DISSERTATIONS IN
HEALTH
SCIENCES

Florinda Ines Maria Ferreri

TMS and TMS-EEG Studies on the Excitability, Connectivity and Plasticity of the Human Motor Cortex

Publications of the University of Eastern Finland
Dissertations in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

FLORINDA INES MARIA FERRERI

TMS and TMS-EEG studies on the excitability, connectivity and plasticity of the human motor cortex

To be presented by permission of the Faculty of Health Sciences, University of Eastern Finland for public examination in ML3, Medistudia Building, on Friday, September 9th, 2011, at 14:00

Publications of the University of Eastern Finland
Dissertations in Health Sciences
Number 69

Department of Clinical Neurophysiology, Institute of Clinical Medicine, School of Medicine, Faculty of Health Sciences
University of Eastern Finland
Kuopio
2011

Kopijyvä
Kuopio, 2011

Distributor:
University of Eastern Finland
Kuopio Campus Library
P.O. Box 1627
FI-70211 Kuopio, Finland
http://www.uef.fi/kirjasto

ISBN (print): 978-952-61-0505-5
ISBN (pdf): 978-952-61-0506-2
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

Author's address: Department of Clinical Neurophysiology, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
KUOPIO
FINLAND

Department of Neurology
School of Medicine
University Campus Biomedico of Rome
ROME
ITALY

Supervisors: Professor Esa Mervaala, MD, PhD
Department of Clinical Neurophysiology, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland Faculty
KUOPIO
FINLAND

Professor Paolo Maria Rossini, MD, PhD
Department of Neurology
Catholic University of Rome
ROME
ITALY

Doctor Sara Määttä, MD, PhD
Department of Clinical Neurophysiology, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland Faculty
KUOPIO
FINLAND

Reviewers: Professor Mark Hallett, MD, PhD
Human Motor Control Section
Medical Neurology Branch, NINDS
National Institute of Health
BETHESDA, MD
USA

Professor Ulf Ziemann, MD, PhD
Department of Neurology
Goethe-University
FRANKFURT
GERMANY

Opponent: Professor John Rothwell, MD, PhD
Department of Neurology
Institute of Neurology, Queen Square
LONDON
ENGLAND

Ferreri Florinda Ines Maria
TMS and EEG-TMS studies on human cortical excitability, connectivity and plasticity, 86 p.
University of Eastern Finland, Faculty of Health Sciences, 2011
Publications of the University of Eastern Finland. Dissertations in Health Sciences Number 69. 2011. 86 p.

ISBN (print): 978-952-61-0505-5
ISBN (pdf): 978-952-61-0506-2
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

ABSTRACT

This thesis, consisting of five original publications (I-V) and a summary, explores the feasibility of combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG) for the examination of the cortical excitability and connectivity of the human cerebral cortex, both in healthy and pathological condition. It addresses some issues crucial for a better elucidation of the mechanisms underlying human brain neuroplasticity. The series of studies clearly demonstrated that TMS -with or without EEG- is a sensitive and objective measure of the effect of different kinds of non-invasive manipulation of the brain's activity. Moreover TMS and TMS-EEG co-registration are valuable methods to study cortical excitability, connectivity and functional organization in healthy subjects and neurological patients such as Alzheimer's disease patients. They are along with clinical, neuropsychological, and neuroimaging data inexpensive measures of disease progression, supplementing traditional methods to assess the effects of therapies.

Taken together, the findings presented in this thesis are important for the adoption of the TMS and TMS-EEG co-registration techniques as a tool for basic neurophysiological research and, in the future, even for clinical diagnostics purposes.

National Library of Medical Classification: WL 141.5.T7, WL 150, WL 307, WL 335, WT 155

Medical Subject Headings (MeSH): Transcranial Magnetic Stimulation; Electroencephalography; Electromagnetic Fields; Electric Stimulation/methods; Motor Cortex; Evoked Potentials, Motor; Brain Mapping; Brain/physiology; Brain/radiation effects; Cellular Phone; Alzheimer Disease

Ferreri Florinda Ines Maria
TMS and EEG-TMS studies on human cortical excitability, connectivity and plasticity, 86 p.
University of Eastern Finland, Faculty of Health Sciences, 2011
Publications of the University of Eastern Finland. Dissertations in Health Sciences Number 69. 2011. 86 p.

ISBN (print): 978-952-61-0505-5
ISBN (pdf): 978-952-61-0506-2
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

TIIVISTELMÄ

Tässä väitöskirjatyössä, joka koostuu viidestä osajulkaisusta ja niiden yhteenvedosta, tutkittiin transkraniaalisen magneettistimulaation (TMS) ja siihen yhdistetyn aivosähkökäyrän (EEG) soveltuvuutta aivokuoren ärtyvyyden ja eri aivokuorialuiden välisten toiminnallisten yhteyksien tutkimisessa. Tutkimus toi uutta tietoa aivokuoren muovautuvuudesta. Tulokset osoittavat, että TMS – joko yhdistettynä EEG:hen tai ilman sitä - mittaa hyvin aivokuoren toiminnallisia muutoksia. Lisäksi tulokset osoittivat, että TMS ja TMS-EEG kuvaavat hyvin aivokuoren ärtyvyyttä, toiminnallisia yhteyksiä ja toiminnan muutoksia niin terveillä koehenkilöillä kuin neurologisilla potilaillakin (tässä tutkimuksessa Alzheimerin tautia sairastavat potilaat). Menetelmää voi käyttää yhdessä neuropsykologisten ja kuvantamistutkimusten kanssa, kun arvioidaan sairauden etenemistä. Lisäksi TMS: n avulla voi arvioida lääkevastetta. Väitöskirjatyön tulokset lisäävät TMS: n ja TMS-EEG: n käytettävyyttä neuropsykologisessa tutkimustyössä ja tulevaisuudessa myös kliinisessä diagnostiikassa.

Yleinen suomalainen asiasanasto (YSA): aivokuori, EEG, transkraniaalinen magneettistimulaatio, aivotutkimus, matkapuhelimet, säteily, Alzheimerin tauti

Acknowledgements

This work represents to a certain extent the synthesis of my journey in the intriguing field of the neuroscience which began in 1999 when, as a young and inexpert medical student, I decided to get some experience in the Department of Neurosurgery at the Children's Hospital, Harvard Medical School, Boston.

When, in 2006, I was a resident in Neurology, attending the Lab of Professor Giulio Tononi in the Center for Sleep and Consciousness of the University of Wisconsin, Madison, and I met Dr. Sara Määttä, I never imagined that I would have a chance to do my PhD in Finland. The idea came in a funny contest in Chicago, and I'm sincerely and profoundly indebted to Sara for that. She helped and amiably supported me on this experience. We have a strong and true friendship and a common love for science, proved over these years on several occasions all over the world from Miami to Rovaniemi via Istanbul, Porto Cervo, Santa Maria di Leuca, Taormina and Ravello. I'm also indebted to Professor Esa Mervaala, who warmly welcomed me to his Department in Kuopio, accepting with enthusiasm the challenge of training an Italian student.

Completing this dissertation took a relatively long time, as most of the work was performed during the weekends, the vacations and the limited time I could spare from the demanding schedule of my daily activity. This thesis collects all my scientific efforts since 2000, when I started my internship in the Department of Neurology at the University Campus Biomedico of Rome under the guidance of my *'Magister'* Professor Paolo Maria Rossini, who supervised my studies and research from the very beginning. I thank him with all my heart: my whole career would not have been possible without his guidance, continuous support, and contributions that go far beyond scientific borders. He has incessantly inspired me with his creative scientific thinking and thoroughness and under his guidance I was able to crystallize my methodological approach for studying the human brain and integrate the results into a framework of known neurological and neurophysiological data. Most importantly, with his unique approach to life, medical science in general and neurological science in particular, Professor Rossini provided me with qualities that will forever remain part of my own style. I'm deeply thankful to Professor Rossini for adding a scientific essence to my thoughts and for being the *'spiritus movens'* of my work.

I am also grateful to Professor Mark Hallett and Professor Ulf Ziemann for reviewing this thesis, and particularly to Professor John Rothwell for accepting to be the opponent in the academic defense of my work. I'm deeply honored by the contributions of these scientists.

I thank Professor Vincenzo Denaro, Professor Flavio Keller, Professor Giordano Dicuonzo and Professor Paola Binetti who trusted me from the beginning.

This work represents not only the efforts made by me over these last years but also the sum of the contribution of many people who helped me in reaching this goal. I have

been extremely fortunate in being given the opportunity to collaborate with top professionals from several parts of the words: I'm indebted to all my coworkers, coauthors, and supervisors, particularly Dr. Patrizio Pasqualetti, my statistical guru, expert advisor and good friend; Dr. Fini, Dr. Dal Forno, Dr. Pauri, Dr. Curcio, Dr. Fabrizio, Mr. Fusco, Professor Vernieri, Professor Babiloni and Professor Miniussi for supporting me when this scientific adventure started; Professor Tononi, Professor Massimini and Professor Huber, who opened my mind; Dr. Esser, Dr. Riedner, Dr. Ferrarelli and Dr. Goldstein, who helped me in the USA; M.Sc. Säisänen who helped me with this thesis, and Dr. Aronica, who polished and improved my poor English.

I also thank my close friends Emma, Alberto, Davide, Federica, Liliana, Mara, Anna and Luigi who sustained me over these years in the international research community and even before, during the hard school time.

A lovely thought goes to all my relatives and particularly to my aunts Alessandra, Cettina and Marilena, to my uncle Franco, and to my sweet grandmother Ines, who always looks forward to my return from my trips all over the world.

I cannot express sufficient gratitude and love to my parents, Mina and Domenico, who taught to me to reach my objectives, however far or arduous, step by step in the right way without taking short cuts and to my little brother Egidio, who is now learning the same lesson and will always be in my heart.

This work would not have been possible without the patience, love and also technical and technological support that my fiancée David has given me over the last sixteen years. He has sacrified his wellbeing innumerable times in favour of my goals. I hope to be able to return at least part of this love in our future life together. Most of the work was performed at home during many hours that would otherwise have been devoted to him and my family.

I fondly dedicate this thesis to my grandfathers Giovanni and Egidio, who look after me since many years and to Anna, Lorenzo, my aunt Angela and my grandmother Florinda, who had a glimpse of the beginning of this way but are not with us anymore.

Kuopio, September 2011

Florinda Ines Maria Ferreri

List of the original publications

This thesis is based on the following five original publications:

I. Ferreri F, Pauri F, Pasqualetti P, Fini R, Dal Forno G, Rossini PM.
Motor cortex excitability in Alzheimer Disease: a transcranial magnetic stimulation study.
Ann Neurol 53(1):102-8, 2003

II. Ferreri F, Curcio G, Pasqualetti P, De Gennaro L, Fini R, and Rossini PM.
Mobile phone emission and human brain excitability.
Ann Neurol 60(2):188-96, 2006

III. Ferreri F, Pasqualetti P, Määttä S, Ponzo D, Ferrarelli F, Tononi G, Mervaala E, Miniussi C, and Rossini PM.
Human brain connectivity during single and paired pulse transcranial magnetic stimulation.
Neuroimage 1;54(1):90-102, 2011

IV. Ferreri F, Pasqualetti P, Määttä S, Ponzo D, Guerra A, Bressi F, Chiovenda P, Del Duca M, Giambattistelli F, Ursini F, Tombini M, Vernieri F and Rossini PM.
Motor cortex excitability in Alzheimer's disease: a Transcranial Magnetic Stimulation follow-up study.
Neurosci Letters 1;492(2):94-8, 2011

V. Ferreri F, Ponzo D, Hukkanen T, Mervaala E, Könönen M, Pasqualetti P, Rossini PM and Määttä S.
Human brain cortical correlates of short-latency afferent inhibition (SAI): a combined EEG-TMS study.
Submitted

The publications were adapted with the permission of the copyright owners. Some unpublished results are also presented.

Contents

Abbreviations

μV	microVolt
ACh	Acetylcholine
AChEIs	Acetylcholinesterase Inibitors
AD	Alzheimer's Disease
ADM	Abductor Digiti Minimi
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid
ANOVA	Analysis of Variance
Aβ	β-amyloid
c/s	cicle/second
CB1	Cannabinoid type 1 *receptor*
CDR	Clinical Dementia Rating
CoG	Center of Gravity
CSP	Cortical Silent Period
dB	decibel
DTI	Diffusion Tensor Imaging
ECD	Extensor Digitorum Communis
EEG	Electroencephalography
EMF	Electromagnetic Field
EMG	Electromyography
EOG	Electro-oculogram
ERD	Event-Related Desynchronization
ERS	Event-Related Synchronization
ERSP	Event-Related Spectral Perturbation
fEPSPs	fast Excitatory Postsynaptic Potentials
fIPSPs	fast Inhibitory Postsynaptic Potentials
fMRI	functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric Acid
GEE	General Estimating Equation
GMFP	Global Mean-Field Power
Hz	Hertz
IADL	Instrumental Activities of DailyLiving
ICF	Intracortical Facilitation
IFCN	International Federation of Clinical Neurophysiology
ISIs	Inter-Stimulus Intervals
ITC	Intertrial Coherence
kHz	kilo Hertz
kΩ	kilo Ohm
LTD	Long-Term Depression
LTP	Long-Term Potentiation
M1	Primary Motor Cortex
MANOVA	Multivariate Analysis of Variance
MEG	Magnetoencephalography
MEP	Motor Evoked Potential
mGluR	metabotropic Glutamate Receptor
mm	millimetre
MMSE	Mini Mental State Examination
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
ms	millisecond
MT	Motor Threshold
mV	milliVolt
N20	Negative SEP deflections
N7,N18,N44, N100,N280	Negative TMS-evoked EEG deflections
NMDA	N-Methyl-D-Aspartato
OP	Opponent Pollicis
P13,P30,P60 P180	Positive TMS-evoked EEG deflections

ppTMS	paired pulse Transcranial Magnetic Stimulation
rMT	resting Motor Threshold
rTMS	repetitive Transcranial Magnetic Stimulation
SAI	Short-latency Afferent Inhibition
SD	Standard Deviation
sEPSPs	slow Excitatory Postsynaptic Potentials
SICI	Short-Interval Intracortical Inhibtion
sIPSPs	slow Inhibitory Postsynaptic Potentials
SnPM	Statistical nonParametric Mapping
STDP	Spike Timing-Dependent Plasticity
T	Tesla
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TNF	Tumor-Necrosis Factor

1 Introduction

It is now well known that one of the most important capabilities of the human brain is changing its organization structurally and/or functionally throughout life in order to cope with normal and abnormal experiences and inputs from the surrounding world. This capacity, named neuroplasticity, has been extensively investigated in the last few decades not only because it supports several brain functions including cognitive processes, but also because of its potential therapeutic applications in neuropsychiatric disorders. The examination of neuroplasticity was initially possible only on animal preparations while the recent development of advanced technologies allows nowadays non-invasive exploration on the living human brains. Among the tools currently available to neuroscientists Transcranial Magnetic Stimulation (TMS) is a non-invasive and painless technique that has opened up completely new and fascinating avenues to study, induce or manipulate the mechanisms underlying plasticity of the human brain.

Firstly, TMS can be used to investigate the functional state of the cerebral cortex (Kobayashi and Pascual-Leone, 2003; Rossini and Rossi, 2007) and the responsiveness of the neuronal population to incoming signals, that is the neuronal excitability, detecting changes which may have occurred through processes such as learning or recovery from a nervous system lesion. Secondly, TMS can interfere with the brain activity, inducing short-lasting 'virtual lesions' which may directly test 'in vivo' the functional relevance and the connectivity of several brain cortices (Oliveri et al., 1999; Oliveri et al., 2000a; Oliveri et al., 2000b; Friston et al., 2003; Ziemann et al., 2008). Thirdly, TMS can manipulate the brain plasticity, inducing by itself changes in excitability and connectivity of the stimulated cortex which might be used therapeutically in neurological and psychiatric diseases (Rothwell, 2010; Platz and Rothwell, 2010). All Publications (I-V) in this thesis used TMS most of all as a probe of cortical excitability.

In TMS the excitation of neurons in deep grey matter can be either direct or indirect through volleys from superficial neurons (Barker et al., 1985). Adequate stimulation of the primary motor cortex (M1) evokes indirect excitation of pyramidal neurons via local inter-neurons with higher probability than direct excitation (Amassian and Cracco, 1987). The volley along the corticospinal pathway can elicit electromyographic (EMG) responses, termed motor evoked potentials (MEPs), in the target muscles contra-lateral to the side of the stimulation (Hallett, 2000). Even though MEPs are used routinely in research and sometimes in clinical settings to study the motor cortex excitability, a proper evaluation should clearly differentiate between the indices of the overall excitability of the corticospinal system (corticospinal excitability), and those directly and specifically reflecting the excitability of the motor cortex (cortical excitability). Indeed the problem with EMG in general, and with MEPs in particular, is that they are affected by a combination of cortical, subcortical, and spinal-cord mechanisms, which usually coincide in time, making their separation very difficult. Amplitudes and latencies of MEPs are in fact parameters resulting from a combination of excitatory and inhibitory events occurring in a complex

synaptic network at different neural levels along the motor pathway (Rossini et al., 1987a; Rothwell et al., 1987; Hallett, 2011; Hallett, 2007; Rossini and Rossi, 2007), and the relative contribution of these events is far from fully elucidated (Baumer et al., 2003; Rothwell, 2010) TMS can be delivered in several paradigms such as single pulse, paired-pulse or repetitive and each of these paradigms has contributed to clarify some aspects of the corticospinal physiopathology (Ziemann, 1999). The results of **Studies I-II-IV** of this thesis complement these findings clearly demonstrating the role of some TMS paradigms (that is TMS mapping and paired pulse TMS (ppTMS)) in the evaluation of corticospinal excitability in healthy and pathological subjects. However, as previously mentioned, if drawn exclusively on MEP recordings, conclusions about cortical pathologies or in general about involvement of M1 in a given process might be uncertain. Recently, a technical device has been introduced that allows the recording electroencephalographic (EEG) responses to different TMS paradigms with millisecond resolution likely without spinal and muscular influences.

Combining TMS of a given scalp site with EEG enables a non-invasive, finally direct, method to study cortical excitability, connectivity and plasticity (Ilmoniemi et al., 1997). A network of neuronal connections is in fact engaged when TMS-evoked activation extends from a stimulation site to other parts of the brain, and the summation of synaptic potentials produces deflections in scalp EEG signals, starting few milliseconds after the stimulus and lasting about 300 ms, first in the form of rapid oscillations and then as lower-frequency waves (Ilmoniemi et al., 1997). The amplitude, latency, and scalp topography of single pulse TMS-evoked EEG responses have been clearly described (Komssi et al., 2004). The characteristics of these responses are thought to depend on the stimulation intensity and functional state of the stimulated cortex as well as the overall brain (Komssi et al., 2004; Siebner et al., 2009). It has been suggested that the very first part of the TMS evoked EEG response reflects the excitability -that is the functional state- of the stimulated cortex while its spatio-temporal distribution over the scalp reflects the spread of activation -that is the effective connectivity of the stimulated area (Lee et al., 2003; Komssi and Kähkönen, 2006)- to other cortical areas -via intra and inter-hemispheric cortico-cortical connections- as well as to sub-cortical structures and spinal cord via projection fibres. The results of **Studies III and V** of this thesis complement these findings by adding new insight into previously delineated functional behaviour of human brain as investigated by EEG oscillations evoked by TMS on M1. In fact, two further early responses -N7 and P13- were observed and characterized in more details; moreover the dependence of the EEG evoked activity on some different TMS paradigms (that is ppTMS and short latency afferent inhibition (SAI)) was described and correlate with the MEPs variability induced by the same paradigms.

Then the present thesis provides evidences on the feasibility of TMS and TMS-EEG for the evaluation of M1 excitability and connectivity, addressing some crucial issues for a better elucidation of the mechanisms underlying brain neuroplasticity in human healthy and pathological subjects.

2 *Review of the literature: basic principles of human brain excitability, connectivity and plasticity*

A rising body of evidence from animal models and neuroimaging and neurophysiologic studies in humans provides evidence for plastic changes and adaptation in central nervous system throughout life (Pascual-Leone et al., 1998; Hallett, 1999; Siebner and Rothwell, 2003; Pascual-Leone et al., 2005; Ziemann et al., 2008).in order to cope with experiences and inputs from the surrounding as well as from the 'interior' environments. While the developing nervous system seems more capable of modifications, dynamic plastic changes can be documented in the adult nervous system as well. The adult central nervous system is indeed emerging as a dynamically adapting and changing system in which modifications are driven by afferent input, efferent demand and environmental and behavioural influences of functional significance (Pascual-Leone et al., 1998). On one hand, neural networks exhibit modularity and carry out specific functions, and on the other they retain the capacity to deviate from their usual functions and to reorganize themselves. The term 'neuroplasticity' encompasses the capacity of synapses, neurons, neuronal circuits and networks in the brain to perceive, adapt and respond to new information, sensory stimulation, development, damage or dysfunction, allowing short-term to long-lasting changes in their connections and behaviour (Pascual-Leone et al., 1998; Rossini and Dal Forno, 2004; Rossini et al., 2003; Pascual-Leone et al., 2005). Neuroplasticity takes place mainly during development when neurons in the young brain sprout branches and form synapses. When the brain initiates to process sensory information, some of these synapses reinforce, some deteriorate and others, if unused, are even completely removed, a process named 'synaptic pruning' (Pascual-Leone et al., 1998). Further forms of neuroplasticity work during the life-span under different circumstances even though sometimes only to a limited extent, underlying the acquisition of new skills, learning, memory, adaptation to new contexts and even recovery of function after nervous system injury (Rossini et al., 2010; Rossini et al., 2011) when the brain attempts to compensate for lost activity leading to positive but also negative or aberrant reorganization. Neocortex is a particularly relevant region for plasticity because it accomplishes sensory, motor, and cognitive tasks with strong learning components.

Investigators have made several important improvements towards understanding the molecular underpinnings of these fundamental plasticity processes and towards defining the learning rules that manage their induction (Buonomano, 1999). Since the 1970s neuroplasticity has gained wide acceptance throughout the scientific community as a essential property of the brain and plastic changes of synapses, neurons, neuronal circuits and networks have been even directly observed thanks to the development of modern molecular, imaging and electrophysiological techniques which can reach as far as the cellular level.

2.1 BRAIN EXCITABILITY, CONNECTIVITY AND PLASTICITY

Fast reorganization and change of the brain's synapses, neurons, neuronal circuits and networks can take place under many different circumstances and in many different forms. Most of the studies about neural plasticity focus on **functional plasticity,** particularly on Hebbian-like long-term synaptic potentiation and depression (respectively LTP and LTD; Hebb, 1949; for reviews see Malenka and Bear, 2004; Raymond, 2007; Massey and Bashir, 2007). In functional plasticity synapses and synaptic strengths are considered as variable amplification factors within a hardwired network structure (Butz et al., 2009). However, functional plasticity can be expressed through synaptic (Bliss and Collingridge, 1993; Malenka and Bear, 2004) and also non-synaptic changes (Hansel et al., 2001; Debanne, 2009). In the former case there are changes in synaptic transmission characteristics while in the latter neuronal intrinsic excitability homeostasis is affected by means of the modulation of the voltage-gated ion channels and passive 'leak' channels, together hosted in neuronal membranes and determining the integrative and excitable properties of neurones. Recent studies tend to integrate these mechanisms at least in part, supporting the idea that homeostatic excitability mechanisms are invoked to ensure that neurons continually operate within a dynamic physiological range of activity (Burrone and Murthy, 2003), possibly acting to normalize overall neuronal activity after synapse-specific Hebbian LTP and LTD (Turrigiano and Nelson, 2004).

In contrast to any forms of functional plasticity, **structural plasticity** changes anatomical connectivity among neurons, modifying synaptic connectivity patterns, synapse numbers and extension, axonal and dendritic branching patterns, axonal fibre densities and even neuronal numbers. A large number of structural plasticity mechanisms are known, many recently discovered (Kim and Linden, 2007; Sjöström et al., 2008). Very rapid structural changes (hours to days) occur continuously at the level of spines and synapses; spine formation and retraction are associated with synapses formation and elimination (Trachtenberg et al., 2002; Holtmaat et al., 2005; Florence et al., 1998; Kleim et al., 1996). Thus, rapid synapses formation and elimination may contribute to rapid experience-dependent plasticity. In contrast, large-scale structural changes involve macroscopic axonal projections including thalamocortical and horizontal, cross-columnar axons and, to a lesser extent, dendrites (reviewed in Fox and Wong, 2005; Broser et al., 2008). These are considered to be slow, as they act in several days or weeks (seeTrachtenberg and Stryker 2001).

2.2 TYPES AND MECHANISMS OF PLASTICITY

In summary, in the last few decades several candidate mechanisms have been proposed and evaluated both at cellular and neuronal population levels to support cortical plasticity (Calford and Tweedale, 1988; Butler and Wolf, 2007; Sanes et al., 1990; Rossini et al., 2010; Rossini et al., 2011). The mechanisms proposed include both functional alteration of synaptic transmission in response to the coincident activity of pre - and post-synaptic

elements in existing synapses and neurons, and dynamic structural mechanisms of physical rewiring of cortical circuits by synapse formation, elimination and morphological change.

Recently, the involvement of LTP and LTD in cortical plasticity has begun to receive direct experimental support (Feldman and Brecht, 2005). However, new sensitive methods have also revealed many novel and already hypothesized mechanisms of cellular plasticity, both in vivo and in vitro. These include multiple forms of LTP and LTD (Sjöström et al., 2008), non-Hebbian plasticity, including homeostatic synaptic scaling and metaplasticity (Abraham and Bear, 1996; Turrigiano and Nelson, 2004), active modulation of intrinsic cellular excitability and plasticity of gamma-aminobutyric acid circuits (GABAergic circuits; Foeller and Feldman, 2004; Hensch, 2005). Moreover, in vivo time-lapse imaging has revealed that rapid structural plasticity of synapses and dendritic spines is widespread (Alvarez and Sabatini, 2007).

2.2.1 Functional mechanisms for cortical plasticity

A large number of functional mechanisms subtending plasticity are known, including the unmasking, uncovering, or activating of structurally pre-existing but functionally silent synapses (Palop et al., 2006; Liao et al., 1995), many of which have been recently discovered (Kim and Linden, 2007; Sjöström et al., 2008). The next sections review many of the known cellular plasticity mechanisms in the cortex (for a complete review see Feldman, 2009).

2.2.1.1 Long-Term Potentiation and Depotentiation

LTP has been proposed to underlie use-dependent and temporal correlation-dependent strengthening of sensory responses in young brains, reinforcement-dependent strengthening of responses in adult brains, and strengthening of spared inputs during deprivation-induced plasticity. Many neocortical excitatory synapses exhibit LTP (Feldman, 2009). Cortical LTP is most often classical NMDA-LTP. In NMDA-LTP, calcium from postsynaptic NMDA receptors and other sources activates kinases which drive specific AMPA receptor phosphorylation, insertion of GluR1-containing AMPA receptors into synapses, and in long-lasting LTP further altered gene expression. NMDA-LTP causes appearance of AMPA receptor currents at immature synapses that express NMDA but not AMPA receptors ('silent synapses'), thus functionalizing these synapses (Isaac et al., 1997; Rumpel et al., 1998). A second form of neocortical LTP is expressed presynaptically by an increase in release probability, which alters short-term synaptic dynamics (Markram and Tsodyks, 1996; Buonomano, 1999; Eder et al., 2002).

Additional forms of LTP may also occur (Daw et al., 2004). NMDA-LTP is strongly implicated in activity and use-dependent strengthening of cortical synapses during early development. The involvement of NMDA-LTP in response to potentiation during

deprivation-induced plasticity has long been hypothesized (Bear et al., 1987; Fox 2002) but remains controversial. In the hippocampus and amygdala, specific molecular and physiological tools have provided direct evidence that LTP occurs during, and is required for, adult learning (Maren, 2005; Sossin, 2008). In contrast, evidence for LTP in adult cortical learning remains inconclusive (Skibinska-Kijek et al., 2008; Feldman, 2009).

Although LTP is remarkable for its stability, numerous studies have revealed that it is initially labile and sensitive to disruption by a variety of interfering events and agents (Huang et al., 2001). Such reversal of synaptic strength from the potentiated state to pre-LTP levels has been termed depotentiation (Huang et al., 2001) and may provide a mechanism of preventing the saturation of the synaptic potentiation and increase the efficacy and the capacity of the information storage of the neuronal networks. Depotentiation of LTP is effectively induced by low-frequency afferent stimulation (<5 Hz), application of adenosine receptor agonists, brief periods of hypoxia and brief cooling shocks. The examples of depotentiation described to date are input specific and not differently expressed during development. The mechanisms responsible for this phenomenon remain to be fully characterized, although some possibilities are dependent on NMDA receptor activation, the increases in intracellular Ca2+, and altered states of protein kinases or phosphatases (Huang et al., 2001).

2.2.1.2 Long-Term Depression

LTD implements use-dependent, homosynaptic and heterosynaptic weakening and therefore may mediate response depression to deprived inputs. Multiple forms of LTD exist and may have different roles in plasticity (for review see Feldman, 2009). In NMDA receptor-dependent LTD (NMDA-LTD), calcium from postsynaptic NMDA receptors activates protein phosphatises, leading to dephosphorylation of specific sites on the AMPA receptor GluR1 subunit and internalization of synaptic AMPA receptors. In the cortex, NMDA-LTD (defined as NMDA receptor involvement and AMPA receptor internalization) has been clearly observed widely (Feldman, 2009). A second major form is metabotropic glutamate receptor-dependent LTD (mGluR-LTD), of which several subforms exist (Egger et al., 1999, Renger et al., 2002). A third form of LTD involves cannabinoid type 1 (CB1) receptors (Chevaleyre et al., 2006). CB1-LTD is independent of post-synaptic NMDA receptors but may require presynaptic NMDA receptors, is able to drive a long-lasting decrease in release probability (Chevaleyre et al., 2006) and occurs at many neocortical excitatory synapses (Feldman, 2009).

2.2.1.3 Spike Timing-Dependent Plasticity

In the past decade, a dizzying variety of LTP/LTD learning rules have been discovered that vary with cell type, synapse location on dendrites, background network activity, and neuromodulation (Bliss and Gardner-Medwin, 1973; Shors et al., 1997; Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997; Huber et al., 2008; Feldman, 2009). Which learning rules are most relevant in vivo, and which spike train patterns or other aspects of neural activity trigger experience-dependent plasticity in vivo, remain largely unknown (Feldman, 2009). One learning rule that appears to mediate some types of experience-dependent plasticity in vivo is spike timing-dependent plasticity (STDP), in which the temporal sequence and interval between pre and postsynaptic spikes drive plasticity. In classical STDP, pre-leading post-firing (0-20 ms interval) drives LTP, and post-leading-pre firing (0 to 20-50 ms interval) drives LTD. Although STDP rules vary considerably across synapses and physiological states, STDP occurs at many neocortical synapses in vitro and can be induced experimentally in vivo by pairing sensory stimulation with precisely timed spikes (Meliza and Dan, 2006; Jacob et al., 2007). STDP mechanisms are surprisingly diverse, involving NMDA-LTP and NMDA-LTD at some synapses, NMDA-LTP and CB1-LTD at others and mGluR-LTD at others (Feldman, 2009).

2.2.1.4 Metaplasticity

To maintain mean cellular activity at a set point level there are slower, non-Hebbian form of plasticity that globally adjust synapse strength and neuronal excitability (Turrigiano and Nelson, 2004). Metaplasticity is the experience-dependent changes in synaptic plasticity rules themselves (Abraham and Bear, 1996; Ziemann and Siebner, 2008; Jung and Ziemann, 2009) as the ability of future stimuli to drive LTP and LTD is modified by experience dependent alterations in inhibitory tone, dendritic excitability, NMDA receptor function, or neuromodulation. This form of plasticity is homeostatic and was hypothesized to counteract the inherently unstable, positive-feedback nature of Hebbian synaptic plasticity (Bear et al., 1987). This homeostatic plasticity was discovered in cortical cultures in vitro, where experimentally increasing (or decreasing) network activity over hours to days causes a uniform, multiplicative decrease (or increase) in excitatory synapse strength, termed homeostatic synaptic scaling. Synaptic scaling is mediated by multiple cellular mechanisms that vary by cell type, time course, brain region, and developmental stage. In neocortex, scaling of excitatory synapses onto principal neurons is expressed primarily by regulating AMPA receptor insertion, similar to NMDA-LTP and LTD (Turrigiano and Nelson, 2004). How cellular or networks activity drives homeostatic plasticity is not clear. Recent studies suggest that glial cells, which detect mean local network activity, trigger one form of synaptic scaling in the hippocampus and visual cortex by secreting the cytokine tumor-necrosis factor-α (TNF-α) (Stellwagen and Malenka, 2006; Kaneko et al., 2008). Homeostatic plasticity occurs in vivo and may explain homeostatic changes in sensory responses with

substantial overuse or deprivation (Feldman, 2009). Intrinsic excitability and excitatory-inhibitory balance is also homeostatically regulated (Feldman, 2009).

2.2.1.5 Plasticity of GABAergic cell and circuits

Although most research has focused on excitatory synapses and circuits as loci for cortical plasticity, recent findings demonstrate that GABAergic inhibitory neurons and circuits play several important roles in sensory map plasticity because they are highly plastic (Feldman, 2009). A major discovery in recent decades was that maturation of specific GABAergic neurons my modulate plasticity editing: for example, pyramidal cell firing patterns to promote excitatory synaptic plasticity. Moreover both inhibitory synapses and excitatory synapses on inhibitory interneurons are capable of activity-dependent, long-term plasticity (Gaiarsa et al., 2002; Kullmann and Lamsa, 2007), which may directly contribute to the expression of receptive field plasticity in target neurons. Finally GABAergic circuits show strong homeostatic plasticity being able to modifying levels of GABA, GABA receptors, as well as to decrease or increase cortical activity in response to over or down-stimulation. These changes are appropriate to preserve both overall cortical activity and excitatory-inhibitory balance, which is tightly regulated to enable proper cortical function (Feldman, 2009).

2.2.2 Structural mechanisms for cortical plasticity

The possibility of determining changes in the synaptic wiring scheme of a neural network which is deleting and/or forming new synapses (Wolff et al., 1993) was first observed by lesion experiments in adult rat brains in the late 1960s (Raisman, 1969). It was then seen that structural plasticity offers pathways of rearranging connectivity that would not be possible by functional plasticity alone (Butz et al., 2009). The aspect that adds a fundamental new dimension to structural plasticity is synaptic rewiring: when a synapse is destroyed due to the loss of its pre - or postsynaptic element, synaptic rewiring can occur by joining the remaining synaptic counterpart with an unengaged synaptic element from another neuron (Butz et al., 2009). Thus, the formation of a synapse between two neurons by synaptic rewiring depends on the breaking of a synapse between another pair of neurons. Therefore, synaptic rewiring involves at least three neurons (Feldman, 2009). In contrast, functional plasticity typically involves just two neurons, since it depends only on the levels of activity in the pre- and postsynaptic cells of the synaptic connection. From a theoretical point of view, structural plasticity therefore allows more complex rules for changing connectivity than functional plasticity (Feldman, 2009). In experimental studies synaptic rewiring is particularly relevant in the context of adult neurogenesis (Butz et al., 2006), since young neurons have to become imbedded into pre-existing networks by the formation of new synapses, that can subsequently be strengthened or weakened by means

of functional plasticity. It is likely that the imbedding of young neurons depends on the synaptic availability of pre-existing neurons in a supply-and-demand fashion (Toni et al., 1999). Synaptic rewiring may therefore substantially contribute to the correct synaptic integration of new cells (Butz et al., 2008). Moreover, synaptic rewiring can result from retraction and reformation of dendritic spines (Trachtenberg et al., 2002) and re-routing of axonal branches within cortical columns (De Paola et al., 2006). Even entirely new anatomic connections can be settled under certain circumstances, as can happen in the recovery after stroke (Nudo, 2007). Intriguingly it can be shown that synaptic rewiring contributes to homeostasis of neuronal and network activity (Butz et al., 2006; Helias et al., 2008; Butz et al., 2009). It should also be noted that chemical neurotransmitters have a strategic role in this frame both as neurotrophic factors and mediators of the bioelectrical activity, initiating or suppressing axonal and dendritic outgrowth (Wolff et al., 1993), spinogenesis and synaptogenesis (Toni et al., 1999).

Even though in vivo assessment of structural plasticity associated with physiological or pathological conditions still requires highly sophisticated experimental approaches and direct observations are usually limited to single synapses or parts of single neurons such as dendritic or axonal branches, it is clear that this way could lead to the key for understanding the role of adult neurogenesis in cortical plasticity. But, there remains a gap between in vivo observations at the single cell level and studies revealing structural plasticity at the network level. This gap might be bridged by novel computational modelling approaches for structural plasticity that implement rules for structural modifications at the cell level and then compute the network dynamics over time (Butz and Teuchert-Noodt, 2006). Clearly not only for experimentalists, but also for theoreticians, structural plasticity raises a wealth of interesting issues, and also offers potential computational properties that can be tested experimentally (Butz et al., 2006; Butz et al., 2009).

Taken together, the data of this paragraph clearly suggest that the structural plasticity concept strongly implies the anatomical brain connectivity concept.

2.3 PRINCIPLES OF BRAIN CONNECTIVITY

When applied to the brain, the term connectivity alludes to many different but interrelated aspects of brain organization (Horwitz, 2003); two potential principles behind it are segregation and integration (Tononi et al., 1994). Segregation concerns the existence of specialized neurons organized into distinct neuronal assemblies and grouped together to form segregated cortical areas. The complementary principle, integration, refers to the coordinated activation of distributed neuronal assemblies, enabling the development of coherent cognitive and behavioural states. The interplay of segregation and integration in brain networks generates information that is concurrently highly diversified and highly integrated, thus creating patterns of high complexity (Sporns et al., 2004; Sporns, 2011).

In general terms connectivity can be studied on many spatial and temporal scales as well as at several levels of complexity. In space, this scale ranges from the axonal wiring

within and between layers of cortex to the connections between remote brain regions based on large fibres or functional coupling (Sporns et al., 2004; Sporns, 2011). In time, it spreads from the functional connectivity based on the existence of active synapses with an instantaneous spiking pattern, to the genetically outlined macroscopic structure of white matter connections throughout the brain, which can be changed on a time-scale of generations (Friston et al., 2003). In complexity these levels include individual synaptic connections that link single neurons at the microscale, networks connecting neuronal assemblies in local circuits (i.e. cortical columns) at the mesoscale, as well as large scale inter-regional brain connections or functional coupling at the macroscale. The accessibility of measurements of connectivity on these different scales is driven by the available measurements (electrophysiological, metabolic or structural imaging) and analysis methods. Today, these measurements and methods still do not provide comprehensive information but at least allow for some conclusions and may be sufficient to speculate about specific features (Friston et al., 2003). On this basis mainly three different types of connectivity can be recognized: anatomical, functional, and effective connectivity.

Anatomical connectivity refers to a network of synaptic connections linking sets of neurons or neuronal elements, as well as their associated structural biophysical attributes condensed in parameters such as synaptic strength or effectiveness (Sporns et al., 2004; Sporns, 2011). As the physical substrate at which all neural information processes happen, it defines the 'functional connectivity space' thus providing plausible biological boundaries for theories and inferences about neural interactions when analysing functional neuroimaging data and developing computer simulations (Lee et al., 2003). That is, because the structural/anatomical input/output connections of a given brain region are the main constraints for its functional properties, the structural brain connectivity does not rigidly determine neural interactions but acts as a dynamic support that reduces the dimensionality of the neural state space. Meanwhile, functional interactions (increasing or decreasing) contribute to modify the underlying structural/anatomical substrate by modifying the synaptic connections (enlarging, forming new synapses, pruning pre-existing synapses. In highly evolved nervous systems, as the human one, within this reduced low dimensional space, neural dynamics remain fluid, variable and sensitive to dynamic perturbations (Sporns, 2011): the concept of *human connectome* is growing up in strict analogy with the genome one. In the human brain the connectome concept is strongly based on the evidence that neuronal populations interact to each other by means of their connections as well as of their temporal related dynamics. This is particularly evident when considering the innumerable brain dynamic states, which vary instantly and continuously on the basis of changing sensory inputs from the internal and external environment (Sporns, 2011).

According to the principles of segregation and integration (Tononi et al., 1994), in human nervous systems, brain anatomical connections are both specific and variable. Specificity is found in the arrangement of individual synaptic connections between morphologically and physiologically different neuronal types, in the organization of axonal arborisations and long-range connectivity between separate cell nuclei or brain regions (Sporns et al., 2004; Sporns, 2011). Variability is found in the configuration of processes of

single neurons, as well as in the size, placement and interconnection of large-scale structures; variability can be evaluated between corresponding structures in brains of individuals of the same species. Moreover, neural structures within the same individual vary across time, as a result of experience, development, aging and so on. It may seem obvious that the function of a network is critically dependent on the pattern of its interconnections and that anatomical variability is one of the main sources for functional variability, expressed in neural dynamics and behavioural performance (Sporns et al., 2004; Sporns, 2011). However comprehensive data of anatomical connectivity is still scarce and difficult to obtain. Nowadays achieving a detailed map of all the neurons and their interconnections in a mammalian brain is simply out of technological reach and only invasive tracing studies are capable of unanimously demonstrating direct axonal connections. By contrast, diffusion weighted imaging techniques, such as Diffusion Tensor magnetic Imaging (DTI), have an inadequate spatial resolution, being difficult to discriminate between crossing and closely running parallel fibres, but are valuable as whole brain in vivo markers of temporal changes in fibre tracts anatomy and help to obtain an initial 'draft' of the human large-scale anatomical connectivity (Sporns et al., 2004; Sporns, 2011).

When evaluating functional integration in neuronal systems, functional connectivity and effective connectivity have to be taken in account. While functional connectivity only describes statistical dependencies between spatially segregated neuronal events, effective connectivity rests on a mechanistic model of how the data were caused. In this frame, functional connectivity is defined as the 'temporal correlations between spatially remote neurophysiological events' (Friston et al., 2003), whereas effective connectivity is defined as 'the influence that one neural system exerts over another either directly or indirectly' (Friston et al., 2003) and requires a causal or a-causal model, in which regions and connections of interest are specified and often constrained by a combination of neuroanatomical, neuropsychological, and functional neuroimaging data (Lee et al., 2003).

Functional connectivity mainly represents a statistical concept being based upon the patterned deviation from the statistical independence between distributed neuronal units, often spatially remote each other. Eventual statistical interdependence can be measured on the basis of correlation or covariance or phase-locking. Differently from the anatomical connectivity, this type is strictly time-dependent. Physiological recordings with multiple electrodes have in fact revealed that distant neurons can synchronize and neuroimaging studies have extensively reported the co-activation of distant brain regions under different experimental conditions, setting the foundations for novel approaches to understand the brain. That is networks of segregated but interacting processes govern neural dynamics on top of the processing of the specialized regions (Tononi et al., 1994). It should be noted that functional connectivity does not make any explicit reference to specific directional effects or to an underlying structural model (Sporns et al., 2004; Sporns, 2011) and is often calculated between all elements of a system, regardless of whether these elements are connected by direct structural links.

Finally effective connectivity represent a liaison between the previous two types and described the causative interrelations between two neuronal systems (Sporns et al., 2004; Sporns, 2011). Several techniques have been used so far to study effective connectivity: between them functional Magnetic Resonance Imaging (fMRI), electroencephalography (EEG), Transcranial Magnetic Stimulation (TMS) and Magnetoencephalography (MEG) are the most widely used. Because temporal correlations between neurophysiological events in distinct neural systems (functional connectivity) may or may not be due to the effect that one neural system exercises over another (effective connectivity), two possible methods of inferring effective connectivity can be considered (temporal precedence and perturbation studies; for review see Lee et al., 2003).

The first method, according to the 'temporal precedence' principle, considers that: if activity in area 1 occurs prior to activity in area 2, then activity in 1 might cause activity in 2 through connections between the two areas. However, in the context of fMRI, temporal precedence at the neuronal level may be covered at the haemodynamic level, because of the temporal smoothing inherent in the coupling between synaptic activity and haemodynamic changes. Even in extreme cases, one region may cause activity in another and, because of different latencies the haemodynamic response in the 'target' region may appear before the haemodynamic response in the 'source' region. Even EEG and MEG studies, which provide a temporal resolution in the order of milliseconds, may not provide a totally adequate solution since temporal precedence is not unequivocally linked to causality. However the time-series causality analysis methods, such as Granger causality or transfer entropy, to these kinds of data seem to resolve this point. Granger causality, for example, has been used both to EEG as well as fMRI time series and has given clear information about directed interactions between neural networks in the course of behavioural and cognitive tasks. The covariance modelling has permitted the recognition of substantial differences in effective connectivity between a given set of brain regions in different cognitive tasks (Brovelli et al., 2004).

The other way of inferring causality encompasses the principle of perturbing the system; the majority of functional neuroimaging experiments involve experimental manipulations of neural activation (i.e., perturbations), in the form of stimuli, be they visual, auditory, psychological, and so on. There are two important benefits of using perturbation studies; the first is that the manipulation is under unequivocal experimental control and can therefore be located exactly in time and space; the second is that it is possible to selectively modify neural activity in definite cortical areas and to evaluate the effect that this modification has on the activity and interactions between the perturbed and non-perturbed areas (for review see Lee et al., 2003). In this frame of particular interest is the combination of TMS with functional neuroimaging (Siebner et al., 2009; Ziemann, 2011). Some are the advantages of such a combination: firstly, TMS permits the use of local perturbations of brain networks while they are involved in the performance of specific tasks; secondly as TMS is independent of behaviour, observed variations in neural activity are not mystified by the subjects' capability to perform a task or by the strategies employed, finally and perhaps of more interest, the combination can be used to change the activity of

one brain area and observe the effects on activity in other areas, either in reaction to further TMS or during behavioural paradigms.

Even though this kind of stimulation is evidently artificial and its effects might never occur naturally, TMS however likely active and then explore existing anatomical circuits, although their physiological activity -if any- is uncertain. Between the functional neuroimaging high-density EEG is important particularly for its millisecond-scale temporal resolution compared to other TMS-imaging approaches. Its high temporal resolution makes the EEG method a perfect complement to the transient perturbations caused by TMS in the brain's processing modes. TMS-EEG carries precise information about the temporal order of activations of distant cortical areas with high signal-to-noise ratio and relatively low cost (see also chapter 2.5.3).

2.4 TOWARDS AN INTEGRATED MODEL OF CORTICAL EXCITABILITY, CONNECTIVITY AND PLASTICITY

There is increasing evidence supporting the notion that brain plasticity consists of distinct functional and structural components, each involving multiple cellular mechanisms working at distinct synaptic loci, time scales and developmental stages in a frame of complexity which presents a tremendous scientific challenge (Feldman, 2009). Available data have begun to identify mechanisms that mediate both components: a common theme is that rapid, homosynaptic, functional components of plasticity involve Hebbian LTP and LTD at cortical synapses, whereas slower, homeostatic or competitive functional components of plasticity are likely to involve homeostatic cellular mechanisms and metaplasticity. In addition to these functional components, rapid experience-dependent structural components play a major role in plasticity of spines and synapses (Feldman, 2009). However, the exact relationship between these functional and structural components is unknown. For example it is not clear which specific functional components of plasticity, if any, mediate which types or sites of rapid structural components of plasticity, or whether chemical signalling is a proximal driver of structural plasticity (Feldman, 2009). Moreover whether novel network dynamics can be obtained from structural plasticity that cannot also be achieved from functional plasticity alone, is also unknown (Feldman, 2009).

Because spine plasticity can accompany experimentally-induced LTP and LTD (Alvarez and Sabatini, 2007), one partial model proposes that experience first induces the formation of new synapses, which then become substrates for the functional selection by LTP and LTD in response to subsequent experience (Alvarez and Sabatini, 2007). An alternative view is that activity rapidly regulates existing synapse strength via LTP and LTD, leading to formation and removal of spines and synapses that effectively rewire cortical microcircuits; in turn, this rewiring may lead to slower macroscopic changes in axons and dendrites (Cline and Haas, 2008).

In conclusion, clarifying the relationship between the functional and the structural components of brain plasticity represents a significant challenge (Tononi et al., 1994; Sporns

et al., 2004; Sporns, 2011) of the modern Neuroscience. In the past 10 years these concepts have also been introduced in clinical settings by means of sensitive physiological and anatomical techniques that have revealed a plasticity driven by many physiological and pathological conditions, as well as many similarities and differences across cortical areas and even human subjects.

It has recently been hypothesized that the increase in cortical networks excitability (Polania et al., 2010; Polania et al., 2011), as seen in several physiological and neuropsychiatric pathological conditions, most of all in M1 by means of TMS (**Ferreri et al., 2003; Ferreri et al., 2006**), could favour the strengthening of existing synapses in a Hebbian sense (Martin et al., 2000; Lisman et al., 2003) and drive axonal outgrowth. Structural plasticity might then hardwire functional changes when new synapses are formed in close apposition to existing synapses enhanced by LTP (Toni et al., 1999), or when synapses are deleted in association with LTD (Becker et al., 2008).

A recent technical advancement allowing the combination of TMS with simultaneous EEG provides us with the possibility to directly and non-invasively probe the brain's instantaneous state, excitability and time-resolved connectivity. Since TMS-EEG can stimulate and record from the cerebral cortex by-passing sensory pathways, sub-cortical structures and motor pathways, the measurement does not strictly depend on the integrity and status of sensory and motor systems and can be applied to any subject (e.g., de-afferentated, paralyzed, unconscious) in many circumstances. More generally, in a clinical context TMS-EEG can be used to prospectively track and monitor the excitability and connectivity changes occurring in any cortical region during rehabilitation, pharmacological therapy, TMS treatment, or spontaneous recovery.

2.5 BASIC PRINCIPLES OF TMS AND TMS-EEG CO-REGISTRATION

2.5.1 Transcranial Magnetic Stimulation

TMS is a non-invasive and painless method to stimulate excitable tissues with an electric current induced by an external time-varying magnetic field (Rossini et al., 1994; Kobayashi and Pascual-Leone, 2003; Rossini and Rossi, 2007). However, it has potential for more sophisticated uses when declined in different paradigms and applied together with several kinds of neuroimaging techniques, such as EEG or fMRI (Siebner et al., 2009; Ziemann, 2011). Both electric and magnetic stimulation methods excite the biological membranes with electric current: the former does it directly, the latter does it with the electric current which is induced within the volume conductor by a time-varying applied magnetic field.

After the revolutionary works of D'Arsonval (1896) and Thompson (1910), describing for the first time the possibility of stimulating the retina by means of magnetic fields, it took some time before the magnetic method was applied again. It was not until 1985, five years after the first presentation of the Transcranial Electrical Stimulation (TES) by Merton and

Morton (Merton and Morton, 1980), that Barker and colleagues presented the TMS at the London Congress of the International Federation of Clinical Neurophysiology (IFCN). They then demonstrated that the stimulation of the human motor cortex and peripheral nerves could be performed by means of a brief but strong, external time-varying magnetic field applied through a wire coil. After stimulation of the corticospinal tract, MEPs can be recorded by a surface electromyography from the connected muscles in an awake and collaborative subject without causing distress or pain (Rothwell, 1993; Rossini et al., 1994). Even though the magnetic pulses are considered to be able to stimulate neural tissues through the cortically-induced electric field depolarizing cell membranes (Barker et al., 1985), it is important to acknowledge that the actual pathways being investigated are not completely known. However, they incorporate the fastest conducting fibers which presumably include the pyramidal tracts (Rossini et al., 1987a; Rossini et al., 1987b).

The Maxwell's equations, formulated by James Clerk Maxwell (1865; 1873), describe the behaviour of time-varying magnetic fields. These equations summarize the mathematical consequences of the classical experiments of Faraday, Ampere, Coulomb, Maxwell, and others. The basic concept is the principle of mutual induction: electrical energy can be transformed into magnetic fields and magnetic fields can be transformed into electric energy. When an electric current is passed through a coil, a magnetic field is produced. If another coil, or length of conductive material such as a nerve fibre, is exposed to a changing magnetic field, a secondary electrical field is induced within the exposed coil or fibres. The intensity of the magnetic field can be represented by flux lines around the coil and is measured in Tesla (T). The magnetic field is oriented perpendicular to the stimulating coil and via currently commercially available devices reaches values around 2 T. The amount of the current induced depends on the strength and rate of change of the magnetic field and on the number of loops in the secondary coil or, in the case of TMS, the anatomical conformation of underlying nerve fibres. The neurons activated depend on the size, shape, orientation, and intensity of the stimulus waveform that is produced by the magnetic stimulator. Biphasic stimulus pulses are more efficient in stimulating the brain than mono-phasic pulses, even when the initial phase of the stimulus is the same size, because the charge transfer is maximal in the swing between the first and second phases of a biphasic pulse (Epstein and Davey, 2002). Macroscopically, the locus of activation in the brain seems to be where the induced field is maximal. Because the total induced electric field is strongest at the boundaries of any homogenous conductor compartment, the stimulating effect of TMS in the brain is thought to be focussed mainly at the cortical surface. Neurons with bent or curved axonal processes, passing at right angles to the lines of force of the magnetic field, are considered to be the most easily activated. However, the precise brain structures stimulated, and the resulting physiological effect of magnetic stimulation, cannot be inferred from the available mathematical models, because the head and brain are irregularly shaped, inhomogeneous volume conductors, and because the spatial orientation and anatomical configuration of underlying nerve fibres are variable (Epstein and Davey, 2002). Moreover the electric field induced in the tissue causes the cell membranes to either depolarize or hyperpolarize; if the depolarization of the membrane overcomes its threshold, an action potential is generated. The classical assumption is that the activation of pyramidal neurons by TMS occurs predominantly via inter-neurons in

superficial cortical layers (Di Lazzaro et al., 1999). Hence when applied over M1 at stimulus intensities currently used in commercial available devices, single-pulse TMS is thought to stimulate the corticospinal tract indirectly (trans-synaptically) through horizontal fiber depolarization.

Since the publication of pioneering works on motor conduction (Rossini et al., 1987a; Rossini et al., 1987b; Rothwell et al., 1987; Hallett, 2011), several other application areas of TMS have emerged (Ziemann, 2004; Hallett, 2011), the main technological innovations of the last few years being the repetitive stimulators and the coils for focal stimulation (Hasey, 1999). In fact a train of pulses seems to be able to produce deeper changes in neuronal activity than those produced by a single pulse. In particular repetitive TMS (rTMS) induces longer-lasting effects which continue past the initial period of stimulation, increasing or decreasing the excitability of the corticospinal tract depending on the intensity of stimulation, coil orientation and frequency. The mechanism of these effects is not clear but it is widely believed to reflect modifications in synaptic efficacy akin to LTP and LTD (Fitzgerald et al., 2006; Esser et al., 2006).

On the other hand relatively focal cortical activation is achieved by using a figure-of-eight coil or a double-cone coil with two loops, in which the current flows in opposite directions. The induced electric field peaks at the intersection of the coil windings (virtual stimulating cathode). Such coils generate a much more focused magnetic field than the original round or teardrop-shaped coils. This permits greater stimulation precision, as a smaller area of the cortex is exposed to the most intense part of the magnetic field. Commercially available devices can produce magnetic fields of intensity greatly exceeding the threshold for the depolarization of neurons, at least in the motor cortex, and improvements in coil design permit focal stimulation of small regions of cerebral cortex. Advances in coil design have led to stimulators capable of activating a small enough region of the cortex to allow mapping of cortical functioning with a degree of accuracy resembling that achieved using direct electrode stimulation of the brain (Hasey, 1999).

Coupled with other devices, such as the electromyography, and declined in several paradigms, Barker's stimulator has then become an significant diagnostic tool in clinical neurology, as well as an investigational tool to study regional cortical involvement in processes as diverse as motor functioning (Hallett, 2000; Ziemann, 2004).

2.5.1.1 Magnetic Resonance Imaging-Guided Stereotactic TMS

As pointed out earlier, coil dimension, shape, stimulus intensity, coil position and orientation relative to the head determine the volume of brain tissue that can be electrically excited by transcranially delivered magnetic pulses. In theory, the reproducibility of phenomena following brain stimulation, both within and between subjects, depends on the capability to excite the same brain regions with the same intensity at different times (Ilmoniemi and Kičić, 2010). Precise targeting of definite brain locations requires a known

system of correspondence between the location and orientation of the coil at the scalp, the stimulus intensity, and the brain volume that is stimulated. This is especially of concern when very focal stimulation is desired using small coils.

A simple way to specify the stimulated region is built on scalp anatomical landmarks. The standardized 10-20 EEG electrode placement system has been widely used in reporting coil position. There are some known correspondences between these locations and underlying cerebral structures (Homan et al., 1987). However, these correspondences are somewhat inconstant due to inter-subject anatomical variability. In fact, individual variability in brain anatomy and the functional reorganization that often accompanies brain pathologies, add imprecision to navigational systems based on scalp landmarks.

Magnetic Resonance Imaging (MRI) based stereotactic navigational techniques have been developed by several laboratories in recent years. The level of accuracy afforded by these navigational techniques makes it feasible to evaluate TMS as a tool for the pre-surgical mapping of eloquent cortical areas (Krings et al., 2001). The navigational techniques essentially determine the transformation required between a coordinate system based on skull or scalp landmarks and one defined in terms of brain anatomy. This is typically based on MRI. Once this transformation is identified, one can compute and display the volume of brain tissue that would receive stimulation given the position and orientation of the coil relative to the head. This minimizes the uncertainty arising from the inconsistent relationship between scalp structures and underlying brain anatomy. A comparison between guided and unguided stimulation based on the measurement of MEPs demonstrated the marked superiority of guided stimulation in being able to revisit specific motor areas accurately (Gugino et al., 2001). Commercial products have recently become available for MRI-guided TMS.

2.5.2 Transcranial Magnetic Stimulation Paradigms

Nowadays TMS can be coupled with several advanced medical devices (Bestmann et al., 2008; Siebner et al., 2009; Ziemann, 2011 see also later) and applied in several paradigms. It can be delivered one stimulus at a time (single-pulse TMS), in pairs of stimuli separated by a variable interval (paired-pulse TMS; Kujirai et al., 1993; Tokimura et al., 2000; Classen et al., 2004), or in trains (rTMS; Hallett, 2000; Walsh and Cowey, 2000; Rossi and Rossini, 2004; Huang et al., 2005; Di Lazzaro et al., 2008; Ziemann et al., 2008). Only TMS paradigms used for the studies on which this thesis is based are reviewed here.

When applied on the scalp overlying the M1, single-pulse TMS produces a series of epidurally recordable corticospinal volleys, reflecting the trans-synaptic activation of superficial cortical neurons, whose temporal summation at the spinal motoneuron level elicits an MEP in contralateral target muscles (Di Lazzaro et al., 2001) consenting routine assessments of the excitability and conductivity of corticospinal motor pathways. The subjective M1 excitability threshold is defined -according to international standardized guidelines as the minimu stimulator's output able to elicit reproducible MEPs (at least 50 μV in amplitude) in at least 50% of 10 to 20 consecutive stimuli (Rossini et al., 1994). This

approach reflects either the excitability and the local density of a central core of excitatory interneurons and corticospinal neurons ('hot spot') or that of small and slow-propagating pyramidal neurons. It is therefore a good parameter to investigate the action of neuroactive drugs as well as the effects of several diseases involving regulatory mechanism of cortical excitability (Rossini et al., 1994; Hallett, 2000; Ziemann, 2004). This approach has been extensively used also in studying causal chronometry in brain-behaviour relations and in studying movement physiology in healthy subjects, in patients with neurological disorders, and in post-lesional follow-up studies of patients with plastic cerebral reorganization (Siebner and Rothwell, 2003; Rothwell, 2007). Single-pulse TMS can be used for the mapping of motor cortical outputs. Cortical mapping procedures with single TMS pulses focally applied on several scalp positions overlying the motor cortex take into account the number of cortical sites eliciting MEPs in a target muscle and its 'center of gravity' (CoG; Rossini et al., 1994). The location of the CoG of the cortical map corresponds to the scalp location at which the largest number of the most excitable corticospinal neurons can be stimulated. Therefore, changes in the CoG should indicate true changes in the topographical organization of motor cortex representations (**Ferreri et al., 2003; Ferreri et al., 2011b**). Other important measures in single-pulse TMS studies include MEP latency, MEP amplitudes (at rest and sometimes with facilitation), and MEP recruitment curves (Liepert et al., 1998). Moreover, all the measured parameters from one peripheral (limb) muscle can be measured in the potential *asymmetry index* when comparing right/left differences (see Rossini et al., 2003). These parameters are useful for tracking plastic changes originating from physiological manipulations, i.e., changing the sensory feedback or during motor learning or secondary to a disease process involving the motor system (Pascual-Leone et al., 1995). Each MEP recorded during a voluntary contraction of the target muscle is followed by a suppression of the electromyographic (EMG) activity; this phenomenon is called the cortical silent period (CSP) and indicates, after an early phase of spinal origin, the activation of inhibitory (mainly GABAB) cortical interneurons (Cantello et al., 1992; Siebner et al., 1998; Werhahn et al., 1999).

Furthermore in paired pulse techniques, TMS stimulation can be delivered to a single cortical target using the same coil, or to two different brain regions using two different coils. Paired pulse techniques can provide measures of intracortical facilitation and inhibition and be used to study cortico-cortical interactions. In the most classical paired pulse paradigm (Kujirai et al., 1993), it is possible to test this intracortical inhibitory/facilitatory balance by means of a sub-threshold conditioning stimulus (S1) followed by a supra-threshold test stimulus (S2). The test responses are inhibited at inter-stimulus intervals (ISIs) of 1-5 ms and are facilitated at ISIs of 8-15 ms; these phenomena are referred to as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). SICI and ICF are likely to be mediated by GABAA and glutamate, respectively (Kujirai et al., 1993; Ziemann, 2004). These measures are fairly symmetrical in the two hemispheres of healthy subjects and are highly reproducible in a test-retest paradigm in the same subject, as well as across different subjects, being only partly influenced by the experimental conditions (Shimizu et al., 1999; **Ferreri et al., 2006**). The effect of S1 on the size of control MEP is thought to originate at the cortical level (Shimizu et al., 1999). It is in fact known that a supra-threshold stimulus determines a corticospinal output leading to an MEP, while

a sub-threshold stimulus only excites local cortical inter-neurons (Di Lazzaro et al., 2002a). Thus, by combining a sub-threshold pulse with a supra-threshold pulse one can assess the effects of inter-neurons on cortical output (Ziemann et al., 1998).

Several other paired pulse paradigms were recently tuned up (Huber et al., 2008). For example, muscle responses recorded in hand muscles after TMS of M1 can be modulated (either inhibited or facilitated) by electrical conditioning stimulation of the peripheral nerve if the time interval between stimulation of the nerve and motor cortex is 2-8 ms longer than the time needed by the peripheral nerve afferent input to reach the cortex (Mariorenzi et al., 1991; Stefan et al., 2000; Tokimura et al., 2000). When this phenomenon refers to the inhibitory part of the curve, it is named short latency afferent inhibition (SAI), and, is thought to depend on neural interactions within the cerebral cortex (Tokimura et al., 2000; Di Lazzaro et al., 2004); the mechanism could involve either direct inhibition of motor cortex from fast conducting afferents, or withdrawal of tonic facilitation from other structures such as the thalamus. Its generation seems to depend on central cholinergic activity being abolished by scopolamine, a powerful muscarinic antagonist (Di Lazzaro et al., 2000).

2.5.3 EEG-TMS co-registration

Besides what has been briefly presented above, TMS has potentiality for far more sophisticated uses when applied together with different kinds of neuroimaging techniques (Siebner et al., 2009; Ziemann, 2011). For example, a promising tool has recently been introduced that permits the co-registration of the EEG activity -which has a temporal resolution of a few milliseconds- during TMS, thus providing the possibility to noninvasively probe the brain's cortical excitability and time-resolved connectivity (Ilmoniemi et al., 1997; Virtanen et al., 1999; Ilmoniemi and Kičić, 2010). This means that combined with EEG, TMS is emerging as a brain research method in which stimulation is navigated into a wanted brain area and the concurrently recorded scalp potentials are processed into source images of the TMS-evoked neuronal activation (Komssi et al., 2004).

The first published effort to measure TMS-evoked brain responses was made in 1989 by Cracco and co-workers (Cracco et al., 1989); in their setup, one scalp electrode was used to record responses to TMS at homologous contra-lateral cortical area to the stimulation site and it was possible to record cortico-cortically mediated activity with an onset latency of 9-12 ms (for a review see Komssi and Kähkönen, 2006). However, this approach at that time was considerably invalidated by serious technical limitations related to the beating down of a strong artefact on the recording system, known already from studies with electrical stimuli (Freeman, 1971). It was necessary to overcome this difficulty to allow multichannel EEG recordings concurrently with TMS. One method to suppress the stimulus artefact was to use a sample-and-hold circuit able to lock the EEG signal for several milliseconds immediately post-TMS, as previously suggested by electrical stimulation experiments (Freeman, 1971). This method, avoiding saturation of the recording amplifiers by the

magnetic stimuli, allowed for the first time the recording of multichannel EEG activity in response to TMS. With this type of amplifier realised by the BioMag Laboratory (Helsinki University Central Hospital, Finland), TMS-evoked brain responses were efficaciously measured in 1997 (Ilmoniemi et al., 1997; Virtanen et al., 1999). Small silver/silver-chloride pellet electrodes with a diameter of 3 mm were used, with no significant heating effects and a stimulation rate of one pulse per second (Roth et al., 1992). The spread of TMS-evoked brain activity was then followed between brain areas starting at a few milliseconds post-stimulus. This amplifier has subsequently been further developed into a commercially available product by Nexstim Ltd. (Helsinki, Finland). Lately, some novel TMS-compatible EEG amplifiers have been described: one with 64 channels, based on a sample-and-hold circuit, another with 128 channels, based on a slow-rate limiter and finally a TMS-compatible EEG apparatus that works in very high time-varying magnetic fields without saturation and does not make use of particular devices to pin the amplifier output to a constant level during and after stimulation (BrainAmp MRplus, BrainProducts GmbH, Munich, Germany; Bonato et al., 2006; **Ferreri et al., 2011a**).

Recently, TMS-EEG studies have started to define the nature of the TMS- evoked EEG responses in order to extend our understanding of the activation mechanisms of TMS; moreover they have confirmed the potentiality of TMS-EEG as a tool for basic neuro-physiological research and possibly for diagnostic purposes.

The electric currents induced in the brain by TMS can depolarize cell membranes so that voltage-sensitive ion channels are opened and action potentials are initiated. Subsequent synaptic activations are directly reflected in the EEG (Ilmoniemi et al., 1997), which records a linear projection of the postsynaptic current distribution on the lead fields of its measurement channels. EEG is not very sensitive to action potentials because of their symmetric current distribution and short duration, so it is believed that postsynaptic currents generate most of the EEG signals. If the conductivity structure of the head is taken into account, the EEG signals can be used to locate and quantify these synaptic current distributions and to make inferences on local excitability and area-to-area functional connectivity in the nervous system (Komssi et al., 2002; Komssi et al., 2004; Komssi et al., 2007; Massimini et al., 2005). The initial TMS-evoked response, although difficult to measure without artefact contamination, appears to result from the activation of the target area whereas later deflections are partially due to activity triggered by axonally-propagated signals. How the signals are transmitted depends strongly on the state of the firing of diffused neuromodulatory systems of the brain (Massimini et al., 2005; Kähkönen et al., 2001), and also on local activation at the time of stimulus delivery (Ilmoniemi and Kičić, 2010).

Understanding of the TMS-evoked activity that is elicited at sites distant from the TMS target can benefit from knowledge of the anatomical connectivity of the brain as seen by Diffusion Tensor Imaging (DTI) studies (Ilmoniemi and Kičić, 2010). In contrast to the high variability of MEPs, the TMS-evoked EEG averaged responses are generally highly reproducible, provided that the delivery and targeting of TMS is well controlled and stable from pulse to pulse and between experiments. Several components of the EEG response to single-pulse TMS in the motor cortex have been described (for a review see Komssi and

Kähkönen, 2006; Ilmoniemi and Kičić, 2010): in particular, single-pulse TMS is able to evoke EEG activity lasting up to 300 ms and composed at the vertex by a sequence of deflections of negative polarity peaking at approximately 7, 18, 44, 100, and 280 ms, alternating with positive polarity peaks at approximately 13, 30, 60 and 190 ms post-TMS (Ilmoniemi et al., 1997; Paus et al., 2001b; Komssi et al., 2002, Komssi et al., 2004, Komssi et al., 2007; Nikulin et al., 2003; Kähkönen et al., 2004, Kähkönen et al., 2005; Kähkönen and Wilenius, 2007; Bonato et al., 2006; Daskalakis et al., 2008; Farzan et al., 2009; Lioumis et al., 2009; **Ferreri et al., 2011a**).

However, these components are not an constant pattern since in addition to inter-individual differences, the responses depend on the exact coil location (Komssi et al., 2002) and orientation, on the state of the cortex (Nikulin et al., 2003) and on the vigilance of the subject (Massimini et al., 2005). In addition to standard evoked responses, TMS may also trigger oscillatory activity (Paus et al., 2001a; Fuggetta et al., 2005; Rosanova et al., 2009) or perturb ongoing rhythms (Rosanova et al., 2009), eliciting event-related synchronization (ERS) or desynchronization (ERD) for example (Pfurtscheller and Lopes da Silva, 1999) or more complicated phenomena (Ilmoniemi and Kičić, 2010).

2.5.3.1 Usefulness of TMS-EEG in studying cortical excitability, connectivity and plasticity

The standard purpose of a topographic plot of TMS-evoked EEG responses is to identify both local and distant effects of TMS, namely to measure both local excitability of the stimulated patch of the cortex and the spreading of TMS-evoked activity in a broader cortical network. Usually the overall response amplitudes are highest under the coil and diminish with increasing distance from the stimulation point. Locally, within one hemisphere, increased EEG activity can be seen in a number of neighbouring electrodes, suggesting the spread of TMS-evoked activity to anatomically interconnected cortical areas (Ilmoniemi et al., 1997; Paus et al., 2001a). An significant feature of TMS-evoked EEG topography is that even though only one cortical hemisphere is stimulated, bilateral EEG responses are evoked with different features. TMS-evoked activity spreads from the stimulation site ipsilaterally via association fibres and contralaterally via transcallosal fibers and to subcortical structures via projection fibres (Ilmoniemi et al., 1997; Komssi et al., 2002, Komssi et al., 2004; Ilmoniemi and Kičić, 2010; **Ferreri et al., 2011a**). Therefore, EEG as a measurement of cortical activity after the TMS pulse provides the opportunity to study cortico-cortical interactions -by applying TMS to one area and observing responses in remote, but interconnected areas- or, more generally to evaluate how the activity in one area affects the ongoing activity in other areas (Ilmoniemi and Kičić, 2010). Moreover in analogy with cortical studies (Krnjevic et al., 1966; Rosenthal et al., 1967), besides activating the large excitatory Betz cells which are found in abundance in the motor cortex, TMS also activates the inhibitory interneurons. Their post-synaptic effects are considered to be represented as the TMS-evoked N44 and N100 components (Nikulin et al., 2003; Bender et al., 2005; Bonato et al., 2006; **Ferreri et al., 2011a**), as it has been seen shown that inhibitory

processes in deeper cortical layers can produce preferentially surface-negative potentials (Caspers et al., 1980). An significant challenge in interpreting TMS results comes from the fact that the effects of brain stimulation spread from the target site ortho- and antidromically in the neuronal network (Ilmoniemi and Kičić, 2010) and likewise, the stimulated area is influenced by connected areas. Because area-to-area modulation is often inhibitory, corticospinal excitability (as reflected by the magnitude of the descending volley elicited by a given cortical stimulus) does not necessarily increase with the general level of cortical activity. Furthermore, virtual lesions (areas where normal operation is disrupted) are not restricted to the stimulated spot but are distributed along the neuronal network (Ilmoniemi and Kičić, 2010).

There is growing evidence, also from stimulation of cortical areas other than the M1, that the effect of TMS on the EEG response is not determined by the characteristics of the stimulus alone, but also definitively by the initial state of the activated brain region. This was preliminarily established by the analysis of the features of the EEG-based pre-stimulus spectrogram to test the hypothesis that fluctuations in neuronal activity have a functional significance and may account for the variability in neuronal or behavioural responses to physically identical stimuli, such as TMS (Ilmoniemi and Kičić, 2010). Besides the evaluation of the general state of the brain (Massimini et al., 2005; Kähkönen et al., 2001), concurrent TMS and EEG have the potential to offer insights into how brain areas interact during sensory processing (Bikmullina et al., 2009; **Ferreri et al submitted**), cognition (Bonnard et al., 2009) or motor control (Nikulin et al., 2003; Kičić et al., 2008; **Ferreri et al., 2011a**). Several recent findings open up promising opportunities to employ this technique to assess directly whether and where in the cortex LTP or LTD plasticity phenomena can be induced with several different paradigms (Esser et al., 2006). Moreover, based on the great interest in the functional role of oscillatory brain activity in specific frequency bands and in specific cortical areas (Rosanova et al., 2009), the use of TMS-EEG studies in this field is especially promising. Deeper comprehension of brain oscillations is expected from combining knowledge on how oscillatory activity in specific frequency bands is related to distinct functions (Komssi and Kähkönen, 2006) with data from TMS-EEG studies (for reviews, see Thut and Miniussi, 2009). Moreover the detection of the natural frequencies with TMS-EEG may also have diagnostic potential and clinical applications, as it opens up possibilities to map the natural frequency of different cortical areas in various neuropsychiatric conditions such as depression, schizophrenia, epilepsy, dementia or disorders of consciousness. Since natural frequencies reflect relevant circuit properties, TMS-evoked EEG may radically extend the window opened by peripherally evoked MEPs. Whereas TMS-MEP is restricted to motor areas, TMS-EEG can access any cortical region (primary and associative) in any category of patients, and may offer a straightforward and flexible way to identify and monitor the state of corticothalamic circuits.

3 Aims of the original studies on motor cortex excitability, connectivity and plasticity in healthy subjects and Alzheimer's disease patients

Although synaptic plasticity was first demonstrated in the hippocampus, it is now well known that the functional organization of the cerebral cortex is plastic; that is, changes in cortical organization take place throughout the life span in response to the several events that characterize experiences (Butler and Wolf, 2007). The potential for reorganization has been demonstrated in both sensory and motor areas of the adult cortex as a result of injury, pathological changes, manipulation of sensory experience or learning and the rules leading this cortical plasticity are progressively more understood (Buonomano and Merzenich, 1998). Alterations in cortical organization, i.e. plasticity, are known to come out through changes in synaptic excitability, efficacy and connectivity both on a microscopic and macroscopic scale (Butler and Wolf, 2007). In motor areas, the horizontal inter-neuron connections within M1 have been suggested as the candidate substrate for plasticity. These collateral connections connect neurons to form neuronal assemblies which create dynamic motor maps and have a capability for long-lasting synaptic change (for a review see Sanes and Donoghue, 2000). Plasticity in M1 has been revealed to be functionally very significant: it guarantees active reorganization of the brain, following brain damage, for example to balance for the function losses of brain networks (Nudo et al., 1996), and has attracted great attention especially in restorative neurology (Butefisch et al., 2006; Duffau 2006). Enlargement or shrinkage of muscle maps can be followed in acute or chronic deafferentations; in an acute experiment, such as with limb anaesthesia (Brasil-Neto et al., 1993; Rossini et al., 1996) or traumatic amputation (Pascual-Leone et al., 1996), as well as in chronic observations, such as after spinal cord lesions (Topka et al., 1991).

The active re-modelling and synaptic modification of M1 circuits also play a crucial role during motor learning and memory consolidation and it is possible to reveal cortical reorganization in, for example, subjects with highly specialized, over-learned skills such as professional musicians or blind persons proficient in Braille reading (Pascual-Leone et al., 1996).

A major challenge in neuroscience is to recognize certain patterns of neuronal activity underlying these plastic changes, disentangling them from the unrelated ongoing electromagnetic brain signals. This issue is further complicated by the fact that all the processes subtending plasticity undergo maturation in infancy, childhood, adolescence and adult aging with dramatic alterations produced by neurodegeneration in dementing disorders (Celesia et al., 1987). It was recently suggested that AD is a plasticity affecting disease from its earliest stages. There is increasing evidence that the pathogenesis and symptoms of AD reflect synaptic dysfunction in the presence of β-amyloid (Aβ) oligomers which depress glutamatergic neurotransmission inhibiting LTP (Klinkenberg et al., 2011) and enhancing LTD, thus leading to a diminished ability for synaptic plasticity (Kim and Linden, 2007). Synaptic plasticity depends largely on glutamate signalling through NMDA

receptors postsynaptic activation and a loss of glutamatergic cortical association fibres -correlating with the level of cognitive impairment- as well as a deficit in NMDA receptors-dependent neocortical plasticity have been found in patients with sporadic AD and in transgenic rodents (Lopes da Silva, 1991), strongly suggesting that an abnormal glutamatergic neurotransmission may intervene in causing AD neurodegeneration.

Much of the work on the basic mechanisms of plasticity in vivo in humans has been done with TMS experiments on the M1, in which the response to each stimulus is relatively easy to quantify through the use of the MEP. TMS provides reliable information about neuronal activity and excitability and can be used in different ways when studying neuroplasticity (Pascual-Leone et al., 1999; Hallett, 2001). For example to reveal plastic changes (**Ferreri et al., 2003; Ferreri et al., 2011b**), to propose mechanisms underlying plasticity changes (**Ferreri et al., 2003; Ferreri et al., 2006**), to modulate neuroplasticity -and to study this manipulation- enhancing or reducing it in order to influence and even guide behavioural changes (**Ferreri et al., 2006**). Recently, the possibility of combining TMS with various neuroimaging techniques has offered complementary information to findings of neuroplasticity, finally making it possible, to directly study the effective connectivity of the horizontal inter-neurons suggested as the candidate substrate for M1 plasticity (**Ferreri et al., 2011a and Ferreri et al submitted**).

The overall general purpose of the studies was to explore the feasibility of TMS and TMS-EEG co-registration for the evaluation of primary motor cortex (M1) excitability, connectivity and plasticity in healthy human subjects and Alzheimer's disease patients.

3.1 SPECIFIC AIMS OF STUDIES I-V

The specific aims of studies I-V were:

1) To test - via TMS - motor function and specific parameters of M1 physiology in patients with early Alzheimer's disease (AD).

2) To test - via ppTMS - the possibility of modulating the excitability of M1 in healthy and young subjects by means of acute external exposure to electromagnetic fields (EMFs).

3) To characterize - via EEG navigated-ppTMS co-registration - the neuronal circuits underlying M1 excitability and connectivity, and investigate whether EEG measures of SICI and ICF are related to the same mechanisms underlying EMG measures of SICI and ICF.

4) To compare - via TMS - M1 functionality in AD patients before and after long-term acetylcholinesterase inhibitors therapy (AChEIs) in order to monitor potential drug-related changes in cortical excitability and organization.

5) To characterize, in line with the few previous EEG-TMS studies focussed on the cortical integration of afferent information, the neuronal circuits underlying the SAI mechanism, particularly evaluating the modulatory influence of SAI on cortical oscillations of healthy human sensorimotor networks.

4 Subject, materials and methods

4.1 SUBJECTS

Altogether, 60 subjects who gave their informed consent participated in Studies I-V. They were distributed as follows: 31 healthy young subjects (**Studies II-III-V**), 13 healthy old control subjects (**Study I**), 16 Alzheimer's patients (**Studies I** and **IV**).

4.2 MATERIALS AND METHODS

4.2.1 Transcranial Magnetic Stimulation and recording systems (EMG and EEG)

4.2.1.1 Studies I and IV

In the **Study I** (**Ferreri et al., 2003**), which aimed to test the motor function and specific parameters of M1 physiology in patients with early Alzheimer's disease, a TMS mapping procedure was conducted in 16 AD patients (1 male, 15 females; age range 59-82 years; mean 75 ± 7 years) and 13 healthy volunteers (8 women, 5 men; age range 56-91 years; mean 72 ± 2 years). In **Study IV** (**Ferreri et al., 2011b**), which aimed to compare motor cortex functionality in AD patients before and after long-term AChEIs therapy, the same procedure was repeated after one year in a subgroup of 10 patients from the original group under therapy. TMS was conducted using MAGSTIM 200 equipment (Magstim Company Limited, Whitland, South West Wales) and an eight-shaped coil with an inner diameter of 70 mm for each wing. The stimulus repetition rate was 0.1 to 0.2 c/s with a stable intensity 10% above the resting motor threshold (rMT) defined according to international guidelines (Rossini et al., 1994), as the stimulator's output that was sufficient to elicit reproducible MEPs (at least 50 μV in amplitude) in at least 50% of 10 to 20 consecutive stimuli. To map the M1 representation on the scalp, the subjects wore a tight elastic cap depicting a grid of 49 numbered squares. The cap was taped in a stable position for scalp anatomical landmarks (nasion, inion, vertex, meatal foramina). Square 1 of the grid corresponded to the designated hotspot, defined as the point from which stimuli at the minimal excitability threshold of TMS triggered MEPs of maximal amplitude and minimal latency. The remaining squares were numbered in a spiral fashion and were tested in a random sequence. Four consecutive MEPs were gathered from each grid position. To maintain alertness, we frequently encouraged the subjects verbally to stay awake. A complete procedure was performed for each hemisphere with a total of 392 stimuli (4 stimuli for 49

positions for two hemiscalps), the duration of the entire session being approximately 90 minutes. The testing procedure was performed with the subject lying supine on a bed to facilitate complete muscular relaxation. Two muscles, namely the extensor digitorum communis (ECD) and abductor digiti minimi (ADM), which are known to share the same 'hotspot' and threshold (Rossini et al., 1994), were examined from each arm via Ag/AgCl disks filled with conductive jelly in a belly/tendon montage. Skin/electrode resistances were less than 10 kΩ. Signal recording was conducted using PHASIS equipment (4 channels; Esaote Biomedica, Genoa, Italy) via a 1 to 2000 Hz filter setting, and a poststimulus analysis time of 50 ms with a 5 kHz sampling rate. Amplitudes of MEPs were measured between the two major and stable peaks of opposite polarity.

4.2.1.2 Study II

In **Study II** (**Ferreri et al., 2006**), which aimed to test, via ppTMS, the possibility of modulating the excitability of M1 by means of external and acute exposure to the electromagnetic field, the paired-pulse procedure was performed on 15 healthy subjects (age range 20-36 years) three times during each session according to standardized methods (Kujirai et al., 1993), with the subject wearing ear plugs and lying supine on a bed to facilitate complete muscular relaxation. All subjects underwent two sessions, 1 week apart, according to a crossover double-blind paradigm. In one session, the signal was turned 'on' (EMF-on, real exposure), whereas in the other session, it was turned 'off' (EMF-off, sham exposure); the duration of exposure was 40 minutes in both cases. MEPs were recorded during motor cortex ppTMS before and immediately after exposure to the EMF (EMF-on or EMF-off), and also after a 1-hour interval, during which time the subject remained in the room quietly (Figure 1). MEPs were recorded after separate TMS of both hemispheres, always starting from the left one. Two magnetic stimulators were connected to a Bistim device (Magstim Company, Dyfed, United Kingdom) and to an eight-shaped coil with an inner diameter of 70 mm for each wing, which was used for stimulation. The centre of the contact between the two circles of the eight-shaped coil was placed over the hand representation area, precisely on the designated hotspot. The hotspot position was marked on the scalp to facilitate an exact repositioning of the coil during the entire experiment.

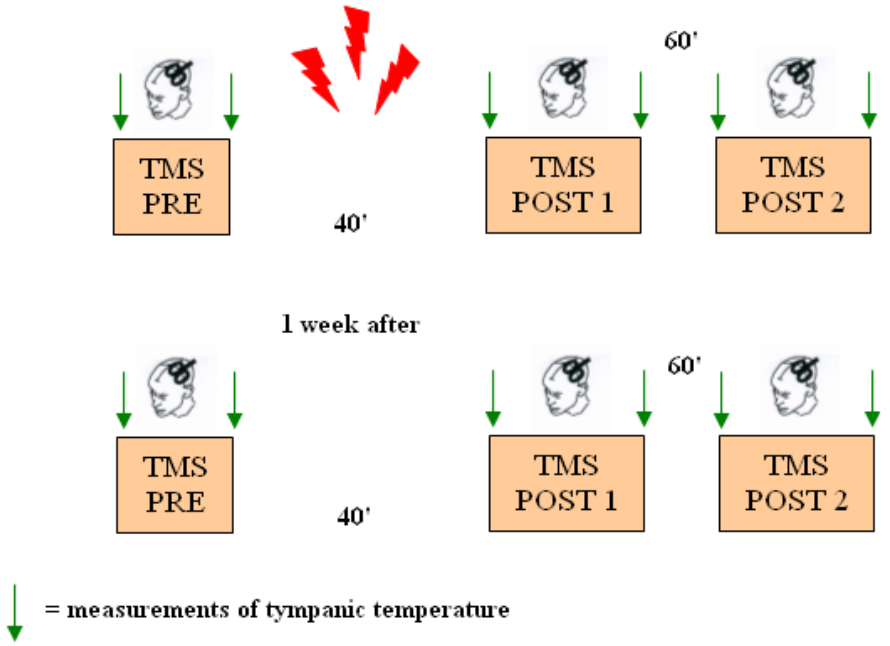

Figure 1. Schematic representation of the experimental design in **study II**. Arrows indicate the timing of tympanic temperature measures. TMS = transcranial magnetic stimulation.

Then the resting motor threshold was identified. Amplitudes of the 'test' MEPs were measured between the two major and stable peaks of opposite polarity and compared with those of a series of 'baseline,' unconditioned (i.e. not preceded by the conditioning stimulus) MEPs. Muscle twitches from either hand triggered by TMS were recorded from the first dorsal interosseus muscle via Ag/AgCl disks filled with conductive jelly in a belly/tendon montage. Skin/electrode resistances were less than 10 kΩ. Signal recording was conducted using PHASIS equipment (Esaote Biomedica, Florence, Italy; 4 channels) via a 1 to 2 kHz filter setting and a poststimulus analysis time of 50 ms with a 5 kHz sampling rate. The stimulus intensity for the first *conditioning* pulse was set at 80% of the resting motor threshold. The second *test* pulse was given suprathreshold with an intensity of 120% of the resting motor threshold. Interstimulus intervals (ISIs) of 1, 3, and 5 milliseconds were selected to test SICI (Sanger et al., 2001). Meanwhile, ISIs of 7, 9, 11, 13, 15, and 17 milliseconds were used to test ICF.

Up to a maximum of eight trials with paired TMS were recorded for each ISI during complete muscle relaxation, and the amplitude of the conditioned MEPs was expressed as the ratio of the control MEP elicited by the test stimulus alone. Finally, tympanic temperature was measured (Braun ThermoScan thermometer, Braun GmBH, Kronberg, Germany) before each of the three TMS recordings from both ears (see Figure 1).

4.2.1.3 Study III

The aim of **Study III** (**Ferreri et al., 2011a**) was to better characterize, via EEG navigated ppTMS co-registration, the neuronal circuits underlying M1 connectivity and the mechanisms regulating its balance between inhibition and facilitation. The paired pulse procedure (Kujirai et al., 1993: see above) was performed on 8 healthy young volunteers (age range 18-30 years) while TMS-compatible EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) was used to continuously record the EEG activity from 19 scalp sites using electrodes positioned according to the 10-20 International System (Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2). Additional electrodes were used as ground and reference and to record MEP. The ground electrode was positioned in Oz in order to have maximal distance from the stimulating coil. The linked mastoid served as the reference for all electrodes. The signal was bandpass filtered at 0.1-500 Hz and digitized at a sampling rate of 2.5 kHz. In order to minimize overheating of the electrodes by the stimulating coil, TMS-compatible Ag/AgCl-coated electrodes were used. Skin/electrode impedance was maintained below 5 kΩ.

Horizontal and vertical eye movements were detected by recording the electro-oculogram (EOG). The voltage between two electrodes located to the left and right of the external canthi recorded horizontal eye movements. The voltage between reference electrodes and electrodes located beneath the right eye recorded vertical eye movements and blinks. The epochs of the TMS-related scalp EEG responses were selected off-line, started 100 ms before and ended 1000 ms after TMS onset. Since this was a stereotactic TMS-EEG experiment, the coordinates of the head, the EEG electrodes, and the coil were determined and transformed to the same coordinate system with magnetic resonance (MR) images (Krings et al., 1997). In this way TMS was continuously targeted to the hot spot. The click associated with the coil's discharge propagates through air and bone, and can elicit an auditory N1-P2 complex at latencies of 100-200 ms (Nikouline et al., 1999). To mask coil-generated clicks, a white noise, obtained from the waveform of the TMS click which was digitized and processed to produce a continuous audio signal with specific time-varying frequencies (Massimini et al., 2005), was continuously delivered through earphones. We adjusted the masking volume until the subjects reported that the TMS click was not audible (always below 90 dB). To ensure wakefulness throughout the recording sessions, subjects were required to keep their eyes open and to fixate on a target on the opposite wall. Additionally, subject's lapses of attention were monitored with a signal detection task during the experiment (Conte et al., 2008; Chee et al., 2008). Brief tones were randomly played through the subject's headphones. These tones were infrequent compared with the number of TMS pulses delivered (from 3 to 6 tones during each block), and were played at irregular intervals. The subject was instructed to count the tones and report their numbers at the end of the block. At the end of the session the global counting error was always below 5%.

4.2.1.4 Study V

The aim of Study V (**Ferreri et al., submitted**) was to characterize, via EEG navigated-TMS co-registration, the neuronal circuits underlying the SAI phenomenon directly from the cortex and to investigate whether EEG measures of SAI are related to the same mechanisms underlying EMG measures of SAI. TMS was performed on 8 healthy young volunteers (age 24-45 years; mean 33 years; four men) with an eXimia stimulator and a biphasic figure-of-eight 70 mm coil combined with a navigation system that enables continuous visualization of the stimulation coil in relation to the individual cortical anatomical structures (Nexstim Ltd., Helsinki, Finland). Three-dimensional T1-weighted MR images (TR 1980 ms, TE 3.93 ms, FOV 256 mm, matrix 179 × 256, slice thickness 1 mm; Siemens Avanto 1.5 T, Erlangen, Germany) were used for the navigation. Three-dimensional scalp surface segmentation of high resolution Dicom MR images at the region of the motor knob was used as the mapping surface. The TMS system delivered trigger pulses that synchronized the TMS, EMG and EEG systems. During TMS, muscle activity was monitored on-line and recorded by continuous electromyography (ME 6000, Mega Electronics Ltd., Kuopio, Finland).

Disposable surface electrodes (circular, diameter 24 mm, Ag-AgCl) were positioned on the right opponent pollicis (OP) and referred to the 1st metacarpal bone in the metacarpophalangeal joint. The EMG signal was recorded at 1 kHz and filtered to a 8-500 Hz band for analysis, amplified and stored for off-line analyses. Median nerve stimulation was performed at the wrist with 0.1 ms electrical rectangular pulses (Digitimer model DS7A, Digitimer, Welwyn Garden City, Herts., UK) using a bipolar electrode. The EEG was recorded with a 60-channel TMS-compatible amplifier (Nextim Ltd., Helsinki, Finland) continuously throughout the experiments. In the EEG system, a sample-and-hold circuit was applied together with blocking of the amplifier input for 2 ms from the stimulus to avoid amplifier saturation. The data were recorded with a 1450 Hz sampling frequency and 16 bit precision. A trigger signal marking the exact stimulation moments was recorded with the EEG. All electrodes were referred to an electrode placed on the right mastoid. To mask coil-generated clicks, a white noise (see above Massimini et al., 2005), was continuously delivered through earphones.

4.2.2 Data Analysis

In each experiment, data analysis was guided and supervised by a biostatistician (PP) and/or a computer science engineer (DP) and/or a biophysicist (MK).

4.2.2.1 Studies I and IV

In **Studies I** and **IV** (respectively **Ferreri et al., 2003** and **Ferreri et al., 2011b**) the mapping procedure gave variables consisting of amplitude and latency values of each hemisphere for both muscles (ADM and ECD), resulting in four data columns. Amplitudes were $\log_e$-transformed to better approximate the Gaussian distribution. When the MEP was not measurable, $\log_e$ (amplitude) was arbitrarily set to 0 (corresponding to amplitude of 1 V). An initial data aggregation was made taking the arithmetic mean of the four repetitions for each scalp position (corresponding to the geometric mean of the untransformed amplitude values). New data files were created successively in which each subject was characterized for the following from each hemisphere: (1) area of muscle representation, i.e. the number of cortical sites from which it was possible to obtain a response in target muscle; (2) volume of active cortical sites, i.e. the sum of the averaged MEP $\log_e$ (amplitude) from each excitable cortical site for the target muscle; (3) excitability thresholds; (4) coordinates of the hotspots expressed in centimetres from Cz (x for the mediolateral axis, y for the anteroposterior axis); and (5) coordinates of the centre of gravity for the hotspots. The centre of gravity for the subject j was expressed as:

$$x_j = \frac{\sum_i x_{ji} \log_e(amp_{ji})}{\sum \log_e(amp_{ji})} \qquad y_j = \frac{\sum_i y_{ji} \log_e(amp_{ji})}{\sum \log_e(amp_{ji})}$$

Whenever the centre of gravity of a subject's motor area was exactly coincident with the hotspot, the coordinates were equal to (0;0). The y was considered positive for forward shifts, negative for backward shifts. The x was considered positive for lateral shifts, negative for medial shifts.

Because the motor area excitability could be measured by various parameters, a doubly multivariate analysis of variance (ANOVA) for repeated measures was chosen as the main statistical procedure. In particular, ADM and ECD areas, ADM and ECD volume and excitability threshold data were considered to be five aspects of the same neurophysiological feature. Hemisphere side (right, left) was entered as a within-subjects factor, whereas group allocation (AD or controls) was entered as a between-subjects factor.

The multivariate ANOVA enabled us to verify whether the two groups could be significantly discriminated in the five-dimensional space. To this end, we used Hotelling's T2, followed by univariate F-tests for the statistical evaluation of the single measures. In each experiment no effect of hemisphere was found for the centre of gravity, allowing us to collapse the two maps. Because the centre of gravity is defined by two coordinates, a multivariate ANOVA was used once again. In this case, Hotelling's T2 should be interpreted as the statistic for the bidimensional assessment of separation between AD and controls.

Throughout the statistical analysis, a *p* value less than 0.05 was considered significant. The software used was SPSS 10.0.7 for Windows (SPSS, Chicago, IL, USA).

4.2.2.2 Study II

In **Study II** (**Ferreri et al., 2006**), because both single and double pulse MEP amplitudes were approximately log-normally distributed, a log transformation was applied before any statistical procedure, allowing for the attainment of a relevant reduction of outliers, a better approximation to gaussianity, and a higher homoscedasticity. For each subject and experimental condition, the ratio between each log-transformed MEP to paired pulses and the mean of log-transformed MEPs to single pulses (with the intensity of the *test* one) was calculated. The distribution of these ratios was analyzed further, because ratios are usually not suitable for treatment by means of general linear models, such as analysis of variance (ANOVA). However, the coefficients of variation of ratios in each cell of the experimental design was low (mean 8%, median 6%) and tended to increase after applying common transformations (log, logit, power, arcsin). No evidence of other serious violations of ANOVA assumptions was found, and simple ratios were therefore the main dependent variable in the statistical analysis. The complete experimental design consisted of the following factors: ISI (9 levels: 1, 3, 5, 7, 9, 11, 13, 15, 17), STIM (type of EMF exposure, 2 levels: sham, real), SIDE (side of TMS stimulation, 2 levels: right, left), TIME (time of TMS stimulation, 3 levels: T0 = baseline, T1 = immediately after, T2 = after 1 hour). Therefore, the experimental design defined a data matrix of 9 x 2 x 2 x 3 = 108 cells. For an individual cell, a maximum of 8 MEPs were recorded for each of the 15 subjects. To take into account the within-subject correlation, we entered 'Subject' as an additional factor. More precisely, subject was entered as a random-effects factor. As is known, this approach is more conservative than a fixed-effects design and is geared toward making inferences about average characteristics of the population (from which our sample of subjects could be assumed to be drawn). Because this design involved four experimental factors (plus the random-effects factor of Subject), a multiway ANOVA was used to identify the significance of each source of variation (main and interactive terms). The working hypothesis of an effect across time (T1 and T2 vs T0) of real EMF (vs sham) on excitability of the ipsilateral (vs contralateral) motor cortex can be formalized statistically by the interaction TIME*STIM*SIDE. Therefore, this triple interaction became the effect of main interest.

Because this effect could vary according to ISI levels, four-way TIME*STIM*SIDE*ISI was also evaluated carefully. In addition, to specifically test whether excitability changes were different in the inhibitory versus facilitatory ranges, we derived a new factor (SICI-ICF) (with 2 levels: 1 = SICI, obtained by averaging 1-3 milliseconds; and 2 = ICF, obtained by averaging 9-13-millisecond ISIs) and entered in the above ANOVA model, replacing ISI with SICI-ICF (TIME*STIM*SIDE*SICI-ICF). Finally, with respect to tympanic temperature, a factorial ANOVA STIM (type of EMF exposure, two levels: sham, real), SIDE (side of TMS stimulation, two levels: right, left), TIME (time of TMS stimulation, three levels: T0 =

baseline, T1 = immediately after, T2 = after 1 hour) was conducted. Throughout the statistical analysis, *p* less than 0.05 were considered statistically significant. However, for each effect of interest, exact *p* values and 95% confidence intervals (CIs) were reported to provide more information on effect sizes.

4.2.2.3 Study III

In **Study III** (**Ferreri et al., 2011a**), data analysis was conducted using MATLAB 2010a version 7.10.0.499 (Math. Works, Natick, MA, USA) and the public license toolbox EEGLAB (Delorme and Makeig, 2004). To compare single trials with paired-pulse trials, in the latter all the signals were aligned to S2 by shifting back the traces by 3 ms or 11 ms respectively. All EEG-TMS evoked activities were visually inspected in each channel, and trials contaminated by environmental noise, muscle activity, or eye movement were rejected together with the corresponding MEPs. Thereafter, EEG signals were baseline corrected (100 ms prestimulus) and average referenced. Time domain was the main aspect of the TMS-evoked potentials investigated. To do this, the global-mean field power (GMFP) - a measure of global brain activation calculated as the root mean-squared value of the EEG signal across all electrodes - was first calculated to identify differences in TMS-evoked activity between the conditions (Lehmann and Skrandies, 1980). Then in each trial and each electrode semi-automatic amplitude/latency measurements of each component of the EEG evoked potentials and corresponding MEP was carried out. We chose not to consider averaged signals in order to have the best chances to investigate eventual correlations linking TMS-induced EEG deflections and MEPs amplitudes. The dataset suitable for statistical analysis was a matrix constituted by 8 (subjects) x 3 (conditions) x 9 (peaks) x 19 (electrodes) x 120 (stimuli) = 492,480 rows and 2 (EEG: latency and amplitude) + 4 (MEP: latency and amplitudes, contra-lateral and ipsilateral) = 6 columns. In addition, 4 subjects also underwent TMS at 80%, which allowed the computing of artificial 80%+120% EEG oscillations to be compared with ISI3 and ISI11 TMS-evoked waves. Therefore, the complete dataset originally comprised 738,720 rows. After cleaning the data, the available dataset consisted of ~600,000 rows. Besides raw EEG and MEP data, their TMS modulations were computed in the following way. MEP values were log-normally distributed and were therefore log-transformed $y=\log_e(MEP+1)$ to achieve a good approximation to gaussianity and to limit the potentially detrimental effect of right-skewed outliers. For each subject i, the average of about 100 single pulse TMS at 120% was computed ($mean_{i,120\%}$) and percentage variations was obtained from each MEP value according to the formula

$$\text{MEP\_modulation}_{i,j,k}\% = 100 \cdot \left[\frac{\log_e(MEP_{i,j,k}+1) - mean_{i,120\%}}{mean_{i,120\%}} \right]$$

where

i=subject number, ranging from 1-8,

j=TMS condition: 120%, ISI3, ISI11,

k=TMS stimulus, ranging 1-≅100

Similarly, for each subject, each latency peak and each electrode

$$\text{EEG\_modulation}_{i,j,l,m,k}\,\% = 100 \cdot \left[\frac{EEG_{i,j,l,m,k} - mean_{i,120\%,l,m}}{mean_{i,120\%,l,m}} \right]$$

where

i=subject number, ranging from 1-8,

j=TMS condition: 120%, ISI3, ISI11,

k=TMS stimulus, ranging from 1-100

l=latency peak, ranging from 1 (N7) to 9 (N280)

m=electrode (1=Fp1,…,19=O2).

It should be noted that EEG data were not previously log-transformed since negative values occurred even for 'positive' peaks. In order to avoid the influence of outliers, especially for the assessment of MEP-EEG correlations, percentiles were computed for each subject and values below the 3rd percentile or above the 97th percentile were discarded. After this procedure, approximately 100 stimuli x 8 subjects = 800 MEP modulations and 100 stimuli x 8 subjects x 9 peaks x 19 electrodes = 136,800 EEG modulations were obtained. The corresponding distributions are represented in Figure 2, B and D.

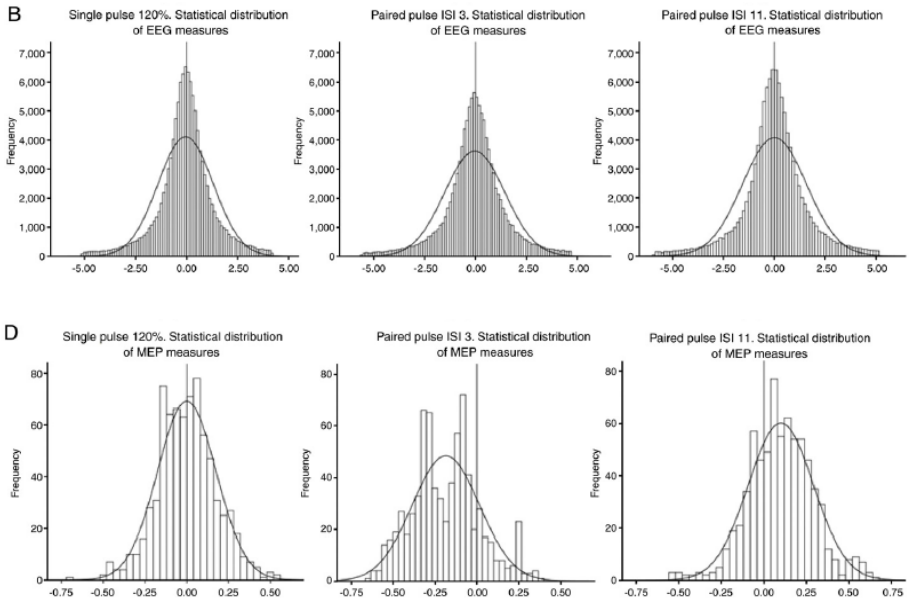

Figure 2 B and D. B: statistical distributions of EEG measures in the experimental conditions of **Study III**. D: statistical distributions of MEP measures in the experimental conditions.

The distributions of all EEG modulations, regardless of peaks and electrodes, are represented for the three TMS conditions (see Figure 2, B). The distributions are quite symmetric but clearly leptokurtic (values at the center and on the tails are more represented than in a Gaussian distribution). In MEP data (see Figure 2, D), the typical Gaussian distribution was obtained for single pulse TMS at 120% (confirmed by a very low Kolmogorov-Smirnov statistic=0.035) and at ISI 11, whereas a peculiar bimodal distribution was observed at ISI 3, probably due to a cluster of MEP amplitudes strongly reduced (or even fully suppressed) and to a cluster with MEP with a partial reduction (never involving the absence of the evoked potential). A more detailed description of the finding is given in the results section. The purpose of statistical analysis was manifold and will be presented in the results section. In order to link tightly the objectives and the statistical procedure, the following assessments were performed: (1) TMS effects (120%, ISI3 and ISI11) on MEP values (as confirmation of well-known literature data); (2) TMS effects (120%, ISI3 and ISI11) on EEG values; and (3) correlation between EEG modulation and MEP modulation.

To obtain the above assessments, a General Estimating Equation model (hereafter, GEE) was applied with Subject as repeated variable. To take into account the possible autocorrelation of the time series in each subject, the auto-regressive structure was chosen as the working correlation matrix. The details of each model were:

1) GEE with MEP as dependent variable and *Condition* as predictor (factor); Sidak's post-hoc procedure was used to assess the significance of the pairwise comparisons between the three factor levels (120%, ISI3, ISI11).

2) For each condition, (120%, ISI3, ISI11) and peak (N7, P13, N18, P30, N44, P60, N100, P190, N280), GEE with EEG amplitude as dependent variable and Electrode as factors. This procedure made it possible to obtain an estimate of the standardized effect size of each peak in each electrode, indicated by a t-statistic (adjusted according to Sidak's procedure). Whenever the t-statistic was between -2 and +2, the null hypothesis of peak absence could not be rejected. In addition, the lack of overlapping between Sidak's adjusted 95% confidence intervals was used as a conservative procedure to evaluate whether (a) a peak was more represented in some areas than in others, and (b) a peak was modulated by ISI. To limit the number of figures, the maps of differences between conditions (ISI) were not represented.

3) Possible correlations between EEG (independent) and MEP (dependent) were also initially assessed by means of GEE. However, we observed that such a procedure was reliable to address points (1) and (2), but not sufficiently robust to investigate point (3), given the limited number (N=8) of subjects. Therefore, sample correlation coefficients between EEG and MEP were computed for each subject, peak and electrode, allowing the examination of the repeatability of each correlation across subjects. Before averaging, correlation coefficients were transformed according to the Fisher transformation:

$$z = \sqrt{n-3}\,\frac{1}{2}\log\frac{1+r}{1-r}$$

4.2.2.4 Study V

In **Study V** (**Ferreri et al., submitted**) data analysis was conducted using MATLAB 2010a version 7.10.0.499 (Math. Works, Natick, MA, USA) and the public license toolbox EEGLAB (Delorme and Makeig, 2004). EEG data were divided into segments of 1000 ms including a 200 ms pre-stimulus baseline. Both in the TMS alone trials and in the SAI trials, all TMS-evoked EEG activity was visually inspected in each channel, and trials contaminated by environmental noise, muscle activity, or eye movement were rejected.

Following this procedure, EEG signals were filtered between 2 and 80 Hz, down sampled from 1450 Hz to 725 Hz, baseline corrected (100 ms prestimulus), average referenced and averaged for each subject. To examine responses in the **time domain**, total EEG activity was assessed using the global-mean field power. GMFP - a measure of global brain activation calculated as the root mean-squared value of the EEG signal across all electrodes - was first calculated to identify differences in TMS-evoked activity between the conditions (Lehmann and Skrandies, 1980). For the analysis of evoked responses, averaged TMS-EEG responses over all the included trials for each electrode and each subject were used, and semi-automatic amplitude/latency measurement of each component of the EEG evoked potentials was carried out. To examine responses in the **frequency domain**, event-related spectral perturbation (ERSP) and intertrial coherence (ITC) between 8 and 50 Hz

were investigated for each subject and for each channel. Event-related spectral perturbation measures the modulation of amplitude induced by a specific event (e.g., TMS pulse), relative to a baseline (e.g., prestimulus condition), whereas intertrial coherence provided a measure of the synchronization of the TMS-evoked potentials across different trials, independent of signal amplitude (Delorme and Makeig, 2004; Ferrarelli et al., 2008). The significance of the results was defined as $P < 0.05$. The following assessments were performed: (1) SAI effects on MEP data, (2) SAI effects on EEG data and (3) correlation between EEG modulation and MEP modulation.

1) The MEP amplitudes were analysed with Bonferroni corrected t-tests comparing MEPs without and with preceding median nerve stimulation (baseline vs. ISI N20+10; baseline vs. ISI N20+3; ISI N20+10 vs. ISI N20+3).

2) EEG data analyses were computed for ISI N20+3 and ISI N20+10 conditions, as we found (see below) that at ISI N20+10 does not produce SAI on MEP amplitudes (Figure 1) and it is therefore suitable to be used as a control condition for the ISI N20+3 (test condition). By using ISI N20+10 as a control condition instead of baseline condition, we wanted to eliminate potential confounding effects induced by the SEPs. TMS-evoked responses amplitude data were analyzed by means of General Estimating Equation model (hereafter, GEE) (**Ferreri et al., 2011a**) to detect significant global effects of condition. GEE (with Sidak's post-hoc procedure) was applied for four peaks (P30, N44, P60 and N100) with two factors: EEG electrodes (n=60) and conditions (2 levels: ISI N20+10 vs. ISI N20+3). This procedure allowed us to determine whether peaks were modulated by ISI. Only results indicating statistically significant between-condition differences are reported. Next, significant topographic differences in EEG data (P30, N44, P60 and N100 and ITC) were assessed by statistical nonparametric mapping (SnPM) (Nichols and Holmes, 2002). For ITC, in addition to the SnPM, two-tailed bootstrap statistics were applied

3) MEP amplitudes were correlated (Pearson, one-tailed) with EEG values. The amplitudes of the EEG responses and ITC and MEPs in ISI20+3 were expressed as a percentage of the corresponding values at ISI20+10.

5 RESULTS

5.1.1 Studies I and IV

Results in AD patients and control subjects from **Study I** (**Ferreri et al., 2003**) are summarised in Table I and Figure 3.

Table 1. Controls and Alzheimer's Disease in **Study I**

		Controls		Patients	
Measure	Hemisphere	Mean	SD	Mean	SD
Excitability threshold (%)[a]	Right	43.7	6.9	40.7	5.1
	Left	42.9	7.4	39.6	3.8
Area ADM (N)	Right	3.6	2.4	4.9	2.9
	Left	3.4	2.9	4.9	3.1
Area ECD (N)	Right	3.9	3.1	5.3	2.9
	Left	3.9	3.1	6.1	4.1
Volume ADM ($\log_e$ mV)	Right	16.4	10.3	25.6	14.6
	Left	14.9	12.9	26.5	18.0
Volume ECD ($\log_e$ mV)	Right	20.4	16.3	30.2	15.9
	Left	19.9	15.2	39.6	27.9

[a] *Percentages refer to stimulator's output at its maximum.*

ADM = abductor digiti minimi; ECD = extensor communis digitorum; N = number of excitable sites; SD = standard deviation.

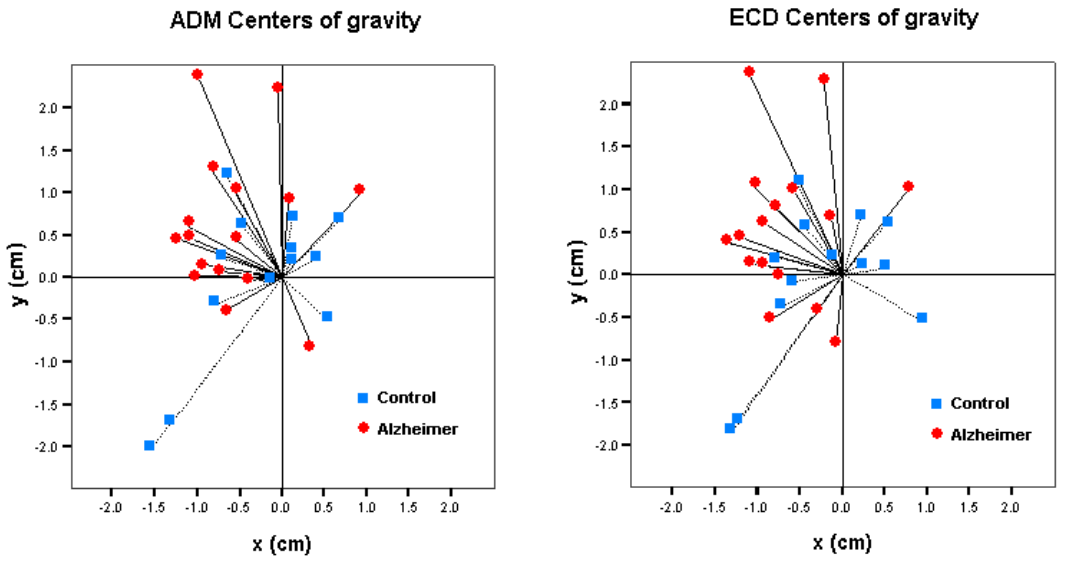

Figure 3, modified from **Study I.** Motor area coordinates for each subject, calculated on the basis of abductor digiti minimi (ADM) (left) and extensor communis digitorum (ECD) (right) output. (squares) Control subjects; (circles) AD patients. Because no interhemispheric asymmetry was found, measures refer to the 'average' hemisphere, although the right hemisphere has been chosen for the Cartesian representation.

No correlation between motor cortex excitability parameters and AD severity (as measured by Mini Mental State Examination (MMSE), Clinical Dementia Rating scale (CDR), Instrumental Activities of Daily Living (IADL)) was found. This might partly be ascribed to the clinical homogeneity of the patients and to the relatively low specificity of the scales. According to statistics, neither the effect of Hemisphere (Hotelling's trace=0.828; F(5,23)=1.599; p=0.200) nor the interaction Group X Hemisphere (Hotelling's trace=0.148; F(5,23)=0.683; p=0.641) were significant, indicating a global symmetry of the motor cortex parameters in the whole sample, without differences between controls and patients. In terms of specific parameters (univariate analysis), the p-value for Hemisphere effect ranged between 0.230 (Thresholds) and 0.909 (ECD Areas). In contrast, the two groups clearly differed, considering together the motor cortex measures (Hotelling's trace=0.828; F(5,23)=3.808; p=0.012), suggesting a global increased motor cortex excitability in AD patients vs. controls. Univariate analysis indicated that the differences were less evident for ADM (p=0.150 for Area, p=0.115 for Volume) and for Threshold (p=0.136), but more extensive for ECD measures (p=0.036 for Area, p=0.029 for Volume).

Table 2 shows the localizations of the hotspot in patients and controls. Statistical analysis did not reveal any inter-hemispheric asymmetry (Hotelling's trace=0.102; F(2,26)=1.327; p=0.283).

Table 2, from **Study I**. Hotspot localization in Cartesian Coordinates on the scalp in controls and Alzheimer's disease patients.

		Controls		Patients	
Location	Hemisphere	Mean	SD	Mean	SD
Medialateral	Right	2.8	1.3	2.5	1.1
axis *x* (cm)	Left	3.1	1.2	3.5	1.3
Anteroposterior	Right	2.3	1.2	2.3	1.3
axis *y* (cm)	Left	2.8	1.1	3.2	1.1

SD = standard deviation.

In addition, an overlap of the hotspot localization in the two groups was found (Hotelling's trace=0.063; F(2,26)=0.824; p=0.450). The centres of gravity of the cortical maps on the scalp are expressed in a Cartesian coordinate axis system. A global consideration of the two examined muscles makes it quite conceivable that in controls the 'hotspot' is coincident with the centre of gravity localized in the middle of the map (Figure 4) with a scalp distribution which is approximately symmetrical on the two hemispheres.

The distance between the hotspot and the map center of gravity can be measured. If the hotspot and centre of gravity coincide, a doubly symmetrical distribution (on both x- and y-axes) should be expected, such as in the case of a bi-variate Gaussian distribution.

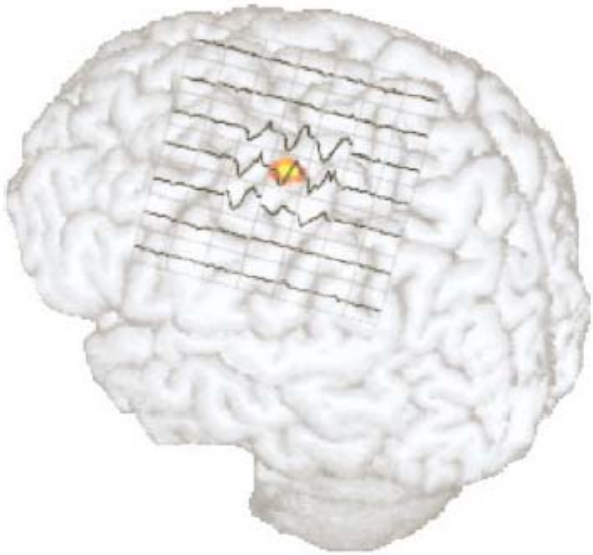

Figure 4, from **Study I**. Control. Superimposition of the grid of stimulation site on a paradigmatic brain model shown as a reference, which is just an approximation for the purpose of display. The area in color represents the hotspot (red) which is also coincident with the center of gravity (yellow).

According to ANOVA results, there was a slight non-significant medial asymmetry (i.e. toward the nasion-inion line) in controls, while in AD patients we observed a significant combined medial and frontal shift (Figure 5).

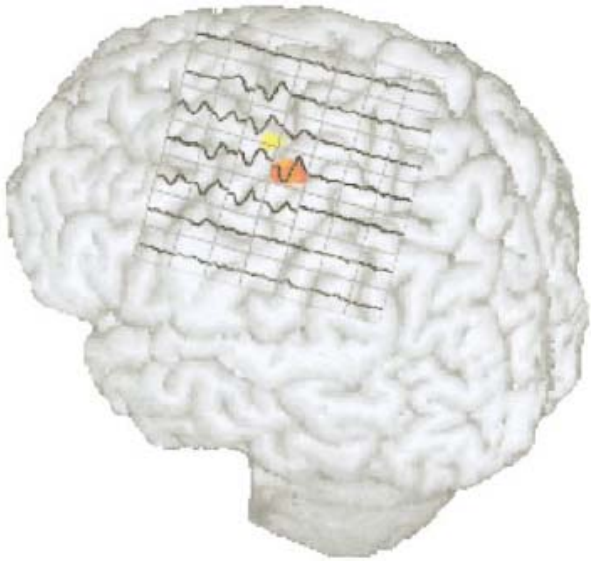

Figure 5, from **Study I.** AD patient. Organization as in Figure 2; notice that in this paradigmatic subject the hotspot (red) is not coincident with the center of gravity (yellow), which is shifted in a frontomedial direction.

The significance of the multivariate test (Hotelling's trace=0.303; F(2,26)=3.933; p=0.032) indicated a bi-dimensional separation between the two groups, although this is not evident when the results are examined in the two dimensions with a univariate type of analysis (p=0.171 for x-axis, p=0.059 for y-axis). The weight of the medial excitable sites was slightly heavier than the weight of the lateral sites, while the hotspot was statistically coincident with the centre of the map, in terms of antero-posterior representation, in the average healthy subject. In the average AD patient, in contrast, we observed a further shift towards medial sites, associated with a clear potentiation of the motor responses of frontal grid sites with respect to parietal ones (Figure 6).

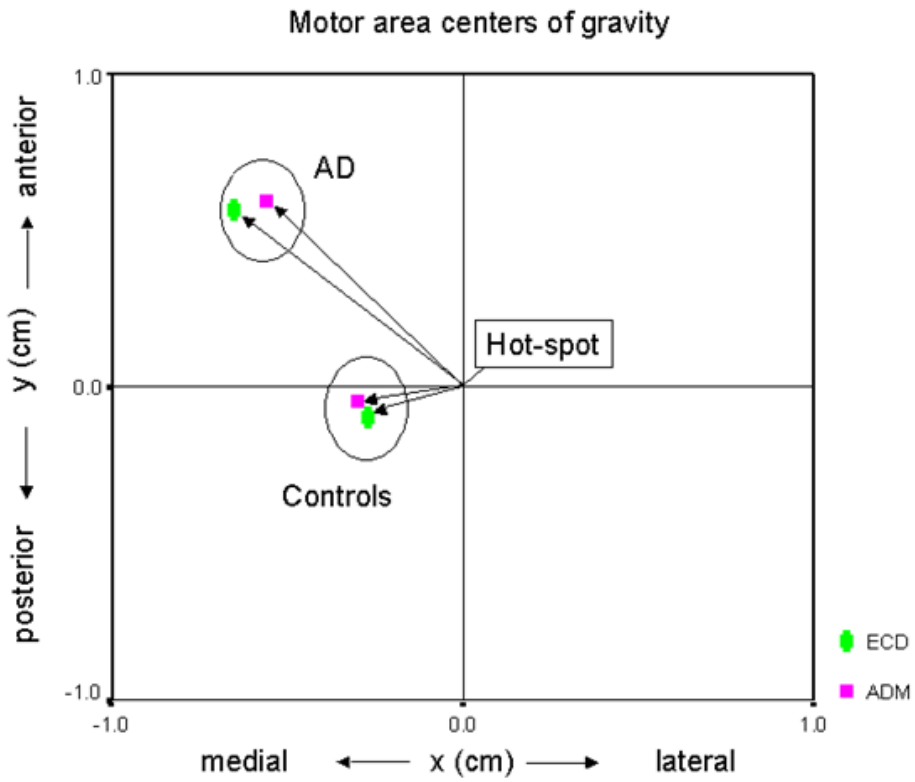

Figure 6, modified from **Study I.** Centers of gravity average coordinates of the abductor digiti minimi (ADM) (squares) and extensor communis digitorum (ECD) (circles) in the two groups. As shown, in controls there is a slight medial shift 'hotspot 3 center of gravity', whereas in AD patients there is a significant anteromedial asymmetry of the motor area. Because no interhemispheric asymmetry was found, measures refer to the 'average' hemisphere, although the right hemisphere was chosen for the Cartesian representation.

In **Study IV** (**Ferreri et al., 2011b**), as reported in the Subjects and Methods section the, 10 patients examined were a subset of the original group of 16 AD patients from the Study I (**Ferreri et al., 2003**). With such a small group, the statistical power of the study is low and we were able to detect as significant a 'large effect size' (Cohen's effect size convention) with a power as low as 0.62. Due to this limitation, this should be considered a pilot follow-up study, potentially useful for planning more statistically powerful studies. In accordance with the follow-up design of the study, the changes in the clinical and neuro-physiological parameters were evaluated by means of ANOVA for repeated measures with Time (T0, T1) as within-subject measures. In addition, since motor thresholds, areas and volumes of the examined muscles (ADM and ECD) were different but related measures of motor excitability, a global MANOVA for repeated measures with Hemisphere (right vs. left) and Time (pre- vs. post- AChEI therapy) was performed. On the basis of the expected minimum annual rate of decline of MMSE score, as estimated in a meta-analysis of 37 studies published between 1988 and 1997 (before widespread use of AChEIs), involving 3492 patients, most untreated AD patients lose at least 2.9 points (3.3 ± 0.4) on the MMSE per year (Han et al., 2000, Nobili et al., 2002). Thus, the expected minimum overall MMSE score change between T1 and T3 in our patients could be computed to be 2.9 x 2 years = 5.8, and if the MMSE score decreased less than this value, or if it remained stable or increased, the patients would be considered stabilized (Nobili et al., 2002). Although statistical significance was reached for the MMSE decrease ($F(2,18)=44.33$; $p<0.001$), our patients both as a group and as individual cases could be considered cognitively stabilized at T2 and at T3 and formed a homogeneous group. They lost on average 1.0 ± 0.7 MMSE points during the first year (no patient lost more than two points) and 1.3 ± 0.7 points during the second year of follow-up (again no patient lost more than two points). Over two years they lost on average 2.3 ± 0.95 points (no patient losing more than three points from examination at T1 to T3; Table 3).

Table 3, from **Study IV**. MMSE trend over two years in patients examined.

PATIENT	MMSE at T1	MMSE at T2	Difference between MMSE at T1 and T2	MMSE at T3	Difference between MMSE at T1 and T3	Total difference	
Mini mental state evaluation							
1	23	22	1	22	0	1	
2	23	21	2	20	1	3	
3	20	20	0	19	1	1	
4	21	20	1	18	2	3	
5	21	20	1	19	1	2	
6	21	20	1	18	2	3	
7	23	21	2	20	1	3	
8	19	18	1	16	2	3	
9	19	18	1	16	2	3	
10	23	23	0	22	1	1	
			1		1.3	2.18	Media
			0.67		0.67	0.95	SD

SD = standard deviation.

In other words, even if in each patient the slight MMSE decrease produced a significant ANOVA finding, clinically our patients did not show a relevant cognitive loss.

This agrees with findings in studies reporting a clinically significant response to AChEI in up to 60% of AD patients (Krall et al., 1999). In this study patient stabilization cannot be ascribed entirely to the effects of the AChEIs. Patient group selection, in fact, was likely biased by the ability of the individuals to tolerate a relatively long procedure in the first place at the time of initial testing, while this subgroup had to be naturally segregated by a more favorable disease progression. Furthermore, appropriate control groups of untreated and of non-stabilized AD patients were not available. Therefore, we cannot exclude the possibility that some patients with a stabilized course would have performed well at 1 year follow-up for other reasons rather than AChEI therapy (e.g. because of a more benign natural course of the disease). Even if a slight decrease of excitability was observed for each parameter, the AChEI therapy effect was not significantly impacting on TMS parameters (Pillai's trace = 0.996; F(5,5)=2.440; p=0.175). Looking at single measures, we consistently did not find any significant change (p=0.154 for threshold, p=0.416 for ADM area, p=0.484 for ECD area, p=0.682 for ADM volume, p=0.368 for ECD volume). Similarly, the center of gravity (with respect to the hotspot) did not show any significant displacement after AChEI therapy (Pillai's trace = 0.450; F(4,6)=1.228; p=0.391). In particular, we found for the ADM (medio-lateral direction) p=0.293, for the ADM (antero-posterior direction) p=0.849, for the ECD (medio-lateral direction) p=0.126, for ECD (antero-posterior direction) p=0.985. The motor excitability parameters are reported in Table 4.

Table 4, from **Study IV.** Motor cortex excitability parameters trend in patients examined.

		Time			
		T1		T2	
TMS parameter	Hemisphere	Mean	SEM	Mean	SEM
Motor excitability parameters					
Threshold (%)	Right	40.8	1.83	38.7	2.15
	Left	39.6	1.54	37.4	1.77
Area ADM (N)	Right	5.3	0.79	4.9	0.75
	Left	5.4	1.10	4.4	1.10
Area ECD (N)	Right	5.7	0.82	5.3	0.79
	Left	6.1	1.35	5.2	1.10
Volume ADM (microV)	Right	26.8	4.07	27.0	4.77
	Left	29.4	5.97	25.8	6.16
Volume ECD (microV)	Right	33.3	4.93	29.7	4.99
	Left	38.4	8.72	31.9	6.64

5.1.2 Study II

In the **Study II** (**Ferreri et al., 2006**), three-way ANOVA (TIME, STIM, SIDE) indicated that resting motor thresholds were not affected by experimental conditions, remaining quite stable during the recording session. In fact, the threshold means ranged between 50.4% and 52.6% of the maximal stimulator's output in the conditions with the lowest and highest values. Single pulse TMS MEP amplitude did not show any dependence on the experimental conditions, being non-significant as regards main effects and for the interactive terms of the three-way ANOVA (STIM, TIME, SIDE), with p-values always > 0.05. Table 1 reports the means and standard deviations of single-pulse MEP amplitudes for

each cell of the experimental design. Inhibition-facilitation TMS curves for each of the six combinations of Time and Hemisphere are illustrated in Figure 7, where the characteristic relationship between ISI and MEP ratios are shown, together with the statistical tests for the difference between real and sham exposure (main effect of STIM) and for the dependence of this difference on ISI (interaction STIM*ISI). The 'real' exposure raised the curve with respect to the 'sham' condition only at T1 and T2 in the exposed left hemisphere ($F(1,14.0)=9.746$; $p=0.007$ and $F(1,14.0)=5.642$; $p=0.032$, respectively). At T1 the lack of a significant STIM*ISI interaction was due to a non-significant difference between the increase in ICF and the decrease in SICI. On the other hand, at T2 a significant STIM*ISI interaction was found in the left hemisphere ($F(8,112.9)=2.073$; $p=0.044$), suggesting that the EMF effect was not homogeneous across ISI, planned comparisons indicating a significant effect only at facilitatory ISI, whilst no difference between real and sham EMF was found at each inhibitory ISI. On first glance, this could indicate that the increased excitability is more lasting for facilitatory than inhibitory ISI. However, these first findings stem from the six patterns of Figure 7, considered separately from each other, like six different snapshots.

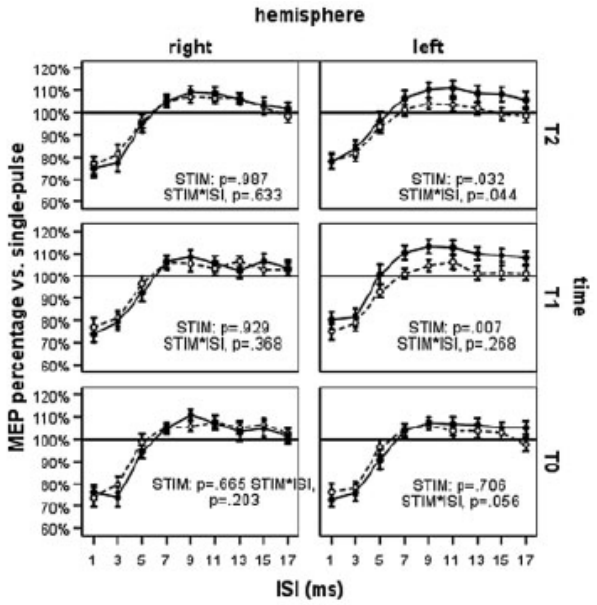

Figure 7, from **Study II.** Inhibition-facilitation curves for each combination of the experimental design. Rows represent time (from bottom: baseline, soon after exposure, 1 hour after exposure). Columns represent hemisphere (right, left) and type of stimulation (continuous line indicates real; dashed line indicates sham). Error bars correspond to 95% confidence intervals after pooling subjects together. MEP = motor-evoked potential; ISI = interstimulus interval.

Therefore, further analyses were carried out in order to (i) use the non-exposed right hemisphere as control for the exposed left hemisphere, and (ii) evaluate changes occurring across the time course of the study.

(i) When both hemispheres were considered, at T1 the real vs. sham excitability changes resulted significantly different in the two hemispheres (STIM*SIDE interaction: $F(1,14)=7.133$; $p=0.018$). This finding endorsed the specificity of an excitability increase in the exposed left hemisphere. As regards T2, STIM*SIDE interaction did not reach the significance threshold ($F(1,14)=2.904$; $p=0.110$), so the excitability increase in the exposed left hemisphere was not strengthened by the comparison with the non-exposed hemisphere. In addition, at T2 the interhemispheric difference after sham exposure was significant ($p=0.022$), so the larger excitability after real exposure of the left hemisphere could be accounted for by the lower (and probably accidental) inhibition-facilitation curve after sham exposure.

(ii) To specifically test the 'increased excitability', the analysis should not be limited to each time (T0, T1, T2) separately, but should be extended on the changes across time (T0-T1-T2). Therefore, the ANOVA model used in (i) was enriched by adding TIME as a factor. The four-way TIME*STIM*SIDE*ISI interaction (testing the homogeneity of the ISI effect across the different TIME*STIM*SIDE interactions) result was non-significant ($F(16, 224.4)=0.854$; $p=0.623$), allowing ISI collapse. In contrast, the triple interaction TIME*STIM*SIDE approached the conventional p-value threshold of 0.05 ($F(2, 28.1)=2.925$, $p=0.070$). As evident in Figure 3, no EMF effect was found in the non-exposed right hemisphere, while a clear change in excitability occurred in the exposed left hemisphere, namely at T1. It seems worth noticing that the 95% confidence intervals for the right hemisphere and for the sham EMF in the left hemisphere were significantly below the reference line at 100%. This simply indicates that, when averaging the ISIs, the weight of the inhibitory effects (mainly due to ISI between 1 and 3) was larger than the weight of the facilitatory effects (mainly due to ISI between 7 and 13). In the left (exposed) hemisphere, the 95% confidence interval at T1 was significantly above the reference line, indicating an inversion of the weights of inhibitory and facilitatory ISIs. When compared with the sham EMF, the computation of the marginal means showed that the net excitatory effect of real EMF in the left hemisphere was clearly significant ($p<0.001$) and resulted equal to 7.6% (95% CI: 6.2%-9.0%). This effect diminished at T2, still remaining statistically significant (5.3%, 95 % CI: 4.1%-6.5%).

This analysis, controlling for both the non-exposed hemisphere (right) and the pre-exposure condition (T0) strongly reduced the differential effect of ISI, as shown in Figure 2 and in the first analysis. In order to better verify a potentially different effect on SICI and ICF, the analysis was re-run replacing the factor ISI (9 levels) with a new factor, namely SICI_ICF, with two levels: 1=SICI, obtained by averaging 1 to 5 ms ISIs, and 2=ICF, obtained by averaging 9 to 13 ms ISI. Again, the TIME*STIM*SIDE*SICI_ICF interaction did not give a significant result ($F(2,28.1)=0.286$; $p=0.754$). Even when analysis was limited to the exposed left hemisphere and to the two contrasts T1-T0 and T2-T0, no evidence of a different real-sham effect on SICI and ICF was found ($F(1,14.1)=0.635$; $p=0.439$ for T1-T0 and

F(1,14.0)=0.009; p=0.927 for T2-T0). Therefore, the null hypothesis of a homogeneous increase in excitability for SICI and ICF could not be ruled out. These findings were not modified when ICF also included ISI=15 and ISI=17. Finally, since ISI=1 and ISI=3-5 have been suggested to have different biological meaning (Hanajima et al., 2003) we tested.

a) whether the average of the ratios at 3 ms and 5 ms modulate the effect; in fact, this was not the case since the SICI-ICF*TIME*SIDE*STIM interaction was again not significant (F(2,28.1)=0.067, p=0.936);

b) whether there was any statistical difference between the MEP ratio at ISI=3-5 ms and the ratio at ISI=1 ms; again, no significant effect was found (F(2,28.3)=0.319; p=0.730).

An important point to be addressed is the homogeneity of such an effect across subjects. When analysed separately, the data from each subject showed an excitability change due to real EMF vs. sham in the left hemisphere in 12 out of 15 subjects. Tympanic temperature showed no significant main effect or interactions (p>0.14).

5.1.3 Study III

In **Study III** (**Ferreri et al., 2011a**), the TMS threshold values ranged between 50.6% and 56.4% of the maximal stimulator's output. The characteristic relationship between ISI and MEP ratios was observed (Kujirai et al., 1993, Ziemann et al., 1996; Figure 8, C). At ISI 3, 83% of MEP was below the mean value at 120% and the corresponding two-modal distribution revealed two peaks of inhibition due to some subjects having relatively high MEP values at 120% and stronger inhibition at ISI 3 and to some others with relatively low MEP values at 120% and slighter inhibition at ISI 3. At ISI 11, 71% of MEP was above the mean value at 120%, indicating facilitation (see Figure 8, C).

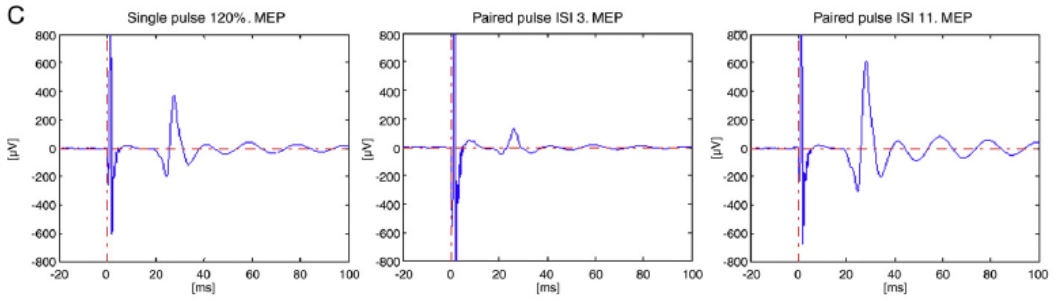

Figure 8 C, from **study III**. Grand average of the MEPs in each condition.

The modulations (percentage variations) of MEP were obtained after log-transformation, thus an inhibition of 20% corresponds roughly to a change from 300 μV to 100 μV and a facilitation of 10% to a change from 300 μV to 530 μV. According to the output of GEE procedure, where MEP was entered as a dependent variable (with log. as link function), TMS condition as factor and Subject as repeated variable, TMS modulated significantly MEP (Wald Chi-square=70.675, df=1, p<0.001). The estimated mean values were **578** (95% CI: 307, 848) for supra-threshold single-pulse with an intensity of 120% of the rMT, **181** (95% CI: 104, 258) for pp ISI 3 ms and **923** (95% CI: 573, 1274) for pp ISI 11ms (see Figure 8, C). Each pair-wise comparison was consistently significant (p<0.007 with Sidak's correction).

As also reported in previous studies (Ilmoniemi et al., 1997; Paus et al., 2001b; Komssi et al., 2002., Komssi et al., 2004; Komssi et al., 2007; Nikulin et al., 2003; Kähkönen et al., 2004 Kähkönen et al., 2005; Kähkönen and Wilenius 2007; Massimini et al., 2005; Bonato et al., 2006; Daskalakis et al., 2008; Farzan et al., 2009; Lioumis et al., 2009), supra-threshold single pulse TMS of the left MI evoked EEG activity lasting up to 300 ms (for a review Komssi and Kähkönen, 2006). In each experimental condition (single and paired pulse TMS, see below) and in each subject, the EEG signals were composed at vertex by a sequence of deflections of negative polarity peaking at approximately 7, 18, 44, 100, 280 ms alternated with positive polarity peaks at approximately 13, 30, 60 and 190 ms post-TMS, as illustrated in Figures 9, A and 10, A. In Figure 10, B the GMFP, which is a measure of global brain activation, is shown for the three experimental conditions (Lehmann and Skrandies, 1980).

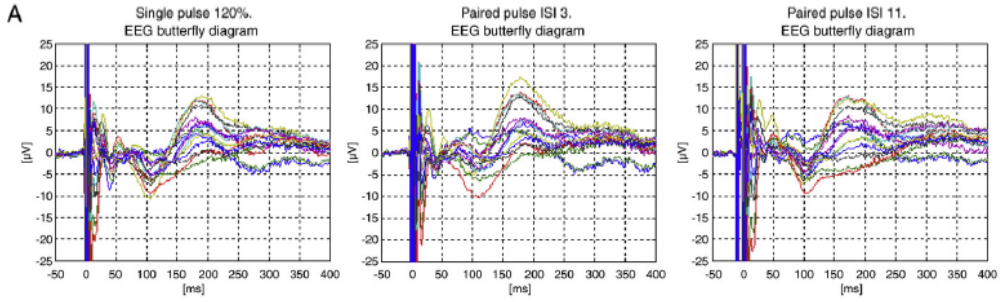

Figure 9 A, from **Study III**. Grand average of TMS-evoked potentials recorded at all electrodes in each condition, superimposed in a butterfly diagram. In the pp sessions all signals were aligned to the second stimulus, shifting back the traces by 3 ms or 11 ms respectively. Polarity of the waveforms is plotted with negative values downward in this and following figures.

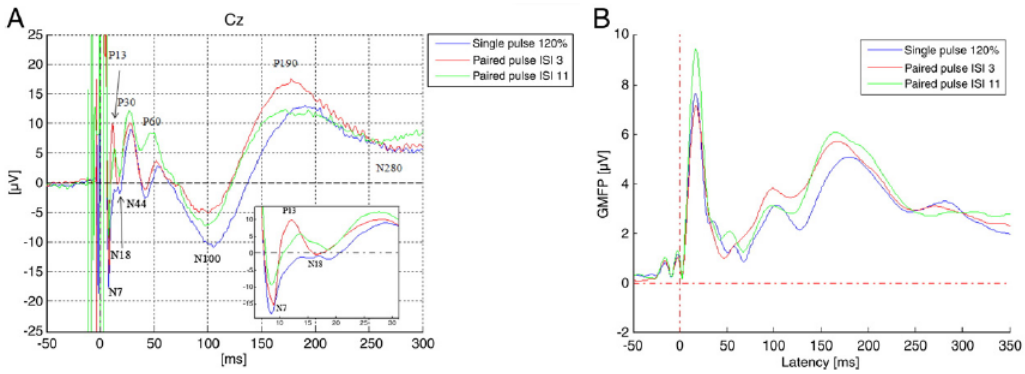

Figure 10 A and B, from **Study III**. A: grand average of the EEG responses recorded at vertex (Cz) in supra-threshold single pulse stimulation and ISI 3 and ISI 11 paired pulse stimulations. In paired pulse sessions all the signals were aligned to the second supra-threshold stimulus, shifting back the traces by 3 ms or 11 ms respectively. B: total activation produced by single and paired pulse TMS over the left M1 as measured by the GMF.

5.1.3.1 Supra-threshold single pulse TMS evoked activity

GEE made it possible to identify specific EEG pattern on the scalp in each peak (Table 4). ***N7*** (mean latency= 7.1; SD= 2.5) was observed in F3, in the stimulated hemisphere (Figure 11, A).

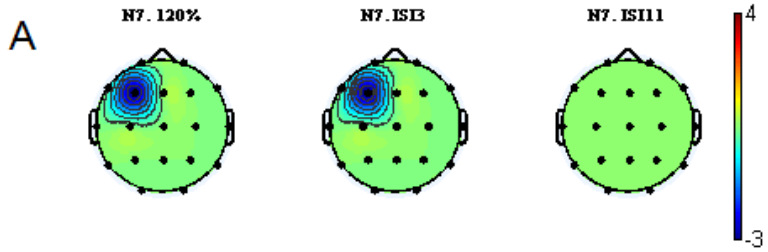

Figure 11 A, from **Study III**. A: N7 was localized in F3 after supra-threshold single pulse TMS and ISI3, after ISI 11 was not statistically evident anywhere.

P13 (mean latency=13.1; SD=2.8) was localized mainly in the contra-lateral hemisphere in (Fp2, F4, F8, C4, T4 and T6. Figure 11, B).

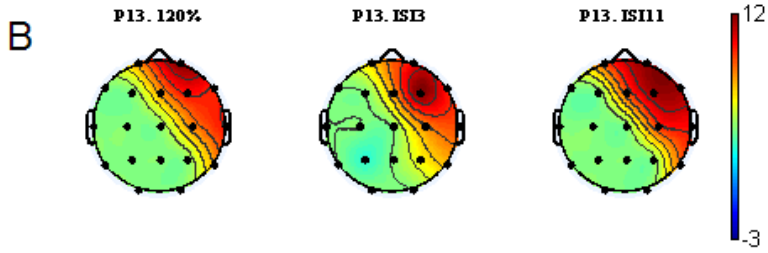

Figure 11 B, from **Study III**. B: in each experimental condition P13 was statistically significant in the anterior regions of the contra-lateral hemisphere and generally stronger at ISI 3.

N18 (mean latency=18.4; SD=3.5) was localized in the posterior areas of the stimulated site, and was strongest in P3 (Figure 11, C).

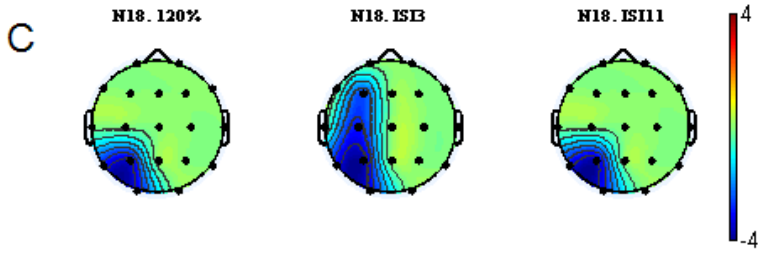

Figure 11 C, from **Study III**. C: N18 was localized in P3 after supra-threshold single pulse TMS and ISI 11 and had a different spatial localization at ISI 3, extending from the posterior regions of the stimulated hemisphere to the more anterior ones.

P30 (mean latency= 28.8; SD= 5.3) was found in most of the electrodes of the non-stimulated hemisphere as well as in Cz, Pz, and T3 (Figure 12, A).

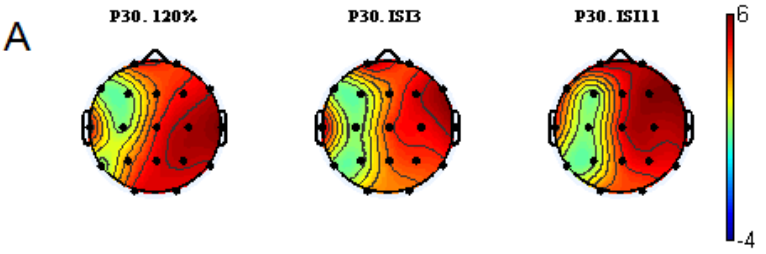

Figure 12 A, from **Study III**. A: P30 had almost the same spatial localization in each experimental condition, being attenuated at ISI 3 and generally stronger at ISI 11 in the unstimulated hemisphere.

N44 (mean latency=44.1; SD=5.8) showed a diffuse spatial distribution, with an antero-posterior amplitude gradient, the posterior mean amplitude being about 20% more than the anterior values (Figure 12, B).

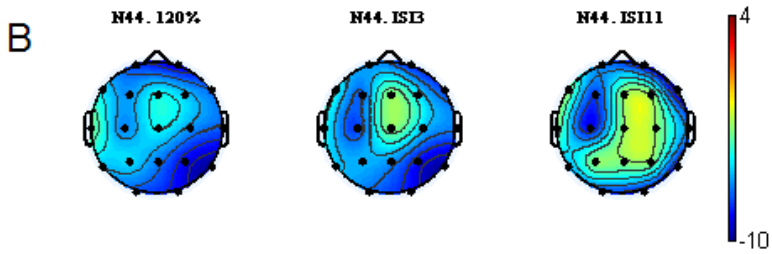

Figure 12 B, from **Study III**. B: N44 peak showed a diffuse spatial distribution, with an antero-posterior amplitude gradient. It was spatially modulated by the paired pulse stimulations and was slightly stronger in C3, P3 and F3 at ISI 3 and virtually absent in the contra-lateral hemisphere at ISI 11.

P60 (mean latency= 62.6; SD= 9.5) was observed posteriorly and contralateral to the stimulation site. Specifically, the largest amplitude was observed in T5, right behind the stimulation site (Figure 12, C).

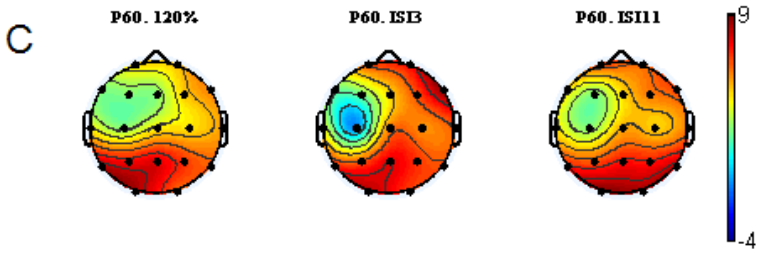

Figure 12 C, from **Study III**. C: P60 was found posterior and contra-lateral to the stimulation site and was modulated by the ppTMS, the polarity of the peak being virtually inverted at ISI 3 in the stimulated area. At ISI 11 it was generally stronger in Cz and in the contralateral hemisphere.

N100 (mean latency=103.3; SD= 19.3) the largest amplitude was observed in C3, the stimulation site with an antero-posterior gradient (Figure 13, A).

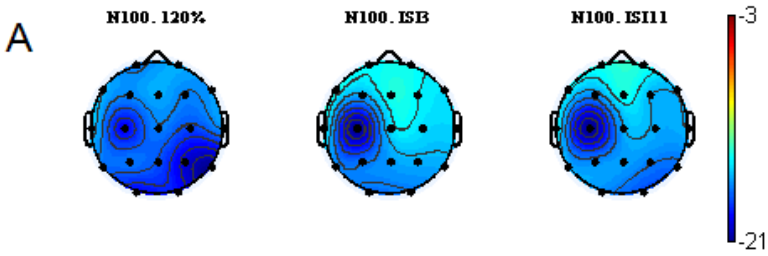

Figure 13 A, from **Study III**. A: N100 largest amplitude was observed in C3 stronger after the ppTMS stimulation.

P190 (mean latency= 189.7; SD= 24.3) showed the highest activation in the centro-parietal region of the non-stimulated hemisphere (C4, P4; Figure 13, B).

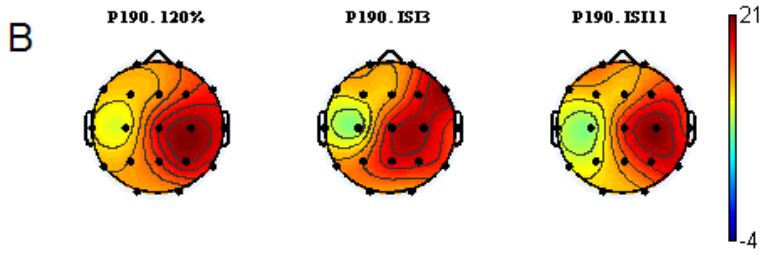

Figure 13 B, from **Study III**. B: P190 was found all over the scalp with the exception of the stimulated area where it was virtually absent after ISI 3.

N280 (mean latency= 266.7; SD= 32.2) showed a diffuse spatial distribution, even though its amplitude was particularly large in relative to F3-C3 (Figure 13, C).

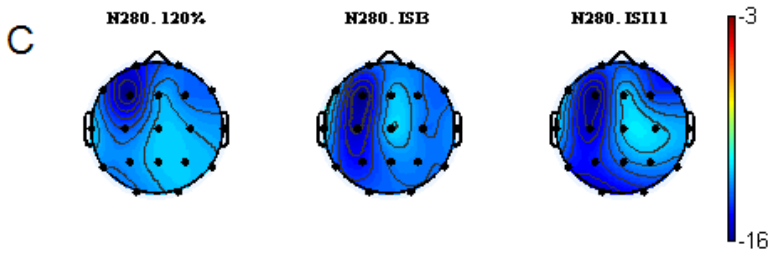

Figure 13 C, from **study III.** C: N280 was found all over the scalp and was generally stronger at ISI 3 and 11 than in supra-threshold single pulse with the exception of T3.

Table 4 from **Study III**. Values are means ± SD; ▲ wave slightly stronger with respect to single pulse; ▲▲ wave stronger with respect to single pulse; ▼ wave attenuated with respect to single pulse;▼▼ wave cancelled with respect to single pulse; ▼▼▼ wave reversed in polarity with respect to single pulse; —— wave stable with respect to single pulse; ○, +, − respectively no correlation, positive and negative EEG-MEP correlation.

		Single pulse	ISI 3	ISI 11
N7	Electrodes	F3	▲F3	▼▼
	Latency	7.1 ± 2.5	7.1 ± 2.5	7.2 ± 2.8
	EEG-MEPc	○	○	○
P13	Electrodes	Fp2,F4,F8,C4,T4,T6	▲▲Fp2,F4,F8,C4,T4,T6	▲Fp2,F4,F8,C4, T4, T6
	Latency	13.1 ± 2.8	12.8 ± 2.3	12.6 ± 3.1
	EEG-MEPc	○	○	+ Fp2,F4,Fz,F8
N18	Electrodes	P3	▲P3,C3,F3	—P3
	Latency	18.4 ± 3.5	18.7, SD = 2.1	17.6, SD = 3.1
	EEG-MEPc	○	○	○
P30	Electrodes	Fp1,Fp2,Fz,F4,F8,Cz,C4, T4,Pz,P4,T6,O1,O2	▼Fp1,Fp2,Fz,F4,F8,Cz, C4,T4,Pz,P4,T6,O1, O2	▲Fp1,Fp2,Fz,F4,F8,Cz, C4,T4,Pz,P4,T6,O1,O2
	Latency	28.8 ± 5.3	28.4 ± 5.1	26.5 ± 5.3
	EEG-MEPc	+ C4,T4,Pz, P4,O2	○	+ F4
N44	Electrodes	Fp1,Fp2,F7,F3,Fz,F4,F8,T3, C3,Cz,C4,T4,T5,P3,Pz,P4, T6,O1,O2	▲F3,C3,P3, ▼Fz,Pz,Cz	▲F3,C3,P3, ▼▼ Fz,F4,Cz,C4,T4, T5,P3,Pz,P4,T6
	Latency	44.1 ± 5.8	43.5 ± 5.3	40.7 ± 5.6
	EEG-MEPc	−F3,T3,C3,Cz,T5,Pz,O1	−T3,T5,O1	○
P60	Electrodes	C3, T5, P3, Pz,O1,O2	▼▼▼C3 — T5 , P3 , Pz , O1 , O2	—C3 , T5 , P3 , Pz , O1 , O2 ▲ Cz
	Latency	62.6 ± 9.5	62.8 ± 9.4	59.2 ± 9.6
	EEG-MEPc	○	○	○
N100	Electrodes	Fp1,Fp2,F7,F3,Fz,F4,F8, T3,C3,Cz,C4,T4,T5,P3,Pz, P4,T6,O1,O2	▲C3	▲ C3
	Latency	103.3 ± 19.3	102.6 ± 19.1	99.9 ± 16.4
	EEG-MEPc	○	○	○
P190	Electrodes	Fp1,Fp2,F7,F3,Fz,F4,F8, T3,C3,Cz,C4,T4,T5,P3,Pz, P4,T6,O1,O2	▼▼ C3 ▲ F8,Cz,C4,Pz	▼▼ C3 — F8 , Cz , C4 , Pz
	Latency	189.7 ± 24.3	185.1 ± 24.1	177.6 ± 25.6
	EEG-MEPc	○	○	○
N280	Electrodes	Fp1,Fp2,F7,F3,Fz,F4,F8, T3,C3,Cz,C4,T4,T5,P3,Pz, P4,T6,O1,O2	▲ Fp1,Fp2, F7,F3,Fz, F4,F8,T3,C3,Cz,C4,T4, T5,P3,Pz,P4,T6,O1,O2	▲ Fp1,Fp2,F7,F3,Fz, F4,F8,T3,C3,Cz,C4,T4, T5,P3,Pz,P4,T6,O1,O2
	Latency	266.7 ± 32.2	269.3 ± 32.0	271.9 ± 33.0
	EEG-MEPc	○	○	○

5.1.3.2 ISI 3 and ISI 11. ISI 3 and ISI 11 versus supra-threshold single pulse. ISI 11 versus ISI 3

The general EEG patterns observed after ppTMS at ISI 3 and ISI 11 are substantially similar to that observed after single pulse (Table 4). However, GEE made possible to identify specific modulations in each peak.

N7 at ISI 3 (mean latency=7.1, SD=2.5) was localized, as in supra-threshold single pulse TMS, only in F3. At ISI 11 (mean latency =7.2, SD=2.8) was not statistically evident anywhere (see Figure 11, A). The subtraction of different maps showed in F3 a slight non-significant difference between single pulse and ISI 11 and between ISI 11 and ISI 3 ($p>0.05$).

P13 both at ISI 3 (mean latency=12.8, SD=2.3) and at ISI 11 (mean latency=12.6, SD=3.1) had the same spatial localization as in supra-threshold single pulse, being statistically evident in the region of contralateral hemisphere and generally more represented at ISI 3 (see Figure 11, B). The subtraction of different maps confirmed higher amplitudes at ISI 3 vs. single pulse in F4 (Sidak's $p=0.032$) and at ISI 11 vs. single pulse in F4 (Sidak's $p=0.041$) and in F8 (Sidak's $p=0.043$).

N18 had a different spatial localization at ISI 3 (mean latency=18.7, SD=2.1) with respect to ISI 11 (mean latency=17.6, SD=3.1) and to single pulse, extending from the

posterior regions of the stimulated hemisphere to the more anterior ones (see Figure 11, C). However, the subtraction of different maps showed no clearly significant difference in this peak ($p>0.05$).

P30 at ISI 3 (mean latency = 28.4, SD=5.1) and at ISI 11 (mean latency = 26.5, SD=5.3) had almost the same spatial localization as in supra-threshold single pulse and was attenuated at ISI 3 and generally stronger at ISI 11 (Paus et al., 2001b, see Figure 12, A) in the unstimulated hemisphere. However, the subtraction of different maps showed no clearly significant difference in this peak ($p>0.05$).

N44 was spatially modulated by the paired pulse stimulations and was virtually absent in the contra-lateral hemisphere at ISI 11 (mean latency=43.5, SD=5.3) (see Figure 12, B). The subtraction of different maps confirmed clear differences between ISI 11 and supra-threshold single pulse (Paus et al., 2001b) in the contra-lateral hemisphere, mostly in the posterior regions (P4: Sidak's $p=0.035$; Pz: Sidak's $p=0.042$) and between ISI 11 and ISI 3 (mean latency = 40.7, SD=5.6) in the same areas (P4: Sidak's $p=0.039$; Pz: Sidak's $p=0.047$).

P60 was also found to be modulated by the paired pulse stimulations, with the polarity of the peak being virtually inverted at ISI 3 (mean latency = 62.8, SD=9.4) in the stimulated area (see Figure 12, C). At ISI 11 (mean latency = 59.2, SD=9.6) it was generally stronger in Cz and in the contra-lateral hemisphere. The subtraction of different maps confirmed clear differences in C3 between ISI 3 and supra-threshold single pulse (Sidak's $p=0.011$) and between ISI 3 and ISI 11 (Sidak's $p=0.015$).

N100 was found all over the scalp being more topographically represented after ISI 3 (mean latency = 102.6, SD=19.1) and ISI 11 (mean latency = 99.9, SD=16.4) than in supra-threshold single pulse on the stimulated area (Figure 13, A). The subtraction of different maps showed significant differences in the stimulated area (C3) between ISI 3 and the supra-threshold single pulse (Sidak's $p=0.044$), and between ISI 11 and the supra-threshold single pulse (Sidak's $p=0.045$).

Also ***P190*** (see Figure 13, B) was also found all over the scalp with the exception of the stimulated area where it was virtually absent after ISI 3 (mean latency = 185.1, SD=24.1) and ISI 11 (mean latency = 177.6, SD=25.6). The subtraction of different maps did not reveal specific areas with significant differences between the three conditions.

N280 was found all over the scalp and was generally stronger at ISI 3 (latency mean=269.3, SD=32.0) and 11 (latency mean=271.9, SD=33.0) than in supra-threshold single pulse with the exception of T3 (see Figure 13, C). The subtraction of different maps confirmed differences between ISI 3 and supra-threshold single pulse and ISI 11 and supra-threshold single pulse in the stimulated area (C3:Sidak's $p=0.039$ and $p=0.049$, respectively).

5.1.3.3 ppTMS-evoked EEG responses latency modulation

Overall, latency was prolonged at ISI 3 by about 2% (2.1; 95% CI=1.9-2.2) and shortened at ISI 11 (Paus et al., 2001b) by about 4% (4.4; 95% CI=4.3-4.5) (for a paradigmatic channel see Figure 10, A). Significant latency modulation ($p<0.01$) was consistently observed in each of the nine peaks considered.

N7 latency slightly but significantly (Sidak's $p=0.007$) increased at ISI 3 versus 120%. No other pairwise difference. The effect size of such modulation was minimal (0.13 ms).

At ***P13***, latency decreased after paired-pulse stimulations versus 120% (ISI 3 $p=0.012$ and ISI 11 $p<0.001$) and was longer at ISI 3 than at ISI 11 ($p=0.011$; less than 0.5 ms).

N18 was slightly prolonged at ISI 3 versus 120% ($p=0.041$), shortened at ISI 11 versus 120% ($p<0.001$) and obviously more shortened at ISI 11 versus ISI 3 ($p<0.001$; about 1 ms).

P30 latency decreased after paired-pulse stimulations (ISI 3 versus 120%, $p<0.001$, ISI11 versus 120% $p<0.001$), and again the minimal latency was found at ISI 11. In this case, the maximal difference (between ISI 11 and 120%) was around 2.2 ms.

A similar pattern was observed at ***N44***, each pair-wise comparison being significant ($p<0.001$). The maximal difference, corresponding to a decrease of 3.9 ms was between ISI 11 and 120%.

P60 did not change between ISI 3 and 120% ($p=0.999$) and strongly decreased at ISI 11 ($p<0.001$) by about 4.5 ms (for a paradigmatic channel see Figure 10, A).

N100 showed a different pattern with respect to P60, since the two paired pulse produced very close latencies ($p=0.999$), clearly shorter than 120% ($p<0.001$) by about 3.9 ms.

P190 was shortest at ISI 11 ($p<0.001$ vs. both other conditions) and longer at 120% versus ISI 3.

N280 did not change between ISI 3 and 120% ($p=0.999$) or between ISI 11 and 120% ($p=0.887$).

5.1.3.4 Correlation between EMG and EEG measures in single and ppTMS

The correlation between EEG, considered as the independent variable, and MEPs, considered as a dependent variable, was statistically significant for some of the peaks considered. In order to reduce the total number of possible correlations, we report here only correlations corresponding to peaks that showed significant changes across conditions. Moreover, to take into account the problem of multiple comparisons, a correlation was considered significant only when its 95% Sidak's confidence intervals did not include the

reference value corresponding to the null hypothesis (r=0, or equivalently z=0, where $z = \sqrt{n-3}\frac{1}{2}\log\frac{1+r}{1-r}$).

In particular, for ***P13*** there were no clear correlations with the MEPs amplitude after the single pulse TMS and paired pulse TMS at ISI 3; however, after ppTMS at ISI 11 a positive correlation was seen in F8 and F4, with a slight involvement of Fp2 and Fz (Figure 14, B).

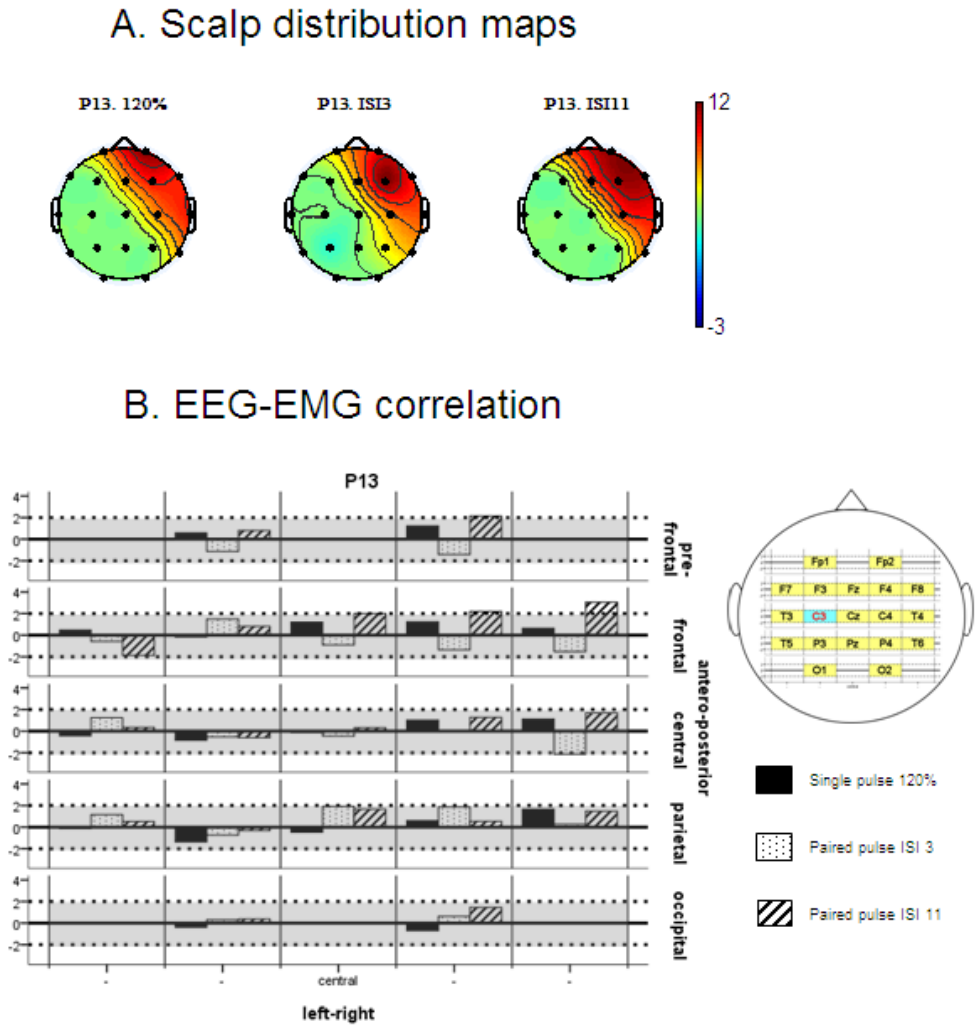

Figure 14 A and B, from **Study III**. A: scalp distribution maps of GEE model EEG pattern at 13 ms after stimulation over the left M1. In each experimental condition P13 was statistically significant in the anterior regions of the contra-lateral hemisphere and generally stronger at ISI 3 B: a positive correlation after ISI 11 was seen in F8 and F4, with a slight involvement of Fp2 and Fz.

At ***P30*** a positive correlation with the MEPs amplitude after the single pulse TMS was observed in the unstimulated hemisphere. No correlation with the MEPs amplitude was observed after ppTMS at ISI 3, while at ISI 11 the main finding was a positive correlation between EEG modulation in F4 and MEP modulation (Figure 15, B).

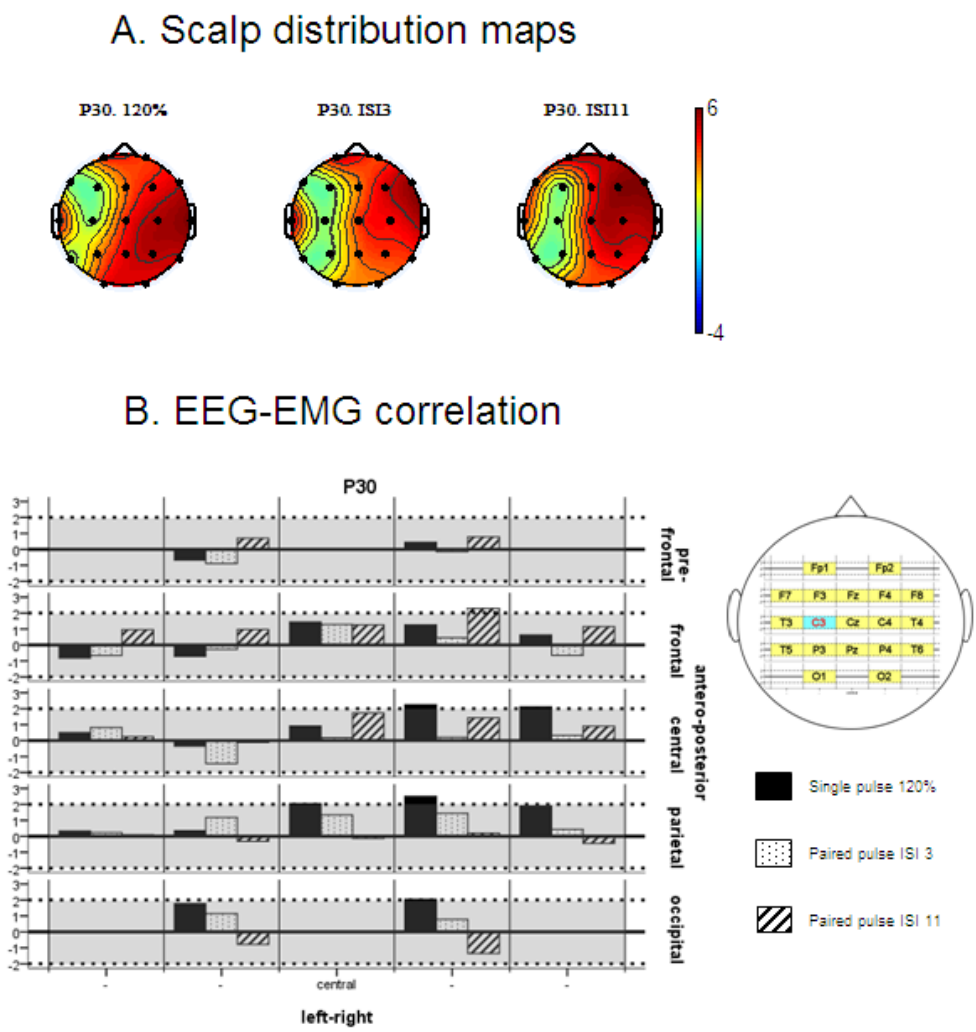

Figure 15 A and B, from **Study III.** A: scalp distribution maps of GEE model EEG pattern at 30 ms after stimulation over the left M1. P30 had almost the same spatial localization in each experimental condition, being attenuated at ISI 3 and generally stronger at ISI 11 in the unstimulated hemisphere. B: positive correlation with the MEP amplitude after the single pulse TMS was observed in the unstimulated hemisphere. No correlation was observed at ISI 3, while at ISI 11 a positive correlation was found in F4.

At ***N44*** a negative correlation with the MEPs amplitude after the single pulse TMS was observed in the central and posterior regions of the stimulated hemisphere. After

paired pulse TMS at ISI 3 the correlation was markedly attenuated, surviving only at T5, T3 and O1. After ISI 11 no clear correlation was observed (Figure 16, B).

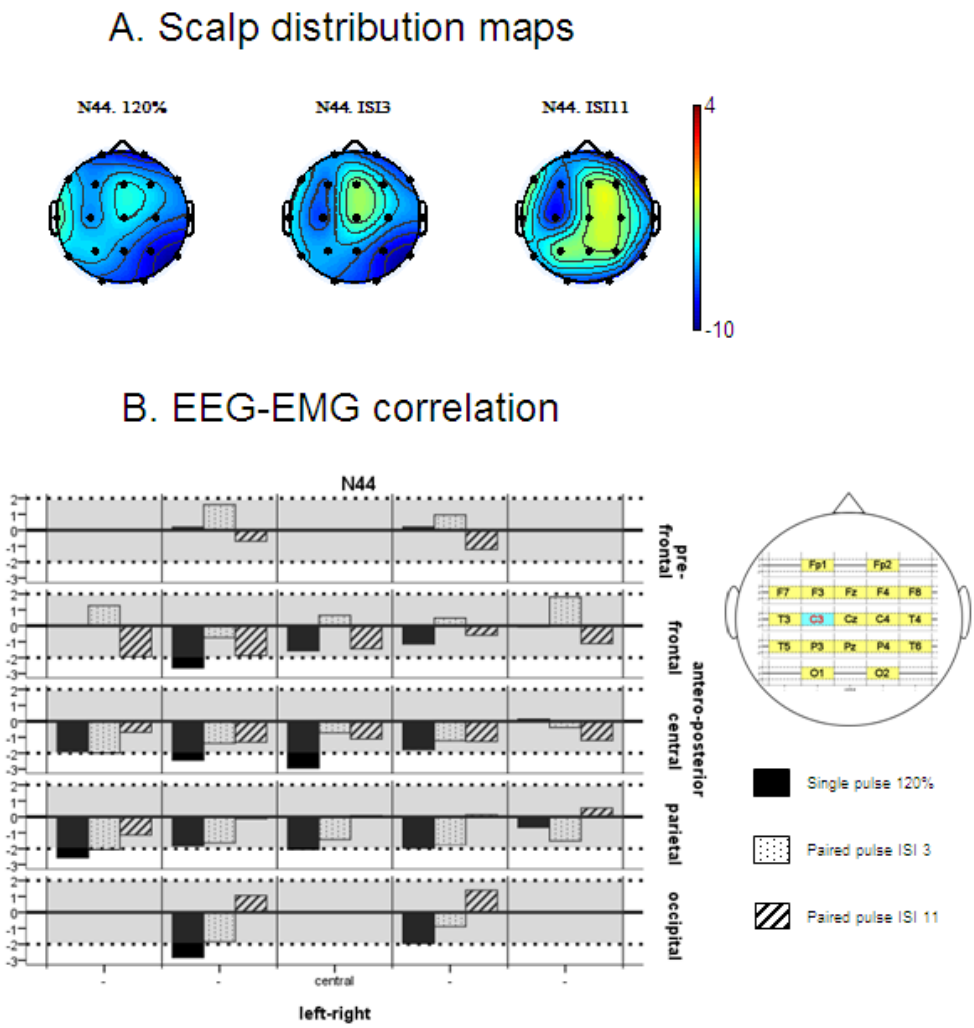

Figure 16 A and B, from **Study III.** A: scalp distribution maps of GEE model EEG pattern at 44 ms after stimulation over the left M1. The N44 peak showed a diffuse spatial distribution, with an antero-posterior amplitude gradient. It was spatially modulated by the paired pulse stimulations, being slightly stronger in C3, P3 and F3 at ISI 3 and virtually absent in the contra-lateral hemisphere at ISI 11. B: a negative correlation after the single pulse TMS was observed in the central and posterior regions of the stimulated hemisphere. After ISI 3, the correlation was markedly attenuated, surviving only at T5, T3 and O1. After ISI 11 no clear correlation was observed.

5.1.4 Study V

In the **Study V** (**Ferreri et al., submitted**), the characteristic relationship between ISIs and MEP ratios in SAI was observed (Tokimura et al., 2000; Di Lazzaro et al., 2004; Di Lazzaro et al., 2005b. Figure 17, A and C). The estimated mean values were 1739 μV (S.D. 1157 μV) for supra-threshold single-pulse with an intensity of 120% of the rMT, 790 μV (S.D. 603 μV) for ISI N20+3 and 1848 μV (S.D. 1134 μV) for ISI N20+10. Pairwise comparison with Bonferroni correction showed a significant difference in MEPs amplitude between ISI N20+3 and supra-threshold single pulse (p=0.009), and between ISI N20+3 and ISI N20+10 (p=0.031). No difference was observed between the baseline condition (supra-threshold single pulse) and ISI N20+10 (p >0.05; see Figure 17, A). In both experimental conditions, i.e. both in ISI N20+3 and N20+10 conditions, and in each subject, the EEG signals were composed at the vertex by a sequence of deflections, as described elsewhere (Ilmoniemi et al., 1997; **Ferreri et al., 2011a**; for a review Komssi and Kähkönen, 2006), of negative polarity peaking at approximately 7 ms, 44 ms and 100 ms, alternating with positive polarity peaking at approximately 30 ms, 60 ms and 180 ms post-TMS, as illustrated in Figures 17 B and 18 A).

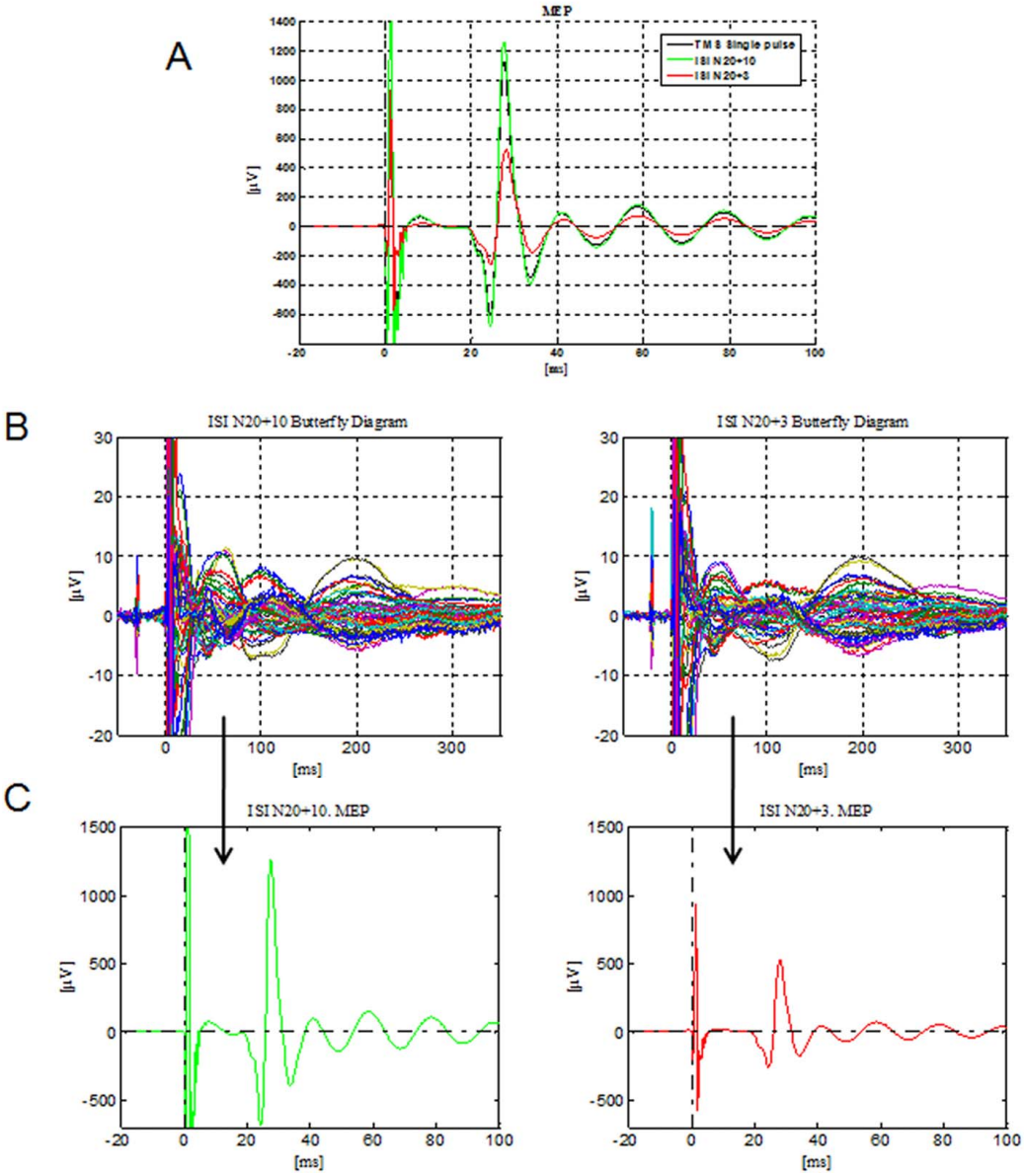

Figure 17 A, B and C, from **Study V**. A: Motor-evoked potentials (MEP). Average of MEPs produced by stimulation at 120% of rMT and at ISI N20+3 and N20+10. Compared with baseline, MEPs were significantly lower in amplitude at ISI N20+3, while no difference was seen at ISI N20+10. B: Butterfly plots. Grand average of TMS-evoked potentials recorded at all electrodes at ISI N20+3 and N20+10 superimposed in a butterfly diagram. All signals were aligned to the magnetic stimulus. The polarity of the waveforms is plotted with negative values downward in this and the following figures. C: Grand average of the MEPs at ISI N20+3 and N20+10.

To quantify the total brain activation evoked by TMS, global-mean field power, calculated as the root mean squared value of the signal across all electrodes, was computed for both conditions under evaluation (see Figure 18, B). The global-mean field power for ISI N20+3, i.e. SAI, revealed a decrease in amplitude between 50 and 130 ms post stimulus relative to ISI N20+10. This decrease was maximal between 60 and 110 ms (Figures 18, 19 and 20).

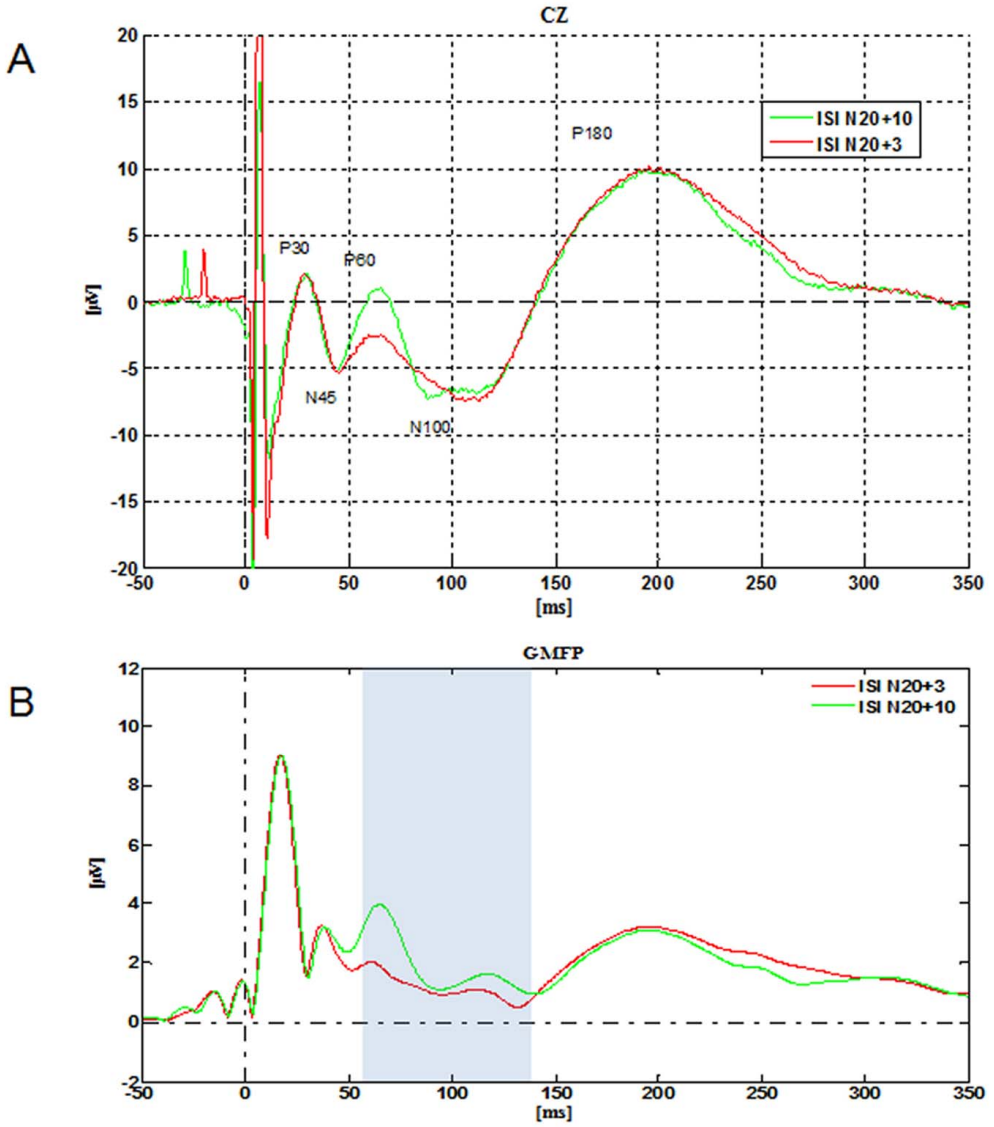

Figure 18 A and B, from **Study V**. A: Single-Channel EEG Responses. Grand average of the EEG responses recorded at vertex (Cz) at ISI N20+10 and ISI N20+3. B: Global-Mean Field Power. Total activation produced by TMS as measured by the global-mean field power derived from all 60 electrodes. Relative to ISI N20+10, the global-mean field power was lower at ISI N20+3, i.e. SAI, between 50 and 130 ms poststimulus (grey area). This decrease peaked at 60 and 110 ms (as evaluated in a two-tailed unpaired t test).

According to the output of the GEE procedure, the ISI N20+3 condition produced a significant **global** attenuation of N100 amplitude compared with the ISI N20+10 condition (main effect of condition, Sidak's p<0.001; Figures 19 and 20 respectively). No significant main effects of condition were found for N7, P30, N44 or P180. In the topographical analysis of the TMS-induced responses, the N7, P30, N44, P60, N100 and P180 amplitudes were assessed by statistical nonparametric mapping (SnPM). After ISIN20+3, we found a significant decrement in P60 amplitude over C1 (left motor cortex; $p < 0.05$, SnPM, Figure 19). Even though a significant **global** attenuation of N100 amplitude was found according to the GEE procedure after ISIN20+3 (see above), the **local** attenuation of the N100 component in the motor cortex did not reach significance when evaluated with the subsequent topographical analysis by SnPM.

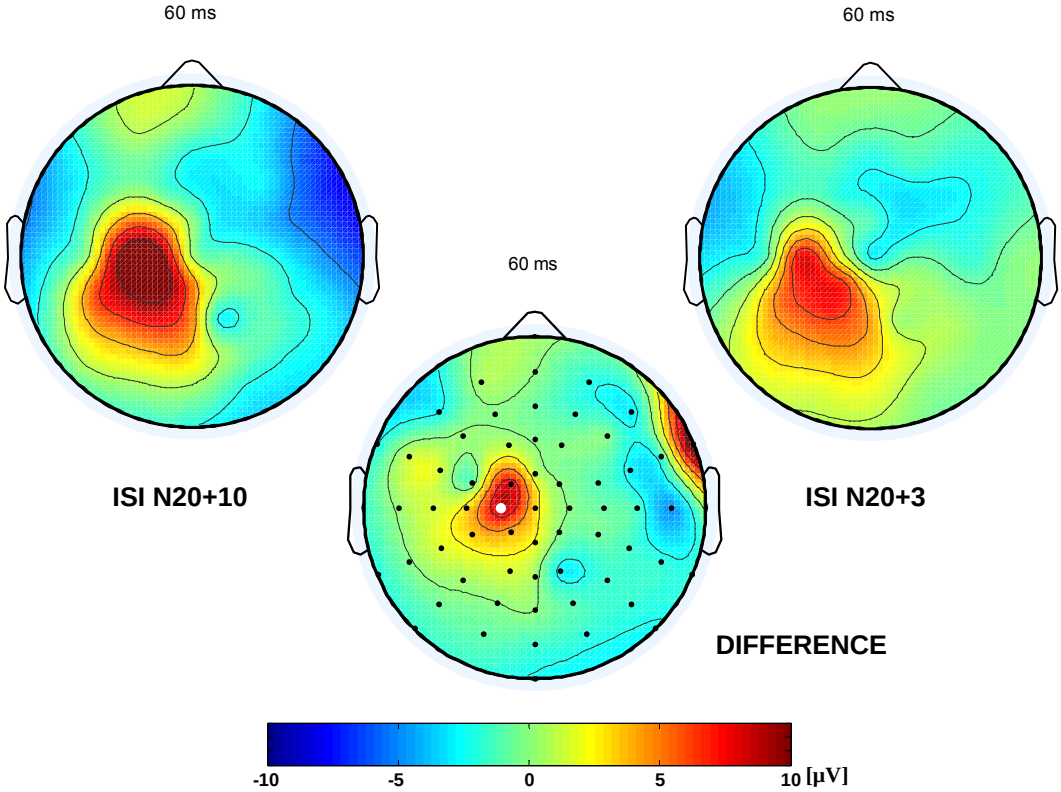

Figure 19, from **Study V**. Topographic distribution of the TMS-evoked activity at P60. Average integrated evoked response at P60 in the two conditions. Comparing the topography of the two conditions revealed lower activity approximately underlying the stimulation site at ISI N20+3, i.e. SAI. The white dot indicates the presence of significant differences (p<0.05; statistical nonparametric mapping, see text for more details).

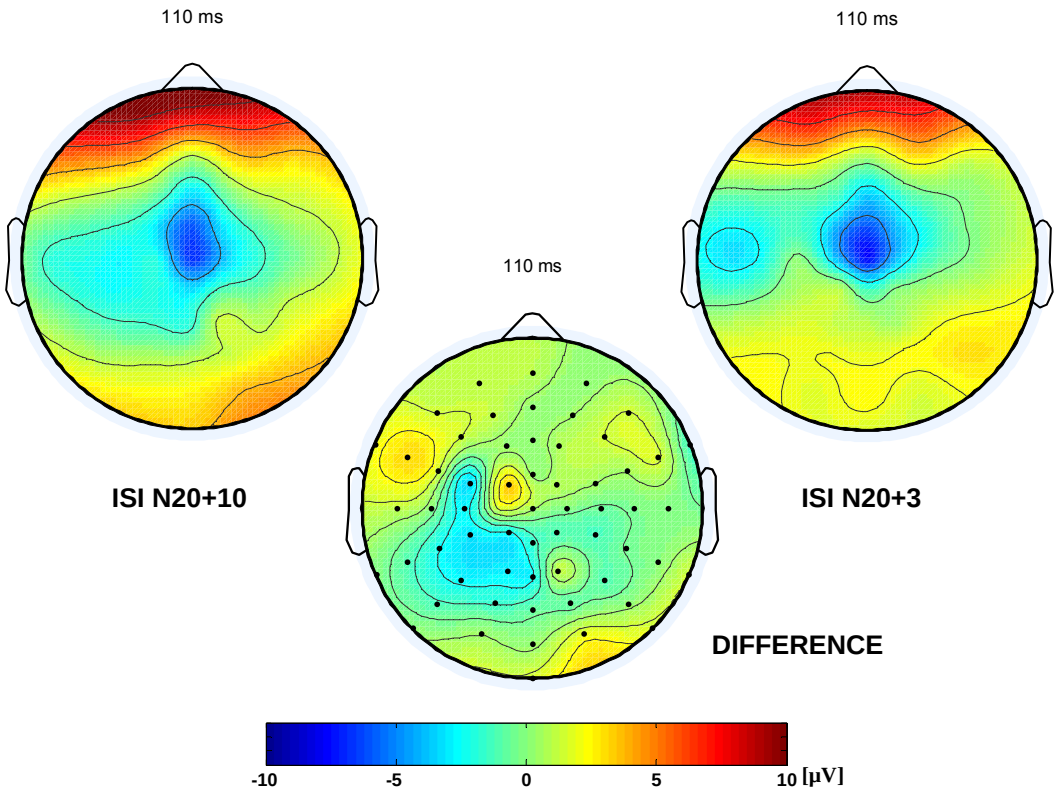

Figure 20, from **Study V**. Topographic distribution of the TMS-evoked activity at N100. Average integrated evoked response at N100 in the two conditions. Comparing the topography of the two conditions revealed a lower activity approximately underlying the stimulation site at ISI N20+3, i.e. SAI.

5.1.4.1 Event-related spectral perturbation and intertrial coherence

Event-related spectral perturbation measures changes in the amplitude of the EEG spectrum relative to an experimental event (e.g., TMS stimulation) and is independent of the phase of EEG-evoked activity. Event-related spectral perturbation values were calculated for different spectrum frequency bands and no significant difference was found for any frequency ranges or channels (data not shown). Intertrial coherence determines the reproducibility of the phase of the EEG-evoked responses across trials, regardless of the amplitude of the responses. Thus, intertrial coherence reflects the intertrial synchronization (phase locking) of the EEG-evoked responses to TMS. We found that ISI N20+3, i.e. SAI, induced a clear reduction in the synchronization of the TMS-evoked responses across trials in the beta band (Figure 21). Intertrial coherence impairment was present in SAI within the first 300 ms following TMS. This impairment was restricted to the beta band ($p<0.05$, statistical nonparametric mapping) and peaked at 2 channels localized in the fronto-central

region (C1 and Cz). No significant differences were detected in the other frequency ranges or in any other channel.

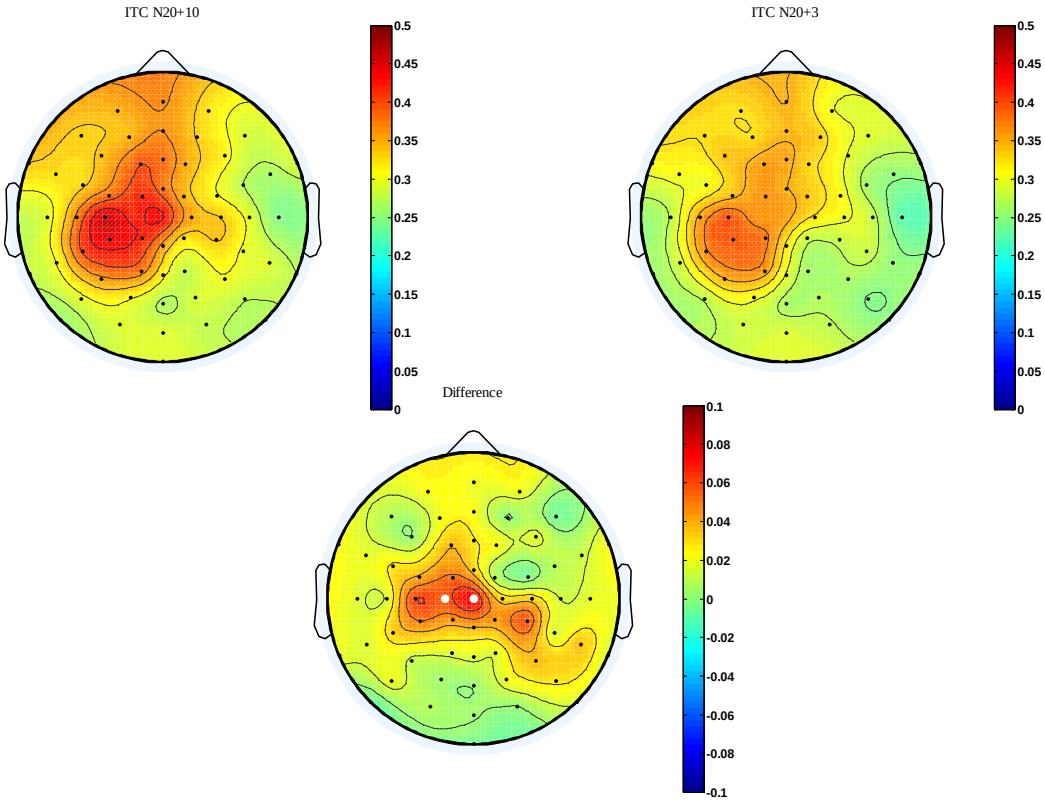

Figure 21, from **Study V**. Intertrial Coherence Topography. Intertrial coherence (ITC) in the two conditions in the beta range between 15 and 25 Hz. The white dots represent the scalp position where intertrial coherence was significantly lower at ISI N20+3, i.e. SAI ($p<0.05$; statistical nonparametric mapping).

5.1.4.2 Correlation between EEG modulation and MEP modulation

To assess the relationship between peripheral and central correlates of SAI, we calculated the correlation between the attenuation of MEPs and modulation of EEG in electrode sites where a significant difference in EEG responses between the conditions had been found (see above; C1 for P60 and C1 and Cz for ITC). For P60, no correlation was found while for ITC in the beta range there was a significant positive correlation with MEP amplitude at C1 ($p<0.05$, $r=0.624$), but no correlation at Cz.

6 Discussion

6.1 SINGLE STUDY DISCUSSION

6.1.1 Studies I and IV

Motor cortex excitability is increased in Alzheimer's disease and the centre of gravity of motor cortical output, as represented by excitable scalp sites, shows a frontal and medial shift, without correlated changes in the site of maximal excitability (hotspot; **Study I**). Motor programming and execution is based on a distributed network with replicated topographic organization of the same body district (particularly for hand and finger control) as has been considered of importance in motor learning and in functional recovery after partial lesions of the motor brain centers (Rossini and Pauri, 2000; Hallett et al., 2000). Higher excitability and fronto-medial 'migration' of the excitable motor areas could be explained by neuronal reorganization, possibly including the dysregulation of the inhibitory frontal centers (the 'suppressory' motor cortex or area 4 S) and their integration in the distributed excitatory network subtending motor output. On one hand fronto-temporal migration of the centre of gravity does not appear to reflect tissue retraction due to brain atrophy; if this were the case, the hotspot should have shifted in a similar way without any dissociation from the centre of the map. This may indicate functional reorganization, possibly after the neuronal loss in motor areas. On the other hand, hyperexcitability might be caused by a dysregulation of the intracortical GABAergic inhibitory circuitries and selective alteration of glutamatergic neurotransmission (Francis et al., 1993; Masliah et al., 1996). The change could be interpreted as a compensatory mechanism allowing the preservation of motor programming and execution over a long period despite disease progression.

When evaluated during long-term AChEI therapy **(Study IV**), the brain hyperexcitability pattern was unchanged in a subset of 10 patients from Study I and their cognitive performance at the MMSE was also stabilized, on the basis of the expected minimum annual rate of decline of the MMSE score (Nobili et al., 2002). Although the question of whether TMS can be used to monitor Alzheimer's disease progression and response to treatment cannot be conclusively addressed by this study as there was no change in any of the parameters analyzed and there was no control group, this hypothesis is supported by previous experiments using the evaluation of SAI (short latency afferent inhibition; Di Lazzaro et al., 2005b) and we can suppose that TMS could be helpful in reducing inter-individual variability and achieving a more direct measure of disease progression. There is increasing evidence (Alagona et al., 2001; Di Lazzaro et al., 2002b; Pennisi et al., 2002) that, together with clinical, neuropsychological and neuroimaging data, TMS could be a useful non invasive, low-cost and repeatable measure of biological progression in AD, and that it may be used in the clinical setting to improve diagnostic power and to monitor and predict the effect of therapy (for example, to see whether a

patient's pre-therapy cortical hyperexcitability changes after being started on drugs, and further determining whether over the long term any change between the pre- and post-TMS actually predict stability). Therefore, the potential clinical value of the TMS evaluation of motor cortex excitability in AD is worthy of further attention, and longitudinal studies with larger number of patients should be planned to substantiate these hypotheses.

6.1.2 Study II

EMFs exposure was able to transiently increase cortical excitability in healthy and young adults, with SICI being reduced and ICF enhanced in the acutely exposed hemisphere compared with the contralateral non-exposed hemisphere or to sham exposure without any thermal effect. This monohemispheric hyperexcitability might depend on decreased GABAA-mediated inhibition or increased NMDA-mediated excitatory activity (Ziemann et al., 1998) or both. Such a disruption in neurotransmitter balance leading to hyperexcitability may also be associated with the monohemispheric increase in regional cerebral blood flow as actually observed via neurometabolic measurements during GSM mobile phone utilization (Huber et al., 2000; Volkow et al., 2011). Neuronal hyperexcitability is considered to play a role in promoting cortical plasticity, either of the adaptive or maladaptive kind, with potential opposite effects on functional recovery, both in healthy people and in neurological diseases (Chen et al., 1998; **Ferreri et al., 2003**, **Study I).** Modifications of cortical excitability as shown in this report should therefore be regarded with interest because it is theoretically possible that exposure to EMFs may provide a new, non-invasive method for modifying brain functioning (Huber et al., 2000). Further studies are needed to better circumstantiate the findings presented and to provide safe rules for the use of this increasingly more widespread device.

6.1.3 Study III

It has been suggested that TMS on M1 predominantly affects the site of stimulation, although it is known that M1 receives input from, and projects output bilaterally to primary motor, supplementary motor, and somatosensory cortices, and the thalamus. Recent NMD and PET studies - which however suffer from a poor temporal resolution (for a review see Bestmann et al., 2008) - have demonstrated some spread of activation from M1 to remote brain areas. The present study adds new insights on the previously delineated (Komssi et al., 2004) functional behaviour of the human brain as investigated by EEG oscillations evoked by TMS on M1. It characterizes in more details two further early responses - N7 and P13 - previously described by Bonato et al., 2006. Moreover, inferring from the existing literature and following M1 anatomical connectivity according to an emerging principle of

reciprocality (Matsumoto et al., 2007), we have extended previous findings about the physiology of the early and late EEG-TMS responses. We have also demonstrated that EEG-ppTMS (Paus et al., 2001b) is a promising tool for characterizing the neuronal circuits underlying human cortical effective connectivity and the neural mechanisms regulating the balance between inhibition and facilitation within the cortices and the corticospinal pathway. We have in fact proved that ppTMS modulates MEPs and EEG early and late evoked responses, and that for some peaks the EEG variability is partly linked with - and therefore might partially explain - MEPs' variability, suggesting that for some TMS-induced EEG peaks the measures of SICI and ICF would be somewhat related to the same mechanisms which mediate EMG measures of SICI and ICF. Additionally, it implies that intracortical inhibition and facilitation, which hitherto can only be indirectly demonstrated in the M1 by measuring MEP amplitude modulation, could be directly evaluated virtually in each cortical area by measuring EEG brain responses to ppTMS (Daskalakis et al., 2008).

6.1.4 Study V

In this study it was possible to confirm previous results showing that the electrical stimulation of peripheral nerve can modulate the excitability of the contra-lateral motor cortex and this effect has a specific time window when it is possible to evoke its clear-cut decrement as evaluable by the inhibition of MEPs amplitude (short latency afferent inhibition, SAI; Tokimura et al., 2000; Di Lazzaro et al., 2003b). It was also possible to demonstrate that this excitability decrement is associate to attenuation of cortical P60 and N100 (Bikmullina et al., 2009), as well as to motor cortex beta rhythm selective desynchronization without changes in the band amplitude. These observations indicate that the mechanisms mediating the inhibition of TMS-evoked brain measures could be in some way related to the same mechanisms mediating inhibition of the EMG measures. They also strongly confirm the widely accepted view that this inhibition has a likely cortico-thalamic origin and exerts its effects on the corticospinal neurons through cortical inter-neurons modulation (Tokimura et al., 2000; Di Lazzaro et al., 2004; Di Lazzaro et al., 2007) i.e. it is affected by the activity of inhibitory or facilitatory circuits in the M1 as well as by the influence of other motor related brain areas.

In view of these results, when combined with those of previous studies from our own (**Ferreri et al., 2011a)** and other groups, it can be hypothesized that the inhibition of MEP, P60 and N100 amplitude as well as beta rhythm de-synchronization in SAI, could reflect an imbalance in GABA-mediated inhibition onto the corticospinal neurons modulated by cholinergic activation. This possibility should be taken into serious consideration because a potential interaction between cholinergic and Gabaergic circuits at several levels in the human brain has been preliminarily suggested by some TMS observations (Sanger et al., 2001; Stefan et al., 2002; Ziemann 2003; Chen 2004; Di Lazzaro et al., 2005c; Alle et al., 2009; Udupa et al., 2009). It is important to understand the functional modulation of cortical inhibitory network dynamics by neurotransmitters and peptides in order to clarify the

physiological basis of human cerebral cortex functioning. Different neurotransmitters interact with a variety of receptors whose activation can produce different effects on the excitability of a diverse group of cortical neurons. This differential modulation of subgroups of inhibitory inter-neurons by ascending cholinergic or other neurotransmitter systems is considered to represent a basis for fine control of functional state and information flow in cortical networks (Steriade et al., 1993). Cholinergic systems have been implicated in several important brain functions, including cortical arousal, sleep-wake cycles, visual information processing, learning, memory, and other cognitive functions (Di Lazzaro et al., 2005a) so detailed information about the effects of cholinergic innervation on the functioning of cortical circuits is of extreme interest.

6.2 GENERAL DISCUSSION

The key feature of TMS is its unique ability to probe changes in the excitability, connectivity and global plasticity of intra-cortical circuits in some cortices (particularly motor and visual ones) both in healthy and pathological human brains by means of a painless though non-physiologically induced neuronal activation (Post and Keck, 2001; Hoffman and Cavus, 2002; Komssi et al., 2004; Rossini and Rossi, 2007). TMS can even be used to induce or guide mechanisms of acute and maybe chronic cortical plastic reorganisation (Platz and Rothwell, 2010). Most of the knowledge about the interaction between magnetic stimuli and cerebral cortex has been obtained through studies that recorded MEPs elicited by single or paired TMS on M1 in basal conditions and after experimental manipulations (Ziemann et al., 1995; Ziemann et al., 1998; see for example **Study II**, **Ferreri et al., 2006**). Moreover in the last two decades advances in TMS functional mapping techniques, offering the possibility to follow the temporo-spatial patterns of local and distal plastic changes in brain function, have provided a sensitive means of identifying brain regions where regional neuronal activity correlates with motor performance and behaviour in both healthy and in pathological conditions (such as Alzheimer's Disease; **Study I Ferreri et al., 2003** and **Study IV Ferreri et al., 2011b**).

For example cortical mapping procedures with single TMS pulses focally applied on several scalp positions overlying the M1 strongly demonstrate in Alzheimer's disease a global cortical hyper-excitability (**Study I, Ferreri et al., 2003**; Di Lazzaro et al., 2004) This possibly parallels cognitive status, and together with clinical, neuropsychological and neuroimaging data could be a measure of biological disease progression (Di Lazzaro et al., 2005b; **Study IV, Ferreri et al., 2011b)**. Cortical hyper-excitability seems to occur independently of GABAergic corticocortical mechanisms dysfunction (Di Lazzaro et al., 2004; Pepin et al., 1999; Liepert et al., 2001). It has been related to an excitatory glutamatergic system dysfunctional activity stemming from an imbalance between non-NMDA and NMDA neurotransmission (**Study I, Ferreri et al., 2003**; Di Lazzaro et al., 2003a) in a complex mosaic involving several neurotransmitter systems in several brain areas (Guerra et al., 2011). Thus multiple lines of evidence suggest that changes in

Acetylcholine (ACh) levels positively modulate glutamatergic function in animal models (Pfurtscheller et al., 1996) and might influence the excitability of cortical and hippocampal glutamatergic networks in several ways. In vitro and in vivo models show that nicotine and other cholinergic agonists enhance glutamate-dependent LTP via an NMDA-dependent mechanism (Feig and Lipton, 1993), suggesting that ACh plays a prominent role on neocortex excitability through a complex interaction with glutamatergic pathways (Veniero et al., 2011).

While these studies can highlight the functional involvement of cortical areas in motor control, the value of TMS as a clinical tool is presently reduced by the partial insights into its mechanisms of action -as revealed by MEP modulation studies- and the incomplete knowledge about its neurobiological effects (Guerra et al., 2011), particularly concerning how this non-physiological stimulation interacts with the intrinsic neuronal activity in the human brain. There are hypotheses about the kinds of interaction, but solid evidence favouring one theory amongst others is still lacking: macroscopically, the locus of brain activation is probably where the induced field is maximal (Krings et al., 1997) but, according to the emerging principle of brain connectivity (Tononi et al., 1994; Sporns et al., 2004; Sporns, 2011; Matsumoto et al., 2007), the existence of spatio-temporal remote activations is now clear (Bestmann et al., 2008). During last decade the persistence of such a doubt motivated the use of neuroimaging techniques to assess the local and remote, as well as the acute and conditioning effects of TMS on brain function (Siebner et al., 2009; Ziemann, 2011; **Study III**, **Ferreri et al., 2011a**).

Combined TMS-neuroimaging studies have greatly stimulated research in the field of TMS (Siebner et al., 2009; Ziemann, 2011). When applied during neuroimaging in an online approach, TMS can be used to test how a focal stimulation acutely changes the activity and connectivity in the stimulated cortical circuits. The temporal and spatial resolution of neuroimaging techniques represents important selection criteria when planning a combined TMS-neuroimaging study: if temporal aspects of neuronal processing are the main focus, the use of a neuroimaging method with a high temporal resolution (millisecond-scale) such as EEG is preferable (Siebner et al., 2009).

The significance of the concurrent multi-channel EEG and TMS resides in its capability to reveal the direct TMS-induced cortical activation, while underlying behavioural changes may be detected with other methods (Komssi et al., 2004). EEG-TMS has several advantages: first, the recordings can be collected at the patient's bedside at a relatively low cost; secondly, if a high density EEG is available, and an advanced source modelling on individual structural neuroimaging is simultaneously performed, a good enough spatial resolution can also be obtained. By combining EEG-TMS recordings with structural neuroimaging, it is also possible to correlate electrophysiological measures of cortical excitability and connectivity with measures of regional brain structure. Moreover, correlational analysis may test for relations between TMS-induced behavioural effects and neuroimaging measures of regional brain structure or evaluate the morphometric assessment of changes in brain structure following diseases or experimental tasks (Siebner et al., 2009). EEG-TMS co-registration, using the high temporal resolution of the EEG method, is then a perfect complement to study or just monitor the transient perturbations

caused in the brain's oscillatory processes by the several TMS paradigms available (Paus et al., 2001a; Van Der Werf and Paus, 2006; Van Der Werf et al., 2006; **Study V**, **Ferreri et al submitted**). In addition, EEG-TMS high temporal resolution can also be exploited to identify critical periods during which the stimulated area and its connections to other remote brain regions make a critical contribution to the experimental task (see for examples **Studies III** and **V**, **Ferreri et al., 2011a and Ferreri et al., submitted**).

TMS-EEG conveys accurate information about the spatio-temporal order of activations of distant cortical areas, being capable of tracing the temporo-spatial dynamics of causal interactions within functional brain networks (Siebner et al., 2009). The spatial distribution of the local and remote TMS-evoked activation is approximately detected from functional imaging studies (for a review see Bestmann et al., 2008), while its temporal sequence cannot be deduced based on the images, which have a poor temporal resolution due to the fact that modifications in blood flow and oxygenation occur a few seconds after changes in neuronal activity (Komssi et al., 2004). Using TMS-EEG recordings it was possible to speculate that the spatio-temporal spreading of the first responses (i.e. N7, P13, N18) after M1 TMS could directly reflect the existence and the time-solved activity -experimentally modifiable- of specific cortico-cortical connections to the ipsilateral and contralateral nonprimary motor cortices and to the ipsilateral posterior parietal cortex. On the other hand, the spatio-temporal spreading of the other responses (P30, N44, P60, N100 and P180) could directly reflect the existence and the time-solved activity of a specific subcortical pathway (via thalamic nuclei and/or basal ganglia) projecting diffusely to the cortex (Ziemann and Rothwell, 2000; **Study III**, **Ferreri et al., 2011a**). Moreover, considering that the firing of cortical neurons enveloped in the EEG signal is associated with the activation of both fast and slow excitatory postsynaptic potentials (fEPSPs and sEPSPs respectively) as well as fast and slow inhibitory postsynaptic potentials (fIPSPs and sIPSPs, respectively), it was possible to speculate about the role of NMDA receptors in the generation and/or modulation of N7 (**Study III**, **Ferreri et al., 2011a**), the role of GABAA postsynaptic receptors in the generation and/or modulation of P13, N18, P30 and N44 (**Study III**, **Ferreri et al., 2011a**), and the role of pre-synaptic and postsynaptic GABAB receptors in the P60, N100, P180 and N280 genesis as well as the modulating role of ascending cholinergic projections (**Studies III and V**, respectively **Ferreri et al., 2011a and Ferreri et al., submitted**).

However, the TMS–EEG co-registration technique also has major disadvantages, such as its limited use to map TMS-induced activations in deep brain structures, its strong susceptibility to artefacts -muscle interference and eye-blinks- and the difficulties in accessing the very immediate cortical responses (i.e. within the first 10 ms). However, this last problem will be overcome by using new devices that do not require pinning the preamplifier output to a constant level during TMS, allowing continuous data recording without saturation of the EEG signals (Veniero et al., 2009). Consequently, it has recently become possible to characterize the very early cortical responses starting from 5-7 ms after TMS (Veniero et al., 2011 ; **Study III, Ferreri et al., 2011a**).

In conclusion, the concurrent use of EEG increases the application areas of TMS to the study of excitability, connectivity and plasticity of the human cortex, offering an intriguing new field for neuroscience research. Furthermore, TMS-EEG offers the possibility of

obtaining detailed information about the state of the brain. However, all this is possible only if appropriate techniques and methods are used to deal with the electromagnetic, electrode- polarization, eye-blink, muscle, auditory and other artefacts. Major progress has been made in fact in solving technical problems caused by the interfering effects of TMS on data acquisition in concurrent TMS-EEG studies. The remaining challenge now is to optimize experimental approaches in a way that it is possible to disentangle the direct effects caused by TMS in the brain from the contribution of non-cortical biological sources, for example those associated with auditory and somatosensory stimulation. A deeper understanding of the real effects of magnetic pulses on the cortex is required before routine clinical use of this technique is possible. The combined TMS-EEG technique is in its early stage and much methodological work is needed in order to fully unfold its potentiality in providing substantial new insights in the pathophysiology of neuro-psychiatric disease.

7 Summary and conclusion

The main findings of Studies I-V are:

1) TMS is a valuable method for studying cortical excitability in healthy subjects and AD patients. In AD patients motor cortex hyperexcitability and reorganization could allow prolonged preservation of motor function during the clinical course of the disease.
2) Brain excitability can be transiently manipulated in healthy subjects possibly modulating the balance between excitatory and inhibitory intracortical neurons. This manipulation can be reliably studied by means of TMS.
3) The combination of TMS and EEG enables the study of the neuronal circuits underlying effective connectivity within the human cortices as well as the mechanisms regulating the balance between inhibition and facilitation within the human cortices and the corticospinal pathway.
4) TMS, along with clinical, neuropsychological, and neuroimaging data, could be an inexpensive tool to follow the biological progression of Alzheimer's disease and it might supplement traditional methods of assessing the effects of therapy.
5) The combination of TMS and EEG is a promising and efficient tool for exploring brain cortical mechanisms integrating afferent information and modulating inhibitory phenomena, such as SAI, in healthy subjects and likely in neuropsychiatric patients.

This thesis presents new findings related to TMS and TMS-evoked brain activity, addressing some crucial issues for a better elucidation of the mechanisms underlying human brain neuroplasticity. The feasibility of the combination of TMS and EEG was clearly confirmed for the examination of the cortical excitability and connectivity of the human cerebral cortex, both in healthy and pathological conditions, also suggesting that TMS - with or without EEG - is a sensitive measure of the effect of different kinds of brain's activity manipulation. The possibility of studying cortical excitability and connectivity and to modulate them is clearly of considerable interest. Neuronal hyper-excitability is generally considered to play a role in promoting brain functional and even structural plasticity, either of the adaptive or maladaptive kind, with opposite effects on functional recovery, both in healthy people and in neurological diseases. This obviously implies the necessity for cautious manipulation, but improved knowledge of the basic mechanisms and awareness of the constrains would strengthen the possibility to guide the plastic potential of the brain, opening a broader field of new therapeutic and research perspectives (Platz and Rothwell, 2010). This may have a tremendous impact on future research efforts in a variety of neuropsychiatric disorders whose patho-physiology has been closely linked to the integrated function of multiple cortical areas. Taken together, these findings are therefore of importance for the adoption of the TMS and TMS-EEG co-registration techniques as a tool for basic neurophysiological research and, in the future, even for diagnostic purposes in clinical settings.

8 References

Abraham W. C. and Bear M. F. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci (1996). 19: 126-130.

Alagona G., Bella R., Ferri R., Carnemolla A., Pappalardo A., Costanzo E. and Pennisi G. Transcranial magnetic stimulation in Alzheimer disease: motor cortex excitability and cognitive severity. Neurosci Lett (2001). 314: 57-60.

Alle H., Heidegger T., Krivanekova L. and Ziemann U. Interactions between short-interval intracortical inhibition and short-latency afferent inhibition in human motor cortex. J Physiol (2009). 587: 5163-5176.

Alvarez V. A. and Sabatini B. L. Anatomical and physiological plasticity of dendritic spines. Annu Rev Neurosci (2007). 30: 79-97.

Amassian V. E. and Cracco R. Q. Human cerebral cortical responses to contralateral transcranial stimulation. Neurosurgery (1987). 20: 148-155.

Barker A. T., Jalinous R. and Freeston I. L. Non-invasive magnetic stimulation of human motor cortex. Lancet (1985). 1: 1106-1107.

Baumer T., Rothwell J. C. and Munchau A. Functional connectivity of the human premotor and motor cortex explored with TMS. Suppl Clin Neurophysiol (2003). 56: 160-169.

Bear M. F., Cooper L. N. and Ebner F. F. A physiological basis for a theory of synapse modification. Science (1987). 237: 42-48.

Becker N., Wierenga C. J., Fonseca R., Bonhoeffer T. and Nagerl U. V. LTD induction causes morphological changes of presynaptic boutons and reduces their contacts with spines. Neuron (2008). 60: 590-597.

Bender S., Basseler K., Sebastian I., Resch F., Kammer T., Oelkers-Ax R. and Weisbrod M. Electroencephalographic response to transcranial magnetic stimulation in children: Evidence for giant inhibitory potentials. Ann Neurol (2005). 58: 58-67.

Bestmann S., Ruff C. C., Blankenburg F., Weiskopf N., Driver J. and Rothwell J. C. Mapping causal interregional influences with concurrent TMS-fMRI. Exp Brain Res (2008). 191: 383-402.

Bikmullina R., Kičić D., Carlson S. and Nikulin V. V. Electrophysiological correlates of short-latency afferent inhibition: a combined EEG and TMS study. Exp Brain Res (2009). 194: 517-526.

Bliss T. V. and Collingridge G. L. A synaptic model of memory: long-term potentiation in the hippocampus. Nature (1993). 361: 31-39.

Bliss T. V. and Gardner-Medwin A. R. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol (1973). 232: 357-374.

Bonato C., Miniussi C. and Rossini P. M. Transcranial magnetic stimulation and cortical evoked potentials: a TMS/EEG co-registration study. Clin Neurophysiol (2006). 117: 1699-1707.

Bonnard M., Spieser L., Meziane H. B., de Graaf J. B. and Pailhous J. Prior intention can locally tune inhibitory processes in the primary motor cortex: direct evidence from combined TMS-EEG. Eur J Neurosci (2009). 30: 913-923.

Brasil-Neto J. P., Valls-Sole J., Pascual-Leone A., Cammarota A., Amassian V. E., Cracco R., Maccabee P., Cracco J., Hallett M. and Cohen L. G. Rapid modulation of human cortical motor outputs following ischaemic nerve block. Brain (1993). 116 (Pt 3): 511-525.

Broser P., Grinevich V., Osten P., Sakmann B. and Wallace D. J. Critical period plasticity of axonal arbors of layer 2/3 pyramidal neurons in rat somatosensory cortex: layer-specific reduction of projections into deprived cortical columns. Cereb Cortex (2008). 18: 1588-1603.

Brovelli A., Ding M., Ledberg A., Chen Y., Nakamura R. and Bressler S. L. Beta oscillations in a large-scale sensorimotor cortical network: directional influences revealed by Granger causality. Proc Natl Acad Sci U S A (2004). 101: 9849-9854.

Buonomano D. V. Distinct functional types of associative long-term potentiation in neocortical and hippocampal pyramidal neurons. J Neurosci (1999). 19: 6748-6754.

Buonomano D. V. and Merzenich M. M. Cortical plasticity: from synapses to maps. Annu Rev Neurosci (1998). 21: 149-186.

Burrone J. and Murthy V. N. Synaptic gain control and homeostasis. Curr Opin Neurobiol (2003). 13: 560-567.

Butefisch C. M., Kleiser R. and Seitz R. J. Post-lesional cerebral reorganisation: evidence from functional neuroimaging and transcranial magnetic stimulation. J Physiol Paris (2006). 99: 437-454.

Butler A. J. and Wolf S. L. Putting the brain on the map: use of transcranial magnetic stimulation to assess and induce cortical plasticity of upper-extremity movement. Phys Ther (2007). 87: 719-736.

Butz M., Lehmann K., Dammasch I. E. and Teuchert-Noodt G. A theoretical network model to analyse neurogenesis and synaptogenesis in the dentate gyrus. Neural Netw (2006). 19: 1490-1505.

Butz M. and Teuchert-Noodt G. A simulation model for compensatory plasticity in the prefrontal cortex inducing a cortico-cortical dysconnection in early brain development. J Neural Transm (2006). 113: 695-710.

Butz M., Teuchert-Noodt G., Grafen K. and van Ooyen A. Inverse relationship between adult hippocampal cell proliferation and synaptic rewiring in the dentate gyrus. Hippocampus (2008). 18: 879-898.

Butz M., Worgotter F. and van Ooyen A. Activity-dependent structural plasticity. Brain Res Rev (2009). 60: 287-305.

Calford M. B. and Tweedale R. Immediate and chronic changes in responses of somatosensory cortex in adult flying-fox after digit amputation. Nature (1988). 332: 446-448.

Cantello R., Gianelli M., Civardi C. and Mutani R. Magnetic brain stimulation: the silent period after the motor evoked potential. Neurology (1992). 42: 1951-1959.

Caspers H., Speckmann E. J. and Lehmenkuhler A. Electrogenesis of cortical DC potentials. Prog Brain Res (1980). 54: 3-15.

Celesia G. G., Kaufman D. and Cone S. Effects of age and sex on pattern electroretinograms and visual evoked potentials. Electroencephalogr Clin Neurophysiol (1987). 68: 161-171.

Chee M. W., Tan J. C., Zheng H., Parimal S., Weissman D. H., Zagorodnov V. and Dinges D. F. Lapsing during sleep deprivation is associated with distributed changes in brain activation. J Neurosci (2008). 28: 5519-5528.

Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res (2004). 154: 1-10.

Chen R., Corwell B., Yaseen Z., Hallett M. and Cohen L. G. Mechanisms of cortical reorganization in lower-limb amputees. J Neurosci (1998). 18: 3443-3450.

Chevaleyre V., Takahashi K. A. and Castillo P. E. Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci (2006). 29: 37-76.

Classen J., Wolters A., Stefan K., Wycislo M., Sandbrink F., Schmidt A. and Kunesch E. Paired associative stimulation. Suppl Clin Neurophysiol (2004). 57: 563-569.

Cline H. and Haas K. The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis. J Physiol (2008). 586: 1509-1517.

Conte A., Belvisi D., Iezzi E., Mari F., Inghilleri M. and Berardelli A. Effects of attention on inhibitory and facilitatory phenomena elicited by paired-pulse transcranial magnetic stimulation in healthy subjects. Exp Brain Res (2008). 186: 393-399.

Cracco R. Q., Amassian V. E., Maccabee P. J. and Cracco J. B. Comparison of human transcallosal responses evoked by magnetic coil and electrical stimulation. Electroencephalogr Clin Neurophysiol (1989). 74: 417-424.

Daskalakis Z. J., Farzan F., Barr M. S., Maller J. J., Chen R. and Fitzgerald P. B. Long-interval cortical inhibition from the dorsolateral prefrontal cortex: a TMS-EEG study. Neuropsychopharmacology (2008). 33: 2860-2869.

Daw N., Rao Y., Wang X. F., Fischer Q. and Yang Y. LTP and LTD vary with layer in rodent visual cortex. Vision Res (2004). 44: 3377-3380.

De Paola V., Holtmaat A., Knott G., Song S., Wilbrecht L., Caroni P. and Svoboda K. Cell type-specific structural plasticity of axonal branches and boutons in the adult neocortex. Neuron (2006). 49: 861-875.

Debanne D. Plasticity of neuronal excitability in vivo. J Physiol (2009). 587: 3057-3058.

Delorme A. and Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods (2004). 134: 9-21.

Di Lazzaro V., Oliviero A., Mazzone P., Pilato F., Saturno E., Insola A., Visocchi M., Colosimo C., Tonali P. A. and Rothwell J. C. Direct demonstration of long latency cortico-cortical inhibition in normal subjects and in a patient with vascular parkinsonism. Clin Neurophysiol (2002a). 113: 1673-1679.

Di Lazzaro V., Oliviero A., Pilato F., Saturno E., Dileone M., Marra C., Daniele A., Ghirlanda S., Gainotti G. and Tonali P. A. Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. J Neurol Neurosurg Psychiatry (2004). 75: 555-559.

Di Lazzaro V., Oliviero A., Pilato F., Saturno E., Dileone M., Marra C., Ghirlanda S., Ranieri F., Gainotti G. and Tonali P. Neurophysiological predictors of long term response to AChE inhibitors in AD patients. J Neurol Neurosurg Psychiatry (2005a). 76: 1064-1069.

Di Lazzaro V., Oliviero A., Pilato F., Saturno E., Dileone M. and Tonali P. A. Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease: evidence of impaired glutamatergic neurotransmission? Ann Neurol (2003a). 53: 824; author reply 824-825.

Di Lazzaro V., Oliviero A., Profice P., Pennisi M. A., Di Giovanni S., Zito G., Tonali P. and Rothwell J. C. Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. Exp Brain Res (2000). 135: 455-461.

Di Lazzaro V., Oliviero A., Profice P., Pennisi M. A., Pilato F., Zito G., Dileone M., Nicoletti R., Pasqualetti P. and Tonali P. A. Ketamine increases human motor cortex excitability to transcranial magnetic stimulation. J Physiol (2003b). 547: 485-496.

Di Lazzaro V., Oliviero A., Saturno E., Dileone M., Pilato F., Nardone R., Ranieri F., Musumeci G., Fiorilla T. and Tonali P. Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. J Physiol (2005b). 564: 661-668.

Di Lazzaro V., Oliviero A., Saturno E., Pilato F., Insola A., Mazzone P., Profice P., Tonali P. and Rothwell J. C. The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. Exp Brain Res (2001). 138: 268-273.

Di Lazzaro V., Oliviero A., Tonali P. A., Marra C., Daniele A., Profice P., Saturno E., Pilato F., Masullo C. and Rothwell J. C. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. Neurology (2002b). 59: 392-397.

Di Lazzaro V., Pilato F., Dileone M., Profice P., Oliviero A., Mazzone P., Insola A., Ranieri F., Meglio M., Tonali P. A. and Rothwell J. C. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol (2008). 586: 3871-3879.

Di Lazzaro V., Pilato F., Dileone M., Profice P., Ranieri F., Ricci V., Bria P., Tonali P. A. and Ziemann U. Segregating two inhibitory circuits in human motor cortex at the level of GABAA receptor subtypes: a TMS study. Clin Neurophysiol (2007). 118: 2207-2214.

Di Lazzaro V., Pilato F., Dileone M., Tonali P. A. and Ziemann U. Dissociated effects of diazepam and lorazepam on short-latency afferent inhibition. J Physiol (2005c). 569: 315-323.

Di Lazzaro V., Rothwell J. C., Oliviero A., Profice P., Insola A., Mazzone P. and Tonali P. Intracortical origin of the short latency facilitation produced by pairs of threshold magnetic stimuli applied to human motor cortex. Exp Brain Res (1999). 129: 494-499.

Duffau H. Brain plasticity: from pathophysiological mechanisms to therapeutic applications. J Clin Neurosci (2006). 13: 885-897.

Eder M., Zieglgansberger W. and Dodt H. U. Neocortical long-term potentiation and long-term depression: site of expression investigated by infrared-guided laser stimulation. J Neurosci (2002). 22: 7558-7568.

Egger V., Feldmeyer D. and Sakmann B. Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. Nat Neurosci (1999). 2: 1098-1105.

Epstein C. M. and Davey K. R. Iron-core coils for transcranial magnetic stimulation. J Clin Neurophysiol (2002). 19: 376-381.

Esser S. K., Huber R., Massimini M., Peterson M. J., Ferrarelli F. and Tononi G. A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. Brain Res Bull (2006). 69: 86-94.

Farzan F., Barr M. S., Wong W., Chen R., Fitzgerald P. B. and Daskalakis Z. J. Suppression of gamma-oscillations in the dorsolateral prefrontal cortex following long interval cortical inhibition: a TMS-EEG study. Neuropsychopharmacology (2009). 34: 1543-1551.

Feig S. and Lipton P. Pairing the cholinergic agonist carbachol with patterned Schaffer collateral stimulation initiates protein synthesis in hippocampal CA1 pyramidal cell dendrites via a muscarinic, NMDA-dependent mechanism. J Neurosci (1993). 13: 1010-1021.

Feldman D. E. Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci (2009). 32: 33-55.

Feldman D. E. and Brecht M. Map plasticity in somatosensory cortex. Science (2005). 310: 810-815.

Ferrarelli F., Massimini M., Peterson M. J., Riedner B. A., Lazar M., Murphy M. J., Huber R., Rosanova M., Alexander A. L., Kalin N. and Tononi G. Reduced evoked gamma oscillations in the frontal cortex in schizophrenia patients: a TMS/EEG study. Am J Psychiatry (2008). 165: 996-1005.

Ferreri F., Curcio G., Pasqualetti P., De Gennaro L., Fini R. and Rossini P. M. Mobile phone emissions and human brain excitability. Ann Neurol (2006). 60: 188-196.

Ferreri F., Pasqualetti P., Määttä S., Ponzo D., Ferrarelli F., Tononi G., Mervaala E., Miniussi C. and Rossini P. M. Human brain connectivity during single and paired pulse transcranial magnetic stimulation. Neuroimage (2011a). 54: 90-102.

Ferreri F., Pasqualetti P., Määttä S., Ponzo D., Guerra A., Bressi F., Chiovenda P., Del Duca M., Giambattistelli F., Ursini F., Tombini M., Vernieri F. and Rossini P. M. Motor cortex excitability in Alzheimer's disease: a transcranial magnetic stimulation follow-up study. Neurosci Lett (2011b). 492: 94-98.

Ferreri F., Pauri F., Pasqualetti P., Fini R., Dal Forno G. and Rossini P. M. Motor cortex excitability in Alzheimer's disease: a transcranial magnetic stimulation study. Ann Neurol (2003). 53: 102-108.

Fitzgerald P. B., Benitez J., Daskalakis J. Z., De Castella A. and Kulkarni J. The treatment of recurring auditory hallucinations in schizophrenia with rTMS. World J Biol Psychiatry (2006). 7: 119-122.

Florence S. L., Taub H. B. and Kaas J. H. Large-scale sprouting of cortical connections after peripheral injury in adult macaque monkeys. Science (1998). 282: 1117-1121.

Foeller E. and Feldman D. E. Synaptic basis for developmental plasticity in somatosensory cortex. Curr Opin Neurobiol (2004). 14: 89-95.

Fox K. Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex. Neuroscience (2002). 111: 799-814.

Fox K. and Wong R. O. A comparison of experience-dependent plasticity in the visual and somatosensory systems. Neuron (2005). 48: 465-477.

Francis P. T., Pangalos M. N., Stephens P. H., Bartlett J. R., Bridges P. K., Malizia A. L., Neary D., Procter A. W., Thomas D. J. and Bowen D. M. Antemortem measurements of neurotransmission: possible implications for pharmacotherapy of Alzheimer's disease and depression. J Neurol Neurosurg Psychiatry (1993). 56: 80-84.

Freeman J. A. An electronic stimulus artifact suppressor. Electroencephalogr Clin Neurophysiol (1971). 31: 170-172.

Friston K. J., Harrison L. and Penny W. Dynamic causal modelling. Neuroimage (2003). 19: 1273-1302.

Fuggetta G., Fiaschi A. and Manganotti P. Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG and TMS study. Neuroimage (2005). 27: 896-908.

Gaiarsa J. L., Caillard O. and Ben-Ari Y. Long-term plasticity at GABAergic and glycinergic synapses: mechanisms and functional significance. Trends Neurosci (2002). 25: 564-570.

Guerra A., Assenza F., Bressi F., Scrascia F., Del Duca M., Ursini F., Vollaro S., Trotta L., Tombini M., Chisari C. and Ferreri F. Transcranial magnetic stimulation studies in Alzheimer's disease. Int J Alzheimers Dis (2011). 2011: 263817.

Gugino L. D., Romero J. R., Aglio L., Titone D., Ramirez M., Pascual-Leone A., Grimson E., Weisenfeld N., Kikinis R. and Shenton M. E. Transcranial magnetic stimulation coregistered with MRI: a comparison of a guided versus blind stimulation technique and its effect on evoked compound muscle action potentials. Clin Neurophysiol (2001). 112: 1781-1792.

Hallett M. Motor cortex plasticity. Electroencephalogr Clin Neurophysiol Suppl (1999). 50: 85-91.

Hallett M. Transcranial magnetic stimulation and the human brain. Nature (2000). 406: 147-150.

Hallett M. Plasticity of the human motor cortex and recovery from stroke. Brain Res Brain Res Rev (2001). 36: 169-174.

Hallett M. Transcranial magnetic stimulation: a primer. Neuron (2007). 55: 187-199.

Hallett M. Neurophysiology of dystonia: The role of inhibition. Neurobiol Dis (2011). 42: 177-184.

Hallett M., Epstein C. M., Berardelli A., Sackeim H. and Maccabee P. Topics in transcranial magnetic stimulation. Suppl Clin Neurophysiol (2000). 53: 301-311.

Han L., Cole M., Bellavance F., McCusker J. and Primeau F. Tracking cognitive decline in Alzheimer's disease using the mini-mental state examination: a meta-analysis. Int Psychogeriatr (2000). 12: 231-247.

Hanajima R., Furubayashi T., Iwata N. K., Shiio Y., Okabe S., Kanazawa I. and Ugawa Y. Further evidence to support different mechanisms underlying intracortical inhibition of the motor cortex. Exp Brain Res (2003). 151: 427-434.

Hansel C., Linden D. J. and D'Angelo E. Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. Nat Neurosci (2001). 4: 467-475.

Hasey G. M. Transcranial magnetic stimulation: using a law of physics to treat psychopathology. J Psychiatry Neurosci (1999). 24: 97-101.

Hebb D. O. Temperament in chimpanzees; method of analysis. J Comp Physiol Psychol (1949). 42: 192-206.

Helias M., Rotter S., Gewaltig M. O. and Diesmann M. Structural plasticity controlled by calcium based correlation detection. helias@bccn.uni-freiburg.de. Front Comput Neurosci (2008). 2: 7.

Hensch T. K. Critical period plasticity in local cortical circuits. Nat Rev Neurosci (2005). 6: 877-888.

Hoffman R. E. and Cavus I. Slow transcranial magnetic stimulation, long-term depotentiation, and brain hyperexcitability disorders. Am J Psychiatry (2002). 159: 1093-1102.

Holtmaat A. J., Trachtenberg J. T., Wilbrecht L., Shepherd G. M., Zhang X., Knott G. W. and Svoboda K. Transient and persistent dendritic spines in the neocortex in vivo. Neuron (2005). 45: 279-291.

Homan R. W., Herman J. and Purdy P. Cerebral location of international 10-20 system electrode placement. Electroencephalogr Clin Neurophysiol (1987). 66: 376-382.

Horwitz B. The elusive concept of brain connectivity. Neuroimage (2003). 19: 466-470.

Huang C. C., Liang Y. C. and Hsu K. S. Characterization of the mechanism underlying the reversal of long term potentiation by low frequency stimulation at hippocampal CA1 synapses. J Biol Chem (2001). 276: 48108-48117.

Huang Y. Z., Edwards M. J., Rounis E., Bhatia K. P. and Rothwell J. C. Theta burst stimulation of the human motor cortex. Neuron (2005). 45: 201-206.

Huber R., Graf T., Cote K. A., Wittmann L., Gallmann E., Matter D., Schuderer J., Kuster N., Borbely A. A. and Achermann P. Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG. Neuroreport (2000). 11: 3321-3325.

Huber R., Määttä S., Esser S. K., Sarasso S., Ferrarelli F., Watson A., Ferreri F., Peterson M. J. and Tononi G. Measures of cortical plasticity after transcranial paired associative stimulation predict changes in electroencephalogram slow-wave activity during subsequent sleep. J Neurosci (2008). 28: 7911-7918.

Ilmoniemi R. J. and Kičić D. Methodology for combined TMS and EEG. Brain Topogr (2010). 22: 233-248.

Ilmoniemi R. J., Virtanen J., Ruohonen J., Karhu J., Aronen H. J., Naatanen R. and Katila T. Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. Neuroreport (1997). 8: 3537-3540.

Isaac J. T., Crair M. C., Nicoll R. A. and Malenka R. C. Silent synapses during development of thalamocortical inputs. Neuron (1997). 18: 269-280.

Jacob V., Brasier D. J., Erchova I., Feldman D. and Shulz D. E. Spike timing-dependent synaptic depression in the in vivo barrel cortex of the rat. J Neurosci (2007). 27: 1271-1284.

Jung P. and Ziemann U. Homeostatic and nonhomeostatic modulation of learning in human motor cortex. J Neurosci (2009). 29: 5597-5604.

Kähkönen S., Kesäniemi M., Nikouline V. V., Karhu J., Ollikainen M., Holi M. and Ilmoniemi R. J. Ethanol modulates cortical activity: direct evidence with combined TMS and EEG. Neuroimage (2001). 14: 322-328.

Kähkönen S., Komssi S., Wilenius J. and Ilmoniemi R. J. Prefrontal transcranial magnetic stimulation produces intensity-dependent EEG responses in humans. Neuroimage (2005). 24: 955-960.

Kähkönen S. and Wilenius J. Effects of alcohol on TMS-evoked N100 responses. J Neurosci Methods (2007). 166: 104-108.

Kähkönen S., Wilenius J., Komssi S. and Ilmoniemi R. J. Distinct differences in cortical reactivity of motor and prefrontal cortices to magnetic stimulation. Clin Neurophysiol (2004). 115: 583-588.

Kaneko M., Stellwagen D., Malenka R. C. and Stryker M. P. Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. Neuron (2008). 58: 673-680.

Kičić D., Lioumis P., Ilmoniemi R. J. and Nikulin V. V. Bilateral changes in excitability of sensorimotor cortices during unilateral movement: combined electroencephalographic and transcranial magnetic stimulation study. Neuroscience (2008). 152: 1119-1129.

Kim S. J. and Linden D. J. Ubiquitous plasticity and memory storage. Neuron (2007). 56: 582-592.

Kleim J. A., Lussnig E., Schwarz E. R., Comery T. A. and Greenough W. T. Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. J Neurosci (1996). 16: 4529-4535.

Klinkenberg I., Sambeth A. and Blokland A. Acetylcholine and attention. Behav Brain Res (2011). 221: 430-442.

Kobayashi M. and Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol (2003). 2: 145-156.

Komssi S., Aronen H. J., Huttunen J., Kesäniemi M., Soinne L., Nikouline V. V., Ollikainen M., Roine R. O., Karhu J., Savolainen S. and Ilmoniemi R. J. Ipsi- and contralateral EEG reactions to transcranial magnetic stimulation. Clin Neurophysiol (2002). 113: 175-184.

Komssi S. and Kähkönen S. The novelty value of the combined use of electroencephalography and transcranial magnetic stimulation for neuroscience research. Brain Res Rev (2006). 52: 183-192.

Komssi S., Kähkönen S. and Ilmoniemi R. J. The effect of stimulus intensity on brain responses evoked by transcranial magnetic stimulation. Hum Brain Mapp (2004). 21: 154-164.

Komssi S., Savolainen P., Heiskala J. and Kähkönen S. Excitation threshold of the motor cortex estimated with transcranial magnetic stimulation electroencephalography. Neuroreport (2007). 18: 13-16.

Krall W. J., Sramek J. J. and Cutler N. R. Cholinesterase inhibitors: a therapeutic strategy for Alzheimer disease. Ann Pharmacother (1999). 33: 441-450.

Krings T., Buchbinder B. R., Butler W. E., Chiappa K. H., Jiang H. J., Cosgrove G. R. and Rosen B. R. Functional magnetic resonance imaging and transcranial magnetic stimulation: complementary approaches in the evaluation of cortical motor function. Neurology (1997). 48: 1406-1416.

Krings T., Reinges M. H., Foltys H., Cosgrove G. R. and Thron A. Multimodality neuroimaging: research and clinical applications. Neurol Clin Neurophysiol (2001). 2001: 2-11.

Krnjevic K., Randic M. and Straughan D. W. Nature of a cortical inhibitory process. J Physiol (1966). 184: 49-77.

Kujirai T., Caramia M. D., Rothwell J. C., Day B. L., Thompson P. D., Ferbert A., Wroe S., Asselman P. and Marsden C. D. Corticocortical inhibition in human motor cortex. J Physiol (1993). 471: 501-519.

Kullmann D. M. and Lamsa K. P. Long-term synaptic plasticity in hippocampal interneurons. Nat Rev Neurosci (2007). 8: 687-699.

Lee L., Harrison L. M. and Mechelli A. A report of the functional connectivity workshop, Dusseldorf 2002. Neuroimage (2003). 19: 457-465.

Lehmann D. and Skrandies W. Reference-free identification of components of checkerboard-evoked multichannel potential fields. Electroencephalogr Clin Neurophysiol (1980). 48: 609-621.

Liao D., Hessler N. A. and Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature (1995). 375: 400-404.

Liepert J., Bar K. J., Meske U. and Weiller C. Motor cortex disinhibition in Alzheimer's disease. Clin Neurophysiol (2001). 112: 1436-1441.

Liepert J., Classen J., Cohen L. G. and Hallett M. Task-dependent changes of intracortical inhibition. Exp Brain Res (1998). 118: 421-426.

Lioumis P., Kičić D., Savolainen P., Makela J. P. and Kähkönen S. Reproducibility of TMS-Evoked EEG responses. Hum Brain Mapp (2009). 30: 1387-1396.

Lisman J., Lichtman J. W. and Sanes J. R. LTP: perils and progress. Nat Rev Neurosci (2003). 4: 926-929.

Lopes da Silva F. Neural mechanisms underlying brain waves: from neural membranes to networks. Electroencephalogr Clin Neurophysiol (1991). 79: 81-93.

Malenka R. C. and Bear M. F. LTP and LTD: an embarrassment of riches. Neuron (2004). 44: 5-21.

Maren S. Synaptic mechanisms of associative memory in the amygdala. Neuron (2005). 47: 783-786.

Mariorenzi R., Zarola F., Caramia M. D., Paradiso C. and Rossini P. M. Non-invasive evaluation of central motor tract excitability changes following peripheral nerve stimulation in healthy humans. Electroencephalogr Clin Neurophysiol (1991). 81: 90-101.

Markram H. and Tsodyks M. Redistribution of synaptic efficacy: a mechanism to generate infinite synaptic input diversity from a homogeneous population of neurons without changing absolute synaptic efficacies. J Physiol Paris (1996). 90: 229-232.

Martin K. C., Barad M. and Kandel E. R. Local protein synthesis and its role in synapse-specific plasticity. Curr Opin Neurobiol (2000). 10: 587-592.

Masliah E., Alford M., DeTeresa R., Mallory M. and Hansen L. Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. Ann Neurol (1996). 40: 759-766.

Massey P. V. and Bashir Z. I. Long-term depression: multiple forms and implications for brain function. Trends Neurosci (2007). 30: 176-184.

Massimini M., Ferrarelli F., Huber R., Esser S. K., Singh H. and Tononi G. Breakdown of cortical effective connectivity during sleep. Science (2005). 309: 2228-2232.

Matsumoto R., Nair D. R., LaPresto E., Bingaman W., Shibasaki H. and Luders H. O. Functional connectivity in human cortical motor system: a cortico-cortical evoked potential study. Brain (2007). 130: 181-197.

McKernan M. G. and Shinnick-Gallagher P. Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature (1997). 390: 607-611.

Meliza C. D. and Dan Y. Receptive-field modification in rat visual cortex induced by paired visual stimulation and single-cell spiking. Neuron (2006). 49: 183-189.

Merton P. A. and Morton H. B. Stimulation of the cerebral cortex in the intact human subject. Nature (1980). 285: 227.

Nichols T. E. and Holmes A. P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp (2002). 15: 1-25.

Nikouline V., Ruohonen J. and Ilmoniemi R. J. The role of the coil click in TMS assessed with simultaneous EEG. Clin Neurophysiol (1999). 110: 1325-1328.

Nikulin V. V., Kičić D., Kähkönen S. and Ilmoniemi R. J. Modulation of electroencephalographic responses to transcranial magnetic stimulation: evidence for changes in cortical excitability related to movement. Eur J Neurosci (2003). 18: 1206-1212.

Nobili F., Vitali P., Canfora M., Girtler N., De Leo C., Mariani G., Pupi A. and Rodriguez G. Effects of long-term Donepezil therapy on rCBF of Alzheimer's patients. Clin Neurophysiol (2002). 113: 1241-1248.

Nudo R. J. Postinfarct cortical plasticity and behavioral recovery. Stroke (2007). 38: 840-845.

Nudo R. J., Wise B. M., SiFuentes F. and Milliken G. W. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. Science (1996). 272: 1791-1794.

Oliveri M., Caltagirone C., Filippi M. M., Traversa R., Cicinelli P., Pasqualetti P. and Rossini P. M. Paired transcranial magnetic stimulation protocols reveal a pattern of inhibition and facilitation in the human parietal cortex. J Physiol (2000a). 529 Pt 2: 461-468.

Oliveri M., Rossini P. M., Filippi M. M., Traversa R., Cicinelli P., Palmieri M. G., Pasqualetti P. and Caltagirone C. Time-dependent activation of parieto-frontal networks for directing attention to tactile space. A study with paired transcranial magnetic stimulation pulses in right-brain-damaged patients with extinction. Brain (2000b). 123 (Pt 9): 1939-1947.

Oliveri M., Rossini P. M., Traversa R., Cicinelli P., Filippi M. M., Pasqualetti P., Tomaiuolo F. and Caltagirone C. Left frontal transcranial magnetic stimulation reduces contralesional extinction in patients with unilateral right brain damage. Brain (1999). 122 (Pt 9): 1731-1739.

Palop J. J., Chin J. and Mucke L. A network dysfunction perspective on neurodegenerative diseases. Nature (2006). 443: 768-773.

Pascual-Leone A., Amedi A., Fregni F. and Merabet L. B. The plastic human brain cortex. Annu Rev Neurosci (2005). 28: 377-401.

Pascual-Leone A., Nguyet D., Cohen L. G., Brasil-Neto J. P., Cammarota A. and Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol (1995). 74: 1037-1045.

Pascual-Leone A., Peris M., Tormos J. M., Pascual A. P. and Catala M. D. Reorganization of human cortical motor output maps following traumatic forearm amputation. Neuroreport (1996). 7: 2068-2070.

Pascual-Leone A., Tarazona F. and Catala M. D. Applications of transcranial magnetic stimulation in studies on motor learning. Electroencephalogr Clin Neurophysiol Suppl (1999). 51: 157-161.

Pascual-Leone A., Tormos J. M., Keenan J., Tarazona F., Canete C. and Catala M. D. Study and modulation of human cortical excitability with transcranial magnetic stimulation. J Clin Neurophysiol (1998). 15: 333-343.

Paus T., Castro-Alamancos M. A. and Petrides M. Cortico-cortical connectivity of the human mid-dorsolateral frontal cortex and its modulation by repetitive transcranial magnetic stimulation. Eur J Neurosci (2001a). 14: 1405-1411.

Paus T., Sipila P. K. and Strafella A. P. Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. J Neurophysiol (2001b). 86: 1983-1990.

Pennisi G., Alagona G., Ferri R., Greco S., Santonocito D., Pappalardo A. and Bella R. Motor cortex excitability in Alzheimer disease: one year follow-up study. Neurosci Lett (2002). 329: 293-296.

Pepin J. L., Bogacz D., de Pasqua V. and Delwaide P. J. Motor cortex inhibition is not impaired in patients with Alzheimer's disease: evidence from paired transcranial magnetic stimulation. J Neurol Sci (1999). 170: 119-123.

Pfurtscheller G., Kalcher J., Neuper C., Flotzinger D. and Pregenzer M. On-line EEG classification during externally-paced hand movements using a neural network-based classifier. Electroencephalogr Clin Neurophysiol (1996). 99: 416-425.

Pfurtscheller G. and Lopes da Silva F. H. Event-related EEG/MEG synchronization and desynchronization: basic principles. Clin Neurophysiol (1999). 110: 1842-1857.

Platz T. and Rothwell J. C. Brain stimulation and brain repair--rTMS: from animal experiment to clinical trials--what do we know? Restor Neurol Neurosci (2010). 28: 387-398.

Polania R., Nitsche M. A. and Paulus W. Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. Hum Brain Mapp (2010).

Polania R., Paulus W., Antal A. and Nitsche M. A. Introducing graph theory to track for neuroplastic alterations in the resting human brain: a transcranial direct current stimulation study. Neuroimage (2011). 54: 2287-2296.

Post A. and Keck M. E. Transcranial magnetic stimulation as a therapeutic tool in psychiatry: what do we know about the neurobiological mechanisms? J Psychiatr Res (2001). 35: 193-215.

Raisman G. Neuronal plasticity in the septal nuclei of the adult rat. Brain Res (1969). 14: 25-48.

Raymond C. R. LTP forms 1, 2 and 3: different mechanisms for the 'long' in long-term potentiation. Trends Neurosci (2007). 30: 167-175.

Renger J. J., Hartman K. N., Tsuchimoto Y., Yokoi M., Nakanishi S. and Hensch T. K. Experience-dependent plasticity without long-term depression by type 2 metabotropic glutamate receptors in developing visual cortex. Proc Natl Acad Sci U S A (2002). 99: 1041-1046.

Rogan M. T., Staubli U. V. and LeDoux J. E. Fear conditioning induces associative long-term potentiation in the amygdala. Nature (1997). 390: 604-607.

Rosanova M., Casali A., Bellina V., Resta F., Mariotti M. and Massimini M. Natural frequencies of human corticothalamic circuits. J Neurosci (2009). 29: 7679-7685.

Rosenthal J., Waller H. J. and Amassian V. E. An analysis of the activation of motor cortical neurons by surface stimulation. J Neurophysiol (1967). 30: 844-858.

Rossi S. and Rossini P. M. TMS in cognitive plasticity and the potential for rehabilitation. Trends Cogn Sci (2004). 8: 273-279.

Rossini P. M., Barker A. T., Berardelli A., Caramia M. D., Caruso G., Cracco R. Q., Dimitrijevic M. R., Hallett M., Katayama Y., Lucking C. H. and et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol (1994). 91: 79-92.

Rossini P. M., Calautti C., Pauri F. and Baron J. C. Post-stroke plastic reorganisation in the adult brain. Lancet Neurol (2003). 2: 493-502.

Rossini P. M., Caramia M. and Zarola F. Central motor tract propagation in man: studies with non-invasive, unifocal, scalp stimulation. Brain Res (1987a). 415: 211-225.

Rossini P. M. and Dal Forno G. Neuronal post-stroke plasticity in the adult. Restor Neurol Neurosci (2004). 22: 193-206.

Rossini P. M., Gigli G. L., Marciani M. G., Zarola F. and Caramia M. Non-invasive evaluation of input-output characteristics of sensorimotor cerebral areas in healthy humans. Electroencephalogr Clin Neurophysiol (1987b). 68: 88-100.

Rossini P. M., Micera S., Benvenuto A., Carpaneto J., Cavallo G., Citi L., Cipriani C., Denaro L., Denaro V., Di Pino G., Ferreri F., Guglielmelli E., Hoffmann K. P., Raspopovic S., Rigosa J., Rossini L., Tombini M. and Dario P. Double nerve intraneural interface implant on a human amputee for robotic hand control. Clin Neurophysiol (2010). 121: 777-783.

Rossini P. M. and Pauri F. Neuromagnetic integrated methods tracking human brain mechanisms of sensorimotor areas 'plastic' reorganisation. Brain Res Brain Res Rev (2000). 33: 131-154.

Rossini P. M., Rigosa J., Micera S., Assenza G., Rossini L. and Ferreri F. Stump nerve signals during transcranial magnetic motor cortex stimulation recorded in an amputee via longitudinal intrafascicular electrodes. Exp Brain Res (2011).

Rossini P. M. and Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. Neurology (2007). 68: 484-488.

Rossini P. M., Rossi S., Tecchio F., Pasqualetti P., Finazzi-Agro A. and Sabato A. Focal brain stimulation in healthy humans: motor maps changes following partial hand sensory deprivation. Neurosci Lett (1996). 214: 191-195.

Roth B. J., Pascual-Leone A., Cohen L. G. and Hallett M. The heating of metal electrodes during rapid-rate magnetic stimulation: a possible safety hazard. Electroencephalogr Clin Neurophysiol (1992). 85: 116-123.

Rothwell J. Transcranial magnetic stimulation as a method for investigating the plasticity of the brain in Parkinson's disease and dystonia. Parkinsonism Relat Disord (2007). 13 Suppl 3: S417-420.

Rothwell J. C. Evoked potentials, magnetic stimulation studies, and event-related potentials. Curr Opin Neurol (1993). 6: 715-723.

Rothwell J. C. Using transcranial magnetic stimulation methods to probe connectivity between motor areas of the brain. Hum Mov Sci (2010).

Rothwell J. C., Thompson P. D., Day B. L., Dick J. P., Kachi T., Cowan J. M. and Marsden C. D. Motor cortex stimulation in intact man. 1. General characteristics of EMG responses in different muscles. Brain (1987). 110 (Pt 5): 1173-1190.

Rumpel S., Hatt H. and Gottmann K. Silent synapses in the developing rat visual cortex: evidence for postsynaptic expression of synaptic plasticity. J Neurosci (1998). 18: 8863-8874.

Sanes J. N., Dimitrov B. and Hallett M. Motor learning in patients with cerebellar dysfunction. Brain (1990). 113 (Pt 1): 103-120.

Sanes J. N. and Donoghue J. P. Plasticity and primary motor cortex. Annu Rev Neurosci (2000). 23: 393-415.

Sanger T. D., Garg R. R. and Chen R. Interactions between two different inhibitory systems in the human motor cortex. J Physiol (2001). 530: 307-317.

Shimizu H., Tsuda T., Shiga Y., Miyazawa K., Onodera Y., Matsuzaki M., Nakashima I., Furukawa K., Aoki M., Kato H., Yamazaki T. and Itoyama Y. Therapeutic efficacy of transcranial magnetic stimulation for hereditary spinocerebellar degeneration. Tohoku J Exp Med (1999). 189: 203-211.

Shors T. J., Gallegos R. A. and Breindl A. Transient and persistent consequences of acute stress on long-term potentiation (LTP), synaptic efficacy, theta rhythms and bursts in area CA1 of the hippocampus. Synapse (1997). 26: 209-217.

Siebner H. R., Bergmann T. O., Bestmann S., Massimini M., Johansen-Berg H., Mochizuki H., Bohning D. E., Boorman E. D., Groppa S., Miniussi C., Pascual-Leone A., Huber R., Taylor P. C., Ilmoniemi R. J., De Gennaro L., Strafella A. P., Kähkönen S., Kloppel S., Frisoni G. B., George M. S. Consensus paper: combining transcranial stimulation with neuroimaging. Brain Stimul (2009). 2: 58-80.

Siebner H. R. and Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res (2003). 148: 1-16.

Siebner H. R., Willoch F., Peller M., Auer C., Boecker H., Conrad B. and Bartenstein P. Imaging brain activation induced by long trains of repetitive transcranial magnetic stimulation. Neuroreport (1998). 9: 943-948.

Sjöström P. J., Rancz E. A., Roth A. and Hausser M. Dendritic excitability and synaptic plasticity. Physiol Rev (2008). 88: 769-840.

Skibinska-Kijek A., Radwanska A. and Kossut M. Alpha calcium/calmodulin dependent protein kinase II in learning-dependent plasticity of mouse somatosensory cortex. Neuroscience (2008). 151: 750-757.

Sossin W. S. Molecular memory traces. Prog Brain Res (2008). 169: 3-25.

Sporns O. The non-random brain: efficiency, economy, and complex dynamics. Front Comput Neurosci (2011). 5: 5.

Sporns O., Chialvo D. R., Kaiser M. and Hilgetag C. C. Organization, development and function of complex brain networks. Trends Cogn Sci (2004). 8: 418-425.

Stefan K., Kunesch E., Benecke R., Cohen L. G. and Classen J. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol (2002). 543: 699-708.

Stefan K., Kunesch E., Cohen L. G., Benecke R. and Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain (2000). 123 Pt 3: 572-584.

Stellwagen D. and Malenka R. C. Synaptic scaling mediated by glial TNF-alpha. Nature (2006). 440: 1054-1059.

Steriade M., McCormick D. A. and Sejnowski T. J. Thalamocortical oscillations in the sleeping and aroused brain. Science (1993). 262: 679-685.

Thut G. and Miniussi C. New insights into rhythmic brain activity from TMS-EEG studies. Trends Cogn Sci (2009). 13: 182-189.

Tokimura H., Di Lazzaro V., Tokimura Y., Oliviero A., Profice P., Insola A., Mazzone P., Tonali P. and Rothwell J. C. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol (2000). 523 Pt 2: 503-513.

Toni N., Buchs P. A., Nikonenko I., Bron C. R. and Muller D. LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature (1999). 402: 421-425.

Tononi G., Sporns O. and Edelman G. M. A measure for brain complexity: relating functional segregation and integration in the nervous system. Proc Natl Acad Sci U S A (1994). 91: 5033-5037.

Topka H., Cohen L. G., Cole R. A. and Hallett M. Reorganization of corticospinal pathways following spinal cord injury. Neurology (1991). 41: 1276-1283.

Trachtenberg J. T., Chen B. E., Knott G. W., Feng G., Sanes J. R., Welker E. and Svoboda K. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature (2002). 420: 788-794.

Trachtenberg J. T. and Stryker M. P. Rapid anatomical plasticity of horizontal connections in the developing visual cortex. J Neurosci (2001). 21: 3476-3482.

Turrigiano G. G. and Nelson S. B. Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci (2004). 5: 97-107.

Udupa K., Ni Z., Gunraj C. and Chen R. Interactions between short latency afferent inhibition and long interval intracortical inhibition. Exp Brain Res (2009). 199: 177-183.

Van Der Werf Y. D. and Paus T. The neural response to transcranial magnetic stimulation of the human motor cortex. I. Intracortical and cortico-cortical contributions. Exp Brain Res (2006). 175: 231-245.

Van Der Werf Y. D., Sadikot A. F., Strafella A. P. and Paus T. The neural response to transcranial magnetic stimulation of the human motor cortex. II. Thalamocortical contributions. Exp Brain Res (2006). 175: 246-255.

Veniero D., Bortoletto M. and Miniussi C. TMS-EEG co-registration: on TMS-induced artifact. Clin Neurophysiol (2009). 120: 1392-1399.

Veniero D., Brignani D., Thut G. and Miniussi C. Alpha-generation as basic response-signature to transcranial magnetic stimulation (TMS) targeting the human resting motor cortex: A TMS/EEG co-registration study. Psychophysiology (2011).

Virtanen J., Ruohonen J., Naatanen R. and Ilmoniemi R. J. Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. Med Biol Eng Comput (1999). 37: 322-326.

Volkow N. D., Tomasi D., Wang G. J., Vaska P., Fowler J. S., Telang F., Alexoff D., Logan J. and Wong C. Effects of cell phone radiofrequency signal exposure on brain glucose metabolism. JAMA (2011). 305: 808-813.

Walsh V. and Cowey A. Transcranial magnetic stimulation and cognitive neuroscience. Nat Rev Neurosci (2000). 1: 73-79.

Werhahn K. J., Kunesch E., Noachtar S., Benecke R. and Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. J Physiol (1999). 517 (Pt 2): 591-597.

Wolff J. R., Joo F. and Kasa P. Modulation by GABA of neuroplasticity in the central and peripheral nervous system. Neurochem Res (1993). 18: 453-461.

Ziemann U. Intracortical inhibition and facilitation in the conventional paired TMS paradigm. Electroencephalogr Clin Neurophysiol Suppl (1999). 51: 127-136.

Ziemann U. Pharmacology of TMS. Suppl Clin Neurophysiol (2003). 56: 226-231.

Ziemann U. LTP-like plasticity in human motor cortex. Suppl Clin Neurophysiol (2004). 57: 702-707.

Ziemann U. Transcranial magnetic stimulation at the interface with other techniques: a powerful tool for studying the human cortex. Neuroscientist (2011). 17: 368-381.

Ziemann U., Chen R., Cohen L. G. and Hallett M. Dextromethorphan decreases the excitability of the human motor cortex. Neurology (1998). 51: 1320-1324.

Ziemann U., Lonnecker S. and Paulus W. Inhibition of human motor cortex by ethanol. A transcranial magnetic stimulation study. Brain (1995). 118 (Pt 6): 1437-1446.

Ziemann U., Paulus W., Nitsche M. A., Pascual-Leone A., Byblow W. D., Berardelli A., Siebner H. R., Classen J., Cohen L. G. and Rothwell J. C. Consensus: Motor cortex plasticity protocols. Brain Stimul (2008). 1: 164-182.

Ziemann U. and Rothwell J. C. I-waves in motor cortex. J Clin Neurophysiol (2000). 17: 397-405.

Ziemann U., Rothwell J. C. and Ridding M. C. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol (1996). 496 (Pt 3): 873-881.

Ziemann U. and Siebner H. R. Modifying motor learning through gating and homeostatic metaplasticity. Brain Stimul (2008). 1: 60-66.

Florinda Ines Maria Ferreri

TMS and TMS-EEG Studies on the Excitability, Connectivity and Plasticity of the Human Motor Cortex

This thesis explores the feasibility of combining transcranial magnetic stimulation (TMS) and electroencephalography (EEG) for the examination of the cortical excitability and connectivity of the human cerebral cortex, both in healthy and pathological condition. It addresses some issues crucial for a better elucidation of the mechanisms underlying human brain neuroplasticity. Taken together, the findings presented in this thesis are important for the adoption of the TMS and TMS-EEG co-registration techniques as a tool for basic neurophysiological research and, in the future, even for clinical diagnostics purposes.

UNIVERSITY OF
EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Health Sciences

ISBN 978-952-61-0505-5