

The primary outcome measure was the amount of motor skill retention at one week (LI of Recall 1), compared between real and sham dual-tDCS with a paired t-test. For the evolution of the circuit game during Training and up to 60 min after, repeated measures analyses of variance (RMANOVA) were used to explore the effect of Stimulation (dual-tDCS, sham) and Time (Baseline, Training, Immediate, 30 min, 60 min). For post-hoc analyses, paired-sampled t-tests were used to compare each LI value between Stimulation (dual-tDCS, sham). Paired sample t-test were also used to compare mean LI and PI of Recall 2 and the New Circuit Game. For PPT and MaxHF, RMANOVA were performed for the Intervention session; paired-sampled t-tests were used for post-hoc analyses. Paired-sample t-tests were also performed between Baseline, Recall 1 and Recall 2.

Correlations analyses were performed to determine whether baseline clinical characteristics (age, mRS and ABILHAND score) predicted the individual percentage of LI improvement at Recall 1 (the primary outcome measure) after dual-tDCS. In order to disclose whether the stroke localisation (cortical / subcortical) influenced the responsiveness to real dual-tDCS, a Student's t-test was calculated to seek for a difference in the LI of Recall 1 between the subgroups (cortical / subcortical).

All statistical tests (both for Recall comparisons and post-hoc analyses of the RMANOVAs) were two-tailed, and corrected for multiple comparisons (Bonferroni) i.e. each observed p-value was multiplied by the number of comparisons performed. A p-value of 0.05 was considered statistically significant. Statistical analyses were performed using SPSS® 15.0 (SPSS Inc., Chicago, IL).

3.3. Results

3.3.1. Primary outcome: Impact of dual-tDCS on long-term motor skill retention

One week after Training (Recall 1), the motor skill learning index (LI) after dual-tDCS (44% ± 25, mean ± SD) was statistically superior to that observed after sham (4% ± 24; $p < 0.001$) (Figure 3.3). A similar effect was

observed at Recall 2 ($p < 0.001$). Moreover, there was a clear performance improvement between Recall 1 and Recall 2 (+13%) one week after dual-tDCS and only a modest improvement after sham (+3%). However, this difference did not reach statistical significance ($p = 0.11$). The order of interventions (real dual-tDCS first or second) did not influence these results ($p = 0.10$: no order effect).

One week after sham dual-tDCS, at Recall 1, seven out of 18 stroke patients exhibited LI degradation (Supplementary Table 3.1). The other eleven patients demonstrated a significant retention of the motor skill (i.e. a positive LI value): six presented a fit pattern and five a shift pattern. In sharp contrast, one week after real dual-tDCS, the 18 stroke patients showed a consistent retention of the LI improvement: seven patients presented a fit pattern and eleven a shift pattern (Supplementary Table 3.1).

3.3.2 Impact of dual-tDCS on online motor skill learning and Early Recalls

Baseline PI values were not statistically different between the nine stroke patients starting with real-dual tDCS and those starting with sham ($p = 0.23$). RMANOVA on the LI during Training and up to 60 min after showed a significant interaction between Time and Stimulation ($p < 0.001$) suggesting that dual-tDCS led to greater online motor skill learning and Early Recalls than sham. RMANOVA also showed a significant effect of Stimulation ($p < 0.001$), suggesting that dual-tDCS induced a greater online motor skill learning since the third block of Training ($p = 0,002$) (Figure 3.3) and a significant effect of Time ($p < 0.001$), suggesting that stroke patients generally improved regardless of intervention.

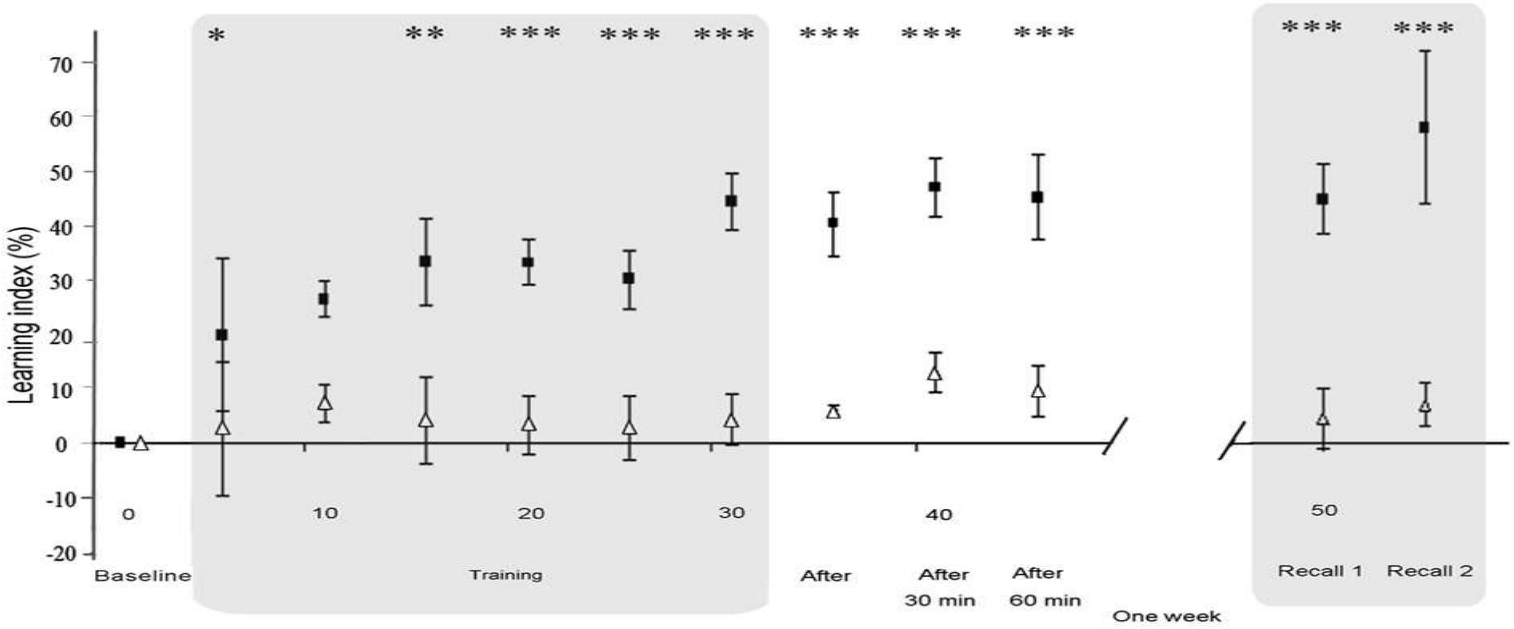

Figure 3.3: Differential evolution of motor skill learning under sham and dual-tDCS Evolution of the Learning Index (LI), expressed as a % change from Baseline during the Intervention session (Baseline, Training, Immediate (After), 30 min and 60 min) and Delayed Recall session (Recall 1 and Recall 2). LI is plotted as the mean ± SD of five consecutive blocks of the circuit game. LI was significantly improved under dual-tDCS compared to sham from the 3rd block of Training until the end of testing. Numbers on the X-axis refer to blocks of the circuit game. White triangles, sham; black squares, dual-tDCS. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$ (all p values corrected for multiples comparisons (Bonferroni)).

Post-hoc analyses demonstrated that dual-tDCS led to a significantly greater and more rapid improvement than sham (Figure 3.3). No order effect was found between the two arms of the crossover design ($p = 0.19$).

Under sham dual-tDCS, eight out of 18 stroke patients exhibited LI degradation during the Training period. The remaining ten patients improved, as shown by the evolution of their velocity and error: seven of them presented a fit pattern and three a shift pattern (Supplementary Table 3.1). In sharp contrast, the 18 stroke patients showed consistent LI improvement after dual-tDCS (i.e. they all achieved motor skill learning). Eight patients presented a fit pattern and ten a shift pattern.

To better depict the trade-off between error and velocity, patients' respective changes from Baseline under dual-tDCS and sham were extracted from the end of the Training period (last five blocks of Training) and from Recall 1 and were displayed as scatter plots in Figure 3.4 (see also Supplementary Table 3.1). The ellipses (computed to contain 90% of the values; Matlab, the Mathworks® R2009b) graphically emphasise that both error and velocity improved more after dual-tDCS than after sham, consistent with an enhanced shift of the SAT.

3.3.3 Generalisation to a New Circuit Game

After completing Recall 1 and 2, the stroke patients performed a New Circuit Game during 5 min on another circuit of identical length and difficulty (see Figure 3.2), to test for a generalisation effect on a novel, untrained circuit.

The PIs from the five consecutive blocks were grouped to compare generalisation of motor performance on a new, untrained circuit between real dual-tDCS and sham. The PI was significantly greater after real dual-tDCS (1.55 ± 1.01) than after sham (1.38 ± 0.87; $p = 0.045$).

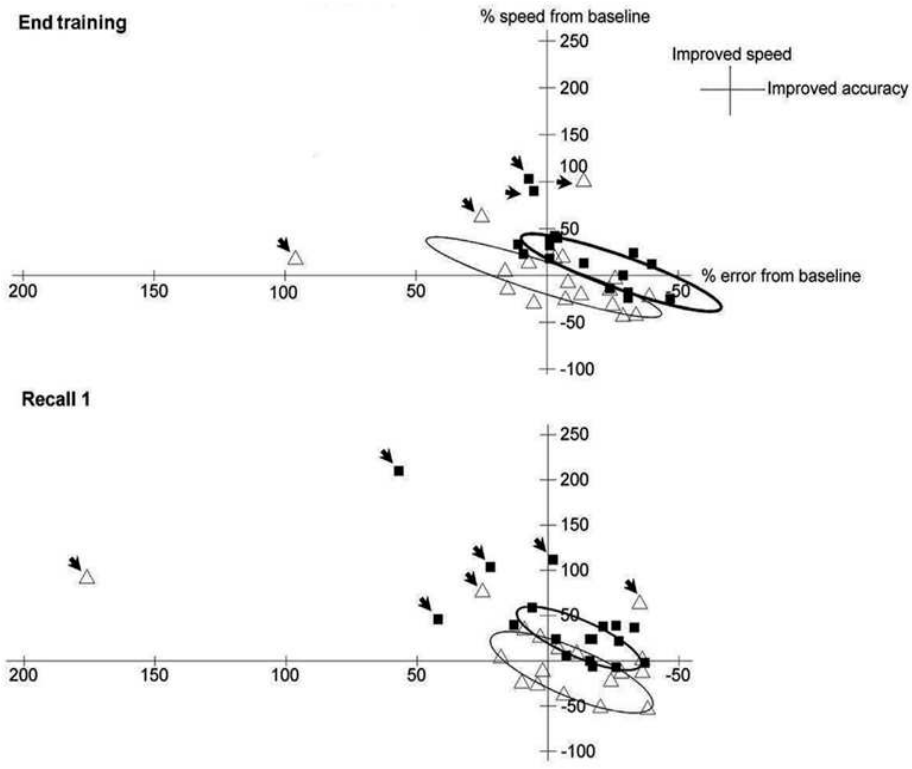

Figure 3.4. Trade-off between error and velocity under sham and dual-tDCS Matlab® (The MathWorks) was used to generate the scatter plots and ellipses. Scatter plot of the trade-off between error (X-axis) and velocity (Y-axis), expressed as % change from Baseline, for each patient after dual-tDCS (black squares) or sham (white triangles) at the end of Training (upper panel) and at Recall 1 (lower panel). The ellipses [contain 90% of the values, outliers (arrows)] show that both error and velocity improved more after dual-tDCS than sham, demonstrating a shift of the SAT, as expected in efficient motor skill learning. Moreover, whereas the ellipse for sham is roughly centred over the equilibrium point, the ellipse for dual-tDCS is clearly shifted from this point, in line with a shift of the SAT.

3.3.4 Generalisation to dexterity and force in the paretic hand

Baseline PPT scores were not statistically different between the nine stroke patients starting with real-dual tDCS and the nine other patients starting with sham ($p = 0.34$). The PPT score of the paretic hand improved over time after dual-tDCS (e.g., +1.4 pegs inserted in 30 s, +19% at 60 min, Table 3.2), but not after sham (+0 pegs in 30 s, 0%). RMANOVA showed a

significant Time x Stimulation interaction ($p = 0.001$), suggesting that dual-tDCS had an impact on the evolution of the PTT score across the Intervention session. Post-hoc analyses demonstrated that at Baseline, there was no significant difference between dual-tDCS and sham ($p = 0.1$) whereas there was a statistically significant difference between dual-tDCS and sham ($p = 0.009$) at the last Early Recall (60 min).

One week after dual-tDCS, PPT scores remained significantly improved at Recall 1 (+0.8 pegs in 30 s, +13%, $p = 0.021$) and Recall 2 (+1.2 pegs in 30 s, +17%, $p < 0.001$) compared to Baseline but not after sham (Recall 1, $p > 0.9$; Recall 2, $p > 0.9$) (Table 3.2). No order effect was found between the two arms ($p = 0.37$).

Baseline MaxHF were not statistically different between the nine stroke patients starting with real-dual tDCS and those starting with sham ($p = 0.73$). For the paretic MaxHF, RMANOVA demonstrated a significant effect of Time ($p = 0.008$), but not of Stimulation ($p = 0.1$) , nor of the Time x Stimulation interaction ($p = 0.2$). Furthermore, post-hoc analyses demonstrated that there were no statistical difference on MaxHF between sham and dual-tDCS at any time (Baseline, Early Recalls, Recall 1 and Recall 2). This suggests a slight progressive improvement of MaxHF, independent of the type of stimulation (Table 3.2).

3.3.5 Dexterity and force in the non-paretic hand

For the PPT score of the non-paretic hand, RMANOVA demonstrated a significant effect of Time ($p = 0.009$), but neither of Stimulation ($p = 0.9$) nor of the Time x Stimulation interaction ($p = 0.3$), suggesting a slight progressive improvement, independent of the type of stimulation. No significant change in PPT score was observed one week later (Table 3.2). The non-paretic MaxHF hand remained unchanged during Intervention and at Delayed Recall (Table 3.2).

3.3.6 Correlation analyses

Correlations analyses were performed to determine whether baseline clinical characteristics predicted the individual percentage of LI

retention at Recall 1 after real dual-tDCS. The patient's age, mRS and ABILHAND scores did not correlate significantly with LI improvement at Recall 1 ($p = 0.68$, $p = 0.55$, and $p = 0.95$, respectively), nor did the localisation of the stroke (cortical versus subcortical: $p = 0.43$ (Student's t-test)).

3.3.7 Carry-over effect

In order to determine whether a carry-over effect could be observed with the cross-over design, statistical comparisons were performed for each parameter (PI, PPT and MaxHF) between the Baselines of the first and second Intervention separately for the two patient's groups. For the nine patients who received sham dual-tDCS during the first Intervention, there was no statistically significant difference between the Baseline performances of Intervention 1 (sham) and 2 (dual-tDCS) (PPT: $p = 0.53$, MaxHF: $p = 0.18$, and PI: $p = 0.06$). For the nine patients who started with real dual-tDCS as first Intervention, there was no statistically significant difference between the Baseline performances of Intervention 1(dual-tDCS) and 2 (sham) for the PPT ($p = 0.07$) and MaxHF ($p = 0.61$). However, Baseline PI of Intervention 2 (i.e. one week after the Delayed Recall session that followed real dual-tDCS) was significantly superior when compared to the Baseline PI of Intervention 1 ($p < 0.001$).

3.4. Discussion

The main findings of this experiment were that 30 min of dual-tDCS applied bilaterally over M1 in chronic stroke patients while they learned a complex motor skill with the paretic hand **i)** rapidly and significantly enhanced online motor skill learning, **ii)** enhanced the *quality* of motor skill learning by increasing the shift of the SAT, **iii)** successfully translated online improvement into long-term retention of the motor skill and **iv)** induced a generalisation of performance improvement to untrained tasks, such as digital dexterity and an alternative version of the motor skill.

Table 3.2. Changes in PPT and MaxHF for paretic and non-paretic hands

Task	**Sham dual-tDCS**					
	Baseline	**After**	**After 30 min**	**After 60 min**	**Recall 1**	**Recall 2**
PPT (PH)	8,0 ± 3.6	7,8 ± 3.5	8,1 ± 3.9	8,0 ± 4.1	8,2 ± 4.0	8.2 ± 4.3
PPT (n-PH)	13,5 ± 2.7	13,2 ± 3.3	13,6 ± 2.9	13,7 ± 3.0	13,5 ± 3.2	13,4 ± 3.3
MaxHF (PH)	32,6 ± 12.1	31,4 ± 12.1	32,7 ± 13.2	34,2 ± 13.2	34,2 ± 13.2	33,4 ± 13.9
MaxHF (n-PH)	37,6 ± 10.6	37,4 ± 11.3	38,1 ± 12.1	39,1 ± 12.1	39,0 ± 11.9	38,9 ± 11.9

Task	**Real dual-tDCS**					
	Baseline	**After**	**After 30 min**	**After 60 min**	**Recall 1**	**Recall 2**
PPT (PH)	7,4 ± 4.4	8,2 ± 4.4	8,8 ± 4.1	8,8 ± 4.3	8,4 ± 4.0	8,7 ± 3.9
PPT (n-PH)	13,1 ± 2.7	13,2 ± 2.8	14,0 ± 2.7	13,9 ±2.8	13,2 ± 3.1	13,2 ± 2.9
MaxHF (PH)	34,0 ± 12.4	33,5 ± 13.2	34,3 ± 13.3	33,8 ± 13.0	34,1 ± 12.0	33,7 ± 11.7
MaxHF (n-PH)	38,5 ± 11.6	38,0 ± 11.6	39,0 ± 12.0	38,7 ± 11.9	39,8 ± 10.3	40,0 ± 11.7

Legend of Table 3.2 Group data (mean ± SD) for the Purdue pegboard test (PPT) and Maximal hand grip force (MaxHF) for each evaluation period (Baseline, Immediate, 30 min, 60 min, Recall 1 and Recall 2) for paretic hand (PH) and non-paretic hand (n-PH) receiving dual-tDCS and sham.

3.4.1 Dual-tDCS enhances the amount and quality of online motor skill learning in stroke patients

Under sham dual-tDCS, ten of 18 chronic stroke patients (56%) achieved online motor skill learning, while eight (44%) showed online deterioration of performance. Of those who deteriorated, four steadily worsened from the beginning and four started to improve but worsened later. This online deterioration of performance could be due to fatigue, lack of attention, or inability to engage the motor skill learning network, possibly secondary to an imbalance of interhemispheric excitability (see below). Only one patient (#12) presented positive retention of the motor skill with a fit pattern after one hour and one week, suggesting that he achieved offline motor skill learning despite online worsening.

In sharp contrast, under dual-tDCS, all the stroke patients (100%) achieved online motor skill learning, showing a dramatic improvement by the end of the Intervention session (dual-tDCS: +44%, sham: +4%). Moreover, dual-tDCS considerably improved the efficiency of online motor skill learning, since the LI was already statistically increased under dual-tDCS compared to sham after a few blocks of training (Figure 3.3). This translated into superior retention of the motor skill 60 min and one week after dual-tDCS.

The current experimental paradigm was designed to involve a SAT for evaluating motor skill learning. This permitted to demonstrate that, in addition to enhancing the amount and speed of online motor skill learning as well as long-term retention, dual-tDCS improved the *quality* of motor skill learning. Among the ten stroke patients (56%) who achieved online motor skill learning under sham dual-tDCS, only three (17%) adopted the most efficient pattern of motor skill learning, the shift pattern; the remaining seven patients (39%) followed a fit pattern. In sharp contrast, all of the stroke patients achieved online motor skill learning under dual-tDCS, ten (56%) with a shift pattern and eight (44%) with a fit pattern. Thus, compared to sham, dual-tDCS also improved motor skill learning *quality* through

increased shift of the SAT (i.e. more efficient motor skill learning), which translated into a successful long-term retention.

Could the observed online and 1-hour post-intervention improvements simply result from modified excitability driven by dual-tDCS? In healthy volunteers, 13 min of anodal tDCS can induce changes in corticomotor excitably lasting up to 90 min (Nitsche and Paulus 2001), and 20 min of cathodal tDCS changes up to 180 min (Di Lazzaro et al. 2012). In stroke patients, 20 min of tDCS can modulate corticomotor excitability up to 60 min after intervention (Zimerman et al. 2012) and enhance motor performance up to 30 min (Hummel et al. 2005). In this type of experiment, it is by definition not possible to disentangle online motor skill learning enhancement and early re-tests from simple motor performance improvement driven by tDCS. However, in the current experiment, the facts that **i)** dual-tDCS translated into successful long-term retention of the motor skill and **ii)** there was no offline improvement unambiguously demonstrate that dual-tDCS indeed enhances online motor skill learning. A similar reasoning applies for the observed improvements of digital dexterity, although there was a limited exposition to the PPT compared to motor skill learning. Since the PPT remained improved one week after dual-tDCS but not after sham, this long-lasting enhancement cannot be attributed to tDCS online effects or after-effects.

3.4.2 Successful long-term retention in stroke patients following online enhancement of motor skill learning under dual-tDCS

This is the first demonstration that enhancement of online motor performance induced by dual-tDCS in stroke patients translated into successful long-term retention of the motor skill learned with the paretic hand, which is a fundamental step forward for neurorehabilitation. Indeed, if a single session of dual-tDCS enhances online motor skill learning and leads to long-term retention of a complex motor skill, then repeated sessions of dual-tDCS combined with neurorehabilitation are likely to improve durably motor recovery. Interestingly, whereas the retention test was not designed to asses continued motor skill learning, the improvement observed between

Recall 1 and Recall 2 was larger after dual-tDCS (+13%) than after sham (+3%). This suggests that Recall of the motor skill acquired under dual-tDCS could reactivate mechanisms that place the brain in an optimal state for subsequent motor skill learning. However, this hypothesis remains to be tested formally. Similarly, whether repeated sessions of motor skill learning coupled with dual-tDCS in stroke patients leads to cumulative improvement, as previously observed in healthy volunteers (Reis et al. 2009), should also be tested.

There was no correlation between the baseline clinical characteristics of the stroke patients (age, mRS, ABILHAND, whether the lesion was cortical or subcortical) and the amount of long-term retention. Since this cohort of 18 patients with mixed stroke subtypes matches well real-life stroke patients, the present results are encouraging for a broad implementation of dual-tDCS as add-on therapy for neurorehabilitation in a large range of stroke patients.

3.4.3 Generalisation of performance improvements and carry-over effect

After the two Recall trials, patients practiced a New Circuit Game during 5 min, with one of the alternative circuit versions of identical length and complexity. Performance on this new, untrained circuit was significantly better after real-dual tDCS than after sham. Thus, dual-tDCS induced a greater generalisation of motor performance improvement than sham, which persisted one week after intervention. Alternatively, Recalling the motor skill learned under dual-tDCS one week before may have placed the motor system in an optimal state for inducing a generalisation of performance improvement to an untrained version of the motor skill.

Dual-tDCS had no impact on paretic hand's grip force. Conversely, digital dexterity of the paretic hand was greatly enhanced immediately after Training under dual-tDCS and kept improving up to +19% 60 min after. This suggests that combination of motor skill learning and dual-tDCS may lead to

generalised improvement on complex or demanding tasks such as the PPT. Alternatively, the protracted improvement of the paretic hand's digital dexterity may reflect a subtle and delayed after-effect of tDCS (Lefebvre et al. 2013b). Interestingly, although there was a slight drop in digital dexterity one week later, the enhancement remained significant at Recall 1 and showed a trend towards improvement from Recall 1 (+13%) to Recall 2 (+17%) after dual-tDCS but not after sham. Thus, dual-tDCS not only enhanced online motor skill learning and long-term retention with the paretic hand but also led to generalisation of motor performance improvements with an alternative version of the motor skill, as well as a lasting improvement of digital dexterity.

Furthermore, in the nine stroke patients who received dual-tDCS as the first Intervention, statistical analysis disclosed a carry-over effect on the Baseline performance (PI) one week after the first Recall session, i.e. two weeks after dual-tDCS. Thus, their second Baseline PI (sham session) was better than the first Baseline PI (dual-tDCS session); this fits with a carry-over effect and further reinforces the idea of lasting generalisation of performance improvement. Could this carry-over effect have induced a ceiling effect during the second (sham) intervention or have skewed the main outcome measure, i.e. the comparison of the LI from Recall 1 between dual-tDCS and sham? Our contention is that the answer is negative for the following reasons. First, the statistical analyses on the primary outcome measure (the LI) did not demonstrate an order effect. Second, as can be appreciated from Supplementary Figure 3.2 and Appendix 3.2, during the second Intervention, the mean LI improved up to 9% 30 min after sham, which demonstrates that motor skill learning did not reach a ceiling. When comparing the two panels of Supplementary Figure 3.2, it appears clearly that dual-tDCS improved online motor skill learning and long-term retention in both groups, and that motor skill learning also took place during and after sham, although to a much lesser extent. Third, although a lasting carry-over effect and/or generalisation were induced by dual-tDCS during the first Intervention, the stroke patients learned an *alternative* version of the circuit during the second Intervention (sham). It would thus be extremely surprising

to observe a ceiling effect on this *new* motor skill, i.e. a *new* circuit of identical length and complexity but arranged in a different order. Anyway, if dual-tDCS indeed induced a ceiling effect, this would be an incredible achievement for neurorehabilitation: a single session of dual-tDCS applied during motor skill learning would have brought these chronic stroke patients to the maximum of their motor potential! This seems very unlikely.

3.4.4 Possible mechanisms underlying improvements induced by dual-tDCS

Several mechanisms may explain the dual-tDCS-induced improvement in motor skill learning and retention in stroke patients. First, dual-tDCS may have re-balanced deregulated interhemispheric interactions. According to the hypothesis of interhemispheric rivalry, deregulated interhemispheric interactions influence residual paretic hand function in stroke patients (Murase et al. 2004). Both rTMS and tDCS have the potential to rebalance these abnormal interhemispheric interactions and to improve motor performances (Hummel et al. 2005; Nowak et al. 2009). In healthy volunteers, dual-tDCS increases excitability on the anodal side associated with a decrease of excitability on the cathodal side (Mordillo-Mateos et al. 2012). A recent study has demonstrated that dual-tDCS could also rebalance abnormal interhemispheric interaction by inducing both a reduction of cortical excitability in the contralesional hemisphere, and an augmentation of excitability in the ipsilesional hemisphere associated with a significant reduction of the transcallosal inhibition from the contra to the ipsilesional hemisphere (Bolognini et al. 2011). Moreover, anodal tDCS applied over the ipsilesional M1 and cathodal tDCS applied over the contralesional M1 both improved fMRI activation during paretic hand movements proportionally to motor function improvement, including enhanced activation of the ipsilesional M1 and connected premotor areas (Stagg et al. 2012). Beyond inducing changes in neuronal membrane excitability, tDCS can modulate glutamatergic and γ-aminobutyric acid (GABA) systems in the motor cortex (Nitsche and Paulus 2000; Nitsche et al. 2005; Stagg et al. 2009b). These modulations are particularly relevant for

motor skill learning and post-stroke neurorehabilitation, as therapeutic manipulation of the glutamatergic and GABAergic systems in the perilesional motor cortex enhances functional recovery in mice after stroke (Clarkson et al. 2010; Clarkson et al. 2011). The beneficial effects driven by modulation of the glutamatergic system may ultimately lead to the release of brain derived neurotrophic factor (BDNF) (Clarkson et al. 2011). It is worth noting that tDCS increases BDNF secretion and synaptic plasticity in animals (Fritsch et al. 2010), which could be a key mechanism underlying tDCS-induced improvements (Reis et al. 2009; Krakauer et al. 2012). Such tDCS-induced modulations of cortical excitability and molecular environment may also underlie the generalisation observed in the current study.

Second, whereas M1 was targeted bilaterally, the current flow delivered by tDCS is not very focal and likely spread to the adjacent dorsal premotor cortex (PMd) and S1. From a neurophysiological point of view, exquisitely focal stimulation is undoubtedly superior. However, from a neurorehabilitation point of view, concomitant stimulation of adjacent cortical areas by tDCS may well be beneficial, since both PMd and S1 are involved in motor skill learning and post-stroke recovery and can be modulated by non-invasive brain stimulation to enhance motor skill learning (Meehan et al. 2011a; Kantak et al. 2012). Furthermore, the effects of tDCS are not circumscribed to the cortical area under the electrodes, but also involve distant interconnected areas (Stagg et al. 2012).

Third, there may be additional mechanisms specific to dual-tDCS due to **i)** a synergic effect of dual stimulation over M1 bilaterally, **ii)** a different current flow direction compared to classical tDCS approach and **iii)** additional effects on interconnected areas. The hypothetical existence of mechanisms specific to dual-tDCS remains to be tested.

Finally, we speculate that dual-tDCS may have non-specifically modulated attention, fatigue or motivation in stroke patients, though this was not formally tested. Despite allowing REST between blocks of Training, eight stroke patients showed online worsening under sham, suggesting a fatigue

effect. Since none of the stroke patients worsened under dual-tDCS, dual-tDCS could have blocked global fatigue or muscle fatigue. Such an anti-fatigue effect has been suggested after tDCS (Cogiamanian et al. 2007) or cTBS (Ackerley et al. 2010). Alternatively, progressive worsening or lack of online improvement may reflect a progressive drift in attention, concentration and/or motivation. Since recent experiments re-emphasised the importance of reward and motivation in motor skill learning (Abe et al. 2011), a high score was displayed during the REST periods to lessen motivation or attention drifts. Moreover, although tDCS can maintain attention and motivation (Kang et al. 2009), electrodes in the current experiment were not placed over the prefrontal cortex, which mediates these functions.

3.4.5 Limitations

This experiment has some limitations. First, most of the stroke patients presented mild to moderate disability (mRS 1-3), although some of them had poor residual digital dexterity and/or bimanual ability (see Baseline PPT and ABILHAND scores, Table 3.1). Nevertheless, all showed training-induced improvement. Thus whether dual-tDCS improves motor skill learning in severely impaired stroke patients remains to be tested. In fact, dual-tDCS or cathodal tDCS of the contralesional M1 might be deleterious in the most severely impaired stroke patients. Indeed, worsening of paretic upper limb performance has been observed in severely impaired stroke patients after inhibitory stimulation of the contralesional hemisphere with cathodal tDCS (Bradnam et al. 2011) or cTBS (Lotze et al. 2006b; Hummel et al. 2008; Ackerley et al. 2010). However, in the current study, dual-tDCS did not cause worsening on any tasks performed with the paretic upper limb, in line with previous reports (Lindenberg et al. 2010; Zimerman et al. 2012). Moreover, dual-tDCS did not worsen the non-paretic hand function. To confirm the potential therapeutic impact of dual-tDCS, a multicentre randomised control trial with a larger range of impairments is required. Furthermore, although the circuit game involved the whole upper limb, no

clinical scales like the Fugl-Meyer or Wolf Motor Function tests were used, and these should also be tested in a large trial[1].

A second limitation was that the stroke patients were relatively heterogeneous in terms of stroke localisation (cortical, subcortical, brainstem), mechanisms (large cortical or subcortical strokes, lacunar infarcts, haemorrhage) and presence of additional vascular injuries. However, this relative heterogeneity may also be strength, as this cohort matches real life[2] stroke patients, emphasising the potentially wide therapeutic impact of dual-tDCS.

Third, this study was based on the premise that dual-tDCS could re-balance abnormal interhemispheric interactions that are known to impair post-stroke recovery (Murase et al. 2004; Bolognini et al. 2011; Stagg et al. 2012). However, no measures of cortical excitability with TMS or changes in activation pattern with fMRI were performed.

Finally, potential confounding effects due to attention, fatigue, concentration and blinding were not evaluated. However, we feel it unlikely that such nonspecific effects might by themselves explain the intensity and nature of the improvements observed in motor skill learning, given the localisation of the electrodes and the demonstration that blinding with sham tDCS is efficient (Gandiga et al. 2006).

3.5. Conclusion

This is the first demonstration that a single-session of dual-tDCS applied during training dramatically enhanced online motor skill learning with the paretic hand in stroke patients, which translated into successful long-

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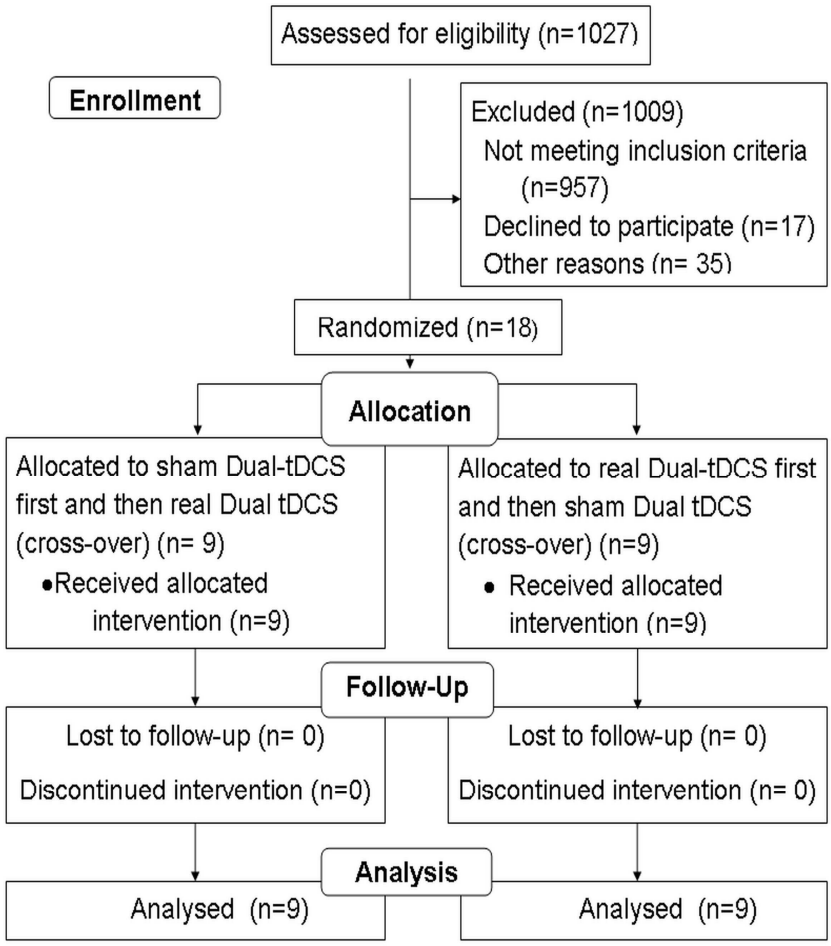
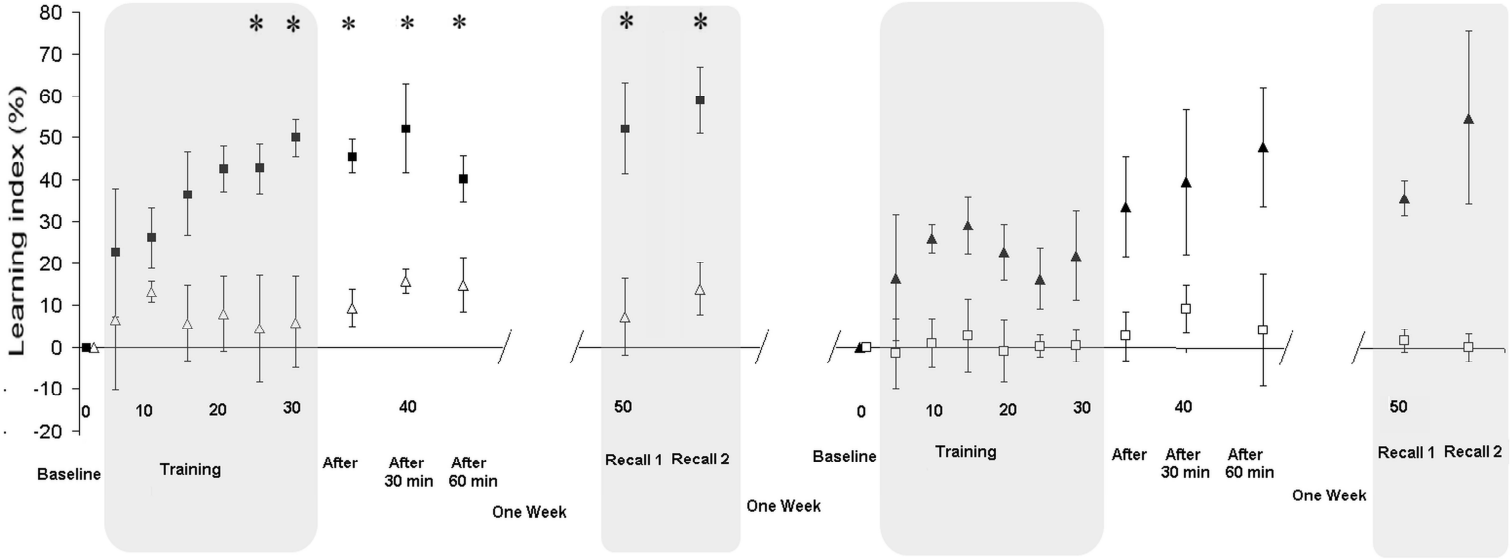
[1] As suggested, in this study the transfer/generalisation of performance improvement to daily life activities have not been tested. This is why additional studies are needed to explore the effect of dual-tDCS and motor skill learning on long term hand motor function with Fugl-Meyer test or with HABILAND scale

[2] This relative heterogeneity of this stroke patients cohort matches real-life population of hemiparetic patients.

term retention of the motor skill. Remarkably, dual-tDCS enhanced the *quality* of motor skill learning by increasing the shift of the SAT. Furthermore, the combination of motor skill learning and dual-tDCS led to a generalisation of motor performance improvements in the paretic hand, without concomitant worsening in the non-paretic hand. Finally, recalling the motor skill learned under dual-tDCS after one week may place the motor system in an optimal state for subsequent improvements in motor skill learning or in complex tasks. This generalisation is particularly attractive in the context of neurorehabilitation. Further studies with TMS and fMRI should explore the mechanisms underlying these improvements mediated by dual-tDCS, as well as whether repeated training sessions combined with dual-tDCS lead to cumulative improvement.



Supplementary Figure 3.1. CONSORT Flow Diagram Method of randomisation: An experimenter established an inclusion list, attributing the Eldith® codes for dual-tDCS and sham to the first and second session in pseudo-randomised, balanced order for each successive patient. A second experimenter applied these codes blindly, and patients were not aware of their treatment, such that dual-tDCS was delivered in a double-blind fashion. Since it was the first time that this paradigm was used in stroke patients to induce long-term retention after motor skill learning, no power analysis was performed.



Supplementary Figure 3.2. Split groups analysis. The left panel di**s**plays the first session (Intervention and Recall) separately for the nine stroke patients randomly allocated to real dual-tDCS as the first intervention and for the nine others allocated to sham; i.e. as if the study had assumed a parallel group design. The LI was significantly improved under dual-tDCS compared to sham from the fifth block of Training (see Appendix 2). The right panel displays the second session (Intervention and Recall) for the two groups of stroke patients (sham or dual-tDCS as the second intervention). Evolution of the Learning Index (LI) is expressed as a % change from Baseline during the Intervention session (Baseline, Training, Immediate (After), 30 min and 60 min) and Delayed Recall session (Recall 1 and Recall 2). LI is plotted as the mean ± SD of five consecutive blocks of the circuit game. White triangles: sham; black squares: dual-tDCS. * $p < 0.05$, (all p values corrected for multiples comparisons (Bonferroni)). Numbers on the X-axis refer to blocks of the circuit game.

Appendix 3.1

Since the motor skill has been designed to involve a SAT, both velocity and error need to be combined into a single parameter that reflects performance improvement (or worsening) over time, the Learning index (LI) (Lefebvre et al. 2012). The computation of the LI requires combining velocity and error values for each subject at each block. By definition, the LI has been designed to increase with improvements in both speed and error, or when one parameter improves and the other does not deteriorate.

The circuit game was displayed and analysed with a dedicated software which expresses error and velocity with arbitrary grid unit (u) as u/s for velocity and u² for error. One arbitrary grid unit (u) displayed on the computer screen is equivalent to a distance of 0.3 cm travelled through in straight line by the computer mouse. Typically, error values ranges from 0.01 to 1.2 u² and velocity values ranges from 1 to 14 u/s. Thus, in order to compute an index **i)** combining these two parameters expressed with different units and **ii)** not skewed by the much greater size of velocity compared to error, the error and velocity were normalised as Pe and Pv. Thus, Pe and Pv were obtained for each block of each stroke patient with the following formula:

Pe = a / patient error

Pv = patient speed / b

This normalisation has been performed using a constant term for velocity (b) and for error (a) obtained on a group of 7 other stroke patients while they trained to perform the circuit game in a pilot experiment.

a = 0.371 u²

b = 4.858 u/s

Next, Pe and Pv were combined to calculate the performance index (PI) for each block of each patient:

PI = Pe x Pv

Finally, the evolution of performance as a percentage from Baseline (i.e. the Learning Index, LI) was calculated with the formula:

LI = [(PI - PI baseline)/PI baseline] x 100

Since the LI (i.e. the main outcome measure) is calculated with the formula LI = [(PI - PI baseline)/PI baseline] x 100, individual normalization of performance improvement over time was embedded in the calculation.

Since a and b are constant values, the normalization of error and velocity could have been calculated with any arbitrary value. Nevertheless, we decided to derive a and b constants from real data obtained in seven pilot stroke patients, having thus a straightforward behavioural significance.

Finally, as depicted in Figure 3.2, the familiarization was performed on a very simple circuit (a square) with a much lower level of complexity, which cannot be compared with the more complex circuits used for motor skill learning. The goal of the familiarization was to allow the stroke patients to be acquainted with the task, to try the computer mouse, to warm up a little bit i.e. to be familiarized with the setup and the task. Therefore, we did not put emphasis on error and speed during familiarization.

Appendix 3.2: Additional analyses of the data from the first session only (parallel-group design).

We performed additional analyses with the data from the first session (Intervention and Recall) only, i.e. as if the study had assumed a parallel group design, with nine stroke patients randomly allocated to real dual-tDCS and the nine others to sham (see Supplementary Figure 3.2).

Baseline characteristics were identical for age ($p = 0.9$), lesion localisation (cortical/subcortical, $p = 0.62$), mRS ($p = 0.1$), ABILHAND ($p = 0.6$), PPT ($p = 0.37$), MaxHF ($p = 0.73$), and Baseline PI ($p = 0.22$).

At Recall 1, the LI was statistically superior one week after real dual-tDCS (52 % ± 25) than after sham (7 % ± 30; $p = 0.006$), as well as for Recall 2 (p

= 0.006), demonstrating that the long-term retention of the learned motor skill was superior after real dual-tDCS than after sham.

The RMANOVA on the LI during Training and up to 60 min after showed a significant Time x Stimulation interaction ($p = 0.017$) suggesting that dual-tDCS led to greater online motor skill learning and superior Early Recalls than sham. RMANOVA also showed a significant effect of Stimulation ($p = 0.026$), suggesting that dual-tDCS induced a greater online motor skill learning since the fifth block of Training ($p = 0.046$) and a significant effect of Time ($p < 0.001$). Post-hoc analyses demonstrated that dual-tDCS led to a significantly greater and more rapid improvement than sham.

Both cross-over and parallel group design have advantages and drawbacks. We believe that using the stroke patients as their own controls in this cross-over study (as done in previous studies (Kim et al. 2006; Zimerman et al. 2012)) is not problematic since the stroke patients trained on *different* circuits (of identical length and complexity), i.e. learned a *different* skill at each session. Since statistical analyses did not demonstrate an order effect (see Result section), the cross-over design allowed us to further characterise the positive effect of dual-tDCS on online motor skill learning and long-term retention, especially by demonstrating an improved shift of the SAT after dual-tDCS in the same stroke patients.

Supplementary Table 3.1 : Behavioural results of motor skill learning

	1			2			3			4			5		
	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)
1	-23	-39	21	-10	-34	35	-11	-30	27	-26	-33	4	-13	-36	29
2	-16	-24	19	-23	34	-36	-26	-9	-1	-8	-5	5	-12	2	-1
3	13	7	3	-22	-24	4	-5	-11	7	5	-6	11	13	-4	17
4	-32	-25	13	-41	-42	27	13	-17	44	11	-18	34	-14	-28	28
5	-43	-34	6	-36	-29	1	-29	-26	13	-22	-26	18	-23	-24	11
6	4	16	-7	-10	-7	-1	-34	4	-32	-36	-19	-17	-38	-6	-30
7	17	96	-14	6	135	-50	22	122	-35	36	147	-45	91	176	-23
8	-21	-13	-8	-20	-15	-1	-25	-22	5	-29	-20	-6	-25	10	-34
9	-44	-29	-17	-51	-36	-6	-15	-12	-7	-48	-31	-19	-54	-38	-16
10	-4	-26	25	19	-23	44	31	-26	60	2	-26	47	1	-36	52
11	19	-6	15	15	-3	10	16	-10	18	1	-10	5	8	-11	14
12	-8	-8	-4	-1	-18	17	4	-9	9	36	6	15	34	9	30
13	62	25	41	78	21	54	104	15	78	74	51	18	76	25	48
14	100	-14	32	15	-44	61	40	-44	53	89	-2	42	63	-35	54
15	-30	5	-27	-33	1	-30	-27	8	-27	-15	11	-12	-27	4	-23
16	-15	15	-22	-2	10	-7	-11	0	-4	22	30	-3	3	18	-10
17	18	-3	23	14	1	16	28	6	19	27	12	10	26	3	20
18	-26	-7	-21	-36	-1	-34	-39	-12	-31	-54	-26	-30	-52	-20	-43
Mean	-2	-4	4	-8	-4	6	2	-4	11	4	2	4	3	1	7
SD	36	31	20	30	41	31	35	35	33	39	42	24	42	48	31

	1			2			3			4			5		
B	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)	V (%)	E (%)	LI (%)
1	12	-40	102	10	-34	137	22	-27	77	33	-20	77	37	-33	119
2	40	-4	49	23	3	-26	32	-15	55	8	10	5	24	-3	30
3	103	7	94	97	13	37	97	18	67	101	26	62	104	22	68
4	-25	-47	65	-28	-39	20	-31	-33	25	9	-42	96	-2	-37	68
5	13	-14	17	44	-20	64	17	-8	9	39	-23	47	39	-26	58
6	39	-1	46	34	9	19	121	15	98	63	-1	67	112	-2	138
7	23	9	10	-11	3	-1	83	9	54	86	60	2	46	42	2
8	-14	-24	31	-17	-27	9	-23	-33	31	-2	-18	44	-7	-26	45
9	42	-3	51	54	2	26	63	3	76	49	-3	50	40	13	43
10	24	-33	81	25	-27	31	29	-16	55	36	-15	62	38	-21	70
11	90	5	78	103	-12	121	68	9	63	73	13	48	210	57	101
12	18	-1	26	22	3	8	14	14	1	8	-2	12	6	-7	22
13	18	-1	17	18	-2	15	16	-6	24	15	-13	37	24	-17	56
14	0	-29	47	-1	-28	-10	27	-19	60	-2	-30	39	22	-27	75
15	33	11	30	33	18	-2	35	12	27	63	10	63	59	6	54
16	-18	-31	15	-10	-28	37	-17	-36	31	7	-20	32	-6	-17	16
17	32	-1	14	20	-2	42	29	-14	31	24	-16	34	24	-16	34
18	-24	-31	19	-27	-23	13	-29	-30	13	4	-9	14	1	-16	26
Mean	23	-13	44	22	-11	30	31	-9	44	34	-5	44	43	-6	57
SD	35	19	29	37	18	42	43	19	27	32	23	25	53	26	36

CHAPTER 4: Brain activations underlying different patterns of performance improvement during early motor skill learning.**

***Chapter 4 is a modified version of a published article similarly named by Stéphanie Lefebvre, Laurence Dricot, Wojciech Gradkowski, Patrice Laloux, Yves Vandermeeren (2012) Neuroimage.(Lefebvre et al. 2012)*

Abstract: **Background / Introduction:** Motor learning plays a central role in daily life and in neurorehabilitation. Several forms of motor learning have been described, among which motor skill learning, i.e. reaching a superior level of performance (a skill) through a shift of the speed/accuracy trade-off (SAT). During the first stage of learning a visuomotor skill, we observed differential patterns of evolution of the SAT in normal subjects. Half of the subjects rapidly achieved successful motor skill learning with an early shift of the SAT leading to a superior level of performance (shift pattern) . The others subjects attained only minimal global improvement due to a converse evolution of speed and accuracy (i.e. a respect of the SAT: fit pattern). Functional magnetic resonance imaging (fMRI) was used to explore the neural substrates underlying these differential patterns during the first stage of motor skill learning in normal subjects. **Methods:** Twenty right-handed normal subjects performed an implicit visuomotor learning task with their non-dominant hand. The task (circuit game) consisted in learning to navigate a pointer along a circuit as quickly and accurately as possible using a fMRI-compatible mouse. Velocity, accuracy, and performance indexes were used to characterise the motor learning pattern (shift / fit) and to perform fMRI correlation analysis in order to find the neural substrate associated with the shift and fit patterns during early motor skill learning. **Results:** Nine subjects showed a fit pattern (fitters) and eleven a shift pattern (shifters)**.** fMRI analyses at whole group level (ANOVA) and at sub-group level demonstrated that the supplementary motor area (SMA) was more activated in shifters than in the fitters groups and that the BOLD activation within the SMA correlated significantly with the online shift of the SAT in the shifters group. **Conclusion:** Despite identical instructions and

experimental conditions, during the first stage of motor skill learning normal subjects spontaneously adopted different patterns that can be differentiated based on distinct fMRI activation patterns. In this implicit visuomotor task, the SMA proper was the key area underlying the achievement of early successful motor skill learning, i.e. online shift of the speed/accuracy trade-off (SAT).

4.1. Background / Introduction:

Motor learning is a generic term encompassing several low and high level processes that co-exist and form a continuum (Krakauer and Mazzoni 2011). These motor learning processes are active during the entire lifespan, from learning to walk to learning how to use a computer or playing tennis. The ultimate purpose of motor learning may be to allow flexible behavioural adjustments while interacting with a changing environment. With regard to a change in motor performance, several forms of motor learning can be distinguished such as use-dependent plasticity, adaptation learning and motor skill learning (Krakauer and Mazzoni 2011). Among these forms of motor learning, motor skill learning is particularly fascinating since it allows the apparently limitless diversification of the motor repertoire by the acquisition of new skills through training. Motor skill learning is defined as an improvement in sensorimotor performance gained through training that involves a shift in the SAT leading to a superior level of performance, i.e. the acquisition of new capabilities or skills (Dayan and Cohen 2011; Krakauer and Mazzoni 2011).

Functional brain imaging studies consistently showed changes in activation in a distributed network of areas involved in motor learning, overlapping with the motor execution and control networks. Several cortical areas such as the primary motor cortex (M1), the supplementary motor area (SMA), the premotor cortex (PM), the dorsolateral prefrontal cortex (DLPFC), and subcortical structures such as the cerebellum and basal ganglia are involved in motor skill learning (Ghilardi et al. 2000; Halsband and Lange 2006; Debas et al. 2010). Other studies suggest a key role for the cerebellum in adaptation (Imamizu et al. 2000; Imamizu et al. 2003; Kawato

et al. 2003). Recent observations using functional connectivity demonstrate a particular involvement of the SMA and prefrontal cortex in learning complex motor skills (Taubert et al. 2011).

During a pilot study involving 18 normal subjects who trained to improve their performance at playing with a circuit game involving a SAT, we noticed that despite identical instructions and experimental conditions normal subjects unconsciously developed different behavioural patterns over the first 15 min of motor skill learning. Three subjects presented a degradation of their performance (no learning). Eight subjects developed rapidly a superior ability involving an online shift in the SAT, i.e. canonical motor skill learning (shift pattern). In the seven other subjects, there was only minimal shift in the SAT since the improvement of one operant characteristic (e.g. speed) was counterbalanced by a concomitant deterioration of the other one (e.g. accuracy), leading to less successful or delayed motor skill learning (fit pattern).

The purpose of this study is to explore with functional magnetic resonance imaging (fMRI) whether different neural substrates underlie the development or the lack of an online SAT shift in normal subjects during the first minutes of learning an implicit visuomotor skill (circuit game).

4.2. Material and Methods

4.2.1 Subjects

The experimental protocol was approved by the local Ethical Committee (Comité d'éthique médicale, CHU Mont-Godinne) and the study has been conducted according to the recommendations of the Helsinki declaration. The normal subjects provided written informed consent, after reviewing the inclusion criteria i) being a healthy volunteer aged 18-80 years, ii) being right-handed, and exclusion criteria i) having a pacemaker or other piece of metal in the body, ii) being pregnant, iii) having suffered from stroke or any brain damage, iv) being unable to perform the task or to understand the instruction. 18 subjects participated in a behavioural pilot study and 25 other subjects in the fMRI study. In the fMRI study, five subjects were excluded from further analysis for the following reasons: technical failure for

one subject, another subject failed to improve any behavioural parameters, two presented deterioration of performance, and in the last subject T1 3D MRI shown the presence of numerous asymptomatic white matter lesions compatible with long-standing leukoaraiosis

4.2.2 Paradigms

4.2.2.1 Behavioural pilot study

During 30 min, 18 subjects trained on a motor learning task (circuit game) with their non-dominant left hand, alternating blocks of learning (30 s) and REST (30 s). The circuit game consisted in moving the pointer with a computer mouse along a circuit under visual control (Figure 4.1 part 3). Subjects were instructed to perform the task as quickly and accurately as possible, accurately meaning keeping the pointer within the track of the circuit. They were informed that the goal of the session was to improve incrementally upon performance. This study was performed to explore the temporal dynamic of the first stage of learning this motor skill in normal subjects.

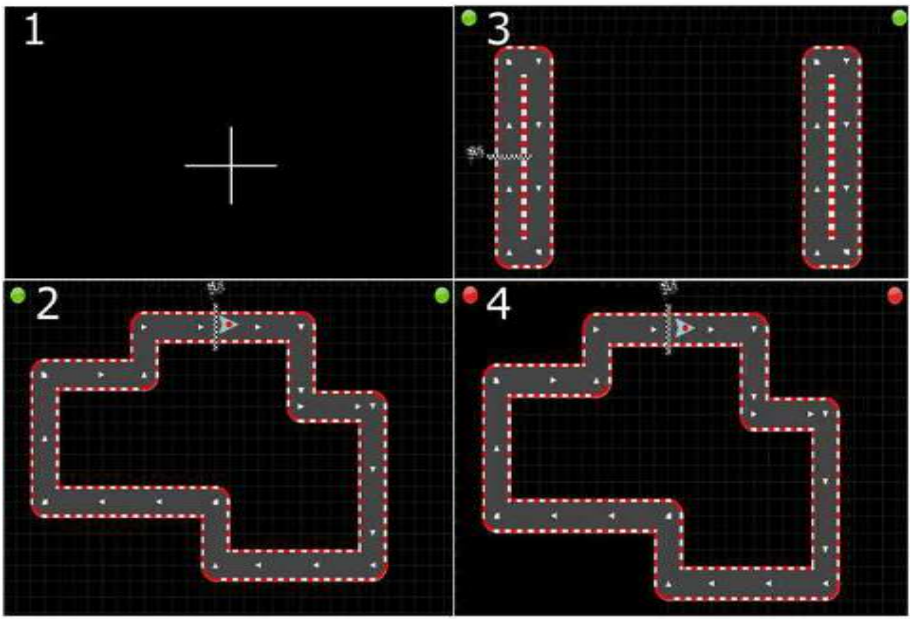


Figure 4.1: fMRI conditions 1: REST: fixation cross, 2: LEARNING: the subjects had to navigate the cursor as quickly and accurately as possible, 3: EASY: the

subjects had to move the cursor between the two targets at comfortable speed (50% trials with vertical movements, 50% with horizontal movements), 4: REPLAY: the subjects had to follow the cursor displacement with their eyes while watching a videoclip of their last LEARNING block, keeping the hands relaxed.

4.2.2.2 fMRI study

The subjects performed three consecutive learning runs of 8 min with a MR-compatible mouse. Each run encompassed 3 conditions and REST (fixation cross): LEARNING (circuit learning), EASY (easy motor task) and REPLAY (Figure 4.1). LEARNING required to perform the circuit game as described previously (4.2.2.1) with exactly the same instructions. EASY required moving the cursor back and forth between two bases, either in horizontal or vertical direction, with the following instructions Move the cursor between the two targets at a comfortable speed, small overshoots and undershoots are allowed. EASY was designed to isolate the activation related to lower aspects of movement execution under visual control.

During REPLAY, a videoclip of the last LEARNING was played; the instruction being Follow carefully with your eyes the cursor displacements, while keeping your hand as relaxed as possible on the MR-compatible mouse. The REPLAY was designed to isolate the activation related to visual and oculomotor activity. Each condition was presented four times during each run, 84 volumes (252 s) of each of the three conditions and REST were analysed. Before these learning runs, the subjects performed an habituation run (40 activation volumes/ 40 REST volumes), which consisted in navigating the cursor on a simple square, in order to familiarize the subject with the MR environment, the concept of the task, and the manipulation of the MR-compatible mouse (this was discarded from further analysis). Visual feedback was projected on a screen; a mirror was placed on the head coil.

4.2.3 Behavioural analysis

For quantifying performance improvements and motor skill learning, the error, velocity and normalized jerk were analysed. Error was defined as the surface area generated by the difference between the real trajectory and

the ideal trajectory in the midline of the track. Velocity was the first derivative of the position. Normalized jerk (NJ) was computed with the formula $NJ = \sqrt{1/2 * \int_{Tstart}^{Tend} jerk^2(t)dt * duration^5 / length^2}$ (Contreras-Vidal and Buch 2003b; Caimmi et al. 2008) where the jerk is the third derivative of the position. The NJ reflects the smoothness of the movements, with the underlying assumption that smoother movements (smaller NJ) are associated with a higher level of skill. Velocity, error and NJ, were averaged in mean error, velocity and NJ using 3 s window (corresponding to the TR) for each block of LEARNING.

Using the mean velocity and error, four indexes were computed to model subjects' behaviour (Table 4.1).

Table 4.1: **Definition of the indexes used in the LEARNING condition**

Index	**Formula**	
Pe	constant error*/subject error	Error Index
Pv	subject velocity*/constant velocity	Velocity index
PI	Pe*Pv	Performance index
LI	(PI-PI initial)/PI initial *100	Learning index [percentage of evolution of PI across the learning session regarding to the first block of learning (30 s)]

- *mean error and mean velocity calculated for the 18 subjects of the behavioural study

From the behavioural pilot study, the error and velocity of the 18 subjects measured during the 15 min of actual training were averaged to extract constant error and constant velocity values. For the fMRI study, the error index (Pe) was computed as Pe = constant error /subject mean error. Pe is a normalized index designed to increase when error diminishes. The velocity index (Pv = subject mean velocity/ constant velocity) is a normalized index designed to increase when velocity increases. The Performance Index (PI= Pv*Pe) was calculated every three seconds and averaged for each learning block. Finally, the Learning Index (LI = [(PI –PI initial)/PI initial] *100)

was calculated for each learning blocks as a percentage of the PI relative to the baseline performance during the first block (PI initial). The LI was only used in order to describe the evolution of the PI over time (% of evolution across the learning blocks), i.e. to quantify the online performance dynamic during early motor skill learning.

Based on these indexes, three different behavioural patterns of motor skill learning were defined (Table 4.2). First, an online shift in the SAT (shift pattern), involving a substantial global performance improvement (LI) with improvement in both speed and accuracy (Pv, Pe) or in one of these two parameters without deterioration of the other one, suggesting an rapid an successful improvement in the motor skill during the first minutes of training. The subjects showing this pattern were refereed as shifters. Second, a fit pattern involving a minimal improvement of the LI without a significant shift of the SAT; i.e. the improvement of one of the two parameters is systematically associated with a concomitant deterioration of the other one. The subjects showing this pattern were refereed as fitters; they did not succeed in achieving consistent online performance improvement during early motor skill learning. Third, a degradation of the LI with deterioration of both speed and accuracy (Pv, Pe), or lack of any change (no change in PI, speed or accuracy). The subjects showing this pattern were excluded from analyses.

4.2.4 fMRI analysis

4.2.4.1. Imaging acquisition parameters

Functional MR images of brain activity were collected using a 3T scanner (Siemens Verio, Erlangen, Germany with a 32-channels head coil) with repeated single-shot echo-planar imaging : echo time (TE) = 23 ms, flip angle (FA) = 90°, matrix size = 64x64, field of view (FOV) = 224x224 mm², slice order descending and interleaved, slice thickness = 2 mm (no gap), number of slices=59 (whole brain). Repetition time (TR) was 3000 ms; the whole brain was scanned 160 times per run. A three-dimensional (3D) T1-weighted data set encompassing the whole brain was acquired to provide

detailed anatomy (1 mm^3) thanks to a ADNI sequence (TR = 2250 ms, TE = 2.6 ms, FA =9°, matrix size = 256 × 256, FOV = 256 × 256 mm^2, 192 slices, slice thickness = 1 mm, no gap).

Table 4.2: **Behavioural patterns of motor skill learning[1]:**

	Motor skill learning					No learning	
	Shift pattern			Fit pattern			
Pe	↗	↗	→	↗↗	↘	↘	→
Pv	↗	→	↗	↘	↗↗	↘	→
PI	↗↗	↗↗	↗↗	→	→	↘	→
LI	↗	↗	↗	→	→	↘	→

Pe: performance error, Pv: performance velocity, PI Performance index, LI learning index

4.2.4.2 Data analysis

fMRI data were analysed using BrainVoyager QX (Version 2.3, Brain Innovation, Maastricht, The Netherlands).

4.2.4.2.1 Pre-processing

Pre-processing consisted of a linear trend removal for excluding scanner-related signal, a temporal high-pass filtering applied to remove temporal frequencies lower than three cycles per run, and a correction for small head movements using a rigid body algorithm rotating and translating each functional volume in 3D space. The data were corrected for the difference between the scan times of the different slices and were not smoothed in the spatial domain. In order to compare the localizations of

[1] *It has to be mentioned that the non-learning behaviour could also be due to a degradation of one of the parameters simultaneously to a stagnation of the other parameter.*

activated brain region across participants, all anatomical and functional volumes were spatially normalized (Talairach and Tournoux 1988) and the computed statistical maps were overlaid on the 3D T1-weighted scans. All the coregistrations were performed automatically and then manually corrected. The functional data were analyzed using one multiple regression model (General Linear Model; GLM) consisting of predictors, which corresponded to the particular experimental conditions, and in which the beta weights quantify the potential contribution of the predictors in explaining each voxel time course. The predictor time courses were computed on the basis of a linear model of the relation between neural activity and hemodynamic response, assuming a rectangular neural response convolved with hemodynamic function (Boynton et al. 1996).

4.2.4.2.2 Contrasts of interest and statistical analyses

First, a random effect group analysis was performed with the 20 subjects. In order to find the areas activated in each condition (LEARNING, EASY, and REPLAY), three basic contrasts of interests (compared to REST) were explored: [LEARNING] (contrast weight: [1 0 0]) (areas involved in motor skill learning), [EASY] [0 1 0] (areas involved in lower aspects of movement control and execution), [REPLAY] [0 0 1] (areas involved in visual and oculomotor activity). In addition, the [(LEARNING + EASY) – REPLAY] [1 1 -2] contrast was computed in order to focus on the areas involved in motor learning and control aspects. Follow up contrasts were averaged over the whole cluster BOLD signal. All the contrasts were balanced.

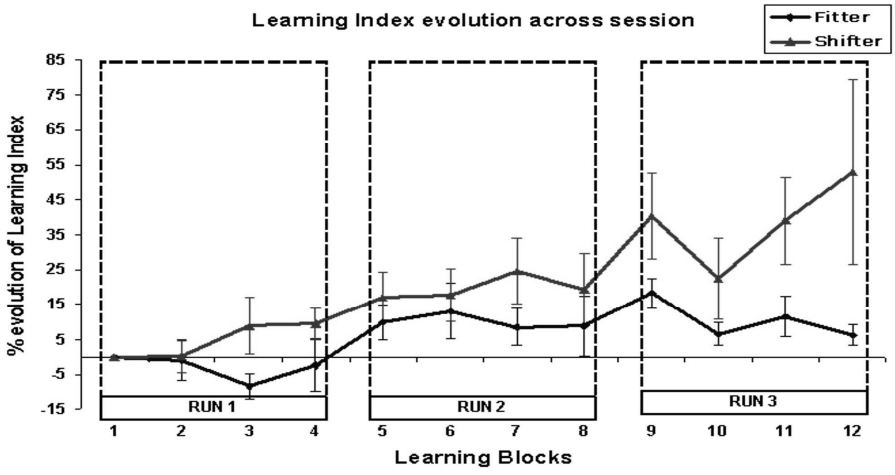
Second, in all the areas found with [LEARNING], correlation analyses were performed between the beta weights of LEARNING and the performance (PI) values, in order to find out the key area(s) explaining performance evolution. For this global correlation, the PI values of the 20 subjects were averaged for each learning block (12 blocks). Then, the correlation was performed between the 12 beta weights LEARNING and the 12 PI values.

Third, to identify the neural substrates underlying the shift pattern versus the fit pattern of motor skill learning, an ANOVA (second level analysis) was computed with one within-subjects factor (conditions) and one between-subjects factor (groups) [1].

4.3. Results

4.3.1 Behavioural results:

The 20 participants were 11 female and 9 male subjects aged from 18 to 62 years (mean ± SD: 33.9±11); all were right handed. Eleven of them displayed a shift pattern (36.7 ± 11.8 years) and 9 a fit pattern (30.7 ± 9.6 years) (Figure 4.2). At the end of the learning session, the performance of the shifters had improved significantly more (LI: 52.8% ± 87.7) than that of the fitters (LI: 6.2% ± 9.9) ($p = 0.002$). Furthermore, the NJ, which reflects the smoothness of the movements, diminished across the learning blocks in the shifters (slope -2799) whereas it increased in the fitters (slope +3202); this differential evolution was statistically significant ($p = 0.048$).



[1] *Fourth, RFX analyses were computed to compare different brain activation patterns between the two subgroups ("shifter"/"fitter" healthy individuals) with [LEARNING-REPLAY] and [LEARNING-(REPLAY+EASY)]. Finally, external Pearson correlation analyses were performed between the beta weights and the PI values evolution in the different ROIs obtained in each subgroup.*

Figure 4.2: Learning Index (LI) evolution across learning blocks: The 12 LI values correspond to the LI during each learning block; triangle: shifters group, diamonds: fitters group. Mean ± SEM.

4.3.2 fMRI results: [1]

4.3.2.1 Whole group analysis

At q(FDR)<0.05 (t_{19}=3.71; $p_{UNCORRECTED}$< 0.0014), the random effect analysis revealed 13 clusters for LEARNING, 14 clusters for EASY and 10 clusters for REPLAY (Figure 4.3, Supplementary Table 4.1). The clusters observed in LEARNING were the right primary motor cortex (M1, Brodmann Area BA 4), bilateral premotor cortex (PM,BA 6), supplementary motor area (SMA, BA 6), bilateral thalamus, left putamen, left anterior cerebellum, and bilateral oculomotor and visual cortical areas. The clusters found in EASY were the right M1 (BA 4), bilateral PM (BA 6), SMA (BA 6), bilateral thalamus, bilateral putamen, left anterior cerebellum, and bilateral oculomotor and visual cortical areas. As expected, the activation in the oculomotor and visual areas found in LEARNING and EASY was also activated in REPLAY, in addition to the bilateral thalamus and PM (BA 6), right limbic lobe (BA 24), and right prefrontal cortex (BA 9). Correlation analyses performed between the performance indexes (PI) and beta weights of each area activated in LEARNING showed a statistically significant effect exclusively in the SMA (r=0.60, p < 0.0052).

The correlations in the other areas were not statistically significant; there was no significant correlation with the NJ (Supplementary Table 4.2).In order to focus on the areas involved in motor control and learning aspects, the [(LEARNING+EASY)-REPLAY] contrast was computed at q(FDR)<0.05 (t_{19}=5.93, $p_{UNCORRECTED}$<0.00001). This contrast revealed significant activation in four areas: the right M1, right thalamus, left anterior cerebellum, and SMA (Table 4.3).

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[1] *The activation observed in the SMA (BA 6) both in the whole-group analysis and in the subgroup analysis was predominantly localized in the right hemisphere, i.e. contralateral to the training left hand.*

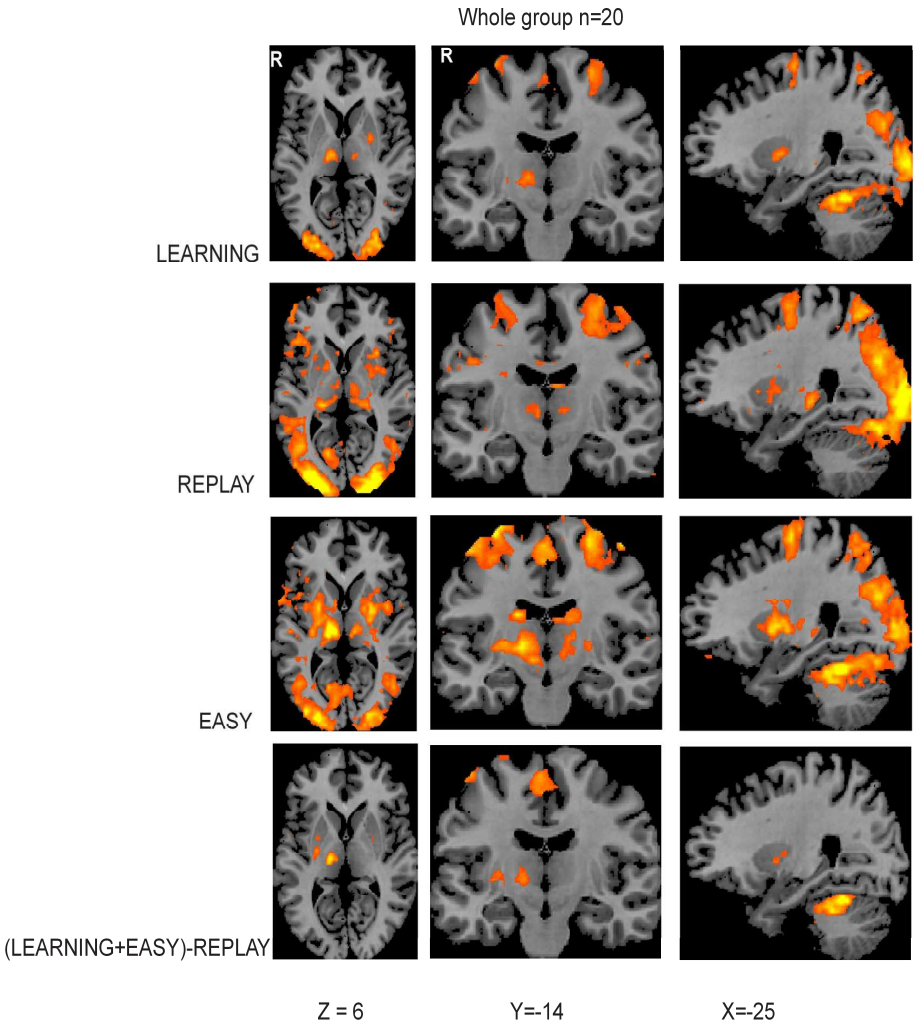


Figure 4.3: Whole group activation: BOLD activation for the 20 subjects contrasting the three basic contrasts ([LEARNING], [REPLAY], [EASY]), and the [LEARNING + EASY) - REPLAY] contrast, (q(FDR) = 0.05 at a t_{19}=3.71, p < 0.0015).

Table 4.3: Whole group comparison:

Brain area / structure	(BA)	mean x	mean y	mean z	mm³
R M1	BA 4	31	-27	55	1 437
R thalamus		13	-19	6	203
L cerebellar hemisphere					
(lobule V-VI)		-16	-49	-18	2 972
SMA proper	BA 6	1	-90	48	117

Contrast [(LEARNING + EASY) - REPLAY]; [q(FDR)<0,05; t_{19}=5.93; $p_{uncorrected}$<0.00001; threshold=100 voxels]. M1: primary motor area, SMA: supplementary motor area, R: Right, L: Left.

A comparison between the shifters and fitters groups was performed in these four regions of interest for the following contrasts: [EASY-REPLAY] [0 1 -1], [LEARNING-REPLAY] [1 0 -1], and [LEARNING-(REPLAY+EASY)] [2 -1 -1] (Figure 4.4). Again, the SMA was the only region where a significant difference between shifters and fitters was found. Moreover, this difference was observed only for the [LEARNING-REPLAY], [LEARNING-(REPLAY+EASY)] and [LEARNING-EASY] [1 -1 0] contrasts (t_{18}=2,47, p < 0.02; t_{18}=2,49 p < 0.02; t_{18}=2.13, p < 0.04 respectively) and not for [EASY-REPLAY] contrast (t_{18}=1.40, p = 0.18). There was no significant difference in the right M1 (BA 4) (respectively for each contrast: t_{18} =1.09, p = 0.29; t_{18}=1.14, p = 0.26; t_{18}=1.09, p = 0.29; t_{18}=0.49, p = 0.63), right thalamus (t_{18}=0.46, p = 0.65; t_{18}=0.99, p = 0.33; t_{18}=1.59, p = 0.13; t_{18}=1.21, p = 0.24), and left anterior cerebellum (t_{18} =1.38, p = 0.18; t_{18}=1.38, p = 0.18; t_{18}=1.04, p = 0.31, t_{18}=0.71, p = 0.48).

4.3.2.2 Whole-brain ANOVA

In order to compare the shifters and fitters groups for each condition, a second level ANOVA with one within-subject factor (conditions) and one between-subjects factor (groups) was computed. The $F_{(4,72)}$ test on the within-subject factor (conditions) showed a significant activation at q(FDR)=0.05, $p_{UNCORRECTED}$ <0.0001. The $F_{(1,18)}$ test on the between-subject factor (groups) showed no significant activation at q(FDR)=0.05, $p_{UNCORRECTED}$ = 0.0002. The $F_{(4,72)}$ test on the interaction between the two factors showed a significant activation in several areas (q(FDR)=0.05, $p_{UNCORRECTED}$ <0.0002). To localize precisely the differences for this interaction, post-hoc analyses with specific contrasts were computed. With the [EASY] and [REPLAY], there were no significant difference between the shifters and fitters groups.

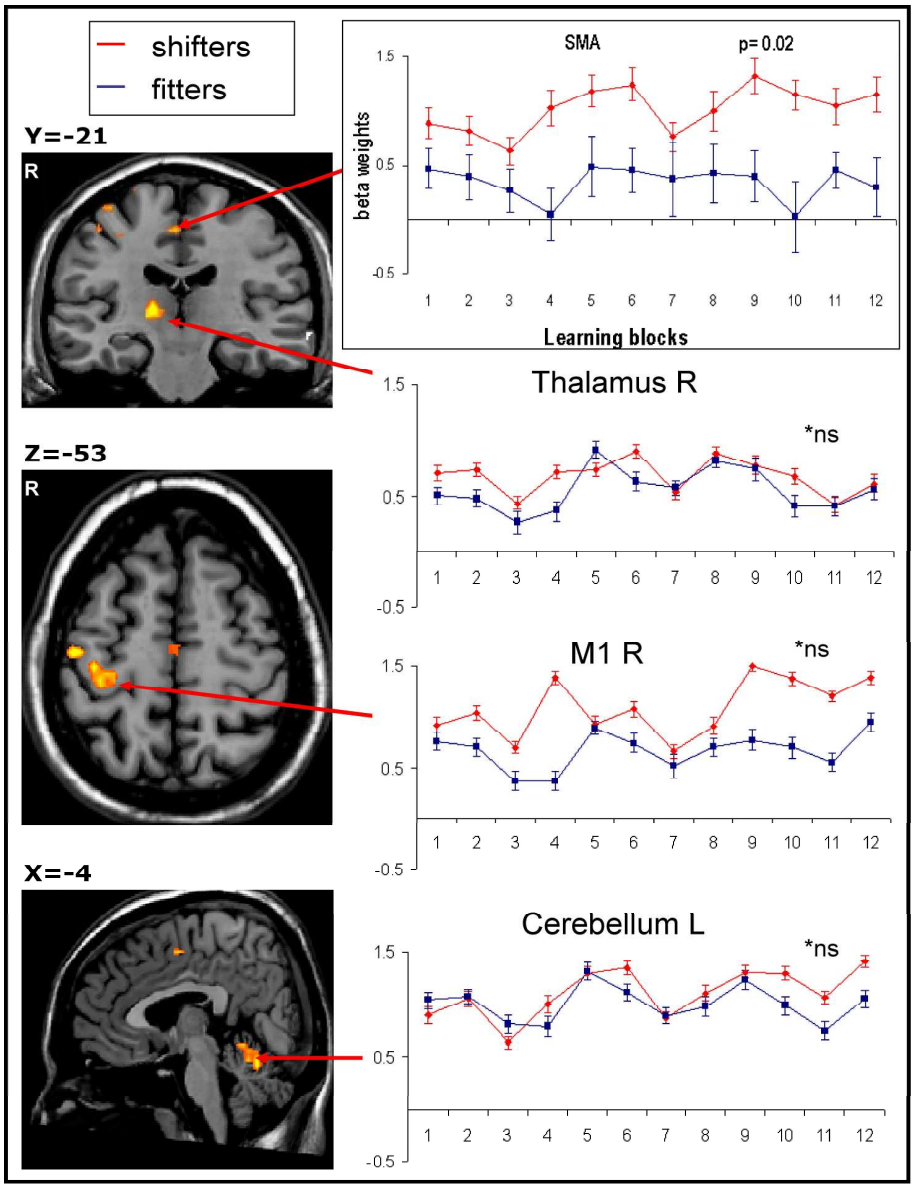


Figure 4.4: Temporal evolution of the beta weights (whole group analysis). [LEARNING - REPLAY] contrast showed four ROIs (Region Of Interest): SMA (BA 6), R thalamus, left M1 (BA 4) and left anterior cerebellar hemisphere (lobule V-VI); (q(FDR)= 0.05; t_{19}=4.49; p < 0.0002; threshold=100 voxels). Each chart plots the evolution of the beta weights (Mean±SEM) across the learning blocks for the shifters (red) and fitters (blue) groups. The difference in beta weights evolution was significantly different between the shifters and fitters groups only in the SMA (p = 0.02).

With [LEARNING], several areas were more activated in the shifters than in the fitters group (q(FDR)=0.05; t_{76}=3.43; p $p_{UNCORRECTED}$ <0.0013): the right hippocampus (BA 48), SMA, left temporal cortex (BA 38), left M1, left posterior cingulate gyrus (BA 31), left putamen, left inferior parietal lobule (BA 40), left premotor cortex (BA 6), left anterior prefrontal cortex (BA 10), left parietal cortex (BA 5), right thalamus (Table 4.4). By contrast, no area was more activated in the fitters than in shifters group. When comparing the shifters and fitters groups, the same regions were found using either [LEARNING-REPLAY] or [LEARNING], and using either [EASY-REPLAY] or [EASY]. This is consistent with the observation that there was no significant difference between the two groups for [REPLAY].

4.3.2.3 Sub-group analyses

In order to detail the activation patterns in the shifters and fitters groups, separate subgroup analyses were computed with the following contrasts focusing on activation related to motor skill learning: [LEARNING-REPLAY] and [LEARNING-(REPLAY+EASY)] (Table 4.5).

Table 4.4: Whole brain ANOVA:

Brain area / structure	**(BA)**	**mean x**	**mean y**	**mean z**	**mm³**
L inferior parietal lobule	BA 40	-45	-34	-43	329
L anterior prefrontal cortex	BA 10	5	-62	5	517
L putamen		-30	-1	9	432
L PMd	BA 6	-42	-9	23	146
R thalamus		23	-13	12	669
R hippocampus	BA 48	33	-34	2	318
L parietal cortex	BA 5	-18	-62	45	601
L temporal cortex	BA 38	-30	3	-28	406
L M1	BA 4	-34	-23	53	144
SMA proper	BA 6	-2	-17	52	124
L posterior cingulate gyrus	BA 31	-25	-27	41	115

Contrast [LEARNING] shifters >fitters; (q(FDR)<0,05; t_{76}=3.62; $p_{uncorrected}$<0,0005; threshold=100 voxels). M1: primary motor area, SMA: supplementary motor area, PMd: lateral dorsal premotor cortex, R: Right, L: Left.

In the shifters group, the random effect analysis (df=10) showed four areas significantly activated with [LEARNING - REPLAY] ((t_{10}= 6.7, qFDR (0.05), $p_{UNCORRECTED}$ <0.0001): the right M1, SMA,, and two areas in the left anterior cerebellar hemisphere (lobule V-VI and VII). The correlation between the PI and the beta weights of these four regions of interest (ROIs) was statistically significant exclusively in the SMA (r=0.63, p < 0.0377) (Supplementary Table 4.2). Individual correlations between the PI and the beta weights were computed, using four ROIs of 50 mm³ (Fox et al. 2009) defined individually for each subject. Individual beta weights were extracted from these ROIs and were correlated with the 12 PI values of each subject. The strongest correlation was observed in the SMA (r=0.33, p < 0.0001); a significant but weaker correlation was found in the left anterior cerebellar hemisphere (lobule V-VI) (r=0.22, p < 0.0113). There was no significant correlation in the right M1 (r=0.1; p = 0.36) and lobule VII of the left cerebellar hemisphere (r=0.1, p = 0.36). With [LEARNING-(REPLAY+EASY)], the shifters conserved significant activation in two areas (t_{10}= 6.7, qFDR (0.05) $p_{UNCORRECTED}$ <0, 0001): the SMA and left cerebellar hemisphere (lobule V-VI).

In the fitters group, the random effect analysis (df=8) showed only one area significantly activated with [LEARNING - REPLAY] (t_8=6.7, qFDR (0.05) $p_{UNCORRECTED}$ <0.0002): the left anterior cerebellar hemisphere (lobule V-VI), where the correlation analysis suggested a non-significant trend (r=0.53, p = 0.13) between the evolution of the PI and the beta weights. Individual correlations were also not significant (r=0.11, p = 0.26). With [LEARNING-(REPLAY+EASY)], the activation in the left cerebellar hemisphere (lobule V-VI) was found exclusively at a t_8 =4.00 ($p_{UNCORRECTED}$ < 0.004).

In order to demonstrate that the performance improvement observed in healthy volunteers training to the circuit game with their left non-dominant hand relies on motor skill learning and implies the retention of the motor skill,

an additional experiment was performed. 18 subjects trained during 12 min on the circuit game with their non-dominant left hand in front of a computer screen, alternating 12 blocks of learning (30 s) and REST (30 s), matching perfectly the fMRI paradigm. A retention test was performed the next day (5 learning blocks of 30 s alternating with REST blocks of 30 s). 13 subjects qualified as shifters (LI: +54% by the end of the learning session), and 5 as fitters (LI: +24%). Compared to baseline, the performance improvement at the retention test on the next day was 44 % for shifters ($p < 0.0005$) and 24% for fitters ($p = 0.0062$) (Supplementary Figure 4.1). Moreover, the slope of the LI evolution across the five retention blocks performed on the next day was steepest for the shifters (7.9) than for the fitters (2.6) ($p = 0.0311$).

Table 4.5: Subgroup analysis

A) Contrast [LEARNING-REPLAY] :

QFDR 0.05 ($t_{10\ AND\ 8}$=6.7; threshold: 40 voxels) M1: primary motor area, SMA: supplementary motor area, R: Right, L: Left

	Brain area / structure	**BA**	**mean x**	**mean y**	**mean z**	**mm**
shifters	R M1	BA 4	34	-29	64	47
	SMA	BA 6	1	-19	49	126
	L cerebellar hemisphere (Lobule V-Vi)		-21	-47	-21	94
	L cerebellar hemisphere (Lobule VII)		-9	-55	-15	57
fitters	L cerebellar hemisphere (Lobule V-Vi)		-20	-45	-20	161

B) Contrast [LEARNING-(REPLAY+ EASY)]

	Brain area / structure	**BA**	**mean x**	**mean y**	**mean z**	**mm³**
shifters	SMA	BA 6	1	-19	49	70
	L cerebellar hemisphere (Lobule V-Vi)		-21	-45	-21	73
fitters*	L cerebellar hemisphere (Lobule V-Vi)		-20	-45	-20	92

*t_8=4.0; $p_{uncorrected}$<0,004

4.4. Discussion

Despite the fact that normal subjects received identical instructions and were studied under identical experimental conditions, they spontaneously exhibited different behavioural patterns of online performance improvement during the early stage of motor skill learning (shift / fit / lack of learning). Comparison of fMRI data between the group of shifters and fitters revealed that differential brain activation underlies these behavioural patterns. The SMA proper was the key area underlying the achievement of online shift of the SAT during early motor skill learning

Which neural processes do reflect the fMRI changes acquired while the volunteers trained to perform the circuit game? On the one hand, these fMRI changes may simply reflect short-term changes related to online motor skill performance improvement. Short-term performances changes and/or transient improvements may be observed during a single training session involving a use-dependent plasticity task such as performing a simple ballistic movement in a specific direction (Classen et al. 1998). This basic form of motor memory mainly involves M1 (Muellbacher et al. 2002) and may serve as a primer for more elaborated forms of motor learning, which mobilize a broader network of cortical areas and subcortical structures(Ghilardi et al. 2000; Baraduc et al. 2004; Floyer-Lea and Matthews 2005). Such an interpretation would by definition imply that the performance improvements gained during training should not be retained in memory as a motor skill, i.e. that no performance gain should remain after a short washout period. The observed fMRI changes would thus simply reflect an online modulation of the network underlying transient performance improvement.

On the other hand, the fMRI changes acquired during the training to perform the circuit game may reflect the early stage of motor skill learning. Previous experiments have demonstrated early neurophysiological modifications underlying the first stages of motor skill learning evaluated over a single session of training (Toni et al. 1998; van Mier et al. 1998; Floyer-Lea and Matthews 2005; Albert et al. 2009; Orban et al. 2011; Tomassini et al. 2011). Indeed, motor skill learning involves at least two

stages developing on different timescales: a fast online learning process leading to large performance improvement over a single training session (as those observed in the current study), and a slower process involving smaller performance gains obtained through repeated training sessions (Dayan and Cohen 2011). Training to perform the circuit game as quickly and accurately as possible not only induces online performance improvements, especially in shifters, but also results in motor skill learning as demonstrated by the retention of the motor skill on the next day in the additional experiment, both for the shifters and the fitters. Thus, the online performance improvements and the related fMRI changes observed during the training blocks reflect the early stage of motor skill learning as demonstrated by the retention of the motor skill on the next day in the additional experiment.

From a behavioural point[1] of view, the shift pattern is superior to the fit pattern since shifting early the equilibrium point of the SAT allows reaching online a superior level of skill. By the end of the learning session, the fit pattern resulted in a smaller improvement of global performance since it did not modify rapidly the equilibrium point of the SAT (Figure 4.2). This was confirmed by the differential evolution of the NJ, which reflects the smoothness of movement, with an improvement in the shifters and deterioration in the fitters. Interestingly, when comparing how much of the skill has been retained on the next day in the additional experiment, the difference was less important between the shifters and the fitters than at the end of the learning session. This could reflect either the maintenance of the skill or the development of slight off-line learning in the fitters group, or some overnight degradation in the shifters group. Nevertheless, both the shifters and the fitters achieved motor skill learning since the test on the next day (from the first block onwards) unambiguously demonstrated retention of the performance improvement gained on the previous day during training. Even if some overnight degradation occurred in the shifters, they retained most of the motor skill they learned the day before. It is also noteworthy that, even

[1] *The rationale for the choice of the non-dominant hand for performing the task is to limit the potential ceiling effect associated with the fact that the task could be too simple with the dominant hand..*

during the very short retention session (five blocks of the circuit game), the shifters maintained again a faster rate of motor skill learning, confirming that their learning strategy was different from that of the fitters.

Neither the shifters nor the fitters were aware of having adopted a particular behavioural strategy, as much as we could determine during informal debriefing. Rather, it seems that the shifters were more efficient from the early phase of motor learning. The stronger fMRI activation in several areas of the shifters compared to the fitters group was present since the first blocks of training. The reason for these differential patterns in normal subjects during the early phase of motor learning is unclear but one could reasonably speculate that, after a longer training period or over several sessions, the fitters would also have achieved a shift pattern.

At the whole-group level (n=20), the activation patterns corresponded to those expected for each condition: predominantly visual and oculomotor activity for REPLAY (Ohlendorf et al. 2010), predominantly motors execution and control areas for EASY[1] (Nair et al. 2003), and motor skill learning network for LEARNING (Grafton et al. 1992; Jenkins et al. 1994; Doyon et al. 2003). The ANOVA demonstrated a lack of significant difference between the shift and fit patterns for EASY (lower-level motor execution and control components) and REPLAY (oculomotor and visual components), strengthening the suggestion that the BOLD activation differences in LEARNING specifically reflect motor skill learning components in addition to simple motor control, oculomotor and visual processing components. In LEARNING, several areas were more activated in the

[1] *As shown in Figure 4.3, the global level of brain activation is higher during the EASY condition than during the LEARNING condition, although the task is easier ("simple motor control"). Two explanations might be proposed. First, since the task is easier during EASY, the subjects make less parasitic movements of the head /arm, which could correlate with the BOLD signal associated to the task. As our functional DATA were motion-corrected, it is possible that the BOLD signal observed in the EASY condition was less corrected (because less contaminated by motion artifacts) and resulted thus in higher levels of brain activation. This could also suggest that some interesting activation was removed from LEARNING by the motion correction of parasitic movements of the arm/head. Second, since the task is easier during EASY, the subjects may perform more movements of the same sort (simple horizontal or vertical movements) in a more constant way (less variability between movements) during this condition, inducing a larger amount of brain activation.*

shifters than in the fitters groups (see Table 4.3). Among these areas, the SMA (BA 6), M1 (BA 4), cingulate gyrus (BA 31), putamen, inferior parietal lobule (BA 40), premotor cortex (BA 6), anterior prefrontal cortex (BA 10), parietal cortex (BA 5), and right thalamus are known to be involved in motor skill learning ((Grafton et al. 1992; Jenkins et al. 1994; Doyon et al. 2003). Interestingly, a differential activation was also observed in the right hippocampus. Traditionally, the hippocampus has been associated with episodic memory formation but not with motor learning, as initially suggested by a lack of deleterious effect of hippocampus lesion on motor skill learning (Spiers et al. 2001; Corkin 2002). However, a recent fMRI study demonstrated that the hippocampus may indeed plays a role in the earlier and later stages of implicit motor sequence learning (Gheysen et al. 2010). Our observations are consistent with such a conclusion, at least when successful learning of a visuomotor skill is involved. Similarly, the temporal cortex (BA 38) has been more classically associated with semantic memory (Clark et al. 2010) but activation has also been observed in the temporal cortex during first stage of bimanual motor skill learning (Ronsse et al. 2011), as well as during motor skill learning with the non-dominant hand (Grafton et al. 2002), suggesting an involvement of the temporal cortex during the first minutes of skill learning; which is consistent with the current observation.

There was no difference between the two groups in the M1 contralateral to the trained hand which is considered as a key area in motor skill learning (Karni et al. 1995; Muellbacher et al. 2002; Kim et al. 2004; Boggio et al. 2006; Tecchio et al. 2010). Recent studies suggested that M1 may be specifically involved in the storage of the low-level executive motor learning components of a task rather than in the higher-order aspects of motor learning (Baraduc et al. 2004; Robertson 2009; Kantak et al. 2010). Therefore, this lack of differential activation in the contralateral M1 suggests that the motor execution, motor control, and lower aspects of motor learning did not significantly differ between the shifters and fitters groups. A stronger activation in the shifters than in the fitters groups in the ipsilateral (left) M1 may suggest that the ipsilateral M1 is also involved in complex motor skill

learning. Alternatively, this may also relate to the proposal that in right-handed subjects the left dominant hemisphere may be more involved in higher-order aspects of motor control than the right hemisphere, and could play a key role in motor learning whatever the hand involved (Goldenberg 2003; Schambra et al. 2011). Thus, whereas lower aspects of motor control and motor learning were similar in terms of recruited neuronal resources between the shifters and fitters, as suggested by a lack of differential activation in the contralateral M1, the shift pattern of motor skill learning was associated with extra fMRI activation in the ipsilateral M1.

In a similar way, there was no statistically significant difference in the areas related to attentional and motivational processes such as the DLPFC or anterior cingulate cortex (Smith and Jonides 1999; Clark et al. 2010) between the shifters and fitters groups. This may suggest that, at least for this task and under these particular experimental conditions, there was no difference in motivational and attentional processes detectable by the current fMRI design that could explain why about half of the normal subjects adopted a shift pattern and half a fit pattern. It is noteworthy that three subjects were excluded from further analysis since their global performance indexes remained stable (one subject) or even deteriorated (two subjects). It is unlikely that these three normal subjects were unable to learn since they did not suffer from neurological nor psychiatric disorder. Their demographical characteristics did not differ from those of the shifters and fitters; and visual comparison of their individual fMRI activation pattern did not differ from those of the shifters and fitters. We can therefore not speculate further about the reasons or neural substrates underlying these behavioural patterns. It is however likely that they lacked motivation and/or attention, or experienced fatigue during the experiment. This may fit with recent observations about the importance of context, motivation, and reward for motor learning (Abe et al. 2011).

No cortical area or subcortical brain structure was significantly more activated in the fitters than in the shifters group. This suggests that the fit pattern was characterised by a globally less intense activation, and that no area outside the network described in the shifters group was specifically

involved in the fitters group. It should also be mentioned that the perception of error (i.e. not keeping the pointer perfectly in the middle of the track) is difficult for the subjects unless they make a broad error such as clear overshoot outside of the track. Moreover, such an error would likely lead to a transient error signal in the brain, since our circuit game requires performing continuous movements. In that sense, it is therefore not surprising that errors in the circuit game do not lead to dedicated brain activations such as in tasks requiring (sequential) key presses or pointing to a small target with a single movement. Thus, either the fitters failed to activate efficiently the key areas involved in the first stage of successful motor skill learning (see below) or this lack of an early activation resulted in a less efficient motor skill learning.

Two areas were of particular importance for achieving early successful motor skill learning of this task: the left cerebellar hemisphere and the SMA. In the shifters group, the BOLD signal was significantly correlated with the evolution of the PI in the left anterior cerebellar hemisphere (lobule V-VI). In the fitters group, this cerebellar zone was the only one disclosed when focusing on motor skill learning, but the correlation with the PI was not significant. Thus, the cerebellar hemisphere is involved in successful motor skill learning as suggested previously (van Mier et al. 1998; Ghilardi et al. 2000; Halsband and Lange 2006; Debas et al. 2010). It is worth noting that neither the whole-brain ANOVA nor the ROI analysis demonstrated a differential activation in the left anterior cerebellar hemisphere between the two groups (Figure 4.3, Table 4.3) in which the same cerebellar zone (lobule V-VI) was activated in the two motor learning patterns (Table 4.5). In lobule V-VI, there was a non-significant trend for a negative correlation between the beta values and the NJ in the two groups; i.e. the stronger the BOLD activation, the smoother the movements. Thus, the lobule V-VI of the ipsilateral left cerebellar hemisphere was particularly important for performing smooth movements, independently of the learning pattern.

All the analyses (whole group, ANOVA, and subgroup analyses) showed that the SMA was the key area for distinguishing the two patterns of

performance improvement during early motor skill learning (shift versus fit). In the shifters group, the temporal evolution of the BOLD signal in the SMA showed the strongest correlation with the temporal evolution of the PI. The SMA is known to be involved in sequence learning such as learning to trace a circuit, the serial reaction time task (SRTT), or finger tapping synchronization (Lee 2004; Lewis et al. 2004; VanMier et al. 2004). In the current experiment, the subjects had basically to learn to perform and to optimise (through a SAT) a complex sequence of precisely timed movements. In that sense, a higher level of activation in the SMA in the shifters is coherent with an early and efficient recruitment of the SMA allowing a better temporal implementation of a sequence of complex movements.

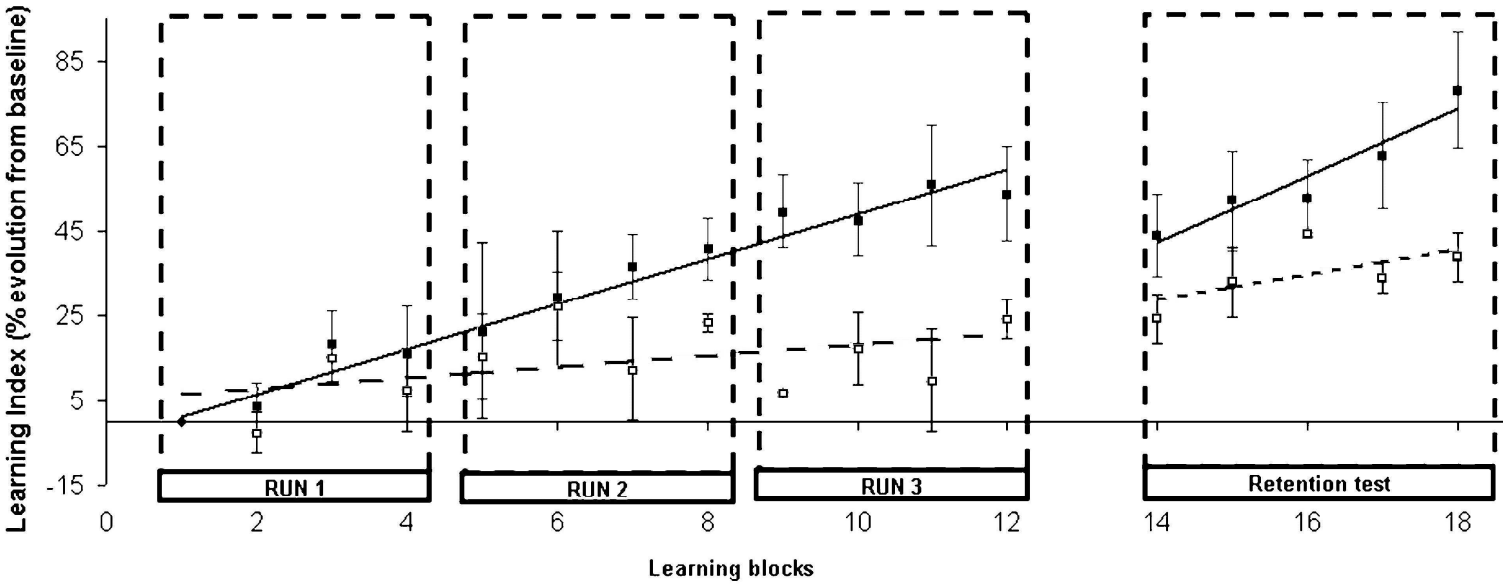
In the present study, the BOLD activity was restricted to the SMA proper, located caudally to the anterior vertical commissure (Picard and Strick 2001; Nachev et al. 2008; Kim et al. 2010b). From a cytoarchitectural and functional point of view, the SMA is separated in two distinct regions: the SMA proper (caudal part) and the pre-SMA (rostral part) (Picard and Strick 2001; Nachev et al. 2008; Kim et al. 2010b). It has been suggested that the SMA proper is involved in implicit motor skill learning and the pre-SMA in explicit motor skill learning (Ashe et al. 2006). The lack of activation in the pre-SMA in the current experiment fits with this observation since the skill to be acquired is implicit by nature. However, the functions of the SMA might be more complex than previously thought (Nachev et al. 2008). It has been suggested that the SMA proper is only involved when the correct sequence is already acquired, and permits to improve the performance of a known sequence; whereas the pre-SMA may be involved during the very first moments when acquiring new sequences (Hatakenaka et al. 2007; Nachev et al. 2008; Nudo 2009). Due to the nature of our task (circuit game), the subjects were immediately aware of the full sequence since the circuit determines the movements to be performed. In that sense, the sequence is known at once and the activation of the pre-SMA might be very transient. Thus, in the shifters group, the rapid activation of the SMA proper and its continuous rise correlating with PI suggest that the SMA proper is the key

area leading to the an early and efficient learning of this motor skill, as suggested previously (Grafton et al. 1992; Toni et al. 1998).

Another hypothesis for explaining the predominant role of the SMA proper may be the involvement of the SMA proper in inter-manual transfer of motor skills (Frings et al. 2006; van Mier and Petersen 2006; Perez et al. 2007; Perez et al. 2008). In the current experience, all the subjects were familiar with computer work; they daily manipulated a computer mouse with their dominant hand. Although we did not specifically tested inter-manual transfer, one can hypothesize that the shifters were more efficient than the fitters in transferring from their dominant towards their non-dominant hand some low-level general (i.e. not task-specific) aptitude to navigate the mouse (inter-manual transfer), and to improve their performance with the non-dominant hand.

4.5 Conclusion

Despite identical instructions and experimental conditions, normal subjects may spontaneously adopt different behavioural patterns (shift/fit) during the first minutes of motor skill learning, which correlate with differential brain activation patterns. On the one hand, the ipsilateral cerebellar hemisphere is involved in the control of movement smoothness independently of the behavioural pattern (shift/fit) . On the other hand, the SMA proper is the key area associated with an early shift of SAT, i.e. the most efficient motor skill learning pattern. This confirms a critical role of the SMA proper in the early stage of motor skill learning, at least when the task requires the performance of a sequence of fast and accurate movements under visual control



Supplementary Figure 4.1: Additional experiment. The 12 LI values (Mean ± SEM) correspond to the LI during each learning block (day 1) and during the retention test blocks (day 2). Black line/squares: shifters group (n = 13), dotted line/white squares: fitters group (n = 5). As demonstrated by the retention of performance improvements on the next day compared to the baseline, the circuit game induces motor skill learning. Note also the steepest slope of LI evolution in the shifters group than in the fitters group even during the five blocks of the retention test.

Supplementary Table 4.1:

A) [LEARNING]

Brain area / structure	(BA)	mean x	mean y	mean z	mm³
R PM	BA 6	49	15	0	262
R occipital lobe	BA 19	22	-75	33	1125
L occipital lobe	BA 19	-26	-77	22	1668
R M1	BA 4	29	-28	56	370
R Thalamus		13	-16	5	1146
SMA	BA 6	3	-20	51	901
L Thalamus		-11	-18	8	151
L Putamen		-26	-3	5	295
L PM	BA 6	-26	-14	57	262
L Inferior parietal lobule	BA 40	-36	-49	50	403
L occipital lobe	BA 18	-27	-86	-5	17115
R occipital lobe	BA 18	19	-84	-7	20395
L anterior cerebellum		-19	-54	-19	9843

B) [EASY]

Brain area / structure	(BA)	mean x	mean y	mean z	mm³
L Inferior parietal lobule	BA 40	-49	-33	35	2795
R M1	BA 4	29	-28	56	5370
R PM	BA 6	53	3	33	209
R Thalamus		13	-19	5	5817
SMA	BA 6	1	-14	50	3044
L Thalamus		-14	-20	9	1836
L Putamen		-25	-4	7	1942
L PM	BA 6	-26	-14	57	1719
L occipital lobe	BA 18	-27	-88	-3	20026
R occipital lobe	BA 18	21	-88	-4	27874
R occipital lobe	BA 19	23	-75	33	4757
L occipital lobe	BA 19	-24	-77	33	3752
R putamen		21	-2	5	2128
L anterior cerebellum		-22	-51	-20	10297

C) [REPLAY]

Brain area / structure	(BA)	mean x	mean y	mean z	mm³
R prefrontal lobe	BA 9	43	4	30	7916
R Thalamus		19	-27	3	2241
R Limbic lobe	BA 24	1	1	29	201
R occipital lobe	BA 19	24	-77	33	15217
L occipital lobe	BA 19	-23	-70	39	10154
L Thalamus		-17	-25	6	1906
R PM	BA 6	36	-4	45	1719
L PM	BA 6	-26	-14	55	1706
R occipital lobe	BA 18	24	-81	-5	36256
L occipital lobe	BA 18	-31	-81	-5	31260

Whole group analysis: q(FDR)= 0,05; t_{19}=3.71; $p_{uncorected}$= 0,0014; threshold= 100 voxels. BA: Brodmann Area, M1: primary motor area, SMA: supplementary motor area, PM: premotor cortex, R: Right, L: Left

Supplementary Table 4.2: correlation analyses

Analysis	Correlation type	Brain area / structure	BA	Mean x	Mean y	Mean z	Correlation with PI		Correlation with NJ	
							r	p value	r	p value
Whole group analysis (n=20) [LEARNING]	Global correlation	R PM	BA 6	49	15	0	0.22	0.35	-0.25	0.43
		R M1	BA 4	29	-28	56	0.39	0.09	-0.02	0.93
		R Thalamus		13	-16	5	-0.22	0.35	-0.33	0.15
		SMA	BA 6	3	-20	51	0.62	0.0035	0.06	0.85
		L Thalamus		-11	-18	8	0.22	0.35	-0.2	0.43
		L Putamen		-26	-3	5	0.22	0.35	-0.2	0.43
		L PM	BA 6	-26	-14	57	0.22	0.35	-0.2	0.43
		L Inferior parietal lobule	BA 40	-36	-49	50	0.32	0.17	-0.06	0.83
		L anterior cerebellum		-27	-52	-21	0.39	0.09	-0.09	0.78
Sub group analysis Shifters group (n=11) [LEARNING-REPLAY]	Global correlation	R M1	BA 4	34	-29	64	0.05	0.88	0.09	0.78
		SMA	BA 6	1	-19	49	0.63	0.0377	0.01	0.97
		L cerebellar hemisphere (Lobule V-Vi)		-21	-47	-21	0.31	0.35	-0.27	0.39
		L cerebellar hemisphere (Lobule VII)		-9	-55	-15	0.05	0.88	-0.12	0.71

Sub group analysis Shifters group (n=11) [LEARNING-(REPLAY+EASY)]	Global correlation	SMA L cerebellar hemisphere (Lobule V-Vi)	BA 6	1 -21	-19 -45	49 -21	0.63 0.53	0.0377 0.09	-0.06 -0.25	0.85 0.43
Sub group analysis Shifters group (n=132) [LEARNING-REPLAY]	Individual correlation	R M1 SMA L cerebellar hemisphere (Lobule V-Vi) L cerebellar hemisphere (Lobule(VII)	BA 4 BA 6	34 1 -21 -9	-29 -19 -47 -55	64 49 -21 -15	0.11 0.33 0.22 0.11	0.21 0.0001 0.0113 0.21	0.09 0.01 -0.12 -0.11	0.3 0.9 0.17 0.21
Sub group analysis Shifters group (n=132) [LEARNING-(REPLAY+EASY)]	Individual correlation	SMA L cerebellar hemisphere (Lobule V-Vi)	BA 6	1 -21	-19 -45	49 -21	0.32 0.22	0.0002 0.0113	-0.06 -0.13	0.49 0.13
Sub group analysis Fitters group (n=9) [LEARNING-REPLAY]										

	Global correlation	L cerebellar hemisphere (Lobule V-Vi)		-20	-45	-20	0.53	0.13	-0.13	0.68
Sub group analysis Fitters group (n=9) [LEARNING-(REPLAY+EASY)]	Global correlation	L cerebellar hemisphere (Lobule V-Vi)		-20	-45	-20	0.53	0.13	-0.11	0.73
Sub group analysis Fitters group (n=108) [LEARNING-REPLAY]	Individual correlation	L cerebellar hemisphere (Lobule V-Vi)		-20	-45	-20	0.11	0.26	-0.09	0.35
Sub group analysis Fitters group (n=108) [LEARNING-(REPLAY+EASY)]	Individual correlation	L cerebellar hemisphere (Lobule V-Vi)		-20	-45	-20	0.11	0.26	-0.07	0.47

Supplementary Table 4.3: Contrasts and their respective weights.

Contrasts	Contrasts Weights
LEARNING	1 0 0
EASY	0 1 0
REPLAY	0 0 1
LEARNING -REPLAY	1 0 -1
EASY- REPLAY	0 1 -1
LEARNING -EASY	1 -1 0
(LEARNING + EASY) -REPLAY	1 1 -2
LEARNING - (EASY+REPLAY)	2 -1 -1

Chapter 5: Neural substrates underlying motor skill learning in chronic stroke patients, a fMRI study.**

***Chapter 5 is a presentation of an on-going study by S. Lefebvre, L. Dricot, W. Gradkowski, P. Laloux, J. Jamart, P. Desfontaines, F. Evrard, A. Peeters, Y. Vandermeeren*. (Lefebvre et al. *in preparation*[1]*)*

Abstract Motor skill learning plays a central role in post-stroke motor recovery, but little is known about its underlying neural substrates. Recently, we utilized a new motor skill paradigm in healthy individuals and identified two subpopulations: shifters and fitters. Shifters showed striking improvements in speed/accuracy trade-off, while fitters did not; the supplementary motor area was the key area underlying efficient motor skill learning. The objectives of this study were to identify with functional magnetic resonance imaging the neural substrates underlying motor skill learning in chronic stroke patients and to determine whether specific neural substrates were recruited in shifter versus fitter stroke patients. During functional magnetic resonance imaging acquisition, 23 chronic stroke patients learned a visuomotor skill with their paretic upper limb, which consisted of using a computer mouse to move a cursor across a circuit as quickly and accurately as possible. At the whole group level, activation during motor skill learning encompassed the primary motor cortex, dorsal premotor cortex, supplementary motor area and dorsolateral prefrontal cortex in the damaged hemisphere, as well as bilateral posterior parietal, primary somatosensory and visual cortices. After subtracting activation related to visual processes and lower aspects of motor control, correlation between activation and motor skill learning was restricted to the dorsal premotor cortex of the damaged hemisphere. In the less efficient fitter stroke patients (subgroup analysis), after subtracting activation related to visual and lower motor aspects, significant activation was restricted to the bilateral posterior parietal cortex and did not correlate with motor skill learning. Conversely, in the more efficient shifter stroke patients, significant activation occurred in the supplementary motor area, primary motor and somatosensory cortex of the damaged hemisphere, as well as bilateral

dorsal premotor cortex. The key area where activation changes correlated significantly with motor skill learning was bilateral dorsal premotor cortex, especially in the damaged hemisphere. These observations suggest a plastic, compensatory reorganisation of brain activation during motor skill learning in chronic stroke patients and point to a key role of bilateral dorsal premotor cortex.

Key words: motor skill learning, stroke, fMRI, neurorehabilitation

5.1. Introduction

Stroke is a devastating pathology that causes life-long upper limb hemiparesis in 30-70% of survivors (Lai et al. 2002; Kwakkel et al. 2003). The biochemical mechanisms triggered by acute stroke (e.g., oedema resolution, inflammation, up- and down-regulation of neurotransmitters) play a prominent role in early recovery (Kreisel et al. 2006; Carey and Seitz 2007). Beyond these biochemical cascades, recovery of motor function also relies on plastic reconfiguration of the cortical motor network and its descending projections, which support transfer of impaired functions towards undamaged areas of the brain (Feydy et al. 2002; Johansen-Berg et al. 2002b; Lotze et al. 2006a). Although this plastic reorganisation may reflect a simple re-routing of information flow through pre-existing, undamaged pathways, stroke patients must learn how to recruit these neuronal resources. To some extent, recovering from hemiparesis might be conceptualised as a particular form of motor skill learning, in other words, learning to use the reconfigured motor network to optimise planning, execution and movement control of the paretic upper limb. Indeed, the idea that motor skill learning plays a central role in post-stroke motor recovery is becoming a major focus in neurorehabilitation (Matthews et al. 2004; Krakauer 2006; Dipietro et al. 2012; Kitago and Krakauer 2013).

The neural substrates of motor skill learning are relatively well elucidated in healthy individuals. Functional magnetic resonance imaging (fMRI) studies demonstrated that motor skill learning relies on a network encompassing the primary motor cortex (M1), supplementary motor area (SMA), premotor cortex (PM), dorsolateral prefrontal cortex (DLPFC),

cerebellum and basal ganglia (Ghilardi et al. 2000; Halsband and Lange 2006; Debas et al. 2010; Hardwick et al. 2013). Recently, the definition of motor skill learning has been refined to a training-induced acquisition and improvement of motor performance (i.e., skills), persisting over time and characterised by a shift of the speed/accuracy trade-off (SAT), automatisation and reduction of performance variability (Reis et al. 2009; Dayan and Cohen 2011; Krakauer and Mazzoni 2011). Using a visuomotor skill learning paradigm involving SAT, we demonstrated that different behaviours can be observed in healthy individuals during the first stages of motor skill learning (Lefebvre et al. 2012). The most efficient subjects were shifters, who achieved a shift of the SAT. The less efficient subjects were fitters, who were characterised by smaller performance enhancement due to opposite changes in speed and accuracy. Finally, a minority of subjects were non-learners, who did not achieve motor skill learning. These different behaviours were observed despite identical instructions and experimental conditions, and they were associated with specific brain activation patterns. Specifically, shifters showed an activation pattern associated with motor skill learning, which involved the SMA, M1 and cerebellum. Furthermore, activation changes in the SMA of shifters correlated with performance improvement, suggesting that the SMA plays a key role in early motor skill learning. The less efficient fitters showed only a non-significant correlation in the cerebellum (Lefebvre et al. 2012).

In stroke patients, functional brain imaging has been used extensively to explore the reorganisation of the network controlling the paretic arm or hand. Early after stroke, this reorganised network is characterised by compensatory recruitment of the undamaged hemisphere, especially the motor and premotor areas (Feydy et al. 2002; Tombari et al. 2004; Jaillard et al. 2005; Ward and Frackowiak 2006) and/or widespread activation in the damaged hemisphere with extensive activation of the somatosensory and premotor cortices (Tombari et al. 2004; Ward et al. 2006). Over time, motor recovery is associated with a shift of activation back toward the damaged hemisphere (Pineiro et al. 2001; Jaillard et al. 2005) and a progressive recruitment of the cerebellum ipsilateral to the paretic

hand (Small et al. 2002). Thus, the more similar the reorganised motor network becomes to that of healthy individuals, the better the recovery. However, the undamaged hemisphere may still play a vicarious role in recovered motor control of the paretic hand (Johansen-Berg et al. 2002b; Werhahn et al. 2003; Tombari et al. 2004; Lotze et al. 2006a). In addition, changes in brain connectivity have been associated with motor function recovery after stroke (Jiang et al. 2013). Early after stroke, both anatomical and functional connectivity decrease within the damaged hemisphere; over time, motor function recovery is associated with gradual recovery of connectivity (Pannek et al. 2009; Golestani et al. 2013).

Since functional reorganisation occurs in the network that supports motor recovery of the paretic upper limb after stroke, it seems logical that similar plasticity should occur in the larger network underlying motor skill learning. However, despite extensive fMRI studies of the functional neuroanatomy of motor skill learning in healthy individuals, very few studies have assessed stroke patients (Ghilardi et al. 2000; Halsband and Lange 2006; Debas et al. 2010; Lefebvre et al. 2012; Hardwick et al. 2013). Using a region of interest (ROI) approach, one study with ten chronic stroke patients performing visuomotor tracking with the paretic hand showed a bilaterally reorganised pattern with a predominance in the undamaged hemisphere during the pre-training fMRI session (Carey et al. 2002). After training, activation was partially transferred back towards the damaged hemisphere, suggesting functional reorganisation (Carey et al., 2002). Another study using ROI showed decreased task-related fMRI activation in the contralesional M1 of nine chronic stroke patients after three days of training on a serial targeting task (Boyd et al. 2010). A recent fMRI study highlighted the differences in brain activation patterns between nine healthy volunteers and nine chronic stroke patients during training over several days on an implicit sequential visuomotor tracking task (Meehan et al. 2011b). Compared to healthy volunteers, motor skill learning and retention in stroke patients relied on a reorganised network involving compensatory activations, especially in prefrontal attentional areas such as the DLPFC. Finally, during baseline performance of a sequential grip-force tracking task, ten chronic

stroke patients showed reduced fMRI activation in the damaged hemisphere compared to healthy controls (Bosnell et al. 2011). After repeated training, fMRI activation decreased in healthy controls but was maintained or increased in stroke patients.

These four initial studies involved small cohorts of mostly high-functioning patients, typically with sub-cortical strokes, and they did not characterise motor skill learning through SAT. Instead, they compared fMRI activation related to motor performance pre- and post-training, and two used an ROI approach (Carey et al., 2002;Boyd et al. 2010), compared fMRI activation related to motor performance pre- and post-training, and did not characterise motor skill learning through a SAT. Since motor learning plays a key role in motor function recovery, a better knowledge of the neurophysiology of motor skill learning after stroke should lead to the refinement of recovery models and translate into the development of specific neurorehabilitation methods based on the principles of motor learning. **i)** to use random effect (RFX) analyses of whole-brain fMRI activation to identify the neural substrates underlying the first stages of motor skill learning in a larger cohort of chronic stroke patients using their paretic upper limb, and **ii)** to determine whether shifter and fitter stroke patients recruit specific neural substrates during early motor skill learning.

5.2. Material and methods

5.2.1 Population

The experimental protocol was approved by the local Ethical Committee (CHU Mont-Godinne, UCL) and was conducted according to the recommendations of the Helsinki declaration. After providing written informed consent, twenty-five chronic stroke patients meeting the following criterion were selected: *Inclusion*: **i)** being a chronic (>6 months) stroke patient aged 18-90 years, **ii)** presenting a chronic motor deficit in the upper limb, **iii)** having a vascular brain lesion demonstrated by cerebral imaging (Figure 5.1); *Exclusion***: i)** being unable to perform the task or to understand instructions, **ii)** presence of intracranial metal, **iii)** alcoholism, **iv)** pregnancy,

v) cognitive impairment or psychiatric disorder, **vi)** any contraindication to MRI.

The following measures were assessed: disability with the modified Rankin Scale (mRS), level of impairment with the National Institutes of Health Stroke Scale (NHISS), residual dexterity with the Purdue Pegboard Test (PPT), maximal hand force (MaxHF) with a whole-hand Jamar dynamometer and manual ability with the ABILHAND scale (Penta et al. 2001) (Table 5.1). Patients #12, 13 and 15 had participated in a previous study at least one year before, exploring motor skill learning enhancement by transcranial direct current stimulation (tDCS) (Lefebvre et al. 2013a). They were included in the present study because they showed no significant different from the other naïve patients on **i)** their new baseline motor performances and **ii)** their new performance evolution (Table 5.2). This analysis was performed using the Crawford & Howell statistical test to compare an individual score to a small population (Crawford and D.C.Howell 1998; Crawford and Garthwaite 2002).

5.2.2. Study design

During fMRI scanning, patients performed two consecutive runs of motor skill learning with their paretic upper limb, using a MR-compatible mouse (NAtA Technologies, Canada). Visual feedback was projected on a screen, which was viewed via a mirror placed on the head coil. As described in a previous study (Lefebvre et al. 2012), each run (duration 8 min 41 s; 172 volumes) contained a REST condition (fixation cross) and three experimental conditions: LEARNING, EASY and REPLAY. LEARNING required performing a motor skill learning paradigm described below. EASY required moving the cursor back and forth at a comfortable speed between two horizontal or vertical targets. This condition was designed to explore brain activation related to simple movement execution under visual control. During REPLAY, a videoclip of the last LEARNING performance was displayed, and patients were instructed to follow the cursor's displacement with their eyes without moving their hand. The REPLAY condition was designed to isolate activation related to visual and oculomotor processes.

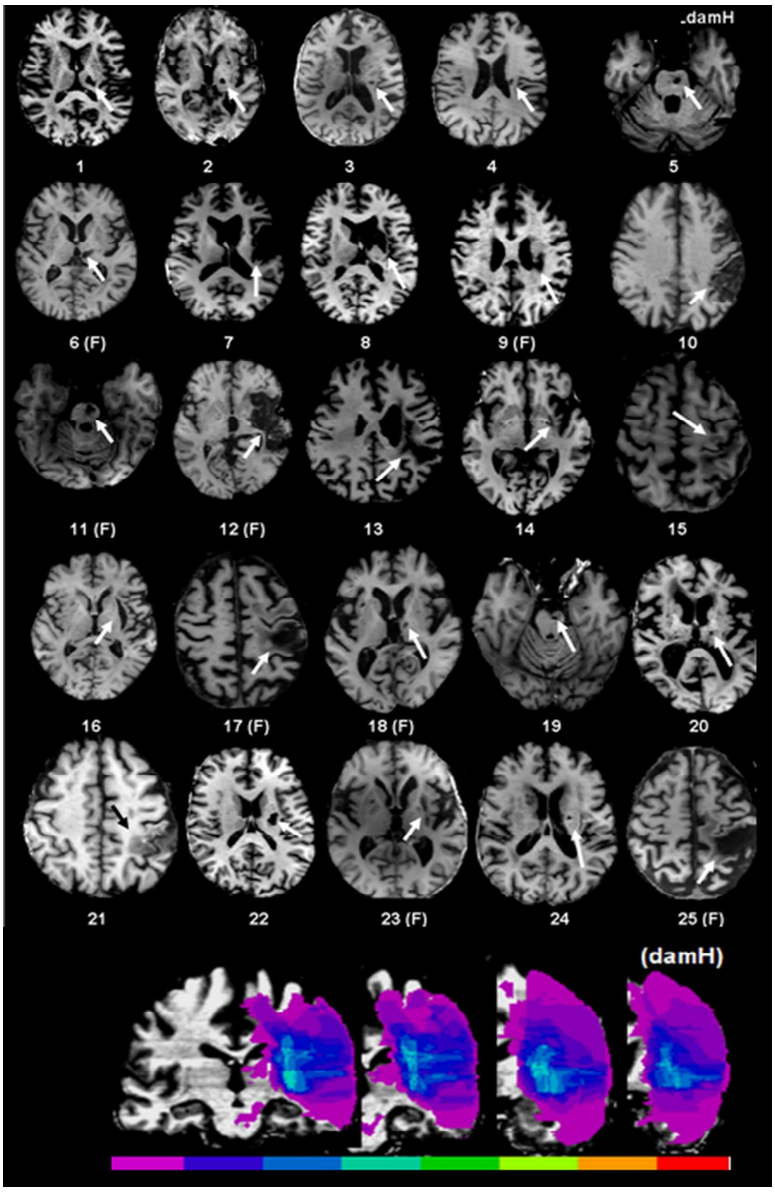


Figure 5.1: Stroke localization and overlap. Upper panel: T1 magnetic resonance imaging (MRI) at the level of the main stroke injury. Lower panel: Lesion overlap in stroke patients symbolised by the colour scale. Purple represents the stroke area of a single patient, green represents localisation shared by half of the patients, and red indicates localisation shared by all of the patients. For patients with lesions on the right side of the brain, the 3D-T1 MRI was flipped. The map of lesion localization and overlap was created with MRIcro 1.4. DamH, damaged hemisphere

			Time since stroke (years)	Main stroke lesion	DH	PH	PH PPT (n)	N-PH PPT (n)	PH MaxHF (Kg)	N-PH MaxHF (Kg)	ABILHAND (logits)	mRS	NIHSS
1	F	66	14	SC	R	R	11,7	14,3	30	30	4,4	1	1
2	M	60	9	SC	R	R	4,3	9	27	33	0,4	3	4
3	M	68	10	SC	R	R	9	7,3	44	33	2,5	2	2
4	M	71	4	SC	R	R	10,6	12,7	22	31	1,7	3	3
5	M	61	2	SC	R	R	11	13	43	57	6	1	1
6	M	64	8	SC	L	L	2	14,7	42	46	0,3	3	4
7	M	58	0,6	C	R	R	3,3	13	19	46	0,4	3	7
8	M	53	3	C	L	R	11,3	12,7	45	35	3,2	1	1
9	F	72	2	SC	R	L	7,3	11	8	21	0,3	2	2
10	M	53	0.5	C	R	R	6.3	14.7	29	44	-0.1	3	4
11	M	63	10	SC	R	L	1.7	8.3	23	47	1.3	1	1
12	M	65	3	C	R	L	3	13.7	26	35	1.9	2	4
13	M	50	5	C	R	L	7	16.3	28	47	1.7	2	2
14	F	68	15	C	R	R	9	12.3	32	34	1.8	2	2
15	M	57	3	C	R	R	9	11.7	55	50	1.9	2	2
16	M	69	3	C	R	R	10	9.7	44	39	1.7	1	0
17	M	57	8	C	R	L	0.3	11	12	37	-0.5	2	/
18	M	82	3	SC	R	L	9	13.7	37	38	3.8	2	2
19	M	74	2	SC	R	R	5.6	9	30	36	2.4	2	2
20	M	62	10	SC	R	R	11	12	44	42	3.8	2	2
21	F	66	2	C	R	R	0	/	0	/	/	3	8
22	M	45	0.6	SC	R	R	8	12	21	41	-0.4	2	4
23	F	72	4	SC	R	L	2.3	8	11	23	-0.3	4	3
24	M	75	0.5	SC	R	R	0	11.3	20	43	-1.0	3	4
25	M	75	4	C	R	L	8,7	12,7	41	44	2,7	2	0
		64 ± 9	4,9 ± 4,3	14SC/ 11C	23 R/ 2 L	16 R/ 9 L	6,7 ± 3,8	11,8 ± 2,4	31 ± 12	39 ± 9	1,8 ± 1,7	2.2 ± 0,8	2.7 ± 2.0

[1]**Legend of Table 5.1: DH: Dominant hand, PH: paretic hand, N-PH: non-paretic hand;** M, male; F, female; SC, subcortical stroke; C, cortical stroke; R, right; L, left; PPT, Baseline Purdue Pegboard Test score; n, number of pegs inserted in 30 s (mean of three trials); MaxHF, Maximal hand grip force; Kg, kilograms; mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Score; /, missing values.

Each condition was presented four times (30 s each; 10 volumes) during each run, and conditions were separated by four volumes (12 s) of REST (Figure 5.2). Beforehand, patients performed one habituation run (4 x 30 s separated by four REST volumes) of a simple circuit square.

5.2.3. Motor skill learning paradigm

The motor skill learning paradigm has been described in detail previously (Lefebvre et al. 2012; Lefebvre et al. 2013a). It consisted of moving the cursor with an MR-compatible mouse along a path under visual control. Patients were asked to follow the track as quickly and accurately as possible and to try to improve their performance over time. Accuracy was defined as keeping the pointer within the track of the circuit.

5.2.4. Behavioural analysis

To quantify motor skill learning, error and velocity were analysed (Lefebvre et al. 2012; Lefebvre et al. 2013a). Error was defined as the surface area between the actual trajectory and the ideal trajectory (i.e., midline of the track). Velocity was the first derivative of the position. Velocity and error during the LEARNING blocks were averaged as mean error and mean velocity over 3 s time bins [corresponding to repetition time (TR)]. Based on mean velocity and error, four indices were computed (for details see (Lefebvre et al. 2012; Lefebvre et al. 2013a)): error index (Pe) = constant error/patient mean error; velocity index (Pv) = patient mean velocity/constant velocity; Performance Index (PI) = Pv * Pe over bins of 3 s,

[1] *Patients who had a 0 score at the PPT or the max hand force were unable to achieve the tasks despite several trials; whereas the patients with missing value had not been tested on the task*

then averaged for each LEARNING block; and Learning Index (LI) = [(PI-PI initial)/PI initial] * 100, which was computed for each LEARNING block as a percentage of the PI relative to the baseline performance during the first block of LEARNING (PI initial).

Based on LI changes over time observed previously in healthy individuals and chronic stroke patients (Lefebvre et al. 2012; Lefebvre et al. 2013a), motor skill learning could follow three behavioural trajectories. First, in the most efficient shifters, large LI improvement was driven by a shift in the SAT, which involved either an improvement of both parameters or improvement of one parameter without concomitant degradation of the other. Second, in the less efficient fitters, smaller LI improvement was due to improvement of one parameter with concomitant degradation of the other. Third, LI degradation or stagnation characterised non-learners.

Student's t-tests were performed to determine whether baseline clinical characteristics (age, time since stroke, mRS, NIHSS, PPT and ABILHAND), predicted emergence of shifters or fitters. Similarly, Chi-square tests were calculated to investigate differences between subgroups (shifter or fitter stroke patients) based on stroke localisation (cortical/subcortical), gender and whether the paretic hand was dominant or non-dominant.

5.2.5. Imaging acquisition parameters

A 3T scanner with a 32-channel head coil (Siemens Verio, Germany) was used to acquire fMRI scans of brain activity with repeated single-shot echo-planar imaging, using the following parameters: TR = 3000 ms, echo time (TE) = 23 ms, flip angle (FA) = 90°, matrix size = 64 × 64, field of view (FOV) = 220 × 220 mm², slice order descending and interleaved, slice thickness = 2 mm (no gap) and number of slices = 59 (whole brain). The whole brain was scanned 172 times per learning run and 60 times during the habituation run. A three-dimensional (3D) T1-weighted data set covering the whole brain was acquired (1 mm^3, TR = 1600 ms, TE = 2.39 ms, FA = 9°, matrix size = 512 × 512, FOV = 256 × 256 mm^3, 176 slices, slice thickness = 1 mm, no gap).

Table 5.2 Baseline comparison

stroke patients	baseline PI	t-value	p-value
naïve (n=20)	0.83 ± 0.11		
patient #12	0.78	-0.444	0.662
patient #13	0.89	0.532	0.601
patient #15	0.99	1.419	0.172

Legend of Table 5.2: Comparison of the Baseline motor performance of the three stroke patients (#12, 13 and 15) who participated in our previous tDCS study (Lefebvre et al. 2013a) to the Baseline performance of the 20 naïve stroke patients. This analysis with the Crawford & Howell statistical test to compare an individual score to a small population did not demonstrate statistically significant difference. PI: Performance Index

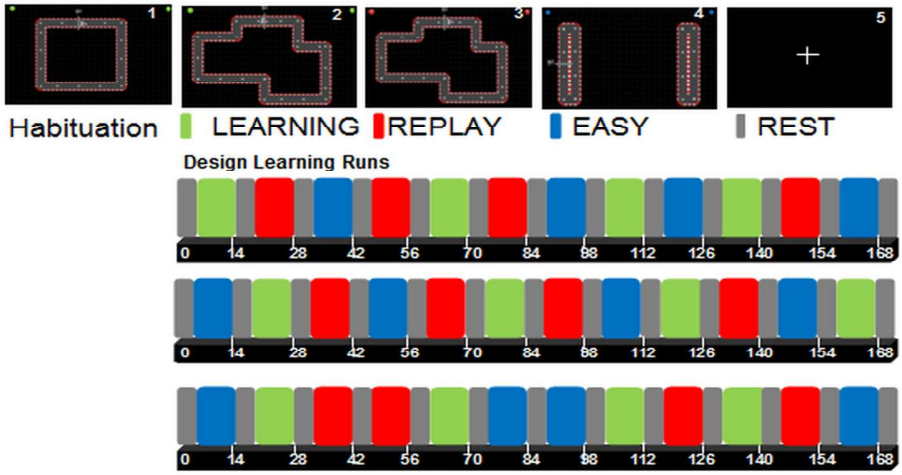


Figure 5.2: fMRI conditions and run design. Upper and lower panel: 1 (green): LEARNING: subjects were instructed to move the cursor as quickly and accurately as possible; 2 (red): REPLAY: subjects were instructed to follow the cursor displacement with their eyes while watching a videoclip of their last LEARNING block, keeping the hands relaxed; 3 (blue): EASY: subjects were instructed to move the cursor between the two targets at a comfortable speed (50% trials with vertical movements, 50% with horizontal movements); 4: REST (grey): fixation cross; 5: simple square circuit for the habituation run *(design not shown)*. Lower panel: During the fMRI session, stroke patients performed two runs of motor skill learning using designs selected in a balanced order from the three designs presented here.

5.2.6. Pre-processing and statistical analyses

fMRI data were analysed using BrainVoyager QX (Version 2.4.2.2070, Brain Innovation, The Netherlands). For patients with stroke lesions on the right side of the brain, both 3D-T1 and functional data were flipped. The pre-processing of the functional data consisted of a slice time scan correction, temporal high-pass filtering (removing temporal frequencies below three cycles/run) and 3D motion correction for head movements using a rigid body algorithm. Functional data were analyzed using the General Linear Model (GLM), consisting of predictors based on specific experimental conditions, in which beta weights measure the potential contribution of the predictors in each voxel time course. Coregistrations between functional runs and 3D-T1 weighted scans of each patient were performed automatically, then manually corrected. All anatomical and functional volumes were spatially normalized in Talairach space (Talairach J 1988) to allow group analysis. The statistical maps obtained were overlaid on the 3D T1-weighted scans. Functional runs were smoothed in the spatial domain with a Gaussian filter of 5 mm.

5.2.7. Contrasts of interest

First, an RFX analysis on the whole group was completed to identify areas involved in each condition. The following balanced contrasts of interest were investigated: [LEARNING] (areas involved in motor skill learning); [EASY] (areas involved in lower aspects of movement control and execution); [REPLAY] (areas involved in visual and oculomotor activity); minus REST activation; [(LEARNING + EASY) - REPLAY] (areas involved in motor skill learning and movement execution minus activation related to visual-oculomotor activity); and [LEARNING - (REPLAY + EASY)] (activation related to motor skill learning minus activation related to visual-oculomotor activity and lower aspects of movement control). To explore whether the whole group activation pattern was influenced by ageing, we performed an external multiple regression analysis combining the healthy individuals from our previous study (n = 22, mean age 33.9 ± 11 years, range: 20-62 years)

(Lefebvre et al. 2012) and the present stroke patients (n = 23, mean age 64 ± 9 years, range: 45-82 years).

Second, in the ROIs found with [LEARNING] and [LEARNING - (REPLAY + EASY)], external Pearson correlation analyses were performed to identify key area(s) where activation changes had the highest correlations with motor skill learning. For this whole-group analysis, performance (PI) values of patients were averaged, and the correlation was performed between the eight beta weights (one value for each block) and the eight PI values.

Third, the beta weights of the ROIs involved in motor skill learning and movement execution (identified with [(LEARNING + EASY) - REPLAY] in the whole-group RFX analysis) were used to perform Student t-tests to compare respective activation in these ROIs between shifter and fitter stroke patients. These comparisons were performed on the beta weights from [LEARNING - (REPLAY + EASY)].

Fourth, RFX analyses were computed to compare different brain activation patterns between the shifter/fitter subgroups [LEARNING - (REPLAY + EASY)]. Finally, external Pearson correlation analyses were performed between the beta weights and the PI values in the different ROIs obtained for each subgroup.

5.3. Results

5.3.1. Behavioural results

Of the 25 stroke patients, two (#2 and 6) were classified as non-learners due to deterioration of motor performance, and these individuals were excluded from further analyses. The 23 remaining patients achieved motor skill learning: nine were classified as shifters and fourteen as fitters (Figure 5.3). At the end of the second learning run, the performance of the shifters had improved significantly more (LI: 49 ± 30%; mean ± SD) than that of the fitters (LI: 13 ± 10%; $p < 0.001$).

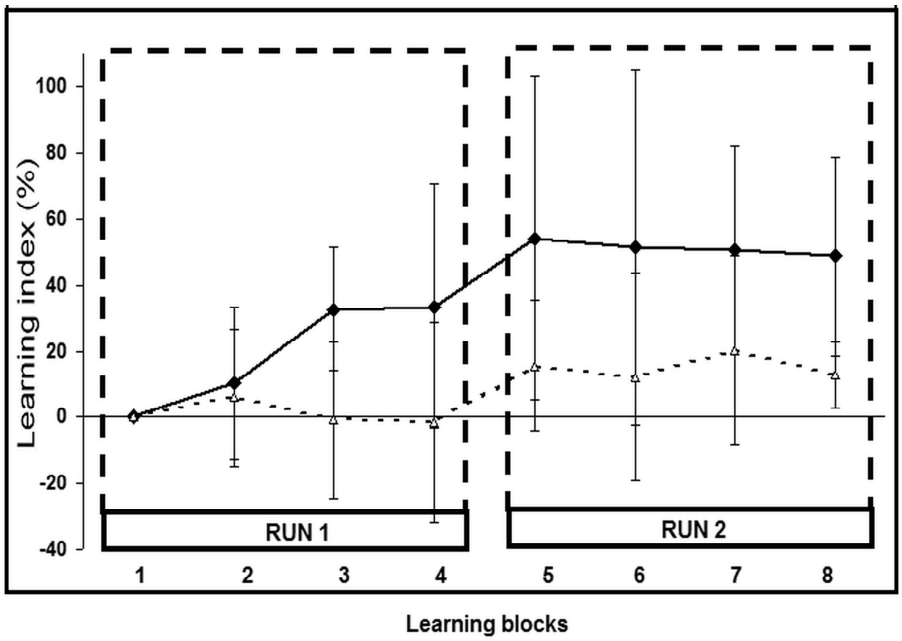


Figure 5.3: Learning Index (LI) evolution across learning blocks. The eight mean ± SD LI values correspond to the LI during each learning block. black squares: shifter stroke subgroup (n=9; LI, 49%± 30), white triangles: fitter stroke subgroup (n=14; LI: 13%± 10; $p < 0.0005$).

5.3.2. Correlation analyses

Correlations analyses were performed to determine whether baseline clinical characteristics could predict the classification of patients as shifters or fitters. Age, time since stroke, mRS, NIHSS, PPT and ABILHAND scores did not correlate significantly with shifter/fitter classification ($p = 0.45$, 0.94, 0.49, 0.99; 0.20 and 0.51, respectively, Student's t-test), nor did type of stroke (cortical versus subcortical; $p = 0.99$, Chi-square), gender ($p = 0.87$, Chi-square) or whether the paretic hand was dominant or non-dominant ($p = 0.29$, Chi-square).

5.3.3. fMRI results

5.3.3.1. Whole-group RFX analysis

As shown in Figure 5.4, whole-group RFX analysis revealed the areas activated during each condition ($t_{22} = 2.19$; $p_{UNCORRECTED} < 0.04$). Areas

activated during LEARNING included: M1 [Brodmann Area (BA) 4] in the damaged hemisphere ($M1_{damH}$), the dorsal premotor cortex (PMd_{damH}, BA 6), SMA_{damH} (BA 6), bilateral posterior parietal cortex (PPC, BA 7), bilateral primary somatosensory cortex (S1, BA 3), $DLPFC_{damH}$ (BA 9) and bilateral visual cortex. Areas activated during EASY included: $M1_{damH,}$ PMd_{damH}, bilateral SMA, bilateral PPC, inferior parietal cortex (IPC_{damH}, BA 40), $S1_{damH}$, bilateral anterior cerebellum and bilateral visual cortex. As expected, the visual area activations during LEARNING and EASY were also observed in REPLAY, as well as activation in bilateral ventral premotor cortex (PMv), bilateral PMd, $thalamus_{undamH}$, bilateral putamen, bilateral PPC and IPC. To highlight the areas involved in motor control and learning, the [(LEARNING + EASY) - REPLAY] contrast was computed (t_{22} = 2.25; $p_{UNCORRECTED}$ < 0.04). The network revealed with this contrast contained the SMA_{damH}, bilateral PMd, $M1_{damH}$ and $S1_{damH}$ (Figure 5.4, Table 5.3). To focus on the areas involved in motor skill learning, the [LEARNING - (REPLAY + EASY)] contrast was computed (t_{22} = 2.10; $p_{UNCORRECTED}$ < 0.04). With this stringent contrast, significant activation was focused in the $M1_{damH}$ (44 mm³) and PMd_{damH} (42 mm³) (Figure 5.4).

Multiple regression analysis showed no statistically significant correlation between ageing (20-82 years) and blood oxygen level-dependent (BOLD) activation in the areas involved in motor skill learning (M1, SMA, PMd, S1, PPC, thalamus, DLPFC and cerebellum) in healthy volunteers and stroke patients.

5.3.3.2. Whole-group RFX correlation analyses

Correlation analyses between the PI and beta weights of each ROI activated in [LEARNING] showed a statistically significant positive correlation in the PMd_{damH} (r = 0.70, p = 0.05) and a negative correlation in the $DLPFC_{damH}$ (r = -0.82, p = 0.01). Correlations in the other areas activated in [LEARNING] (i.e., $M1_{damH,}$ $SMA_{damH,}$, bilateral S1, bilateral PPC, bilateral visual cortical areas) were not statistically significant. Correlation analyses performed based on [LEARNING - (REPLAY + EASY)] showed a statistically significant correlation exclusively in the PMd_{damH} (r = 0.71, p = 0.048).

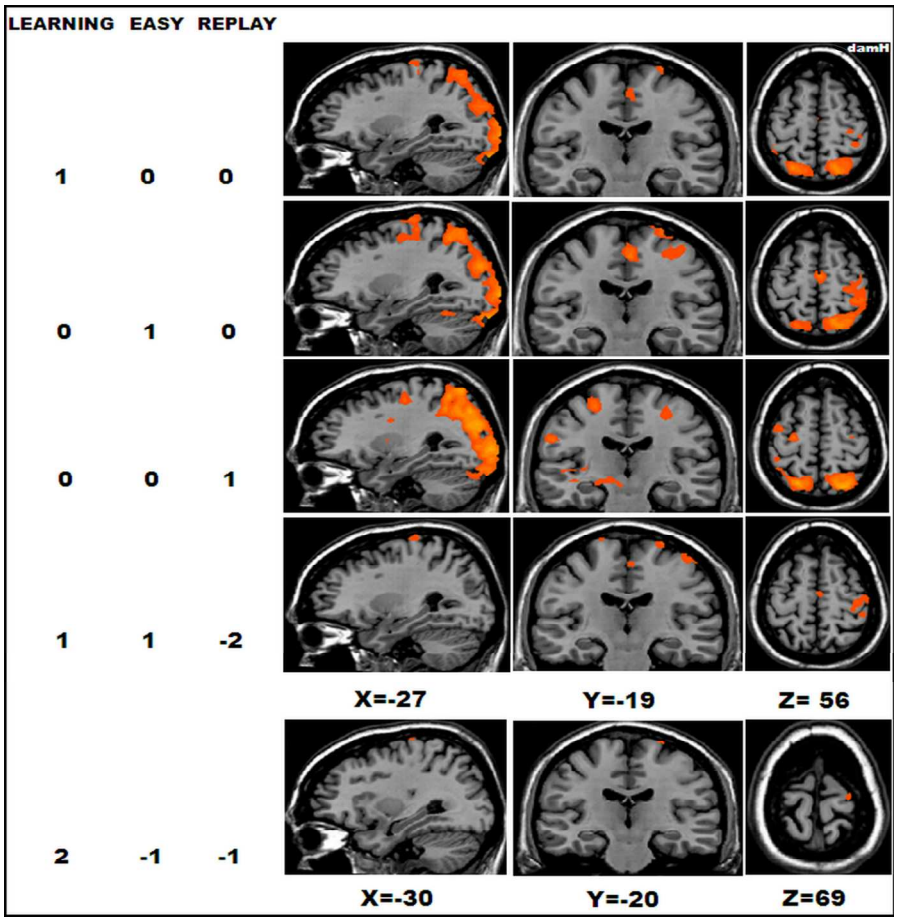


Figure 5.4: Whole-group activation: BOLD activation for the 23 chronic stroke patients comparing the three basic contrasts, [LEARNING], [REPLAY] and [EASY] (RFX t_{22} = 2.19; $p_{UNCORRECTED}$ < 0.04), as well as two additional contrasts, [(LEARNING + EASY) - REPLAY] (RFX t_{22} = 2.25; $p_{UNCORRECTED}$ < 0.04) and [LEARNING - (REPLAY + EASY)] (RFX t_{22} = 2.10; $p_{UNCORRECTED}$ < 0.04).

Table 5.3: Whole-group RFX analysis for the [LEARNING + EASY) - REPLAY] contrast

Brain area	BA	mean x	mean y	mean z	mm³
SMA_{damH}	6	-3	-15	55	494
PMd_{damH}	6	-35	-20	60	597
$M1_{damH}$	4	-34	-28	61	1 654
$S1_{damH}$	3	-40	-36	57	233
PMd_{undamH}	6	17	-18	72	59

Legend of Table 5.3: BA: Brodmann area, SMA: supplementary motor area, M1: primary motor cortex, PMd: dorsal premotor cortex, S1: primary somatosensory cortex; damH: damaged hemisphere; undamH: undamaged hemisphere; mm^3: activated volume (equivalent to the number of activated voxels since voxels were isotropic ($1mm^3$), see Methods). Whole-group RFX analysis for the [LEARNING + EASY) - REPLAY] contrast, (t_{22}=2.25; $p_{UNCORRECTED}$<0.04).

5.3.3.3. Comparisons between fitter and shifter patients based on whole-group RFX analysis

In each ROI involved in motor skill learning or movement execution obtained with the whole-group RFX analysis, external Student t-tests were performed to compare the BOLD activation between shifter and fitter patients for [LEARNING - (REPLAY + EASY)]. These comparisons, summarized in Table 5.4 and in Supplementary Table 5.1 (for additional contrasts), showed statistically significant differences exclusively in the PMd_{damH} (shifters > fitters, p = 0.004) and PMd_{undamH} (shifters > fitters, p = 0.03).

Table 5.4: Differential activation between fitter and shifter stroke patients (whole-group RFX analysis).

brain area	contrasts			beta weights (mean ± SD)		Student t-test
	LEARNING	EASY	REPLAY	shifters	fitters	p value
PMd_{damH}	2	-1	-1	0.90 ± 0.86	-0.003 ± 0.57	0.004
PMd_{undamH}	2	-1	-1	0.39 ± 0.33	0.002 ± 0.42	0.03
SMA_{damH}	2	-1	-1	0.34 ± 0.55	0.03 ± 0.58	0.23
$M1_{damH}$	2	-1	-1	0.31 ± 0.78	0.14 ± 0.60	0.59
$S1_{damH}$	2	-1	-1	0.23 ± 0.81	0.04 ± 0.59	0.52

Legend of Table 5.4 SMA: supplementary motor area, M1: primary motor cortex, PMd: dorsal premotor cortex, S1: primary somatosensory cortex; damH: damaged hemisphere; undamH: undamaged hemisphere.

5.3.3.4. RFX subgroup analyses

To compare the activation patterns in shifter and fitter patients, separate RFX subgroup analyses were computed with [LEARNING - (REPLAY + EASY)] (Figure 5.5, Table 5.5). In the shifter stroke subgroup (t_8 = 2.31; $p_{UNCORRECTED}$ < 0.05), five areas were activated: SMA, bilateral PMd, $M1_{damH}$ and $S1_{damH}$. The correlation analysis between the PI and beta weight changes over time revealed a significant correlation exclusively in the PMd_{damH} (r = 0.91, p = 0.002) and PMd_{undamH} (r = 0.79, p = 0.02). In the fitter stroke subgroup (t_{13} = 2.17; $p_{UNCORRECTED}$ < 0.05), only the bilateral PPC was significantly activated with no significant correlation between PI and beta weight changes. In both the fitter and shifter stroke subgroups, in each area where a significant correlation between the beta weight and the PI was found, there was also a positive correlation with the change of velocity over time and a negative correlation with the change of error over time. No areas showed correlations exclusively with error or velocity.

Some areas that have been implicated in motor skill learning in healthy volunteers (e.g., cerebellum ipsilateral to the paretic hand (Lefebvre et al. 2012)) and in stroke patients (e.g., $DLPFC_{damH}$ (Meehan et al. 2011b)) were not revealed by subgroup RFX analyses. Furthermore, no significant difference in the beta weights between shifter and fitter patients was found in the cerebellum [ROI from (Lefebvre et al. 2012): x = -21, y = -45, z = -21, 73 mm³; p = 0.39] nor in the $DLPFC_{damH}$ (ROI from whole-group analysis of [LEARNING]: x = -40, y = 42, z = 30, 363 mm³; p = 0.24). Finally, there were no significant correlations between the beta weights and PIs in these ROIs.

Table 5.5: RFX subgroup analysis with [LEARNING − (REPLAY + EASY)]

	BA	mean x	mean y	mean z	mm³	r value	p value
shifter stroke subgroup RFX at t_8=3.31; $p_{UNCORRECTED}$<0.05							
SMA_{damH}	6	-4	-24	67	98	0.29	0.48
PMd_{damH}	6	-18	-18	68	109	0.91	0.002
PMd_{undamH}	6	15	-17	72	10	0.79	0.02
$M1_{damH}$	4	-10	-29	68	64	0.28	0.51
$S1_{damH}$	3	-43	-35	53	18	-0.22	0.59
fitter stroke subgroup RFX at t_{13}=2.17; $p_{UNCORRECTED}$<0.05							
PPC_{damH}	7	-16	-71	51	287	0.43	0.29
PPC_{undamH}	7	17	-65	55	107	0.19	0.64

Legend of Table 5.5: BA: Brodmann area, SMA: supplementary motor area, M1: primary motor cortex, PMd: dorsal premotor cortex, S1: primary somatosensory cortex; PPC: posterior parietal cortex; damH: damaged hemisphere; undamH: undamaged hemisphere; mm^3: activated volume (equivalent to the number of activated voxels since voxels were isotropic ($1mm^3$), see Methods).

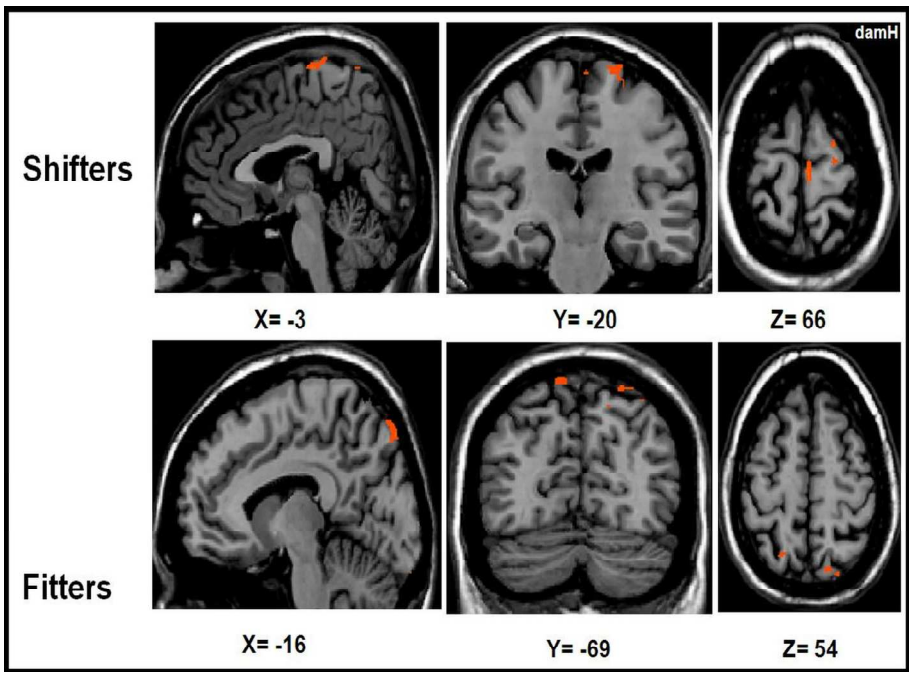


Figure 5.5 Shifters and fitters stroke subgroups activation BOLD activation for [LEARNING - (EASY + REPLAY)] for shifter stroke patients (RFX t_8 = 2.31; $p_{UNCORRECTED} < 0.05$) and fitter stroke patients (RFX t_{13} = 2.17; $p_{UNCORRECTED} < 0.05$). Note that the activation in the shifter stroke subgroup is distributed in a sensorimotor/premotor network (SMA, bilateral PMd, $M1_{damH}$ and $S1_{damH}$), whereas significant activation in the fitter stroke subgroup is limited to the bilateral PPC.

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5.4. Discussion

In chronic stroke patients, early motor skill learning with the paretic upper limb was associated with a bilateral fMRI activation pattern characterised by a positive correlation in the PMd_{damH} and a negative correlation in the $DLPFC_{damH}$. After subtracting activation related to visual processes and lower aspects of motor control, correlation with motor skill learning was restricted to the PMd_{damH}. Comparison between the fitter and shifter stroke subgroups revealed two distinct brain activation patterns. In the less efficient fitters, significant fMRI activation was restricted to the bilateral PPC, but did not correlate with motor skill learning. In contrast, in the more efficient shifters, fMRI activation encompassed the SMA_{damH}, bilateral PMd, $M1_{damH}$ and $S1_{damH}$. The bilateral PMd, especially PMd_{damH}, showed the most significant correlation between fMRI activation changes and early motor skill learning in shifters.

Learning a motor skill with the paretic upper limb

As in healthy individuals under similar experimental conditions (Lefebvre et al. 2012), the shifter/fitter dichotomy during early motor skill learning was found in chronic stroke patients, with a different distribution between shifters (50% in healthy individuals versus 36% in stroke patients) and fitters (41% versus 56%), but not in non-learners (9% versus 8%). In a previous study using the same motor skill learning paradigm, chronic stroke patients behaved preferentially as fitters rather than as shifters after 30 min of motor skill learning under control condition (sham tDCS) (Lefebvre et al. 2013a). These observations suggest that human subjects, whether healthy individuals or stroke patients, spontaneously adopt different strategies or behaviours during motor skill learning and that shifters are intrinsically more efficient than fitters, at least during the early stages of motor skill learning. Demographic factors, stroke characteristics and level of impairment did not predict whether stroke patients would behave as shifters or fitters. It remains to be determined whether a genetic background, such as a polymorphism of the brain-derived neurotrophic factor (BDNF) (Bath and Lee 2006; Kleim et al. 2006; McHughen et al. 2010), underlies these different behaviours.

A progressive deterioration of LI was observed in two patients (8%). There were no obvious differences in demographic or stroke characteristic between these two non-learners and the other patients. We speculate that a lack of motivation or excessive fatigue may have been a factor for non-learners, but these measures were not assessed. Alternatively, strokes in these individuals may have destroyed key areas that support motor skill learning. However, this seems unlikely for two reasons. First, their strokes were similar in extent and location to those of the 23 other patients. Second, to date, there is no compelling evidence that a specific brain injury may abolish motor skill learning with the paretic upper limb. Indeed, stroke patients remain able to learn new motor skills (Platz et al. 1994; Carey et al. 2002; Boyd et al. 2010; Meehan et al. 2011b), and motor skill learning is not abolished, only impaired, after a focal lesion of the basal ganglia (Vakil et al. 2000; Exner et al. 2001) or prefrontal cortex (Gomez Beldarrain et al. 2002).

Reorganised motor skill learning network after stroke

In chronic stroke patients, the evolution of fMRI activity correlated positively with successful motor skill learning in the PMd_{damH} and negatively in the $DLPFC_{damH}$. In healthy individuals, only a positive correlation in the SMA was found (Lefebvre et al. 2012). These differences suggest a plastic recruitment of additional areas to achieve motor skill learning after stroke.

Although the SMA_{damH} was also recruited, the PMd_{damH} was the key area driving motor skill learning, suggesting a plastic reorganisation. Previous motor skill learning studies in stroke patients led to conflicting observations about PMd. After extensive tracking training, performance improvement was associated with a shift of activation in PMd_{damH} at the expense of PMd_{undamH} (Carey et al. 2002). In contrast, in another study, activation in PMd_{undamH} correlated with motor sequence accuracy at retention (Meehan et al. 2011b). The current study clearly showed that PMd_{damH} plays a key role during the early stages of motor skill learning with the paretic hand. This compensatory activation in PMd_{damH} is coherent since PMd is a crucial area for motor skill learning in healthy individuals (Kantak et al. 2012; Hardwick et al. 2013) and is involved in recovery of motor function after

stroke (Carey et al. 2002; Fridman et al. 2004; Tombari et al. 2004; Lotze et al. 2006a).

A recent study in stroke patients (Meehan et al. 2011b) showed strong compensatory activation of the bilateral DLPFC and another prefrontal area (BA 46) in the undamaged hemisphere during motor sequence learning, and of prefrontal area (BA 8) in the undamaged hemisphere during the retention test. In contrast, in the current study, activation in the $DLPFC_{damH}$ correlated negatively with performance improvement during early motor skill learning. This may suggest a transient recruitment of prefrontal attentional areas (here, $DLPFC_{damH}$) in parallel with a transition towards increasing recruitment of the motor learning network in stroke patients.

In chronic stroke patients, the bilateral cerebellum was activated during the simple motor condition EASY, consistent with the role of the cerebellum in motor control (Sakai et al. 1998; Miall et al. 2001) and recovered motor function after stroke (Small et al. 2002). In contrast, during motor skill learning, there was neither significant activation nor correlation related to the cerebellum in stroke patients. The heterogeneity of the strokes might have prevented reliable recruitment of the cerebellum. However, this seems unlikely since consistent cerebellar activation was observed during simple motor performance (EASY). It is worth noting that no change in cerebellar activation associated with motor learning was reported in previous whole-brain studies in subcortical stroke patients (Bosnell et al. 2011; Meehan et al. 2011b). We speculate that, in contrast to healthy individuals, the lack of consistent cerebellar activation associated with motor learning in stroke patients might reflect a plastic reorganisation of the motor skill learning network, possibly with a preferential recruitment of the premotor and/or prefrontal areas rather than the cerebellum.

Differential brain activation in shifter and fitter stroke patients

In the shifter stroke subgroup, the activation pattern involved the SMA_{damH}, bilateral PMd, $M1_{damH}$ and $S1_{damH}$.; in shifter healthy individuals (Lefebvre et al. 2012), significant activation was observed in the SMA and

cerebellum. In shifter healthy individuals, significant correlation between motor skill learning and BOLD signal changes was restricted to the SMA whereas, in stroke patients, a significant correlation was found exclusively in the bilateral PMd. This suggests that the shifter stroke patients recruited neuronal resources from both hemispheres but predominantly from the damaged hemisphere to achieve efficient motor skill learning. The stronger involvement of bilateral PMd (especially PMd_{damH}) compared to the SMA_{damH} likely reflects a plastic reorganisation of the motor skill network in shifter stroke patients. Fitter stroke patients seemed unable to efficiently engage the motor learning network observed in shifter stroke patients: significant activation was restricted to the bilateral PPC, but it did not correlate with motor skill learning. The PPC is involved in the planning of visuomotor tasks (Desmurget et al. 1999; Torres et al. 2013). This may suggest that the fitter stroke patients were focused (or jammed) on the visuospatial aspects of the task, possibly reflecting a protracted exploratory phase.

The current fMRI results may shed new light on a previous observation in chronic stroke patients. Compared to sham, tDCS applied bilaterally over M1 (dual-tDCS) enhanced motor skill learning in 100% of chronic stroke patients (n = 18) (Lefebvre et al. 2013a). In sharp contrast, after sham dual-tDCS, 44% of the stroke patients (n = 8) showed performance worsening (non-learners). Furthermore, the SAT was shifted more consistently after dual-tDCS (56%, n = 10) than after sham (17%, n = 3) (Lefebvre et al. 2013a). Given the spread of the direct current delivered through tDCS electrodes centred over M1, it is possible that bilateral PMd were also stimulated. If PMd are indeed crucial for efficient motor skill learning in chronic stroke patients, as suggested by the current fMRI observation, stimulation of PMd could partially explain the enhancement of motor skill learning and long-term retention by dual-tDCS in stroke patients (Lefebvre et al. 2013a).

Post-stroke plasticity or aging-related modifications?

This activation pattern could reflect ageing-related reorganisation, rather than post-stroke plasticity. Previous studies demonstrated a

modification of the motor control/execution network associated with ageing (Mattay et al. 2002; Heuninckx et al. 2008). It is thus logical to infer that ageing would also influence the motor skill learning network, even though such a reorganisation has yet not been formally demonstrated (Daselaar et al. 2003). Due to the differences between cohorts and number of runs, no direct comparisons could be made between the current study and our previous fMRI study with healthy individuals (Lefebvre et al. 2012)[1]., However, the external multiple regression analysis combining the healthy individuals (Lefebvre et al. 2012) and stroke patients did not demonstrate a statistically significant correlation between age and fMRI activation.

Furthermore, studies comparing fMRI activation between stroke patients and age-matched healthy individuals consistently found striking differences suggestive of post-stroke plastic reorganisation, both for motor skill learning (Carey et al. 2002; Bosnell et al. 2011; Meehan et al. 2011b) and motor performance (Zemke et al. 2003; Ward and Frackowiak 2006; Schaechter and Perdue 2008). This clearly demonstrates that the impact of stroke on the re-organisation of brain activation is considerably greater than the impact of ageing. Therefore, we conclude that the observed differences in fMRI patterns between chronic stroke patients and healthy individuals (Lefebvre et al. 2012) predominantly reflect post-stroke plastic reorganisation rather than ageing. In other words, chronic stroke patients recruited a reorganized (likely compensatory) network to achieve motor skill learning.

Limitations of the study

This study had three main limitations. First, patients were relatively heterogeneous in terms of stroke localisation (cortical, subcortical, brainstem) and aetiology (large arteries, lacunar infarcts, intracerebral

[1] *At a behavioural level, it is interesting to mention that at the end of the second learning block, the healthy volunteers presented a LI improvement of 15 ± 31 % (fitters : 9 ± 23 %; shifters 19 ± 36 %), whereas stroke patients presented a LI improvement of 27 ± 27 % (fitters 13 ± 10 %, shifters 49 ± 30 %). This suggests that i) the stroke patients involved in this study were not impaired in their motor skill learning ability overall, ii) the stroke patients were more prone to achieve relatively large performance improvements on this task with their paretic hand.*

haemorrhages). However, this relative heterogeneity may also be considered to be a strength, since this cohort represents real-life hemiparetic stroke patients.

Second, to limit the time in the MRI scanner and movements artefacts, we acquired only two runs, whereas three runs were acquired in our study with healthy volunteers (Lefebvre et al. 2012). Thus, direct comparison between these two studies could not be made. Further studies are required to unveil the full dynamic evolution of motor skill learning in stroke patients compared to matched healthy controls.

Third, although the analysis including all of the healthy individuals (Lefebvre et al. 2012) and stroke patients suggested that altered brain activation patterns were not age-dependent, we acknowledge that such an analysis does not have the strength of an age- and sex-matched comparison. However, the reorganisation of the motor skill learning network we observed in stroke patients is coherent with previous studies showing post-stroke compensatory reorganisation of the motor control network (Carey et al. 2002; Feydy et al. 2002; Jaillard et al. 2005; Ward et al. 2006).

5.5. Conclusions

Compared to previous studies based on ROI analyses and/or smaller cohorts of subcortical stroke patients, the current study represents several important advances. First, the use of whole-brain fMRI analysis unveiled a network dynamically engaged during early motor skill learning in chronic stroke patients. Second, application of RFX analyses to a relatively large cohort of chronic patients with various types of stroke identified more general reorganisation mechanisms that are likely shared by most hemiparetic chronic stroke patients. Third, careful behavioural dissection of this motor skill learning paradigm involving an SAT provided an unprecedented level of precision to investigate the fine neuronal mechanisms underlying motor skill learning in stroke patients.

When learning a new visuomotor skill with the paretic upper limb, chronic hemiparetic stroke patients presented a reorganised brain activation pattern involving both hemispheres with a predominant recruitment of the

damaged hemisphere. In stroke patients, the key area underlying efficient motor skill learning was bilateral PMd and especially PMd_{damH}, in contrast to the SMA in healthy individuals. This suggests a plastic, compensatory recruitment of additional areas during motor skill learning in chronic stroke patients. A better understanding of the neural substrates underlying motor learning in stroke patients is a crucial step forward to design the next generation of neurorehabilitation paradigms

Supplementary Table 5.1:

brain area	contrasts			beta weights (mean ± SD)		Student t-test
	LEARNING	EASY	REPLAY	shifters	fitters	p value
PMd_{damH}	1	0	0	0.67 ± 0.52	0.17 ± 0.56	0.04
PMd_{undamH}	1	0	0	0.30 ± 0.31	0.13 ± 0.52	0.39
SMA_{damH}	1	0	0	0.35 ± 0.54	0.26 ± 0.44	0.68
$M1_{damH}$	1	0	0	0.27 ± 0.61	0.27 ± 0.51	0.99
$S1_{damH}$	1	0	0	0.25 ± 0.58	0.08 ± 0.43	0.42
PMd_{damH}	0	1	0	0.41 ± 0.47	0.26 ± 0.56	0.49
PMd_{undamH}	0	1	0	0.21 ± 0.22	0.16 ± 0.44	0.74
SMA_{damH}	0	1	0	0.35 ± 0.45	0.33 ± 0.39	0.90
$M1_{damH}$	0	1	0	0.32 ± 0.36	0.31 ± 0.50	0.93
$S1_{damH}$	0	1	0	0.27 ± 0.35	0.11 ± 0.42	0.35
PMd_{damH}	0	0	1	-0.02 ± 0.29	0.09 ± 0.56	0.59
PMd_{undamH}	0	0	1	0.005 ± 0.26	0.11 ± 0.47	0.56
SMA_{damH}	0	0	1	0.01 ± 0.33	0.16 ± 0.43	0.34
$M1_{damH}$	0	0	1	-0.09 ± 0.24	0.08 ± 0.52	0.35
$S1_{damH}$	0	0	1	0.003 ± 0.12	0.02 ± 0.34	0.92

Supplementary Table 5.1: Comparisons of the contrasts [LEARNING], [EASY] and [REPLAY] between the shifter and fitter stroke patients in the activated network (whole-group RFX analysis). For the [LEARNING] contrast, a statistically superior activation in the shifters compared to fitters was found only in PMD_{damH}. Levels of activation for other areas and contrasts were similar between shifters and fitters.

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CHAPTER 6: Neural substrates underlying the dual-tDCS-induced motor skill learning retention improvement in chronic stroke patients (a fMRI study)**

***Chapter 6 is a presentation of an on-going study by S. Lefebvre, L. Dricot, W. Gradkowski, P. Laloux, P. Desfontaines, F. Evrard, J. Jamart, Y. Vandermeeren* (Lefebvre et al. *in preparation*[2]*).*

6.1. Introduction

Motor skill learning is defined as a practice-dependent motor performance improvement that persists over time and is associated with a shift in the speed/accuracy trade-off (SAT), some degree of automatisation and a reduction of variability (Reis et al. 2009; Dayan and Cohen 2011; Krakauer and Mazzoni 2011). As motor skill learning permits the acquisition of new motor abilities (skills) and the enhancement of motor performance (Dayan and Cohen 2011; Krakauer and Mazzoni 2011), it is a crucial element in motor function recovery after stroke. In a recent study, we demonstrated that dual-hemispheres transcranial direct current stimulation (dual-tDCS) improves online motor skill learning and retention in chronic stroke patients (Lefebvre et al. 2013a).

A better comprehension of **i)** the neural substrates underlying motor learning in stroke patients and **ii)** on which neural substrates tDCS acts to enhance motor learning after stroke could potentially have a strong impact in neurorehabilitation. Whereas numerous studies investigated the neural substrates underlying motor skill learning in healthy individuals (Karni et al. 1995; Halsband and Lange 2006; Lefebvre et al. 2012; Hardwick et al. 2013), only few studies explored the neural substrates underlying motor skill learning in stroke patients (Bosnell et al. 2011; Boyd et al. 2010; Carey et al. 2002; Meehan et al. 2011b). In addition, using functional magnetic resonance imaging (fMRI), we demonstrated that motor skill learning in chronic stroke patients relied on a reorganised network requiring especially the activation of the dorsal premotor cortex in the damaged hemisphere (PMd_{damH}) (Chapter 5, Lefebvre et al., *in preparation*[1]). In the present study combining dual-tDCS and fMRI, we explored the neural substrates

underlying the tDCS-enhanced long-term motor skill retention during the delayed Recall session and the neural substrates underlying successful continued motor learning.

6.2. Material and methods

Population

Nineteen chronic stroke patients provided written informed consent and were included in this study, which was conducted according to the recommendations of the Helsinki declaration after being approved by the local Ethical Committee (Comité d'éthique médicale, CHU Mont-Godinne, UCL). The inclusion criteria were the following 1) being a chronic (>6 months) stroke patient aged 18-80 years, 2) presenting a chronic motor deficit in the upper limb, 3) having a vascular brain lesion demonstrated by cerebral imaging (Figure 6.1). The exclusion criteria were: 1) having a contraindication to magnetic resonance imaging (MRI) or to tDCS, 2) being unable to perform the task or to understand instructions, 3) suffering from epilepsy, alcoholism, cognitive impairment or psychiatric disorder, and 4) being pregnant. At inclusion, their impairment was evaluated means of the Purdue Pegboard Test (PPT) (Tiffin and Asher 1948), residual maximal hand force (MaxHF) with a whole-hand Jamar dynamometer, residual manual ability with the ABILHAND scale (Penta et al. 2001), and the National Institutes of Health Stroke Scale (NIHSS) (Kasner et al. 1999); their overall degree of disability with the modified Rankin Scale (mRS) (Bonita and Beaglehole 1988) (Table 6.1). Except patient #8, all of them participated in a previous study of motor skill learning during a single fMRI session, at least one week before (Lefebvre et al. personal communication). Four patients (# 2, 3, 4, 8) participated in a previous study exploring the impact of a single session of dual-tDCS on precision grip and dexterity, at least one year before (Lefebvre et al. 2013b).

Study design

The general design was similar to that of a previous study exploring the impact of dual-tDCS on motor skill learning in stroke patients (Lefebvre

et al. 2013a), except that the Intervention sessions were performed in the supine position and the Recall session (one week later) during fMRI scanning (Figure 6.2).

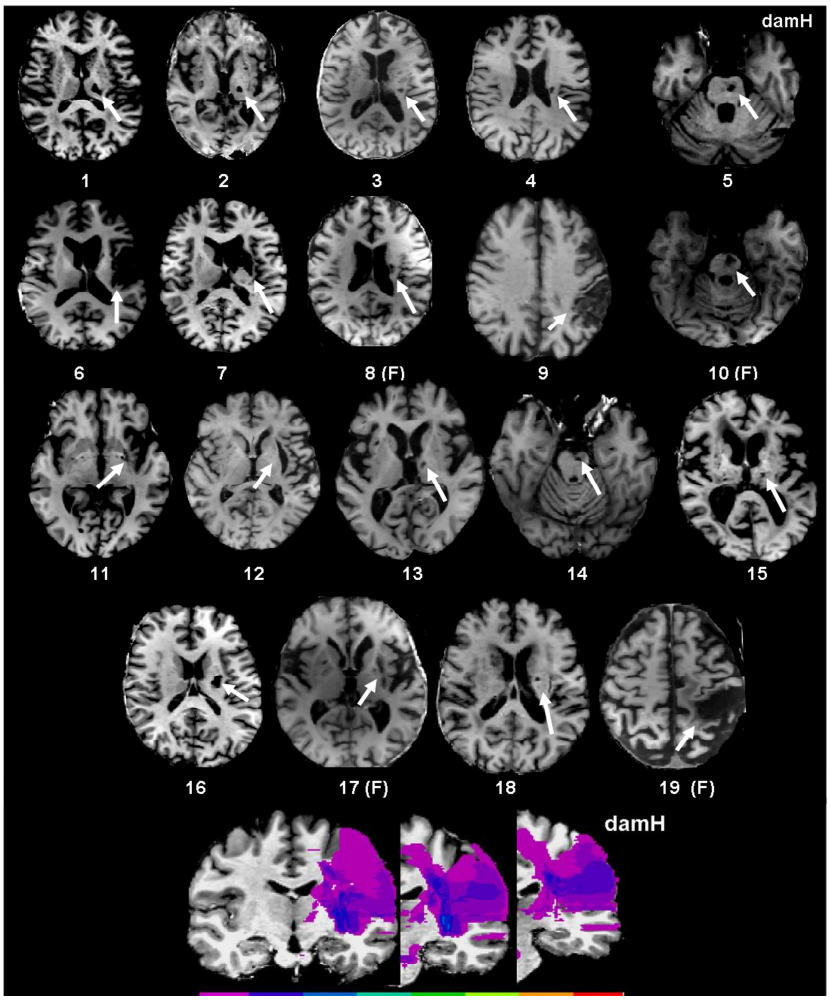


Figure 6.1: Stroke localisation and overlap. Upper panel: T1 magnetic resonance imaging (MRI) at the level of the main stroke injury. Lower panel: patient's lesion overlap symbolised by the colour scale (created with MRIcro 1.4). Purple represents the stroke area of a single patient, green represents localisation shared by half of the patients, and red indicates localisation shared by all of the patients. For patients with lesions on the right side of the brain, the 3D-T1 MRI was flipped. The map of lesion localization and overlap was created with MRIcro 1.4. damH, damaged hemisphere. F: flipped scan.

		Age (years)	Time since stroke (years)	Main stroke lesion	DH	PH	PH PPT (n)	N-PH PPT (n)	PH MaxHF (Kg)	N-PH MaxHF (Kg)	ABILHAND (logits)	NIHSS	mRS
1	F	66	14	SC	R	R	11,7	14,3	30	30	4,4	1	1
2	M	60	9	SC	R	R	4,3	9	27	33	0,4	4	3
3	M	68	10	SC	R	R	9	7,3	44	33	2,5	2	2
4	M	71	4	SC	R	R	10,6	12,7	22	31	1,7	3	3
5	M	61	2	SC	R	R	11	13	43	57	6	1	1
6	M	58	0,6	C	R	R	3,3	13	19	46	0,4	7	3
7	M	53	3	C	L	R	11,3	12,7	45	35	3,2	1	1
8	M	56	6	SC	R	L	7,7	12,4	27	44	2,4	2	2
9	M	53	0,5	C	R	R	6,3	14,7	29	44	-0,1	4	3
10	M	63	10	SC	R	L	1,7	8,3	23	47	1,3	1	1
11	F	68	15	C	R	R	9	12,3	32	34	1,8	2	2
12	M	69	3	C	R	R	10	9,7	44	39	1,7	0	1
13	M	82	3	SC	R	L	9	13,7	37	38	3,8	2	2
14	M	74	2	SC	R	R	5,6	9	30	36	2,4	2	2
15	M	62	10	SC	R	R	11	12	44	42	3,8	2	2
16	M	45	0,6	SC	R	R	8	12	21	41	-0,4	4	2
17	F	72	4	SC	R	L	2,3	8	11	23	-0,3	3	4
18	M	75	0,5	SC	R	R	0	11,3	20	43	-1	4	3
19	M	75	4	C	R	L	8,7	12,7	41	44	2,7	0	2

		65 ± 10	**5,22 ± 4,62**	**13SC/6C**	**18R/1L**	**14R/5L**	**7,4 ± 3,7**	**11,5 ± 2,3**	**31,6 ± 10,3**	**39 ± 8,1**	**2,1 ± 1,8**	**2,3 ± 1,8**	**2,1 ± 0,9**

Legend of Table 6.1 (stroke patients). M, male; F, female; SC, subcortical stroke; C, cortical stroke; R, right; L, left; PPT, Baseline Purdue Pegboard Test score; n, number of pegs inserted in 30 sec (mean of three trials); MaxHF, Maximal hand grip force; Kg, kilograms, mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Score, PH: paretic hand, N-PH: non- paretic hand.

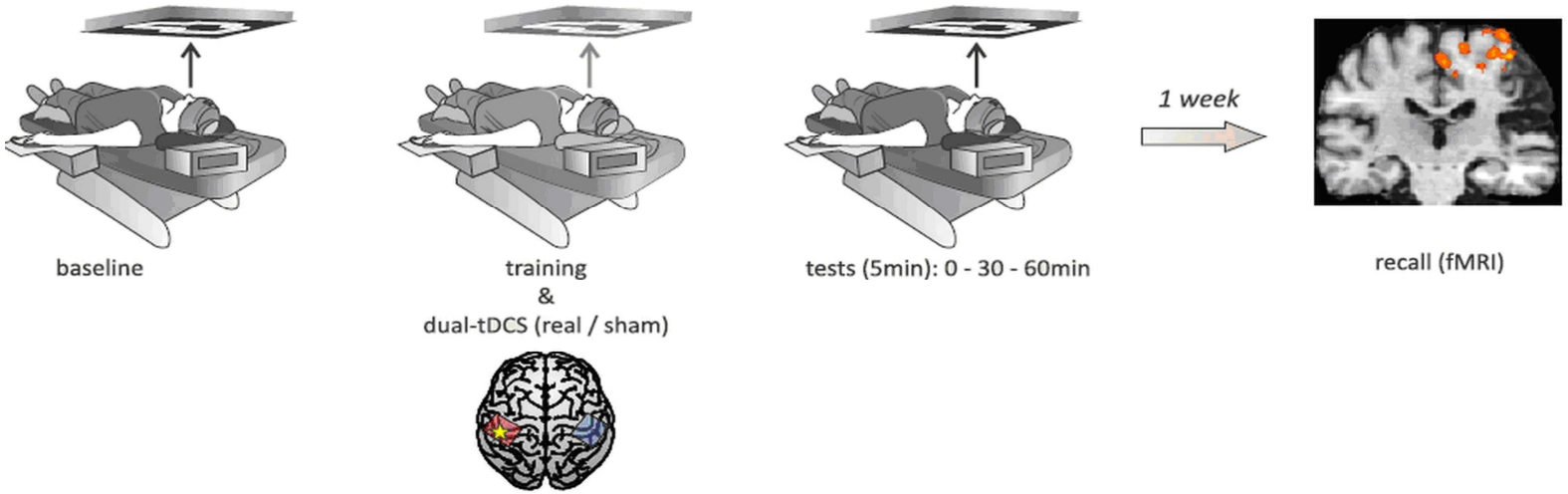


Figure 6.2 Study design

Dual-tDCS

Dual-tDCS (1 mA, 30 min) was applied over the two primary motor cortices (M1) using an Eldith DC-Stimulator® (NeuroConn, Ilmenau, Germany). The stimulation parameters, electrode size, and the hot spot localisation method have been detailed previously (Lefebvre et al. 2013a).

Behavioural analysis

The motor skill learning paradigm (circuit game and its analysis) has been described in detail previously (Lefebvre et al. 2012; Lefebvre et al. 2013a). Briefly, the circuit game consisted of moving a cursor with an MR-compatible mouse held by the paretic hand along a path as quickly and accurately as possible. During the Intervention session, the patients performed the task in the supine position with the circuit projected on the ceiling (Figure 6.2), to match the position in the MR environment. The evolution of motor skill learning was explored by the Learning Index (LI), which was designed to quantify the (evolution of the) SAT compared to Baseline.

In order to compare the amount of motor skill retention one week after learning under real/sham dual-tDCS, paired sample t-tests were used to compare LI of Recall 1 and 2 between Intervention.

To explore a potential transfer towards unspecific motor performance improvement, a RMANOVA was performed on the PPT and MaxHF scores during the Intervention sessions, with Bonferroni t-test post-hoc analysis (see Supplemental Appendix 2); , and a paired t-test for the Recall sessions.

During the delayed Recall sessions, two runs of the circuit game were successively performed by the stroke patients during fMRI acquisition. The performance evolution during the Recall sessions could thus also be evaluated in terms of continued learning. In other words, a Recall session could be considered as a second learning session of the same circuit trained one week before during Intervention. To quantify continued learning evolution, the LI was recalculated using the first circuit block of the Recall session as the new Baseline. Based on LI changes over time (Lefebvre et al.

2012; Lefebvre et al. 2013a), motor skill learning could follow three behavioural trajectories. First, in the most efficient shifters, large LI improvement is driven by a shift in the SAT, which involves either an improvement of both parameters or improvement of one parameter without concomitant degradation of the other. Second, in the less efficient fitters, smaller LI improvement is due to improvement of one parameter with concomitant degradation of the other. Third, LI degradation or stagnation characterises non-learners. Based on LI evolution, the stroke patients were classified as shifters, fitters or non-learners during continued learning. Their respective proportions were compared one week after real/sham dual-tDCS using a Chi-square test.

Pearson correlations analyses were performed to determine whether baseline clinical characteristics (age, mRS, NIHSS, PTT score, time since stroke and ABILHAND score) predicted the individual percentage of LI improvement at Recall 1 after real dual-tDCS. In order to disclose whether the stroke localisation (cortical/subcortical), the fact that the paretic hand was dominant influenced the responsiveness to real dual-tDCS, a Student's t-test was calculated to seek for a difference in the LI of Recall 1 between these subgroups.

fMRI design, acquisition and analysis

The Recall sessions were performed during fMRI acquisitions which consisted of one habituation run (2 min 40 s; 4 blocks of practice on a simple square circuit alternating with 4 blocks of REST) and of two runs of the visuomotor skill learned one week before during Intervention (Figure 6.2) (8 minutes 41 s, 172 scans). Each run contained three conditions (1) LEARNING (performing the circuit game learned one week before as quickly and accurately as possible), (2) EASY (simple motor task condition: moving the cursor back and forth at a comfortable speed between two horizontal or vertical targets, without speed or accuracy constraint) and (3) REPLAY (visual and visuomotor condition: a videoclip of the last LEARNING performance was displayed, and patients were instructed to follow the

cursor's displacement with their eyes while keeping the hands motionless); as well as REST (fixation cross).

fMRI data were analysed using BrainVoyager QX (Version 2.4.2.2070). The fMRI acquisition and pre-processing have been detailed previously (Lefebvre et al. 2012); Lefebvre et al., *personal communication).* A random effect (RFX) analysis was computed for each Recall session (Recall after real dual-tDCS and Recall after sham dual-tDCS).

First, the network involved in the retention of the specific motor skill was compared between the two Recall sessions (one week after real/sham dual-tDCS) with a RFX whole-group analysis of the [LEARNING - REPLAY] contrast, i.e. the brain activation underlying the performance of the motor skill learned one week before *minus* activation related to visual-oculomotor activity. The numbers of activated voxels in [LEARNING – REPLAY] obtained at a p value of 0.05 for each stroke patient were compared between the two Recall sessions both for whole brain activation and separately for each region of interest (ROI) with paired Student t-tests. Next, in the ROIs found with [LEARNING - REPLAY], external Pearson correlation analyses were performed to identify key area(s) where activation had the highest correlations with retained motor skill performance. For this whole-group analysis, the LI of each patients were averaged across the two runs (overall mean LI, reflecting the general level of performance enhancement specific to the motor skill learned one week before). The external Pearson correlation was performed between these overall mean LI and the mean beta weights of each stroke patients across the two runs.

Second, a control whole-group RFX analysis was computed to seek for differential brain activation during [RELAY] between the two Recall sessions. In order to look out for a possible involvement of REPLAY in motor skill performance at Recall, the same correlation analysis used for [LEARNING – REPLAY] was computed in the ROIs found with [REPLAY].

Third, to identity the neural substrates underlying the performance of unspecific, untrained movements performed with the paretic upper limb without performance constraint, a whole-group RFX analysis compared the

brain activation during [EASY] between the two Recall sessions. The numbers of activated voxels in [EASY] obtained at a p value of 0.05 for each stroke patient were compared between the two Recall sessions (whole brain and ROI) with paired Student t-tests. The amount of simple movements (total distance), their speed and normalised jerk [with the formula $NJ = \sqrt{1/2 * \int_{Tstart}^{Tend} jerk^2(t)dt * duration^5 / length^2}$ (Contreras-Vidal and Buch 2003a; Caimmi et al. 2008)] were compared between the two Recall sessions by paired Student's t tests.

Fourth, RFX whole-group analyses were computed with [LEARNING - (REPLAY + EASY)] to compare the brain areas involved in continued learning during the Recall sessions one week after real/sham dual-tDCS. The [LEARNING - (REPLAY + EASY)] contrast reflects the activation related to continued motor skill learning *minus* activation related to visual-oculomotor activity and lower aspects of movement control. The patients failing to achieve continued motor skill learning were excluded from this analysis; the others were categorized as shifters or fitters (see Behavioural analysis). Finally, external Pearson correlation analyses were performed between the beta weights and the PI values in the different ROIs obtained for each Recall session (one week after real/sham dual-tDCS).

6.3. Results

Behavioural results

The impact of real dual-tDCS on online motor skill learning and retention is similar to that described previously (Lefebvre et al. 2013a). Compared to sham, real dual-tDCS improved both the magnitude (Figure 6.3) and the quality of motor skill learning (details in Supplemental Appendix 1). One week after Intervention the LI at Recall 1 after dual-tDCS (42 ± 28%, mean ± SD) was statistically superior to that observed after sham (2 ± 19%; p=0.002) (Figure 6.3); a similar effect was observed at Recall 2 (p=0.005).

As previously observed (Lefebvre et al. 2013a), the specific performance enhancement of the motor skill driven by dual-tDCS transferred on (untrained) dexterity of the paretic hand. One week after real dual-tDCS,

PPT scores remained significantly improved (+0.88 pegs in 30 sec, +12%, p=0.001) compared to Baseline.

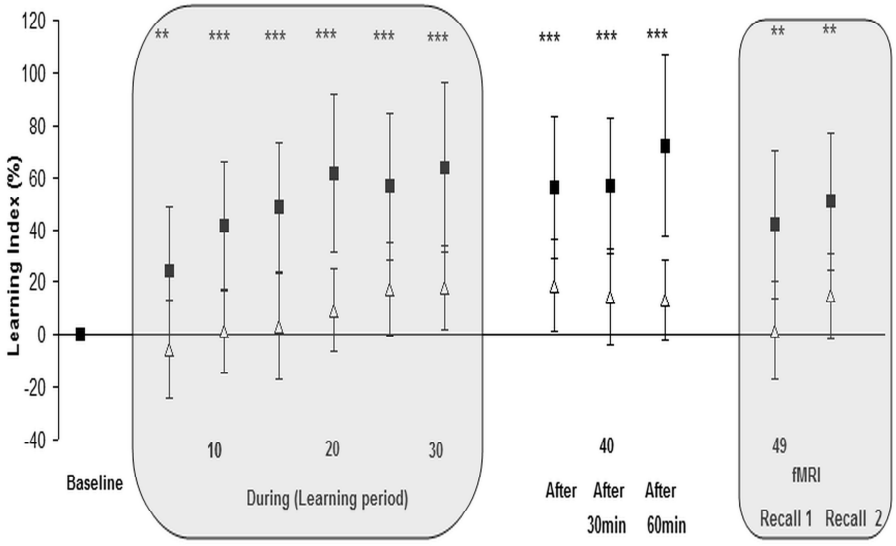


Figure 6.3 Differential evolution of motor skill learning under sham and real dual-tDCS. Evolution of the Learning Index (LI), expressed as a % change from Baseline during the Intervention session (Baseline, Training, Immediate (After), 30 min and 60 min) and Delayed Recall session (fMRI run 1 (Recall 1) and run 2 (Recall 2)). LI is plotted as the mean ± SD of five consecutive blocks of the circuit game. Numbers on the X-axis refer to blocks of the circuit game. White triangles: sham; black squares: real dual-tDCS. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

There was a significant deterioration after sham (-0.58 pegs, -8%, p=0.01) and a significant difference between sham and real dual-tDCS (p=0.001). The MaxHF remained unchanged (see details in Supplemental Appendix 2).

There was no significant correlation between LI improvement at Recall 1 after real dual-tDCS and age (p = 0.97), time since stroke (p = 0.54), mRS (p = 0.12), ABILHAND (p = 0.08), NIHSS (p = 0.9), baseline PPT scores (0.41); nor whether the paretic hand was dominant or not (p = 0.30, Student's t-test). There was a non-significant trend for larger improvement in patients with a cortical stroke (n=6; LI Recall 1: 72 ± 23%) compared to

those with a subcortical stroke (n=13; LI Recall 1: 29 ± 48%; p = 0.052, Student's t-test).

fMRI results

Activation underlying tDCS-enhanced motor skill performance with the paretic upper limb at retention

The whole-group RFX analyses with [LEARNING - REPLAY] revealed different activation patterns between Recall sessions after sham/real dual-tDCS. These patterns were associated with a different level of performance of the retained motor skill: the mean LI was significantly greater after real (52% ± 29) than after sham dual-tDCS (12% ± 20, p=0.002).The brain activation was both less intense and more focused on the motor/premotor network one week after real compared to sham dual-tDCS (Figure 6.4 and Table 6.2).

Group comparisons were performed between the numbers of voxels activated in each patient at the whole brain level and in bilaterally specific ROIs : the SMA proper, pre-SMA, M1, PMd, S1, PPC, DPFC, visual areas and cerebellum. At the whole brain level, the number of activated voxels one week after sham (74 307 ± 65 080 voxels) was greater than one week after real dual-tDCS (35 993 ± 31 203 voxels, p=0.02) and encompassed both hemispheres (Figure 6.5). In the damaged hemisphere, the fMRI activation was more widespread one week after sham (45 432 ± 40 894 voxels) than after real dual-tDCS (15 062 ± 13 748 voxels; p=0.01), with the following specific differences (ROIs comparisons): SMA_{damH} proper (1 670 ± 2 254 voxels versus 521 ± 575 voxels; p=0.02, after sham versus real dual-tDCS, respectively), pre-SMA_{damH} (578 ± 963 voxels vs 69 ± 109 voxels; p=0.03), $M1_{damH}$ (5 282 ± 4 614 voxels vs 2 092 ± 2 282 voxels; p=0.004), PMd_{damH} (6 181 ± 6 479 voxels vs 2 263 ± 2 608 voxels; p=0.002) and $S1_{damH}$ (1 353 ± 1 541 voxels vs 494 ± 640 voxels; p=0.01). In the undamaged hemisphere, there was also a (non-significant) trend for a larger activation one week after sham compared to real dual-tDCS (31 497 ± 29 205 versus 16 499 ± 16 492 voxels; p=0.09), mainly driven by a significantly larger activation in the SMA_{undamH} proper (856 ± 892 vs 352 ± 552 voxels p=0.04) and the pre-

SMA_{undamH} (317 ± 510 vs 82 ± 163 voxels p=0.058) (Figure 6.5). There was no significant difference in the other ROIs.

Correlation analyses were performed between the mean LI (reflecting the general level of performance enhancement specific to the motor skill learned one week before) and mean beta weights of each patient. The mean LI over the two fMRI runs was significantly greater after real (52 ± 29 %) than after sham dual-tDCS (12 ± 20 %, p=0.002). One week after sham dual-tDCS, there was a significant correlation between the mean LI and the mean beta weights of each patient exclusively in $M1_{undamH}$ (r = 0.61, p = 0.005). By contrast, one week after real dual-tDCS, there was a significant correlation exclusively in PMd_{damH} (r = 0.63, p = 0.004).

Visual and visuomotor activations during Recall sessions

Interestingly, there was no difference in the activation pattern for the [REPLAY] contrast between the Recall sessions (one week after real/sham dual-tDCS). Similarly, in the ROIs found with [REPAY], there was no statistically significant correlation between the mean LI and the mean beta weights. Finally, the group comparisons performed between the numbers of voxels activated with [REPLAY] at the whole brain level and in specific ROIs (the same as for [LEARNING –REPLAY]) showed no statistically significant difference between the Recall sessions (one week after real/sham dual-tDCS).

Activation underlying the performance of simple, untrained movements by the paretic upper limb at retention

During the performance of simple, untrained movements (EASY condition), there was a significant difference between the two Recall sessions neither in speed (17 ± 3 u/s after sham versus 18 ± 4 u/s after real, p= 0.28) nor in the total amount of movement (479 ± 92 u versus 503 ± 112 u, p= 0.5) or in the NJ (353 070 ± 201 347 versus 513 756 ± 513 766, p = 0.18) . Despite the fact that EASY motor performance was the same one week after real and sham dual-tDCS, the activation patterns were different. There was more activation in the sensorimotor/premotor network after sham than after real dual-tDCS (Figure 6.4, Table 6.2).

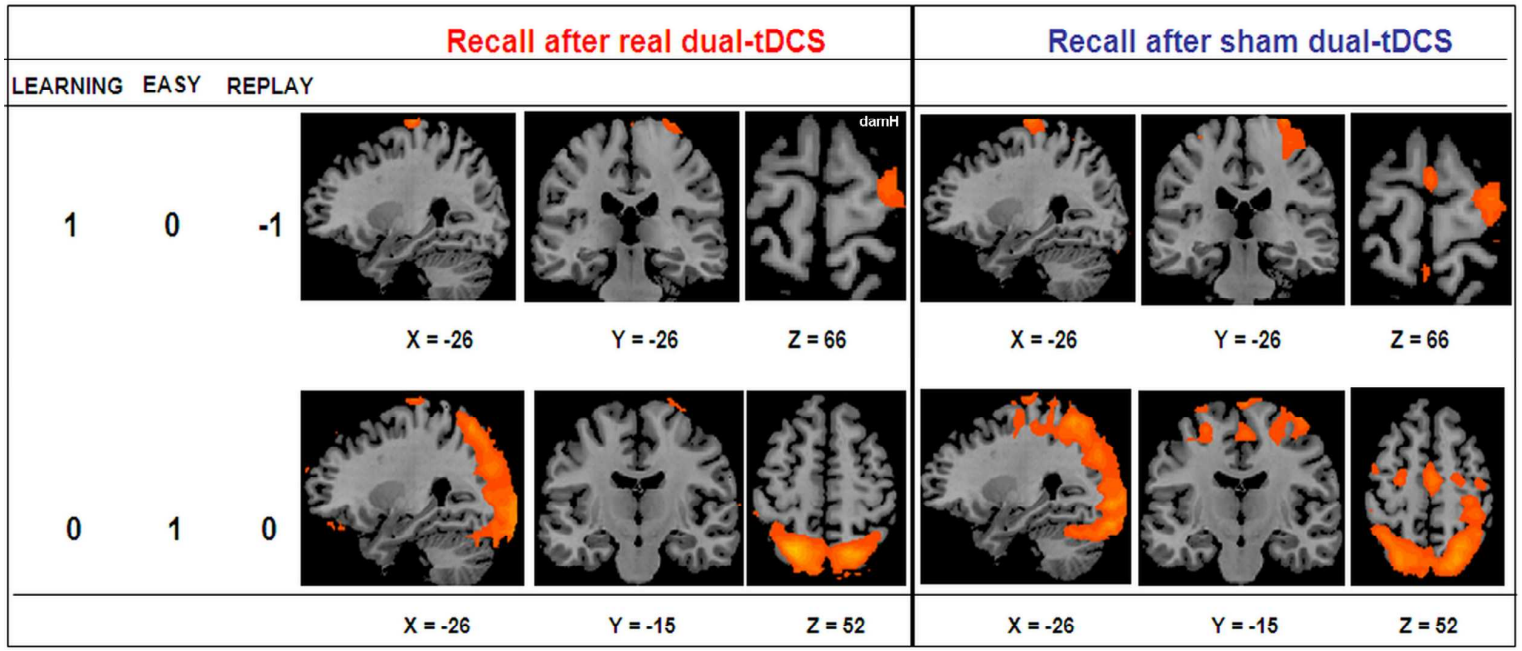


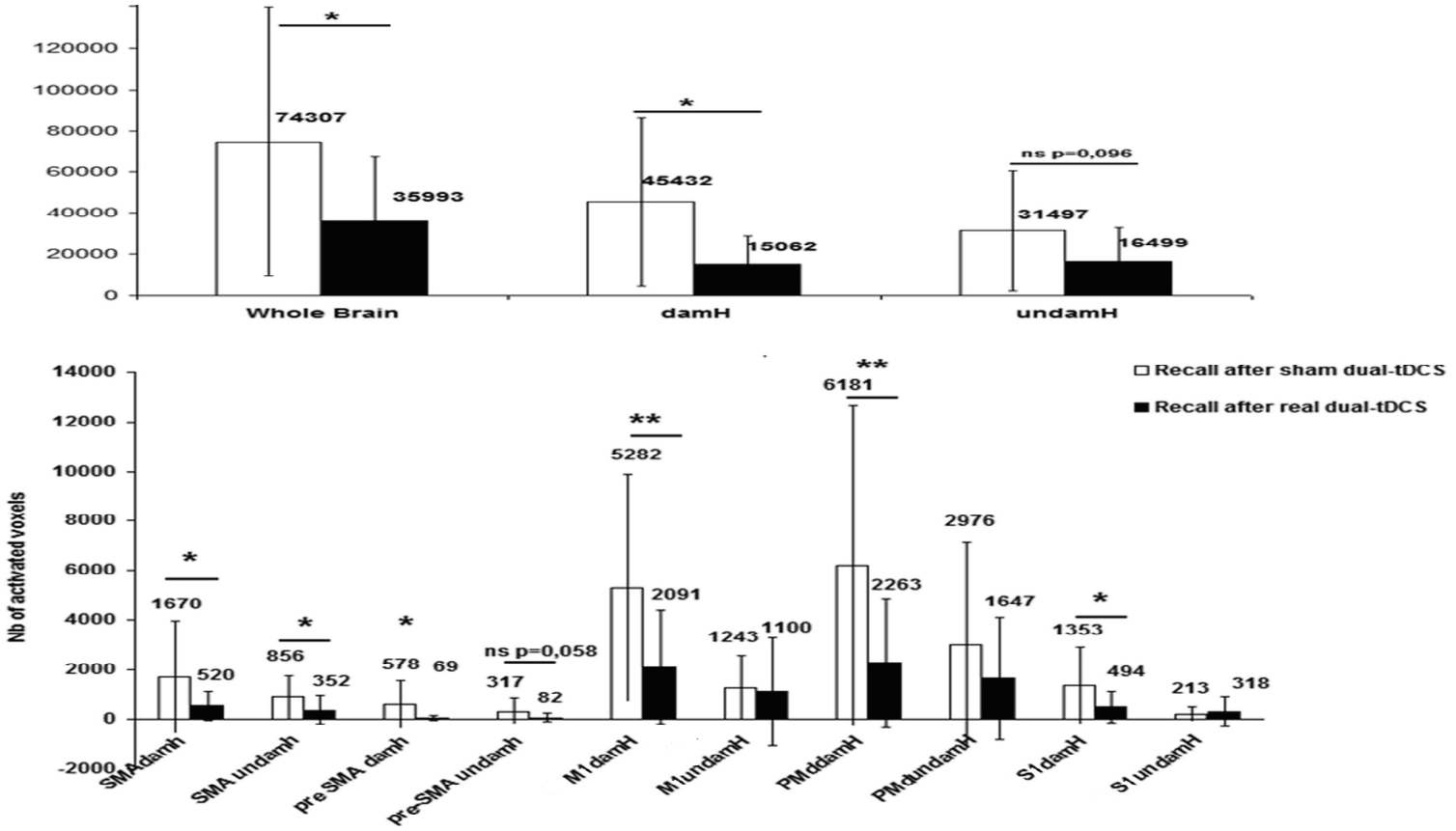
Figure 6.4. Activation underlying tDCS-enhanced motor skill performance and simple motor performance at retention. Whole-group **brain** activation of the 19 stroke patients for the [LEARNING - REPLAY] and [EASY] contrast, RFX at a t_{18}=2.13; $p_{UNCORRECTED}$< 0.05 one week after real/sham dual-tDCS). damH: damaged hemisphere

Table 6.2 Activated areas during the Recall sessions for [LEARNING - REPLAY] and [EASY], RFX: $p_{UNCORRECTED} < 0.05$

	BA	mean x	mean y	mean z	mm³	activation peak (t)
real [LEARNING - REPLAY]						
SMA_{damH}	6	-2	-13	69	89	3.13
SMA_{undamH}	6	1	-10	70	59	2.92
$M1_{damH}$	4	-28	-25	68	381	3.80
PMd_{damH}	6	-27	-18	68	773	3.91
PMd_{undamH}	6	22	-12	69	68	2.61
Cerebellum ipsilateral to paretic hand		51	-63	-30	93	2.44
sham [LEARNING - REPLAY]						
SMA_{damH}	6	-3	-14	60	510	4.26
SMA_{undamH}	6	2	-11	69	289	4.01
pre-SMA_{damH}	6	-1	-4	69	4	2.51
pre-SMA_{undamH}	6	1	-4	69	11	2.56
$M1_{damH}$	4	-33	-29	59	2 810	3.66
PMd_{damH}	6	-30	-19	65	980	3.77
PPC_{damH}	7	-16	-60	59	91	2.91
$S1_{damH}$	3	-37	-37	57	575	3.09
real [EASY]						
SMA_{damH}	6	-4	-28	70	4	2.39
SMA_{undamH}	6	2	-10	71	28	2.33
$M1_{damH}$	4	-30	-27	67	476	3.03
PMd_{damH}	6	-26	-18	69	386	3.22
PMd_{undamH}	6	22	-12	69	91	2.46
PPC_{damH}	7	-20	-68	49	11 313	6.02
PPC_{undamH}	7	18	-65	50	16 816	6.81
cerebellum contralateral to paretic hand		-16	-71	-22	4 789	3.84
cerebellum ipsilateral to paretic		18	-65	-21	10 278	5.80

hand						
visual areas$_{damH}$	18-19	-29	-82	0	21 688	4.73
visual areas$_{undamH}$	18-19	28	-79	-1	36 035	6.55
lentiform nucleus$_{damH}$		-19	-6	1	93	3.16
lentiform nucleus $_{undamH}$		17	-3	3	18	2.66
sham [EASY]						
SMA$_{damH}$	6	-4	-15	56	1 527	3.81
SMA$_{undamH}$	6	2	-20	59	842	3.14
pre-SMA$_{damH}$	6	-1	-2	57	32	2.56
pre-SMA$_{undamH}$	6	4	-1	46	500	3.34
M1$_{damH}$	4	-30	-31	55	3 334	3.59
PMd$_{damH}$	6	-29	-15	58	2 967	3.60
PMd$_{undamH}$	6	33	-8	51	2 145	3.43
S1$_{damH}$	3	-32	-39	56	1 850	4.34
S1$_{undamH}$	3	33	-40	58	201	2.53
PPC$_{damH}$	7	-22	-63	48	16 866	5.55
PPC$_{undamH}$	7	21	-63	47	16 998	4.99
cerebellum contralateral to paretic hand		-20	-70	-24	9 800	4.03
cerebellum ipsilateral to paretic hand		22	-65	-25	12 906	5.10
visual areas$_{damH}$	18-19	-32	-80	-1	19 968	5.21
visual areas$_{undamH}$	18-19	28	-79	1	35 937	5.86
lentiform nucleus$_{undamH}$		16	-5	4	277	2.97

Legend of Table 6.2 BA: Brodmann area, M1: primary motor area, SMA: supplementary motor area PMd: dorsal premotor cortex; PPC: posterior parietal cortex; S1: somatosensory cortex; DLPFC: dorsolateral prefrontal cortex; damH: damaged hemisphere; undamH: undamaged hemisphere, mm³: number of activated voxels.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40000
20000
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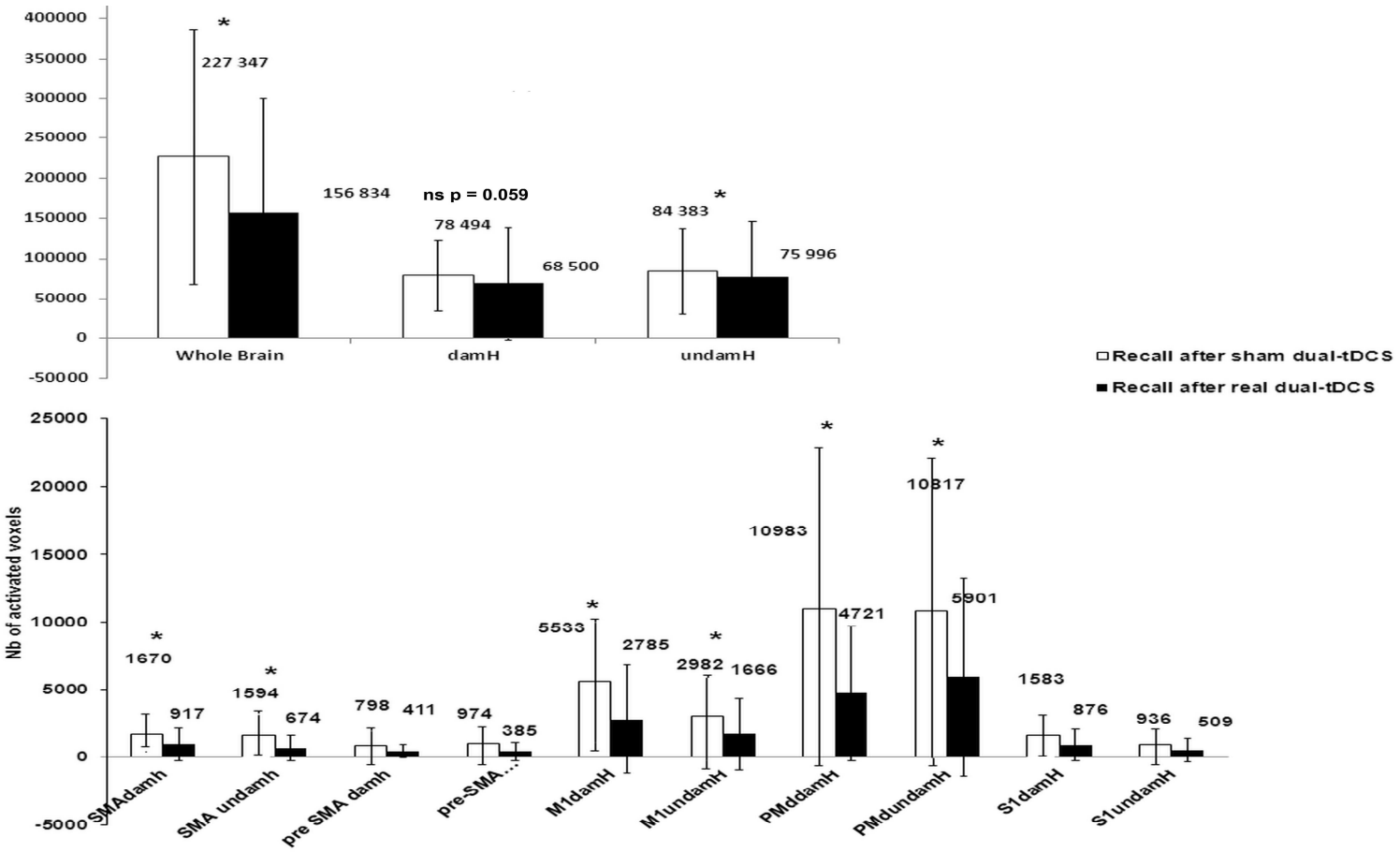
*
74307
35993
*
45432
15062
ns p=0,096
31497
16499
Whole Brain
damH
undamH
14000
12000
10000
8000
6000
4000
2000
0
-2000
Nb of activated voxels
Recall after sham dual-tDCS
Recall after real dual-tDCS
*
1670
520
*
856
352
*
578
69
ns p=0,058
317
82
**
5282
2091
1243
1100
**
6181
2263
2976
1647
*
1353
494
213
318
SMAdamH
SMA undamH
preSMA damH
preSMA undamH
M1damH
M1undamH
PMddamH
PMdundamH
S1damH
S1undamH

Figure 6.5. Comparison of the number of activated voxels one week after real/sham dual-tDCS with [LEARNING - REPLAY]. The number of activated voxels in each ROI was obtained for each patient at a p value of 0.05 for the [LEARNING - REPLAY] contrast, and compared between the two Recall sessions. White/black bars (mean ± SD): one week after sham/real dual-tDCS, respectively. SMA: supplementary motor area; BA Brodmann area; PM: premotor cortex; damH: damaged hemisphere, undamH: undamaged hemisphere.

Group comparisons were performed between the numbers of voxels activated with [EASY] at the whole brain level and in specific ROIs (the same as for [LEARNING –REPLAY]). At the whole brain level, the number of activated voxels one week after sham (227 347 ± 159 109 voxels) was greater than one week after real dual-tDCS (156 834 ± 143 694 voxels, p=0.049) and encompassed both hemispheres (Figure 6.6). In the damaged hemisphere, the fMRI activation tended to be more widespread one week after sham (number of activated voxels: 78 494 ± 44 312) than after real dual-tDCS (68 500 ± 70 242 voxels; p=0.059), with significant differences in the following ROIs: SMA_{damH} proper (1 670 ± 1320 voxels (after sham) versus 917 ± 1 225 voxels (after real); p=0.023), $M1_{damH}$ (5 533 ± 4 449 voxels versus 2 785 ± 3 952 voxels; p=0.019), PMd_{damH} (10 983 ± 11 666 voxels versus 4 721 voxels; p=0.028). In the undamaged hemisphere, the fMRI activation was more widespread one week after sham (84 383 ± 53 121 voxels) than after real dual-tDCS (75 996 ± 70 650 voxels; p=0.048), especially in the following ROIs: SMA_{undamH} proper (1 59 3± 1 500 voxels versus 674 ± 936 voxels; p=0.019), $M1_{undamH}$ (2 982 ± 3 248 voxels versus 1 665 ± 2 638 voxels; p= 0.020), PMd_{undamH} (10 817 ± 11 666 voxels versus 5 901 ± 7 334 voxels; p= 0.047) (Figure 6.6). There was no significant difference in the other ROIs (bilateral pre-SMA, S1, PPC, DPFC, cerebellar hemispheres and visual areas (BA 18 and BA 19)) (Figure 6.6).


400000
350000
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250000
200000
150000
100000
50000
0
-50000
*
227 347
156 834
ns p = 0.059
78 494
68 500
84 383
*
75 996
Whole Brain
damH
undamH
Recall after sham dual-tDCS
Recall after real dual-tDCS
Nb of activated voxels
25000
20000
15000
10000
5000
0
-5000
*
1670
917
*
1594
674
798
411
974
385
*
5533
2785
*
2982
1666
*
10983
4721
*
10817
5901
1583
876
936
509
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SMA undamh
pre SMA damh
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M1damH
M1undamH
PMddamH
PMdundamH
S1damH
S1undamH

Figure 6.6. Comparison of the number of activated voxels one week after real/sham dual-tDCS with [EASY]. The number of activated voxels in each ROI was obtained for each patient at a p value of 0.05 for the [EASY] contrast, and compared between the two Recall sessions. White/black bars (mean ± SD): one week after sham/real dual-tDCS, respectively. SMA: supplementary motor area; BA Brodmann area; PM: premotor cortex; damH: damaged hemisphere, undamH: undamaged hemisphere.

Activation underlying continued motor skill learning with the paretic upper limb one week after Intervention

At the behavioural level, during continued learning one week after sham, there were six non-learners, nine fitters and four shifters. One week after real dual-tDCS, there were three non-learners, four fitters and twelve shifters (Chi-square; p=0.05).

The non-learners were excluded from further analysis. During continued motor skill learning one week after sham dual-tDCS, activation at a RFX t_{12}=2.23 (p<0.05) with the contrast [LEARNING - (EASY + REPLAY)] was observed in the bilateral M1, SMA_{undamH}, $S1_{damH}$, PPC_{damH}, IPC_{damH} (Figure 6.7, Table 6.3). Correlation analyses between the PI and beta weights of each ROI activated showed a statistically significant positive correlation in $M1_{damH}$ (r = 0.74, p = 0.04), $M1_{undamH}$ (r = 0.82, p = 0.01), PPC_{damH} (r = 0.85, p < 0.01) and in IPC_{damH} (r = 0.85, p < 0.01).

During continued motor skill learning one week after real dual-tDCS, activation at a RFX t_{16}=2.23 (p<0.05) was observed in $M1_{damH}$, SMA_{damH}, PMd_{damH}, and in the cerebellum ipsilateral to the paretic hand (Figure 6.7, table 6.3). Correlation analyses showed a significant positive correlation exclusively in PMd_{damH} (r= 0.81, p= 0.02).

6.4. Discussion

The main results of this double-blind, sham-controlled, cross-over RCT are that 30 min of real dual-tDCS compared to sham dual-tDCS applied bilaterally over M1 in chronic stroke patients while they learned a complex motor skill with the paretic hand **i)** enhanced both quantitatively and qualitatively online motor skill learning, **ii)** induced a transfer of performance

improvement to an untrained task (the PPT), **iii)** successfully translated online improvement into long-term retention of the motor skill, and **iv)** that the long-term enhancement driven by real dual-tDCS compared to sham was associated with a normalisation of the fMRI activation pattern when performing the same task (specific component) and when performing a simple non-trained task (unspecific component), **v)** when the patients performed continued-learning on the circuit game at the delayed Recall session, real dual-tDCS improve the quality of the behaviour (more shifters and less non-learners) compared to sham, this improvement is associated with a more efficient recruitment of brain areas in a network which is more similar to the one attempt in healthy individuals compared to sham.

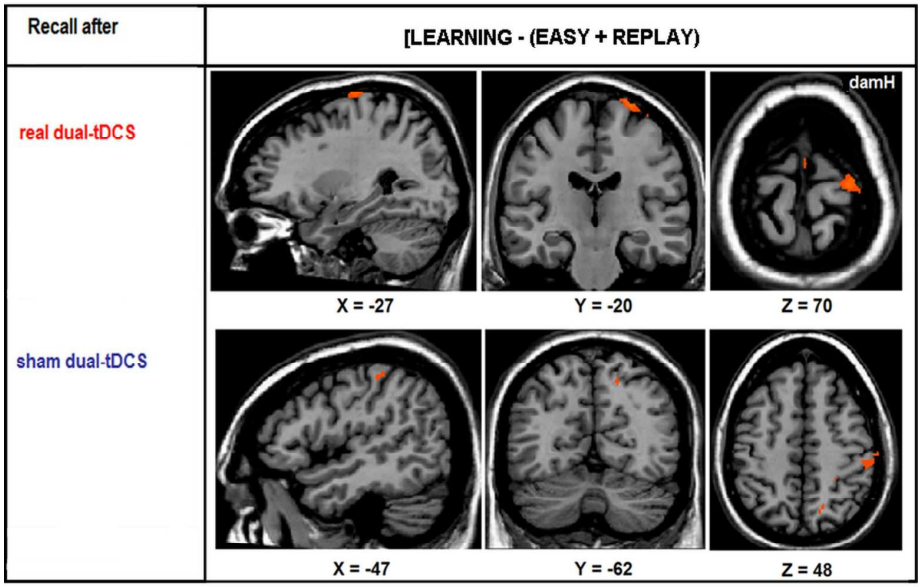

Figure 6.7. Brain activation underlying continued motor skill learning one week after sham/real dual-tDCS. Group activation with the [LEARNING – (EASY + REPLAY] contrast for the patients who achieved successful continued motor skill learning (i.e. fitters and shifters), RFX; $p_{UNCORRECTED} < 0.05$)) one week after real dual-tDCS (n=16 at a t_{15}=2.23) and after sham dual-tDCS (n=13 at a t_{12}= 2.23). damH: damaged hemisphere.

Table 6.3 Activated areas during the Recall sessions for continued learning : [LEARNING- (EASY+REPLAY)] RFX; $p_{UNCORRECTED} < 0.05$

	BA	mean x	mean y	mean z	mm³	correlation	
real [LEARNING - (EASY + REPLAY)]						r	p value
$M1_{damH}$	4	-25	-23	70	56	0.67	0.07
SMA_{damH}	6	-1	-8	69	13	-0.55	0.16
PMd_{damH}	6	-27	-18	68	377	0.81	0.02
cerebellum ipsilateral to the paretic hand		48	-61	-31	43	0.32	0.44
sham [LEARNING - (EASY + REPLAY)]							
$M1_{damH}$	4	-33	-29	40	101	0.74	0.04
$M1_{undamH}$	4	29	-30	37	26	0.82	0.01
SMA_{undamH}	6	2	-9	72	8	0.29	0.49
PPC_{damH}	7	-24	-56	47	65	0.85	0.001
$S1_{damH}$	3	-45	-27	46	188	0.69	0.006
IPC_{damH}	7	-33	-36	37	66	0.85	0.001

Legend of Table 6.3. BA: Brodmann area, M1: primary motor area, SMA: supplementary motor area PMd: dorsal premotor cortex; PPC: posterior parietal cortex; S1: somatosensory cortex; DLPFC: dorsolateral prefrontal cortex; damH: damaged hemisphere; undamH: undamaged hemisphere, mm³: number of activated voxels.

Quantitative and qualitative impact of dual-tDCS on online motor skill learning

Real dual-tDCS induced a significantly greater improvement of online motor skill learning (69%) compared to sham (20%). Under sham dual-tDCS, six patients were unable to achieve online motor skill learning (non-learners). Among the thirteen stroke patients who achieved online motor skill learning under sham dual-tDCS, only four adopted the most efficient behaviour of motor skill learning, the shift behaviour. The nine other patients followed a fit behaviour. In sharp contrast, there was only one non-learner patient whose performance deteriorated under real dual-tDCS. Among the eighteen stroke

patients who achieved online motor skill learning, two were classified as fitters and sixteen as shifters.

Thus, these observations confirmed the results of our previous study (Lefebvre *et al.* , 2013) and demonstrated that compared to sham, dual-tDCS improved both online motor skill learning quantitatively and *qualitatively* through increasing the shift of the SAT (i.e. more efficient motor skill learning). After real dual-tDCS, this improvement was maintained during the Early Recall tests (0, 30 min after the end of the stimulation) and enhanced after 60 min to 75%, whereas there was a little drop during the Early Recall after sham dual-tDCS to 13%. The little drop observed after sham dual-tDCS could be explained by a fatigue or a weariness effect even if this has not been formally tested.

Impact of dual-tDCS on long-term motor performance improvement

In the ten stroke patients who started with real dual-tDCS during the first Intervention, the motor performance improvement driven by real dual-tDCS resulted in a carry-over effect, i.e. an increase of the motor performance that persisted more than one week and influenced the Baseline motor performance at the next (sham) session. However, the motor skill learning behaviour (learning and improvement of a different circuit) was not compromised by the order of the interventions. Indeed, online motor skill learning improved much more during real dual-tDCS compared to sham, and the long-term motor retention remained better one week after real dual-tDCS than after sham. In addition, this motor performance improvement induced by real dual-tDCS led to a transfer of the improvement to digital dexterity quantified with a non-trained task (PPT).

Long-term impact of dual-tDCS on continued motor skill learning

When they were subjected to a second (shorter) training session (the delayed Recall session) on the same circuit as that presented one week before during Intervention session (continued motor skill learning), more stroke patients adopted the more efficient shift behaviour (n= 12) after real dual-tDCS compared to sham (n=4), less stroke patients adopted the fit

behaviour after real dual-tDCS (n=4) compared to sham (n=9), and less stroke patients did not achieved continued learning after real dual-tDCS (non-learners, n=3) compared to sham (n= 6).

As the patients received the same instructions, there is no obvious reason explaining these different behaviours, although the non-learners seemed to experience some fatigue and the fitters seemed to adopt a less efficient motor skill learning strategy than the shifters. One could speculate that after several training sessions, the fitters would also adopt a shift behaviour.

Since there was no identified clinical or demographic reason for this distinction, and since this dichotomy between “shifters” and “fitters” has also been observed in healthy individuals learning the same skill (Lefebvre et al. 2012), one could suppose that there is a fundamental distinction between the populations who adopted spontaneously a “fit” or a “shift” behaviour. Hypothetically, this distinction could be explained by the genetic background. E.g., the BDNF polymorphism is known to modulate the capacity to successfully achieve motor learning such as experience dependent plasticity (Kleim et al. 2006; McHughen et al. 2010) or motor skill learning (Fritsch et al. 2010; Li Voti et al. 2011). In terms of neurorehabilitation, the application of dual-tDCS during training could increase the quality and the recovery of motor function.

Brain activation related to the retention of (tDCS-enhanced) motor performance: specific component

To uncover the brain network correlating with retention of motor performance on the trained task one week after Intervention, we used the [LEARNING – REPLAY] contrast. This contrast focuses on the activation involved in the performance of the learned skill (retention: LEARNING) subtracting the activation associated to visual-oculomotor activity (REPLAY). This contrast allowed observing the brain activation associated with the amount of long-term motor performance improvement.

Thus, the improved long-term motor skill retention observed after real dual-tDCS compared to sham was associated with a reorganised

network during the retention test, namely much weaker fMRI activation in the undamaged hemisphere and focusing on M1-PMd in the damaged hemisphere. This observation is reminiscent of the inter-hemispheric transfer of brain activation associated with motor function recovery after stroke, with an enhancement (decrease) of activation in the damaged (undamaged) hemisphere (Chollet et al. 1991; Nelles et al. 2001; Johansen-Berg et al. 2002a; Calautti and Baron 2003). It is remarkable that this reduced, focused brain activation observed one week after motor skill learning under real dual-tDCS compared to sham correlated with better motor performance, suggesting a more efficient recruitment of neural resources that persisted one week after real dual-tDCS.

Brain activation related to a simple non-trained task: unspecific component

To uncover the brain network correlating with the motor on a simple non-trained task one week after Intervention, we used the [EASY] contrast. This contrast focuses on the activation involved when performing a very simple motor control task with the paretic hand which could be considered to the unspecific component of motor skill retention.

In this very simple task, without any constraint (of velocity or accuracy) the performance of the patients was similar during the two conditions (recall after real or sham dual-tDCS). Nevertheless, despite the similar performance, the performance of the patients at the recall after real-dual tDCS was associated with a reorganised network compared to the one observed after sham dual-tDCS. Actually there is a bilateral reduction of the recruitment and a focusing on simple motor-premotor network after real-dual tDCS.

Brain activation related to continued motor skill learning

To explore the brain activation correlating with the continued learning of the motor skill trained one week before during Intervention, we used the [LEARNING – (EASY + REPLAY)] contrast. This contrast focuses on the areas involved in continued motor skill learning, by subtracting the

activation associated with “lower” aspects of motor control/execution (EASY) and visual-oculomotor activity (REPLAY).

During continued learning one week after real dual-tDCS, more stroke patients (n=12) adopted the more efficient shift behaviour compared to sham (n= 4). This enhanced continued learning after real dual-tDCS was associated with a more focused, restrained brain activation pattern, more similar to the network observed during motor skill learning in healthy individuals (Lefebvre et al. 2012), compared to the widespread activation found after sham. The network related to continued learning after real dual-tDCS encompassed activation especially in the PMd_{damH}, the SMA_{damH}, $M1_{damH}$ and the cerebellum ipsilateral to the paretic hand. In addition, in this network, the continued motor skill learning behaviour correlated significantly with brain activation in $PMd_{damH.}$ By contrast, the network related to continued learning after sham dual-tDCS encompassed more widespread activation in bilateral M1, SMA_{undamH} and sensorimotor and parietal areas in the damaged hemisphere. In this network, the continued motor skill learning behaviour correlated significantly with brain activation in $M1_{undamH.}$

Strikingly, the specific correlation observed after real dual-tDCS is also observed in stroke patients with the most efficient motor skill learning behaviour (shift) during early motor skill learning without tDCS (Lefebvre et al. *in preparation*[1]). These networks and correlations showed that dual-tDCS applied during motor skill learning improved the neural network associated with continued learning one week later (transfer to an efficient network in the damaged hemisphere) and leads to an improvement in the quality of continued learning behaviour.

We acknowledge that the subgroups of stroke patients who achieved continued motor skill learning (either as shifters or fitters) during the delayed Recall sessions were not the same one week after real dual-tDCS and sham. Indeed, **i)** some of them could not achieve continued motor skill learning and were thus excluded from analysis as non-learners (n= 3 after real dual-tDCS, n= 6 after sham). Furthermore **ii)**, as already mentioned, the proportions of continued shifters and fitters were not the same one week after real dual-tDCS and sham. However, despite this limitation, the striking

focusing of fMRI activation onto PMd_{damH}, $SMA_{damH,}$ $M1_{damH}$ and cerebellum ipsilateral to the paretic hand during continued motor skill learning one week after real dual-tDCS compared to sham suggest again a sustained and more efficient recruitment of neural resources.

General conclusion

The motor function improvement associated with real dual-tDCS in chronic stroke patients using their paretic hand was associated with a (relative) "normalisation" of the brain activation pattern compared to sham, specifically **i)** a lesser activation of the undamaged hemisphere and **ii)** a focusing of the activation in the damaged hemisphere onto M1-PMd. Such a re-balancing of brain activation towards the damaged hemisphere has been observed longitudinally during motor recovery (a shallow concept) after stroke (Nelles et al. 2001; Johansen-Berg et al. 2002a; Calautti and Baron 2003). Our study also confirms that chronic stroke patients relied on a reorganised brain activation network compared to healthy individuals (Lefebvre et al. 2012), involving especially the PMd_{damH}, to achieved continued motor skill learning. This network is similar to that observed in shifter stroke patients during early motor skill learning without tDCS (Lefebvre et al. *in preparation*[1]). This study confirms the potential of dual-tDCS as a tool to improve neurorehabilitation benefit for stroke patients and raises the question of the key target area for brain stimulation.

Supplemental Appendix 1: on-line motor skill learning

A repeated measures analyse of variance (RMANOVA) was used to explore the effect of Intervention (training with real/sham dual-tDCS) and Time (Baseline, Training, After 0 min, 30 min, 60 min) on the LI evolution. Bonferroni t-test post-hoc analyses were used to compare each LI value between Intervention.

RMANOVA demonstrated that the LI during Training and up to 60 min after showed a significant interaction between Time and Intervention ($p < 0.001$) (Figure 6.3), suggesting that real dual-tDCS enhanced motor skill learning online and during Early Recall compared to sham (end of Training: 64% ± 32 for real dual-tDCS versus 18% ± 16 for sham; Early Recall 60 min: 72% ± 35 for real dual-tDCS versus 13% ± 16 for sham). Post-hoc analyses demonstrated that dual-tDCS led to a significantly greater and more rapid improvement than sham since the beginning of the Training. No order effect was found between the two arms (crossover design) for the LI evolution ($p = 0.298$). At the end of the Training under sham dual-tDCS, there were 6 non-learners, 9 fitters and 4 shifters ; whereas under real dual-tDCS there were 16 shifters, 2 fitters and only 1 non-learner.

Supplemental Appendix 2. Evolution of the PPT score at the Early Recall test.

The PPT score of the paretic hand improved over time after dual-tDCS (e.g., 60 min later: + 1.97 pegs inserted in 30 sec (+28%)) but not after sham (+0.25 pegs (+3%)). RMANOVA showed a significant interaction between Time and Intervention ($p < 0.001$), suggesting that dual-tDCS had an impact on the evolution of the PTT score across the Intervention session. Post-hoc analyses demonstrated that the Baseline PPT was not significantly different between dual-tDCS and sham ($p = 0.24$), whereas there was a statistically significant difference ($p < 0.001$) since the Early Recall (30 min).

Chapter 7: Conclusions

7.1 Purposes overview

The global aging of the population has two main consequences on stroke care management. First, aging people are more vulnerable to develop stroke, therefore, the incidence of stroke will likely keep rising during the next decades (Lopez et al. 2006; Donnan et al. 2008). Second, the stroke survivors live longer with their motor deficits, which constitute a heavy burden for them and for the community. Therefore, the development of new neurorehabilitation strategies to improve motor recovery is essential. Since motor learning is one essential component of motor recovery (Krakauer 2006; Dipietro et al. 2012), neurorehabilitation methods or techniques increasing motor learning could improve post-stroke motor function recovery.

The present work has four main goals. **i)** To explore the ability of dual-tDCS to improve short-term motor performances in chronic stroke patients. **ii)** To explore the ability of dual-tDCS to improve both online motor skill learning and its long term retention in chronic stroke patients. **iii)** To identify the brain areas involved in motor skill learning with a SAT in both chronic stroke patients and healthy individuals, by means of fMRI. **iv)** To explore with fMRI the neural substrates underlying the motor skill learning improvement induced by dual-tDCS.

7.2. Contribution of this work to the field : Summary of Chapters 2-6

The main results and conclusions gathered from the six experimental parts of the present work (Chapters 2 to 6) could be summarised as follows:

In the first experiment (Chapter 2, (Lefebvre et al. 2013b)), the impact of real versus sham dual-tDCS on digital dexterity (evaluated by the PPT) and precision grip (quantified with a dedicated manipulandum) were compared in a randomised, double-blind, sham-controlled, cross-over study in 19 chronic stroke patients using their paretic hand. Chronic stroke patients showed an improvement in paretic hand digital dexterity (PPT score) during real dual-tDCS; this improvement kept on growing up to 20 min after the end

of the real dual-tDCS compared to sham. In contrast, a protracted improvement after the termination of the real dual-tDCS compared to sham was observed for the precision grip with the paretic hand, as quantified with several parameters. These delayed improvements are in line with the previous studies using rTMS or cTBS on similar tasks (Mansur et al. 2005; Ackerley et al. 2010) and using classical tDCS to improve motor function of the paretic hand (Hummel et al. 2005; Hummel et al. 2006). This is the first study to demonstrate the ability of dual-tDCS to improve digital dexterity and precision grip with the paretic hand in chronic stroke patients. It is worth noting that the magnitude of these improvements seemed to correlate inversely with the level of impairment: the more impaired patients experienced the greatest benefit from real dual-tDCS-induced motor improvement compared to the less impaired patients. This study demonstrates the ability of dual-tDCS to induce a lasting enhancement of motor performance with the paretic hand in chronic stroke patients. These lasting improvements of the paretic hand's motor function were observed without concomitant detrimental effect on the non-paretic upper limb and any degradation in the paretic upper limb. However, in a recent study, some impairment such as deterioration of the proximal upper limb control were observed in the paretic upper limb after uni-hemispheric cathodal stimulation of the undamaged hemisphere in the more impaired patients (Bradnam et al. 2011; Bradnam et al. 2013). In the present study (Chapter 2, (Lefebvre et al. 2013b)), we did not observed similar impairment, neither on the grip lift task nor on the PPT, even in the more impaired patient. It should however be mentioned that the stroke patients included in this study were not severally impaired (i.e. they were able to make a precision grip with the paretic hand).

A pilot clinical trial, involving 18 chronic stroke patients is detailed in Chapter 3 (Lefebvre et al. 2013a). For the first time in chronic stroke patients, a complex motor skill learning task based on a recent definition of motor skill learning (the SAT (Reis et al. 2009; Krakauer and Mazzoni 2011) was used. Spontaneously, three distinct behaviours could be adopted during this task. First, the most efficient is the shift behaviour characterized by a large shift in the SAT, driven by improvement of both speed and accuracy or

by the improvement of one parameter without concomitant deterioration of the second parameter. Second, the fit behaviour is characterized by a modest global performance improvement, due to the combination of improvement of one parameter and a concomitant deterioration of the second parameter. Third, the non-learning behaviour is characterized by a degradation of performance over time. Compared to sham, real dual-tDCS applied during training on the circuit game induced an online improvement in both quantity and quality of motor skill learning (switch of the behaviour from fit to shift, or from a lack of learning to fit or shift behaviour). This online improvement translated into an enhanced motor performance one week later, at a Recall session. Furthermore, the motor performances on non-trained tasks such as the PPT and a new version of the circuit game were improved at the Recall session following real dual-tDCS, compared to sham. These improvements were independent of the level of impairment, since both the less and the more impaired stroke patients benefited from real dual-tDCS. This study is the first to demonstrate the ability of dual-tDCS to improve online motor skill learning and its long-term retention in chronic stroke patients. No degradation was observed in either the paretic or non-paretic hand during or after real dual-tDCS, bringing another argument for the use of the dual-tDCS. In the present study, dual-tDCS improved not only the quality and quantity of motor skill learning and its retention after one week, but also induced a carry-over effect. After real dual-tDCS, the motor performance at the next intervention session (sham session) was significantly improved (carry-over effect). As the ability to learn a new version of the circuit game was not inhibited, we can assume that after a single session of dual-tDCS, there was a strong and lasting improvement of motor performance not only on a trained task. This is thus an important step in neurorehabilitation, since it suggests that dual-tDCS could be used in clinical practice as an add-on tool to physiotherapy and occupational therapy to improve as well long term motor performances and the quality of the movement.

In the third experiment (Chapter 4, (Lefebvre et al. 2012)) the neural substrates underlying motor skill learning during the newly developed circuit

game were explored using fMRI in a group of 20 healthy individuals using their non-dominant left hand. Despite the fact that the same instructions were given, the healthy individuals adopted distinct behavioural patterns: 11 individuals adopted the shift behaviour and 9 individuals adopted the fit behaviour. Each of these behaviours was associated with a specific brain activation pattern. During motor skill learning, brain activation at the whole-group level was observed in the SMA, the contralateral right M1, the ipsilateral left anterior cerebellum and the contralateral thalamus. Within this motor skill learning network, there was a significantly greater activation in the SMA in the shifters compared to the fitters. In addition, the SMA was the only area in which the fMRI activation correlated with the motor performance improvement in the shifters.

In the fourth experiment (Chapter 5 (Lefebvre et al. *in preparation*[1]), the neural substrates underlying early motor skill learning during the circuit game were explored using fMRI in a group of 25 chronic stroke patients using their paretic hand. At the behavioural level, stroke patients spontaneously adopted three distinct behaviours: 9 the shift behaviour, 14 the fit behaviour and 2 the non-learning behaviour. As we previously observed in healthy individuals (Lefebvre et al. 2012), these distinct behavioural evolutions (shift / fit) were associated with specific brain activation patterns. During early motor skill learning, at the whole group level, brain activations were observed in the SMA_{damH} proper, the $M1_{damH}$, the PMd_{damH}, the bilateral S1, the $DLPFC_{damH}$ and bilateral IPC (BA7). The fMRI activation correlated positively with the global motor performance evolution (i.e. with motor skill learning) only in the PMd_{damH} whereas a negative correlation was observed in the DLPFC $_{damH.}$ In addition, there was a significantly greater activation in the bilateral PMd in the shifters stroke subgroup compared to the fitters subgroup. Finally, the fMRI activation changes in the bilateral PMd presented the strongest correlation with motor performance evolution in the shifter stroke patients. In the fitter stroke patients the bilateral PPC were associated with motor skill learning, even if, no significant correlation was found between these areas and the motor performance improvement. Spontaneously, more chronic stroke patients

adopted the less efficient fit behaviour (14/25); similar observation has been done in previous study (Chapter 3, (Lefebvre et al. 2013a)). This suggests that stroke patients, like healthy individuals, spontaneously adopt different strategies or behaviours to learn a new visuomotor skill and that shifters are intrinsically more efficient than fitters, at least during the early stages of motor learning. In addition, the functional network underlying motor skill learning in chronic stroke patients encompassed a larger network compared to healthy individuals. This reorganised, supposedly compensatory, network involved especially PMd which is known to be of particular importance in motor skill learning (Kantak et al. 2012; Hardwick et al. 2013) and in motor function recovery after stroke (Carey et al. 2002; Tombari et al. 2004; Lotze et al. 2006b).

In the fifth experiment (Chapter 6 (Lefebvre et al. *in preparation*[2]*)*), the neural substrates underlying dual-tDCS-induced motor skill learning improvement were explored using fMRI in a group of chronic stroke patients (n=19). In this study, the behavioural results presented in Chapter 3 (Lefebvre et al. 2013a) were confirmed in a new cohort of chronic stroke patients: compared to sham, dual-tDCS improved significantly online motor skill learning with the paretic hand both in quantity and quality, and enhanced its long-term retention after one week. In addition, the motor skill learning behaviour during the delayed Recall session (continued learning) was also modified in terms of quality: more chronic stroke patients adopted the (more efficient) shift behaviour one week after real dual-tDCS than after sham. We observed that the motor skill enhancement retention one week after real dual-tDCS was associated with a reorganised network compared to sham, i.e. a transfer of fMRI activation from the undamaged hemisphere to a focusing on M1-PMd in the damaged hemisphere. A similar observation was made for the network involved during the continued learning behaviour. The normalisation of the brain activation pattern was characterised by a transfer of the brain activation from the undamaged towards the damaged hemisphere, and a focusing within the damaged hemisphere into M1 and PMd, as observed longitudinally during motor function recovery after stroke (Nelles et al. 2001; Johansen-Berg et al. 2002a; Calautti and Baron 2003).

This study confirms the potential use of dual-tDCS as a tool to improve neurorehabilitation benefit for stroke patients, and starts to unveil the neural substrates of tDCS-induced long-term enhancements.

7.3. Motor skill learning

7.3.1. Motor skill learning in post stroke recovery

Post-stroke recovery has to improve patients' motor function in daily life activities and relies on the ability to use the spared neural resources to compensate for motor planning, execution, feedback, control and manual ability. Although recovery relies on the resolution of metabolic events, the adaptive recruitment of spared neuronal resources and the functional and/or structural reconfiguration of the motor system, the stroke patients will somehow have to learn how to use these spared neuronal resources and reconfigured motor network. In other words, after stroke, motor recovery, among all other mechanisms, is based on a form of motor skill learning (Krishnan 2006; Schubring-Giese et al. 2007; Hosp and Luft 2011). So motor skill learning is an essential component in the recovery process of the daily life activities. It should be acknowledged that the motor skill learning paradigm used in this thesis (the circuit game) permits only to explore "functional limitation" with a specific laboratory task and is not designed to quantify "activity limitation" (see ICF Figure 1.1) (Arnould et al. 2007). In addition, in the studies presented in this thesis, a crucial component has not been evaluated: the transfer of motor performance improvement from specific laboratory task to daily life activities. Actually, these studies are a first step to explore the neuronal substrates of motor skill learning after stroke and the impact of dual-tDCS on motor recovery and motor skill learning. Future studies should focus on "activity limitations" and, by example, on bimanual tasks (Gordon et al. 2007), in order to address the patient's specific needs, because stroke patients are impaired in hands coordination and bimanual activities. Additional studies should also explore the transfer of motor performance improvement to something useful for the patients, such as long-term measurement of manual ability improvement

with the ABILHAND scale or with more clinical scales such as the Fugl-Meyer Test.

7.3.2. Modern motor skill learning paradigms: SAT function

Motor skill learning is defined as a change in motor performance inducing a change in the capability of the motor system, i.e. in its operating characteristic. An operating characteristic is related to the relationship between different movement parameters, such as speed and accuracy (Hallet 2005). Spontaneously, motor performances follow the Fitt's law, i.e. slower movements are more accurate whereas faster movements are less accurate (Fitts 1954). The Fitt's law is a way to formulate the SAT function (Figure 7.1). Motor skill learning induces a change in this pre-established relationship, a change in the operating characteristic, i.e. faster movements become also more accurate.

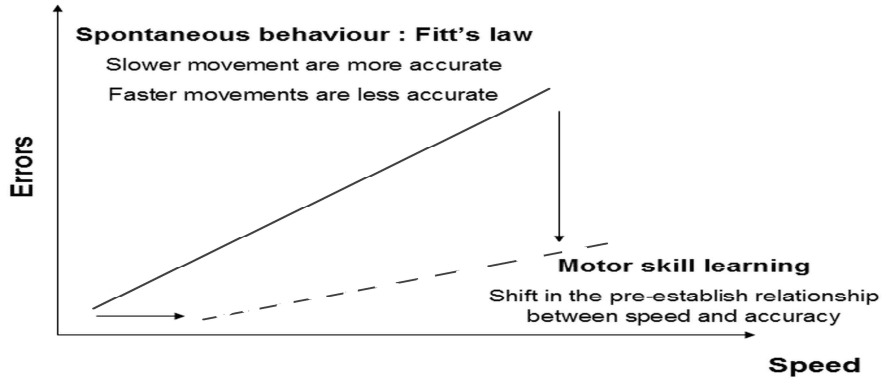

Figure 7.1: Schematic representation of the relationship between speed and accuracy (error) in a movement task (**SAT function).** The black line represents the spontaneous relationship (the Fitt's law), and the dotted line represents the modification of this relationship after motor skill learning. The abscise represents the speed of the movement and the ordinate represents the accuracy of the movement (high error = less accurate movement)

The majority of motor skill learning studies explored speed and accuracy separately (Karni et al. 1995; Walker et al. 2003; Kim et al. 2004; Kim et al. 2006). This might have lead to some ambiguity if the changes were too subtle. New motor skill learning paradigms have been designed

especially to capture the evolution of concomitant evolution of speed and accuracy (Reis et al. 2009; Krakauer and Mazzoni 2011). The circuit game developed for this thesis, (Chapters 3-6),(Lefebvre et al. 2012; Vandermeeren et al. 2012; Lefebvre et al. 2013a); Lefebvre et al. *in preparation*[1]; Lefebvre et al. *in preparation*[2]), relies on this modern definition of motor skill learning. With this paradigm, we could explore both global motor performance evolution (LI, combining both speed and accuracy) and the evolutions of speed and accuracy separately. With this paradigm, subjects could spontaneously adopt three distinct behaviours. The shift behaviour, which is more efficient, involves a global performance improvement with no degradation of speed or accuracy. The fit behaviour, which is less efficient, involves a smaller global performance improvement with a strong improvement of one of the operating characteristic (e.g. speed) concomitant to a small degradation of the second one (e.g. accuracy). Finally, the non-learning behaviour involves a degradation of global performance over time, with either a simultaneous degradation of the two operating characteristics or a strong degradation of one operating characteristic concomitant to a small improvement of the second one. The non-learning behaviour also refers to a stagnation of the global motor performance over time. Figure 7.2 illustrates these different possibilities via the behaviour adopted by chronic stroke patients in the two dual-tDCS studies presented in this thesis (Chapters 3 and 6. (Lefebvre et al. 2013a); Lefebvre et al. *in preparation*[2]) (Figure 7.2).

The reason why both healthy individuals and stroke patients spontaneously adopted one of these different behaviours (shift, fit and non–learning) is open to debate. On the one hand, one could reasonably assume that in some healthy individuals and stroke patients, the fit behaviour is an intermediate exploratory step before achieving the shift behaviour. After a longer training period or if they were subjected to several learning sessions, maybe the fitters would also have achieved a shift behaviour. Do the fitters need more time to familiarise with the task or to explore the dimensions of the task? This remains to be determined. It is however worth mentioning that although possible, this could not fully explain this observation. Indeed, **i)** a

familiarization was always provided before learning, and **ii)** the fit behaviour was also observed in chronic stroke patients during the second sham session, i.e. after they already trained during 30 min under dual-tDCS (and achieved shift motor skill learning for some of them) and performed the Recall session one week later. Alternatively, the fit pattern may truly reflect less efficient neural processes, leading to smaller increment in online motor skill learning.

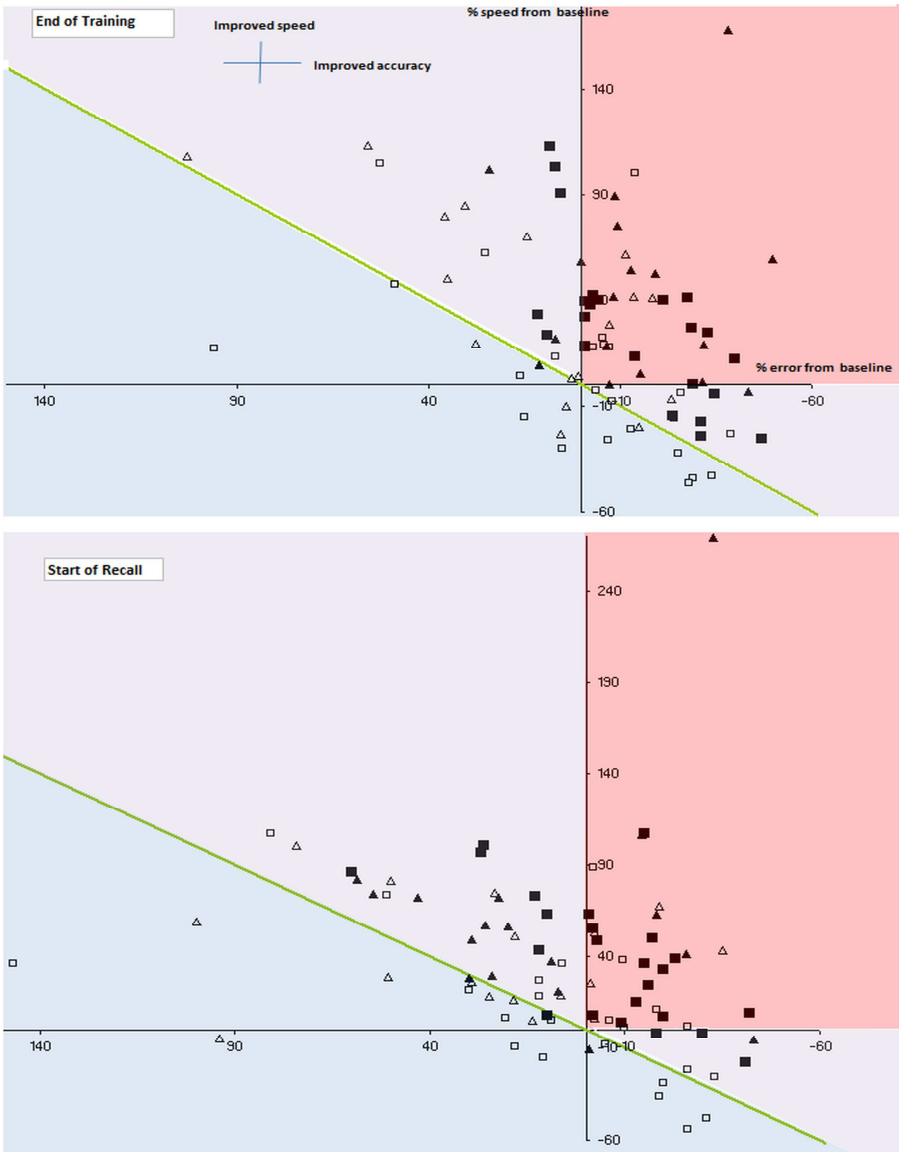

Figure 7.2: Example of SAT. Scatter plot of the trade-off between error (abscise, i.e. the inverse of accuracy) and velocity (ordinate) for the 42 chronic stroke patients expressed as percentage of change from Baseline, for each patient after real dual-tDCS (black) or sham dual-tDCS (white) at the end of Training (upper panel) and at the start of the Recall session (lower panel). Squares: patients who trained in the sitting position. Triangles: patients who trained in the supine position and in whom Recall was performed in the MRI scanner. The green line reflects the Fitt's law: sliding along this line represent a strict respect of the Fitt's law ("perfect fitter"), below this line, motor skill learning is not achieved (deterioration or of the LI; light blue zone), above the green line, motor skill learning is achieved since the LI is increased, either as the less efficient fit behaviour (light purple zone) or as the more efficient shift behaviour (pink zone).

In healthy individuals, the fit behaviour is associated with a brain pattern showing less activation in the SMA, which is essential for this kind of motor skill learning as suggested previously (van Mier et al. 1998). In addition, in chronic stroke patients, the fit behaviour during early motor skill learning (in the MRI scanner, in the absence of real or sham dual-tDCS) is also associated with a (supposedly) less efficient fMRI pattern involving only the bilateral PPC in fitter stroke patients. In addition, less activation was observed in bilateral PMd in fitters than in shifter stroke patients. This suggests that the fitters might still be in an exploratory phase of early motor skill learning.

On the other hand, the fit and shift behaviours could be considered as two distinct behavioural strategies which could be adopted by subjects (healthy individuals or stroke patients) depending on their motor abilities or motor/cognitive potential. Actually in both healthy individuals and stroke patients, the fMRI activation patterns were different between shifters and fitters from the very first blocks of motor skill learning, suggesting an intrinsic difference in the recruitment of neural resources rather than delayed motor skill learning processes in fitters compared to shifters. If the shift and fit behaviours indeed reflect two distinct strategies, they might be linked to the subjects' cognitive profile or genetic background. There are some evidence that subjects with distinct genetic background (such as BDNF polymorphism)

present different cognitive abilities (Bath and Lee 2006), memory aptitudes and especially motor learning acquisition such as experience-dependent plasticity (Kleim et al. 2006; McHughen et al. 2010) or motor skill learning (Fritsch et al. 2010; Li Voti et al. 2011). One could speculate that *the majority of the* shifters could likely present the most favourable polymorphism of the BNDF gene at codon 66 (*BDNF Val66Val*), whereas the *majority of the* fitters may be heterozygote (*BDNF Val66Met*), and the *majority of the* non-learners would present the less favourable polymorphism of BDNF (*BDNF Met66Met*). Nevertheless, the hypothetic genetic differences could not fully explain the observations in stroke patients. Actually, if the genetic background defines the motor skill learning behaviour (fit/shift/non-learning), why did some patients experience spontaneous modifications of their behaviour [i.e some patients moved spontaneously from a fit to a shift behaviour and vice et versa]; even with sham dual-tDCS? In addition to the possibility that BDNF polymorphism could affect motor learning, other genetic factors such as the polymorphisms of serotonin transporter, or other proteins involved in neurotransmitter regulation could also be involved (Pearson-Fuhrhop et al. 2009; Pearson-Fuhrhop and Cramer 2010; Pearson-Fuhrhop et al. 2012). Additional experiments are needed both in healthy individuals and stroke patients, including longer and multiple training sessions as well as longer retention tests, to explore these issues. Additionally, experiments using the dominant hand instead of the non-dominant could be a way to settle whether the fit behaviour is an independent strategy or only a step before the shift behaviour. Actually, if the fit behaviour is a personal strategy it could also be used when subjects trained with the dominant hand.

7.3.3. Neural substrates of motor skill learning in healthy individuals

The overall fMRI brain activation pattern observed during early motor skill learning in healthy subjects (Chapter 4, (Lefebvre et al. 2012)) is concordant with the networks observed in previous studies (Grafton et al. 1992; Jenkins et al. 1994; Doyon et al. 2003; Hardwick et al. 2013). The M1 contralateral to the working (non-dominant) hand is known to be an essential

area for motor learning (Karni et al. 1995; Muellbacher et al. 2002; Kim et al. 2004; Boggio et al. 2006; Tecchio et al. 2010), the SMA is a crucial area for sequential motor skill learning (van Mier et al. 1998), and a recent study confirmed that activation in the lobules V and VI of the cerebellum correlated with sequential motor skill learning (Bernard and Seidler 2013). This study confirms that the circuit game is a motor skill learning paradigm and that it elicits a fMRI activation pattern similar to those observed with other motor skill learning paradigms such as SRTT, sequential finger tapping or visuomotor tracking task (Grafton et al. 1992; Jenkins et al. 1994; van Mier et al. 1998; Doyon et al. 2003; Halsband and Lange 2006). Since the main result suggests a key role of the SMA in motor skill learning, it raises the question of defining the best target for NIBS to improve motor skill learning in both healthy individuals and stroke patient. In the majority of the NIBS studies (Kim et al. 2006; Reis et al. 2009; Zimerman et al. 2012), M1 was the target area but maybe the SMA could be a better choice, since this could also potentially improve the *quality* of motor skill learning.

Additional experiments are needed in healthy individuals to confirm the key role of the SMA in efficient motor skill learning (shift behaviour). For example, interferential experiment could be realized to determine whether NIBS (e.g. rTMS) applied over the SMA of a shifter individual (who typically recruits the SMA according to our observations) deteriorate the motor skill learning behaviour (fitter or non-learner) .

7.3.4. Neural substrates of motor skill learning in stroke patients

In chronic stroke patients, online motor skill learning with the paretic hand is supported by a distributed network which is both **i)** globally similar to that observed in healthy individuals (SMA, $M1_{damH,}$), but **ii)** also reorganised since it involves additional activations (supposedly compensatory) in the bilateral premotor, somatosensory, motor and parietal cortices (Chapter 5 (Lefebvre et al. *in preparation*[1])). These additional activations – compared to healthy individuals - suggest that the motor skill learning network is reorganised in chronic stroke patients, with a more distributed (bilateral) fMRI activation and a recruitment of areas involved in higher-order cognitive

process such as the DLPFC and visuomotor planning process such as the bilateral PPC (Bremmer et al. 2001; Turken et al. 2008; Hauschild et al. 2012; Torres et al. 2013). Alternatively, these additional activations may suggest that the task is more demanding for stroke patients than for healthy individuals, and thus requires the recruitment of additional neural resources. Furthermore, we demonstrated that, in chronic hemiparetic stroke patients, the temporal evolution of the BOLD signal in the bilateral PMd showed the strongest correlation with the temporal evolution of the motor performance (PI) during early motor skill learning. This is in line with previous studies suggesting that PMd plays a key role in motor skill learning in healthy individuals (Kantak et al. 2012; Hardwick et al. 2013) and, furthermore, plays a key role in motor function recovery after stroke (Carey et al. 2002; Fridman et al. 2004; Tombari et al. 2004; Lotze et al. 2006b).

7.4. NIBS as a therapeutic tool

7.4.1. tDCS *versus* rTMS

A crucial point is the choice of the NIBS method: rTMS (including TBS) versus tDCS. Grossly, so far, similar improvements of motor function and motor learning have been reported with both NIBS methods (Table 1.2 and 1.3). The use of rTMS is limited by several factors such as the risk of inducing an epileptic seizure especially in patients with a brain lesion (Nowak et al. 2006; Lomarev et al. 2007), the relative difficulty of use, the price of the devices and neuro-navigation systems and the uncomfortable sensations for the patients. Nevertheless, rTMS delivers a more focal stimulation. The maximum of induced electrical current density is spatially centred around the area targeted with a (focal) coil (Wassermann 1998) With tDCS, the larger electrodes stimulate a larger cortical zone (typically on both hemispheres). However, stimulating a larger network could potentially induce greater improvement in many functions. In addition, given its safety (Nitsche and Paulus 2001; Merrill et al. 2005), portability, user-friendly and patient-friendly features, existence of convincing sham stimulations (Gandiga et al. 2006) and lower price, tDCS seems more likely than rTMS to rapidly become a therapeutic adjuvant in neurorehabilitation.

7.4.2. Why dual-tDCS rather than uni-hemispheric tDCS ?

A recent study showed that the improvement in motor performance and cortical plasticity in healthy individuals was similar between dual-tDCS and uni-hemisphere tDCS (Kidgell et al. 2013). However, previous studies suggested that the motor function improvement is greater after dual-tDCS than after classical tDCS (uni-hemispheric tDCS) in healthy individuals (direct comparison) (Vines et al. 2008) and chronic stroke patients (indirect comparison) (Lindenberg et al. 2010). In addition, recent studies demonstrated that dual-tDCS induced a stronger modulation of the connectivity between the two M1 than did uni-hemispheric tDCS (Lindenberg et al. 2013; Sehm et al. 2013). These results suggest that the behavioural effects driven by dual-tDCS could be associated with a modulation of interhemispheric inhibition. However, Linderberg and collaborators (Lindenberg et al. 2013) recently suggested that the after-effects of uni-hemispheric tDCS relied on a re-balancing of interhemispheric inhibition, whereas the after-effects of dual-tDCS relied not only on this re-balancing effect but also on a neuromodulation of distinct, additional and remote areas such as the posterior cingulate cortex.

In our studies (Chapter 2 (Lefebvre et al. 2013b), 3 (Lefebvre et al. 2013a),5 (Lefebvre et al. *in preparation*[1]) and 6 (Lefebvre et al. *in preparation*[2])), we did not observe motor performance degradation of the paretic or of the non-paretic arm. This contrasts with the deterioration of motor function in the paretic upper limb observed after uni-hemispheric cathodal tDCS (Bradnam et al. 2011) and TBS (Ackerley et al. 2010) over the undamaged hemisphere. This could suggest that, with dual-tDCS, the concomitant increase of excitability in the damaged hemisphere (anodal stimulation) and decrease of excitability in the undamaged hemisphere (cathodal stimulation) was better at restoring the balance between the two hemispheres than uni-hemispheric stimulation, or has activated additional mechanisms which need to be explored.

These observations do not invalidate the interhemispheric rivalry model but suggest that the after-effect of tDCS (and especially of dual-tDCS)

are indeed supported by more complex interactions and mechanisms than suggested initially.

Nevertheless, larger studies are needed to determine whether dual-tDCS could improve motor function / motor skill learning in all types of stroke patients or only for a specific population. Actually, patients with large cortical lesion including M1/PMd will probably not take benefit from dual-tDCS. Maybe a quantification of the CST integrity by DTI could help to identify dual-tDCS responders.

7.4.3. What are the best parameters for NIBS?:

7.4.3.1. What is the ideal timing of stimulation for improving motor learning with regards to motor training/learning?

Is the ideal time to apply NIBS before training to put the brain in an optimal state, during training to reinforce training-induced plasticity, or after training to improve consolidation? A recent study (Stagg et al. 2011) demonstrated that “during training” is the best time in healthy individuals. Larger trials are required to confirm these results in stroke patients.

7.4.3.2. What is the ideal target for NIBS?

The demonstration has been made that both **i)** uni-hemispheric stimulation of $M1_{undamH}$ (Takeuchi et al. 2008b; Meehan et al. 2011a; Avenanti et al. 2012; Zimerman et al. 2012), **ii)** uni-hemispheric stimulation on the $M1_{damH}$ (Kim et al. 2006), and **iii)** dual-hemispheric M1 stimulation ((Takeuchi et al. 2009); Chapter 3,(Lefebvre et al. 2013a), Chapter 6 Lefebvre et al. *in preparation*[2]) improve motor learning in stroke patients. However, given the tDCS electrodes’ size used in our studies (Chapter 3, (Lefebvre et al. 2013a), Chapter 6, Lefebvre et al. *in preparation*[2]) (35 cm²) and the current spread (Crawford and D.C.Howell 1998), PMd and S1 may also have been stimulated through local current spread since tDCS was centred over M1 (the hotspot was determined with focal TMS in the vast majority of stroke patients, or assigned as the C3-C4 positions in a few patients).

Nevertheless, based on the fMRI patterns related to motor skill learning we observed in healthy individuals and in stroke patients, the SMA or the bilateral PMd may potentially be considered as better targets. Actually, the fMRI activation in the SMA was associated with a better motor skill learning in (shifter) healthy individuals, whereas the fMRI activation in the bilateral PMd was associated with a better motor skill learning in (shifter) chronic stroke patients. So, the bilateral PMd are additional areas that should be considered as potential NIBS targets, compared to the network observed in healthy individuals (Chapter 4 and 5 (Lefebvre et al. 2012), (Lefebvre et al. *in preparation*[1])). The bilateral PMd belong to the reorganized fMRI network associated with efficient (shift) motor skill learning in chronic stroke patients. So, stimulating the bilateral PMd may potentially reinforce this reorganised fMRI pattern. However, since "*the more the reconfigured network is similar to the original undamaged network, the better the recovery"* (Denny-Brown 1950; Ward et al. 2003; Krakauer 2004), stimulating areas which are involved spontaneously during motor skill learning in healthy individuals such as the SMA or the $M1_{damH}$ could be more effective.

Additional studies are needed to determine the best NIBS target area in stroke patients. Maybe the best target area should be defined on an individual basis (with fMRI, DTI and/or TMS) to tailor NIBS to each patient, rather than applying a one-size-fits-all approach centred over M1.

7.4.3.3. How to localize the target area?

The localization of the target area with focal TMS, (evoking movements or MEPs in the paretic hand) could be used only for M1 localisation (the hotspot). For other areas, alternative approaches have to be used. One method could be the localisation with the EEG 10-20 system (DaSilva et al. 2011; Garin et al. 2011; Ladeira et al. 2011; Teo et al. 2011; Dubois et al. 2012; Borckardt et al. 2013). An alternative option would be the localisation by neuro-navigation systems, based either on anatomical landmarks or on individual fMRI activation foci (Herwig et al. 2001). Using the EEG 10-20 system is a rather crude and imprecise approach, whereas

fMRI neuro-navigation permits to specifically identify the target area on an individual basis. Theoretically, due to brain reorganisation after stroke, customised localisation for each patient might be a more efficient strategy. Another - time-consuming - alternative would be to use neuro-navigated interferential TMS to identify the best cortical target.

7.4.3.4 What is the ideal timing for NIBS after stroke with regards to time since stroke?

The studies presented in this thesis were performed in chronic stroke patients with the aim to avoid interferences due to spontaneous recovery during the (sub)acute stroke period. In a population of chronic stroke patients with stable deficits, an improvement could be more certainly attributed to the intervention.

Nevertheless, after a stroke, the most dynamical period is the (sub)acute phase, when the fastest and largest brain reorganisations occur and when the recovery curve is the steepest (Kreisel et al. 2006; Wieloch and Nikolich 2006). The practical consequences of this time-dependent potential for brain plasticity are twofold: **i)** any intervention taking advantage of the dynamical changes during the acute stroke stage should have a strong and lasting impact on recovery; **ii)** neurorehabilitation programs should be adapted specifically to each stage of recovery, since brain states and biochemical events are different (Kreisel et al. 2006; Nudo 2006).

One could speculate a differential impact of stroke on motor learning depending on the moment of intervention (Figure 7.3). Given the profound hemodynamic and electrophysiological perturbations observed during acute stroke (Kreisel et al. 2006; Wieloch and Nikolich 2006; Cramer 2008), the motor learning capacity should be depressed, as is motor performance. Then, during the subacute stage, motor learning capacity should re-appear in parallel with recovery of synaptic activity. To date, it is unknown whether the capacity for motor *learning* (and not just motor *performance*) during the subacute phase is impaired, transiently enhanced during a critical period due to a permissive plastic state, or simply unchanged but hidden by the slowly recovering sensorimotor and cognitive impairments. Finally, whereas motor

deficits are supposed stable during the chronic stage, task-specific neurorehabilitation improves motor function through motor learning even years after stroke (Carey et al. 2002). Thus, the capacity to achieve motor (skill) learning recovers at least partially in the chronic stage.

Even if the ability of dual-tDCS to improve motor skill learning in chronic stroke patients has been demonstrated (Chapters 2-3 and 6), one of the next step for inducing a larger benefit for patients might be to apply dual-tDCS during the acute stroke phase (Figure 7.3).

7.5 Impact of a carry-over effect in the studies with cross-over design

As detailed in the Introduction (section 1.8.1, page 58), the studies presented in this thesis have been all performed with a cross-over (randomised, balanced, double-blind and placebo-controlled) design. The main criticism about cross-over design is that the performance of the second session might be contaminated by the first session, especially in case of patients who received the real dual-tDCS combined with motor skill learning during the first intervention session (carry-over effect). Potentially, a carry-over effect could have induced a ceiling effect during the second intervention or have skewed the results of this second intervention session. Theoretically, when real dual-tDCS was applied first, the stroke patients could have reached their maximal level of performance (ceiling effect) and could not be able to improve further during the second intervention session (sham dual-tDCS). Nevertheless as the patients are trained on different circuits in each session, it seems unlikely that their performance on the new sequence of movement is influenced by the first circuit. On the other hand, the first intervention session (either sham or real dual-tDCS) could be considered as an extensive familiarisation session, so the performance could improve during the next session “simply” because of such a potential extensive familiarization.

As demonstrated in the Chapters 3 and 6, a carry-over effect and a transfer of performance improvement to non-trained tasks (PPT, entirely new circuit game) were induced by real dual-tDCS, as suggested by the higher

performance level (PI) at Baseline of the second intervention session (sham). First, this suggests that the effect of a single session of dual-tDCS during motor skill learning leads to a sustained improvement of motor performances not only one week after the end of the stimulation (formal Recall session) but also up to at least three weeks later (i.e. the beginning of the second recall session). Second, since the stroke patients learnt an *alternative* version of the circuit during the second Intervention, it would be surprising to observe a ceiling effect on this *new* motor skill. Actually, each version of the circuit consists on a new sequence of paths (see Figure 1.5) and so needs a new sequence of movements to perform the task.

Finally, since a carry over effect has been observed in these two studies (Chapters 3 and 6), a supplemental analysis based only on the first arm (First Intervention session + First recall) was performed, as if the study had been done with a parallel group design. This analysis demonstrated that based only on the first arm, the stroke patients who received the real dual-tDCS presented a significant on-line motor skill learning improvement which was maintained at the Recall session. This improvement was significantly superior to the slight improvement observed with sham dual-tDCS. These additional analyses confirmed the ability of real dual-tDCS to improve online motor skill learning and its long-term retention

To conclude, even if the non-specific carry-over effect could be considered as a limitation from a strictly scientific point of view, by opposition it could also be considered as an advantage for the future therapeutic use of tDCS since a single session of dual-tDCS improved not only the performance on the trained task by also induced a non-specific transfer to non-trained tasks.

7.6 Translation into real clinical setting

Are we ready for a bench-to-beside translation with generalised use of tDCS in post-stroke neurorehabilitation program, during physical and occupational therapy, as a routine add-on treatment? Several studies already used tDCS in RCT or in (pre-)clinical trial (Kim et al. 2010a; Adeyemo et al. 2012; Khedr et al. 2013; Wu et al. 2013), with successful

results in enhancing the effect of neurorehabilitation. For example, tDCS applied during six days concomitantly with neurorehabilitation improves the motor performance of stroke patients, with improvements lasting at least up to 3 months after intervention (Khedr et al. 2013).

Therefore, what is the rationale to get one step back and to study motor learning in chronic stroke in pre-clinical studies, as we did in this thesis? As a matter of fact, several questions about the mechanisms of the tDCS-induced performance improvements in stroke patients and about the precise neural substrates of motor learning after stroke (which is central in recovery) have to be addressed before launching large multi-centre RCT applying NIBS in a clinical setting. The current thesis provided key milestones for the implementation of motor learning and NIBS in stroke neurorehabilitation but much work has still to be done.

7.6.1. Target population

The cohorts of chronic stroke patients included in the different studies presented in this thesis were heterogeneous, with different stroke localization and nature (cortical/subcortical, ischemic/haemorrhagic), large range of delay since stroke onset (from 0.5 to 15 years), different patient's age (35 to 82 years), and different level of impairment (from very mild to severe, even if no patient was hemiplegic). However, these cohorts fairly matched the characteristics of non-hemiplegic stroke patients met in real life, which suggest that dual-tDCS may be able to improve motor skill learning in a large variety of stroke patients, at least in those who conserved voluntary mobility in the upper paretic limb, and not only in a small hyper-selected laboratory population (e.g. 50-years old stroke patients with a single small subcortical lacuna in the internal capsule, and no co-morbidities). The demonstration has been made that dual-tDCS in small cohorts has a beneficial effect on fine paretic hand motor function (n=19) and on motor skill learning enhancement (n=18 and n=24) in patients with mild to severe impairment with different types of stroke and with a slight to extensive lesion burden.

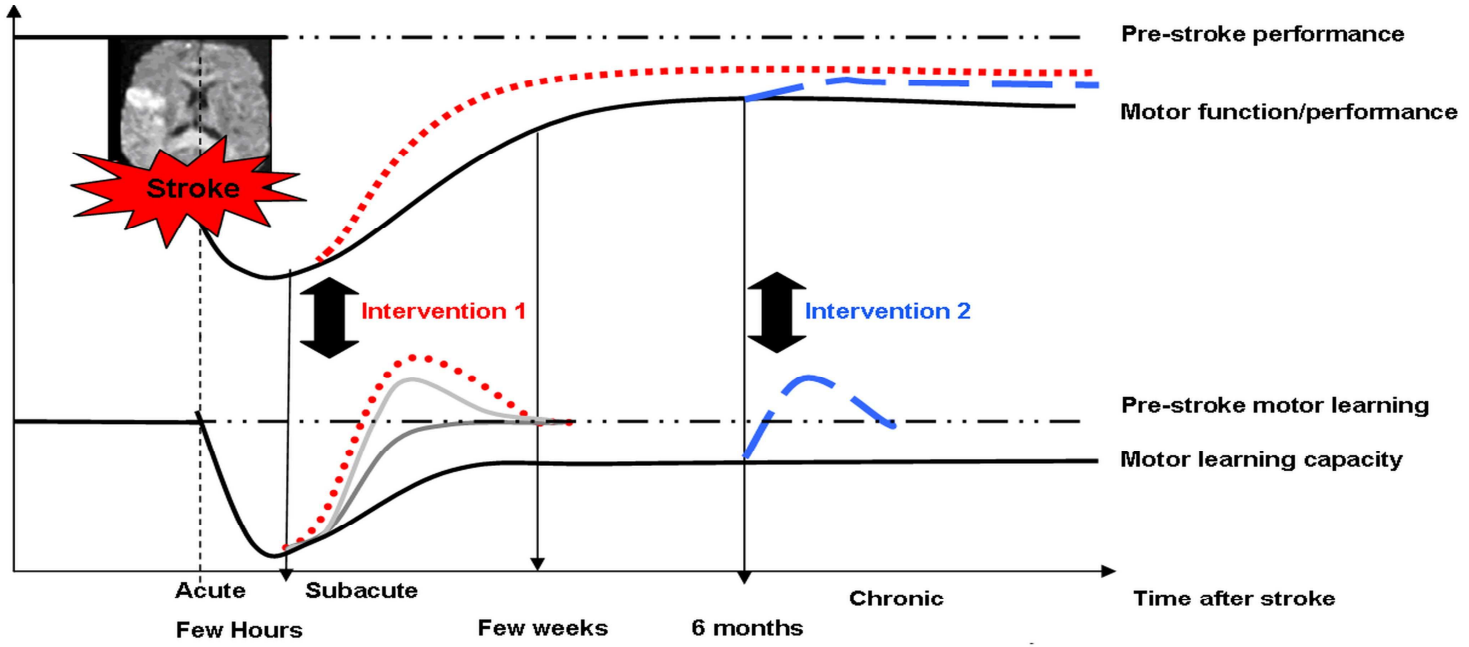

Figure 7.3: Enhancing motor learning during post-stroke motor recovery. When a stroke affects the CST or motor areas, motor function and performance reach a deep immediately or during the first hours (acute stage, upper black curve: *motor function/performance*). During the first days and weeks after stroke ((sub)acute stage), dynamic metabolic and neurophysiological changes place the brain in a highly plastic state, which is permissive for functional and structural remodelling. Afterwards, recovery progressively slows and – putatively - reaches a plateau after a few months; this is the chronic stage during which no or little spontaneous changes are expected to occur in the absence of

training. The spontaneous evolution of *motor learning capacity* (lower black curve) after stroke is currently unknown. Given the dramatic metabolic and electrophysiological perturbations that occur early after stroke, it is logical to hypothesise that during the acute stage, the motor learning capacity is depressed just as motor function/performance. In contrast, during the subacute stage the motor learning capacity might be either depressed (black curve), normal (dark grey curve) or enhanced (light grey curve). On the one hand, motor learning capacity may remain depressed due to enduring metabolic changes, the difficulty to mobilise and optimise the spared neuronal resources or the lower efficiency of the reconfigured networks supporting recovery. On the other hand, motor learning capacity may be restored to a normal level but may remain partly hidden by the importance of functional impairments. Finally, motor learning capacity might theoretically be *enhanced* during the subacute permissive stage, which consists in a temporal window for increased cerebral plasticity. Along the same line, it is unknown whether the motor learning capacity during the chronic stage is depressed, normal or enhanced. Any therapeutic intervention, and especially those targeting motor learning, has to take into account the highly dynamical evolution of post-stroke motor recovery since each stage is characterised by specific metabolic and electrophysiological changes, which condition the potential of cerebral plasticity, motor function and likely motor learning capacity. The most dynamical and permissive phase is the subacute stage; interventions could potentially benefit from this critical period of enhanced cerebral plasticity (Intervention 1, red curves). This is especially true if the therapeutic intervention enhances motor learning and its long-term retention. During the chronic phase, intervention also improves motor function/performance but to a lesser extent, although motor learning capacity is enhanced (Intervention 2, blue curves).

Will tDCS induce benefit to all stroke patients? And if not, how to identify the potential responders? In the present work, no differential effect has been observed based on the nature and localisation of stroke. However, larger clinical trials including extensive cohorts of stroke patients with a complete range of deficits are needed to seek predictors that could discriminate NIBS responders and non-responders. Interestingly, recent findings demonstrated that BDNF polymorphism could be used to predict the efficiency of the tDCS in healthy individuals (Fritsch et al. 2010; Wang et al. 2011a). As a first step, this correlation between BDNF polymorphism and the

response to NIBS has to be demonstrated in chronic stroke patient. If so, a possible way to improve the efficiency of NIBS would be to realize a genetic screening (BDNF polymorphism) in order to identify the stroke patients who should be NIBS responders. Although attention has been focused on BDNF, there are probably other genes that may influence the efficacy of NIBS.

7.6.2. Role of attention and fatigue

In the studies presented in this thesis, we observed a degradation of motor performances in some stroke patients and healthy individuals. We assume that this degradation was associated with a fatigue effect or an attention decrease. However, no specific measurement of fatigue and attention has been done. Thereby, could the improvement on motor performance/motor skill learning driven by real dual-tDCS be attributed to an anti-fatigue effect (Ackerley et al. 2010) or an increased attention paid to the task (Gladwin et al. 2012)? Actually, the stimulation of M1 by tDCS is known to decrease muscular fatigue in healthy volunteers (Cogiamanian et al. 2007); such an effect could clearly support performance improvement. Even if these effects are probably present during the application of tDCS, it is unlikely that they are the main/only cause of the observed improvement. First, although tDCS applied with an hotspot on M1 will modulate brain excitability in a larger adjacent network than only M1, there is no specific stimulation of attentional areas. Second, dual-tDCS application over M1 is associated with an increase of MEP amplitude as measured in the paretic hand (Bolognini et al. 2011), suggesting in a direct effect on the motor system. Third, in the present work, even if patients were not specifically asked, no patients spontaneously reported a muscular fatigue during the task under the sham condition. Finally, although reduction or improved attention paid to the task could improve on-line motor performance and lead to greater retention of the motor skill, it seems unlikely that these effects would be sufficient to induce the kind of long-term improvement we observed. However, dissociating the potential effects of tDCS on attention and fatigue from the effects on motor performance is essential to refine the knowledge about tDCS mechanisms.

7.7 Concluding remarks and futures directions

Is it possible to increase functional benefit by applying repeated NIBS sessions? And if so, what is the best delay between NIBS sessions? Daily stimulations during a few days induce a cumulative effect on off-line motor skill learning enhancement in healthy individuals (Reis et al. 2009). Nevertheless, the best timing in stroke patients has to be determined. In addition, repetitive sessions of NIBS have to be applied during occupational or physical therapy, and the potential benefits in daily life remains to be explored, in order to confirm the potential use of dual-tDCS as an add-on tool to neurorehabilitation. As previously suggested, the choice of the target area remains a crucial question. Studies exploring the effect on motor skill learning and its long term retention of tDCS over the SMA, the bilateral PMd or the cerebellum ipsilateral to the paretic hand are needed.

The studies presented in this thesis, as the majority of the studies already published, relied on relatively small numbers of patients, and mono-centric NIBS setting. Large multi-centres RCT using the same NIBS protocol and the same tasks are needed to corroborate the current results and to evaluate the real potential benefits of these techniques.

Finally, although this thesis brings new key insight about the neural substrates underlying motor skill learning in stroke patients and how to improve motor skill learning with NIBS in stroke patients, many questions have to be addressed before NIBS can be widely used to improve post-stroke recovery. A better understanding of the contribution of motor (skill) learning to stroke recovery/neurorehabilitation is of paramount importance to move ahead, as well as a better understanding of the mechanisms underlying NIBS effect on post-stroke motor function. Identifying the key structures, understanding the mechanisms of motor skill learning after stroke and developing efficient methods to enhance residual motor learning capacities are among the greatest challenges for modern neurorehabilitation.

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