NON INVASIVE INVESTIGATION OF SENSORIMOTOR CONTROL FOR FUTURE DEVELOPMENT OF BRAIN-MACHINE-INTERFACE (BMI)

by

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Non invasive investigation of sensorimotor control for future development of Brain-Machine-Interface (BMI) by Leo Tomasevic

Abstract

My thesis focuses on describing novel functional connectivity properties of the sensorimotor system that are of potential interest in the field of brain-machine interface. In particular, I have investigated how the connectivity changes as a consequence of either pathologic conditions or spontaneous fluctuations of the brain's internal state. An ad-hoc electronic device has been developed to implement the appropriate experimental settings.

First, the functional communication among sensorimotor primary nodes was investigated in multiple sclerosis patients afflicted by persistent fatigue. I selected this condition, for which there is no effective pharmacological treatment, since existing literature links this type of fatigue to the motor control system. In this study, electroencephalographic (EEG) and electromyographic (EMG) traces were acquired together with the pressure exerted on a bulb during an isometric hand grip. The results showed a higher frequency connection between central and peripheral nervous systems (CMC) and an overcorrection of the exerted movement in fatigued multiple sclerosis patients. In fact, even though any fatigue-dependent brain and muscular oscillatory activity alterations were absent, their connectivity worked at higher frequencies as fatigue increased, explaining 67% of the fatigue scale (MFIS) variance ($p=.002$). In other terms, the functional communication within the central-peripheral nervous systems, namely involving primary sensorimotor areas, was sensitive to tiny alterations in neural connectivity leading to fatigue, well before the appearance of impairments in single nodes of the network.

The second study was about connectivity intended as propagation of information and studied in dependence on spontaneous fluctuations of the sensorimotor system triggered by an external stimulus. Knowledge of the propagation mechanisms and of their changes is essential to extract significant information from single trials. The EEG traces were acquired during transcranial magnetic stimulation (TMS) to yield to a deeper knowledge about the response to an external stimulation while the cortico-spinal system passes through different states. The results showed that spontaneous increases of the excitation of the node originating the transmission within the hand control network gave rise to dynamic recruitment patterns with opposite behaviors, weaker in homotopic and parietal circuits, stronger in frontal ones. As probed by TMS, this behavior indicates that the effective connectivity within bilateral circuits orchestrating hand control are dynamically modulated in time even in resting state.

The third investigation assessed the plastic changes in the sensorimotor system after stroke induced by 3 months of robotic rehabilitation in chronic phase. A functional source extraction procedure was applied on the acquired EEG data, enabling the investigation of the functional connectivity between homologous areas in the resting state. The most significant result was that the clinical ameliorations were associated to a 'normalization' of the functional connectivity between homologous areas. In fact, the brain connectivity did not necessarily increase or decrease, but it settled within a 'physiological' range of connectivity.

These studies strengthen our knowledge about the behavioral role of the functional connectivity among neuronal networks' nodes, which will be essential in future developments of enhanced rehabilitative interventions, including brain-machine interfaces. The presented research also moves the definition of new indices of clinical state evaluation relevant for compensating interventions, a step forward.

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment. Noteworthy, the InPresS device (Chapter 2) was objective of the bachelor thesis (Italian Laurea in Electronic Engineering) of Federica Riccardi who worked under my supervision.

This study was undertaken at the Italian National Research Council (CNR), in the Laboratory of Electrophysiology for Translational neuroScience (LET'S).

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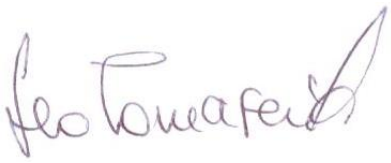
Signed 

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INTRODUCTION

This thesis is the fruit of personal curiosity and of the fascinating attraction that the studies over the brain can produce. My electronic engineering background and being part of a laboratory that has years of experience in sensorimotor system studies gave me the opportunity to investigate relevant aspects of the way our brain connects to external world, in future perspectives of Brain Computer Interface (BCI). The communication between the human being and the machine is important because it could hug the society from all points of view, from the pure gaming to communication, to improvements in everyday life and jobs. Even more important the BCI could become a therapy as complement to standard neurorehabilitation methods or even a mean to substitute the entire body structures with alternative devices. BCI could then give alternative solutions for patients with severe neuromuscular disorders such as spinal cord injury, amyotrophic lateral sclerosis, stroke and cerebral palsy. Having in the mind the final result, still a chimera, this study has the ambition of exploring the brain from different points of view and expanding our knowledge about brain structures preferential for the BCI development.

It is necessary to explain that despite BMI term is used in the title of this thesis, in the body only BCI term will be used. Even though the two words nowdays are synonyms, we could consider BMI as a direct and analogic connection between the brain and a mechanical device, and BCI as including an intermediate digital/electronic step. Nowdays it is hard to imagine the technological evolution that skips the electronics, for this BMI in the title of the present work wants to remember the challenge of days in which the actual digital world was not available.

Aims

Since the sensorimotor system is the most direct connection door for the control of external devices, this project is mainly focused on the corresponding brain areas and their connectivity. Among the cortical representation of the human body, brain areas devoted to hand motor control and its sensory perception are the most appropriate for their importance and size. A larger size, in fact, allows a better evaluation via non-invasive techniques and allows the perception of changes due to different states.

The primary aims of the present study are therefore the identifications of aspects of the sensorimotor system for a deeper knowledge of a so interesting brain area and its functional organization in healthy people as in patients. To achieve this goal standard analysis procedures and innovative methods are applied. The idea is to study the sensorimotor system from different points of view, it means in different conditions and different stimulation states, to hone knowledge of how the sensorimotor system works, adapts or suffers.

The secondary aim is to develop experimental and analysis tools to be used in different contexts by mine and other laboratories.

These findings could be useful in future for the development of neurorehabilitation or stimulation BCI systems. These are attracting interests in the recent years since they are completely non-invasive and they could fascinatingly allow the brain to ‘cure itself’.

Contributions

The scientific contributions of my PhD work are:

- A new interactive device for experimental protocols that contains a pressure sensor (to monitor, to record and to give the feedback of the exerted pressure) and triggers used as stop and go signals or to pilot stimulation machines;

- The identified dependence between fatigue scores in multiple sclerosis (MS) and frequencies of central-peripheral functional connectivity, plus their link with the overcorrection of the performed movement. These findings were used to develop a compensating intervention efficacious against MS fatigue. Prospectively, an objective measure of fatigue in MS could be introduced;

- The localisation stability method, enabling single-subject extraction of active sources in noisy event related data;

- The assessed intra-cerebral functional connectivity changes due to trial-to-trial spontaneous fluctuations in response to identical stimuli, which can propagate for decades of milliseconds involving distant regions;

- The correlation of functional connectivity between homologous cortical regions at rest and the clinical recovery induced by robotic rehabilitation in stroke patients; this seems to provide an intra-cortical connectivity index at rest, sensitive to brain plasticity changes and relevant for motor control amelioration in stroke patients. It can be exploited to ameliorate rehabilitative protocols.

Thesis structure

My thesis is subdivided in six chapters. The first contains an overview to introduce the field, the used technology and data analysis methods of the present project. The last part of the Introduction is dedicated to BCI and its development to make a link between the present results and future possible developments. The hope is that all the new knowledge about brain functional activity can one day be used with a translational approach where BCI could play a lead role.

In the second chapter, I describe an interactive device (InPresS) that I projected and realized to control the sensorimotor tasks under investigation and to assess their

execution quality. The multimodal usage of InPresS made possible to monitor and record the pressure exerted by subjects, to send and record the stimuli as explained in detail in the experimental chapters.

In Chapter 3 a study about the cortico-muscular connectivity changes due to the presence of fatigue in multiple sclerosis is exposed. This investigation shows how a physiological voluntary control of a handgrip changes in fatigued MS patients, involving changes of both brain-muscle communication and an overcorrection of the movement.

The Chapter 4 faces the single epoch variability, a crucial element for BCI applications, in a passive condition of activation induced by direct central magnetic stimulation (transcranial magnetic stimulation, TMS). In fact, BCI aims to associate the same intended final movement to the same intention to move of the subject, whereas human brain displays a –more or less– different activity (called single epoch variable activity) while performing the same action. Therefore, the second study documented the brain connectivity changes secondary to the spontaneous involuntary fluctuations of the motor corticospinal pathway excitation. Within the same study, a method is provided to assess the space of sources instead of sensors' space in single subjects allowing to investigate small differences in strongly artefacted conditions.

The last study (Chapter 5) is about the connectivity at rest of interhemispheric functionally homologous areas that can be modulated in stroke patients, together with the hand control ability, through a robotic rehabilitation. As will be shown, a data analysis tool, in-house developed by my laboratory, permitted to identify the sensorimotor regions involved in the plastic changes and to study them at rest.

Chapter 6 is subdivided in a part regarding the conclusions of the project, a part regarding the influence of the presented studies and finally a part about possible future development of the researched topics.

CHAPTER 1

NERVOUS SYSTEM, EXPERIMENTAL APPROACH, BRAIN COMPUTER INTERFACE (BCI)

Central and peripheral nervous system

My thesis relates to the sensorimotor system in humans and therefore it involves the human nervous system. The entire nervous system can be considered as composed by two main parts, the central nervous system (the brain and the spinal cord) and the peripheral nervous system (nerves and ganglia outside of the brain and spinal cord). The central nervous system is the final destination of all the sensory information that our body collects (afferent information) and it analyses and processes that information to send proper instructions to our body to suitably behave in the environment (efferent information). The peripheral nervous system is made by two counterparts, the motor one, which delivers the instructions to translate in action by the addressee organ; and the sensory one, which collects signals from every sensory channel (visual, auditory, somatosensory, gustative and sense of smell) and sends it to the central nervous system. The brain can be considered the dynamic and plastic balance of this action-reaction interaction of the being with the environment. This bottom-up and top-down interplay shows how both central and peripheral nervous systems are inseparable sub-parts of the sensorimotor control system. Final station and part of the input-output balance occurs within the primary sensorimotor areas, which I will focus in my thesis.

On a more microscopic level the nervous system is mostly composed by two kinds of cells, nerve cells or neurons and supporting cells, called neuroglia (or simply glia from Greek γλία, γλοία that means glue). While the glial cells are of essential

importance in supporting and protecting the neurons, the nerve cells are specialized in electrochemical signal transmission through synaptic transfer (or neuronal “firing”; Niedermeyer and Lopes da Silva 1999).

Neurons are cells made by: a cell body (soma), that contains the nucleus and the cytoplasm; dendrites, short branches that receive signals from other (pre-synaptic) neurons; an axon, that is a single filament which transports electric signals at short (millimetres for intra-cortical neurones) or even very long distances (meters, for example from the big toe to spinal cord) to other neurones (post-synaptic); a cell membrane that contains them all.

The cells of the nervous system are organized in ensembles called neural circuits (neuronal networks), which can be defined as neuronal pools (network nodes) with direct axonal fibres’ connection (network structural connectivity), or as neuronal pools connected by a specific process (functional network), where the nodes are functionally connected through the synchronization of their activities. In all cases, it can be recognized an inflow and an outflow of a specific network, where the input-output association represents the network function.

In my thesis, all investigation is focused on the hand control. The afferent input starts from the skin and junctions’ touch and proprioceptive receptors and travels through spinal cord to the brain, by a three-neurons relay with the three synaptic stations at midbrain, thalamus and cortical primary sensory areas. After primary cortical area activated by the sensory input (S1, McLaughlin and Kelly, 1993), the secondary somatosensory cortex (SII, Mauguière et al., 1997) and associative areas enter the perceptive somatosensory process.

From the structural point of view, the primary somatosensory and motor areas constitute the central sulcus (Latin, ‘furrow, wrinkle’). The central -or Rolandic- sulcus is a groove running in the medio-lateral direction that divides each hemisphere in two

regions, which can be considered motor (anteriorly to the central sulcus, frontal lobe) and sensory sections (posteriorly, including parietal, occipital and temporal lobes) of the cortical mantle. The primary sensory region lies in the parietal lobe, including the posterior wall of the central sulcus and the bump posterior to the sulcus named post-central gyrus (Latin, from Greek guros ‘a ring’) and the motor primary area is immediately frontal to the sulcus, including the anterior wall of the central sulcus and the pre-central gyrus. The cytoarchitecture provides further division of S1 identifying areas 1, 2, 3a and 3b (Brodmann, 1909; Vogt and Vogt, 1919): area 1 is located on the gyral surface of the post-central gyrus, area 2 on the posterior portion of the post-central gyrus and in the post-central sulcus, while areas 3a and 3b respectively on the fundus and posterior bank of the central sulcus (Geyer et al., 1999).

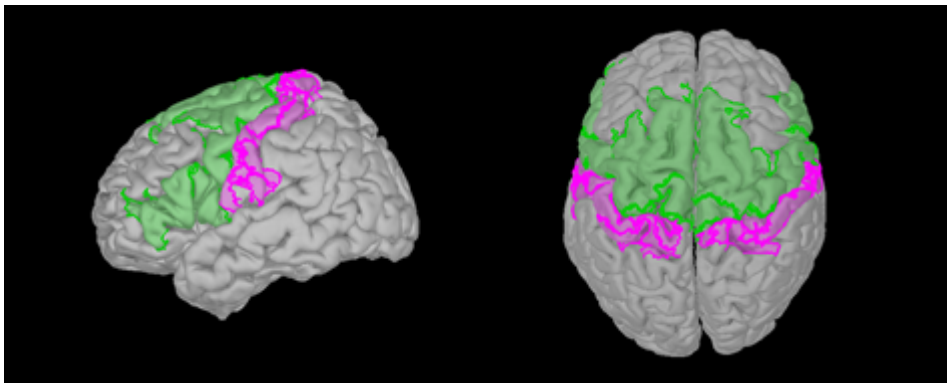

Figure 1.1 Sensorimotor areas

From left: left and top view of brain cortex with highlighted the sensory (magenta) and motor (green) areas

The somatosensory system is composed by diverse receptors that are dislocated throughout the whole body, from the skin to the internal organs, from bones to muscles. These receptors are the transducers of perception of touch, proprioception, nociception and temperature. Proprioception (mix of the Latin word proprius, "one's own" and perception) is feeling of our body, the relative position of neighbouring parts of it and

the strength used to perform a movement. Nociception (Latin noxius from noxa ‘harm’) reveals stimuli that potentially could damage tissue. The cutaneous receptors of the skin are connected through the spinal cord to the thalamus and the primary somatosensory cortex. Adjacent areas on the skin are represented by adjacent neurons and this correspondence of an body area with areas in the central nervous system is called somatotopy. Moreover some body areas are more sensitive corresponding to finer control (i.e. digits) and are innervated by more neurons, so that their cortical representation occupies more extended cortical areas (magnification principle of central representations). Other areas need less control (i.e. trunk) and have a smaller dedicated area in the primary sensory region. All this somatotopy rules can be represented through a sensory homunculus that is an illustration of how much primary sensory area is dedicated to each body part central representation (Penfield and Boldrey, 1937). The brain plasticity is expressed also in the somatosensory area by the reorganization of neurons, the less used areas are partially or totally (for example because of an amputation) reassigned to the adjacent body parts, such as the more used areas tend to expand their “territory”. The cerebral or synaptic plasticity, in fact, is defined as the brain areas’ reorganization that produces different response to the same stimulus (Tecchio et al., 2007a).

Similarly to the sensory region representation, the motor system also has its relative homunculus and in this case it is distorted in dependence of the movement complexity that can be performed by the correspondent body part. The homunculus representation changes over time in dependence of the person’s development, his/her life experience (Nudo et al., 2001). As an example the hand representation in the brain is different for a concert pianist (Elbert et al., 1995) or for a Braille reader (Pascual-Leone and Torres, 1993). The motor system is responsible for voluntary movements and the activation of a complex net devoted to hand control is required for the fine control of exploration and

manipulation. This network involves a wide area in the primary motor region, with a large number of pyramid-shaped neurons (pyramidal neurons) that project long axons down the corticospinal tract. The somatotopic organization is maintained along the entire path of the voluntary movement activation. The whole way the information has to cover starts from the cortex and passes through Betz cells, the corticospinal tract, motor neurons up to the voluntary muscles.

Data recordings

Some of the most used techniques for data recordings in neuroscience have been previously mentioned, here follows a description of data recording technologies utilized in the present thesis.

Electroencephalography

Electroencephalography (EEG) records the difference between two scalp sites of the electric potential generated by the electric currents produced by neurons. EEG is the oldest, the cheapest and the easiest method to record the brain activity. It has also the advantage of an excellent temporal resolution, of being non-invasive, it is adequate for multimodal investigations in combination with fMRI, TMS and others, while its main limits are the quantity of recorded artefacts, the poor spatial resolution and the sensitivity which dramatically decreases for deep and subcortical structures. In fact, the electric field propagates and reaches the whole space inversely (in proportion to the second or third power) to the distance from the originating current, so that many electrodes sense the activity of many sources. In other words, each electrode records the summation of the activity of all 'enough strong' neuronal pools. What non-invasive EEG senses is the activity of large population of neurons. Each neuron repeatedly fires bursts of spikes that are the way neurons use to communicate each other. As mentioned above, the EEG acquired signal is the difference of the potentials that electrodes placed

in different positions of the scalp sense. Electric potentials produced by single firing of a neuron are very small and they have to cross different layers, including the scalp and the skull, that attenuate their propagation, so they hardly reach 100 μV. Moreover, internal and external noise, stronger than signals generated by neurons, would completely cover their activity. For all this reasons EEG system that uses electrodes mounted over the scalp needs the summation of the synchronous activity of thousands or millions of neurons with similar spatial orientation and as close as possible to the electrodes. This is the case of the Pyramidal neurons of the cortex that are probably the greatest source of the EEG signal as they are well-aligned (dendrites oriented parallel to each other perpendicularly to the cortical surface) and fire together and therefore the summation of their activity can be strong enough to be detected (Niedermeyer and Lopes da Silva 1999). For the same reason, the necessity of summation of a large quantity of activities from contemporary active neurons, the postsynaptic potential (tens of milliseconds) is more probable to be influent than the action potentials (1-2 milliseconds), since it lasts longer permitting the overlapping of potentials. The action potential is the change of polarity flowing through the axon, while the post-synaptic potential change happens when the signal passes from a neuron to the sequent by a synaptic contact.

EEG is a direct measure of neuronal activity unlike other techniques that use brain metabolism information to reconstruct the information about neuronal functions (fMRI, PET, SPECT). Since hemodynamic response takes times of the order of second to react to the neuronal functional demand, electro-physiological recordings have a much higher temporal resolution, up to milliseconds, while the above mentioned metabolic techniques depend on blood flow and are thus limited to time scales larger than seconds. Furthermore, all of them assess the neuronal activity mediated by the neurovascular

coupling, which can be deeply affected in pathological conditions (D'Esposito et al., 2003; Iadecola, 2004).

Another strength of studying brain activity by EEG is that it is totally non-invasive, not requiring any exposure to magnetic fields, X-rays or the injection of contrast substances, as isotopes.

Electromyography

Electromyography (EMG) is the technique that allows recording of electrical activity produced by skeletal muscles. The surface EMG, which is set placing electrodes over the skin, is a non-invasive way to detect electrical potential generated by activated muscle cells, that during the muscle contraction ranges between 50 μV and up to 20 to 30 mV (Reaz et al., 2006).

One motor neuron and the muscle fibres it innervates are defined as motor unit. When the neuron fires through impulses, that are called action potentials, the relative muscles are activated. The produced electrical activity is called motor unit action potential (MUAP, Kandel et al., 2000). This electrophysiologic activity from multiple motor units is the signal typically evaluated during an EMG. The composition of the motor unit, the number of muscle fibres per motor unit, the metabolic type of muscle fibres and many other factors affect the shape of the motor unit potentials in the myogram. EMG is the indirect measure of the activity of the spinal motor neurons since they have a one-to-one correspondence with muscle fibres (Kandel et al., 2000; Beck et al. 2007; Yao et al. 2007).

In the presented studies the surface EMG was recorded over the fingers muscles using the belly-tendon montage, which consists of the active electrode placed on the skin over the belly of the muscle in the thenar eminence, the reference electrode on the tendon at a distance of 2.5 cm.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a noninvasive tool for the investigation of the functional state of the human cerebral cortex (Barker et al., 1985). The magnetic stimulation is obtained producing a brief current high intensity pulse in a coil of wire, the magnetic coil, placed over the scalp. The current produces a magnetic pulse perpendicular to the plane of the coil that passes undistorted through extracerebral tissues and induces electric currents within the superficial cortical layers. These currents can activate the pyramidal neurons (Di Lazzaro et al., 1998).

In the set of possibilities, the single-pulse TMS targeting the primary motor cortex (Barker et al., 1985) is an efficacious tool to test the excitability state of the corticospinal tract. In fact, when the pulse is delivered over the motor cortex, it induces an efferent signal through the corticospinal pathway till the relative muscle producing its contraction (motor evoked potential - MEP). This peripheral effect, recorded with electromyographic systems, provides a clinically used parameter of the quality of the primary motor pathway (for a review see Rossini & Rossi 2007).

TMS simultaneous to EEG

Thanks to the recent technical developments of the electroencephalographic amplifiers, it became possible to combine TMS brain stimulation with EEG recordings (Ilmoniemi et al., 1997). This gives new perspectives about the central effects of TMS stimulation and enables to test cortical excitability, functional connectivity and also brain activity modulation (Komssi et al. 2002, 2004; Massimini et al. 2005; Bonato et al 2005) analysing the transcranial evoked potentials (TEPs). This is a great advancement compared to the sole TMS that, as explained previously, could give information only through peripheral or secondary responses. Finally the responses to stimulations of all brain areas can be studied with high spatiotemporal specificity directly on the areas of interest, giving a new insight on their cortical reactivity, effective connectivity, and

synchronized neuronal firing between them (Ilmoniemi et al., 1997; Paus et al., 1997; Siebner et al., 2009; Virtanen et al., 1999). Therefore, TMS-EEG is one of the most promising neurophysiological methods and EEG responses at precise and repeatable latencies after stimulation have already been observed in physiological conditions (Lioumis et al., 2009), proving that specific topographies are relative to the stimulation site (for the primary motor area: Bonato et al., 2006; Ilmoniemi et al., 1997; Komssi et al., 2002, 2004; Komssi and Kähkönen 2006; Nikulin et al., 2003; Paus et al., 2001).

Brain properties for data analysis

Brain connectivity and synchrony

EEG activity is characterized by oscillations at some specific frequency ranges and spatial distributions defining different brain states and networks (Buzsáki G and Draguhn A, 2004). Cortical neuronal activity sensed by EEG expresses mainly in five frequency bands: delta (δ), theta (θ), alpha (α) (mu (μ)), beta (β), gamma (γ) (Buzsáki, 2006).

Delta rhythm is in the range 0.5-4 Hz and usually with high amplitudes.

Theta rhythm belongs to the range of 4-7.5 Hz and the origin of the name maybe comes from the previous belief that it is originated in the thalamus.

Alpha waves lie within 8 and 13 Hz and they show highest power from the occipital lobe. Alpha power shows a clear reactivity (decrease) when people open their eyes while they were relaxed at closed eyes. This is the first oscillatory activity observed and registered by Berger in 1929.

Mu rhythm expresses in the alpha band ranges and it is generated by the somatosensory cortices. As all resting state oscillatory activities, the amplitude is related to levels of relaxation of the generating system. Thus mu rhythm modulations express the level of

involvement of the motor system. Importantly, for BCI systems, it decreases both when movements are executed and imagined (Pfurtscheller and Lopez da Silva, 1999).

Beta activity is in the 14-32 Hz range and is usually located over the frontal lobe. The central beta rhythm is related to the mu rhythm and it is also sensitive to motor activity, in addition to the tactile stimulation. Since it decreases with movement, it has been defined anti-kinetic (Joundi et al., 2012, Pogosyan A et al., 2009).

Gamma is the rhythm that considers frequencies above 30 Hz, it increases during movement and therefore is considered pro-kinetic (Joundi et al., 2012).

Synchronization within different bands are used by the brain in coordinating the role of neuronal pools that can be even spatially far from each other (Nunez et al., 2001). The fact that some bands are more linked with some functions and structures can be thought as a kind of information encoding that permits to each region to identify the information that is of its responsibility and to fix inter-areas connectivity.

For these reasons the frequency analysis is often used to identify particular activities and it has been also proved that they are sensitive to age changes and that they also can give information about the cortical state and presence of disease.

The temporal synchrony between activities of even distant neuronal pools can dynamically form functionally coherent assemblies in sensorimotor contexts. This spatially distributed set of cells activated in a coherent fashion becomes part of the same representation or executed function (Roskies, 1999; Singer, 1999; Engel et al., 2001, 2005). A measure of this connectivity is coherence, which is a normalized measure of phase-locking between two oscillators at a given frequency (Nunez et al., 1997).

Coherence

Moreover, as mentioned above, it has been shown that there is a synchronization inside the beta range rhythm between the cortex in the rolandic area and the motor

neurons at spinal and muscular districts during sensorimotor tasks in both monkeys (Lemon RN et al., 1998; Baker SN et al., 2003) and in humans (Feige B et al., 2000). This connectivity can be also measured by coherence which is suitable for studying brain at work during a simple muscle contraction (Baker SN et al., 1997; Kristeva R et al., 2007).

The coherence is defined as:

$$Coh(f) = \frac{\left|\sum_{i=1}^{L} X_i(f) * Y_i^*(f)\right|^2}{\sum_{i=1}^{L}|X_i(f)|^2 * \sum_{i=1}^{L}|Y_i(f)|^2}$$

Where Xi(f) and Yi(f) are the Fourier transform of the i^{th} epoch of the signals, L is the number of data epochs available and * as apex denotes complex conjugate.

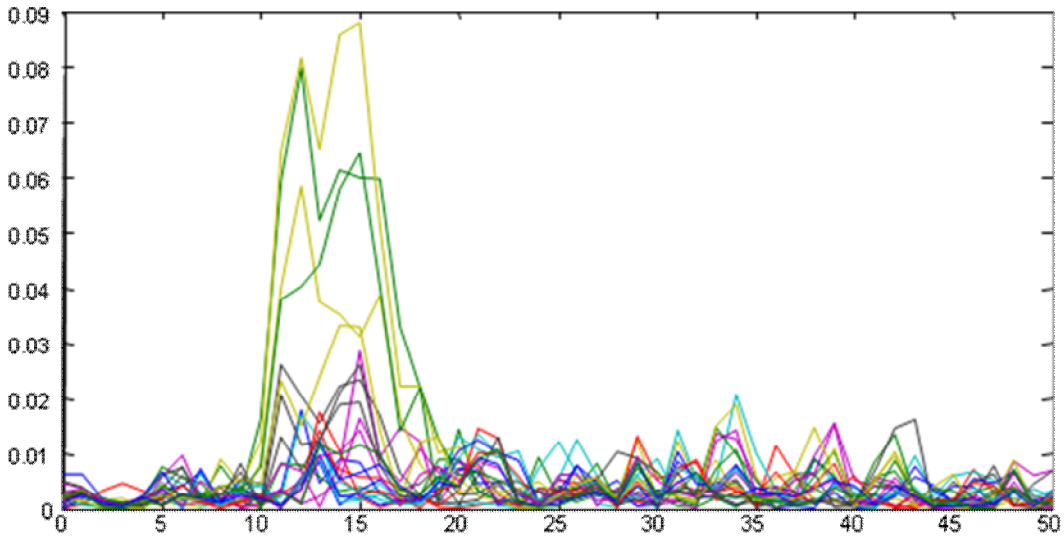

Figure 1.2 – Example of Cortico Muscolar Coherence (64 channels EEG system)

Cortico-muscolar coherence (CMC) measures the oscillatory coupling between cortex and moto-neuronal firing recorded by the EMG (Mima and Hallett, 1999a) and in particular it represents a direct cortical oscillatory drive to maintain steady force output (Mima and Hallett, 1999b; Gross et al., 2000). Good performance areas showed higher beta band CMC between active muscle EMG and sensors situated over the sensorimotor

area corresponding to that muscle (Kristeva et al., 2007) and CMC amplitude has been shown dependent of precision, attention and fatigue (Kristeva-Feige; Kristeva et al., 2007; Tecchio et al., 2006a).

Spectral coherence can also been used to estimate connectivity between two different regions, thus identifying communicating brain areas (Cortico-Cortical Coherence, CCC). In physiological organization the low or high ranges of physiological coherence can be caused by reduced connectivity of the areas (Wheaton et al 2008) or by the necessity to compensate (Strens et al. 2004) respectively.

Event-related potentials

Event-related potentials are EEG repeatable signals due to internal or external stimulations. While the internal stimuli are relative to a processing step or preparation to movement, the external stimuli are always considered the ones provoked by the sensory inputs. The lasts are produced by tactile, electric (Penfield and Boldrey, 1937), visual (Spehlmann, 1965; Jeffreys and Axford, 1972) and auditory (Joos et al., 2014) stimulation. The brain response to the stimuli (called evoked response) depends on the area involved and its properties and is usually expressed by an amplitude between 0.1-20 μA within the interval of 2-500 ms. Being the brain responses in the same range of the background activity and of external noise, which can be even many times larger, the brain activity phase-locked with the external stimulus, the evoked response, is typically obtained averaging a big number of repetitions (reducing noise in proportion to square of N, number of averaged response epochs).

Evoked potentials (MEP, TEP, SEP)

Motor evoked potentials (MEPs) are quantitative measures used also for clinical purposes to examine cortico-spinal pathway. The technique force the state of the brain with externally induced magnetic stimuli in order to study the nervous system response.

MEP is obtained recording the target muscle EMG activity after a TMS pulse applied over a the cortical area corresponding to the primary representation of that muscle. The relatively new technology for EEG amplifiers allows the recording of cortical activity during TMS stimulations, TMS-evoked potentials (TEPs). Therefore while MEP studies have a long and deep tradition, TEPs are still to be adequately elaborated. Their investigation is crucial to have real-time information on cortical reactivity and connectivity and information about the high inter-subject and inter trials variability of TMS stimulation effects.

Somatosensory evoked potentials (SSEPs) or sensory evoked potentials (SEPs) are brain electrical potentials that arise in response of an electrical stimulation of a peripheral nerve, with typical stimulus duration between 100 and 300 μs. They are easy to elicit and repeatable and have been used in clinical setting to check the integrity of the afferent pathways. The analysis of the evoked potential is performed by averaging, usually more than 300 stimulus trials, in order to maximize the signal to noise ratio. The obtained waveform is the effect of the repeated response from both the gray and white matter in phase with the stimulation. In the case of stimulus' intensity above the motor threshold, the sensory stimulation provokes a muscle twitching which is used as a marker of the intensity level of stimulation.

Brain Computer Interface – BCI

This part is an introduction to BCI technology to contextualize the possible future direction of the research presented in this thesis.

Introduction - Background

The dream of connecting our thoughts to the computer, brain-computer interface (BCI), exists from many years. Our technologic environment made possible to transform this dream to a target to pursuit. In fact, a proper connection is necessary in

order to have a proper BCI, devices that can receive, store and elaborate a huge quantity of data, that can then transform the received information in execution of a task. In the past century the technology dealt with big challenges. The computers were born as simple mechanical calculation machines capable to store a very small amount of data on punched papers, while the recent technological improvements, with computers becoming more performing, treating and exchanging everyday a higher amount of data, and the simultaneous miniaturization of the electronic devices, changed our possibilities. At the same time the sociological advancement and new requirements of the advanced technology made it of spared usage, reducing its expenses. The technologic improvement towed also the research to capture more sophisticated knowledge about the most complex device to be connected: the brain.

First BCI studies started on monkeys at the Regional Primate Research Center and Department of Physiology and Biophysics, University of Washington School of Medicine in Seattle (Fetz EE, 1969), while first large BCI study on humans started at the University of California, Los Angeles, with the Brain Computer Interface project in 1973. Approximately a decade later a group showed that it is possible to move vertically a rocket image across a screen modulating slow oscillating activity (Elbert et al., 1980) and some more years later the event related component P300 was used to spell words (Farwell and Donchin, 1988).

Technological and conceptual advances permitted to study our thinking organ in a more deep and exhaustive manner. The first sight about its real time functioning was given in 20s by a rudimental electroencephalography (EEG). Over the decades diverse techniques were invented and used to investigate the cerebral structure and functionality from different perspectives. Interestingly, the first mean, EEG, is mainly used yet to connect the brain to the computer.

But nowadays what do we really define a BCI?

A BCI is a communication system between the brain and the computer that transforms the received information in commands. The computer does not only acquire and analyse brain signals, but also translate them into information useful for a device, which will consequentelly perform an action. The main points to be stressed in order to avoid confounds are three: BCI is relative only to central nervous signal in loco, peripheral nerves and muscles are not contemplated; an EEG system is not a BCI because it does not change the environment of the user; BCI exists to permit the control of an external device by mean to the clear willing of the user. In fact, BCI is not a machine that can or want to read the thoughts or control the mind of a person (Shih et al., 2012).

With the growth of interest in BCI, mainly for clinical purposes, in the last decade the intra-cranial studies, microelectrode arrays and electrocorticographic (ECoG) systems, gave their fundamental contribution. Nevertheless the high level achievements are still far enough to allow the usage of intra-cranial systems in everyday life. So far the studies have been essentially focused on healthy subjects, but some good results obtained in case of patients with severe diseases, where BCI could play a fundamental role, are a first step in the translational direction.

The contribution to BCI development can be given from different fields because diverse are the disciplines involved. Each part of the process needs its specific experts as engineers, computer scientists, physicists, neuroscientists, neurologists and others. As the BCI technology improves, many new questions arise about the best choices regarding each aspect, i.e. the electrode type, the invasive/non-invasive connection etc. Each field has still to be improved, as the hardware for data treatment, connection for data transfer, proper electrodes, devices for command accomplishment, and brain knowledge. All these aspects have to be updated simultaneously to contribute constantly to the advancements. More is possible to do with BCI and more will be pretended by the BCI systems. Therefore in future the systems will be more complex, able to deal with an

enormous quantity of information and therefore it is important to know as much as possible about the brain behaviour to choose which of its properties to use for BCI, which to discard as noise or useless and which to use to adapt the recording system to highly variable brain state.

Acquired signals

Since the time in which first brain signal was recorded, with an EEG rudimental system by Hans Berger in 1929, many things have changed. New possibilities to record and treat signals came from the development of new acquisition systems and methodologies. Roughly, any system that is able to record a brain activity can be used for BCI, but all of them have their advantages and disadvantages. While the scalp recorded EEG is easy, safe, inexpensive, it has the big disadvantage of acquiring electric signals strongly attenuated and distorted by meninges, cerebrospinal fluid, skull and scalp (Akhtari et al., 2000). To overcome this limitation Intracranial Electrocorticography (ECoG) and Stereoelectroencephalography (SEEG) have been considered. The last is able to record action potentials of individual neurons and local field potentials of a small group of neurons through a small intracortical array of electrodes, but this is also its limit (Krusienski and Shih, 2011b; Shih et al., 2012). ECoG can record the signal from larger areas through a grid or strip of electrodes on the cortical surface (Krusienski and Shih, 2011a). The main disadvantages of these techniques are that they are strongly invasive and that the electrodes undergo strong alterations in long-term usage. Also other types of signal were used for BCI purpose, as the magnetic signal originated by electrical currents sensed through magnetoencephalography (MEG; Mellinger et al., 2007; van Gerven et al., 2009), or functional magnetic resonance imaging (fMRI) that measure the neuronal activity through the blood oxygenation (Lee et al., 2009).

The fMRI has an excellent spatial resolution, but also a low temporal resolution for the slow response of the hemodynamic process. On the opposite the MEG is characterized by a high temporal resolution (measuring directly the neural activity) but a lower spatial resolution. Nevertheless the main problem limiting their use in BCI is that they are both not movable, very bulky and expensive.

Summarizing, the EEG results as the most reliable acquisition technique for the BCI systems for being not expensive as first purchase and during maintenance, for being portable, non-invasive and having a high time resolution with short time constant.

One of the main problems in BCI is the reliability of the information due to the contemporary active sources, the complexity of the brain structure and the not totally explained trial-to-trial variability of the system. At the present, the sites and properties and the functional relationships of many EEG rhythms and evoked potentials have been identified, but, nevertheless, there are still secrets that our brain is keeping undiscovered, and that we need to unveil to permit the BCI improvement.

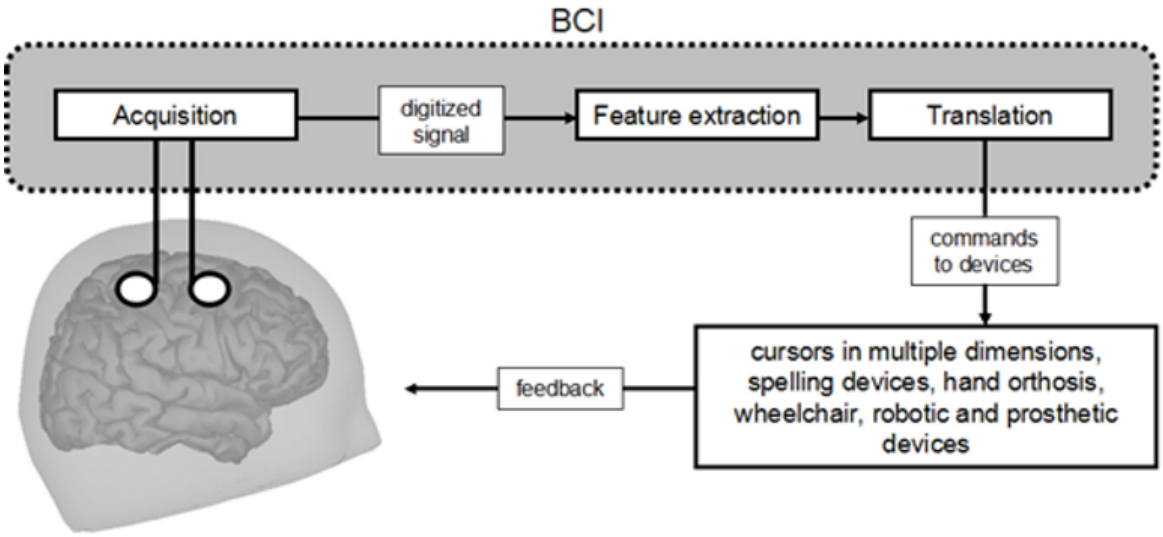

Figure 1.3 BCI system schema

BCI functioning

As previously explained, BCI is a system in which the information flows from the brain to a device via computer in a cascade of consecutive operations (Wolpaw et al., 2002): (1) signal recording, (2) feature extraction, (3) feature translation, and (4) output device control. Once the signal has been acquired, there are some preprocessing steps to be executed by the system in order to enhance the feature, in which is held the subject's intent, and to minimize all other signals, from the environmental noise to the biological ones like the muscular artefact or non pertinent cortical activities. It is necessary then to identify a feature adequate to be easily tuned by the subject and which changes can be easily and in real time recognized by the external system. In fact, at each moment the brain signal is composed by a summation of contemporary active sources and noise of various origins. One way to improve the system sensitivity to the subject's voluntary commands is to consider a training period with two goals: the feedback training can enhance one selected property, for example it has been documented the ability of voluntarily enhance/inhibit the rhythmic activity generated by the primary cortical region controlling the body movements (mu rhythm, Kuhlman, 1978); the BCI training can lead to a better setup of communication/control between the user and the machine.

The brain signals used so far are the time-triggered responses (amplitudes and latencies), or the amount of power in defined frequency bands or neuronal firing rates. The identified feature is then monitored to sense its changes and to translate them into commands for the external device. One of the most important properties of the BCI system is that the algorithm devoted to the feature control have to be flexible and dynamic. It must adapt to the behaviour of brain activity that can change over the time for both spontaneous activity or modifications due to the training. Diverse external

devices have been used to execute an action and provide a feedback to the user, such as the control of a cursor on a monitor, of a robotic arm and others.

Brain features and output devices

The sensorimotor system, which controls our body, is the preferential choice also for controlling an external device by BCI. In fact, the EEG changes with movement and movement imagery (Gastaut, 1952) have been used to control cursors in multiple dimensions, spelling devices, hand orthosis (Pfurtscheller et al., 2000), a wheelchair (Galan et al., 2008; Tanaka et al., 2005) and robotic and prosthetic devices (Di Pino et al., 2012; Tombini et al., 2011). My thesis will be devoted to crucial principles of the sensorimotor system, in future exploitable for BCI feature extraction.

As previously mentioned for the BCI, the comprehension of the brain function is an interdisciplinary challenge and needs contribution of expertise from diverse fields, as mathematics, engineering, computer science, psychology, neurology and others. The contribution of the present study is in the neurophysiological field through the descriptions of phenomena that happen in our brains and could be important for BCI usage. In fact, more are the possibilities given by new technologic devices and more accurate the knowledge of areas chosen for BCI control, and the brain as a whole, have to be. A better understanding of the system functionality is necessary to monitor adequately its changes and to estrapolate only the signals relative to the selected feature, whether they are evoked potentials, spontaneous rhythms, or neuronal firing rates. Inside the whole range of possible directions, this work is thus focused on brain mechanisms relevant for motor control and methods relative to the recognition and extraction of the proper information.

For all the considerations explained in previous paragraphs two choices have been made. First, the EEG was chosen as acquisition system, because at the moment it is still the most suitable for BCI purposes, it is not invasive and it is easy to use. Second, the

primary sensorimotor areas were selected to be studied because they represent the gate between brain and action and have more repetitive activity properties, thus providing indices with more suitable reference ranges, especially for applications in neurological diseases.

Plan of work

The aim of my thesis is to refine the knowledge about sensorimotor areas' contribution to behaviour control. The sensorimotor areas and their integration and communication are represented through a complex equilibrium. Discovering parts of the interplay of communicating brain areas can contribute not only to the overall knowledge of the system, but also give crucial information about possible intervention in case of diseases or damages of the system itself. It's worth to mention that in the last five years a big effort has been done with the Human Connectome Project (HCP, http://www.humanconnectomeproject.org/), a five-year project sponsored by components of the National Institutes of Health of United States. An appropriate knowledge about the system state and communication could provide researchers and clinicians with objective indices for diagnosis or clinical situation definition in rehabilitation processes. Exactly in the path of this research an elaborate set of experiments have been planned in the present project.

Firstly, since the cortico-muscolar coherence showed to be sensitive to changes in peripheral fatigue, we wanted to investigate if the central-peripheral functional connectivity holds the information about central fatigue in multiple sclerosis. In the experimental session the compliance of sustained pressure task was required by two groups of patients, one with high and the other with low perception of fatigue. The idea was that an erroneous communication of cortical areas devoted to movement control would affect also the connectivity with peripheral nerves.

Maintaining the focus on connectivity, the second experiment was set in order to discover the difference in the connectivity path caused by a different response of the sensorimotor system to identical external stimulus. We took advantage of an already known property of the trial-to-trial variability caused by the motor pathway excitability fluctuations during the transcranial magnetic stimulation of the primary motor area. Reproducing this experiment we wanted to discover which regions are involved in the process after the stimulus delivery. For the scope it was necessary to introduce a newly developed data analysis procedure, named localisation stability.

The last experiment was planned on the bases of a strong background of the laboratory in diverse fields, but still in the research of the communication between areas. In this case sensorimotor homologous, and also distant, neuronal pools and their synchrony. The idea was to investigate the changes in connectivity that occur with rehabilitation and thanks to the plasticity after stroke. Using an algorithm, developed in-house, it was possible to study the sensorimotor sources at rest. The resting state is a very sensitive condition to study connectivity and its changes. The study unveiled another possible objective index to be refined and then used in clinical definition of the patients' state in stroke recovery.

All the previous experiments were performed using the newly developed device that is part of the present project. The device was built and programmed using only open source electronic parts and programming environments.

In this context, we assume that BCI will technologically improve in the next years, thanks to the overall technological improvements, and thanks to the increasing interest in the whole spectrum of possible usages of BCI systems: from the most noble, which is the enhancement of the quality of life for persons with severe injuries of the corticospinal system, to the most recreational, as the console games. BCI could be interesting to a so wide audience that also the investments should grow equivalently to

the advancements of the BCI itself. As previously mentioned, BCI needs to be followed from different points of view and each field must contribute, this is the secondary aim of the presented thesis. In fact, considering that the sensorimotor system is one of the most eligible areas for external devices control and that the technological improvements make possible each day to deal with more information, more fine behaviour of the sensorimotor system, that have not been taken into account so far, starts to be of growing importance for the future BCI systems.

CHAPTER 2

STUDY 1 - INTERACTIVE DEVICE FOR EXPERIMENTAL SETUP

To equip the laboratory with a multi-usage device, suitable for the experiments and studies performed generally and in particular for this thesis, an ad-hoc device was developed: **In**teractive **Pres**sure **S**ensor (InPresS). Its goal is to ease a complete and clean experimental setup with a tool useful in diverse acquisition protocols, being easy to set and easily repeatable among different sessions, and even among different laboratories.

InPresS functions are the following:

- Programmed automatic stimuli delivery (trigger);
- Stimuli delivery by operator (trigger);
- Handgrip exerted pressure acquisition;
- Visual feedback of exerted pressure (and eventually of go and stop trigger);
- Required pressure set up with respect to subject's maximal voluntary handgrip contraction.

For the purpose, the project was developed choosing open-source solutions and already existent electronic board, to make, as mentioned above, its replication as easy as possible and available. The device is composed by a hardware box and two software codes one to be installed on the box itself and the other on the operator's computer. The box converts the exerted pressure (over a silicone bulb) into electric analogical or digital signal, sends triggers to acquisition or stimulation devices and both of them to the software installed on the computer (Figure 2.1). The operator's computer pilots the box via an USB cable, receives and stores the data from it and then gives the feedback to the subject. InPresS has been planned for studies involving control of the exerted pressure

by the handgrip over an easy to handle elastic bulb, which both permits sensitive manipulation tasks in healthy people and is suitable for people with motor deficits.

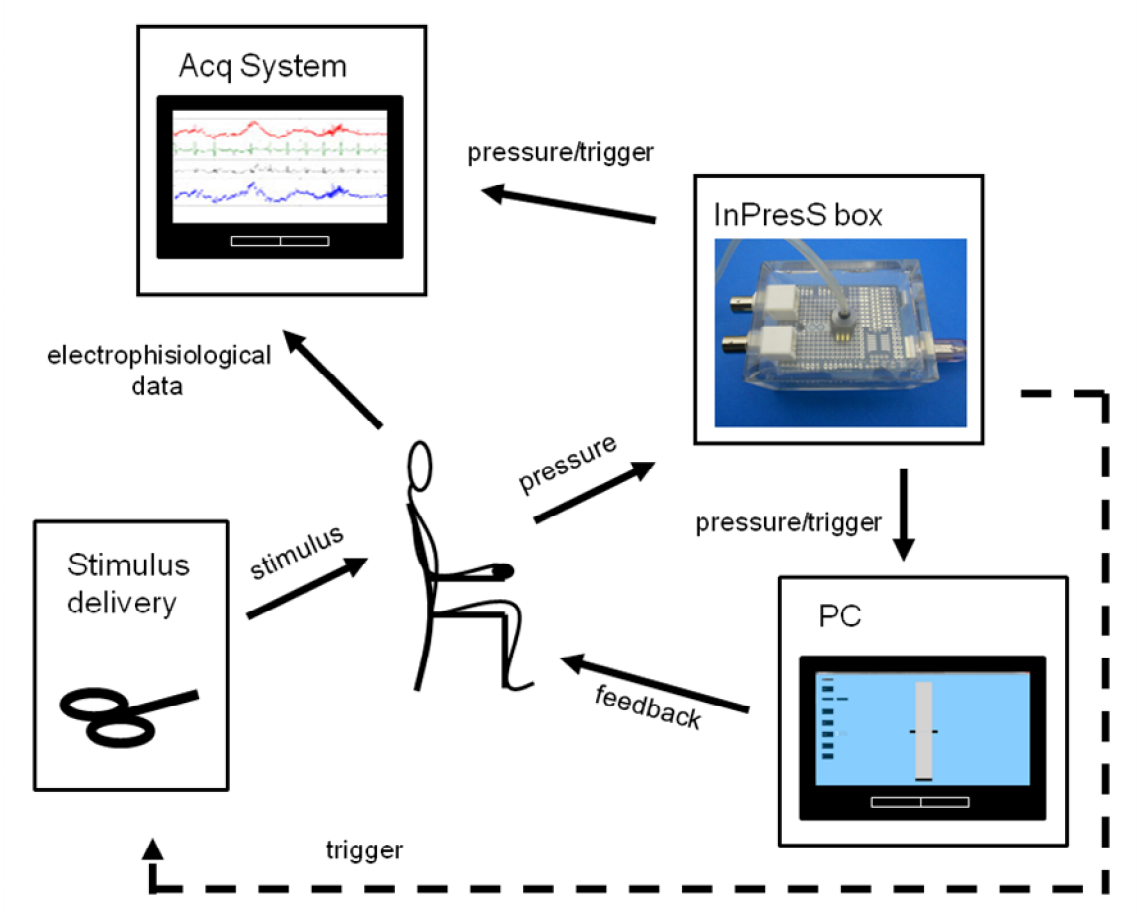

Figure 2.1 InPresS connections

InPresS – stimulation piloting

InPresS was developed for multimodal usage following the ambitions of the present research. For this it can send triggers through BNC ports to pilot the stimulation instrumentation, as the somatosensory stimulator or the transcranial magnetic stimulator (Figure 2.1). For example, there is the possibility to send a predefined trigger train–with either fixed of randomly varying inter-stimulus interval – or single triggers by pressing a button or a key on the keyboard. It is worth to mention that the trigger does not compromise the pressure acquisition and feedback, allowing the stimulation contemporary to the pressure task.

InPresS – exerted pressure monitoring

InPresS has been designed to process, visualize and acquire the exerted pressure (Figure 2.2). The device allows to monitor precise hand gripping of a bulb, a movement typically spared till the severer conditions of movement disorders.

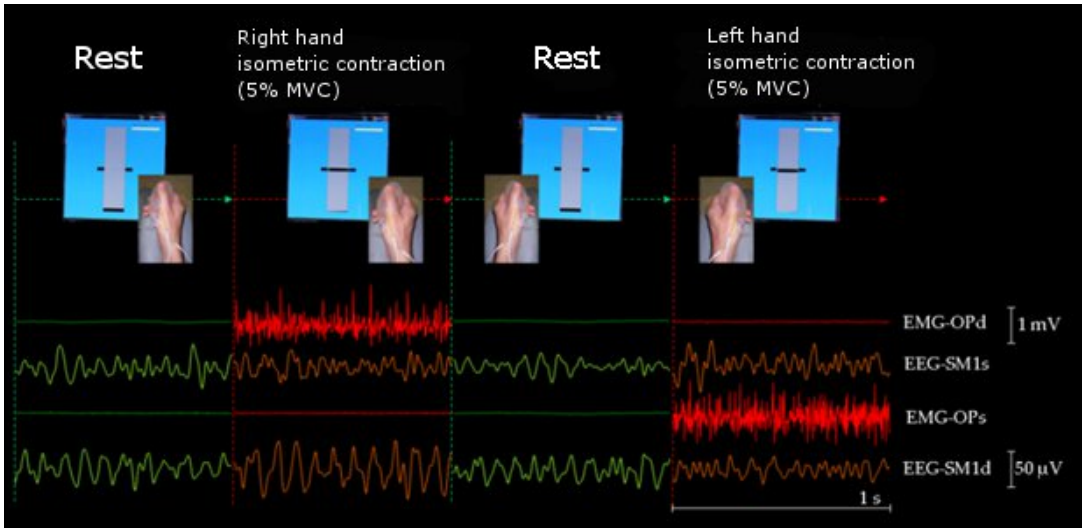

Figure 2.2 Experimental setup investigating hand grip control*

From top: Visual feedback while performing the task with the right and the left hands: period of rest were intermingled to isometric contractions; representative 1-sec signals of EMG from left and right opponens pollicis muscle (rOP-EMG and lOP-EMG) and contralateral bipolar derivation (lSM1-EEG and rSM1-EEG).

At the beginning of the task, InPresS software starts with the acquisition of the subject's maximal voluntary contraction (MVC), which is used to fix the percentage level of required pressure. The MVC is obtained as the mean of the values that are major than 80% of the maximum value reached during three short (less than half a second) repetitions of maximal pressure that the subject is able to execute. The three repetitions

* Reprinted from Tomasevic L, Zito G, Pasqualetti P, Filippi M, Landi D, Ghazaryan A, Lupoi D, Porcaro C, Bagnato F, Rossini P, Tecchio F. Cortico-muscular coherence as an index of fatigue in multiple sclerosis. Mult Scler. 2013 Mar;19(3):334-43 with permission from SAGE Pubblications Ltd.

are planned to be short to not induce fatigue before the start of the pressure task. Once the MVC is obtained, the experimenter sets the required level of pressure as a MVC percentage, which is displayed on the monitor as required pressure level. The motor task is designed with rest periods interleaved by the isometric contraction ones, to avoid the arising of fatigue during the task. The intervals are driven by a trigger sent also to the EEG and/or EMG acquisition system to have all the acquisition systems synchronized (Figure 2.1). All the triggers are sent to external devices through a BNC port, for being easily connectable to any acquisition/stimulation device. The trigger is sent also through USB connection to the InPress software and is stored in the pressure acquisition file. Triggers can be displayed as visual feedback together with the exerted and the required pressure. In all protocols the synchronization between all the devices has an extreme importance for further data analysis.

In addition to the required level, InPresS can display, or not, the pressure exerted by the subject, to provide, or not, a visual feedback of the quality of execution. In case, the feedback is represented by an horizontal bar moving up and down in proportion to the exerted pressure (Figure 2.4).

To realize a stable system some solutions have been implemented. One is the baseline correction of the feedback to take into account that some air could enter into the hose or the bulb. Another is the use in visualization of the online sliding windows averaging to avoid the flicker of the feedback due to various noise sources.

InPresS – hardware

The device is connected to the computer by a USB cable. A flexible silicone hose connects the hardware box and a bulb, which shape is suitable for studying movement control also in patients suffering of partial motor control loss.

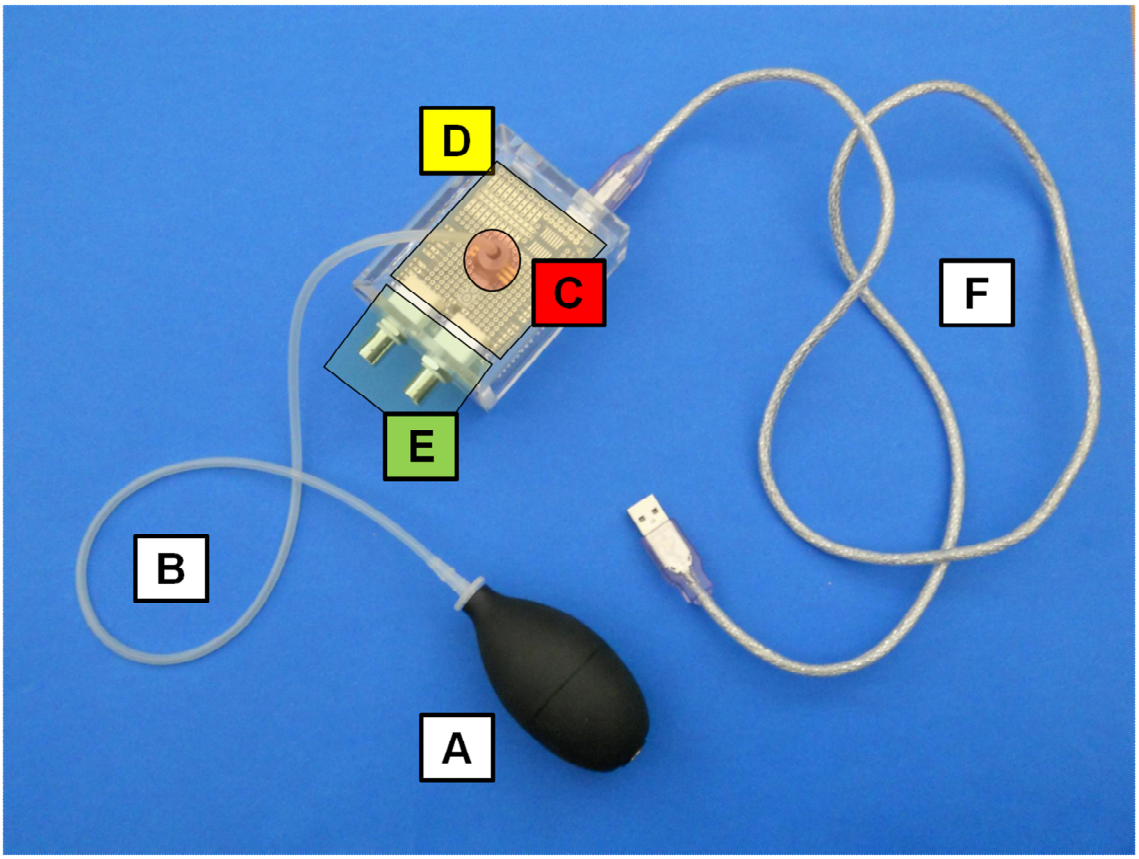

Figure 2.3 Hardware composition of InPresS device

InPresS: A) Silicone bulb; **B)** Silicone hose; **C)** pressure sensor (40PC100G1A, Honeywell Sensing and Control); **D)** Arduino board; **E)** USB connection cable; **F)** BNC connectors

The hardware box is composed by a pomp, a pressure sensor (40PC100G1A, Honeywell Sensing and Control) and an electronic board (Arduino) (Figure 2.3). The pressure sensor is connected both to the Arduino board and to a BNC. The last is to

acquire the output also with an EEG or EMG acquisition system. The best solution is to have all the signals acquired by the same acquisition system.

The choice of each part was made with respect to the experimental necessities, the facility to obtain them and the mutually required properties. For this reason the pressure sensor is adequate to be used with forces expressed by human hand (0÷15 psi). The board, then, has the appropriate power supply connection (5 V) and input channels with the right range for the acquisition of the electrical activity produced by the pressure transducer (0.5÷4.5 V).

Inside the box, on Arduino board, there is an ad hoc software that receives commands from the software installed on the computer and executes the adequate operations. It has been written in Arduino software based on Wiring programming language that is used with microcontrollers. The software installed on the computer is programmed in Processing, apt to communicate with programs written in Wiring and projected to realize easily graphical effects.

Once the device was realised it was used in all the experimental settings described below.

Arduino (http://arduino.cc/)

Arduino is an open source board, cheap, easy to purchase, with also the possibility to adapt it as necessary or even to build it entirely. The schemes and data sheets are all provided on the website. It has been conceived to receive inputs from various sensors and to send outputs to the computer or to control devices, motors, lights, and others. Arduino can be easily programmed by Wiring (programming language which instructions are loaded directly on the board). In the stimulation protocols Arduino can be used as a standalone device, while in the isometric compression task it has been used with the PC. In fact the software that handles via USB with the Arduino

board is installed on the PC and can ‘order’ to the hardware part to act as a stimulator or to give the visual feedback during isometric contraction.

There are diverse versions of the board and in the present study Arduino UNO has been used. It’s main properties are the following:

- Microcontroller ATmega328;
- 14 digital input/output pins (of which 6 can be used as PWM outputs);
- 6 analog inputs;
- 16 MHz ceramic resonator;
- a USB connection;
- a power jack;
- an ICSP header;
- a reset button;
- 5V operating voltage;
- Recommended input voltage 7-12V (limits 6-20V);
- DC current for I/O pins 40 mA (for 3.3 V pin 50 mA);
- Memories on ATmega328: Flash (32 KB), SRAM (2KB), EEPROM (1 KB).

There are diverse ways to supply the board with power: DC power jack (7 - 12V, 2.1mm center-positive plug), the USB connector (5V), the VIN pin of the board (7-12V). As indicated the board works properly with the external supply in the range of 7 to 12 Volts and maximum current draw is 50 mA. Also a battery can be connected through cables to the Gnd and Vin pins of the board.

Diverse are the ways in which the board can deliver the power to external circuits: VIN in case the board is connected to power supply by power jack, 5V and 3V3 pins provide respectively regulated 5 and 3.3 Volts supplies generated by the on-board regulator.

InPresS – software

Wiring (http://wiring.org.co/)

As previously mentioned, Arduino behaviour can be programmed by users through a software based on Wiring, that is an open-source programming environment. It is freely available in internet and made to handle microcontrollers, in this case ATmega, and their interactions with other devices and sensors. It has been developed to give to everybody, even newbies, the possibility to create active objects for many purposes, from artistic to lower level engineer fields.

Main properties:

- Free to download, open source and open hardware
- For GNU/Linux, Mac OS X, and Windows
- Over 100 libraries extend the software

There are several libraries that can be used to control the board, in the present project we used the serial library that handles the data transmission through USB ports.

For InPresS project, an ad-hoc software has been developed. The complete code is in Appendix while below there is a brief recap of the main points.

After a declaration and initialization part, there is a setup block (always present Wiring program structure) that includes the initialization of the serial communication and of the variable with the start time of the program.

At this point starts the loop that executes the main code and which actions depend on the input from the PC. This is performed by the serial library that contains the function *available.* This function listens for the arrival of an information from the serial port. In dependence of the information from the serial port, the possible instructions are listed in the switch/case structure as follows:

case 'S': Imposes to Arduino to send a rect signal to one of BNC outputs (pin 4);

case 'T': Trigger (20 μs duration) sent from the BNC by the operator with a button or key. Useful for TMS;

case 'P': Pressure sent to PC (uses the function *Pressione*);

case 'Q': Quit;

case 'R': Pressure task with 20 seconds of contraction and 10 seconds rest as explained above (uses the function *Pressione*).

The *Pressione* function acquires the analog signal from the pressure sensor, adds the value of the trigger and sends the result to the serial port. The summation of a constant value to the pressure doesn't compromise the acquired signal, but saves the transmission rate. It is possible to send also the trigger and the pressure separately, on two different channels, but this splits in half the transmitted signal. The software running on PC identifies the presence of the trigger (a signal many times higher of the possible pressure) and separates it from the pressure data. This part is treated later in the Processing paragraph.

Processing (http://www.processing.org/)

Processing is an open source language and has its own environment for the development of the code. It has been made for teaching purposes in computer programming, but now it is used for visual effects inside technological projects. For this and for its communication feasibility with Arduino, it is also suitable to be the graphical user interface and stimuli delivery software in experimental protocols. The main programming rules are very similar to Wiring.

Its main properties are:

- free to download
- open source
- interactive programs with 2D, 3D or PDF output
- OpenGL integration for accelerated 3D

- for GNU/Linux, Mac OS X, and Windows

When launched a program written in Processing, it opens a window where the planned graphical effects are contained, but for creating controllers it is necessary to use a compatible GUI library.

GUI library - controlP5 (http://www.sojamo.de/libraries/controlP5/)

For the graphical user interface (GUI) the controlP5 library was selected, which has been written for the Processing programming environment. It has a variety of possible and easily to add controllers such as: Sliders, Buttons, Toggles, Knobs, Text fields, Radio Buttons, Check boxes and others. They are superimposed on top of the Processing window and it is possible to organize them in groups, tabs or to hide them all (or show) through keyboard command combinations. This is very useful to not confound the subject during motor contraction with unnecessary images. In case of a not proper visualization it is possible to move a controller to another position (with ALT-key pressed). The library has also the option to create multiple separate windows for controllers or parallel double sketches.

ControlP5 allows the change of the Processing variable values online because controlEvent function is able to detect the use of a controller while the program is running.

The platforms supported are OS X, Windows and Linux.

Key-commands

ALT-mouseMove move controllers

ALT-h show and hide controllers

ALT-shift-s save controller setup in an properties-document

ALT-shift-l load a controller setup from a properties-document

The InPresS program

The software developed for InPress follows the standard Processing program structure and it is divided in declaration, setup and draw parts.

The declaration part contains the code lines necessary to connect the software with external libraries, such as the graphical or the serial port communication library. The serial port communication is important for the connection to Arduino via USB. Other declarations are of variables dedicated to:

- data
- time
- control
- graphics

The setup part contains the activation of all the structures used by the software. It means that it contains the initialization of the graphics (size of the main window, background colour, properties of the used font, frame rate), of the used elements of the external graphical library (check-box, drop-down list, buttons) and of serial port connection (the COM port number, transmission rate).

The declaration and setup parts are performed only once and, after this step, a loop starts that contains graphical visualization, the *draw* function.

For this project, where the data received from Arduino control the visual feedback, all the main part of data handling is inside the *controlEvent* function. This in fact senses the changes of the values of the controlP5 library and the received instruction is then sent to the switch/case structure.

The first graphical controller is a dropdown menu for the selection of the appropriate serial communication on a list of possible COM ports.

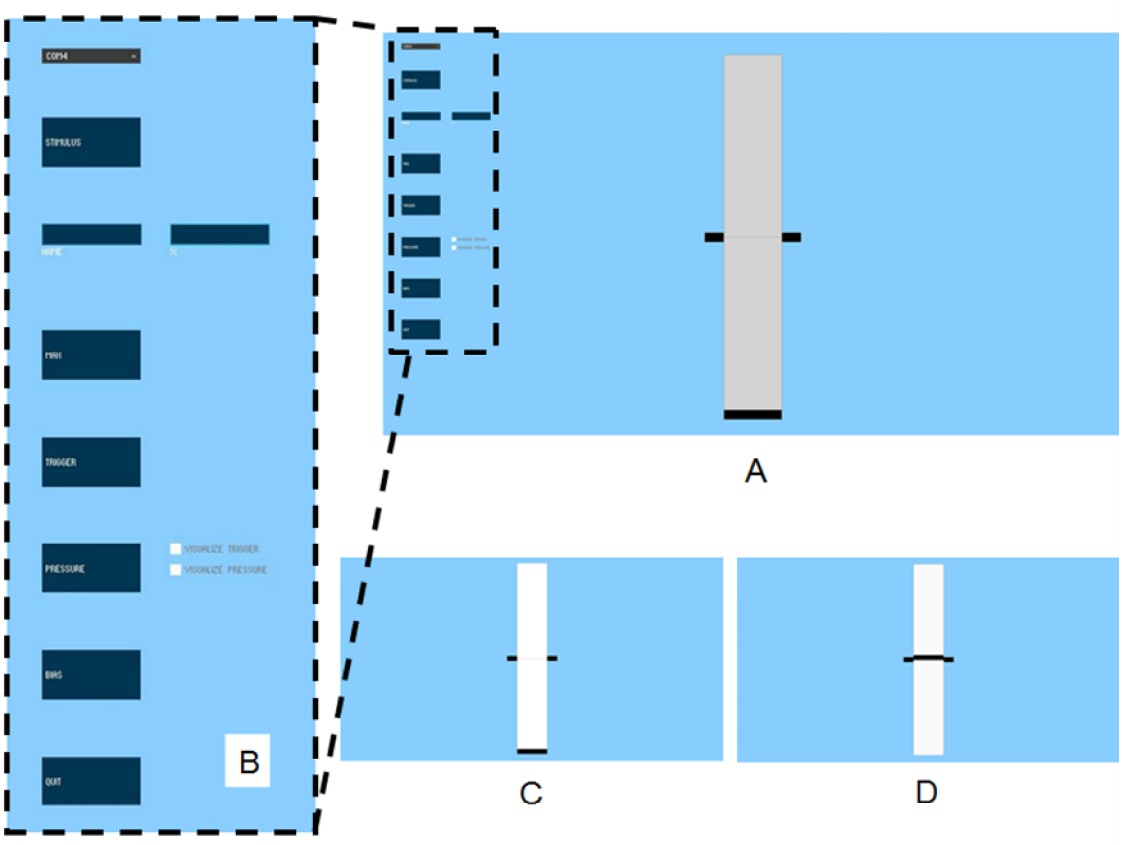

Figure 2.4 GUI of InPresS device software

A) The GUI is ready for operators commands; **B)** Button part enlarged; **C)** Visual feedback at rest; **D)** Visual feedback during contraction.

Then two fields to compile are (Figure 2.4):

- **Name –** this involves also the creation of the output files which name is composed by the subject's name and the current date (nameYYYYMMDD_max.txt and nameYYYYMMDD.txt). The first file will save the exerted maximum voluntary contraction while the second the pressure and eventually the triggers sent by Arduino during the experiment.
- **Percentage** of MVC requested to be maintained by the subject.

Once the Name and Percentage are both inserted, the other control buttons appear to the operator (Max, Trigger, Pressure) with the other already present (Stimulus, Bias, Quit; Figure 2.4).

- **Max** calculates the maximal voluntary contraction as explained below.
- **Quit,** the program saves all the pending values to the output file (nameYYYYMMDD.txt) and closes it.
- **Pressure** task with 20 seconds of contraction and 10 seconds rest.
- **Bias** calculates the new bias value to be subtracted to the incoming data.
- **Stimulus** sends the command to Arduino to start a stimulation protocol by repeated trigger pulses to be delivered to a stimulator through the BNC port.
- **Trigger** the command to Arduino to send one trigger pulse through the BNC port for each button/key pressure.

Two check boxes are also present near the Pressure button, one for pressure and the other for trigger visualization. If the boxes are checked the visualization is suppressed.

Auxiliary functions

Display - defines the feedback to be displayed to the subject (a horizontal bar sensitive to the exerted pressure).

customize - defines the style of the drop down list and adds a row for each available COM connection.

keyPressed (default function) - is sensitive to the keyboard typing, it can be programmed to react at each key pressure. In this case it sends the command to arduino for a single trigger sending (‘t’) or adjusts the bias (‘b’).

Bias - calculates the average value of 100 inputs from the pressure sensor whale there is no pressure exerted over the bulb. This value will be subtracted from each acquired value from the input sensor.

Move - function that moves the cursor bar relatively to the exerted pressure. The central value (to be maintained) over the screen is calculated with respect to the MVC and the imposed percentage. The obtained value is then averaged (100 points) to stabilize the visualization avoiding flicking. A limitation is also implemented for the cursor which excurtion is limited to the designed area.

The *serialEvent* function (part of the serial port library) listens the serial port for incoming data that are read and processed.

MVC evaluation

In case Max button has been pushed, firstly three maximal handgrips are required and then MVC calculation is performed.

When the signal overpasses a first sold (higher than any observed noise fluctuation and chosen to identify the voluntary contraction) the pressure acquisitions starts. A second sold was defined to avoid the stop of the acquisition for noise fluctuations.

All the values over the first sold are recorded inside an array and when the signal definitely goes under the second sold, all the values that are higher than 80% of the maximum value present in the array are averaged.

This procedure is repeated three times and numbered by an increasing number over the Max button.

Once the 3 numbers representing the three short maximal contractions are calculated, their average is computed for the final MVC value. Afterwards the header is created in the output file containing the following values:

- Name
- Date (day/month/year)
- Time (hour:minute)
- Bias
- MVC

At this point the file with the pressure data acquired for MVC is closed and saved (nameYYYYMMDD_max.txt).

Triggers

During the data arrival it is necessary a pre-processing step with the identification of the trigger. In case it is present, it is extracted from the data and saved to a new variable. There are two triggers, one positive and the other negative to mark the start and the end of the contraction task. Then the bias function is called.

The trigger visualization is set up to show a green colour for the start (in correspondence of the arrival of the positive trigger) or a red colour for the stop (negative trigger). In both cases the colour lasts on the screen only for 1 second. It is used to signal the contraction and the relaxation periods (STOP&GO). Bias function is called 3 seconds before each GO signal to calculate the new bias value that is then subtracted from each value that arrives from Arduino.

In case the visualization is set up, the Move function is called, otherwise the cursor bar is kept fix at the centre of the screen.

Two columns of data are constantly saved on the file: the pressure value and the trigger.

Summarizing, InPresS triggers other stimulation devices and shows the exerted pressure during motor task sessions.

CHAPTER 3

STUDY 2 - FUNCTIONAL CONNECTIVITY IN MULTIPLE SCLEROSIS FATIGUE

Introduction

The fatigue is one of the physical conditions responsible for motor control impairing, especially in pathological conditions. Since it can almost certainly occur in any BCI application, we investigated the involved mechanisms. We considered its occurrence in multiple sclerosis (MS), because fatigue can be the most disabling symptom in this brain autoimmune disease when the clinical impairment is mild. For this purpose, two groups of patients were recruited with mild MS and presenting no differences except for fatigue perception.

Fatigue

Fatigue has been defined as a decrease in generating force or performing work at the peripheral (muscular) and/or central (cortical) level (Asmussen, 1979; Bigland-Ritchie, 1981, 1986; Gandevia, 1998; Maton, 1991). Peripheral fatigue is relative to a muscles difficulty to produce force, while the central fatigue, defined as the failure to initiate and/or sustain attentional and physical tasks (Chaudhuri et al., 2000), refers to the inability to properly control the execution of voluntary movements.

Multiple sclerosis

Multiple sclerosis is a disease affecting 2,1 million of people in the world. Its causes are in chronic inflammatory demyelination and axonal degeneration manifested prevalently in late adolescence and early adult life (Gilgun-Sherki et al., 2004). MS goes with a wide range of clinical symptoms in most patients: weakness or diminished

dexterity in one or more limbs, a sensory disturbance, monocular visual loss (optic neuritis), double vision (diplopia), gait instability, ataxia, bladder dysfunction, fatigue and heat. As a disease consequence 60% of patients experience depression. MS diagnosis has been improved by MRI technology evidencing multiple, asymmetrically located white matter lesions distributed throughout the CNS. Four clinical courses have been described in multiple sclerosis: relapsing remitting, secondary progressive, primary progressive, progressive relapsing. Since the relapsing-remitting subtype has prolonged periods without disease activity, nor clinical worsening, between two relapses, this has been chosen as the most relevant for the study.

Moreover the patients cohort has been chosen also on the base of a low disability level, assessed through a standardized method in MS: Kurtzke Expanded Disability Status Scale (EDSS) <2.

Fatigue in multiple sclerosis

The fatigue in MS is defined as a "subjective lack of physical and/or mental energy that is perceived by the individual or caregivers to interfere with usual and desired activities" (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998).

Fatigue in MS is present in up to 70% of disease cases (Krupp et al., 1988; Freal et al., 1984) being perceived even in earliest phases of mild disability (Krupp et al., 1988) and may impact on emotional, physical and social functions reducing the overall quality of life (DeLuca et al., 2008).

Being the fatigue a subjective and non-specific symptom it may be mixed up with weakness or depressed mood. In MS the difference between the central and peripheral fatigue plays a fundamental role, patients in fact may suffer of mental fatigue not sustained by parallel physical fatigue (Ford et al., 1998). Another evidence of the central origin of MS fatigue and of the importance of the sensorimotor system results from studies which reported a hyperactivity during the motor task and inhibitory mechanisms

failure afterwards in frontal areas (Leocani et al., 2001) and primary motor (M1) areas (Liepert et al, 2005) and an increased M1 excitability (Thickbroom et al, 2006).

Other studies by diverse techniques have showed that fatigue involves multiple brain regions part of the sensorimotor system, including the internal capsule, periventricular trigone (Colombo et al, 2000), right parieto-temporal and left frontal regions (Sepulcre et al, 2009) and associates to structural and functional connection alterations, evidenced by diffuse axonal pathology (Tartaglia et al, 2004; Tellez et al, 2008), the disruption of the cortico-subcortical circuits linking the cortex to the basal ganglia (Roelcke et al, 1997; Filippi et al, 2002).

Fatigue scales

Being the fatigue definite 'subjective' the self-reports have been used to quantify it. Among the existing fatigue scales some of the most frequently used are: the Fatigue Impact Scales (FIS) which is 40-item questionnaire that assesses the impact of fatigue on cognitive, psychosocial and physical functioning (Vucic et al., 2010); the Modified Fatigue Impact Scale (MFIS) is a shorter version of the FIS with 21 items chosen to assesses the impact of fatigue on everyday life; the Fatigue Severity Scales (FSS) is a 9-item questionnaire that assesses the severity, frequency and impact of fatigue on daily life (Comi et al., 2001).

Corticospinal tuning changes in multiple sclerosis (MS) depending on fatigue levels

Our study (Tomasevic et al., 2013) of motor system involvement in MS fatigue focused on cortico-muscular coherence (CMC), a measure well known to assess the physiological and pathological organization of movement control through the cortico-muscular coupling between the neuronal activities of the brain areas controlling the execution of a movement and the contracted muscles. CMC and its sensitivity to the sensori-motor area alterations has been introduced in Chapter 1. The investigation has been performed thanks to the collaboration of various experts, as clinicians for recruitments, and MRI analysis expert for structural assessment via volumetric MRI-derived metrics (MD Zito).

Patients cohort was recruited to enhance only the fatigue difference between the two groups of subjects and, for the proved involvement of the sensorimotor system and of the connectivity to the fatigue perception, the cortico-muscular coherence (CMC) was chosen as a possible sensitive index (Conway et al, 1995; Piper, 1912). In fact, it is the measure of the central-peripheral coupling of neural/muscular oscillating rhythmic firing, and contains information about primary motor area (M1) connectivity. Being M1 the final and most frequently used cortical station before the movement command delivery to the rest of the body, it includes all the impairments of the preceding stations. Studies in MS showed also that nodes within networks that are more frequently used have higher possibility of being influenced by remote damage (Dell'Acqua et al, 2010; Tecchio et al, 2008c).

Previous studies have demonstrated that CMC amplitude in healthy subjects is sensitive to the precision needed to perform a hand gripping task. In fact, the number of corrections increased to maintain the performance of the motor task and this corresponded to higher level of CMC (Graziadio et al, 2010). On the other hand the muscular fatigue, that can be also due to a fatiguing task, showed a shift of the

coherence to higher frequencies. Our investigation was designed not to induce muscular fatigue within the probing task execution. We employed InPresS (Chapter 2), not only for the feedback through which the patients were able to monitor the quality of the precision in maintaining the required pressure, but also to record the pressure all along the task. This measure was integrated with other parameters to control for the possible occurrence of fatigue along the experimental task (pressure, EMG power, EMG median frequency).

Radiology: MRI exam and measure estimate

To test the two groups for homogeneity also from brain structural perspective, several analysis have been performed by the MRI expert on the brain as a whole: total lesion load, lesion relative fraction, brain parenchymal fraction; and on the primary motor control system: thalamic volume, rolandic cortical thickness. None of them showed differences between the two groups.

METHODS

Patients

Twenty mildly disabled RR-MS20 patients were recruited from the MS center of the department of neurology of the Fatebenefratelli Hospital in Rome, Italy, after the approval by the Ethical Committee and following the ethical standards noted in the 1964 Declaration of Helsinki. Prior to their inclusion to the study, all the patients signed the informed consent.

For each patient, the clinical history was collected inclusive of disease duration, annual relapse, ongoing disease-modifying therapy (DMT) and other scores as Beck Depression Inventory (BDI; Beck et al, 1961), Expanded Disability Status Scale (EDSS; Kurtzke et al, 1983) and Nine Hole Peg Test (9-HPT; Mathiowetz et al, 1985).

Since a correlation between higher perception of fatigue and severe depression in MS

patients has been demonstrated (Pittion-Vouyovitch et al., 2006), the EDSS results have been used in exclusion criteria. It is important to non confound the fatigue level with the mood state while comparing the two groups.

The immunomodulatory treatments are another point that has been taken into account since they may induce feeling of tiredness (Leocani et al., 2001).

For all this reasons, and considering the importance of having a homogeneous cohort of patients, the exclusion criteria included:

- presence of clinical relapse or radiological evidence of disease activity over the last three months;
- ability to rule out physical disability or depression as confounding factors by EDSS score ≤ 2 and BDI score <13 as cut-off in recruiting patients;
- assumption of symptomatic drugs which may affect the level of fatigue, depression and anxiety within the past three months (Rietberg et al, 2011);
- epilepsy or other central/peripheral nervous system comorbidities;
- any systemic conditions that may cause fatigue (e.g. anemia or pregnancy).

For fatigue definition, the Fatigue Severity Scale (FSS; Krupp et al, 1989) and the Modified Fatigue Impact Scale (MFIS; Kos et al, 2006) were used, where MFIS identifies the physical, cognitive and psychosocial influence of fatigue. The physical sub-score (MFIS_phys, total range (0–36)) was considered for defining the belonging of a patient to the fatigued (FatigYes ≥ 16) or non-fatigued group (FatigNo < 15; Riccitelli et al, 2011).

Within a few days after MRI recordings and after the confirmation of absence of acute lesions, EEG sessions were performed.

Electrophysiological investigation

EEG, EMG, and electrocardiogram (EKG) were recorded band pass filtered between 0.48 Hz and 256 Hz, sampled at 1024 Hz with Micromed System Plus SAM32

(Micromed S.p.A., Mogliano Veneto, Italy) using Ag/AgCl electrodes. EEG montage was of 23 channels, to the standard 19 channels of the 10-20 International EEG system, 4 more channels were added in the sensorimotor area (FC4, FC3, CP4, CP3; Figure 3.1). The reference was medio-frontal and the ground occipital.

The belly-tendon montage (2.5 cm inter-electrode distance) was used for surface EMG recording of the left and right opponens pollicis muscle (EMG_{OPr} and EMG_{OPl}).

The subjects, sitting comfortably in a chair, executed the motor task using the InPresS device. As previously explained, firstly the maximum voluntary contraction (MVC) was estimated and after a pause of 2 minutes started the motor task. This was composed by 12 blocks of 20 seconds of isometric contraction at 5% of MVC during visual feedback interleaved by blocks of 20 seconds of relaxation of muscle tone. The low level of force and the pauses between blocks were defined in order to minimize weariness related to the task. Artifacted intervals were excluded, such as the transactions from rest to isometric contraction and vice versa (2 seconds per interval), obtaining not less than 200 s of data per subject. The quality of the execution was calculated as the difference between the required and the exerted pressure, normalised by the mean.

Cortico-muscular coherence

The CMC is an adequate measure of the brain and muscle coupling as explained previously (Chapter 1). In this case the coherence was calculated between the rectified EMG of opponens pollicis (EMG_{OPr}) and the EEG bipolar derivation (EEG_{SM1}). The bipolar derivation has been chosen as the one with maximal coherence with EMG (Graziadio et al, 2010), then the maximal amplitude and the relative frequency of the coherence were used in further statistical analysis. Both the CMC and the power spectral density (PSD) of the two signals, EMG_{OPr} and EEG_{SM1}, during the motor task were calculated through the windowing method (2048 ms duration, Hanning window, no overlap, number of artefact-free trials fixed across patients). CMC was identified in

all patients and none of them was excluded from the study for data quality reason.

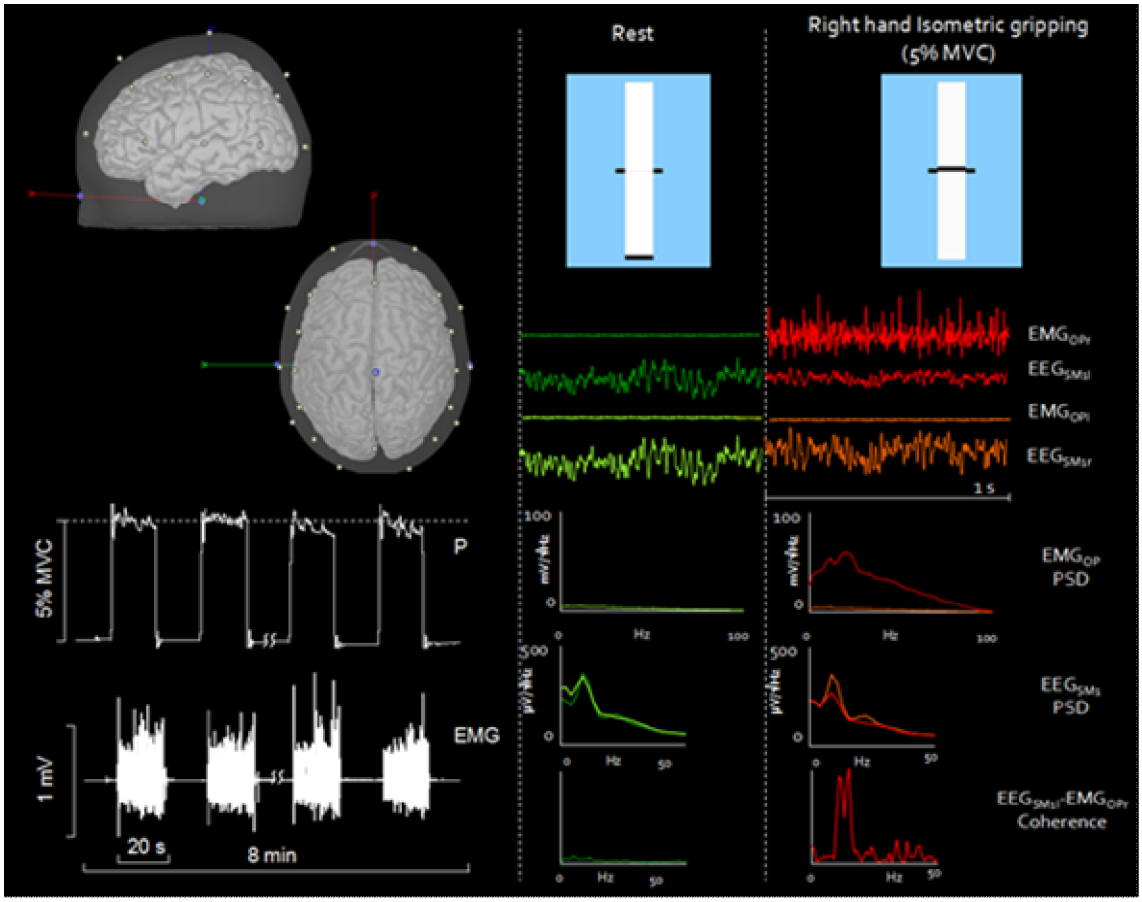

Figure 3.1 Experimental setup investigating hand grip control*

Left top EEG montage; **Left bottom** the exerted pressure acquired by InPresS and the EMG of the relative opponens pollicis during the first and last two contractions; **Right from top** the visual feedback, the EMGs of the opponens pollicis relative to both hands and EEG bipolar derivations maximally coherent with contralateral EMG activity, the PSDs of the EMG and bipolar EEG, the CMC in the two conditions.

Statistical analysis

Fatigue-dependent group analysis - After checking for the normal distribution of the variables, the appropriate transformation has been applied when necessary. Then

* Reprinted from Tomasevic L, Zito G, Pasqualetti P, Filippi M, Landi D, Ghazaryan A, Lupoi D, Porcaro C, Bagnato F, Rossini P, Tecchio F. Cortico-muscular coherence as an index of fatigue in multiple sclerosis. Mult Scler. 2013 Mar;19(3):334-43 with permission from SAGE Pubblications Ltd.

independent samples t-test was used to identify the between group differences related to fatigue. The Analysis of variance (ANOVA) was applied to between-subjects factor FatigGroup (FatigNo, FatigYes) and the within-subjects factor Band (Delta to High gamma) to investigate EEG_{SM1} and the rectified EMG_{OPr} in the frequency domain through their PSDs.

Fatigue-dependent regression analysis – To determine the fatigue score variance explained by the electrophysiological variables, a multiple regression model has been set up with fatigue score as dependent factor and significant variables as the independent factors.

To identify the possible confounding factors in the regression analysis, the correlation coefficients between fatigue scores and all the other variables (performance, exerted pressure, EMG area, EMG median frequency and heart rate) has been calculated and factors with $p<.200$ have been introduced as covariates into the model.

Results

Groups homogeneity

As previously explained, the two groups of patients were selected with respect to the fatigue-dependent difference criterion, maintaining them fully comparable in terms of all demographic and clinical features.

Fatigue scores, evaluated by the FSS, MFIS_tot and MFIS_phys, did not correlate with any of the clinical and demographic variables (no correlation with age, disease duration, annual relapse rate, EDSS, or BDI) nor neuroradiological variables (total lesion volume, lesion relative fraction or brain parenchymal fraction, mean between left and right thalamic volume and cortical thickness). There were no differences between the two groups either in the fine hand motor control assessed by 9-HPT test (independent sample *t*-test $p=.949$).

Weariness

We evaluated whether the fatigue has been induced by the experimental task (which we called *weariness* for the sake of clarity) to exclude that the observed fatigue-dependent results would depend on the probing task instead of the individual condition. The weariness during the motor task was estimated trough indices known to score fatigue: pressure (reduced), EMGOP amplitude (increased) and median spectral frequency (lowered), heart rate (increased) (Figure 3.2). Weariness within trial was calculated using the first and the last 4 seconds of each period of contraction and weariness along session was estimated considering the first and the last two periods of contraction.

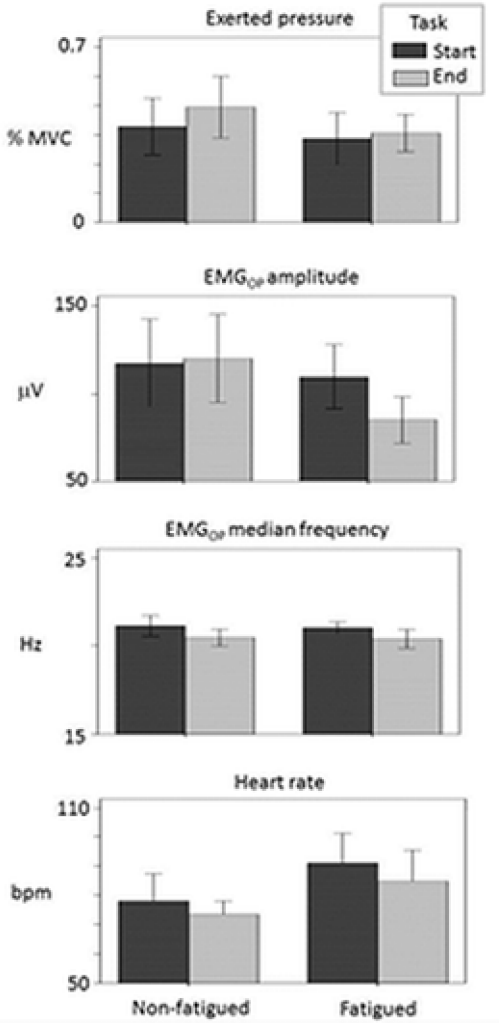

Figure 3.2 Weariness[*]

Vertical bars indicate one standard error. No difference was found between the two groups for any variable.

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To identify possible different task-induced weariness of patients in dependence on their condition of fatigue, models within trial and along session were estimated. For each of the above indices, ANOVA included *Weariness* (first epoch, last epoch) as within-subject factor and *FatigGroup* (Fatigues, Non-fatigues) as between-subjects factor.

The interaction *FatigGroup*Weariness* was not found for any of the fatigue indices (p>.200 consistently for the pressure level, the amplitude or median spectral frequency of the EMGOPr or heart rate), indicating that both fatigued and non-fatigued patients were similarly prone to weariness secondary to the motor task. No main effect of *FatigGroup* (p>.200) indicated that the two groups were comparable for overall indices values.

Performance

The quality of the execution of the pressure task was studied through the distance between the required pressure level, normalized by the mean pressure along the contraction periods from the whole task. The transition ramps were excluded.

The two groups did not differ regarding the performance.

CMC during right handgrip

After excluding epochs with artefacts, at least 226 seconds of clean data were used for to have a fixed number of epochs (110) across subjects for CMC and PSD estimation. The analysis lead to the result that fatigued patient compared to non-fatigued patients expressed CMC at higher frequencies (independent sample t-test <0.001), while no difference was found for CMC amplitude (Figure 3.3).

PSD

Power spectral densities of EMG_{OPr} and the EEG_{SM1} were estimated and then studied in physiological CNS bands. No effects were found for ANOVAs with within-subject factor Band and between-subject factor Fatigue (p>.500 consistently).

None of task performance, exerted pressure, EMGOP amplitude, EMGOP

median frequency or heart rate correlated with fatigue scores (p>.300 consistently). This analysis was performed for weariness variables either inside periods of contraction and along the whole session .

	CMC		Task performance	Correction rate
	Freq (Hz)	Amp		
FatigNo	**16.7** (3.6)	.06 (.05)	0.05 (0.04)	**2.20** (0.55)
FatigYes	**27.5** (4.8)	.07 (.02)	0.03 (0.02)	**1.40** (0.38)
t test p	*<.001*	*.720*	*.412*	*.006*

Table 1. Motor execution indices in the two Fatigue-dependent groups during right isometric handgrip[*]

Mean (sd) of CMC frequency (Freq) of the maximal amplitude peak (Amp, dimensionless between 0 and 1) and of the ratio between the exerted pressure power at low and high frequencies (Correction rate). Values differing between the two groups are bolded, according to independent t test 2-tails significance.

Correction

The working hypothesis of the study was that the fatigue symptoms were related to a neuronal communication fail. As a measurable result of the impaired sensorimotor communication, we selected an overcorrection of the voluntary controlled movement. We defined an index as the ratio between pressure Power Spectral Density (PSD) at low and high frequencies (CorrRate), quantifying the relationship between less frequent and

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more frequent corrections of the exerted pressure. Lower index values correspond to more frequent corrections.

While the fatigued and non-fatigued patients maintained the same level of performance, fatigued patients showed a more frequent correction of the exerted pressure (Table 2; Figure 3.3).

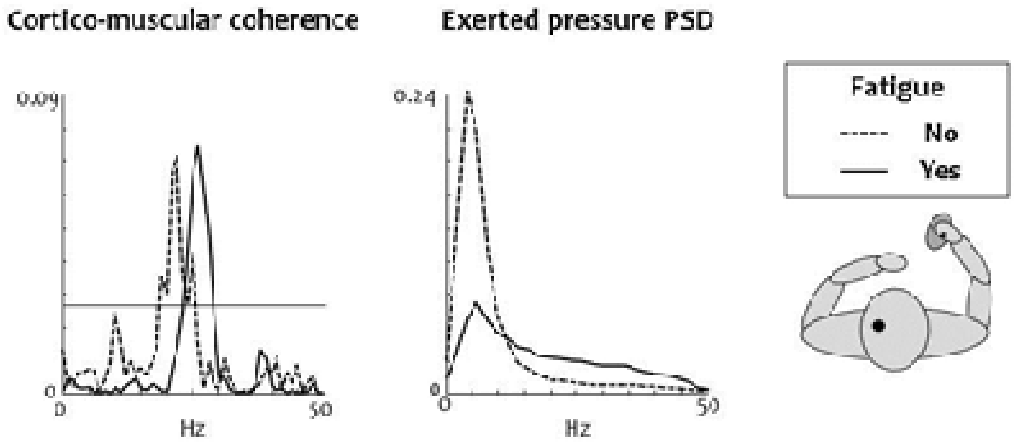

Figure 3.3 CMC and task execution dependency on MS fatigue[*]

For patients with fatigue (full line) and with no fatigue (dotted line): **left -** the coherence function between EEG_{SM1l} and EMG_{OPr}, horizontal line is the significance level; **right -** power spectral density function (PSD) of the pressure exerted on the InPresS bulb. The discriminating point between low and high frequencies was selected for each subject when the first derivative became stable within 10% of the difference between its maximum and minimum in 0–90 Hz.

Fatigue-explaining regression analysis

The frequency of the CMC peak and the pressure correction index were also studied in regression with the collected fatigue scores to better investigate which factor is more relevant. They were included as independent variables in the regression model

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with the dependent variable $MFIS_{phys}$ and only the CMC frequency fitted into the model:

$$MFIS_{phys} = -8.374 + 0.968\ CMC_{Freq}$$

with 67% of the $MFIS_{phys}$ variance explained by the maximum peak frequency of CMC [F(1,9)=18.059, p=.002]. Analysing whether CMC_{Freq} was associated to CorrRate, we found that as CMC expressed at higher frequencies also the pressure was corrected more frequently (Pearson's correlation between CorrRate and CMC_freq *r*=-.643, *p*=.033).

		MFIS_tot	MFIS_Phys	FSS
CMC_Freq	r	**.773**	**.771**	**.737**
	(p)	(.005)	(.001)	(.003)
Correction rate	r	-.307	***-.551***	**-.645**
	(p)	(.247)	(.027)	(.007)

Table 2. Motor execution indices in relationship with fatigue scores during the right isometric handgrip*

Correlation between the fatigue scale scores and the cortico-muscular coherence frequency (CMC_Freq) and Correction rate. In the table the pearson coefficient (r) and 2-tailed significance (p) are shown (in bold values with significance at the .01 and italic at .05).

Discussion

The main discovery of the present study is that only variables related to movement execution showed dependence on the fatigue, while the morpho-structural

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measures related to the rolandic sensorimotor area didn't show any association. In fact, in fatigued patients, the cortico-muscular connectivity arose at higher frequencies and also the exerted pressure during steady state handgrip was corrected more frequently.

In this study, not only the CMC amplitude was not different between fatigue-dependent groups, but it showed also similar behaviour to the amplitude of previous studies on CMC in young adults (here .07 ± .02 in fatigued patients and.06 ± .05 in non-fatigued ones vs. .08 ± .04 in 10 subjects with age range 21–35 years, Graziadio et al, 2010).

In previous studies on healthy subjects the CMC shifted to higher frequencies when the task required force level changing over time compared to constant ones (Kristeva et al, 2007). If compared to the results obtained in the present study, it suggests that for MS-fatigued patients the isometric contraction is perceived as a more complex movement continuously changing. At the same time the continuous correction was not the origin of fatigue, which was mostly explained by CMC frequency. Also the correction rate was associated with fatigue, but it did not enter the regression model, indicating that movement control corrections are less responsible of fatigue compared to the functional connection alteration. In our study, the latter, expressing an impairment of the central-peripheral communication, was sensitive to motor system functional changes that are more evident with more severe fatigue symptoms.

Previous studies on dystonic patients showed a reduced M1 responsiveness to peripheral nerve stimulation similar to those we observed in MS patients, but no changes of CMC frequency (Dell'Acqua et al, 2010; Tecchio et al, 2008c). This finding could confirm the specificity of the present picture for MS fatigue, also related with the difficulty of performing everyday motor tasks in fatigued MS patients.

Furthermore, we compared CMC after vs. before motor fatigue, i.e. a maximal voluntary contraction maintained as long as possible, in healthy people while executing a similar hand gripping non inducing intra-task weariness and we observed CMC

amplitude increasing with no changes in frequency (Tecchio et al, 2006a). This also strengthens that the mechnisms subtending MS-fatigue are not those of muscle fatigue in healthy subjects (Bigland-Ritchie et al, 1984; Sheean et al, 1996).

The presented results also showed that power properties of central motor area and muscles were not dependent on fatigue, opposite of their connectivity. Therefore CMC frequency increase may reflect a non-matching feedback counterpart within the sensori-motor control network, with the functional communication between the involved nodes more impaired than each node's activity alone.

CHAPTER 4

STUDY 3 - BRAIN STIMULATION TO ASSESS MOTOR SYSTEM FUNCTIONAL CONNECTIVITY

The idea of the study was to investigate the perturbation of cortical communication, caused by the spontaneous fluctuations of the excitability of brain areas. Therefore here we analysed whether intra-subject and intra-session spontaneous fluctuations of motor pathway excitability can influence the cortico-cortical, cortico-subcortical, and spinal pathways impinged by M1 projections (Giambattistelli and Tomasevic et al., 2014).

Introduction

The coregistration of the EEG activity during the stimulation of the primary motor cortex (M1) relative to a muscle and its electromyographic (EMG) recordings of the motor evoked potential (MEP) allows the study of M1 cortico-cortical and intrahemispheric and interhemispheric connections (Bender et al., 2005; Bonato et al., 2006; Ferreri et al., 2010; Huber et al., 2007; Ilmoniemi et al., 1997; Komssi et al., 2002, 2004; Nikulin et al., 2003; Paus et al., 2001). In fact, MEPs responses and amplitudes are the result of the combination of the cortical, spinal and neuromuscular states, i.e. the excitatory/inhibitory phenomena occurring along the whole motor pathway. MEPs properties, latency and amplitude, contain the information about the functional state of the corticospinal motor pathway (CSMP) and its transfer capabilities, useful for both physiological and pathological conditions evaluation (Barker et al., 1985b; Rossini and Rossi, 2007 for a review). While the onset latencies are relatively stable, the amplitudes, on the other hand, are highly variable within the same

experimental session with the same subject. This trial-to-trial variability occurs with no apparent changes in the experimental setup, in the delivered stimulus, nor in the subject parameters (Ellaway et al., 1998; Starr et al., 1988). Even minimum shifting of the background tone of the target muscle can affect the MEP amplitudes, but for example cardiac or respiratory phases are not influent with respect to the TMS pulse (Amassian et al., 1989; Filippi et al., 2000; Kiers et al., 1993; Sparing et al., 2008). Therefore, with spatially and physically stable stimulus, MEPs variability analysis counts as studying the spontaneous fluctuations of neuronal and trans-synaptic excitation at cortical (Adrian and Moruzzi, 1939) and spinal levels. TMS/EEG is a technique that can lead to an explanation of the origin and consequences of these fluctuations at cortical level (Amassian et al., 1989; Magistris et al., 1998; Rossini et al., 1991; Steriade and Llinas, 1988).

The aim of this study was to take advantage of the TMS/EEG technique to quantify whether the state of the CSMP in terms of excitation level, evaluated through MEP amplitude, influences the cortical connectivity. Previously other laboratories approached similar investigation through EEG channel-derived measures (Bonato et al., 2006; Mäki and Ilmoniemi, 2010a; Nikulin et al., 2003; Paus et al., 2001). Here, also the sensory and proprioceptive feedback, due to different MEP amplitudes, was taken into account considering separately the recruitments that occurred before and after the peripheral inflow arrival. The study revealed that intrahemispheric and interhemispheric cortico-cortical and subcortico-cortical connections recruited ipsilateral and contralateral cortical areas.

The second aim was to identify a method to follow the signal fluctuations in each individual subject. Since the TMS/EEG data are strongly impacted by the magnetic pulse, the single subject signals are very difficult to manage, especially when the expected magnitude of the effect is relatively small as in this case. Contrasting subject

by subject two different conditions it was possible to minimize all the common signals (artefact and brain response common to the two conditions); then, taking advantage of the grandaverage, it was possible to localize the involved regions, goal not achievable in individual data (sLORETA; Fuchs et al., 2002; Pascual-Marqui, 2002). With the introduction of the concept of localization stability the areas were defined in relation to a temporal interval and then used to extract the single subject activity. In fact, the current density peaks were used as measure of the recruitment strength of the identified areas (Casali et al., 2010; Massimini et al., 2005) and analysed for statistical significance.

Methods

After giving the written informed consent to the experimental protocol, previously approved by the institutional Ethics Committee, 10 healthy volunteers (6 males, age range 19–35 years; four females age range 18–30 years) were enrolled for the experiment. All of them were instructed to abstain from caffeine, alcohol, and medication and to maintain their regular sleep-wake schedule for 3 days before the experimental session. The followed exclusion criteria were those established by international safety standards for TMS (Rossini et al., 1994; Rossi et al., 2009). For homogeneity the subjects were all right-handed as evaluated by the Handedness Questionnaire (0.70±0.08). The EEG recordings were performed using the TMS-compatible equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) with electrodes mounted on an elastic cap and placed on 32 scalp sites following the 10-20 International System (Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T7, T8, P7, P8, Fz, Cz, Pz, FC1, FC2, CP1, CP2, FC5, FC6, CP5, CP6, TP9, TP10, FT9, FT10, FCz) with two more electrodes used as ground (Oz to have maximal distance from the stimulating coil) and reference (linked mastoids). TMS-compatible

Ag/AgCl-coated electrodes were used to minimize overheating of the electrodes located in the vicinity of the stimulating coil. Skin/electrode impedance was maintained below 5 kΩ. MEPs were acquired recording the EMG activity from the dorsal interosseous (FDI) muscle of the right hand via surface electrodes in belly tendon montage. TMSwas performed with the Magstim SuperRapid magnetic stimulator with a figure-of-eight coil having an outer wing diameter of 7 cm (Magstim Company, Whitland, UK). Once the EEG cap was mounted, the coil was placed tangentially to the scalp over the site corresponding to C3 with the handle pointing backward and laterally at about a 45° angle from the midline. In order to identify the coil position that induces maximal MEPs, the coil was moved in steps of approximately 0.5 cm searching for the position that induces maximal MEPs from the right FDI. Then the coil position and orientation with respect to the subject's head was monitored online by a neuronavigation system (NBS system, SofTaxicOptic, Bologna, Italy) (Fig. 1). The FDI resting motor threshold (RMT) was determined as the lowest stimulus intensity eliciting at least five MEPs of 50 mV out of 10 consecutive stimuli (Rossini et al., 1994, 1999). All the signals were recorded at the sampling rate of 5 kHz and band-pass filtered (50–1,000 Hz EMG and 0.1–500 Hz EEG). Considering that the coil-generated clicks evoke cortical responses, that are part of the summation of effects of the stimulation, and considering also that in this case these responses can be considered as cortical noise, a white noise was continuously delivered through earphones. The volume of the white noise was risen until a level that covered the TMS click, as reported by the subjects (always below 90 dB). Once the experimental session was set up, about 120 magnetic stimuli were delivered at 120% RMT (supra-threshold stimulation) over the left M1 region. The stimulation was triggered with InPresS using a random interstimulus interval of about 5 s. The subjects were asked to keep their eyes open and the gaze on a fixation point

during subperiods of 2-3 minutes to ensure wakefulness and reduce the impact of the ocular artefact.

Data analysis

Offline analysis of the EEG and EMG data were performed using MATLAB (The Mathworks, Natick, MA). The preprocessing steps consisted in:

- removal of excessively noisy channels and trials after visual inspection;
- removal of trials with a muscle contraction in the 300 ms preceding the stimulation;
- filtering of EEG data using a second-order Butterworth band-pass (1-100 Hz) filter;
- reduction of non-biological (TMS) and biological (eye and muscular) artefacts through independent component analysis (Barbati et al., 2004).

As previously explained, the analysis of cortical connectivity influenced by CSMP excitation was studied through the variability of MEP amplitudes. Therefore firstly peak-to-peak MEP amplitudes were determined on EMG data and then TMS-evoked EEG responses were averaged separately for trials corresponding to the smallest (EEG_low) and the largest (EEG_high) thirds of MEP amplitudes (Mäki and Ilmoniemi, 2010a; Tecchio et al., 2008b). For each subject two averages from 100 ms pre-stimulus to 500 ms post-stimulus were obtained with baseline correction in the (-100, -10) ms interval.

A new procedure to identify relevant cortical sources was used:

1) Computation of the difference between the individual averages: $(EEG\_high)_i$ - $(EEG\_low)_i$, where *i* indicates each subject. This step minimizes the artefact common to the whole session, mainly due to the TMS pulse and facial muscle contraction.
2) Evaluation of the grand average across all subjects of the individual differences obtained at the previous step.
3) Localization of sources relative to the difference of the two states.

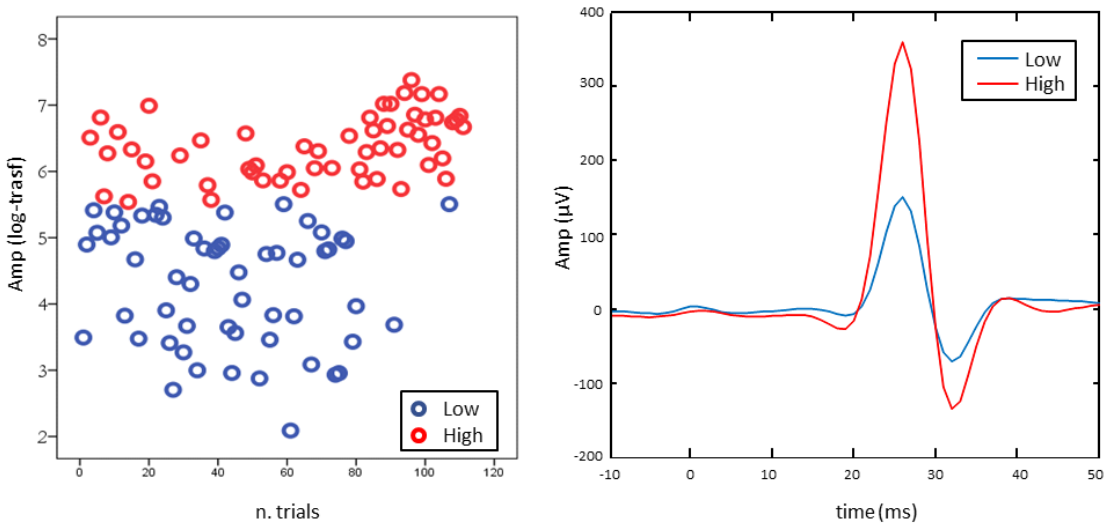

Figure 4.1 High and low motor pathway excitation levels*

Left: MEP peak-to-peak amplitude for each trial (representative subject), with largest/smallest third (red/blue) of trials used to identify trials with high/low CSMP excitation level. **Right:** Average of largest/smallest third (red/blue) MEPs in the [-10, 50] ms time window.

To face with the last point it was necessary to define a new procedure of source identification, based on localization "stability". The grand average was submitted to a source localization algorithm (sLORETA, Pascual-Marqui, 2002) which localized the active voxels at each millisecond in the interval between 3 and 100 ms after the pulse. For each localization, the 200 voxels were extracted with highest intensity among the 6,239 cortical grey matter voxels scanned by sLoreta in the whole brain volume (MNI - Montreal Neurological Institute - coordinates, at 5 mm resolution). The RoI (Region of Interest) was identified by the localization stability property (Figure 4.1): 1) at least 75% of voxels were common for localizations of two consecutive milliseconds and 2) this happened for at least 3 consecutive milliseconds.

* Reprinted from Giambattistelli F, Tomasevic L, Pellegrino G, Porcaro C, Melgari JM, Rossini PM, Tecchio F. The spontaneous fluctuation of the excitability of a single node modulates the internodes connectivity: a TMS-EEG study. Hum Brain Mapp. 2014 Apr;35(4):1740-9. with permission from John Wiley & Sons Ltd.

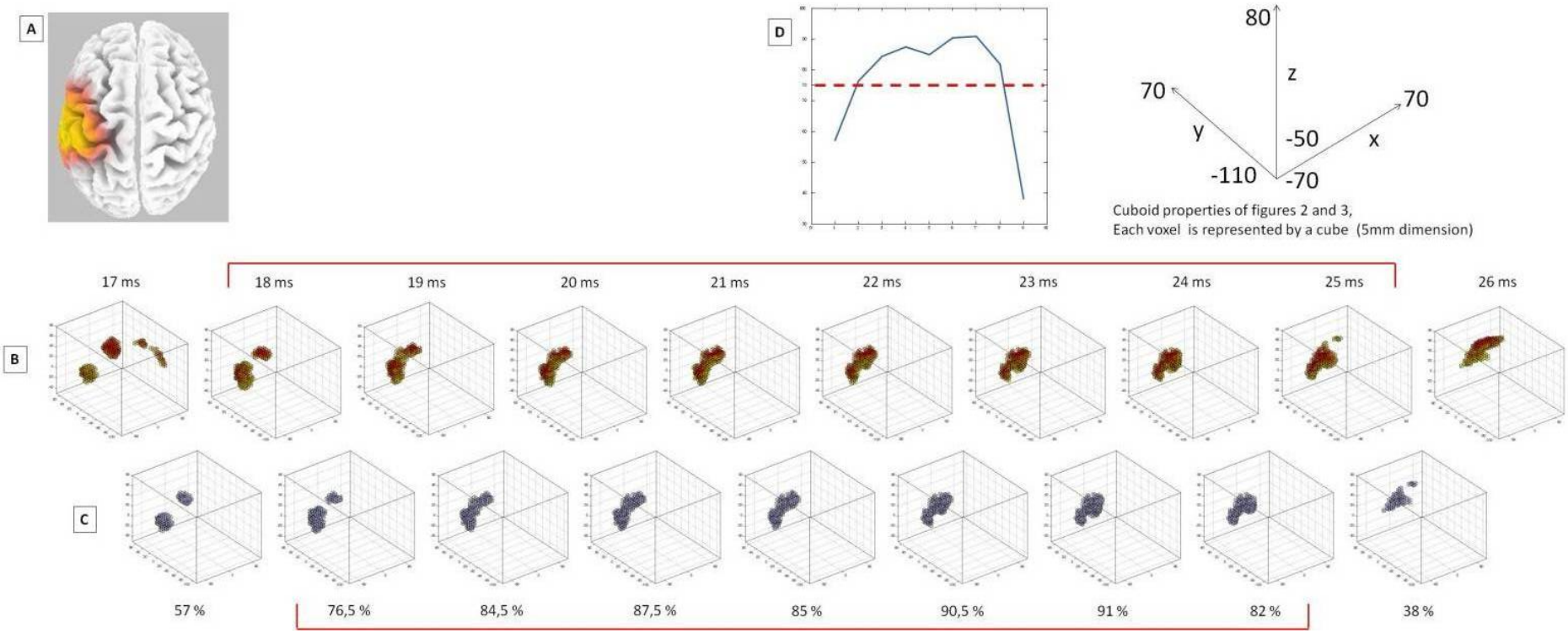

Figure 4.2 Exemplificative case of localization stability - The procedure for the 18-25 ms RoI: **A)** The electric field potential distribution of the High-Low difference grandaverage projected on the cortical surface; **B)** the distribution of 200 higher intensity voxels among the 6239 cortical grey matter voxels (sLoreta) over the interval of interest (Cuboid position and dimension indicated upper right); **C)** the voxels common to 2 consecutive milliseconds (the percentage is reported below each cuboid); **D)** the percentages plotted over the analysis interval.

From this analysis two parameters were obtained: the RoI spatial position (average of contiguous points weighted for the current density, RoI_pos), that could include also more cortical regions, and the starting and final time points of position stability (RoI_lat). The last can include more than three milliseconds in case the localisation was stable for a longer time, so all the corresponding milliseconds were considered in the analysis and the time ranges RoI_lat were different (Table 3). RoI_pos was then used to extract the time courses of current densities for each subject which were then averaged among high and low trials. For each subject and for each RoI the current density peak was identified inside the RoI_lat time interval and the mean value of that peak ± 2 ms was used to estimate the strength of cortical recruitment at high and low CSMP excitability for subsequent statistical analysis (RoIi low; RoIi high).

Statistical analysis

The correlation between MEP amplitudes and their timing order (performed by Sperman's Rho correlation coefficients) was studied to reveal a possible MEP modulation caused by consecutive stimuli repetition. The current density distributions of RoIs were checked for normality and the appropriate transformation was applied if necessary.

To study the dependence of cortical responses on the TMS efficacy, a repeated measure ANOVA model was set up with within-subjects factors Connected Node (RoI1, RoI2, RoI3, etc.) and CSMPexc (RoIlow, RoIhigh). Thanks to the stability method used in data analysis it was possible to deal with cerebral recruitments corresponding to high and low CSMP excitation levels in single subjects. The results were considered as statistically significant only with $p<0.05$ and trends if $p<0.10$.

Results

CSMP Excitation

Mean RMT was 45.7 ± 8% of maximal stimulator output. An average of 110 ± 6 EEG/MEP trials were obtained per subject after data cleaning.

MEP amplitudes did not have a normal distribution and therefore before further statistical analysis the log-trasformation was applied (y = log(MEP+1)). In this way the data had a good approximation to Gaussianity proper for general linear model analyses. As expected the MEP latency was stable across trials for each subject (peak MEP latency 24.0 ± 1.6 across subjects) while the amplitude confirmed high variability (5.65 ±0.24) through the variation coefficient (ratio between standard deviation and mean across subjects). No relation was found between amplitude (independent variable) and MEPs chronological order (dependent variable) with the correlation analysis (P = 0.39).

Brain areas recruited by left M1-TMS for low and high CSMP excitation levels

To identify the brain areas influenced by the high and low CSMP excitation levels, first the average for each group of trials was calculated for each subject separately. Then for each subject the difference between the two averages was performed. This gave as result a response free of the signal common to the both responses, including the remaining artefacts. Since the obtained data were not robust enough to be analysed separately, the grand-average of the subjects was done leading to a response suitable for localization of sources involved by different CSMP behaviour. The localization procedure was based on sLoreta algorithm. The previously explained stability method for localizations produced eight RoIs in correspondence to latencies and sites already emerged by other studies (Bonato et al., 2006; Daskalakis et al., 2008; Ferreri et al., 2010; Lee et al., 2007; Rizzo et al., 2011). It is worth to mention that no RoI was unveiled with this procedure in the prestimulus interval ((-10, 0) ms).

For each RoI and for each subject, the individual current densities were extracted for post hoc comparisons. Therefore with this method we studied the single subject response passing through a more robust grand-average localization analysis. The procedure can be thought as a special filter defined by the behaviour of a population and then applied to each component of the population itself.

Different responses in current corresponded to different RoIs, as showed through the ANOVA model with Component (RoI1, RoI2, RoI3, etc.) and CSMPexc (RoIlow, RoIhigh) as within-subjects factors (Component*CSMPexc interaction factor ($F = 6.648$, $P < 0.001$); Table 3, Figure 4.3).

Cerebral recruitments preceding sensory feedback arrival

The list of identified intervals:

6-10 ms: the involved area was the right non-stimulated hemisphere, central parietal and frontal (BA5, 4, and 3). The post hoc comparison between EEGlow and EEGhigh, in this RoI and in this interval, showed a trend of the association between lower current densities and higher CSMP excitation ($P = 0.08$).

13-16 ms: the identified RoI is in the frontal lobe bilaterally (BA6 bilaterally), but the post hoc comparison revealed no significant differences ($P = 0.24$).

18-25 ms: the localization procedure evidenced the parietal lobe of the left stimulated hemisphere (BA3, 2 and 40) with stronger recruitment for lower CSMP excitation ($P = 0.02$) in the post hoc comparison.

26-32 ms: the RoI is positioned in the frontal BA6 bilaterally and expressed higher current densities for higher CSMP excitation ($P = 0.03$).

35-39 ms: centro-frontal areas of the right non-stimulated hemisphere (BA6 and 4) were identified, but with no statistical significance for the correspondent activations ($P = 0.23$).

RoI_lat (ms)	RoI_pos (mm)	Cytoarchitectonic structure			Recruitment change
		B. A.	Lobe	Gyrus	
6-10	30, -40, 67 35, -26, 66 35, -21, 66	5 R 4 R 3 R	Parietal Frontal Parietal	Postcentral Precentral Postcentral	~—
13-16	-5, 13, 64 -10, 13, 64 5, 22, 59	6 L and R	Frontal	Superior frontal	=
18-25	-64, -23, 20 -54, -23, 33 -64, -13, 24	40 L 2 L 3 L	Parietal	Postcentral	—
26-32	-5, 13, 64 -10, 13, 64 5, 22, 59	6 L and R	Frontal	Superior frontal	+
35-39	54, 2, 46 50, 2 46 50, -7, 51	6 R 6 R 4 R	Frontal	Middle Frontal Precentral	=
44-47	-25, 32, 49 -45, 12, 50	8 L 6 L	Frontal	Sup. Frontal Mid. frontal	+
68-75	-15, -6, 65 -10, -7, 60 10, -6, 65	6 L and R	Frontal	Superior, Middle and Medial	~+
79-85	-5, 13, 64 -10, 13, 64 5, 22, 59	6 L and R	Frontal	Superior frontal	=

Table 3 Low vs. High CSMP excitation Regions of Interest (RoIs)[*]

From left: starting and final time points (RoI_lat, t=0 ms), positions (RoI_pos) in Talairach coordinates and corresponding cytoarchitectonic structure (Brodmann area, BA:, Left and Right, L and R). RoI's multiple cortical areas are presented in order of their current density. Areas activated more (less) for high CSMP excitation are indicated by +(-), ~ added for p<.10, and = is reported with no statistical difference.

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Cerebral recruitments after sensory feedback arrival

44-47 ms: the activity is localized in the left sensory-motor area (BA6, 4 and 8) which expressed a higher level of current densities values for higher MEP values (P = 0.01). This time interval is concomitant with the sensory feedback arrival from the activated hand muscle, but no correlation was observed between the MEP latencies and the RoI activation (r = 0.120, P = 0.734).

The last two identified RoIs were in the left and right frontal lobes (BA6 bilaterally) in the 68-75 ms and 79-85 ms intervals but while the first showed a trend of stronger activation for higher CSMP excitation (P = 0.08), the second did not show statistical significance (P = 0.23) in post hoc comparison.

Discussion

Changes of CSMP, expressed by the MEP amplitude, correspond to different effective connectivity patterns impinged by M1 (Table 3; Figure 4.3). The main effects were found corresponding to stronger CSMP excitation (MEPs of larger amplitude): (1) the right non stimulated primary sensorimotor region homotopic to the stimulated region showed a trend of weaker activation at 6–10 ms; (2) the left parietal area was less activated at 18–25 ms; (3) bilateral motor areas showed a stronger activation at 26–32 ms; (4) the left sensory-motor area was more activated at 44–47 ms and a trend of stronger activation of bilateral frontomedial lobes was found at 68–75 ms.

Stronger contra-lateral and ipsi-lateral inhibitory effects can follow a stronger response of the target site and this study shows an inhibited central-parietal projection efficacy at early latencies (<25 ms) followed by stronger centro-frontal recruitments (26–47 ms) relative to a higher peripheral response.

RoIs recruitments preceding sensory feedback arrival

SM1 recruitment in right non-stimulated hemisphere

All subjects showed neuronal excitation in the region homologous to the stimulated one at 6-10 ms after the left M1 stimulation, probably due to mediation by transcallosal fibres (Daskalakis et al., 2008; Lee et al., 2007; Rizzo et al., 2011, Rizzolatti and Luppino, 2001). The involvement of homotopic areas was already confirmed by a TMS/fMRI study (Bestmann et al., 2004), while the explanation for the trend of lower recruitment could be that minor inhibitory effects aroused contralaterally with higher MEPs. This could be due to a hemispheric hierarchy present in resting state and during motor relaxation, as it has already been proved for tasks with unimanual motor execution (Bender et al., 2005; Hoppenbrouwers et al., 2013; Kicić et al., 2008; Nikulin et al., 2003; Voineskos et al., 2010). In these in fact the interhemispheric connectivity tunes inhibition levels on contralateral homologous areas.

There was not statistically significant dependence of neuronal recruitment on CSMP excitation at 13-16 ms in accordance with a previous study which studied the same correlation with EEG peaks at 15 ms after TMS stimuli.

Left parietal recruitment

A stronger CSMP excitation caused a weaker parietal recruitment ipsilateral to the TM-stimulation at 18-25 ms. Previous studies have observed that there are direct connections between the posterio-parietal cortex area and M1 of the same hemisphere (Jones, 1978; Rizzolatti and Luppino, 2001) and recent functional studies confirmed it (Ferreri et al., 2010; Koch et al., 2007; Rizzo et al., 2011). In this study, however, the high latency suggests a thalamo-cortical communication implication that is weaker in case of prevalent projection to peripheral motor nerves.

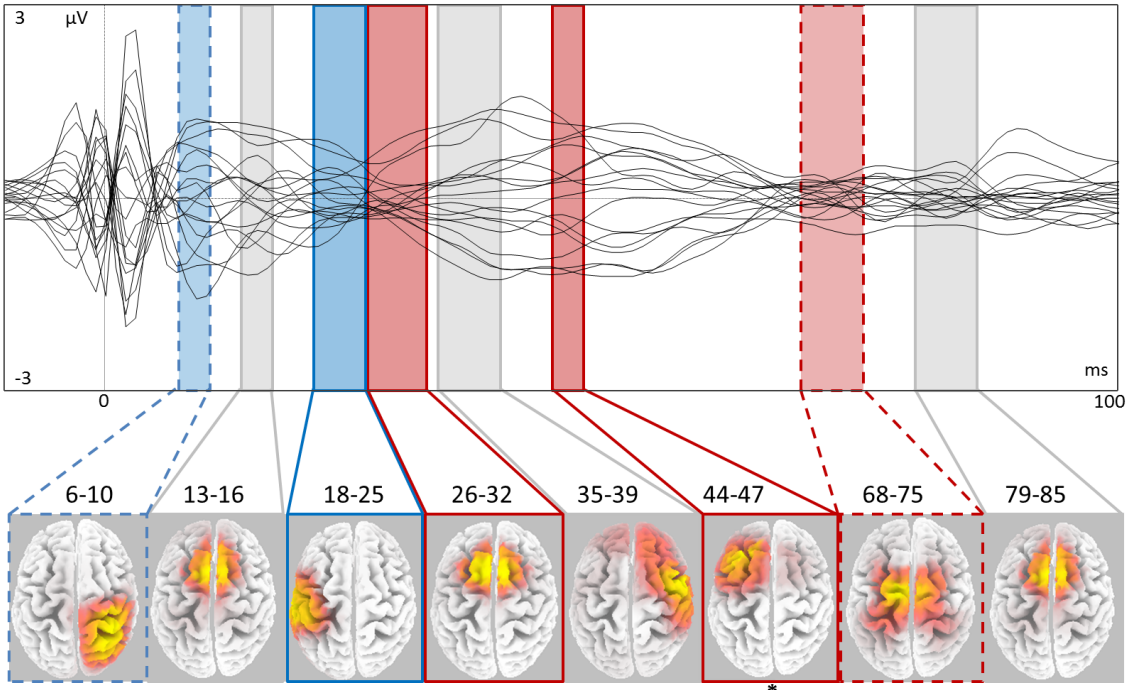

Figure 4.3 – RoI recruitments*

Top Butterfly plot obtained from grand average of the differences between high and low CSMP of the TMS evoked potentials in the [-10, 100] ms interval. **Bottom** Region of activation at corresponding latencies (ms), with color code referring to lower/higher (blue/red) activation corresponding to higher/lower CSMP excitation level, trends indicated by dashed lines and significant differences by solid lines.

In TMS/EEG data there is always a strong risk of influence of the head muscular twitch artefact on the first response latencies (Kujirai et al., 1993; Mäki and Ilmoniemi, 2010b). But in this analysis, since the MEP responses should not be correlated to the amplitude of the artefact and since the two first latencies have opposite behaviour, it can be assumed that muscular activities do not conceivably contribute to RoI identification.

Bilateral centro-frontal recruitment

The behaviour at 26-32 ms is comparable to previous studies which showed a stronger recruitment of bilateral motor areas for stronger CSMP excitation (Mäki and

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Ilmoniemi, 2010a). This centro-frontal cortical activation could be due to inhibitory-excitatory phenomena from contralateral primary motor to premotor and supplementary motor areas ipsilateral to the delivered stimulus.

RoIs recruitments after sensory feedback arrival

Higher CSMP excitation at 44-47 ms elicited a higher recruitment of the primary sensorimotor areas of the stimulated hemisphere. At these latencies the arrival of the sensory feedback to the muscular twitch provoked by the stimulation is expected (Desmedt, 1987; Kawamura et al., 1996; Rossini et al., 1987; Siniatchkin et al., 2007). It can be supposed that a stronger muscle contraction should evoke a stronger feedback and contribute to this RoI response summed to contributions from central projections. At the same time, the lack of correlation between MEP and activation latency in this RoI could imply a lower incidence of the feedback in the summation of contributions.

No effects for neuronal recruitments were found after 80 ms, in line with other studies that reported contradictory results about the association between MEP amplitude and the response at around 100 ms. Association that was described by Paus et al., 2001and not by Nikulin et al., 2003.

Conclusions

The results of the study showed two findings. The first is about the changes provoked by the state of the cortico-spinal neuronal excitation, expressed through spontaneous and involuntary fluctuations, in the primary motor network in the first 75 ms. In this specific case we investigated the primary motor area devoted to hand control obtaining different behaviour in the early latencies (<25 ms) with lower cortical recruitments (central-parietal) compared to later latencies (>26 also before the feedback arrival at around 45-50 ms) where a higher level of CSMP corresponded to higher response (centro-frontal recruitments).

The second main result is relative to the method of information extraction from the recordings. In very noisy data, such as TMS/EEG, a first individuation of involved RoI through grandaverages can allow the study of single subject's cortical activity using the spatial filtering.

CHAPTER 5

STUDY 4 - EFFECTS ON FUNCTIONAL CONNECTIVITY OF STROKE ROBOTIC REHABILITATION

This study (Pellegrino and Tomasevic et al., 2012) focused on plasticity processes and functional connectivity changes of the sensorimotor area in stroke patients after robotic rehabilitation. The sensorimotor area was identified thanks to a source extraction algorithm, developed at my lab, which was able to unveil the neuronal pools responding to peripheral sensory stimulation.

Introduction

Stroke is one of the main causes of mortality and provokes neurological damages which involve motion, force, sensory perception, sensori-motor integration (Roger et al., 2011). Stroke is also the first cause of disability considering that more than 1 of 3 stroke survivors is strongly disabled despite many attempts that have been made so far to improve their clinical state. In this contest the brain plasticity could have a fundamental function (Cramer, 2008; Cramer et al., 2011; Rossini et al., 2003). Since the stroke lesions relative to the middle cerebral artery (MCA) can provoke the sensori-motor impairments, it has been proven that, in case of hand representation areas involvement, the plasticity can change the activity, position and representation of sensori-motor areas bilaterally (Rossini et al., 2003; Tecchio et al., 2007a). This is evidenced by changes of the responsiveness to median nerve stimulation and the involvement of other, not usual, brain areas (Forss et al., 1999; Rossini et al., 1998; Rossini et al.; 2001). These changes are due to plastic reorganization linked to motor functions recovery (Altamura et al., 2007; Rossini et al., 1998; Rossini et al., 2001;

Tecchio et al., 2007a). The interhemispheric interactions are also modulated, as confirmed through TMS (Grefkes et al., 2008; Shimizu et al., 2002; Takeuchi et al., 2010; Traversa et al., 1998) and fMRI (Grefkes et al., 2008). To investigate the plasticity in sensori-motor areas, this study benefits of techniques that do not request subject's voluntary movements, which can be highly impacted by the disability condition. We focused our analysis on the brain connectivity at rest between ipsilesional hemisphere (ILH) and contralesional hemisphere (CLH) brain areas reactive to median nerve stimulation, which were previously used to reveal plasticity changes in stroke patients (Wikstrom et al., 2000; Rossini et al., 2004). The experimental setup is easily repeatable, usable among subjects with different level of motor impairment, it is not influenced by the attention degree (Allison et al., 1991).

Motor practice, somatosensory input and pharmacological agents (Dimyan and Cohen, 2011; Laufer and Elboim-Gabyzon, 2011; Nudo et al., 1996) such as neurorehabilitation techniques (Cramer, 2008; Dimyan and Cohen, 2011) can be fundamental for motor recovery after ischemic stroke influencing positively the neuronal reorganization. In this direction robotic systems have also been developed as alternative mean for motor recovery (Brochard et al., 2010) with a repeatable, programmable, controlled rehabilitation and to assess motor features in a quantitative manner (Volpe et al., 2009). There is still an open debate about their real effectiveness with respect to the standard rehabilitation methods (Kwakkel et al., 2008). A multi-centric, randomized, controlled trial on stroke patients with long-term disability tested the method and declared that results are comparable to those obtained by a therapist providing intensive therapy (Lo et al., 2010).

The aim of this study is to investigate in a cohort of chronic stroke patients the effects of a robot-aided rehabilitation program on clinical and brain plasticity changes using InMotion2 and InMotion3 robots.

To identify the network devoted to the sensori-motor hand control, in an unpredictable situation as it is the brain rearrangement after stroke, a procedure named Functional Source Separation (FSS) was used (Porcaro et al., 2008). Like other component decomposition procedures, FSS assumes that EEG is a linear combination of active brain sources, but it extracts one source a time using as input a functional property of the source itself. In fact, if the behaviour of a neuronal pool is known, this is used to discriminate the desired source from the remaining ones. The strength of this procedure lies in the fact that it can extract the activity of a cortical source in a certain experimental condition and then unveil the activity of that source in other conditions of interest. In this experiment the selected functional property is the well known first cortical response to the median nerve stimulation, located in the primary sensory area at about 20 ms after the stimulus delivery (N20, Allison et al, 1991). This procedure offers the excellent opportunity to identify sources on the basis of the information provided by the signal dynamics, instead of the previous solving of the inverse problem (single and multiple dipoles: Scherg & Berg, 1991; Multiple Signal Classification (MUSIC): Mosher et al. 1992; recursively applied and projected-MUSIC (RAP-MUSIC): Ermer, 2000; minimum norm estimates: Hamalainen & Ilmoniemi, 1994; Low resolution brain electromagnetic tomography (LORETA): Pascual-Marqui et al. 1994) to spatial filtering like beam forming (for example synthetic aperture magnetometry, (SAM): Vrba & Robinson, 2001).

Materials and methods

Patients and clinical assessment

All the subjects gave their written consent and the study was approved by the local Ethical Committee. The enrolled subjects were 7 (age 60±18y, 5 males) between 1 to 5 years after the first ischemic stroke in the MCA territory (5 right side, 2 left side)

that provoked upper limb sensory/motor impairment. Before the start of the study, for the clinical conditions stability evaluation, the patients accomplished the Upper Limb Fugl-Meyer Assessment Scale (FMA, Sivan et al., 2011) for three times, one every six weeks. MRI registration was performed to confirm the diagnosis and have lesion site characteristics. The exclusion criteria were: peripheral neuropathy, dementia, severe aphasia or an impairment that could bias the correct execution of the task. A multimodal evaluation of the overall status of the patients (clinical status, quantitative motor performance and neurophysiological features) were performed before (Tpre) and after (Tpost) the 12-week upper-limb robot-aided neurorehabilitation program (Figure 5.1).

Isometric contraction of the thumb against index and middle finger was used as simple task for motor performance evaluation. For the purpose the ALLADIN Finger Device (AFD) module was used, which is composed by a rigid hand orthesis for isometric tasks and three force/torque sensors (JR3 model No. 50M31A-I25) located on the outer side of the hand. While the forearm lies on the arm support the three fingers are blocked to the module by Velcro straps (Figure 5.1).

The acquisition protocol was organized as follows:

MVC - maximum voluntary contraction for each hand, for residual ability evaluation;

Rest - 5 minutes of rest to avoid fatigue;

Isometric contraction task - 20 seconds of isometric contraction alternated to 20 seconds of rest for 15 times repeatedly, therefore 300 s of contraction for each hand.

Three short maximal contractions were averaged for MVC evaluation. The required isometric contraction was set to 20% of MVC. This value was chosen higher than in standard isometric contraction experiments in our lab because stroke patients perform a lower MVC with the paretic hand. Therefore a lower percentage of MVC would have resulted in a very low requested pressure level, with major difficulty for the patients in fine motor control and for data analysis with poor signal-to-noise ratio. The required

and exerted pressure were both provided through a visual feedback to the subject.

The paretic and non-paretic hand motor control was evaluated through:

1) MVC;

2) Contraction level, the average of the applied force during all contraction periods of the whole task;

3) Contraction quality, the time that the subject was able to maintain the force level within ±5% of the requested level of contraction calculated.

Robotic rehabilitation

A 12-week robotic rehabilitation program was arranged for all the subjects, 1 hour a day for three days a week (Figure 5.1). The 12 weeks were subdivided in 6 weeks dedicated to shoulder- and -elbow with the InMotion2 (or MIT-Manus) and and 6 weeks to wrist with the InMotion3 robots (Zollo et al., 2011; Zollo et al., 2011). MIT-Manus is a planar, two degree-of-freedom (DOF) machine projected to assist the patient's movements of the upper limb during the execution of tasks with visual feedback (Krebs et al., 2007). The robot for wrist rehabilitation has 3 DOF: abduction–adduction; flexion–extension; pronation-supination (Krebs et al., 2007).

EEG recordings

The EEG recordings were performed with a 32 channels acquisition system mounted in accordance with the 10-20 international system (Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, P7, P8, T7, T8, FZ, CZ, PZ, FC1, FC2, CP1, CP2, FC5, FC6, FT9, FT10, FCZ, CP5, CP6, TP9, TP10) and with the binaural reference. Electrodes for vertical and horizontal electro-oculogram recording were mounted bipolarly for the identification of eye movement related artefacts. The impedances of the electrodes were under 5 kΩ during the experiment with the acquisition of 1024 samples per second and pre-sampling analogical band-pass filter set at 0.48–256 Hz (BrainAmp System).

Figure 5.1 Experimental setup.[*]

Left: Robot of the rehabilitation program. ***Centre:*** Experimental Flow-chart. ***Right:*** Hand motor control and brain activity and connectivity evaluations pre- and post-robotic rehabilitation program.

[*] Reprinted from Pellegrino G, Tomasevic L, Tombini M, Assenza G, Bravi M, Sterzi S, Giacobbe V, Zollo L, Guglielmelli E, Cavallo G, Vernieri F, Tecchio F. Inter-hemispheric coupling changes associate with motor improvements after robotic stroke rehabilitation. Restor Neurol Neurosci. 2012;30(6):497-510 with permission from IOS Press.

The EEG recording protocol consisted on two conditions: 5 minutes resting state with open eyes and median nerve stimulation with 0.2 ms short painless electric pulses with 631 ms interstimulus interval. As already described in Chapter 1, the stimuli were delivered separately to right and left median nerve at the wrist through a pair of nonmagnetic, 2.5-cm spaced, Ag–AgCl disc electrodes filled with conductive jelly. The intensity was set to three times the individual sensory threshold to have a clearly visible thumb twitch. Before further analysis on the recorded resting state data, the influences of extra-cerebral sources (heart, eyes, muscles) were reduced by a semi-automatic artefact rejection procedure (Barbati et al., 2004). For the sensorimotor area identification, all the trials of the SEP were averaged to identify the stimuli related response.

Primary sensorimotor hand area identification

The primary sensorimotor area was identified from 32 EEG channels recordings through the previously introduced in-house made algorithm, FSS (Porcaro et al., 2009; Tecchio et al., 2007b). In this study, the algorithm was applied to the response of the median nerve stimulation to extract the source of first cortical response which corresponds to hand representation sensori-motor area (N20, Allison et al, 1991). Repeating the procedure for the paretic and non-paretic hand, bilateral temporal trace and channel weights of S1 neuronal pools were achieved (ILH S1 for ipsilesional hemisphere and CLH S1 for contralesional hemisphere). Finally, with the weights we obtained the position (expressed in the Talairach coordinates system) of the sources by the inverse problem algorithm sLoreta (Pascual-Marqui, 2002) and also the opportunity to investigate the sources' behaviour (activity and connectivity) at rest.

FSS procedure

Independent Component Analysis model (fastICA, Hyvarinen et al., 2001) assumes the set of X signals as a linear combination of statistically independent non-

Gaussian sources S through an unknown mixing matrix A:

$$X = A*S$$

Sources S are estimated, up to arbitrary scaling and permutation, by independent components Y as:

$$Y = W*X$$

where the un-mixing matrix W is estimated along with the independent component Y.

Functional Source Separation (FSS, Tecchio et al., 2007b) is a procedure that, starting from the ICA model, uses additional information implemented in the contrast function to identify a single solution that satisfies physiological assumptions (functional source). Therefore the new contrast function becomes:

$$F = J + \lambda R$$

where J is kurtosis generally used in fastICA, λ is a parameter to weigh the two parts of the contrast function, and R contains information typical for the source chosen to be extracted. Different R corresponds to a different source and each extraction starts from the original data without imposing orthogonality to the extracted sources, but the F maximization through the simulating annealing algorithm. Moreover once the source is identified, it is possible to study its behaviour in other conditions using the weight matrix on the original X data.

FSS has already been successfully used to extract the cortical pools relative to the hand representation using as its property the first response (around 20ms) to the median nerve stimulation (Barbati et al., 2006; Porcaro et al., 2008; Porcaro et al., 2009).

The evoked response (EA) was obtained by averaging the trials linked to the stimulus trigger, then the functional coefficient R was calculated as:

$$R = \int_{20}^{40} |EA(t)|dt - \int_{-30}^{-10} |EA(t)|dt$$

with t = 0 corresponding to the stimulus delivery to the median nerve at wrist. The two

intervals correspond to the maximum activation due to the median nerve stimulation (20÷40 ms; Tecchio et al., 1997) and to the chosen baseline in the pre-stimulus time range (-30÷-10 ms). Once the sources were defined, they were studied at rest by multiplying the mixing matrix by the artefact-cleaned resting state data (Y = W*X).

Sensorimotor area properties at rest

The performed measures at rest were the power spectral density (PSD) relative to each hemisphere (ILH and CLH) and inter-hemispheric coherence (IHCoh). Both the estimates were obtained through Bartlett's method with Hanning window (2048 ms), for IHCoh the number of windows was fixed to 120. PSD frequency bands were estimated with respect to the individual alpha frequency (IAF) and then normalized by dividing them by the total power in the (2, 90 Hz) frequency range: delta (2, 3.5), theta (4, IAF−2.5 Hz), alpha (IAF−2, IAF+2 Hz), low beta (IAF+2, 23.5 Hz), high beta (24–32.5 Hz), low gamma (33–47.5 Hz), high gamma (52.5, 90 Hz).

Statistical analysis

The statistical analysis was set to derive the effects of robotic rehabilitation on FMAS scores and motor performance indices (clinical state), and on the cerebral reorganization. When necessary, adequate transformations were applied for a better gaussianity approximation and outliers control. Since FMAS scores have a single value for each session and subject, the paired samples t-test was used. The statistical analysis was performed considering for each patient the motor performance obtained from 15 trials, for each hand (paretic and non-paretic) and for the two sessions (pre- and post-robotic rehabilitation). For the purpose, the General Estimating Equation model was adopted with the patient as the Subject (or cluster) variable, motor performance values as dependent variable and *Moved Hand* (paretic, non-paretic) and *Robotic Rehab* (pre-, post-robotic rehabilitation) as predictors. The autoregressive (lag=1) working correlation within subjects was used because motor performance indices were estimated

on consecutive epochs. The correction for multiple comparison was performed by Sidak's procedure. For plastic brain reorganization described by S1 position and responsiveness to median nerve stimulation (N20), and for spectral features and inter-hemispheric coherence of primary somatosensory areas were applied similar models with *Robotic rehab* (pre-, post- rehabilitation) and *Hemisphere* (ILH, CLH) as predictors. For coherences and PSDs, the *Frequency band* (delta, theta, alpha, low beta, high beta, low gamma, high gamma) predictor was added.

To investigate the relevance of brain connectivity reorganizations for motor and clinical recovery through robotic rehabilitation, the bivariate parametric correlation between neurophysiological measures, impacted by robot-aided rehabilitation and the clinical conditions of patients (FMA and motor performance), was adopted.

Results: ***Effects of robotic rehabilitation***

Clinical

At stroke onset NIHSS was 8.4±3.0. The patients started the rehabilitation process with a moderate to severe residual impairment and concluded with ameliorations of both both shoulder (15.0±7.2 at Tpre 17.6±7.0 at Tpost ($p = 0.007$) and wrist (Tpre 16.8±12.4 and Tpost 19.4±13.8, $p = 0.041$).

Motor hand control

The performance in executing the required motor task improved. In fact, the analysis of the mean level of contraction (Contraction level) during the task revealed that the patients showed the ability of maintaining a higher level of contraction after robotic rehabilitation (T_{post}) compared to before (T_{pre}) [Figure 5.2, *Robotic Rehab* factor Wald chi-square = 6.775, df = 1, $p = 0.009$] bilaterally [non statistically significant interaction *Moved Hand*Robotic Rehab* $p = 0.974$]. Only for completeness of information, the non-paretic hand had better performance during both sessions [Moved

hand effect Wald chi-square = 52.893, df = 1, p < 0.001].

The MVC analysis showed a strong *Moved Hand*Robotic Rehab* interaction effect [Wald chi-square = 8.159, df = 1, p = 0.004], with higher level of improvement for the rehabilitated paretic hand [Figure 5.2, post-hoc analysis Sidak's corrected p = 0.001] than for the non-paretic hand [Figure 5.2, p = 0.050] and a lower MVC ability disparity between the two hands at Tpre [p < 0.001] and Tpost [p = 0.035].

The Contraction quality of the task increased [interaction Moved hand*Robotic rehab effect , Wald chi-square = 6.824, df = 1, p = 0.009] for the paretic hand [Figure 5.2, post-hoc analysis Sidak's corrected p < 0.001] and not for the non paretic [p = 0.711], till both hands had a more similar execution quality levels [post-hoc analysis Sidak's corrected p = 0.571], compared to how different they were before [p < 0.001].

Sensorimotor hand representation

There were no effects on the position of S1 source in ILH or CLH [*Robotic rehab* and interaction factors p > 0.200 consistently] nor on resting state sources' activities. On the contrary the robotic rehabilitation changed the inter-hemispheric coherence between homologous S1s [Robotic rehab factor Wald chi-square = 4.971, df = 1, p = 0.026, Figure 5.2]. The effect depended on the oscillatory frequency domain [*Robotic rehab*Frequency band* factor Wald chi square = 54.575, df = 6, p < 0.001]. In fact, the reduction due to rehabilitation was present in high beta and gamma bands [high beta p = 0.027, low gamma p = 0.019, high gamma p = 0.015]. It is important to note that there was a big difference in spread of S1coherences between the two sessions as evidenced by Levine test on the differences between IHCoh standard deviations pre- and post-rehabilitation (Figure 5.3): delta (p = 0.008), theta (p = 0.022), alpha (p = 0.001), low beta (p = 0.002), high beta (p = 0.015), low gamma (p = 0.009), high gamma (p = 0.011) and on the whole spectrum (p = 0.019). These results show also sensitivity to spread differences between two sessions in bands that did not show

difference in means (delta, theta and alpha bands).

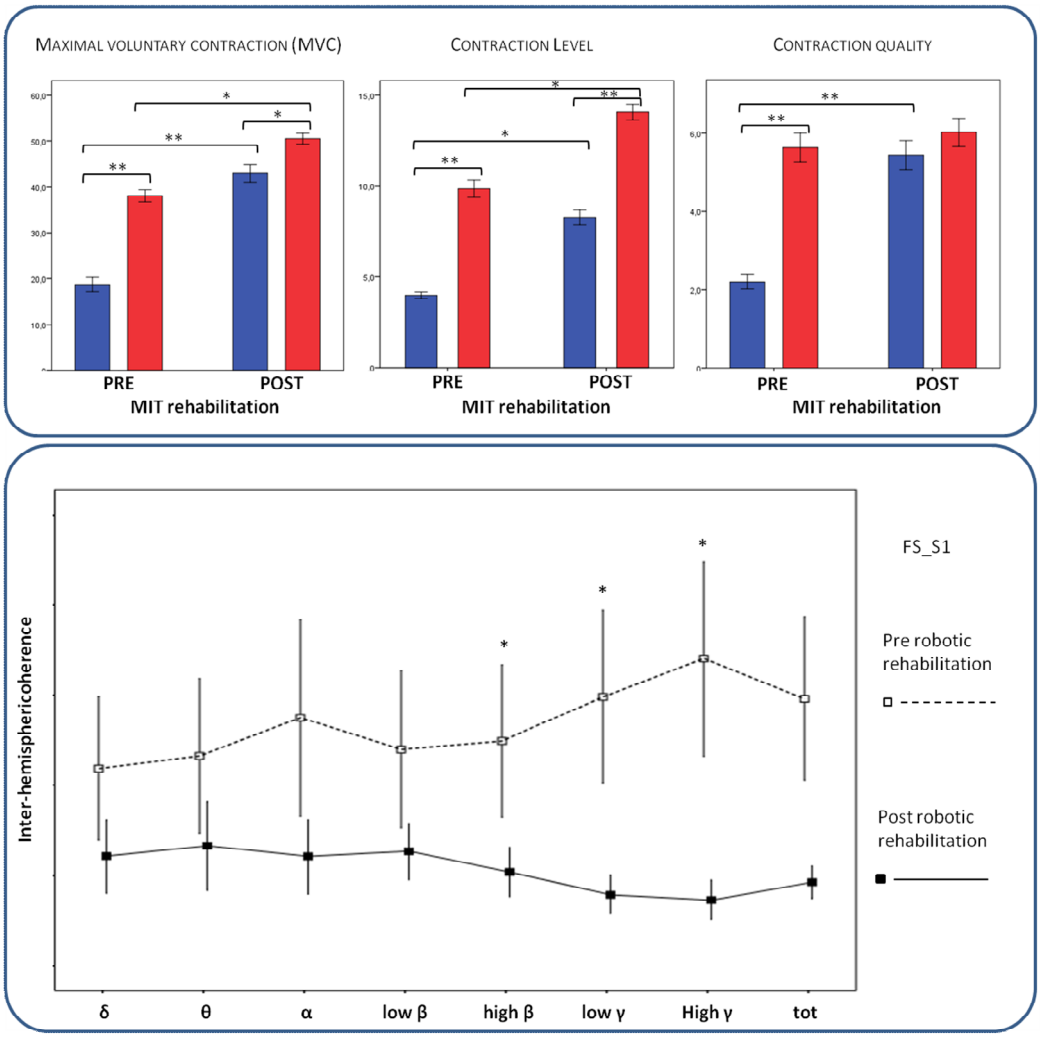

Figure 5.2 Robotic rehabilitation effects on hand motor control and on inter-hemispheric sensorimotor connectivity*

Top: Error bars (mean ± 1 SE) for the 3 indices: MVC and Contraction Level (N); execution quality strength within ±5% of the established level of contraction and its duration (N*sec). Bars: Blue - Paretic Hand; Red - Non Paretic Hand. Significant differences: * $p<0.05$; ** $p<0.001$. **Bottom:** IHCoh for FS_S1 at T_{pre} and T_{post}. Error bars ± 1 SE. Frequency bands defined on the individual alpha frequency (IAF): delta (2, 3.5), theta (4, IAF-2.5 Hz), alpha (IAF-2, IAF+2 Hz), low beta (IAF+2, 23.5 Hz), high beta (24-32.5 Hz), low gamma (33, 47.5 Hz), high gamma (52.5, 90 Hz). Significant differences: * ($p<0.05$) FS_S1 IHCoh difference between T_{pre} and T_{post}.

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Relationship between robotic rehabilitation effects on inter-hemispheric sensorimotor connectivity and hand motor control

The improvement of the contraction level of the paretic hand correlated with the changes in S1 IHCoh [r = 0.758, p = 0.048 in high beta band, Figure 5.4] and there was a trend in that direction for the non-paretic hand [r = 0.739, p = 0.058; high beta band].

Discussion

The results showed that the upper limb robotic rehabilitation treatment in chronic stroke patients for 12 weeks influences the inter-hemispheric functional connectivity relative to the primary sensori-motor areas of the hand. Moreover this connectivity changes correlated with the motor control improvement of the paretic hand. In particular the inter-hemispheric connectivity had a spread distribution before rehabilitation treatment and tended to less distant final values after. Considering that the patients showed a clear motor improvement thanks to the robotic rehabilitation, it could be hypothesized that the value, to which the connectivity values tended, represents a functional 'best state', a more 'physiological' condition. The results of the study were in line with previous findings about the improvements obtained through robotic rehabilitation (Brochard et al., 2010), but with the novel finding on two important links: the central reorganization depends on rehabilitation and the inter-hemispheric connectivity of homologous sensori-motor areas depends on a finer hand control.

Cortical connectivity in stroke patients has been studied previously through EEG task-related protocols describing a clear reduction of communication and the involvement of a reduced number of brain areas in beta and gamma bands (de Vico Fallani et al., 2009), with an increase of low beta inter-hemispheric coherence between sensory-motor regions in well recovered patients (Gerloff et al., 2006). The decrease of inter-hemispheric coupling could be due to the functional recovery mechanisms (Strens

et al., 2004), while the increase of connectivity, mainly in beta band, could be a consequence of compensatory activity secondary to stroke (Wheaton et al., 2008).

Resting state

In this study, differently from the just mentioned ones, the connectivity was studied at rest between sensorimotor areas relative to paretic and non-paretic hand. In fact, resting state is suitable for the investigation of properties of networks related to specific functional domains (Deco and Corbetta, 2011) and even for the evaluation of the health of brain networks with determinant role of inter-hemispheric interactions (Carter et al., 2010). It is important to notice that the study of the sensorimotor region in resting state permits to compare the non-paretic and paretic hand in stroke patients and to compare them to healthy controls because tasks that involve motor activity with disabled limb are avoided. For this, the use of FSS was determinant for the proper identification of areas devoted to motor control, since stroke can determine the functional shifting of areas thanks to plastic reorganization as consequence of an ischemic lesion (Tecchio et al., 2006b, Tecchio et al., 2007a). We were thus able to determine functionally homologous areas devoted to hand perception and study them in resting state. In fact, hand sensorimotor areas were identified through their passive response to a peripheral stimulation. Our study evidenced that the robotic treatment had effects on both the hand control improvement and on the interhemispheric connectivity in resting state. The cortico-cortical coherence is unable to discriminate if the coupling between the sensorimotor areas is due to excitatory or inhibitory activity. But, since the main part of transcallosal projections activate local inhibitory networks, being the medium for the interhemispheric connectivity (Lee et al., 2007), it could be reasonably supposed that local hemispheric inhibition circuits are linked with the cortico-cortical coherence between the areas of the two hemispheres. At the same time the unaffected

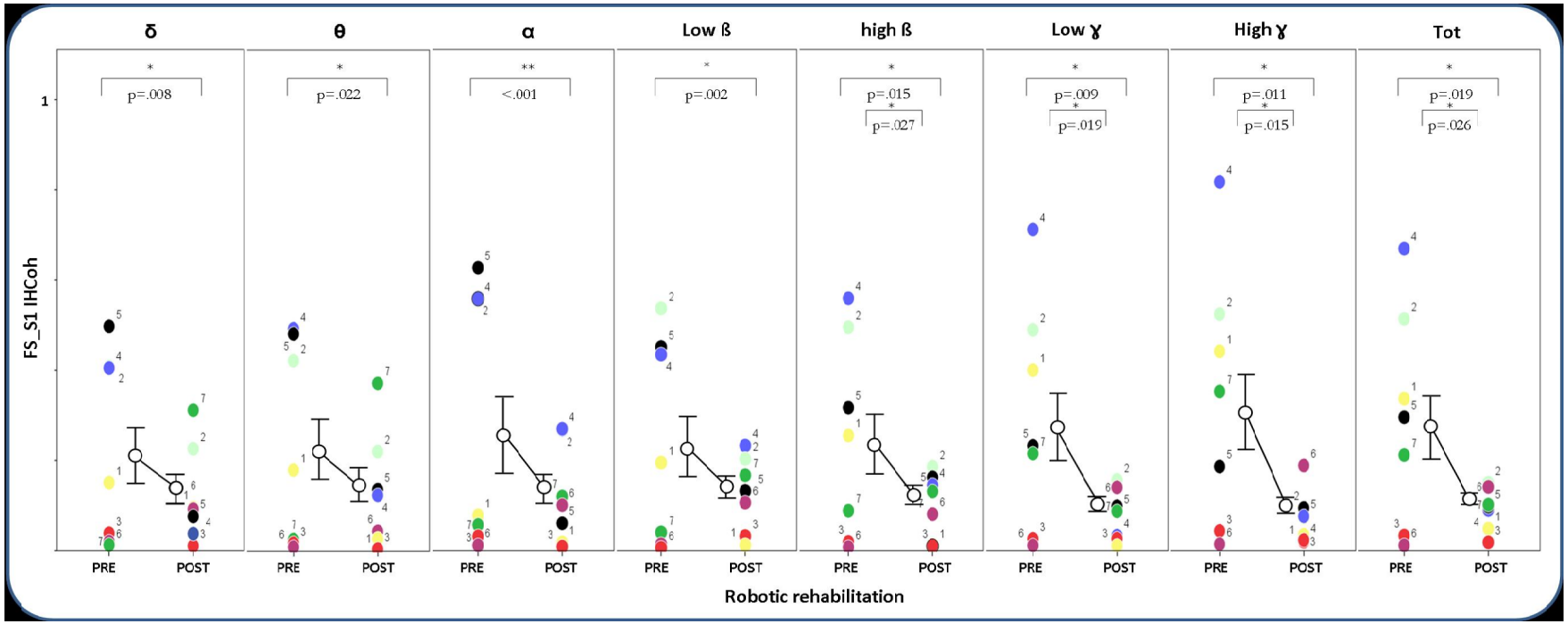

Figure 5.3 FS_S1 IHCoh values distribution before and after robotic rehabilitation*

FS_S1 IHCoh values distribution (colored dots) and level (empty dots, mean ± 1SE) pre and post rehabilitation program. At the top of the figure, the upper line refers to Levene test applied to T_{pre} and T_{post} values distribution, the lower one refers to T_{pre} and T_{post} FS_S1 IHCoh difference. IHCoh was distributed across a wide range before and tightly to a final level after treatment. Significant differences: * corresponds to $p<0.05$; ** corresponds to $p<0.001$.

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hemisphere tends to excessively inhibit the affected one provoking the sensori-motor interhemispheric imbalance (Murase et al., 2004; Oliveri et al., 1999; Traversa et al., 1998) with the involvement of both the motor (Shimizu et al., 2002; Takeuchi et al., 2010) and somatosensory cortex (Floel et al., 2004). The prove was also obtained through the use of inhibitory rTMS applied over the unaffected hemisphere in order to obtain a reduction of its over-activation (Nowak et al., 2008) and the its inhibitory action over the affected one. The last consequently increases its activity (Conchou et al., 2009) improving motor function (Mansur et al., 2005). This process produces jointly the interhemispheric connectivity reduction and clinical improvement (Grefkes et al., 2010; Landi and Rossini, 2010; Rossini et al., 2003). Therefore the modulation of connectivity described in present study could be originated by changes in local and interhemispheric excitatory/inhibitory balances.

Inter-hemispheric connectivity at rest is crucial for behaviour

Functional recovery after ischemic stroke can be improved by brain plasticity (Cramer et al., 2011; Rossini et al., 2003; Tecchio et al., 2006b; Tecchio et al., 2007a) where, in addition to localized changes, the connectivity of distributed areas is fundamental (Grefkes and Fink, 2011; Siegel et al., 2012). The brain connectivity depends on more factors: time of evaluation from stroke (Wang et al., 2010), on the condition (rest vs. task related) and on analysis procedure (Muthukumaraswamy and Singh, 2008). A lower connectivity is present in acute phase, matched with severe clinical state, in animal models (van Meer et al., 2010) and humans (Carter et al., 2010) while it tends to increase with progressive motor control recovery. In fact, acute stroke patients studied through fMRI along one year showed dependency between motor improvements and the resting state inter-hemispheric coupling between BOLD signals of homologous primary motor areas (Wang et al., 2010).

FSS procedure when extracting sources is able to extract also their high rhythms that are

difficult to identify from EEG scalp activity (Porcaro et al., 2009). Making advantage of this property in the proposed study the variations in beta and gamma bands were associated to interhemispheric connectivity changes. These findings are supported by previous studies where the beta band synchronization is involved in sensory motor integration (Engel and Fries, 2010; Tombini et al., 2009) and gamma-band connectivity plays a role for the dynamical connection of small- and large-scale neuronal pools of sensori-motor integration (Alonso et al., 1996; Szurhaj and Derambure, 2006; Tecchio et al., 2007c; Tecchio et al., 2008a).

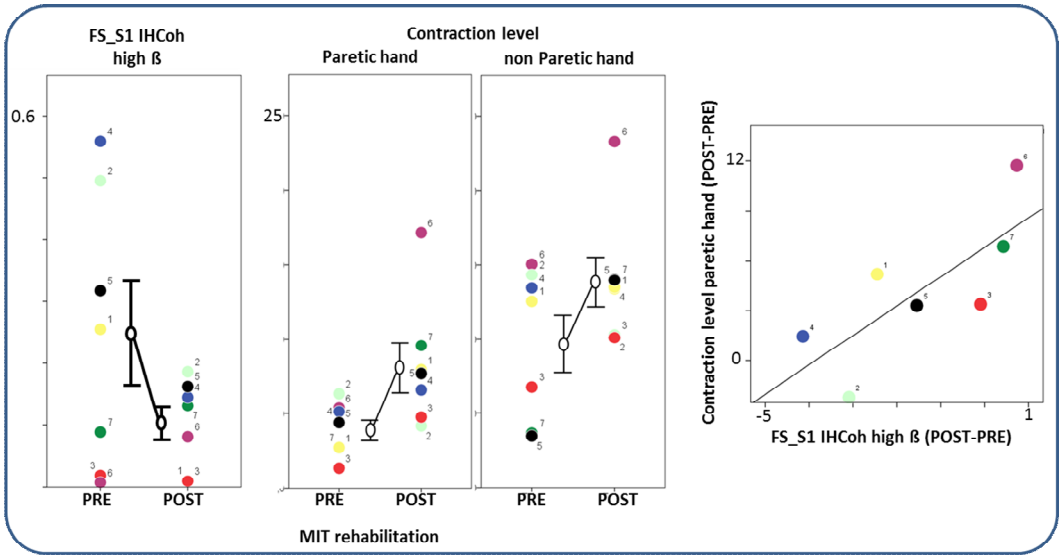

Figure 5.4 Relationship between robotic rehabilitation effects on inter-hemispheric sensorimotor connectivity and hand motor control*

Left: FS_S1 IHCoh distributions and levels at the start and at the end of the rehabilitation program (High beta band has been chosen as indicative). **Centre**: Contraction Level distributions and levels at the start and at the end of the rehabilitation program. Both Paretic and Non Paretic Hand improve their hand motor control. **Right**: Graphical representation of correlations between the change of FS_S1 interhemispheric coherence (T_{post}-T_{pre}, X Axis) and the improvement of Contraction Level (T_{post}-T_{pre}, Y Axis).

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Conclusion

As the study in the previous chapter, this study is important in two fields, a methodological/experimental and a biological one. The first is obtained with the use of the FSS algorithm, that firstly permitted to extract the sources relative to the sensori-motor system even after consistent plastic changes. Then FSS weights were used to study the identified areas at rest. The biological result is represented by the newly identified connectivity behaviour that involves also beta and gamma bands and describes how the robotic rehabilitation is capable of modulating the connectivity in sensori-motor homologous regions. Therefore the plastic changes at rest are linked to the improvement of motor control. It is worth to mention that all the investigation was performed during the passive participation of the patients to the study. In fact, through the peripheral sensory stimulation we identified the target areas and through the resting state the levels of the connectivity between them.

CHAPTER 6

CONCLUSIONS

Summary of work

The aim of my research was to unveil some properties of the sensorimotor system that would be useful for a deeper knowledge of our most complex and mysterious organ, the brain. The sensorimotor system constitutes our connection with the external world and it is also one of privileged areas for the connection with external devices. But in the first place it is important to discover the connections within the sensorimotor system itself. For this purpose three different experiments have been run to show three aspects of the connectivity relative to the studied neuronal pools and a device was developed for the accomplishment of the experiments.

As described in Chapter 2, the device (InPresS) provides the monitoring, acquisition and feedback of the pressure exerted by the subject on a compliant bulb and also the piloting of other stimulation devices. The importance of the device has been highlighted by its usage in the experimental setup for fatigued MS patients, but it has been also used in other laboratories that have active collaborations with us about common research interests.

In Chapter 3 a study about central-peripheral communication relative to fatigue in MS patients has been described. It has been implemented by comparing two groups of MS patients sensing or not fatigue. The two groups have been selected to avoid any other difference, from the clinical to radiological point of view to prevent confounds about results linked to their CMC and motor control behaviour.

Chapter 4 focused on the areas involved by different motor pathway excitability during a stable, constant external stimulation. The study was performed using TMS/EEG recordings and the intra-cortical connectivity was analyzed in dependence on brain

excitability, which spontaneously fluctuates. A new analysis procedure, to identify region of interest in the brain source space, was proposed and successfully applied enabling single-subject assessment.

In the study proposed in Chapter 5 another source extraction algorithm was utilized to identify involved neuronal pools even when plasticity processes modified their spatial-anatomical properties with respect to standard landmarks. This made possible the observation of changes of the brain organization associated to the improvement of the paretic hand motor control induced by three months of robotic rehabilitation. Moreover, this study reveals that the functional connectivity – as opposed to neuronal pools activity – in the resting state is associated to the motor control improvement becoming more physiological, underling that brain features at rest are relevant for its functional abilities.

Contribution to knowledge

Also for the importance of the topic, the described studies have already obtained some important results: through the publication of the work of this thesis in international peer reviewed journals the novel methodology and findings have impacted the work at the lab and among the wider international comunity.

The study presented in Chapter 3 described the connectivity behaviour in case of central fatigue in MS providing a possible objective measure of fatigue perception. It has been published in the Multiple Sclerosis Journal (Tomasevic et al., 2013) and is part of a more complex and wide research that is held in my laboratory about multiple sclerosis. It also contributed, among other papers, to continue our investigation in this field leading to two new recently published papers (Tecchio et al., 2013; Zito et al., 2014). Moreover the result of the study has been used by other groups for developing their investigation in MS (Paoloni et al., 2013; Dawes et al., 2014).

The study in Chapter 4 has been published in Human Brain Mapping journal (Giambattistelli and Tomasevic et al., 2014). It has been the first step into TMS/EEG data analysis in my laboratory and our first result in understating the effects of the spontaneous variability in cortical excitation on the subsequent intra-cortical functional communication. It introduced a new procedure to identify relevant Regions of Interest based on sources' localization in group-analysis which enabled the single-subject evaluation in the study. This procedure could be promising also for BCI purposes, since the identification of proper areas from a group, allows to capture useful information from each subject individually.

The study in Chapter 5 about cortical connectivity at rest of functionally homologous sensorimotor areas in the lesioned and contral-lesioned hemispheres in stroke patients was published in Restorative Neurology and Neuroscience (Pellegrino and Tomasevic et al., 2012). Stroke involving the sensorimotor system is highly studied in my laboratory, mainly in the direction of identifying prognostic indices of recovery as guide for the selection of the proper intervention. This paper contributed to the knowledge about the brain reaction to perturbations that impact on its normal functioning and about the direction in which the connectivity expresses the overall system improvement. The results have been considered of importance among the scientific community and the paper has been cited by the Review published in Neuron which sustains that the oscillations and couplings in specific frequencies of brain areas are fundamental in determining the functional networks in ongoing activity (Engel et al., 2013). Other two Reviews about rehabilitation after stroke considered our study (Bola et al., 2013; Poli et al.,2013). Others used our results to reinforce their findings relative, for example, to brain areas connectivity in different bands and the influence of one band over another (Vukelić et al., 2013) or the difference between functional coupling in skilled surgeons

performing laparoscopy or robotic surgery (Bocci et al., 2013). This last presented an analysis over the interaction between the human brain and robotic devices.

Future work

This thesis faced the complex and fascinating issue of the connections inside the sensorimotor system and opened new possible paths in exploring and using them. Each of the proposed studies can be continued leading to a deeper comprehension of the behavioural relevance of the functional communication inside our brains. By the study in MS patients we illustrated how a central dysfunction emerges with both an altered central-peripheral communication and movement control overcorrections associated to fatigue. The next natural step would be to investigate the intra-cortical correlate of the cortico-muscular observed dysfunction. Prospectively these studies could lead to the definition of an objective index of fatigue, which is presently scored by subjective tests. The second study about spontaneous fluctuations in cortical excitability and intra-cortical connectivity will be continued analysing the brain state that induces such fluctuations and using this information to deepen understanding of cerebral phenomena in neurological patients. The third study on stroke recovery induced by robotic intervention could be continued investigating the optimal rehabilitative protocol in inducing 'physiological' functional connectivity and thus enhancing motor recovery.

This thesis has the also the prospective aim to support the research on BCI. The functional communication between the brain and the muscles and its changes due to fatigue are interesting for the BCI point of view, since the modifications of the system, due to its internal state, should be taken into account in feature definition. The same consideration should be dedicated to the trial-to-trial variability of the response to external stimuli since it involves more regions and for many milliseconds. But two are the main achievements that the presented studies want to highlight for BCI applications:

1. The importance of moving from extra-cranial recorded signals to properly extracted source activities. If this point is not adequately treated, it could be impossible to detect the activity of an area because is covered by other activities (Chapter 4) or because it changed its position and activity (Chapter 6).

2. The importance of focusing on cerebral areas' connections, instead of single areas alone, keeping trace of networking nature of the brain. The network connectivity emerges as a inescapable variable for identification of control abilities or internal wishes contained in brain activity. This empowers the concept that an efficient BCI feature extraction would benefit from simultaneous assessment of more collaborating areas to obtain an efficient effector control in physiological and neuro-rehabilitation contexts.

APPENDIX

InPresS software

ARDUINO

Declaration part

```
int sensorPin = 0;                                  // pin variables
int trigPin1 = 7;
int trigPin2 = 4;
int VarOut = 0;                                     // input/output variables
int VarPress = 0;
int val = 0;
int VarTrig = 0;                                    // trigger variables
unsigned long TrigTime = 20000;
int Trig = 0;
unsigned long time1, time2, VarDif;
unsigned long ExeTime =20000000;                    // 20 s of compression
unsigned long RestTime = 10000000;                  // 10 s of motor relaxation
unsigned long ExeGap = ExeTime – TrigTime;          // GO – STOP interval
unsigned long RestGap = RestTime – TrigTime;        // STOP – GO interval
int control = 0;                                    // control variables
int cctt = 0;
const int dim = 40;
long myArray[dim];
int i = 0;
int j = 0;
```

```
long sumPress = 0;                                    // pressure variables
long medPress = 0;
```

setup

```
void setup() {
  Serial.begin(9600);                                 // the serial communication
  pinMode(trigPin1, OUTPUT);                          // definition of pin usage
  pinMode(trigPin2, OUTPUT);
  digitalWrite(trigPin1, LOW);
  time2 = micros();                    // time variables initialization to the current time
  time1 = time2;
}
```

loop function

```
void loop() {
  if (Serial.available()) {                           // wait for the operator
    val= int(Serial.read()); }
switch (val) {
    case 'S':                          // automatic stimulation condition: in this case 4ms
      digitalWrite(trigPin2, LOW);      trigger is sent each 223 ms to one BNC connector
      delay(223);
      digitalWrite(trigPin2, HIGH);
      delay(4);
      break;
    case 'T':                          // pulse controlled by the operator and sent to the BNC
      if (control == 0){
        control=1;
        time1=micros();
```

```
  }
  if (control == 1) {
    time2 = micros();
    VarDif = time2 - time1;
    if (VarDif <= TrigTime){
      digitalWrite(trigPin1, HIGH);
      VarTrig = 10000;
    }
    else {
      digitalWrite(trigPin1, LOW);
      VarTrig = 0;
      control = 0;
      val = 'P';
    }
  }
  Pressione();                          // call of the function for the pressure acquisition
  break;
case 'P':
  Pressione();
  break;
case 'Q':                                                                  // quit
      digitalWrite(trigPin1, LOW);              // when quitting the pins are set to LOW
      digitalWrite(trigPin2, LOW);
  break;
 case 'R':              // acquisition protocol for 10s relax and 20s of contraction
```

```
time2 = micros();
VarDif = time2 - time1;
if (VarDif >= RestGap && control == 0)
{
 digitalWrite(trigPin1, HIGH);                    // GO trigger
 VarTrig = 10000;
 time1 = time2;
 control = 1;
}
VarDif = time2 - time1;
if ( VarDif >= TrigTime && control == 1)
{
  digitalWrite(trigPin1, LOW);
  VarTrig = 0;
  time1 = time2;
  control = 2;
}
VarDif = time2 - time1;
if (VarDif >= ExeGap && control == 2)
 {
  digitalWrite(trigPin1, HIGH);                   // STOP trigger
  VarTrig = -10000;
  time1 = time2;
  control = 3;
}
VarDif = time2 - time1;
```

```
        if ( VarDif >= TrigTime && control == 3)  {
          digitalWrite(trigPin1, LOW);
          VarTrig = 0;
          time1 = time2;
          control = 0;
        }
      Pressione();                    // call of the function for pressure acquisition
      break;
  }
}
```

Pressione

```
void Pressione() {                                  // pressure acquisition function
  VarPress = analogRead(sensorPin);                 // pressure from the sensor
  VarOut = VarPress + VarTrig;                      // sum of pressure and the trigger
  Serial.print(VarOut, DEC);                        // data sent to the serial port
  Serial.print('\n');                                                  // newline
}
```

PROCESSING

Declaration part

```
import processing.serial.*;                         // importing of the serial library
import controlP5.*;                                 // importing of the GUI library
ControlP5 controlP5;                                // initialization of variables
ControlFont font;
String pippo[];
DropdownList p1;
```

```
CheckBox checkbox;
int buttonValue = 0;
float myPress,myPress4;
float trig = 1;
float myData;
int cc1=0;                                  // variables for buttons control
int cc2=0;
int fbt, fbp;                               // variables for checkbox control
int db = 0;                                 // bias function variables
int db2 = 0;
int ib = 0;
int controlBias= 0;                         // initialization of control variables
int controlMax = 0;
int controlTime = 0;
int controlVisual = 0;
int col1 = 211;                             // Display function variables
int col2 = 211;
int col3 = 211;
int[] press = new int[0];                   // variables used for MVC
int nmax = 3;
float mmv = 0;
int cmax = 0;
int maxmax = 0;
int MM = nmax;
int dim = 50;
int vid = 0;                                // visualization variables
```

```
float[] mvid = new float[dim];
int conta = 0;
int sw=screen.width;
int sh=screen.height;
float k;                                                    // Move function variables
float ypos = 0;
int contay = 0;
int dimy = 100;
float[] Yposs = new float[dimy];
float medypos = 0;
int pozi=sh-(sh/16+12);
float yypos = pozi;
int ypixel = pozi;
int timeS1, timeS2, timeS3, timeS4, timeS5, difT, difT2, difT3;          // time variables
int maxVal;
float perc = 0;
Serial myPort;                                                  // input/output variables
int numPort;
PrintWriter output, mass;
String nome = "";
```

setup

```
void setup() {
                                                    // initialization of graphical properties
  size(sw, sh);                          // size of the window is equal to the size of the screen
  background(135, 206, 255);
  PFont pfont = createFont("Arial",20,true);
```

```
font = new ControlFont(pfont);
smooth();
frameRate(30);
pippo=(Serial.list());
controlP5 = new ControlP5(this);                    // definition of GUI's objects
checkbox = controlP5.addCheckBox("FBack",-120,5*sh/10);          // checkbox
checkbox.setColorForeground(color(120));                  // checkbox's layout
checkbox.setColorActive(color(255));
checkbox.setColorLabel(color(128));
checkbox.setItemsPerRow(1);
checkbox.setSpacingColumn(30);
checkbox.setSpacingRow(10);
checkbox.addItem("Visualize trigger",'t');                  // checkbox's items
checkbox.addItem("Visualize pressure",'p');
checkbox.activate("Visualize trigger");
checkbox.activate("Visualize pressure");
p1 = controlP5.addDropdownList("PORT",50,sh/20,100,120);      // dropdown list
customize(p1);                                  // call the customize function
                                                                  // buttons
controlP5.addButton("stimulus",'S',50,sh/10,100,50).setId(3);    // automatic stimulus
controlP5.addButton("max",'M',-120,3*sh/10,100,50).setId(4);     // MVC estimation
controlP5.addButton("trigger",'T',-120,4*sh/10,100,50).setId(6);     // manual trigger
controlP5.addButton("pressure",'P',-120,5*sh/10,100,50).setId(6);          // pressure
                                                        feedback and acquisition
controlP5.addButton("bias",'B', 50,6*sh/10,100,50).setId(7);          // bias adjustment
controlP5.addButton("quit",'Q', 50,7*sh/10,100,50).setId(5);                // quitting
```

```
                                                    // text fields
controlP5.addTextfield("name",50,2*sh/10,100,20).setId(1);          // subject's name
                                                                    or code
controlP5.addTextfield("%",180,2*sh/10,100,20).setId(2);            // required percentage
                                                                    of the MVC
}
```

draw

```
void draw() {                                                       // the draw function (loop)
  background(135, 206, 255);
    Display();                                                      // Display function call
}
```

Display

```
void Display() {                                                    // function that displays the feedback
  stroke(150);
  rectMode(CENTER);                                                 // central box
  fill(col1,col2,col3);
  rect(sw/2, sh/2, 150, sh-sh/8);
  line(sw/2-75, sh/2, sw/2+75, sh/2);
  fill(0, 0, 0);
  rect(sw/2-100, sh/2, 50, 24);                                     // reference bars
  rect(sw/2+100, sh/2, 50, 24);
  rect(sw/2, ypixel, 150, 24);                                      // mobile bar
}
```

controlEvent

```
public void controlEvent(ControlEvent theEvent) {                   // function, part of the
                                                                    ControlP5 library, that waits for operator's input
```

```
if (theEvent.isGroup()) {                    // Group refers to dropdown lists and checkboxes
  if (theEvent.group().name()=="FBack"){                    // checkboxes for feedback
    fbt = (int)theEvent.group().arrayValue()[0];
    if ((fbt==1) && (buttonValue=='P')) {                    // pressure with triggers
     myPort.write('R');
    }
    else if ((fbt==0) && (buttonValue=='P')) {                    // pressure without triggers
     myPort.write('P');
    }
    fbp = (int)theEvent.group().arrayValue()[1];
  }
  else if (theEvent.group().name()=="PORT"){                    // dropdown list for COM port
   numPort=int(theEvent.group().value());
   myPort = new Serial(this, Serial.list()[numPort], 9600);
   myPort.bufferUntil('\n');
  }
}
else if(theEvent.isController()) {                    // controller refers to textboxes and buttons
  switch(theEvent.controller().id()) {
   case(1):                                                    // 'name' textbox
    nome=theEvent.controller().stringValue();
    mass = createWriter(nome + year() + month() + day() + "_max.txt");  // output files
    output = createWriter(nome + year() + month() + day() + ".txt");
    cc1=1;
    if ((cc1+cc2)==2) {
    controlP5.controller("max").position().x=50;
```

```
  controlP5.controller("trigger").position().x=50;
  controlP5.controller("pressure").position().x=50;
  checkbox.setPosition(180, 5*sh/10);
  }
break;
case(2):                                          // 'percentage' textbox
  perc=float(theEvent.controller().stringValue())/100;
  cc2=1;
  if ((cc1+cc2)==2) {
    controlP5.controller("max").position().x=50;
    controlP5.controller("trigger").position().x=50;
    controlP5.controller("pressure").position().x=50;
    checkbox.setPosition(180, 5*sh/10);
  }
break;
case(3):                                          // automatic stimulus
  buttonValue=int(theEvent.controller().value());
  myPort.write(buttonValue);
break;
case(4):                                          // MVC estimation and acquisition
  maxVal=int(theEvent.controller().value());
  controlBias= 1;
  controlMax = 1;
  myPort.write('P');
break;
case(5):                                          // 'quit' button
```

```
      buttonValue=int(theEvent.controller().value());
      myPort.write(buttonValue);
      if (cc1==1) {
        output.flush();                    // write data in memory to the file
        output.close();                    // close the file
      }
    break;
    case(6):                               // pressure
      buttonValue=int(theEvent.controller().value());
      if (fbt==1) {
        myPort.write('R');
      }
      else {
        myPort.write(buttonValue);
      }
      controlVisual = 1;
    break;
    case(7):                               // 'bias' button
      controlBias= 1;
    break;
 }
}
}
```

customize

```
void customize(DropdownList ddl) {          // customizes the dropdown list, which
  ddl.setBackgroundColor(color(190));       contains the identified COM ports
```

```
  ddl.setItemHeight(20);
  ddl.setBarHeight(15);
  ddl.captionLabel().style().marginTop = 3;
  ddl.captionLabel().style().marginLeft = 3;
  ddl.valueLabel().style().marginTop = 3;
  for(int i=0;i<pippo.length;i++) {
    ddl.addItem(pippo[i],i);
  }
  ddl.setColorBackground(color(60));
  ddl.setColorActive(color(255,128));
}
```

keyPressed

```
void keyPressed() {                    // function that receives the  input from the  keyboard
  if (key == 't') {
    myPort.write('T');                                    // trigger sent to the BNC port
  }
  if (key == 'b') {                                                    // bias adjustment
    controlBias= 1;
  }
}
```

Bias

```
void Bias(float myPre)  {                 // function that calculates the bias as mean of 100
  if(ib < 100 && controlBias== 1){                                    consecutive values
    float db1 = myPre;
    db2 = db2 + int(db1);
    ib = ib + 1;
```

```
  }
  if (ib>=100){
    db2 = int(db2 / 100);
    db = db2;
    ib = 0;
    if(controlMax == 1)  {
      controlMax = 2;
    }
    controlBias= 2;
  }
}
```

serialEvent

```
void serialEvent(Serial myPort) {     // function, part of the 'serial library', that receives
  String  myString = myPort.readStringUntil('\n');                  in input the values sent by
  myData = float(myString);                                                  the Arduino board
  if (myData > 10000) {                // identification and extraction of the 'START' trigger
    myPress = myData - 10000;                                                  from the data
    trig = 10;
  }
  if ( 0 < myData && myData < 10000 ){   // data acquisition during standard data flow
    myPress = myData;
    trig = 0;
  }
  if (myData < 0) {     // identification and extraction of the 'STOP' trigger from the data
    myPress = myData + 10000;
    trig = -10;
```

```
}
Bias(myPress);                                                    // bias calculation
if(maxVal == 'M' && nmax > 0 && controlMax == 2){   // maximal values calculation
 float mmm = myPress;
  mmm = mmm - db;                                                 // bias subtraction
  mass.println(myPress);                                          // data saving to file
  if (mmm > 60){                          // threshold that starts the acquisition of the data
    press = append(press, int(mmm));
    if (mmm < 60 && mmm> 50){
      press = append(press, int(mmm));
    }
    mmv = mmm;
  }
  if (mmv > 50 && mmm < 50) {                       // threshold that stops the acquisition
    int M = max(press);                                  // maximal value identification
    int sumax = 0;
    int cont = 0;
    for (int i = press.length-1; i >=0; i = i-1) {
      if (press[i]>=(0.8*M)) {            // identification of all the values higher than 80%
        sumax = sumax + press[i];                                 of the maximal value
        cont = cont + 1;
      }
      press = shorten(press);
    }
    cmax = cmax + 1;
```

```
    controlP5.controller("max").setCaptionLabel("max "+cmax);          // the number of
    nmax = nmax - 1;                    exerted pressure repetitions is showed on the button
    maxmax = maxmax + int(sumax / cont);
    int[] press = new int[0];
    mmv = mmm;
   }
  }
  if (maxVal == 'M' && nmax <= 0 && controlMax == 2) {
   maxmax = int(maxmax / MM);                                          // MVC calculation
   output.println("Name: " + nome + "\t" + "Date: " + day() + "/" + month() + "/" +
year() + "\t" + "Time: " + hour() + ":" + minute() );                  // writing on file
   output.println("Bias:" + db + "\t" + "MVC:" + maxmax);
   mass.flush();                                          // write on file data still in memory
   mass.close();                  // close the file with pressure data for MVC estimation
   maxVal = 'Q';
   controlMax = 3;
  }
  if(controlVisual == 1){                                              // trigger visualization
    if ( trig == -10 && controlTime == 0) {          // if the trigger is negative, then the
    controlTime = 1;                                    central box colour is red for 1 s
    timeS1 = second();
   }
   if (controlTime == 1) {
    timeS3 = second();
    difT2 = timeS3 - timeS1;
    if (difT2 <= 1) {                                                                  // red
```

```
    col1 = 238;
    col2 = 0;
    col3 = 0;
  }
  else {
    controlTime =2;
    col1 = 250;
    col2 = 250;
    col3 = 250;
  }
}
if( trig == 0 && controlTime == 2) {                    // 10 seconds of relaxation
  col1 = 250;
  col2 = 250;
  col3 = 250;
  timeS2 = second();
  difT = timeS2 - timeS1;
  if (difT >= 7 )  {
    controlBias= 1;
    Bias(myPress);                    // before the new trial starts the bias is corrected
    controlTime = 3;
  }
}
if( trig == 10 && controlTime == 3 ) {        // if trigger is positive, the 'GO' signal is
  controlTime = 4;                                        green and il lasts for 1 second
  timeS4 = second();
```

```
}
if (controlTime == 4) {
  timeS5 = second();
  difT3 = timeS5 -timeS4;
  if (difT3 <= 1) {
    col1 = 0;                                                    // green
    col2 = 238;
    col3 = 0;
  }
  else {
    controlTime = 0;
    col1 = 250;
    col2 = 250;
    col3 = 250;
  }
}
if (trig == 0 && controlTime == 0) {                    // 20 seconds of bulb compression
  col1 = 250;
  col2 = 250;
  col3 = 250;
}
float vid = myPress;
vid = vid - db;
mvid[conta] = vid;                          // the software can provide different visualization
if (conta == dim-1) {              sensitivity in dependence of the window size (dim) used
  conta = 0;                                                     for sliding window average
```

```
    }
    else {
      conta = conta + 1;
    }
    float sumvid = 0;
    for (int zz = 0; zz < dim; zz = zz+1) {
      sumvid = sumvid + mvid[zz];
    }
    float medvid = sumvid / dim;
    if (fbp==1) {
     Move(medvid);              // the final value of pressure is sent to the Move function
    }
    else {
      ypixel=sh/2;
    }
    output.println(myPress + "\t" + trig + "\t" + vid);          // the data are sent to the file
  }
}
```

Move

```
void Move(float pre)    {                              // the function moves the cursor bar
  k = ((sh-sh/8)/2 / (maxmax * perc));                 // k adapts the cursor movement with
  ypos = (k * pre);                                    respect to the MVC percentage and screen height
  Yposs[contay] = ypos;
  if (contay == dimy-1) {
    contay = 0;
  }
```

```
  else {
    contay = contay + 1;
  }
  float sumypos = 0;
  for (int zy = 0; zy < dimy; zy = zy+1) {        // the average is performed at this level to
    sumypos = sumypos + Yposs[zy];                                    avoid flickering of the bar
  }
  medypos = sumypos / dimy;
  yypos = pozi - medypos;                                                          // cursor position
  if (yypos < 50) {                          // upper and lower limits imposition to the cursor
    yypos = sh/16+12;
  }
  if (yypos > pozi) {
    yypos = pozi;
  }
  ypixel = int(yypos);
}
```

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