Cortical plasticity and motor learning:

Variability in response to non-invasive brain stimulation and its relation with motor learning.

Plasticidad cerebral y aprendizaje:

Variabilidad inter- e intra-individual en la respuesta a la estimulación cerebral no invasiva y su relación con el aprendizaje motor.

Author/ Autora: Virginia López Alonso

Doctoral thesis/ Tesis de doctorado UDC / 2015

Supervisors/ Directores: Miguel Ángel Fernández del Olmo

Binith Joseph Cheeran

Programa de doctorado en Deporte, Educación Física y Ocio Saludable

UNIVERSIDADE DA CORUÑA

Name of department: Departamento de Educación Física y Deportiva

Research group: Motor Control Group

University of A Coruña

Author: Virginia López Alonso

Title: CORTICAL PLASTICITY AND MOTOR LEARNING:
Variability in response to non-invasive brain stimulation and its relation with motor learning.

Supervisors: Prof. Miguel Ángel Fernández del Olmo

Dr. MD. Binith Joseph Cheeran

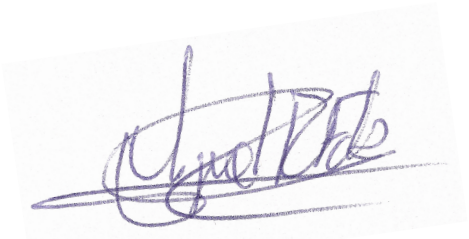
Submitted: January 29th, 2015.

Miguel Ángel Fernández del Olmo, PhD in Physical Education and Proffesor in the Department of Physical Education and Sport in the University of A Coruña, and Binith Cheeran, PhD and MD in the John Radcliffe Hospital, Oxford.

DECLARE:

That the Bachelor in Physical Education and Sport Sciences Virginia López Alonso, has developed under their supervisión the work called "CORTICAL PLASTICITY AND MOTOR LEARNING: *variability in response to non-invasive brain stimulation and its relation with motor learning*". This work satisfies all the requirements for a dissertation to aim for the International PhD in the University of A Coruña.

La Coruña, January 27th, 2015.



Miguel Ángel Fernández del Olmo

PhD. Proffessor.

Binith J Cheeran

PhD.MD.

A mi marido
y a mis padres

ACKNOWLEDGEMENTS

Me gustaría empezar dando las gracias a, más que un compañero, un amigo. Al que ha estado ahí desde mi primer minuto como doctoranda. Siempre dándome ánimos para que no desistiese y que, aunque en los últimos meses, no hemos coincido tanto, siempre he podido contar con él cuando lo he necesitado. Mil gracias ***Jose***.

A mis dos directores. ***Miguel***, no podría haber elegido a otro mejor. Aunque muchas veces por mi orgullo, mi estrés y sobre todo, mi cabezonería no te haya demostrado mi agradecimiento, soy lo que soy profesionalmente hoy por "tu culpa". Gracias jefe por no haber perdido la paciencia conmigo aunque sé que muchas veces te lo puse difícil. ***Bin***, you are one of the better professionals, better boss and overall better person that I have known. Thanks so much for all you taught me and for be always available when I needed.

A los que eran doctorandos en el grupo cuando llegué, pero que ya son unos exitosos PhD. ***Olalla***, gracias por tu amistad y tu apoyo durante todos estos años. ***Ángel***, compi de congresos, de despacho y, sobre todo, "psicólogo" personal, gracias por escucharme y darme consejo siempre.

Juako, Luis, Mila, gracias por hacerme sentir una más y por todo el cariño que me habéis dado durante esta larga aventura. En muchos momentos fue imprescindible para no tirar la toalla.

Gracias ***Rafa*** por esas sobremesas tan productivas en las que tanto me has enseñado sobre la vida.

Gracias a mis compis de doctorado, de risas, de llantos, de cafés y de tirarnos las cosas a la cabeza dependiendo del momento. Estos años no hubiesen sido lo mismo si no los hubiese compartido con vosotros, ***Dan, Helena, Edu, Xián, Jorge*** y ***Vero***. Gracias a todos por esas inolvidables (e interminables, muchas veces) horas compartidas.

Gracias ***Leo*** por abrirme las puertas del HCPS en el NIH. Fue una experiencia inolvidable en la que maduré como investigadora y como persona. Thanks to all the great people and awesome PhDs that I knew there, I learned so much science from them, ***Lei, Marco, Wole, Eran, Ethan, Marta, Ben, Rita, Nguyet, Sylvie, Joseph*** and thanks ***Steph*** for help me with all the paperwork. I also wanted to thanks ***Mark Hallet*** and ***Eric Wassermann*** for their help and support during my stance. Gracias ***Óscar***, por acogerme en Silver Spring y hacer mi estancia tan agradable desde el primer día.

Gracias a todos los que he conocido en el camino, de todos ellos he aprendido algo, y todos ellos han aportado un pequeño granito de arena para que hoy pueda presentar este trabajo. ***Eli, Lalo, Cela, Raed, Francesco, Alessia, María…*** los ***alumnos*** que os habéis prestado como sujetos para los estudios, muchas gracias por las desinteresadas horas invertidas. Los ***PAS***, sobre todo ***Luis, Mª Carmen y Gloria***, gracias por recibirme siempre con una sonrisa.

Al ***INEFg*** por ser la casa en la que me he formado durante más de 10 años.

Y los últimos y más importantes. Gracias a mi familia. ***Papá, mamá,*** gracias por apoyarme siempre y confiar siempre en mí. Gracias, porque gracias a vosotros estoy aquí, porque gracias a vosotros soy la persona que soy. Gracias por ser como sois. Os quiero. ***Go, Anita,*** porque aunque últimamente estamos lejos, siempre estamos cerca para lo que haga falta. Gracias ***abuela*** por llamarme por las noches a INEF para mandarme para casa a descansar.

Gracias ***Gabi***. Para ti no tengo palabras. Eres el que realmente ha sufrido todo este proceso, el que me aguanta a diario y el que se traga todos mis gritos cuando llego a casa en esos días frustrantes que tiene el mundo de la investigación. Gracias cariño por quererme tanto como para aguantarme.

Cells that fire together, wire together ...

… espero que sigamos "disparando" juntos mucho más tiempo

Gracias a todos.

"Todo hombre puede ser, si se lo propone,
escultor de su propio cerebro".

Santiago Ramón y Cajal.

ABSTRACT

Human brain is plastic, i.e. it has the ability to make changes in its structure and function. The key mechanisms involved in these changes at the synaptic level are the long-term potentiation (LTP) and the long-term depression (LTD). LTP and LTD have been induced by practice (learning processes) or artificially through electrical stimulation in cortical and hippocampal slices. In the last decades, several techniques have been developed to stimulate the awake human cerebral cortex safely and non-invasively. The two commonly used stimulation techniques are transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) produce changes in cortical excitability, with several properties in common with LTP/LTD in cortical slices. These changes in cortical excitability have been used to justify the utilization of these techniques both in rehabilitative processes of different patients populations and in the potentiation of learning in healthy subjects. However, there is controversy regarding effects on small groups of subjects and whether the effects of these techniques are effective or safe for the individual. In the last years some studies suggest that some subjects do not respond as expected to the stimulation.

As these techniques are very recent, there are some questions that remain still unresolved. In the present work we wanted to address the following:

1) The huge inter-individual variability in response to the different non-invasive brain stimulation techniques.
2) The intra-subject reliability of the non-invasive brain stimulation techniques.
3) The relationship between cortical plasticity and motor learning capacity.

In this work we demonstrated first, that there are different patterns of response to each of the facilitative non-invasive brain stimulation protocols tested. There is a group of subjects that respond as expected (i.e. increasing

cortical excitability), whilst there is another group of subject who do not show this expected response. Furthermore, an expected response to one protocol, do not imply such response to another protocol.

Second, our results show a fair intra-subject reliability in response to non-invasive brain stimulation during the half-hour post-stimulation (tested with tDCS).

And third, we observe no correlation between the plasticity induced in the motor cortex by non-invasive brain stimulation and the motor learning capacity. However, we found a relationship between the pattern of response to some non-invasive brain stimulation protocols and the reaction time, i.e. the group of subjects who respond as expected to the stimulation is faster than those subjects in the group with a non-expected response.

Therefore, due to the huge inter-individual variability in response to non-invasive brain stimulation, and the large amount of stimulation protocols and motor learning task, it seems very important to take into account all of this for the design of programs involving stimulation. So, is important to know which protocol and/or motor task are more suitable depending on the therapy and depending on the subject. Furthermore, as the intra-individual reliability of stimulation seems to be fair, once a technique is successfully probe in a subject, is expected similar success in successive sessions.

Future studies are needed to further establish the optimal protocols and parameters for each intervention in which non-invasive brain stimulation is involved.

RESUMEN

El cerebro humano es plástico, tiene la capacidad de producir cambios en su estructura y función. Los mecanismos principales que explican dichos cambios a nivel sináptico son la potenciación a largo plazo (LTP) y la depresión a largo plazo (LTD). Ambos mecanismos pueden ser inducidos mediante la práctica (procesos de aprendizaje), pero también pueden ser inducidos de manera artificial a través de estimulación eléctrica tanto en la corteza cerebral como en el hipocampo. En las últimas décadas se han desarrollado numerosas técnicas que permiten estimular la corteza cerebral humana de manera segura y no invasiva. La estimulación magnética transcraneal (TMS) y la estimulación transcraneal por corriente directa (tDCS) son las dos técnicas más utilizadas. Numerosos estudios han demostrado que ambas técnicas inducen cambios en la excitabilidad cortical y, por ende, en la plasticidad (compartiendo propiedades con el LTP y el LTD inducido en preparaciones corticales). Estos cambios en la excitabilidad cortical se han utilizado para justificar el uso de estas técnicas tanto en la mejora de los procesos rehabilitadores de diferentes poblaciones de pacientes como en la potenciación del aprendizaje en sujetos sanos. Sin embargo, hay una gran controversia en cuanto al efecto de estas técnicas a nivel individual, ya que en los últimos años se han publicado artículos en los que se observa que algunos sujetos no responden como es esperado a la estimulación.

Al ser estas técnicas de estimulación relativamente nuevas, existen todavía varias cuestiones que no han sido resueltas completamente. En el presente trabajo tratamos de abordar las siguientes:

1) La gran variabilidad inter-individual que parece existir en la respuesta a las diferentes técnicas de estimulación.

2) La fiabilidad intra-sujeto de las técnicas de estimulación.

3) La relación entre plasticidad cortical y la capacidad de aprendizaje motor.

En este trabajo demostramos que, efectivamente, existen diferentes patrones de respuesta a cada uno de los protocolos de facilitación testados. Un grupo de sujetos responde como se espera (incrementando la excitabilidad cortical), mientras que otro grupo de sujetos no muestra la respuesta esperada. Además, el que una persona responda a un protocolo no es indicativo de que vaya a responder a otro.

En segundo lugar, los resultados nos han mostrado que hay una fiabilidad intra-sujeto moderada durante la media hora siguiente a la estimulación transcraneal.

Y en tercer lugar, nuestros datos no muestran relación entre la plasticidad inducida en el área motora con estimulación transcraneal y la capacidad de aprendizaje motor. Sin embargo, hemos encontrado una relación entre el patrón de respuesta a la estimulación y el tiempo de reacción. Nuestros resultados muestran que el grupo de sujetos que responde como se espera a determinados protocolos de estimulación, tienen un tiempo de reacción menor que aquellos del grupo que no responde como es esperado.

Por lo tanto, debido a la gran variabilidad inter-individual en la respuesta a la estimulación transcraneal no invasiva, junto con la gran cantidad de protocolos de estimulación y de tareas de aprendizaje motor existentes, parece necesario contemplar todos estos aspectos a la hora de diseñar programas en los que se utilicen estas técnicas. Es decir, es importante saber qué protocolo y/o qué tarea de aprendizaje es más adecuada para según qué tipo de terapia y según qué tipo de sujeto. Además, parece que la fiabilidad intra-sujeto de las técnicas de estimulación es aceptable, por lo que una vez comprobada la eficacia de una técnica en un sujeto, podemos esperar que sucesivas sesiones muestren similares efectos.

Futuros estudios deben ser realizados para seguir concretando los protocolos y parámetros óptimos para cada intervención en la que se utilicen técnicas de estimulación transcraneal no invasiva.

RESUMO

O cerebro humano é plástico, ten a capacidade de producir modificacións na súa estructura e na súa función. Os principais mecanismos que explican estas modificacións a nivel sináptico son a potenciación a longo prazo (LTP) e a depresión a longo prazo (LTD). Ambos mecanismos poden ser inducidos mediante a práctica (procesos de aprendizaxe), mais tamén poden ser inducidos de maneira artificial a través de estimulación eléctrica tanto na corteza cerebral coma no hipocampo. Nas últimas décadas véñense desenvolvendo numerosas técnicas que permiten estimular o córtex humano de maneira segura e non invasiva. A estimulación magnética transcraniana (TMS) e máis a estimulación transcraniana por corrente directa (tDCS) son as dúas técnicas máis empregadas. Numerosos estudios demostraron que ámbalas dúas técnicas inducen modificacións na excitabilidade cortical e, polo tanto, na plasticidade (compartindo propiedades co LTP e mailo LTD inducido en preparacións corticais). Estas modificacións na excitabilidade cortical utilízanse para xustificar o uso destas técnicas tanto nos procesos rehabilitadores de diferentes poboacións de doentes coma na potenciación do aprendizaxe en suxeitos saudables. Porén, existe unha grande controversia en relación ó efecto destas técnicas a nivel individual, xa que nos últimos anos publicáronse artigos nos que se observa que algúns suxeitos non responden como é de esperar á estimulación.

Debido a que estas técnicas son relativamente novas, aínda existen varias cuestións sen resposta. No presente traballo preténdense abordar as seguintes:

1) A grande variabilidade inter-individual que parece existir na resposta ás diferentes técnicas de estimulación.

2) A fiabilidade intra-suxeito das técnicas de estimulación.

3) A relación entre plasticidade cortical e a capacidade de aprendizaxe motora.

Neste traballo demostramos que, efectivamente, existen diferentes padróns de resposta a cada un dos protocolos de facilitación testados. Un grupo de

suxeitos responde como é de esperar (incrementando a excitabilidade cortical), namentres que outro grupo de suxeitos non amosa a resposta esperada. Ademais, que unha persoa responda a un protocolo non é indicativo de que responda a outro.

En segundo lugar, os resultados amosaron unha fiabilidade intra-suxeito moderada durante a media hora seguinte á estimulación transcraniana.

E, en terceiro lugar, os nosos datos non amosan relación entre a plasticidade inducida na zona con estimulación transcraniana e a capacidade de aprendizaxe motora. Porén, encontramos unha relación entre o padrón de resposta á estimulación transcraniana e o tempo de reacción. Os nosos resultados amosan que o grupo de suxeitos que responde como é de esperar a determinados protocolos de estimulación, teñen un tempo de reacción menor que aqueles do grupo que non responde como é de esperar.

Por tanto, debido á grande variabilidade inter-individual na resposta á estimulación transcraniana non invasiva, xunto coa grande cantidade de protocolo de estimulación e de tarefas de aprendizaxe motora existentes, semella necesario contemplar todos estes aspectos á hora de deseñar programas nos que se empreguen estas técnicas. É dicir, é importante saber o protocolos e/ou a tarefa de aprendizaxe máis adecuada para segundo o tipo de terapia e segundo o tipo de suxeito. Ademáis, semella que a fiabilidade intra-suxeito das técnicas de estimulación é aceptable, por tanto, unha vez comprobada a eficacia dunha técnica nun suxeito, é de esperar que sucesivas sesións amosen efectos similares.

Futuros estudios deben ser realizados para seguir concretando os protocolos e parámetros óptimos para cada intervención na que sexan empregadas as técnicas de estimulación transcraniana non invasiva.

PREFACE

The present work, the thesis titled *Cortical plasticity and motor learning: variability in response to non-invasive brain stimulation and its relation with motor learning* contains experimental work performed between 2011 and 2015 at *Faculty of Sports Science and Physical Education of University of A Coruña, Department of Sports Science*. Also, some work was performed during an stance in the *laboratory at the Charles Wolfson Clinical Neuroscience Facility, Nuffield Department of Clinical Neuroscience, John Radcliffe Hospital, Oxford* under the supervision of Dr.MD. Binith Cheeran from July to August 2013, and in the *Human Cortical Physiology and Stroke Rehabilitation Section, Institute of Neurological Disorder and Stroke, National Institutes of Health (NIH), Bethesda* under the supervision of Dr.MD. Leonardo G Cohen from April to October 2014.

Three original experimental studies are included, one already published in the international peer-review journal *Brain Stimulation*. The second under review in the international peer-review journal *Clinical Neurophysiology*. The third study is presented as a preliminary manuscript and would be submitted in the following months.

These studies were supported by grants from:
Pre-doctoral Fellowship from University of La Coruña.
Pre-doctoral Fellowship from Xunta de Galicia.
FPU fellowship from “Ministerio de Educación, Cultura y Deporte” of Spain.
Mobility fellowship from University of La Coruña.
FPU mobility fellowship.

CONTENTS

FIGURES INDEX

Introduction

Study I

Study II

Study III

TABLES INDEX

Introduction

Study I

Study II

Study III

Appendix

ABBREVIATIONS

AE – Angular error

AMT – Active motor threshold

AtDCS – Anodal trancranial direct current stimulation

BDNF – Brain-derived neurotrophic factor

cAMP – Cyclic adenosine monophosphate

CMCT – central motor conduction time

CNS – Central nervous system

cTBS – Continuous theta-burst stimulation

EF – Electric fields

EMG – Electromiography

FDI – First dorsal interosseous

GABA – Gamma-aminobutyric acid

ICF – Short intracortical facilitation

IL – Implicit learning

ISI – Interstimulus interval

iTBS – Intermittent theta-burst stimulation

LM – Lateral to medial

LTD – Long-term depression

LTP – Long-term potentiation

LR – Learning rate

M1 – Motor cortex

MEP – Motor evoked potentials

NIBS – Non-invasive brain stimulation

NMDA – N-methyl-D-aspartate

$NMDA_r$ – N-methyl-D-aspartate receptors

PA – Posterior to anterior

PAS – Paired associative stimulation

PAS_{25} – Paired associative stimulation with an interstimulus interval of 25ms

PPS – Paired pulse stimulation

QPS – Quadripulse stimulation

RMT – Resting motor threshold

RT – Reaction time

rTMS – Repetitive transcranial magnetic stimulation

SAF – Speed-accuracy trade-off function

SICI – Short intracortical inhibition

SPM – Synaptic plasticity and memory (hypothesis)

SRTT – Serial reaction time task

STDP – Spike-timing-dependent plasticity

SVIPT – Sequential visual isometric pinch task

TBS – Theta-burst stimulation

tDCS – Transcranial direct current stimulation

TES – Transcranial electric stimulation

TMS – Transcranial magnetic stimulation

VAT – Visuomotor adaptation task

LIST OF PUBLICATIONS INCLUDED IN THIS THESIS

Study I: **Inter-individual Variability in Response to Non-invasive Brain Stimulation Paradigms**

Virginia López-Alonso, Binith Cheeran, Dan Río-Rodríguez, Miguel Fernández-del-Olmo.

Brain Stimulation 2013, 7(3): 372-80.

Study II: **Intra-individual variability in the response to anodal Transcranial Direct Current Stimulation**

Virginia López-Alonso, Miguel Fernández-del-Olmo, Alessia Costantini, Juan Jose Gonzalez-Henriquez, Binith Cheeran

Under review in Clinical Neurophysiology.

Study III: **Relationship between NIBS-induced plasticity and capacity for motor learning.**

Virginia López-Alonso, Binith Cheeran, Miguel Fernández-del-Olmo.

Under review in Brain Stimulation.

OTHER SCIENTIFIC CONTRIBUTIONS

During these four years of PhD training, I also participated in other six studies with the Motor Control Group (University of A Coruña), and in other in collaboration with a group from the Oxford University:

Fernandez-del-Olmo M, Bello O, **Lopez-Alonso V**, Sanchez JA, Santos-García D, Valls-Solé J (2012). The effect of auditory startle and non-startle stimuli on step initiation in Parkinson's disease. *Mov Disord, 27(12):1570-3.*

Joundi RA, **Lopez-Alonso V**, Lago A, Brittain JS, Fernandez-Del-Olmo M, Gomez-Garre P, Mir P, Jenkinson N, Cheeran B, Brown P (2012). The effect of BDNF val66met polymorphism on visuomotor adaptation. *Exp Brain Res*, *223(1):43-50.*

Bello O, Sanchez JA, **Lopez-Alonso V**, Marquez G, Morenilla L, Castro X, Giraldez M, Santos-García D, Fernandez-del-Olmo M (2013). The effects of treadmill or overground walking training program on gait in Parkinson's disease. *Gait and Posture. 38(4):590-595*.

Lago A, **Lopez-Alonso V**, Fernandez-del-Olmo M (2013). Mirror neuron system and observational learning behavioural and neurophysiological evidence. *Behav Brain Res. 248(C):104-113*.

Fernandez-del-Olmo M, Bello O, **Lopez-Alonso V**, Marquez G, Sanchez JA, Morenilla L, Valls-Solé J (2013). The effects of startle and non-startle stimuli on wrist flexion movement in Parkinson's disease. *Neurosci Lett. 548:56-60.*

Fernandez-del-Olmo M, Bello O, Sanchez JA, **Lopez-Alonso V**, Marquez G, Morenilla L, Castro X, Giraldez M, Santos-García D (2014). Treadmill training improves overground walking economy in Parkinson's disease: A

randomized, controlled pilot study. *Frontiers Neurology. 5:191 - Movement Disorders.*

Fernandez-Lago H, Bello O, **Lopez-Alonso V**, Sanchez JA, Morenilla L, Fernandez-del-Olmo M. Attentional mechanisms involved in treadmill walking in parkinson´s disease (under review). *Eur J Phys Rehabil Med.*

I also contributed with communications/posters to the following international congresses:

López-Alonso V, Cheeran B, Del-Olmo MF, Lago A, Gomez-Garre P, Koch G, Teo J, Rothwell J. The RODIL proyect: understanding variability in the response to rTMS. *Magstim Summer School*. Oxford, 2011.

López-Alonso, V; Lago, A; Fernández-del-Olmo, M. Relationship between TBS-induced plasticity and learning of two motor tasks. *VII International Congress of the Spanish Sport Sciences Association*. Granada, 2012.

Morenilla L, Márquez G, Sánchez-Molina JA, Bello O, **López-Alonso V**, Fernández-del-Olmo M. Postural stability in parkinson's disease patients: effect of a simultaneus cognitive task performance. *VII International Congress of the Spanish Sport Sciences Association*. Granada, 2012.

Lago-Rodríguez A, **López-Alonso V**, Fernández-del-Olmo M. Beneficios perceptivos y comportamentales del aprendizaje motor por observación. *VII International Congress of the Spanish Sport Sciences Association*. Granada, 2012.

López-Alonso, V; Lago, A; Cheeran, B; Joundi, R; Teo, J; Mir, P; Fernández-del-Olmo, M. Does the response to rTMS predict potential for Implicit Motor Learning or for Visuomotor Adaptation?. *2nd Workshop on Synaptic Plasticity: from Bench to Bed Side*. Taormina, 2012.

López-Alonso, V; Lago, A; Cheeran, B; Teo, J; Mir, P; Fernández-del-Olmo, M. Does the response to rTMS predict potential for Motor Learning?. *8th FENS Forum of neuroscience and Satellite Event "New strategies to optimize the acquisition and consolidation of motor skills"*. Barcelona, 2012.

López-Alonso V, López-Bermúdez G, Sánchez-Molina JA, Fernández-del-Olmo M. Estudio piloto sobre el efecto de la tDCS en el aprendizaje de un gesto deportivo. *VIII Congreso Internacional de la Asociación Española de Ciencias del Deporte*. Cáceres, 2014.

Ferrer-Uris B, Busquets A, **López-Alonso V**, Fernández-del-Olmo M, Angulo-Barroso R. Effects of a single bout of intense endurance exercise on the adaptation and retention of a perceptual-motor task. Program No. 171.04/LL22. 2014 Neuroscience Meeting Planner. *Society for Neuroscience (SfN)*. Washington DC, 2014.

Chapter 1

Introduction

1. INTRODUCTION

1.1. PLASTICITY

Historically, it was thought that the role of the synapse was to simply transfer information from one neuron to another neuron or from a neuron to a muscle cell. Moreover, it was thought that these connections, once established during development, were relatively fixed in their strength. One exciting development in neurobiology over the last decades is the realization that most synapses are extremely plastic.

In general, plasticity is defined as the ability of the nervous system to make changes in its structure of function to adapt to alterations in its environment. These changes occur throughout life, and can occur at various levels of brain organization: from the ultrastructural to synaptic level (Duffau, 2006). Synaptic plasticity specifically refers to the activity-dependent modification of the strength or efficacy of synaptic transmission at pre-existing synapses. Synaptic plasticity has been proposed to play a central role in the capacity of the brain to incorporate transient experiences into persistent memory traces. This assumption of storage of information in the brain as changes in synaptic efficiency emerged over a century ago following the demonstration by the Spanish Nobel laureate Santiago Ramón y Cajal that neurons are independent elements but communicate with each other at the specialized junctions. Sherrington called these junctions synapses.

> *"(…) Las células nerviosas son elementos independientes jamás anastomosados ni por sus expansiones protoplasmáticas (dendritas) ni por las ramas de su prolongación de Deiters (axón), y que la propagación de la acción nerviosa se verifica por contactos al nivel de ciertos aparatos o disposiciones de engranaje, cuyo objeto es fijar la conexión, multiplicando considerablemente las superficies de influencia".*
>
> *(Cajal, 1899)*

Donald Hebb further advanced this idea in 1949 establishing that:

> *"When an axon of cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased".*
>
> *(Hebb, 1949)*

So, he proposed that associative memories are formed in the brain by a process of synaptic modification that strengthens connections when presynaptic activity correlates with postsynaptic firing.

Bliss and colleagues in 1973 were the firsts describing in detail and with experimental support the existence of such long-lasting, activity-dependent changes in synaptic strength in rabbits (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973). They reported that brief trains of high-frequency stimulation to monosynaptic excitatory pathways in the hippocampus cause an abrupt and sustained increase in the efficiency of synaptic transmission that could last for hours or even days. This effect is called long-term potentiation (LTP) (Bliss and Collingridge, 1993). Long-term depression (LTD) was also reported in response to brief trains of low-frequency stimulation (Dudek and Bear, 1992; Stanton and Sejnowski, 1989). LTD induces weakness in the synaptic strength (Dudek and Bear, 1992; Dudek and Bear, 1993; Stanton and Sejnowski, 1989).

1.1.1. Long-term potentiation (LTP) and long-term depression (LTD)

As defined in the previous paragraph, LTP is an increase in the synaptic strength that could last for days, weeks or even months (figure 1). Since the studies of Bliss and colleagues, LTP has been widely investigated because its suggested role in memories formation (Bear, 1996; Citri and Malenka, 2008; Martin, Grimwood, et al., 2000; Pastalkova, Serrano, et al., 2006). Furthermore, most synapses that exhibit LTP also express its opposite phenomenon, the long-term depression. LTD is a long-lasting weakening of a neuronal synapse (Malenka and Bear, 2004). Hence, synaptic strength at excitatory synapses is bidirectionally modifiable by different patterns of activity (Citri and Malenka, 2008).

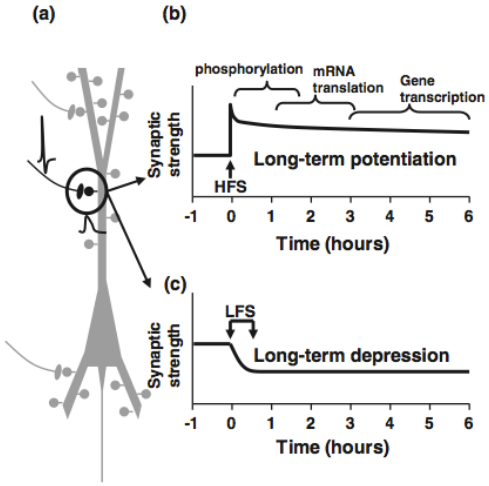


Figure 1. Basic features of synaptic plasticity. (a) The spike illustrates the afferent activity, which produces postsynaptic potentials (shown below highlighted synapse). (b) High frequency stimulation (HFS) results in long-term potentiation. (c) Low frequency stimulation (LFS) results in long-term depression. Modified from (Nathan, Cobb, et al., 2011).

Although LTP and LTD are more frequently studied in the CA1 region of the hippocampus, they are not unitary phenomena. Mechanisms underlying these plastic changes vary depending on the synapses and circuits in which they operate (Malenka and Bear, 2004). Also, most of the studies are focused on the LTP that is called associative, or "Hebbian".

As introduced by Hebb, the property of associativity relies upon a mechanism that detects coincident pre- and postsynaptic activity. However, this does not means that the induction requires perfectly synchronous activation of the converging systems, but means that the order of the trains is crucial. This was called spike-timing-dependent plasticity (STDP). LTP is induced when the presynaptic neuron is stimulated prior to the postsynaptic neuron within a window of tens of milliseconds, whereas stimulation in the reverse order induces LTD (Hoogendam, Ramakers, et al., 2010; Levy and Steward, 1983).

This function of associativity is performed by the N-methyl-D-aspartate (NMDA) sub-class of glutamate receptor at most glutamatergic synapses in the central nervous system (CNS) (Collingridge, Kehl, et al., 1983). Therefore, NMDA receptor ($NMDA_r$) seems to be the cellular basis of LTP and LTD mechanisms. This receptor is placed postsynaptically and has an intrinsic cation channel, which is blocked by magnesium ions (Mg^{2+}) when the cell is at its normal resting membrane potential. Only when the postsynaptic cell is sufficiently depolarized the Mg^{2+} is expelled from the cation channel, allowing an influx of sodium (Na^+) and calcium (Ca^{2+}) ions into the cell. It is this Ca^{2+} influx that is thought to initiate LTP induction (figure 2) (Cooke and Bliss, 2006).

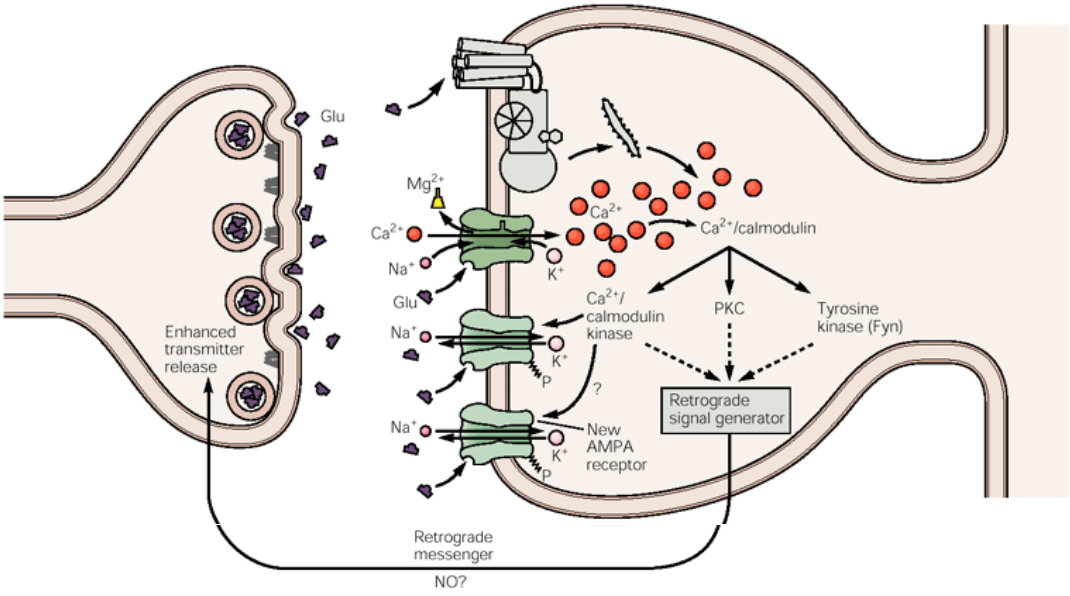


Figure 2. A model for the induction of the early phase of long-term potentiation. In the normal resting membrane Mg^{2+} blocks potential NMDA channels. When the postsynaptic membrane is depolarized this Mg^{2+} is relieved, allowing the influx of Ca^{2+}. The resulting rise in Ca^{2+} in the dendritic spine triggers calcium-dependent kinases (Ca2+/calmodulin kinase and protein kinase C) and the tyrosine kinase Fyn that together induce LTP. Modified from (Kandel, Schwartz, et al., 2000).

This $NMDA_r$-dependent mechanism explains several characteristics of LTP that besides its long-term effect make it an attractive candidate mechanism for the storage of information. Two of these characteristics of LTP are (Barrionuevo and Brown, 1983; Cooke and Bliss, 2006; Levy and Steward, 1979):

1) Synaptic LTP is an input-specific process, such that a single pathway can be potentiated without effect on inactive neighbouring inputs to the same cell.
2) The associativity property of LTP ensures that a weak tetanus, which is not by itself capable of initiating LTP, can become potentiated through association with a strong tetanus.

So, just to conclude this section, as memory formation and synapses strengthening are the bases of learning, learning seems to rely also on LTP-

and LTD-like mechanisms. So far, to support this hypothesis we could assume the three premises we were talking about from the beginning: 1) synapses are modifiable, 2) they modify with learning, and 3) they strengthen through an LTP-like mechanism (Rioult-Pedotti, Friedman, et al., 2000).

Interestingly, these LTP- and LTD-like mechanisms can be induced artificially in the human brain by non-invasive brain stimulation (NIBS).

1.2. NON-INVASIVE BRAIN STIMULATION (NIBS)

In the 1950's there were already many attempts to stimulate the human brain through the scalp using trains of stimuli similar to those conventionally used to stimulate the exposed cortex during neurosurgery (Gualtierotti and Paterson, 1954; Rothwell, 1997). However, this procedure was extremely painful and inefficient, since most of the current flowed through the scalp rather tan into the brain. Merton and Morton performed the first clinical applicable method of transcranial electric stimulation (TES) in 1980. They succeeded in stimulate motor areas of the human brain through the intact scalp. They used a brief, high-voltage electric shock over the primary motor cortex (M1) producing a brief, relatively synchronous muscle response, the motor evoked potential (MEP) (Merton and Morton, 1980). However, the pain induced by this type of stimulation, was still strong. Five years later, Barker et al. showed that it was possible to stimulate the brain (and also peripheral nerves) with magnetic stimulation with little or no pain (Barker, Jalinous, et al., 1985). This new NIBS technique is called transcranial magnetic stimulation (TMS).

1.2.1. Transcranial magnetic stimulation (TMS)

For transcranial magnetic stimulation a brief, high-current pulse is produced in a coil of wire, called the magnetic coil, which is placed above the scalp. A magnetic field is produced with lines of flux passing perpendicularly to the plane of the coil. An electric field is induced perpendicularly to the magnetic field. In a homogeneous medium, the electric field will cause current to flow in loops parallel to the plane of the coil. The loops with the strongest current will be near the circumference of the coil itself. The current loops become weak near the

centre of the coil, and there is no current at the centre itself (Hallett, 2000). In clinical use, the advantage of the technique is that magnetic fields of the frequencies used pass through all body structures without significant attenuation and hence the presence of structures with a high resistance to electrical current such as bone and fat do not affect the magnetic field distribution beneath a stimulating coil (Rothwell, Thompson, et al., 1991).

There are different shaped coils for TMS. Smaller coils enable focal stimulation. Unfortunately, increased focality of stimulation is offset by a decrease in the effectiveness of stimulation (Rothwell, Thompson, et al., 1991). *Circular coil* (the simplest TMS coil, and historically the first to be used) has particularly poor focality. *Figure-eight-shaped* or *butterfly coil* is much more focal, producing maximal current at the intersection of the two round components (figure 3) (Cohen, Roth, et al., 1990; Epstein, 2008; Hallett, 2000).

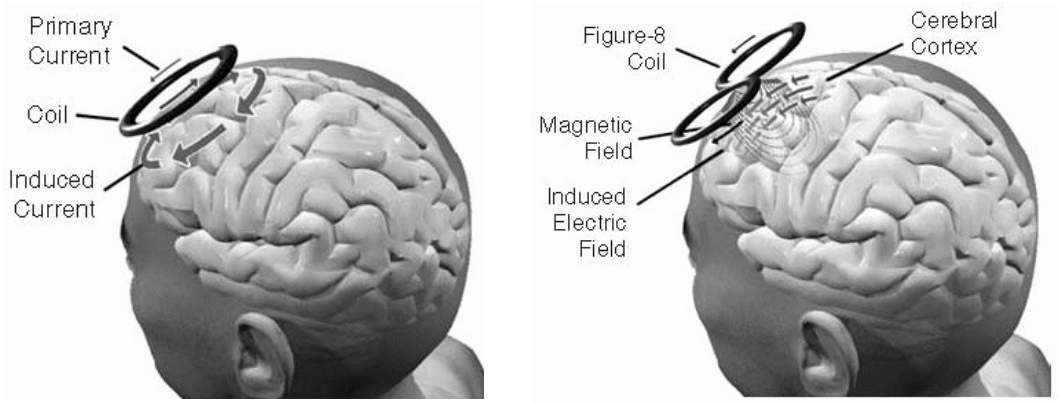


Figure 3.- Two of the most used TMS coils: the circular coil (on the left) and the figure-eight-shaped or butterfly coil (on the right). Black arrows show the current direction. The grey arrows the direction of the current induced in the brain (i.e. the magnetic field). The size of the arrows does not reflect the size of the current. Modified from (Epstein, 2008).

TMS introduced a novel research tool for studying the functionality, morphology and connectivity of several cortical regions (Terao and Ugawa, 2002). We can summarize the applications of TMS relating to four types of studies (Hallett, 1996; Hallett, 1996; Kobayashi, Hutchinson, et al., 2004;

Pascual-Leone, Tarazona, et al., 1999; Reis, Swayne, et al., 2008; Rosler, 2001):

- Demonstration of plastic changes.
- Elucidation of mechanisms underlying plasticity.
- Providing functional information to findings of neuroplasticity with other neuroimaging techniques.
- Modulating neuroplasticity to enhance it or reduce it in order to influence behavioural consequences.

The more common output of TMS is the motor evoked potential (MEP). MEP monitoring requires transcranial stimulation of the motor cortex to produce a descending response that traverses the corticospinal tracts and eventually generates a measurable response in the form of muscle activity that can be recorded by electromyography (EMG) (figure 4). There are several parameters of MEPs that can be studied, such as the latency providing the central motor conduction time (CMCT), the size of the MEP (amplitude, duration and area), and others (such as stimulation thresholds, silent period, facilitation…) (Rosler and Magistris, 2008).

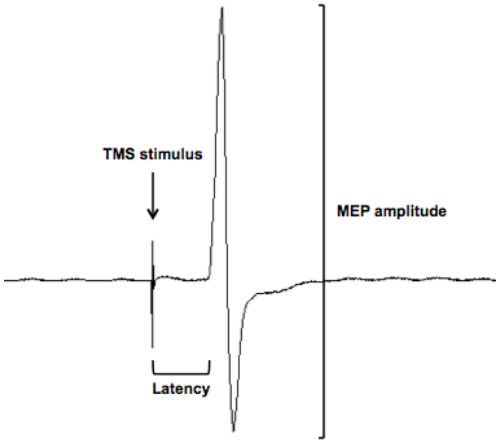


Figure 4. Motor evoked potential (MEP). This figure illustrates the resulting MEP of a TMS pulse over the motor cortex, recorded from the first dorsal interosseous (FDI) with EMG. Latency is the time between the TMS stimulus and the appearance of the MEP. MEP amplitude is the peak-to-peak size of the wave.

1.2.1.1. Physiologic mechanisms of single pulse TMS

Although the exact mechanisms underlying the physiological effects of TMS are not yet totally defined, it is usually assumed that initial TMS neural

activation is restricted to superficial brain regions, subcortical areas may be secondarily activated through brain networks (McKinley, Bridges, et al., 2012).

Di Lazzaro and colleagues has been studied the physiological basis of the effects of the TMS since the 1990´s (Di Lazzaro, Oliviero, et al., 2004; Di Lazzaro, Oliviero, et al., 1998; Di Lazzaro, Profice, et al., 2012; Di Lazzaro and Ziemann, 2013). Using a figure-of-eight coil and a monophasic posterior to anterior (PA) induced current in the brain they observed that at the lowest intensity to evoke a MEP, TMS evokes a single descending wave called I1 wave. This descending wave is produced by indirect trans-synaptic activation of pyramidal tract neurones. At higher stimulus intensities later volleys appear, these are called late I-waves (numbered in order of their appearance). An interesting characteristic of these late I-waves is that they occur at a fairly regular of around 1.5 ms interval apart and it is unclear if the different I-waves (I1, I2, I3, and so forth) represent distinct populations of excitatory interneurons or the repetitive discharge of pyramidal tract neurones through reverberating activation in a microcircuit of highly connected excitatory cells (Cheeran, Koch, et al., 2010; Di Lazzaro, Profice, et al., 2012). A further increase of TMS intensity (approximately 180-200% of the active motor threshold) leads to a direct excitation of the pyramidal tract neurone axons resulting in a D-wave, that is though to result from the direct activation of corticospinal axons because of it short latency (1.0 to 1.4 ms shorter than the I1 wave) (Di Lazzaro, Oliviero, et al., 1998). When the orientation of the figure-of-eight coil is changed, so that monophasic currents in the brain are induced in a lateral to medial (LM) direction, TMS recruits a D-wave even at MEP threshold intensity (figure 5) (Di Lazzaro, Oliviero, et al., 1998; Di Lazzaro and Ziemann, 2013).

Therefore, MEP is a summation of multiple motor units depolarizing in response to D-wave and I-waves arriving onto spinal motor neurons (Cheeran, Koch, et al., 2010). Furthermore, although theoretically, the size of a MEP should relate to the number of activated corticospinal motor neurons, this relation is obscured by some particular characteristics of MEPs, making the interpretation of MEP size measurements difficult. Three basic physiological mechanisms may influence the size of MEPs (Rosler and Magistris, 2008):

- The number of recruited motor neurons in the spinal cord.

- The number of motor neurons discharging more than once to the stimulus.
- The synchronization of the TMS-induced motor neuron discharges.

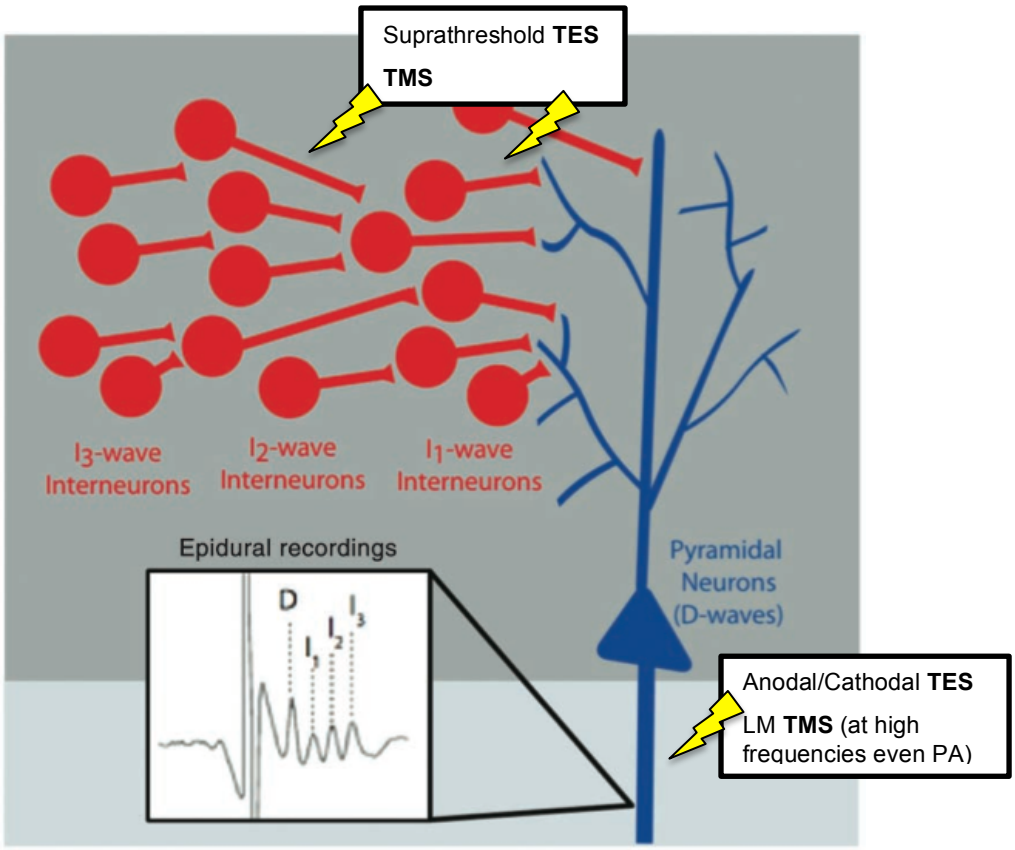


Figure 5.- D-waves and I-waves. D-waves result from the depolarization of pyramidal neurons. The following depolarization of several populations of excitatory interneurons is believed to produce the I-waves. Subcortical D-wave activation at the proximal part of the pyramidal cell can be produced with TES, LM TMS and, at high intensities, even PA TMS (Di Lazzaro, Oliviero, et al., 2004). Recordings from peripheral muscles will demonstrate a motor-evoked potential, which is a summation of multiple motor units depolarizing in response to D-wave and I-waves arriving onto spinal motor neurons. Modified from (Cheeran, Koch, et al., 2010).

1.2.1.2. Paired pulse

Paired pulse TMS techniques can be used to study intracortical excitability and the level of activity of different cortico-cortical connections and neurotransmitter systems (Pascual-Leone, Tarazona, et al., 1999). Two common measures of interneuron influences in the cortex obtained by paired-

pulse techniques are short intracortical inhibition (SICI) and facilitation (ICF) (Ziemann, Rothwell, et al., 1996).

Kujirai and colleagues described SICI for first time in 1993. They shown that a magnetic conditioning stimulus given over the motor cortex at intensities below threshold for obtaining EMG responses in relaxed hand muscles can suppress responses evoked in the same muscles by a suprathreshold magnetic test stimulus given approximately 1-6 ms later (Kujirai, Caramia, et al., 1993). SICI is likely largely a GABAergic effect, specifically $GABA_a$ (Di Lazzaro, Oliviero, et al., 2000; Hallett, 2007).

ICF follows the same methodology. An initial conditioning stimulus given at an intensity enough to activate cortical neurons, but small enough so that no descending influence on the spinal cord can be detected and there is no MEP, precedes a second test stimulus, at suprathreshold level. The ISI for ICF should be between 8 and 30 ms. Intracortical influences initiated by the conditioning stimulus increases the amplitude of the MEP produced by the test stimulus (Hallett, 2007).

So far, we have seen the utility of TMS as a powerful tool for investigating several cortical regions. Other important application of TMS is its ability to induce plasticity. The two more noted TMS modulatory protocols are the repetitive (r)TMS and the paired associative stimulation (PAS). Moreover, the rediscovery of electrical stimulation almost two decades ago has introduced another NIBS technique able to modulate cortical excitability in the human brain. This technique is called transcranial direct current stimulation (tDCS).

1.2.1.3. Repetitive transcranial magnetic stimulation (rTMS)

Repetitive (r)TMS, when applied to the motor cortex or other cortical regions of the brain, may induce effects (increase or decrease of cortical excitability) that outlast the stimulation period (Classen and Stefan, 2008). The duration of these effects could vary depends on the rTMS protocol used. Some of the variables that influence such effects are stimulus frequency, stimulus intensity, shape of the magnetic pulse, duration of the application period, and the total number of stimuli (Classen and Stefan, 2008; Hoogendam, Ramakers, et al., 2010).

High frequency stimulation (>5Hz), which is thought to act through the stimulation of glutamatergic neurons, produces facilitatory aftereffects (Pascual-Leone, Valls-Sole, et al., 1994; Quartarone, Bagnato, et al., 2005). Conversely, low frequency rTMS (≤1Hz), which generally induces inhibitory effects, seems to rely more selectively on GABAergic neurons (Chen, Classen, et al., 1997; Gilio, Rizzo, et al., 2003; McKinley, Bridges, et al., 2012; Muellbacher, Ziemann, et al., 2000).

Furthermore, we can differentiate rTMS protocols in simple or patterned. Simple protocols consist on individual stimuli spaced apart by an identical interstimulus interval (ISI), whereas in patterned protocols, different ISIs are used (figure 6) (Classen and Stefan, 2008; Hoogendam, Ramakers, et al., 2010).

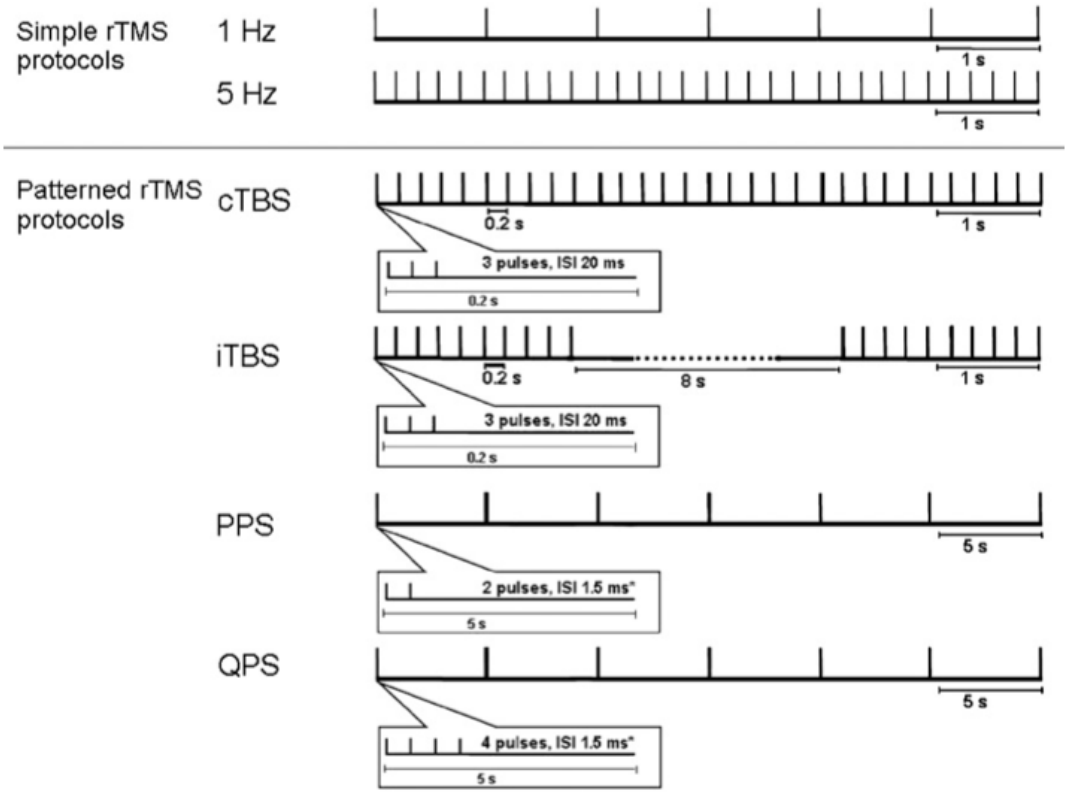


Figure 6. Repetitive TMS protocols. cTBS: continous theta-burst stimulation. iTBS: intermittent theta-burst stimulation. PPS: paired pulse stimulation. QPS: quadripulse stimulation. From (Hoogendam, Ramakers, et al., 2010).

As for the present work we only used the intermittent theta burst stimulation (iTBS), between all the rTMS protocols, we will focus deeper in this patterned protocol.

1.2.1.3.1. Intermittent theta burst stimulation (iTBS)

Huang and colleagues introduced theta burst stimulation (TBS) in 2005 (Huang, Edwards, et al., 2005). TBS is a patterned rTMS protocol based on the naturally occurring theta rhythm (5Hz) of the hippocampus (Hoogendam, Ramakers, et al., 2010; Huang, Edwards, et al., 2005).

The patterns of TBS all consisted of bursts containing 3 pulses at 50 Hz and an intensity of 80% AMT repeated at 200 ms intervals (i.e., at 5 Hz). In the intermittent theta burst stimulation pattern (iTBS), a 2 s train of TBS is repeated every 10 s for a total of 190 s (600 pulses) (figure 6) (Huang, Edwards, et al., 2005).

TBS is particularly attractive because of the short duration of its intervention. The excitability changes induced by iTBS outlast the effects seen in many other rTMS protocols. It last, at least, 15 minutes. TBS is also remarkable since its stimulus intensities are subthreshold for activation of descending pathways. This low intensity also indicates that TBS-induced effects are likely generated exclusively cortically, a notion supported by the absence of changes in H-reflex amplitudes after TBS conditioning (Classen and Stefan, 2008; Huang, Edwards, et al., 2005).

Pharmacological studies have demonstrated that facilitatory effects induced by iTBS are blocked by an $NMDA_r$ antagonist, suggesting that the aftereffects of iTBS relied in some kind of LTP-like mechanisms (Di Lazzaro, Pilato, et al., 2008; Huang, Chen, et al., 2007; Siebner and Rothwell, 2003).

1.2.1.4. Paired associative stimulation (PAS)

Paired associative stimulation (PAS) was first described by Stefan and colleagues in 2000 (Stefan, Kunesch, et al., 2000). This protocol consists on paired repetitively low-frequency peripheral stimulation of somatosensory afferents with TMS over the contralateral motor cortex. PAS resembles models of associative LTP in animals and is based on the Hebbian concept of spike-timing-dependent plasticity (Hoogendam, Ramakers, et al., 2010). In almost all

cases, electrical peripheral nerve stimulation precedes TMS over the cortex. Depending on the interestimulus interval, synchronous (ISI ~25 ms) or asynchronous (ISI ~10 ms) activation of somatosensory-motor cortical connections is induced, leading to increase or decrease cortical excitability, respectively (reflecting in the MEPs) (Nathan, Cobb, et al., 2011; Stefan, Kunesch, et al., 2000; Stefan, Wycislo, et al., 2006).

These changes in cortical excitability displays rapid evolution, long duration, reversibility, topographical specificity, and timing dependency (Stefan, Kunesch, et al., 2002).

For all of the above mentioned reasons and for pharmacological studies showing PAS-induced changes dependency on NMDA (Stefan, Kunesch, et al., 2002; Wolters, Sandbrink, et al., 2003), there is enough evidence to support that the increase or decrease of motor cortical excitability by PAS may represent associative LTP/LTD or a closely related phenomenon in the human motor cortex (Carson and Kennedy, 2013; Stefan, Kunesch, et al., 2002; Stefan, Kunesch, et al., 2000; Stefan, Wycislo, et al., 2006).

1.2.2. Transcranial direct current stimulation (tDCS)

Transcranial direct current stimulation (tDCS) is a kind of NIBS technique that could enhance or reduce cerebral excitability by the use of low current delivered through the skull via two small electrodes (Nitsche and Paulus, 2000).

Although tDCS is capable of produce similar physiological and behavioural effects to TMS, they are believed to operate by different mechanisms. However, the mechanisms by which tDCS exerts its effect are yet to be fully determined (Dayan, Censor, et al., 2013; Stagg and Nitsche, 2011). There are two mechanisms by which tDCS modulates brain activity that are widely accepted. The first proposes that tDCS acts by the alteration of resting membrane potentials of neuronal populations via ionic adjustment of extracellular space. Neurons proximal to the anode are thought to become de-polarized whilst neurons near the cathode are thought to be hyper-polarize at a subthreshold level, thereby modulating spontaneous firing frequency (Paulus, Antal, et al., 2012). This shift in resting membrane potential is believed to occur both during stimulation and for a short period of time (less than 5 minutes) following stimulation (Horvath, Forte, et al., 2015). The second proposes that tDCS

modulates synaptic activity in a similar way as LTP (under the anode) and LTD (under the cathode). This mechanism is believed to be active for an extended period of time (up to 2 hours) following the cessation of at least 7 minutes of stimulation (Horvath, Forte, et al., 2015; Stagg and Nitsche, 2011).

Therefore, anodal stimulation increases cortical excitability whilst cathodal stimulation decreases it.

tDCS applied for a critical period of time produces aftereffects on cortical activity that last for hours after the stimulation ended, i.e. aftereffect of 90 minutes duration has been reported with only 13 minutes of stimulation (Nitsche and Paulus, 2000; Nitsche and Paulus, 2001).

Other factors that could be modified in order to influence the aftereffects of tDCS are the intensity of the stimulation, the size of the electrodes (smaller the electrode, bigger the focality) and the position of the reference electrode (could be cephalic or extracephalic) (Nitsche, Cohen, et al., 2008).

One of the biggest advantages of tDCS, besides its easy application during task performance or during imaging, is its efficient sham (Gandiga, Hummel, et al., 2006).

For anodal stimulation, these aftereffects are protein-synthesis-dependent, are accompanied by intracellular calcium accumulation, and enhance cyclic adenosine monophosphate (cAMP)-levels (Paulus, Antal, et al., 2012). Besides that the physiological and cellular characteristics of the effects of tDCS share some characteristics with LTP, Fritsch and colleagues recently directly confirmed in mouse M1 slices the synaptic effect of DCS in inducing LTP. Furthermore, they concluded that this induction is polarity specific, NMDA receptor dependent, and requires coupling of DCS with repetitive low-frequency synaptic activation (Fritsch, Reis, et al., 2010).

1.2.3. iTBS, PAS and tDCS

After introduce briefly three of the most used NIBS techniques (iTBS, PAS and tDCS), we can conclude that all of them are able to induced cortical plasticity through LTP-like mechanisms. They share some common underlying mechanisms to induce such plasticity, however, they differ in many others (figure 7). iTBS increases cortical excitability mimicking the frequency of discharge of hippocampal neurons during learning (Huang, Edwards, et al.,

2005). PAS enhance excitability pairing peripheral nerve stimulation (that activates somatosensory fibbers) with TMS pulse over the correspondent primary motor cortex. Following the principles of the Hebbian Rule, both stimuli reach the synapsis synchronically (or approximately) and enhance excitability. Anodal tDCS increases cortical excitability, possibly by depolarizing neurons (Nitsche and Paulus, 2001), is non-focal and affects primarily areas near the surface of the brain (layer II-III). Both PAS and iTBS are focal and affect deeper areas than tDCS (layer V-VI). Otherwise, PAS-induced plasticity is associative-dependent, whilst tDCS and TBS do not induce associative plasticity (for a review and comparison of the techniques, see (Nathan, Cobb, et al., 2011).

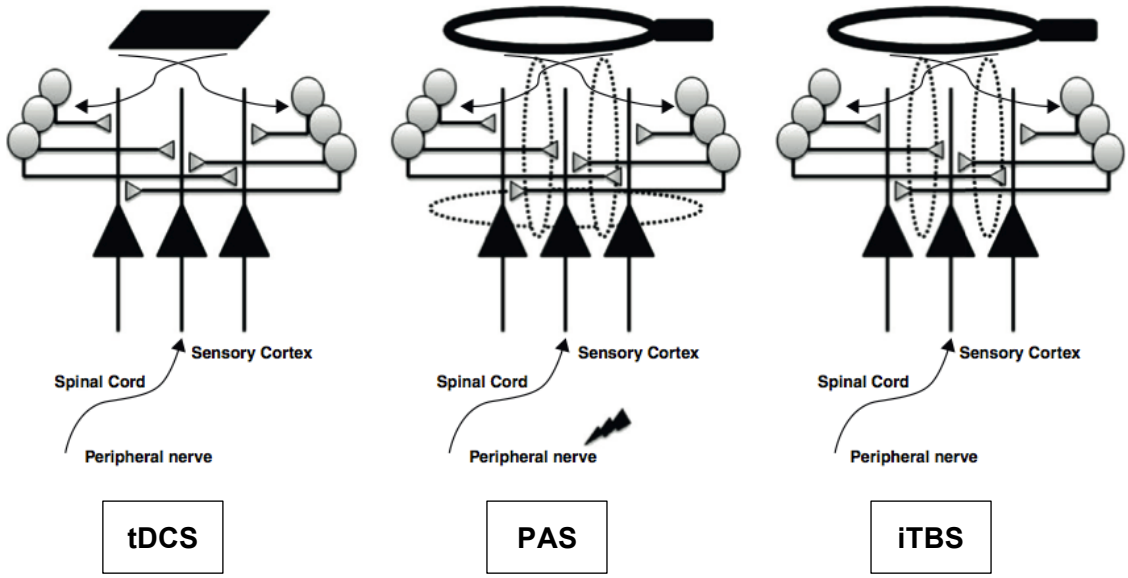


Figure 7. Physiological basis of cortical excitation by tDCS, PAS, and TBS. Black triangles represent pyramidal cells, grey circles represent GABA interneurons and dashed lines show the current induced by TMS. Modified from (Nathan, Cobb, et al., 2011).

The changes in cortical excitability induced by iTBS, PAS and tDCS have been shown to be NMDA receptor dependent, therefore involving LTP/LTD like changes in the human brain (Nathan, Cobb, et al., 2011; Ziemann, Reis, et al., 2014).

Table 1. Strengths and weakness of plasticity inducing transcranial stimulation protocols (modified from (Ziemann, Paulus, et al., 2008)

	tDCS	PAS	iTBS
Rooting in basic research	+	+++	+++
Knowledge of physiology	++	++	++
Knowledge of parameter variation	+	+	+
Tool to test plasticity in humans	+	+++	++
Diagnostic applications	+	++	-
Therapeutic applications	++	?	++

- weak, + moderate, ++ strong, +++ excellent, ? no sufficient data

At this point, is important to noteworthy that although TMS and tDCS can be used to probe, to facilitate, and to suppress or inhibit the cortical network, these tools are artificial and neither is able to imitate in full the engagement of the same network that occurs during a voluntary movement (Di Lazzaro, Ziemann, et al., 2008).

On one hand, as described by Cheeran and colleagues, artificially induced plasticity shows more similarities with LTP/LTD, besides be NMDA-dependency (Cheeran, Koch, et al., 2010):

- Frequency-dependency: rTMS increases or decreases MEP amplitude depending on frequency of stimulation.
- Spike-timing dependency: precise timing of stimuli can produce changes in MEP amplitude in paired-associative stimulation.
- Hebbian plasticity: the changes in MEP amplitude have a degree of somatotopy in paired-associative stimulation.
- Consecutive sessions of PAS produce an effect similar to the Bienenstock, Cooper and Munro (BCM) rule and metaplasticity.
- The effect of brain-derived neurotrophic factor (BDNF) polymorphisms in human plasticity.

Nevertheless, on the other hand, NIBS-induced plasticity shows some differences with LTP/LTD (Cheeran, Koch, et al., 2010):

- The change in MEP in some induction protocols does not always occur immediately after induction.
- Changes in the excitability of corticospinal neurons, rather than just the synaptic efficacy of the excitatory interneurons synapsing onto corticospinal neurons, can also produce changes in MEP amplitude.
- High degree of inter-individual and intra-individual variability.

This last characteristic of NIBS-induced plasticity, the high inter- and intra-individual variability is the aim of study of the first and second experiments in the present work.

1.2.4. Variability in response to NIBS

Already in simple TMS pulses the stimulus-response relationship varies considerably between subjects (van der Kamp, Zwinderman, et al., 1996) and also between muscles in the same subject (Ziemann, Ilic, et al., 2004). Furthermore, MEP size and shape varies from stimulus to stimulus, even if the stimulus parameters are kept constant (Ellaway, Davey, et al., 1998; Kiers, Cros, et al., 1993). Varying numbers of excited motor neurons, varying numbers of repetitive discharges, and varying synchronization are three mechanisms that may contribute to this variability.

Moreover, this inter-individual variability exists also in NIBS-induced plasticity. Recently several studies have reported that not all people respond as expected to NIBS protocols (Gangitano, Valero-Cabre, et al., 2002; Hamada, Murase, et al., 2013; Hinder, Goss, et al., 2014; Maeda, Keenan, et al., 2000; Muller-Dahlhaus, Orekhov, et al., 2008; Wiethoff, Hamada, et al., 2014). It is very important to understand this variability in response to NIBS in order to establish efficient strategies of rehabilitation. Knowing whether a person will respond positively or negatively to a certain protocol could afford time and unnecessary stimulation and could allow designing more effective programs or treatments to each subject. A recent study has demonstrated that application of NIBS may be detrimental to some people, rather than merely less effective, depending on the trait (Sarkar, Dowker, et al., 2014), so this approach appears to be essential.

1.3. MOTOR LEARNING

> *"Motor learning is a set of processes associated with practice or experience leading to relatively permanent changes in the capability for skilled movements".*
>
> *(Schmidt and Lee, 2011)*

It is out of the scope of the current thesis to perform a systematic review about the motor learning domain, the theories, hypothesis and procedures. Therefore, we focus on the terminology and motor paradigms relevant for the current thesis, being aware of this basic approach to the motor learning field.

To begin this section is important to distinguish between two key concepts in the motor learning domain, learning of a new motor skill and adaptation to an external perturbation (Krakauer and Mazzoni, 2011).

Acquisition of a new motor skill involves the acquisition of new movement qualities and/or muscle synergies that enhance performance beyond pre-existing levels (Reis, Robertson, et al., 2008).

Adaptation may not require this acquisition of new motor synergies or movement patterns. Adaptation engages movements that were achieved throughout life, to return to baseline levels of performance in response to external perturbations (Reis, Robertson, et al., 2008).

Furthermore, traditional classifications of learning and memory distinguish between learning of explicit memories and learning of implicit memories. Implicit learning involves the acquisition of knowledge independently of awareness of both the process and the products of acquisition (Seber, Allen, et al., 1999) . An example of implicit learning is the natural language acquisition. On the other hand, explicit learning is that we are conscious about its acquisition. Whereby various mnemonics, heuristics, and strategies are engaged to induce a representational system (Dekeyser, 2008).

Finally, we should differentiate measurements of motor performance (i.e. speed or accuracy in isolation) from motor skill. Motor skill involves a change in the speed-accuracy trade-off function (SAF) and is task-dependent (Reis,

Robertson, et al., 2008). To better explain motor skill we will provide the simple example described by Reis and colleagues, *if a tennis player hits 125-mph serves but only gets 25% of the balls in the service box, is he more, less, or equally skilled in comparison to a player who hits the ball at 100 mph but gets 50% of the balls in? Answering this question in general requires the ability to distinguish between whether 1) the SAF has changed (which would mean that skill has changed) or 2) performance has been sampled at a different place on the same SAF (which would mean that skill has not changed)* (Reis, Schambra, et al., 2009).

1.3.1. Paradigms of motor learning

There are several paradigms to test the different aspects of motor learning. In this section are described those utilized in the studies of this thesis.

1.3.1.1. Sequence learning

Motor sequence learning is the acquisition and optimization of a novel series of inter-related movements (Penhune and Steele, 2012).

Nissen and Bullemer introduced one of the first and more used paradigms of sequence learning, the serial reaction time task (SRTT) (Nissen and Bullemer, 1987). In this task, subjects learn a sequence of key-press movements in response to a visual cue. With the repetition, the reaction time (RT) becomes progressively faster. Then a random series of button presses is presented and the reaction time would be slower. Improvements in performance on this task are measured by shortening in reaction times (comparing repeating sequences with random series). There are many variants of the task. In this thesis we used a similar task as the original described by Nissen and Bullemer. Subjects learn the sequence without awareness. So we used it primarily as a measure of implicit learning.

Other kind of sequence learning tasks could be, for example, sequential visual isometric pinch tasks (SVIPT). These tasks measure accurate motor performance. Reis and colleagues used a SVIPT in which squeezing a force transducer moves a cursor in the screen depending on the intensity of the squeeze. The goal of the task was to move the cursor quickly and accurately

between the start position and a numbered order of targets (Reis, Schambra, et al., 2009).

1.3.1.2. Visuomotor adaptation

As already described, one of the fundamental properties of the human brain is its ability to adapt to changing conditions.

Visuomotor adaptation tasks (VAT) are for example reaching tasks that involve environmental changes during the performance, such as, artificially rotating and/or scaling the visual space via manipulation of the real-time visual feedback of hand movements displayed on a computer monitor. In visually guided reaching, the location (or spatial direction) of an object with respect to the initial position of the hand needs to be transformed into motor commands that move the arm toward the target (Krakauer, Ghilardi, et al., 1999). This visuomotor transformation needs to be updated if environmental conditions change which requires the adaptation (Buch, Young, et al., 2003).

In summary, the visuomotor adaptation paradigm can be viewed as an active task that requires transformation, integration, modification, and storage of visuospatial and kinesthetic information (Buch, Young, et al., 2003).

1.3.2. Role of M1 in motor learning

Motor learning engaged the activation of a wide network in the cortex. This network includes primary motor, premotor and supplementary motor cortices, cerebellum, thalamic nuclei and striatum (Karni, Meyer, et al., 1998; Penhune and Steele, 2012; Reis, Robertson, et al., 2008; Sanes and Donoghue, 2000; Ungerleider, Doyon, et al., 2002). Depends on the learning paradigm and the stage of learning, the involvement of each area is different (Smyth, Summers, et al., 2010). Between all these cortical areas, most of the TMS and tDCS studies studying motor learning have focused on M1. This is the case of the present work as well.

M1 has been demonstrated to be critical in different motor learning paradigms, although there is some controversy about the weightiness of its role. M1 involvement has been demonstrated in humans for example in the encoding of elementary motor memories (Butefisch, Khurana, et al., 2004), in sequence motor learning, mainly in the implicit process (Kantak, Mummidisetty, et al.,

2012; Wilkinson, Teo, et al., 2010) and in motor adaptation (Hadipour-Niktarash, Lee, et al., 2007; Riek, Hinder, et al., 2012).

Furthermore, the involvement of M1 in motor learning is also essential in the offline learning, i.e. the acquisition of skill that continues after practice ends, also called consolidation (Galea, Vazquez, et al., 2011; Kantak, Mummidisetty, et al., 2012; Reis, Schambra, et al., 2009; Robertson, Press, et al., 2005).

1.4. RELATIONSHIP BETWEEN PLASTICITY AND MOTOR LEARNING

As already described, changes in synaptic plasticity induced by all the three aforementioned protocols have been shown to be NMDA receptor-dependent, therefore involve LTP/LTD like changes in the human brain. Furthermore, LTP and LTD remain prime candidates for mediating learning and memory (Collingridge, 1987; Malenka and Bear, 2004; Martin, Grimwood, et al., 2000; Moser, Krobert, et al., 1998). This suggests that the iTBS-, PAS- and tDCS-induced strengthening of glutamatergic neurotransmission should subsequently enhance the performance on learning tasks (Nathan, Cobb, et al., 2011).

Martin and colleagues established an hypothesis, which called synaptic plasticity and memory (SPM) hypothesis, joining those of previous authors (Hebb, 1949; Kandel and Schwartz, 1982; Lynch and Baudry, 1984; McNaughton and Morris, 1987) that shared a common core: *Activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed (Martin, Grimwood, et al., 2000).*

According to this suggestion, a number of studies have shown improvements in motor learning following iTBS (Teo, Swayne, et al., 2011), PAS (Jung and Ziemann, 2009; Rajji, Liu, et al., 2011) and tDCS (Nitsche, Schauenburg, et al., 2003; Reis and Fritsch, 2011; Reis, Schambra, et al., 2009).

So, seems clear that is possible to improve motor learning through NIBS, however, the relation between these two concepts, i.e. motor learning and

NIBS-plasticity is still not fully understood. There are several studies questioning this relationship.

In fact, as suggested by Martin and colleagues (Martin, Grimwood, et al., 2000), the SPM hypothesis should be distinguished from others about LTP or LTD. These include the null hypothesis, called the plasticity/pathology continuum hypothesis, which states that synaptic plasticity has nothing to do with memory (McEachern and Shaw, 1996), and the notion that synaptic plasticity plays a role in attentional rather than memory processes (Shors and Matzel, 1997).

More recently, behavioural studies have thrown controversial results about this topic. Some of them concluded that the plasticity induced by NIBS over M1 does not correlate directly with motor learning (Agostino, Iezzi, et al., 2008; Li Voti, Conte, et al., 2011; Muellbacher, Ziemann, et al., 2000; Vallence, Kurylowicz, et al., 2013), whilst others succeeded in found such relation (Witte, Kurten, et al., 2012).

The extent number of motor learning paradigms and the variety of NIBS protocols make difficult to establish a relationship between both concepts. Each motor learning task involves different demands of brain regions; and mechanisms of NIBS protocol, although similar, differed in some aspects. Furthermore, the huge inter-individual response both to NIBS and motor learning, could also contribute to complicate the observation of such relation.

Chapter 2

Questions of relevance

2. QUESTIONS OF RELEVANCE

After the brief review of the current data about the topic of this thesis, it still remain unresolved some questions of interest:

1) Do all the people respond equal to each of the NIBS protocols?

2) Are all the NIBS techniques equally effective?

3) Is there any predictor of the response to NIBS protocols?

4) Does a subject respond in the same way to the same protocol in separate sessions?

5) Is there a relationship between cortical plasticity induced by NIBS and motor learning capacity?

With this work we tried to address these questions. Questions 1, 2 and 3 will be addressed in the first study. Questions 4 and 5 will be addressed in the second and third study, respectively.

Chapter 3

Hypothesis and main aims of the studies

3. HYPOTHESIS AND MAIN AIMS OF THE STUDIES

3.1. Study I: Inter-individual Variability in Response to NIBS

3.1.1. Hypothesis

Inter-individual variability in response to NIBS paradigms would be better explained if a multimodal distribution was assumed.

3.1.2. Aims

- To address inter-individual variability of the three more used facilitatory protocols of NIBS used to induce increments of excitability (PAS_{25}, AtDCS, and iTBS).
- To test whether baseline TMS measures, change in inhibitory interneuronal activity or response to another NIBS paradigm could predict the pattern of MEP amplitude response for each individual.
- To compare the effectiveness of the three NIBS protocols tested (number of responders and intensity of the aftereffects).

3.2. Study II: Intra-individual Variability in Response to AtDCS

3.2.1. Hypothesis

Intra-individual variability in response to two separate sessions of anodal tDCS would be lower than inter-individual variability.

3.2.2. Aims

- To test the intra-individual reliability in response to Anodal Transcranial Direct Current Stimulation (AtDCS).

3.3. Study III: M1 modulation and motor learning

3.3.1. Hypothesis

Changes in cortical excitability of the primary motor induced by non-invasive brain stimulation are related with motor learning capacity.

3.3.2. Aims

- To explore whether the cortical plasticity induced by NIBS protocols on M1 correlates with the motor learning capacity as measured by performance on established lab-based motor learning tasks.

Chapter 4

Studies

Study I:

Inter-individual Variability in Response to Non-invasive Brain Stimulation Paradigms

4.1. Study I: Inter-individual Variability in Response to Non-invasive Brain Stimulation Paradigms

4.1.1. Abstract

Background: Non-invasive Brain Stimulation (NIBS) paradigms are unique in their ability to safely modulate cortical plasticity for experimental or therapeutic applications. However, increasingly, there is concern regarding inter-individual variability in the efficacy and reliability of these paradigms. *Hypothesis:* Inter-individual variability in response to NIBS paradigms would be better explained if a multimodal distribution was assumed. *Methods:* In three different sessions for each subject (n=56), we studied the Paired Associative Stimulation (PAS_{25}), Anodal Transcranial DC Stimulation (AtDCS) and intermittent Theta Burst Stimulation (iTBS) protocols. We applied cluster analysis to detect distinct patterns of response between individuals. Furthermore, we tested whether baseline TMS measures (such as Short Intra Cortical Inhibition (SICI), Resting Motor Threshold (RMT)) or factors such as time of day could predict each individual's response pattern. *Results:* All three paradigms show similar efficacy over the first hour post stimulation- there is no significant effect on excitatory or inhibitory circuits for the whole sample, and AtDCS fares no better than iTBS or PAS_{25}. Cluster analysis reveals a bimodal response pattern – but only 39%, 45% and 43% of subjects responded as expected to PAS_{25}, AtDCS, and iTBS respectively. Pre-stimulation SICI accounted for 10% of the variability in response to PAS_{25}, but no other baseline measures were predictive of response. Finally, we report implications for sample size calculation and the remarkable effect of sample enrichment. *Conclusion:* The implications of the high rate of 'dose-failure' for experimental and therapeutic applications of NIBS lead us to conclude that addressing inter-individual variability is a key area of concern for the field.

4.1.2. Introduction

Non-Invasive Brain Stimulation (NIBS) paradigms remain the principal tool to probe and modulate cortical plasticity in the awake human cortex. The effects of NIBS manifests as an increase or decrease in cortical excitability, as measured

by the change in amplitude of Motor Evoked Potentials (MEPs), that outlasts the period of stimulation (Nitsche and Paulus, 2000; Priori, Berardelli, et al., 1998; Ziemann, Paulus, et al., 2008). Moreover, NIBS-induced changes in cortical excitability may be sub-served by mechanisms similar to those of NMDA receptor (NMDAR) dependent long-term potentiation (LTP) or long-term depression (LTD), the synaptic currency by learning occurs and memory is encoded (Cooke and Bliss, 2006; Moser, Krobert, et al., 1998; Ziemann, Ilic, et al., 2004). This characteristic has underpinned the application of NIBS as a therapeutic adjunct, for example in rehabilitation after neurological diseases such as stroke (Demirtas-Tatlidede, Vahabzadeh-Hagh, et al., 2013; Di Lazzaro, Profice, et al., 2010; Zimerman, Heise, et al., 2012).

As a result this broad utility, there has been a proliferation the number of NIBS protocols and proposed applications of each protocol. The most established protocols to increase cortical excitability (by recent citation records) are excitatory Paired Associative Stimulation (PAS) (Stefan, Kunesch, et al., 2000), anodal Transcranial Direct Current Stimulation (AtDCS) (Nitsche and Paulus, 2000) and intermittent Theta Burst Stimulation (iTBS) (Huang, Edwards, et al., 2005).

Despite the widespread adoption of the NIBS protocols, there appears to be little consensus (or data) regarding the relative merits of each protocol with regards to efficacy (in terms of the magnitude or duration of the aftereffects) (Di Lazzaro, Dileone, et al., 2011; Player, Taylor, et al., 2012; Vallence, Kurylowicz, et al., 2013). Recently, studies have also questioned the reliability (percentage of subjects that respond as expected) of PAS and TBS when analysed with an ‘intention to treat’-like approach (i.e. where the study sample was not enriched by omitting subjects that did not show the expected response), and reported significant inter-individual variability in the response for these paradigms (Hamada, Murase, et al., 2013; Muller-Dahlhaus, Orekhov, et al., 2008). To date, there are no studies reporting a similar lack of efficacy or significant inter-individual variability in the response to tDCS. However, knowledge of the efficacy, time course of effects and reliability (or failure-rate) for each individual NIBS protocol is crucial for the sample size calculation, choice of NIBS paradigm, design and analysis of experiments.

In this study we compared the efficacy and reliability of the three most established excitatory NIBS protocols (PAS_{25}, AtDCS and iTBS) on excitatory and inhibitory intracortical networks, in the same cohort of 56 subjects. We hypothesized that inter-subject variability could be explained if the response to NIBS was not unimodal, and therefore cluster into distinct populations. If distinct patterns of response were found, we wished to test if baseline TMS measures, change in inhibitory interneuronal activity or response to another NIBS paradigm could predict the pattern of MEP amplitude response for each individual.

4.1.3. Methods and Materials

4.1.3.1. Subjects

The experiments were approved by the Ethics Committee of University of A Coruña. A total of 56 Caucasian subjects (6 women; 53 right-handed), aged between 19-24 years (mean age±SD: 20,52±1,52) were recruited for this study after giving written informed consent. Subjects were screened for contraindications to TMS (Wassermann, 1998) (no neurological (including a past medical history of head injury or seizures), psychiatric or other significant medical problems). Each subject participated in all three stimulation protocols.

4.1.3.2. General procedure

The order of stimulation sessions (for each protocol) was counterbalanced (to avoid an ordinal effect) and sessions for each subject were at least one week apart (to avoid cumulative effects). Each individual subject took part in all three sessions at the same time of day. 36% of the subjects were tested in the morning.

4.1.3.3. EMG recordings

Electromyographic (EMG) traces were recorded via Ag-AgCl, 9mm diameter surface cup electrodes, from the right first dorsal interosseous (FDI) muscle. Signals were filtered (30 Hz to 2 kHz) with a sampling rate of 5 kHz and amplified with a Digitimer D360 amplifier (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK), and then recorded using SIGNAL software (Cambridge

Electronic Devices, Cambridge, UK).

4.1.3.4. TMS procedure

TMS were delivered through a figure-of-eight coil with outer diameter of 70 mm (Magstim Co., Whitland, Dyfeld, UK) over the left motor cortex. The coil was held with the handle pointing backwards and laterally to evoke an anteriorly directed current in the brain, and was optimally positioned to obtain MEPs in the contralateral FDI. Single and paired pulses were delivered from a monophasic Magstim BiStim.

For all three protocols, baseline and outcome data was collected in an identical fashion (see figure 8). For all the protocols, we first localized the "hot spot" (defined as the point on the scalp at which single pulse TMS elicited MEPs of maximal amplitude from the right FDI) and established the resting motor threshold (RMT) (minimum stimulation intensity over the motor hot-spot, which can elicit a MEP of no less than 50 μV in 5 out of 10 trials in the relaxed FDI) and active motor threshold (AMT) (intensity necessary to evoke a 200 μV MEP while subjects maintained approximately 10% contraction of the FDI). Active motor thresholds were obtained with both the BiStim and Super Rapid Magstim packages in the case of iTBS protocol (AMT and AMT_r, respectively, and in this order).

For the baseline, we recorded 20 MEP's (at SI_{1mV}) and SICI measures. After each protocol, 12-MEPs amplitude (inter-trial interval 5 s, vary 10%) was measured at 5-minute intervals for 60 minutes. Two blocks of SICI (10 test stimulus (TS) and 10 conditioned stimulus (CS) each, randomised) were recorded at minute 6 and minute 46 post-stimulation.

SICI was measured using the technique described by Kujirai et al. (1993) (Kujirai, Caramia, et al., 1993) -a subthreshold conditioning stimulus at the 80% of AMT (Orth, Snijders, et al., 2003) precedes a TS by 2 ms (Fisher, Nakamura, et al., 2002). The mean peak-to-peak amplitude of the conditioned MEP was expressed as a percentage of the mean peak-to-peak amplitude of the unconditioned MEP. The design of the protocol, with MEP measures each 5 minutes, did not allow us to measure a SICI response curve (combining different interestimulus intervals (ISIs) and intensities), neither the individual adjustment of the TS intensity. However, this limitation in no way detracts from

the major aim of the study.

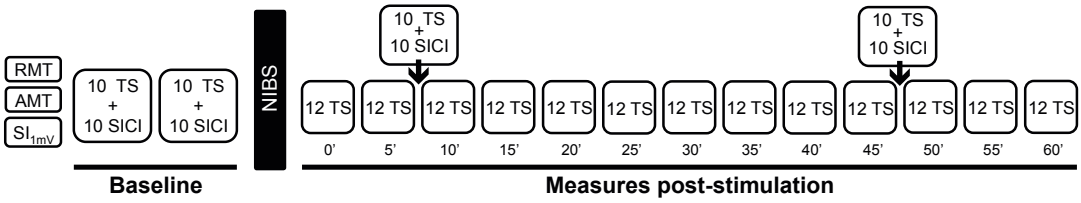


Figure 8. Common Protocol for each NIBS session. Resting Motor Threshold (RMT), Active Motor Threshold (AMT), Stimulus intensity to elicit a 1mV (SI_{1mV}) peak to peak amplitude Motor Evoked Potential (MEP) were recorded. 20 Baseline MEP's (at SI_{1mV}) and SICI measures were recorded. After each protocol was delivered, MEP amplitude was measured at 5-minute intervals for 60 minutes. Two blocks of SICI were recorded at minute 6 and minute 46 post-stimulation.

4.1.3.5. Paired associative stimulation (PAS_{25})

PAS consisted on 200 electrical stimuli (at 300% of the perceptual threshold (PT)) over ulnar nerve at the right wrist, paired with TMS pulses (interstimulus interval of 25 ms) over the left hemisphere FDI hotspot at a rate of 0,25 Hz (total protocol duration approximately 13 minutes). Subjects were asked to count the number of stimuli given to ensure their attention did not vary.

PAS protocols commonly pair ADM and ulnar nerve or APB and median nerve. We opted to use a less frequently employed PAS protocol, pairing FDI and ulnar nerve, in order to record the FDI muscle across all three NIBS protocols. Although the ulnar nerve innervates the FDI, the ulnar nerve does not supply the cutaneous area over FDI. However, several studies have reported that this protocol induces significant changes in MEP amplitude (Dileone, Profice, et al., 2010; Player, Taylor, et al., 2012). We acknowledge this may impact the direct comparison with previous studies and interpretation of PAS_{25} protocol results as no direct comparison has been made between these PAS protocol variants.

4.1.3.6. Anodal transcranial direct current stimulation (AtDCS)

tDCS was delivered at 1 mA for duration 13 min through a pair of saline-soaked sponge surface electrodes (35 cm^2) connected to a DC stimulator

(neuroConn). Active electrode (anode) was placed over the hotspot of the left M1 (as determined by TMS), the reference electrode (cathode) was placed over the contralateral supraorbital region. The current was faded in and faded out 8 seconds each.

4.1.3.7. Intermittent theta-burst stimulation (iTBS)

A biphasic stimulator, a Super Rapid Magstim package (Magstim Co., UK), was used to deliver TBS. iTBS was applied over the left motor cortex hot-spot as described by Huang et al. (2005) (Huang, Edwards, et al., 2005). Each burst consisted of three stimuli (at 80% AMT_r stimulator intensity) given at 50 Hz, repeated at 5Hz. iTBS involves giving a 2s train repeated every 10s for 20 repetitions (600 stimuli).

4.1.3.8. Statistical analysis

All statistical analyses were performed using SPSS (SPSS, Chicago, IL).

4.1.3.8.1. Effect of NIBS on MEP Amplitude and SICI

Henze-Zirkler test was applied to explore the normality of the multivariate dataset.

Repeated measures analyses of variance ($ANOVA_{rm}$) was conducted for the absolute values of baseline MEP amplitude with PROTOCOL as main factor.

$ANOVA_{rm}$ was also performed for the absolute values of MEP amplitude with PROTOCOL (PAS_{25}, AtDCS and iTBS) and TIME (baseline, 0, 5, …. 60 min) as factors. $ANOVA_{rm}$ was also conducted for the absolute values of SICI for each protocol, with PROTOCOL (PAS_{25}, AtDCS and iTBS) and TIME (baseline, 6 and 46 min) as factors.

4.1.3.8.2. Cluster Analysis

Due to the high interindividual variability in the response to each NIBS protocol, we used the SPSS TwoStep cluster analysis to determine if there are patterns of response to each protocol. This clustering method determines the optimal number of clusters that best explains variance in the data automatically. MEP amplitudes of each block (0, 5,… 60), normalised to the baseline, were used for this analysis. TwoStep analysis resulted in a two-cluster distribution for

each of the three paradigms, and we termed these clusters “responders” (showing the expected response) and “non-responders” (those who don’t show an expected response).

$ANOVA_{rm}$ was conducted for the absolute values of MEP amplitude for each protocol with TIME (baseline, 0, 5, … 60) as factor and CLUSTER (the variable obtained in cluster analysis; “responders” and “non-responders”) as inter-subject factor. Although a good quality cluster analysis would be expected to result in clusters that are significantly distinct on an ANOVA, we performed this to detect the time points where responders and non-responders differed significantly (both from baseline MEP amplitude within cluster and between clusters).

Grand average analysis was also conducted to look for the percentage of “responders” and “non-responders” using the mean grand average post-stimulation. Subjects with grand average > 1 were classified as “$responders_{GA}$” and subjects with grand average < 1 were classified as “$non\text{-}responders_{GA}$”.

McNemar analyses were conducted to look for differences in frequency of responders to each protocol regarding to MEP amplitude. Cohen’s Kappa coefficient was calculated to test for associations between the responses induced by the three stimulation protocols.

4.1.3.8.3. Predictors of response to PAS_{25}, AtDCS and iTBS

To look for predictors, forward binary logistic regression was conducted for each protocol with CLUSTER (for each protocol) as the dependent variable and measures listed in Figure 12 as independent variables.

$ANOVA_{rm}$ was conducted for the absolute values of SICI for each protocol with TIME (baseline, 6 and 46 min) as factor and CLUSTER (responders and non-responders) as inter-subject factor. Contingency analyses were also conducted with clusters for each protocol and change in SICI (increase or decrease from baseline to minute 6). Pearson chi-square test was used to test the independence of the two variables.

When a significant main effect was found (p value < 0.05), post hoc t-test with Bonferroni corrections were conducted. Greenhouse-Geisser correction was used for non-spherical data.

4.1.4. Results

No adverse effects were reported.

Henze-Zirkler test confirmed normality in the set of data (p=0.2092).

4.1.4.1. Effect of NIBS on excitatory and inhibitory intracortical interneuronal circuitry

$ANOVA_{rm}$ revealed no significant differences in baseline MEP amplitude between protocols (F=0.687; p=0.505).

$ANOVA_{rm}$ for MEP amplitude at each timepoint in the whole sample revealed that there was no effect of PROTOCOL (F=0.235; p=0.791) or PROTOCOL x TIME (F=1.274; p=0.226) interaction. Although there was an effect of TIME (F=2.405; p=0.015), post hoc analysis showed no differences between baseline and any time point (see Figure 9).

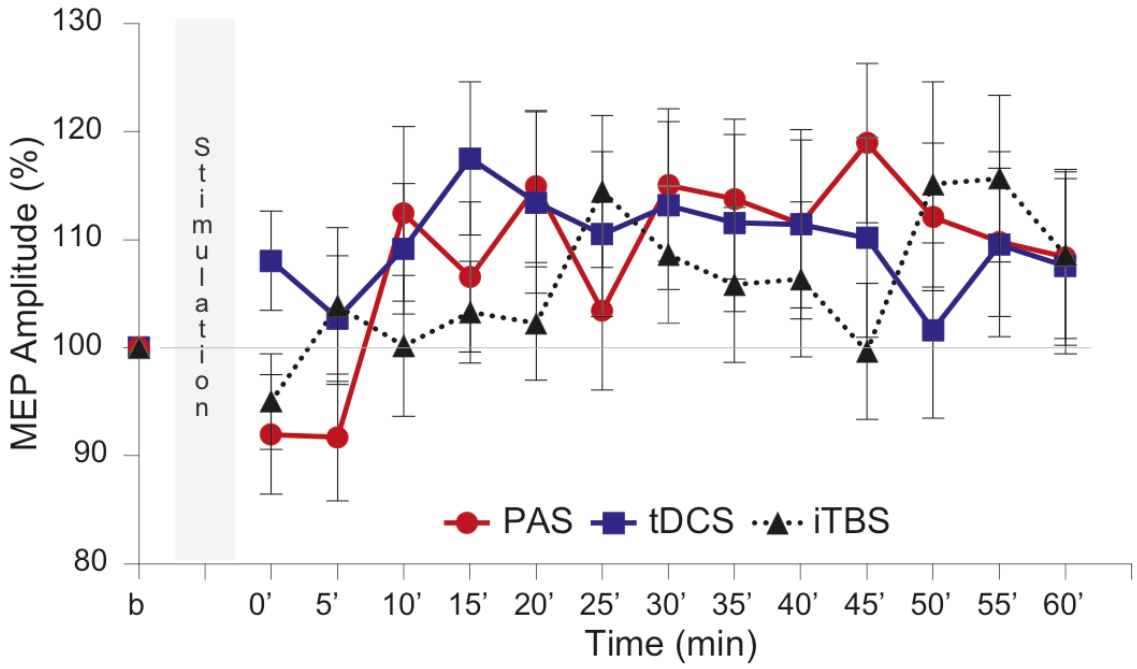


Figure 9. Effect of NIBS on MEP amplitude: Change in MEP amplitude (normalised to baseline MEP amplitude (b)) for the whole sample (n=56) for PAS_{25}, anodal AtDCS and iTBS. Error bars represent standard error. ANOVA of repeated with absolute values revealed significant main effect of TIME (F=2.405; p=0.015) but not PROTOCOL (F=0.235; p=0.791) or TIME x PROTOCOL (F=1.274; p=0.226) interaction. Post hoc pairwise comparisons analysis showed no significant differences between baseline and any time point.

In one subject the data recording was incomplete due to a technical issue with delivering the conditioning stimulus in the tDCS session and this subject was removed from the SICI analysis. $ANOVA_{rm}$ for absolute values of SICI did not revealed significant effect of TIME (F=0.367; p=0.571), effect of PROTOCOL, (F=0.564; p=0.57) or PROTOCOL x TIME (F=2.4; p=0.051) interaction (Figure 10).

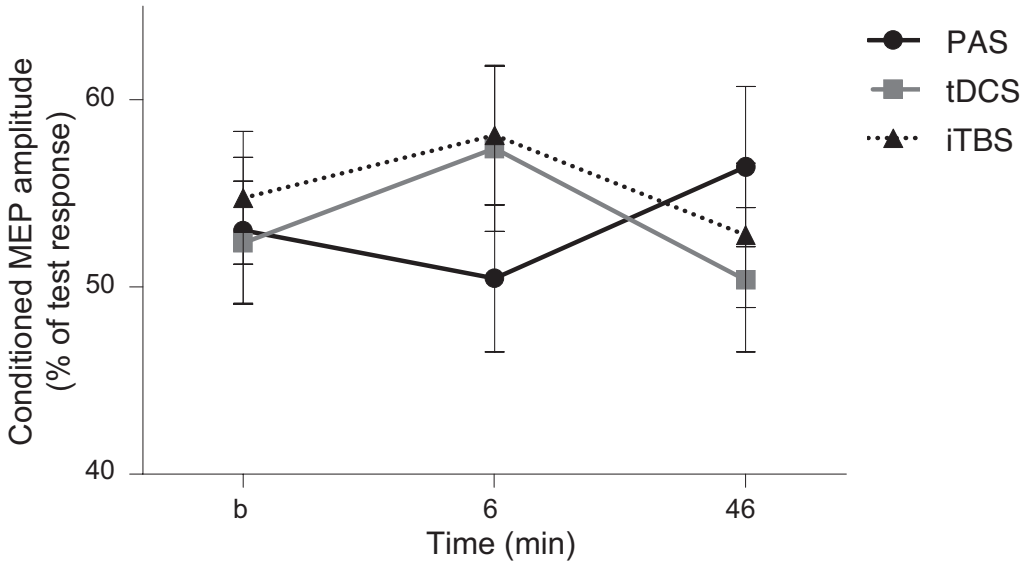


Figure 10. Effect of NIBS on SICI amplitude: Change in SICI (shown as paired pulse conditioned MEP amplitude normalised to test MEP amplitude) at baseline (pre-NIBS), minute 6 and minute 46 post-NIBS, for the whole sample (n=56). Larger SICI amplitude implies less GABAaR mediated inhibition. Error bars represent standard error. ANOVA of repeated measures with absolute values showed a lack of main effect for TIME (F=0,367; p=0.694) and PROTOCOL (F=0.564; p=0.571), but a trend in the TIME x PROTOCOL interaction (F=2,4; p=0.051). Post hoc pairwise comparisons analysis showed no significant differences between baseline and any post NIBS SICI measure.

TwoStep cluster analysis revealed two clusters for each protocol. We have termed the cluster showing the expected response to the protocol, an increase in MEP amplitude, “responders (R)”, and the cluster showing no increase in MEP amplitude “non-responders (NR)”.

39%, 45% and 43% of the subjects responded as expected to PAS_{25}, AtDCS and iTBS, respectively.

The percentage of responders for each protocol in the GRAND AVERAGE analysis were 53.6%, 50% and 46.4% for PAS, tDCS and iTBS, respectively.

Only 12.5% of the total sample responded as expected to all the three protocols and 25% showed an unexpected response to all the three protocols. McNemar analysis revealed no significant differences between the numbers of responders to each protocol.

Kappa (κ) for each pair of protocols was $\kappa_{tDCS/PAS}$= 0.23; $\kappa_{tDCS/iTBS}$= 0.165; $\kappa_{PAS/iTBS}$= 0.041. Kappa lower than 0.4 is considered as poor agreement (Fleiss, 1981).

$ANOVA_{rm}$ of MEP amplitude for each protocol with CLUSTER as inter-subject factor revealed significant main effect of TIME ($F=4.320$; $p<0.001$), CLUSTER ($F=17.108$; $p<0.001$) and TIME x CLUSTER ($F=3.884$; $p<0.001$) interaction for PAS_{25}. There was no effect of TIME ($F=1.020$; $p=0.419$) but significant effect of CLUSTER ($F=18.175$; $p<0.001$) and TIME x CLUSTER ($F=6.728$; $p<0.001$) interaction for AtDCS. There was no effect of TIME ($F=1.617$; $p=0.122$) but significant effect of CLUSTER ($F=19.402$; $p<0.001$) and TIME x CLUSTER ($F=4.734$; $p<0.001$) interaction for iTBS.

Post hoc with Bonferroni correction revealed significant differences between CLUSTERS from minute 5 onwards for PAS_{25} (Figure 11.A, hashes indicate significant differences between clusters); significant differences between CLUSTERS in the baseline and from minute 10 onwards for tDCS (Figure 11.B); and significant differences between CLUSTERS from minute 0 onwards (lack of significant differences between CLUSTERS in minute 45) for iTBS (Figure 11.C).

Post hoc with Bonferroni correction revealed that in the PAS_{25} protocol responder cluster showed significant differences between baseline and all time points from minute 10 onwards (Figure 11.A, asterisks indicate significant differences between baseline and time point below the asterisk). Similarly significant differences are shown between baseline and all time points from minute 10 onwards (except minute 50) for AtDCS, (Figure 11.B). The iTBS protocol responder cluster alone showed significant differences between baseline and all time points from minute 5 onwards (except minute 45) (Figure 11.C).

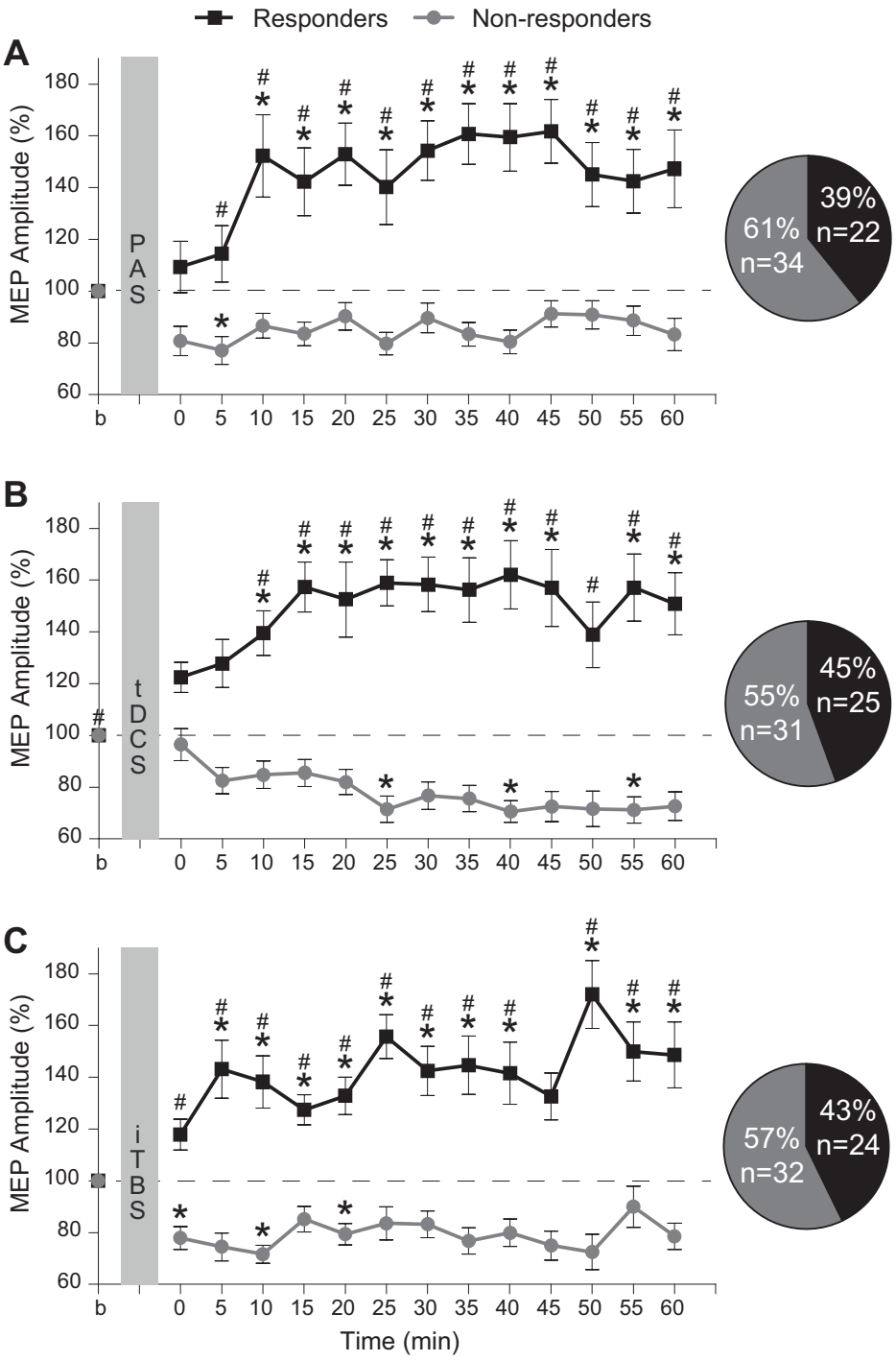


Figure 11. Cluster analysis of Effect of NIBS on MEP amplitude: Change in MEP amplitude (normalised to baseline (b)) for each cluster of (A) PAS_{25}, (B) anodal AtDCS and (C) iTBS. Errors bars represent standard error. ANOVA of repeated measures was conducted with absolute values. Asterisks indicate statistical significance between the MEP amplitude at that time point and the baseline MEP amplitude. Hash symbol indicates statistical significance between clusters ($p<0.05$). Pie charts to the right of (A), (B) and (C) indicate the number (and percentage) of subjects in each cluster for PAS_{25}, anodal tDCS and iTBS respectively. The segment shaded black shows the number of subjects in the cluster with (the predicted) increase in MEP amplitudes for each NIBS paradigm.

Post hoc with Bonferroni correction in the non-responders cluster to each protocol revealed significant differences only from baseline and minute 5 after stimulation for PAS_{25}, only between baseline and minutes 25, 40 and 55 after stimulation for AtDCS and significant differences between only between baseline and minutes 0, 10 and 20 after stimulation for iTBS.

4.1.4.2. Predictors for PAS_{25}, AtDCS and iTBS: Baseline measures

Binary logistic regression between CLUSTER for each protocol and baseline measures, revealed positive correlation only between CLUSTER and normalised conditioned stimulus before stimulation (Baseline SICI) for PAS_{25} (Cox and Snell's R^2=0.097; p=0.023) (Figure 12). Baseline SICI odds ratio was 1.024 (95% CI, 1.00 to 1.04).

Baseline measures tested as predictors

PAS_{25}	AtDCS	iTBS
Time of day	Time of day	Time of day
Age	Age	Age
SI_{1mV}/RMT	SI_{1mV}/RMT	SI_{1mV}/RMT
Baseline SICI	Baseline SICI	Baseline SICI
PT		AMT_{rapid}

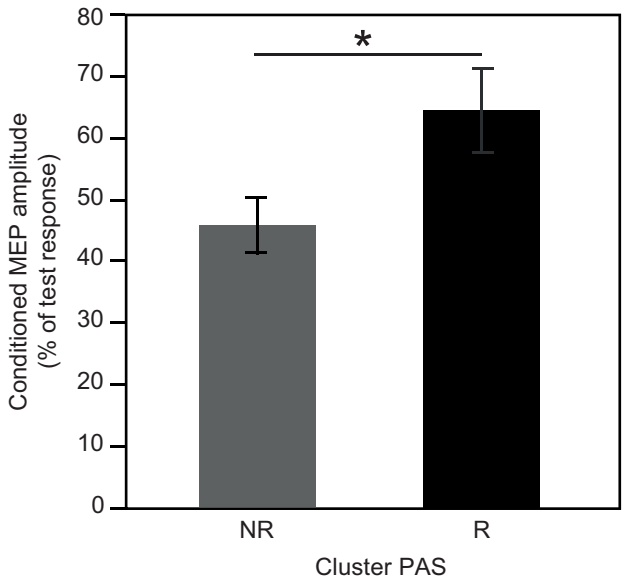


Figure 12. Baseline TMS measures as predictors of NIBS response. Top table shows measures tested as predictors for each protocol and p values from logistic regression (time of day: a.m. vs. p.m.; age; SI_{1mV}/RMT: stimulus intensity to get 1mV amplitude MEP/resting motor threshold; Baseline SICI: SICI before stimulation; PT: perceptual threshold; AMT_{rapid}: AMT measured with the biphasic stimulator). Logistic regression only shows a positive correlation between cluster and Baseline SICI for PAS_{25}. As illustrated in the figure (bottom), subjects that respond to PAS_{25}, have lower SICI (lower GABAaR inhibitory interneuronal activity resulting in larger % of test response) immediately before stimulation.

Binary logistic regression did not revealed any predictors for AtDCS or for iTBS. It is worth noting that although baseline MEP amplitude in AtDCS was different between clusters in our results (non-responders group has a significantly higher mean MEP amplitude compared to the responders group), in logistic regression baseline MEP amplitude did not show significant correlation with the response to AtDCS.

4.1.4.3. Predictors for PAS_{25}, AtDCS and iTBS: Intracortical inhibition

$ANOVA_{rm}$ for SICI with CLUSTER as inter-subject factor, for PAS_{25} revealed an effect of CLUSTER (F=5.402; p=0.024), but not TIME (F=1.148; p=0.321) or TIME x CLUSTER interaction (F=0.732; p=0.484) (Figure 13.A). $ANOVA_{rm}$ for AtDCS did not revealed significant effect of TIME (F=2.258; p=0.11), CLUSTER (F=0.741; p=0.393) or TIME x CLUSTER interaction (F=0.592; p=0.555) (Figure 13.B). $ANOVA_{rm}$ for iTBS reveal an effect of CLUSTER (F=4.751; p=0.034) but not TIME (F=1.519; p=0.224) or TIME x CLUSTER interaction (F=0.301; p=0.741) (Figure 13.C).

Contingency analysis with Pearson chi-square showed that response to each protocol is independent of the change in the SICI for the same protocol (PAS: χ^2= 0.014; p=0.906; tDCS: χ^2= 0.488; p=0.0.485; iTBS: χ^2= 1.542; p=0.214).

4.1.5. Discussion

To our knowledge this is the first and largest single center study prospectively comparing the effects of the PAS_{25}, AtDCS and iTBS NIBS paradigms on cortical excitability and inhibition, and in the same cohort of subjects. Our study confirms considerable interindividual variability in the response to all three protocols tested. There was no significant effect of any of the three NIBS protocols tested on MEP amplitude or SICI over a one-hour time period for a sample of 56 subjects. Cluster analysis based on changes in MEP amplitude revealed distinct groups of responders and non-responders to each protocol.

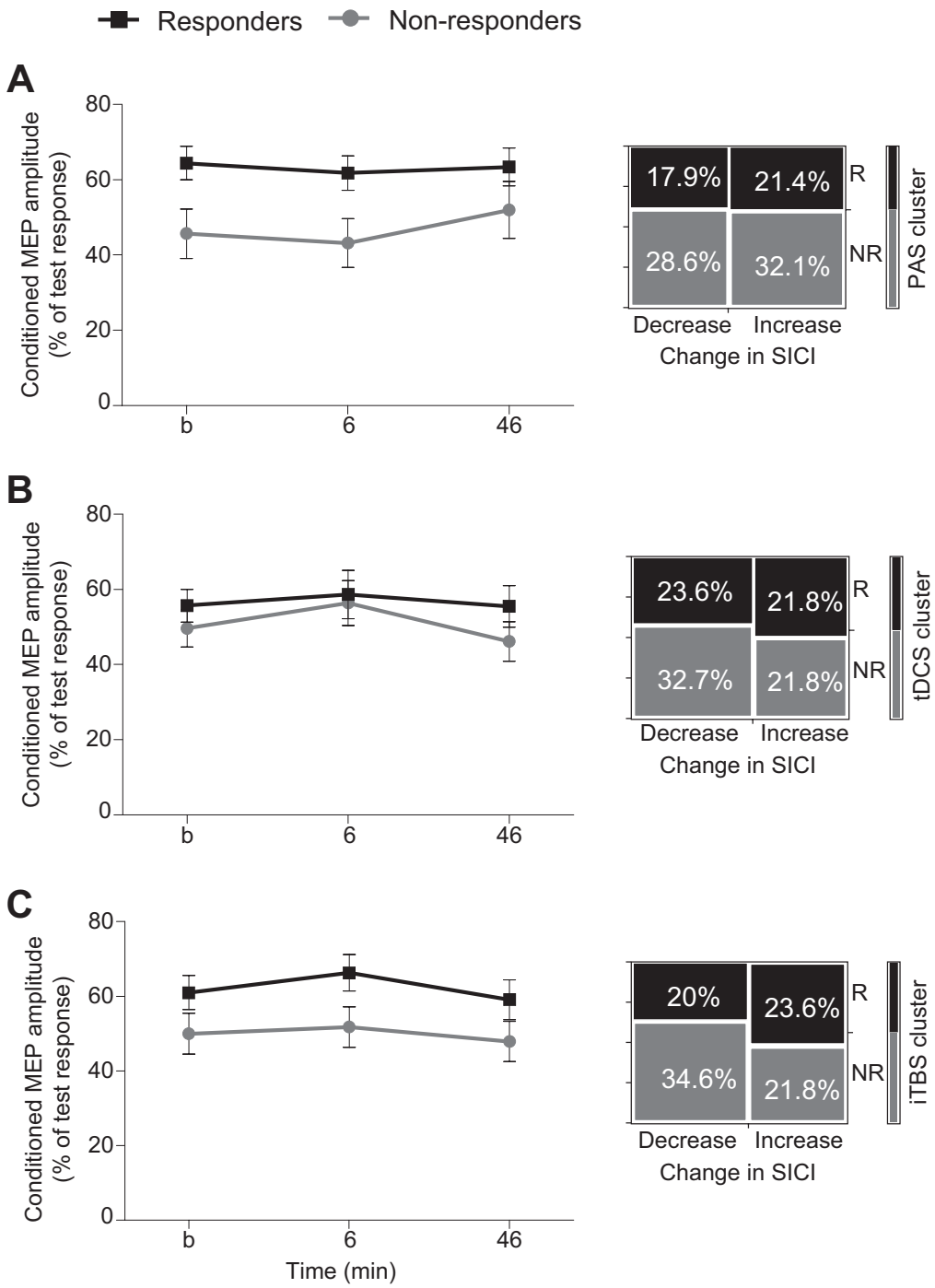


Figure 13. Change in SICI as a determinant of NIBS response. Change in SICI (shown as paired pulse conditioned MEP amplitude normalised to test MEP amplitude) (larger SICI amplitude implies less GABAaR mediated inhibition), for each cluster of (A) PAS_{25}, (B) AtDCS and (C) iTBS. Errors bars represent standard error. Contingency charts to the right of (A), (B) and (C) indicate the frequency distribution of subjects that increase or decrease SICI (x-axis) and responders and non-responders subjects (y-axis) for PAS_{25}, anodal AtDCS and iTBS respectively. The segment shaded black shows the number of subjects in the cluster with (the predicted) increase in MEP amplitudes for each NIBS paradigm.

4.1.5.1. Measuring outcomes after NIBS

We used both MEP amplitude and SICI as outcome measures, to probe effects on both excitatory and inhibitory intracortical circuits as therapeutic, lesional or behavioural effects of NIBS could be the result of direct excitatory effects or the permissive effect of altering inhibitory interneuronal circuitry. A change in MEP amplitude after NIBS reflects the larger corticospinal volley reaching the spinal motor neuron pool. At higher stimulus intensity the corticospinal volley becomes more complex and consists of multiple 'I-waves' (I2-I4 in addition to I1) (Di Lazzaro, Oliviero, et al., 2004), produced by activation of a chain of cortical excitatory interneurons (Amassian, Stewart, et al., 1987; Ziemann and Rothwell, 2000) projecting onto the pyramidal cell. Intracortical inhibition was measured by changes in SICI induced by paired pulses of TMS reflecting inhibition through gamma-aminobutyric acid-A ($GABA_aR$) (Kujirai, Caramia, et al., 1993). Studies in rats have shown that level of GABAergic inhibition affects susceptibility to induce LTP-like effects in the motor cortex, and LTP is increased when $GABA_aR$ receptor are blocked by an antagonist (Hess, Aizenman, et al., 1996). It is also feasible that NIBS exerts therapeutic or behavioural effects without any measurable effect on excitatory or inhibitory circuitry, for example by effecting haemodynamic changes (Khaleel, Bayoumy, et al., 2010; Thomson, Maller, et al., 2012). We are also not able to estimate the effects of chronic (repeated sessions of) stimulation, and whether this has a positive impact on the number of responders. However, we have designed this as a pragmatic study, that tests NIBS protocols and outcomes as they were originally described.

4.1.5.2. Effect of NIBS on excitatory and inhibitory intracortical interneuronal circuitry

Our results for iTBS and PAS are in line with Hamada et al. (2013) (Hamada, Murase, et al., 2013) and Muller-Dahlhaus et al. (2008) (Muller-Dahlhaus, Orekhov, et al., 2008), and show that these protocols failed to induce a significant increase in cortical excitability after stimulation when the whole sample is analysed. We are able to confirm additionally, that AtDCS shows a similar failure to induce a significant increase in cortical excitability after stimulation when the whole sample is analysed. Furthermore, there has been

disagreement about which NIBS protocol is more efficacious, since Player et al. (2012) (Player, Taylor, et al., 2012) found that PAS is more effective than iTBS to induce plasticity, whilst Di Lazzaro et al. (2011) and Vallence et al. (2013) (Di Lazzaro, Dileone, et al., 2011; Vallence, Kurylowicz, et al., 2013) failed to find differences between these two protocols. As there have been no studies to date reporting a lack of effect of tDCS or comparing the effect of tDCS and other stimulation protocol in the same sample, this NIBS technique has been considered by many to be the most effective and reliable NIBS protocol. Our results also show that none of the stimulation protocols induces significant changes in the intracortical inhibitory circuits assayed by SICI in the whole sample. Previous studies have reported that SICI did not change after PAS (Cirillo, Lavender, et al., 2009) or after iTBS (McAllister, Rothwell, et al., 2009), which is in line with our results. However, Kidgell et al. (2013) and Stagg et al. (2009) (Kidgell, Daly, et al., 2013; Stagg, Best, et al., 2009) had suggested that SICI diminishes after AtDCS. However, the design of the protocol, with MEP measures each 5 minutes, did not allow us to measure a SICI response curve (combining different ISIs and intensities), or the individual adjustment of the test stimulus intensity. We acknowledge this is a limitation, since it has been demonstrated that MEP amplitude of the test stimulus influences the amount of SICI. Smaller test MEP amplitudes result in lower SICI (Roshan, Paradiso, et al., 2003). The failure to adjust TS intensity individually to maintain test MEP amplitude could obscure a real change in the SICI after NIBS protocols in our data.

4.1.5.3. Cluster Analysis of MEP Amplitude response after NIBS

It will come as no surprise to experienced NIBS operators that we also found the high interindividual variability reported by Muller-Dahlhaus (2008) for PAS_{25} (Muller-Dahlhaus, Orekhov, et al., 2008) and Hamada et al (2013) for iTBS (Hamada, Murase, et al., 2013). In addition, it was evident from our raw data (Table 2) that although tDCS also had no effect on average across the whole sample, there were clearly individuals with significant changes in MEP amplitude after tDCS. Given the number of data points and subjects available, we were able to employ cluster analysis to detect differing patterns of response across all timepoints.

Table 2. Absolute mean (±SD) MEP amplitude values for the whole sample (n=56) in each time point for each protocol. (B: baseline).

	B	0′	5′	10′	15′	20′	25′	30′	35′	40′	45′	50′′	55′	60′
PAS_{25}	0.96 (±0.26)	0.87 (±0.44)	0.85 (±0.40)	1.07 (±0.60)	1.00 (±0.50)	1.10 (±0.57)	0.98 (±0.54)	1.10 (±0.57)	1.06 (±0.50)	1.06 (±0.58)	1.14 (±0.62)	1.05 (±0.53)	1.05 (±0.58)	1.04 (±0.64)
AtDCS	1.00 (±0.28)	1.06 (±0.40)	1.03 (±0.54)	1.09 (±0.51)	1.16 (±0.57)	1.12 (±0.65)	1.06 (±0.56)	1.09 (±0.55)	1.10 (±0.66)	1.07 (±0.64)	1.05 (±0.60)	1.01 (±0.70)	1.06 (±0.64)	1.05 (±0.62)
iTBS	1.00 (±0.32)	0.94 (±0.50)	1.00 (±0.58)	0.99 (±0.56)	1.01 (±0.45)	1.00 (±0.49)	1.11 (±0.59)	1.06 (±0.54)	1.05 (±0.71)	1.03 (±0.56)	1.01 (±0.68)	1.12 (±0.77)	1.10 (±0.61)	1.03 (±0.54)

We found that under half of the sample responded as expected to each protocol (39%, 45% and 43% for PAS_{25}, AtDCS and iTBS respectively). Only 12.5% responded as expected to all the protocols, whilst 25% showed an unexpected response to all the three protocols. Thus, for the individual subject, a significant response to one protocol does not imply an increased likelihood of significant response to another NIBS paradigm. This result supports the idea of different mechanisms underling the facilitatory effect for each NIBS protocol (see (Ziemann, Paulus, et al., 2008) and (Nathan, Cobb, et al., 2011) for a review).

The number of responders (showing a significant response in the predicted direction) is slightly lower to that reported by Hamada et al. (2013) (Hamada, Murase, et al., 2013) and Muller-Dahlhaus et al. (2008) (Muller-Dahlhaus, Orekhov, et al., 2008), but this could be because grouping in these papers was based on the grand average of normalised MEP amplitude at all time points after stimulation- a single time point showing a very high response could change the classification of a subject as a "responder" or vice versa.

The group that responded as expected to each protocol showed an increase in MEP amplitude from minute 10 after stimulation compared to baseline MEP amplitude for both PAS and AtDCS. iTBS alone showed an earlier effect, from minute five after stimulation compared to baseline MEP amplitude in the responders group.

4.1.5.4. Predicting the pattern of response from baseline measures

Several reasons have been put forward for the considerable variability in the response to NIBS protocols, such as time of day (Ridding and Ziemann, 2010), anatomical aspects as cortical thickness (Conde, Vollmann, et al., 2012), coil

orientation (Talelli, Cheeran, et al., 2007), genetic variation (Cheeran, Talelli, et al., 2008). Other than time of day (no effect of PAS_{25}, AtDCS or iTBS), we are unable to address any of these factors in this study. There have been a few previous studies that have attempted to link baseline TMS measures with the subsequent response to NIBS protocols. PAS_{25} has been reported to correlate negatively with RMT and SI_{1mV} (Muller-Dahlhaus, Orekhov, et al., 2008) and posetively with the thickness of the underlying sensorimotor cortex (Conde, Vollmann, et al., 2012). iTBS seems to correlate with latency of MEPs evoked by TMS pulses that induced an anterior-posterior directed current across the central sulcus (Hamada, Murase, et al., 2013).

We tested time of day, age and several TMS baseline measures as possible predictors of response for all the protocols. We found that only Baseline SICI correlated with the response to the PAS_{25} protocol, and this predictor only accounted for around 10% of the variability in the data. This result implies that subjects with less $GABA_aR$-mediated inhibition immediately pre-stimulation are more likely to have a greater increase in MEP amplitude after PAS_{25} stimulation. This result echoes animal studies (Hess, Aizenman, et al., 1996) showing that blockade of $GABA_aR$ with the antagonist bicuculline methiodide allows the increase in stimulation-induced plasticity.

4.1.5.5. Role of SICI response after NIBS

Similar to the changes in cortical excitability, we found a remarkable interindividual variability in the change of SICI after stimulation. Around 50% of the subjects increase SICI after stimulation whilst the other half of subjects decreases SICI. To test if the two patterns of MEP amplitude response to each protocol of stimulation (responders and non-responders) were driven by changes in cortical inhibition, we performed contingency plots with SICI response (increase or decrease in SICI). We concluded that changes in cortical inhibition are not responsible for the direction of change in cortical excitability to each protocol.

4.1.5.6. Enrichment of Responders

We performed a sample size calculation with the results obtained from our study (using G*Power 3.1). For the iTBS protocol, given the mean difference

between Baseline MEP and the grand average across all timepoints of 0.04, SD for this difference of 0.35 mV and alpha set at 0.05, the sample size required to detect significant effects is 830 subjects. We acknowledge that large effect sizes in small N studies (that form the vast body of NIBS literature) are at odds with the results of this study.

Given that baseline measure appear to be unable to predict NIBS outcomes, we looked at whether using the early response to each protocol could enrich the number of responders in a given sample. At the same time we wished to establish the minimum sample size needed to get a significant effect on cortical excitability from each protocol. For example, if the mean of MEP Amplitude at timepoint 0, 5 and 10 minutes post iTBS stimulation is used for enrichment, 88.5% (23/26) of subjects with a mean response greater than Baseline MEP amplitude are in the Responders cluster for iTBS. Only 3 subjects from the Non-Responders cluster are included. Only one subject from the Responders cluster is falsely rejected. Recalculating a sample size for this enriched cohort of 26 subjects shows that just 18 subjects are required to detect significant effects.

We have constructed a table to show the predictive effect of each timepoint for each protocol (Table 3).

4.1.5.7. Should we worry about variability?

For many, the inter-individual variability in the response to NIBS protocols reported here would not come as an unexpected finding. NIBS protocols, and especially NIBS protocols designed to facilitate cortical excitability, have to tread the fine line between safety and efficacy. They remain unique tools, and their utility in the fields of neuroscience, neurology, psychology and psychiatry will no doubt remain undiminished. The variability described here amounts to a high rate of 'dose-failure' for these protocols and highlights the need for better ways to optimise NIBS protocols on an individual basis. Certainly, addressing the inter-individual variability of NIBS is key to solving issues such as adequate sample sizes in NIBS studies, the poor record of to replication of NIBS/TMS results, and the failure to consistently translate NIBS interventions showing promise in pilots studies to clinical practice. Elucidating pre-test predictors such as SICI for PAS_{25} or AP-PA latency (Hamada, Murase, et al., 2013) is one approach. Formally agreed methods for enrichment of responders to NIBS may

also be a viable approach. Among the subjects that respond to each NIBS protocol, there are individuals with a 300% increase in MEP Amplitude at 60 minutes post stimulation- addressing inter-individual variability will no doubt be an important factor in improving the safety of NIBS protocols. On a final note, it is worth considering the variability in response to NIBS as an opportunity to gain a unique insight into factors that determine variability of NMDAR-dependent LTP-like plasticity in the awake human cortex.

Table 3. Enrichment of subjects using MEP amplitude (normalised to Baseline MEP) >1 at single time points. This table illustrates the effect of enrichment of subjects (selection of subjects that respond to NIBS) on sample size for the three NIBS protocols. The first row for each protocol shows the number of subjects with a normalised MEP (MEP_n) > 1 at each time point.. The second row shows the number of subjects in the Responder cluster with the normalised MEP > 1 at each time point. In the third row we have the sample size needed to get a power of 0.8 when only subjects that have a normalised MEP amplitude greater than 1 at that timepoint are included. The fourth row reflects the percentage reduction in the sample size needed without (wt) enrichment and with (w) enrichment (*(sample size wt enrichment-sample size w enrichment)*100/(sample size wt enrichment)*) from the sample size needed if all the points after stimulation were taken (number in brackets). (n: number).

	Effect of enrichment on sample size												
	0′	5′	10′	15′	20′	25′	30′	35′	40′	45′	50′	55′	60′
PAS													
N of subjects with MEP_n >1	21	20	29	27	31	23	26	29	28	33	31	26	25
N of responders included	13	12	18	16	19	15	17	19	19	18	17	14	13
Sample size (power = 0.8)	7	7	11	9	7	9	5	7	7	8	8	6	9
Reduction in sample size (from 165)	95.8%	95.8%	93.3%	94.5%	95.8%	94.5%	97.0%	95.8%	95.8%	95.2%	95.2%	96.4%	94.5%
tDCS													
N of subjects with MEP_n >1	36	25	29	30	23	27	29	27	25	27	25	26	26
N of responders included	21	19	20	22	17	23	22	23	22	21	18	21	20
Sample size (power = 0.8)	7	6	7	6	7	5	6	9	8	9	9	8	7
Reduction in sample size (from 224)	96.9%	97.3%	96.9%	97.3%	96.9%	97.8%	97.3%	96.0%	96.4%	96.0%	96.6%	96.4%	96.9%
iTBS													
N of subjects with MEP_n >1	22	25	20	33	23	32	26	27	25	27	29	29	23
N of responders included	17	19	17	21	19	23	20	20	19	18	23	21	17
Sample size (power = 0.8)	12	8	7	6	6	6	6	11	7	10	8	7	6
Reduction in sample size (from 475)	97.5%	98.3%	98.5%	98.7%	98.7%	98.7%	98.7%	97.7%	98.5%	97.9%	98.3%	98.5%	98.7%

Study II:

Intra-individual variability in the response to anodal Transcranial Direct Current Stimulation

4.2. Study II: Intra-individual variability in the response to anodal Transcranial Direct Current Stimulation

4.2.1. Abstract

Objective: To test the intra-individual reliability in response to Anodal Transcranial Direct Current Stimulation (AtDCS). *Methods:* 45 healthy subjects received AtDCS (1mA, 13 min) in two separate sessions, 6-12 months apart. Motor evoked potentials were collected at baseline and then at 5-minute intervals after AtDCS for 1 hour. *Results:* AtDCS increased cortical excitability over minutes 0-30 post-stimulation in both sessions, with fair intra-individual reliability. 60% and 64% of subjects responded with the expected increase in cortical excitability in each session, respectively. 69% of the subjects maintained their response pattern between sessions during this timeframe. However, there were no significant effects on cortical excitability over the full hour post AtDCS in either session. *Conclusion:* A change in cortical excitability in the first half-hour post-AtDCS may be a good predictor of the response in a subsequent session. Furthermore, minute 15 post-stimulation showed the maximum increase in cortical excitability in both sessions, and minutes 5-15 post AtDCS may be the most reliable window for effects on cortical excitability. *Significance:* We show for the first time that intra-individual variability is lower than inter-individual variability, and with fair intra-individual inter-sessional reliability for 30 minutes after AtDCS- subjects are likely to maintain their response patterns to tDCS between sessions, with implications for experimental and therapeutic applications of tDCS.

4.2.2. Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that modulates cortical excitability, and consequently cortical function, by delivering relatively weak currents through scalp electrodes placed over target cortical areas. Previous studies (Nitsche and Paulus, 2000; Priori, Berardelli, et al., 1998) have reported modulation in human cortical excitability either during or after tDCS stimulation, by measurement of changes in motor evoked potentials (MEPs) elicited with transcranial magnetic

stimulation (TMS). According to the polarity of the current delivered by the tDCS, the effects over the cortical excitability can result in facilitation or inhibition of the MEPs. There remain gaps in our understanding of the mechanistic underpinnings of tDCS on cortical excitability. During tDCS the current injected through scalp electrodes induces electric fields (EF) in the cortex, which is believed in turn to modulate neuronal excitability. Therefore, less synaptic input may be needed in order to produce an action potential. This on its own may not explain the effects of tDCS on behaviour. Anodal tDCS (AtDCS) may exert some effects by inducing long-term potentiation (LTP)-like mechanisms (Fritsch, Reis, et al., 2010). Both facilitatory and inhibitory effects (with anode or cathode over the area of interest) may last longer than the period of stimulation (i.e. aftereffect of 90 minutes duration has been reported with only 13 minutes of stimulation) (Nitsche and Paulus, 2000; Nitsche and Paulus, 2001).

tDCS may have several advantages over other non-invasive brain stimulation techniques (NIBS) such as paired associative stimulation (PAS) or theta-burst stimulation (TBS). tDCS is technically easier to apply than the above mentioned NIBS, more effective when used as sham stimulation (Gandiga, Hummel, et al., 2006), and requires less expensive equipment. Moreover, tDCS can easily be applied concurrently during the performance of cognitive or motor tasks (Reis, Schambra, et al., 2009).

It is therefore unsurprising that, in the last decade, tDCS has been increasingly favoured in studies pairing stimulation with learning or memory tasks. Several studies have shown that tDCS can improve motor performance and motor learning in healthy subjects, both during (Galea, Vazquez, et al., 2011) and after stimulation (Boggio, Castro, et al., 2006). In addition, tDCS holds promise as a therapeutic tool in neurologic diseases such as stroke (Hummel and Cohen, 2006; Zimerman, Heise, et al., 2012) or epilepsy (Fregni, Thome-Souza, et al., 2006); psychiatric diseases such as depression (Boggio, Rigonatti, et al., 2008) or drug addiction (Boggio, Zaghi, et al., 2010); and in chronic pain (Lefaucheur, Antal, et al., 2008).

However, a source of increasing concern has been that despite initially promising results, a number of studies attempting to replicate findings of prior tDCS studies have not found the same effects (Meesen, Thijs, et al., 2014; O'Connell, Wand, et al., 2014; Polanowska, Lesniak, et al., 2013; Wrigley, Gustin, et al., 2013). One reason may be the inter- and intra-individual variability in the response to tDCS (Horvath, Carter, et al., 2014). Recent studies have attempted to define the large inter-individual variability in response to NIBS protocols (Hamada, Murase, et al., 2013; Lopez-Alonso, Cheeran, et al., 2014; Muller-Dahlhaus, Orekhov, et al., 2008), and specifically in response to tDCS (Lopez-Alonso, Cheeran, et al., 2014; Wiethoff, Hamada, et al., 2014). The consensus from these studies appears to be that only a percentage of the population respond as expected to NIBS protocols. In addition to this inter-individual variability in response, the intra-individual variability across a temporal window is also a relevant issue in the use of the NIBS protocols, particularly when these protocols are applied across days or weeks. Intra-individual variability has been explored for repetitive TMS (Sommer, Wu, et al., 2002); PAS (Fratello, Veniero, et al., 2006; Sale, Ridding, et al., 2007); continuous (Huang, Rothwell, et al., 2008; Vernet, Bashir, et al., 2014) and intermittent theta burst (Hinder, Goss, et al., 2014; Huang, Rothwell, et al., 2008), but to date, no data about tDCS has been reported.

Therefore, the aim of the present study is to explore the reliability (intra-individual variability) of anodal tDCS across two separate sessions for a sample of 45 healthy subjects. Our findings could be of relevance for a more rational application of the tDCS in fields such as rehabilitation.

4.2.3. Methods and materials

4.2.3.1. Subjects

The Local Ethics Committee of the University of La Coruña approved the experimental protocols, in accordance with Declaration of Helsinki. A total of 45 Caucasian subjects (39 men, 6 women) aged between 19 and 24 years (mean age±SD: 20.51±1.5) were consented. 42 subjects were right-handed. Subjects were screened for contraindications to TMS (Wassermann, 1998). None of the

subjects reported any neurological (including a past medical history of head injury or seizures), psychiatric or other significant medical problems. All subjects participated in both tDCS sessions. Subjects were asked to abstain from caffeine or alcohol from the day before the experimental session, and to get a good night sleep the night before the session, to minimise the effects of these factors on responses evoked by TMS.

4.2.3.2. General procedure

Subjects were seated in a comfortable chair with their eyes open and were asked not to engage in conversation. Sessions for each subject were administrated between 6 and 12 months apart (mean±SD: 287.2±46.8 days), to minimise any potential cumulative effects. Time between sessions has been shown to not influence reproducibility of other NIBS protocols (Vernet, Bashir, et al., 2014).

4.2.3.3. EMG recordings

Electromyographic (EMG) traces were recorded via Ag-AgCl, 9mm diameter surface cup electrodes, placed over the right first dorsal interosseous (FDI) muscle. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified with a Digitimer D360 amplifier (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) through filters set at 30 Hz and 2 kHz with a sampling rate of 5 kHz, then recorded using SIGNAL software (Cambridge Electronic Devices, Cambridge, UK). The Magstim stimulators were triggered using Signal software.

4.2.3.4. TMS procedure

TMS was delivered through a figure-of-eight coil with outer diameter of 70 mm (Magstim Co., Whitland, Dyfeld, UK) over the left motor cortex. A monophasic Magstim BiStim was used to deliver single pulses to measure changes in cortical excitability, and paired pulses to measure short-interval intracortical inhibition (SICI).

We first localized the “motor hot spot” (defined as the point on the scalp at which single pulse TMS elicited MEPs of maximal amplitude from the right FDI)

by functional localization in both sessions. The starting point for hotspot hunting was marked 4cm lateral and 2cm anterior to the vertex. Single TMS pulses starting at 40% of the maximum output of the stimulator were delivered in a approx. 3x3cm grid until FDI contraction was observed. If no FDI contraction was observed, stimulator intensity was increased in 5% steps until FDI contraction was observed. The coil was then moved in 5mm steps from this point around a 2x2 cm grid until all points where stimulation evoked a MEP over FDI were located. Stimulation intensity was then reduced in 1‑2% steps at these points until 3 MEPs were observed out of 3 trials at a given position, while stimulation of adjacent positions did not evoke reliable MEPs on 3 trials. If no MEPs were evoked at any position at a given intensity, while at an intensity 1% higher, 3 MEPs were still observed out of 3 trials in more than one point, the "hot spot" was defined as the position in which the largest mean MEP amplitude was detected (Schluter et al., 1998).

We determined resting motor threshold (RMT) (minimum stimulation intensity over the motor hotspot that elicited a MEP of no less than 50 μV in 5 of 10 trials in the relaxed FDI) and active motor threshold (AMT) (intensity necessary to evoke a 200 μV MEP while subjects maintained approximately 10% contraction of the FDI).

The baseline block consisted of 20-test stimulus (TS) (pulses delivered at a stimulator intensity adjusted to evoke a MEP of approximately 1mV peak-to-peak amplitude in the FDI) and 20 SICI paired pulses, delivered in random order. SICI was measured using the technique described by Kujirai et al. (1993) (Kujirai, Caramia, et al., 1993), where a sub-threshold conditioning stimulus (CS) is followed by a TS. CS was delivered at the 80% of AMT and the interstimulus interval (ISI) was 2 ms. Peak-to-peak MEP amplitude of the 20 test stimuli and 20 SICIs were averaged separately.

After baseline measures, anodal tDCS was administered for 13 minutes. MEP amplitudes were recorded from minute 0 to minute 60 after stimulation at fixed five minutes intervals (0, 5, 10… 60) over the same hotspot. MEP amplitudes were normalized with baseline MEP amplitudes. Each block of post-stimulation measures consisted of 12 TS. After the MEP measurement block at minute 5 and minute 45, we measured a SICI block (10 TS + 10 SICI, delivered

in random order). SICI was expressed as a percentage of the mean peak-to-peak amplitude of the unconditioned MEP.

4.2.3.5. Anodal transcranial direct current stimulation (AtDCS)

tDCS was delivered at 1 mA for duration 13 min through a pair of saline-soaked sponge surface electrodes (35 cm^2) connected to a DC stimulator (neuroConn). Active electrode (anode) was placed over the hotspot of the left M1, the reference electrode (cathode) was placed over the supraorbital contralateral. Current was faded in and faded out for 8 seconds.

4.2.3.6. Statistical analysis

4.2.3.6.1. Effects of anodal tDCS

Repeated measures ANOVA was used to compare absolute MEP values before and after AtDCS with SESSION (session 1 and session 2) and TIME as main factors. Three ANOVAS were conducted for three time bins, from baseline to minute 60 (TIME factor with 14 levels), from baseline to minute 30 (TIME factor with 8 levels), and baseline together with minutes from 35 to 60 (TIME factor with 7 levels).

A repeated measure ANOVA with SESSION (session 1 and session 2) and TIME (baseline, minute 6 and minute 46) was conducted on SICI values.

When a significant effect was found, post hoc t-test with Bonferroni corrections were conducted. Greenhouse-Geisser correction was used for non-spherical data.

4.2.3.6.2. Reliability of AtDCS-induced changes

Paired t-tests and intraclass correlation coefficient (ICC (2,1) (Shrout and Fleiss, 1979)) was calculated to estimate reliability of AtDCS-induced changes during the two stimulation sessions for the following variables: normalized-to-baseline MEP in each time point post-stimulation, averages of normalized-to-baseline MEP from minute 0 to minute 60, from 0 to 30 minutes, and from 35 to 60 minutes; maximal average MEP amplitude obtained in a time point from minute 0 to minute 60, from 0 to 30 minutes, and from 35 to 60 minutes; SICI at minute 6 and minute 46.

ICC results are reported according with the criteria established by Cicchetti and Sparrow (1981) (Cicchetti and Sparrow, 1981) and Fleiss (1981) (Fleiss, 1981): intraclass reliability can be considered *poor* (ICC values < 0.40), *fair* (ICC values between 0.40 – 0.59), *good* (ICC values between 0.60 – 074) or *excellent* (ICC values > 0.74). Negative ICC values indicate that the measure is *not reliable* (Lahey, Downey, et al., 1983).

Forward binary logistic regression was conducted with "change in average of normalised MEP amplitude from session 1 to session 2" (for the whole hour, and the first half-hour post-stimulation) as the dependent variable and "sex", "handedness", "smoking status", "days between sessions" and "baseline MEP amplitude differences between sessions" as independent variables.

We also calculated the number of "responders" and "non-responders" to the AtDCS for each and both sessions, and also for each time bins (0-60, 0-30 and 35-60). To determine the number of "responders" and "non-responders" we used the classification based on the grand average MEP. We classified subjects as "responders" or "non-responders" based on an normalised to baseline average MEP amplitude >1 or <1, respectively (Hamada, Murase, et al., 2013; Lopez-Alonso, Cheeran, et al., 2014; Wiethoff, Hamada, et al., 2014).

4.2.3.6.3. Intra- and inter-individual variability

To assess the contribution of intra- and inter-individual components to the MEP amplitude variability, we calculated a variance component analysis (ANOVA type), similar to Sommer et al. (Sommer, Wu, et al., 2002). We used MEP amplitude as the dependent factor, "day" and "subject" as random factors, and "Time_Bin" as fixed factor. By adopting the analysis reported by Sommer et al. (Sommer, Wu, et al., 2002), we are able to provide a direct comparision with earlier (and now less commonly used) non-patterned NIBS paradigms.

All statistical analyses were calculated using SPSS (SPSS, Chicago, IL). None of the data violated the normality assumption necessary to conduct parametric statistical tests. A p value ≤ 0.05 was considered statistically significant.

4.2.4. Results

No adverse effects were reported during or after tDCS. None of the subjects reported any significant discomfort and no tDCS experiments had to be discontinued. In one subject the data recording was incomplete due to a technical issue (too few valid trials in baseline block) during the second session and this subject was removed from the analysis.

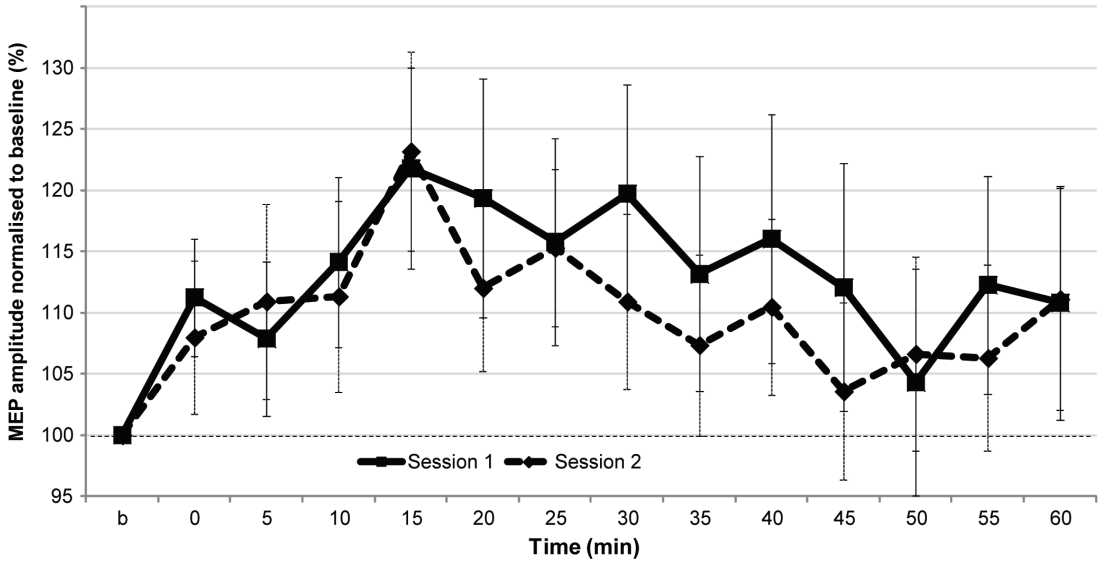
4.2.4.1. Effects of anodal tDCS

The ANOVA of the absolute MEP amplitudes from baseline to 60 minutes post-stimulation did not show significant SESSION or TIME main effects, or a SESSION*TIME interaction (figure 1). The same results were found for the analysis of the MEP amplitudes from baseline and 35-60 minutes. However, the ANOVA reported a significant TIME effect ($F = 2.41$ $p = 0.036$), but not SESSION or SESSION*TIME interaction for the analysis of the MEP amplitudes from baseline to 30 minutes. Post-hoc analysis without correction for multiple comparisons revealed significantly larger MEP amplitudes than baseline for minutes 15, 20 and 25 post-stimulation ($p = 0.020$, $p = 0.037$ and $p = 0.049$, respectively). When Bonferroni correction was applied, the change in MEP amplitude between baseline and minute 15 post-stimulation was the only time point to remain significant ($p = 0.048$).

4.2.4.2. Reliability of AtDCS-induced changes

Paired t-tests did not show any significant difference between sessions for all the variables analysed (average and maximal MEP amplitudes and SICI values).

ICC for MEP amplitudes each time point post-stimulation shown poor intra-individual reliability for the first half hour and lack of reliability (negative values) during the second half hour (figure 14).



	0	5	10	15	20	25	30	35	40	45	50	55	60
S1	111.3% ±32.2	107.9% ±42.3	114.2% ±46.7	121.8% ±54.9	119.4% ±65.6	115.8% ±56.7	119.7% ±59.5	113.2% ±64.3	116.0% ±68.2	112.1% ±67.8	104.3% ±62.1	112.3% ±59.7	110.8% ±64.1
S2	108.0% ±42.2	110.9% ±53.6	111.3% ±52.6	123.2% ±54.4	112.0% ±45.6	115.3% ±43.1	110.9% ±48.0	107.4% ±48.5	110.5% ±48.4	103.6% ±48.5	106.6% ±53.3	106.3% ±51.0	111.1% ±60.5
ICC	0.253	0.114	0.430	0.264	0.177	0.237	0.304	-0.092	0.036	-0.008	-0.018	-0.009	-0.062

Figure 14. Effect of anodal tDCS on MEP amplitude in both sessions: Change in MEP amplitude (shown MEP amplitudes normalised to baseline) for the whole sample (n=44) in the first and second session. Error bars represent standard errors. ANOVA for the absolute values revealed no significant SESSION and TIME main effects or SESSION*TIME interaction. In the table below, MEP amplitude values (Mean+-SD) normalised to baseline at each time point after stimulation (%) and Intraclass Correlation Coefficient (ICC) for MEP change at each time point between both sessions are displayed.

ICC values for average and maximal MEP amplitudes from 0 to 60 minutes time bin showed poor intra-individual reliability (table 4). The negative values of ICC for these variables in the 35-60 minutes time bin indicated lack of reliability. However, the average and maximal MEP amplitudes for the 0-30 minutes time bin showed fair intra-individual reliability. ICC for the SICI values indicated a fair reliability at 6 minutes post-AtDCS stimulation but no reliability at 46 minutes post-AtDCS stimulation.

Binary logistic regression did not revealed any influence of “sex”, “handedness”, “smoking”, “days between sessions” or “baseline MEP amplitude

differences between sessions" in the reliability of tDCS effects, for both the effects during the whole hour or the first half-hour post-stimulation.

Table 4. Average change in cortical excitability, maximum change reached and SICI in each time bin: This table shows the averaged amplitude of post tDCS MEP's normalized to baseline amplitude (%MEP_n) from minute 0 to minute 60, from 0 to 30 minutes, and from 35 to 60 minutes. We also show the maximum MEP amplitude change from baseline reached and change in SICI in each measured time point (shown as paired pulse conditioned MEP amplitude normalized to test MEP amplitude; larger SICI amplitude implies less GABAaR mediated inhibition). T-test p-values and Intraclass Correlation Coefficient (ICC) between both sessions for each variable are shown.

	%MEP_n			MÁX MEP amplitude			SICI		
	0-60	**0-30**	**35-60**	**0-60**	**0-30**	**35-60**	**Bas**	**Min 6**	**Min 46**
Session 1	113.7% ±45.7	116.4% ±39.0	111.5% ±55.8	177.9% ±65.2	162.0% ±58.2	155.4% ±74.2	51.9% ±26.17	59.3% ±34.91	51.1% ±30.92
Session 2	110.5% ±36.1	113.1% ±36.1	107.6% ±40.6	175.0% ±62.4	161.3% ±53.3	152.3% ±61.0	55.5% ±28.46	55.4% ±34.07	54.8% ±39.34
t-test p-value	0.679	0.539	0.714	0.804	0.916	0.834	0.334	0.447	0.598
ICC	0.242	0.565	-0.028	0.265	0.438	-0.041	0.598	0.465	0.147

The results of the grand average MEP analysis of the number of "responders" and "non-responders" are summarised in table 5. The time bin with highest number of subjects maintaining their response over both sessions was the first half-hour post-stimulation.

4.2.4.3. Inter- and intra-individual variability

We have intentionally used an analysis as performed by Sommer et al. (Sommer, Wu, et al., 2002) to enable comparison with variability in response to non-patterned rTMS. As shown in figure 15, inter-individual variability contributes much more than intra-individual to the total variance, similar to the results for non-patterned rTMS reported by Sommer et al. (Sommer, Wu, et al., 2002).

Table 5. "Responders" and "non-responders" to anodal tDCS: This table illustrates the number and percentage of "responders" (subjects with MEP amplitudes post-stimulation larger than baseline MEP amplitude,) and "non-responders" (subjects with MEP amplitudes post-stimulation lower than baseline MEP amplitude) to each and both sessions of anodal tDCS. Table also shows the percentage from responders in session 1 (S1) that are responders in S2, and the percentage from non-responders in S1 that are non-responders in S2.

	0-60		**0-30**		**35-60**	
	R	**NR**	**R**	**NR**	**R**	**NR**
Session 1	24 53.3%	21 46.7%	27 60%	18 40%	23 51.1%	22 48.9%
Session 2	26 57.8%	19 42.2%	29 64.4%	16 35.6%	24 53.3%	21 46.7%
Both sessions	15 33.3%	10 22.2%	21 46.7%	10 22.2%	12 26.7%	10 22.2%
% maintaining response type between sessions	62.5%	47.6%	77.8%	55.6%	52.2%	45.5%

	Sum of Squares	df	Mean Square
Subject (inter-subject)	27.474	44	0.624
Day (intra-subject)	0.082	1	0.082
Time_Bin	0.121	2	0.06
Subject*Day	17.16	44	0.39
Subject*Time_Bin	1.161	88	0.013
Day*Time_Bin	0.0005	2	0.0002
Subject*Day*Time_Bin	2.472	88	0.028

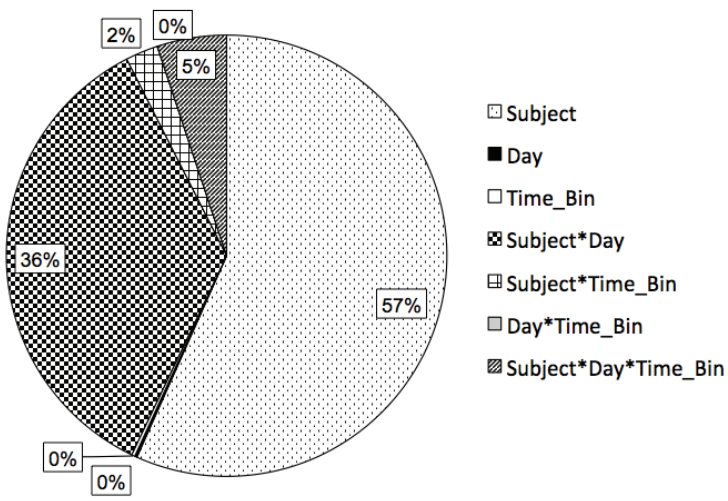


Figure 15. Inter- and intra-individual variability contribution to the total variance: A pie chart illustrating the contribution of the sum of squares to the total sum of squares illustrates the greater contribution of inter-individual variability compared to intra-individual variability to total variance in response to AtDCS (calculated for the first half-hour post-stimulation).

4.2.5. Discussion

To our knowledge, this is the first study examining the intra-individual reliability of anodal tDCS stimulation over primary motor cortex in two separate sessions. Our results indicate a fair intra-individual reliability and significant excitatory effect of anodal tDCS in the first half-hour post-stimulation.

4.2.5.1. Effects of anodal tDCS

In our study, 13 minutes of 1mA anodal tDCS did not induce a significant increase in M1 cortical excitability during the whole hour post-stimulation, in a sample of 44 subjects. The lack of long-lasting effect of the AtDCS over M1 cortical excitability was consistent across the two stimulation sessions. It should be noted that the absence of effect at the group level over one hour was characterized by highly variable inter-individual responses. Our results show that 53% and 58% of the sample responded as expected (excitatory effect) in the first and second session, respectively. The choice of MEP's as an outcome measure may contribute to the variability, although the effects of tDCS (and other NIBS paradigms) on cortical excitability have largely been defined on the basis of changes in MEP amplitude. Another potential confound is the lack of stereotactic localisation of hotspot between sessions, which may also contribute to the variability recorded – FDI hotspot is determined functionally in both sessions, and variance may be present in coil position and tilt between sessions. While stereotactic localisation may reduce the variance in coil position between sessions, we concluded that stereotactic localization of the FDI hotspot in session 2 (based on the hotspot recorded using functional localization in session 1) would not be appropriate- if the stereotactically determined coil position was stimulated, and this hotspot varied in session 2 from a carefully determined functional hotspot, the results may be rendered meaningless. Finally, the high inter-individual variability in response to AtDCS in this study is in accordance with several previous studies that showed that around 50% of the sample responded as expected (excitatory effect), while other 50% showed an unexpected response (no effect or inhibitory effect) (Lopez-Alonso, Cheeran, et al., 2014; Wiethoff, Hamada, et al., 2014).

It is plausible that the duration (13 minutes) or intensity (1mA) of stimulation in our study was not enough to induce effects lasting for one hour and consequently mask an effect on earlier timepoints. For this reason, we analysed the effects of the AtDCS during an early (from minute 0 to 30) and late (from minute 35 to 60) time window. Our results show that AtDCS induced a significant increase in cortical excitability only during the first half-hour post-stimulation, for both stimulation sessions. Averaged across both sessions during these first 30 minutes post-stimulation, there was a mean 14.75% increase in MEP amplitude from baseline values (table 4). Our data revealed that the build-up of excitability peaked around minute 15, and a 22.5% increase in MEP amplitude from baseline values averaged across both sessions was recorded at this time-point (figure 14). These results, showing a cut-off for potential clinically or experimentally relevant changes in cortical excitability after AtDCS may be relevant to planning interventions (Hinder, Goss, et al., 2014).

The SICI did not vary after anodal tDCS at the two timepoints recorded (minute 6 and 46 post-stimulation), in accordance with previous results (Lopez-Alonso, Cheeran, et al., 2014). In our study we did not adjust the MEP size of the test pulse for SICI, which could explain the discrepancy with several studies that have reported an effect of tDCS on SICI (Kidgell, Daly, et al., 2013; Stagg, Best, et al., 2009).

4.2.5.2. Reliability of anodal tDCS-induced changes

As previously discussed, the excitatory effect induced by AtDCS was constrained to the first half-hour post-stimulation, and this effect was obtained for both stimulation sessions. In order to test the reliability of this effect between sessions we conduct an analysis for each time-point post-stimulation. The results indicated poor reliability for each time point, although the ICC values were higher for the minutes during the first than during the second half-hour post-stimulation. However, the analysis of each time point may not be very informative of the reliability as it would require each subject to show an identical time course of response to the AtDCS between two sessions in order to obtain high ICC values. The result suggests that it is unlikely that subjects show an identical response on different days at any given time point. Each time point is

an average of 12 test-stimuli, and the intrinsic variability in MEP response to single TMS pulse may also contribute to this variability (Kiers, Cros, et al., 1993; Sommer, Wu, et al., 2002).

An alternative approach to evaluate the consistency of the AtDCS effects between sessions is to calculate the reliability of the average of all the time-points along the hour post-stimulation. Since we have shown than AtDCS only increase the cortical excitability the first 30 minutes post-stimulation, we calculate separately this reliability for the average values during the first and second half-hour post-stimulation. Our results indicated a fair intra-individual reliability for the average of MEP amplitudes and maximal MEP values obtained during the first 30 minutes post-stimulation. This intra-individual reliability is similar to that one reported by Hinder et al., (2014) (Hinder, Goss, et al., 2014) for iTBS but contrasts with the excellent reliability reported by Huang et al., (2008) (Huang, Rothwell, et al., 2008) for both iTBS and cTBS. This discrepancy could be the result of different analysis procedures between studies, since Huang et al. calculated the coefficient of correlation rather than the ICC. It is not the scope of this study to compare the reliability between different NIBS techniques, but to provide the first values of intra-individual reliability for the AtDCS. As we observed a higher reliability during the first half-hour post-stimulation, we also performed ICC calculations on rolling time-point each 10 minutes to further refine the most reliable window for serial observations (figure 16). tDCS effect between minute 5 and 15 showed the highest reliability, with a mean 14.9% increase in MEP amplitude from baseline values, averaged across both sessions (extracted from figure 14).

In contrast with the results for the first half-hour post-stimulation, the ICC values of the second half-hour for MEP and SICI values were not reliable. This lack of reliability could be due to fluctuations of the physiological state of the participant along the session due to fatigue, as previously suggested (Vernet, Bashir, et al., 2014). Although, in our experiment subjects were not allowed to move or talk during the hour post-stimulation, we could not ensure that they did not engage in mental activities that could produce subtle modification in their brain excitability.

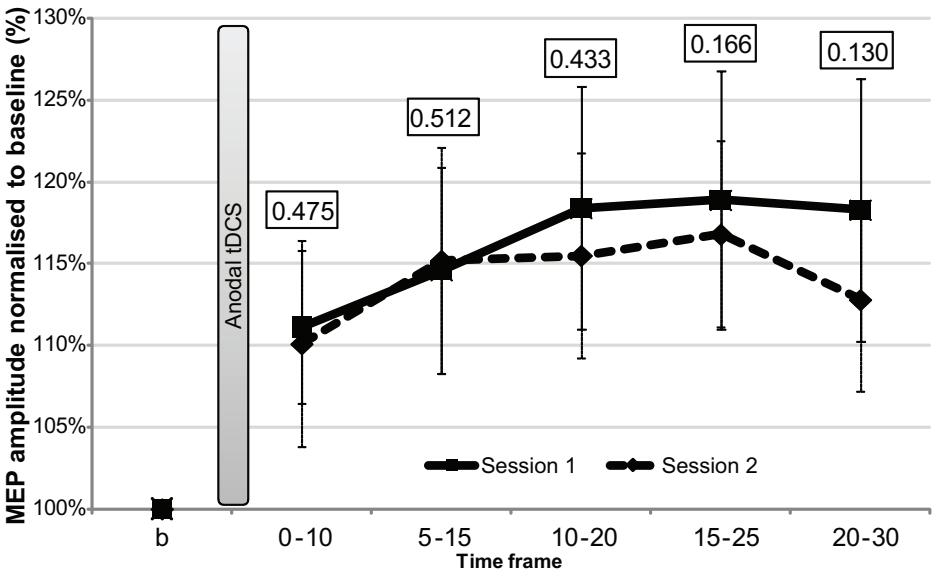


Figure 16. Average change in cortical excitability in 10-minutes bins: This figure shows the running average of normalized MEP to baseline and the Intraclass Correlation Coefficient (ICC) for the first half-hour post-stimulation in 10-minutes bins. Error bars represent standard errors.

This study shows inter-individual variation contributes much more than intra-individual variation to the total variance. In our results, 60% or more of the subjects responded in each of the two stimulation sessions during 30 minutes after stimulation. Around half of the sample maintained this facilitatory response in both sessions (table 5). It is important to note that 78% of the responders to the first tDCS session displayed the same response (increase in cortical excitability) in the second session (figure 17). These findings are of relevance when tDCS needs to be applied in several sessions, and suggest using a first session of stimulation in order to know whether the subject is a responder or not.

In summary, AtDCS has a significant effect of the on cortical excitability for the first half-hour post-stimulation. Furthermore, around minute 15 post-stimulation seems to be the time point with maximum increase in cortical excitability on average. Intra-individual reliability in response is maximum between min 5 and 15 (figure 16). The response to one AtDCS session, particularly between minute 0-30 could predict to a certain extent the response

to following sessions. These assumptions should be tested in patient populations if tDCS is to be used successfully in clinical programs.

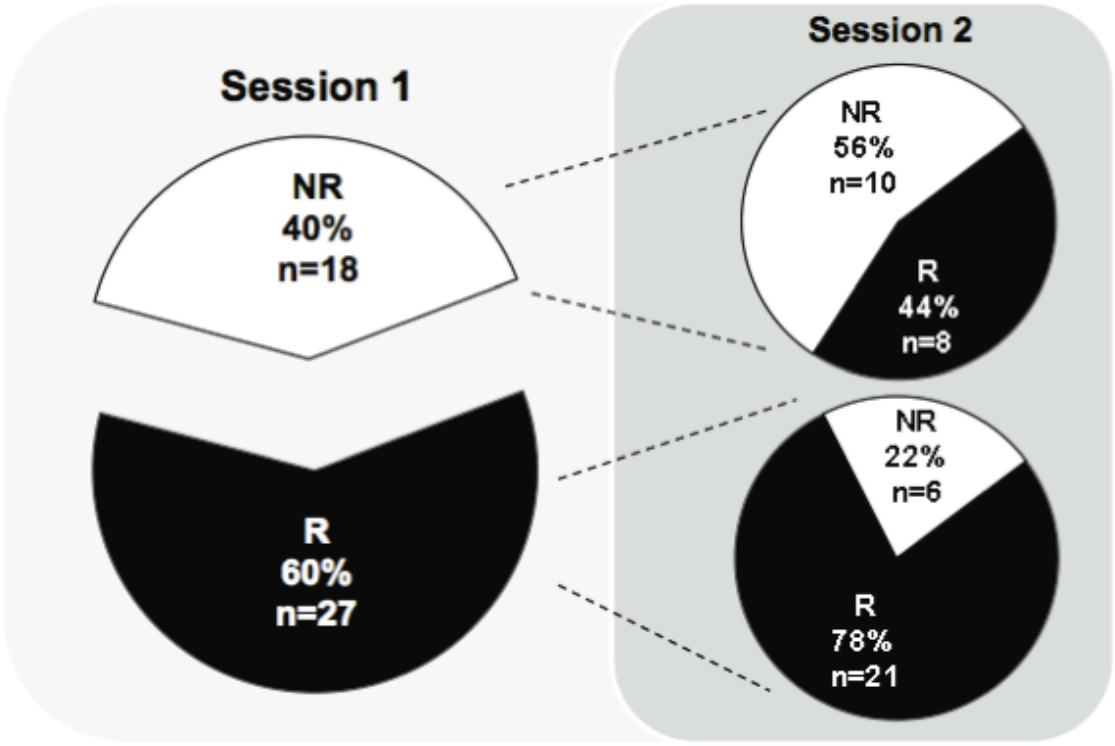


Figure 17. Enrichment: The figure illustrates the effect of cohort enrichment using the average post AtDCS MEP between minutes 0-30 normalised to baseline MEP.

Study III:

Relationship between NIBS-induced plasticity and capacity for motor learning

4.3. Study III: Relationship between NIBS-induced plasticity and capacity for motor learning.

4.3.1. Abstract

Background: Cortical plasticity plays a key role in motor learning. Several non-invasive brain stimulation (NIBS) protocols have been used to induce such plasticity in the human motor cortex in order to facilitate motor learning. However, little is known about the relationship between plasticity induced by these NIBS protocols over M1 (by convention assessed by the change in Motor Evoked Potentials (MEP)) and motor learning capacity. *Hypothesis:* MEP changes induced by non-invasive brain stimulation are related to motor learning capacity. *Methods:* We recruited 56 subjects for six experimental sessions. Three sessions were stimulation sessions (testing paired associative stimulation (PAS), anodal transcranial direct current stimulation (AtDCS) and intermittent theta-burst stimulation (iTBS)), and the other three were lab-based motor learning task sessions (serial reaction time task (SRTT), a joystick visuomotor adaptation task (VAT) and a sequential visual isometric pinch task (SVIPT)). *Analysis:* After clustering the patterns of response to the different protocols of stimulation, we compared the motor learning variables between the different patterns found. We also used a stepwise linear regression analysis to explore further the relationship between motor learning capacity and a number of summary measures of the change in MEPs (0-30 minutes, 5-15 minutes and max change 0-30 minutes) of each NIBS. *Results:* Cluster analysis revealed two patterns of response ("responders" and "non-responders"). We found no differences in motor learning variables between the two clusters of response. Stepwise regression suggests that greater response to facilitatory NIBS protocols may be predictive of poor performance within certain blocks of the VAT task. However, the physiological significance of this result is uncertain. "Responders" to AtDCS and to iTBS showed significantly faster reaction times than "non-responders" in a choice reaction time task. *Conclusion:* MEP changes induced in M1 by PAS, AtDCS and iTBS, by and large, appears to have no association with the motor learning capacity tested with SRTT, VAT and SVIPT

tasks. However, cortical excitability changes induced in M1 by AtDCS and iTBS are related with reaction time performance.

4.3.2. Introduction

The ability to learn new motor skills is dependent on brain plasticity, the ability of the human brain to make changes in its structure or function (Classen, Liepert, et al., 1998; De Beaumont, Tremblay, et al., 2012; Iezzi, Suppa, et al., 2010). Long-term potentiation (LTP) and long-term depression (LTD) have been proposed as the principal mechanism of such learning (Rioult-Pedotti, Friedman, et al., 2000; Ziemann, Ilic, et al., 2004). LTP and LTD-like changes in cortical excitability can be induced by non-invasive brain stimulation techniques (NIBS) such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) (Di Lazzaro, Pilato, et al., 2008; Huang, Chen, et al., 2007; Stagg and Nitsche, 2011; Stefan, Kunesch, et al., 2002). The above mentioned NIBS protocols have been applied to different cortical areas, but mostly commonly to the primary motor cortex (M1) due to the putative role of this area in the motor learning processes (Muellbacher, Ziemann, et al., 2001). The common procedure to evaluate the effects induced by those techniques is to measure the changes in the amplitude of the motor evoked potentials (MEPs) on the primary motor cortex (M1) before and after NIBS paradigms. Excitatory paired associative stimulation (PAS) (Stefan, Kunesch, et al., 2000), anodal transcranial direct current stimulation (AtDCS) (Nitsche and Paulus, 2000) and intermittent theta burst stimulation (iTBS) (Huang, Edwards, et al., 2005) are some examples of NIBS protocols that have been reported to induce a facilitation in the MEPs for periods up to one hour post-stimulation.

Stimulation of M1 by NIBS has been reported to enhance performance and learning in healthy subjects in a variety of motor tasks such as implicit learning (Nitsche, Schauenburg, et al., 2003), visuomotor learning (Antal, Nitsche, et al., 2004) or accurate motor performance (Reis, Schambra, et al., 2009) tasks (for a review see (Krakauer and Mazzoni, 2011; Reis, Robertson, et al., 2008). These effects of NIBS are believed to involve or augment the same mechanisms involved in the motor skill learning process, and are a key argument in utilizing NIBS in rehabilitation (e.g. in Stroke) (Liew, Santarnecchi, et al., 2014).

However, little is known about the relationship between the plasticity induced by these NIBS protocols and performance on motor learning tasks. Therefore, the main goal of this study is to explore whether the cortical plasticity induced by NIBS protocols on M1 correlates with the motor learning capacity as measured by performance on established lab-based motor learning tasks.

We applied anodal tDCS, PAS and iTBS over the left motor cortex in a total of 56 subjects. We then measured performance on three well-established motor learning capacity measures: implicit motor learning, visuomotor adaptation and motor accuracy. We used the serial reaction time task (SRTT) (Nissen and Bullemer, 1987) (a widely used tool to measure implicit learning in which subjects learn without awareness a sequence of finger movements), a joystick task (VAT) (Joundi, Lopez-Alonso, et al., 2012) to measure the visuomotor adaptation and a sequential visual isometric pinch task (SVIPT) (Reis, Schambra, et al., 2009) to measure accurate motor performance.

4.3.3. Methods

4.3.3.1. Subjects and general procedure

A total of 56 Caucasian subjects (50 men; 6 women; 53 right-handed), aged between 19 and 24 years (mean age 20.52±1.52) who had already participated in a previous NIBS study in our lab (Lopez-Alonso, Cheeran, et al., 2014) were recruited after giving written informed consent. The experiments were approved by the Ethics Committee of the University of La Coruña and are in accordance with Declaration of Helsinki.

The subjects participated in 3 sessions of NIBS with at least a one week interval between them. The order of the NIBS sessions was counterbalanced between subjects. A minimum of one week after the last NIBS session, subjects participated in the SRTT, VAT and SVIPT motor tasks, at least one week apart. 100% of the sample (56 subjects) performed the SRTT and VAT while 78.6% (44 subjects) completed the SVIPT. The order of motor learning studies was counterbalanced between subjects.

Each individual subject took part in all sessions at the same time of day.

4.3.3.2. EMG recordings

Electromyographic (EMG) traces were recorded via Ag-AgCl, 9-mm-diameter surface cup electrodes, from the right first dorsal interosseous (FDI) muscle. Signals were filtered (30 Hz to 2 kHz) with a sampling rate of 5 kHz and amplified with a Digitimer D360 amplifier (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK), and then recorded using SIGNAL software (Cambridge Electronic Devices, Cambridge, UK).

4.3.3.3. TMS procedure

TMS were delivered through a figure-of-eight coil with an outer diameter of 70 mm (Magstim Co., Whitland, Dyfeld, UK) over the left motor cortex. The coil was held with the handle pointing backwards and laterally to evoke an anteriorly directed current in the brain, and was optimally positioned to obtain MEPs in the contralateral FDI. Single and paired pulses were delivered from a monophasic Magstim BiStim.

For all three protocols, baseline and outcome data were collected in an identical fashion (see figure 8). For all the protocols, we first localized the “hot spot” (defined as the point on the scalp at which single pulse TMS elicited MEPs of maximal amplitude from the right FDI) and established the resting motor threshold (RMT) (minimum stimulation intensity over the motor hot-spot, which elicits an MEP of no less than 50 μV in 5 of 10 trials in the relaxed FDI) and active motor threshold (AMT) (intensity necessary to evoke a 200 μV MEP while subjects maintained approximately 10% contraction of the FDI). Active motor thresholds were obtained with both the BiStim and Super Rapid Magstim packages in the case of iTBS protocol (AMT and AMT_r, respectively).

For the baseline, we recorded 20 MEPs (at SI_{1mV}) and SICI measures. After each protocol, 12-MEPs amplitude (inter-trial interval 5 s, vary 10%) was measured at 5-minutes intervals for 60 minutes. Two blocks of SICI (10 test stimulus (TS) and 10 conditioned stimulus (CS) each, randomised) were recorded at minute 6 and minute 46 post-stimulation.

SICI was measured using the technique described by Kujirai et al. (1993) (Kujirai, Caramia, et al., 1993) – a subthreshold conditioning stimulus at the 80% of AMT (Orth, Snijders, et al., 2003) precedes a TS by 2 ms. The mean peak-to-peak amplitude of the conditioned MEP was expressed as a

percentage of the mean peak-to-peak amplitude of the unconditioned MEP.

4.3.3.4. Paired associative stimulation (PAS_{25})

PAS consisted on 200 electrical stimuli (at 300% of the perceptual threshold (PT)) over ulnar nerve at the right wrist, paired with TMS pulses (interstimulus interval of 25 ms) over the left hemisphere FDI hotspot at a rate of 0.25 Hz (total protocol duration approximately 13 minutes). Subjects were asked to count the number of stimuli given to ensure their attention did not vary.

4.3.3.5. Anodal transcranial direct current stimulation (AtDCS)

tDCS was delivered at 1 mA for duration 13 minutes through a pair of saline-soaked sponge surface electrodes (35 cm^2) connected to a DC stimulator (neuroConn). Active electrode (anode) was placed over the hotspot of the left M1 (as determined by TMS), and the reference electrode (cathode) was placed over the contralateral supraorbital region. The current was faded in and faded out 8 seconds each.

4.3.3.6. Intermittent theta-burst stimulation (iTBS)

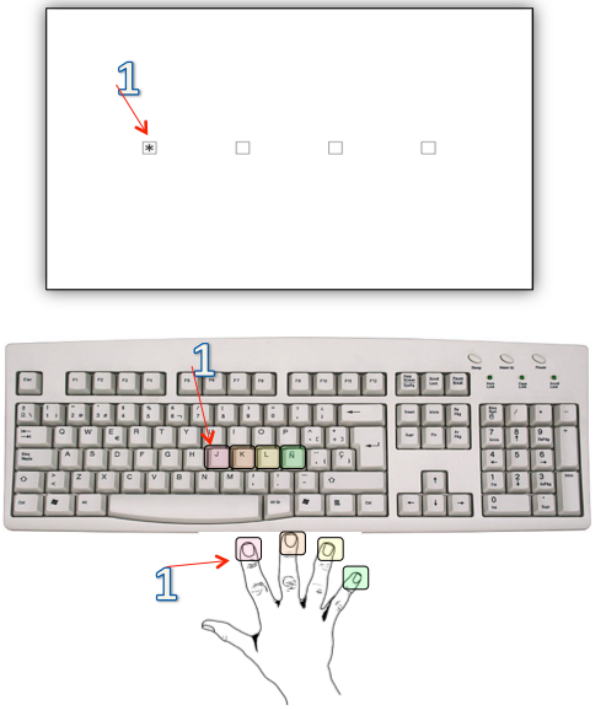
A biphasic stimulator, a Super Rapid Magstim package (Magstim Co., UK), was used to deliver TBS. iTBS was applied over the left motor cortex hot-spot as described by Huang et al. (2005). Each burst consisted of three stimuli (at 80% AMT_r stimulator intensity) given at 50 Hz, repeated at 5Hz. iTBS involves giving a 2 seconds train repeated every 10 seconds for 20 repetitions (600 stimuli).

4.3.3.7. Serial reaction time task (SRTT)

Subjects were seated in front of a computer screen (46 x 29 cm) at eye level behind a keyboard on the table with four coloured keys (letters “j”, “k”, “l” and “ñ”; from now on we will refer to them as “1”, “2”, “3” and “4”, respectively). They performed a SRTT (Nissen and Bullemer, 1987) running on SuperLab (version 4.0). They were instructed to push each key with a different finger of the right hand (index finger for “1”, middle finger for “2”, ring finger for “3”, and little finger for “4”).

An asterisk appeared in one of four positions that were horizontally spaced on a computer screen and permanently marked by black squares on a white screen background.

Each screen position corresponded to a key on the keyboard. The spatial configuration of the keys was fully compatible with the screen positions. Subjects were instructed to press the correspondent key as fast as possible. The stimuli disappeared immediately after pushing any key, and appeared again after 500 ms (Figure 18).



SRTT							
R1	S1	S2	S3	S4	R2	S5	S6

Figure 18. Serial reaction time task (SRTT). After the appearance of the asterisk subjects should press as fast and as accurately as possible the corresponding key in the keyboard, with the corresponding finger. In the table on the bottom, the order of blocks is shown (R: random block; S: sequence block).

Before starting the SRTT experiment, a practice block with 60 trials in random order was administered to ensure that participants understood the instructions.

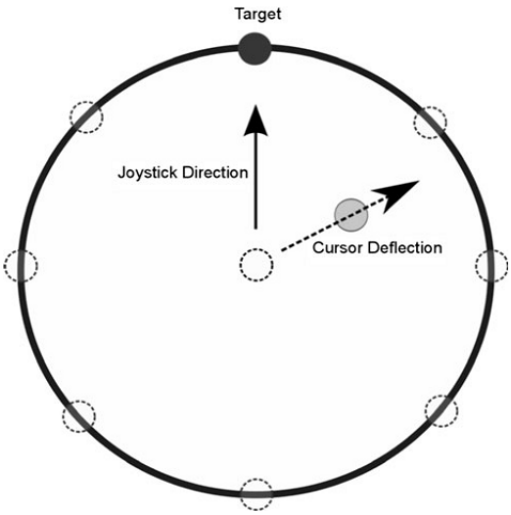
SRTT consisted on eight blocks of 120 trials (with an extra practice block of 60 trials in pseudorandom order). In Blocks 1 and 6 (random "R" blocks), the sequence of asterisks followed a pseudorandom order. For both blocks asterisks were presented equally frequently in each position, the sequence could not contain runs of four units (e.g., 1234 or 4321) or trills of four units (e.g., 1212). In Blocks 2 to 5 and 7 and 8 (sequence "S" blocks), the same 12-trial sequence of asterisk positions repeated itself 10 times (121423413243). Subjects were not told about the repeating sequence (Figure 18).

4.3.3.8. Visuomotor adaptation task with a joystick (VAT)

Similar to Joundi et al., 2012 (Joundi, Lopez-Alonso, et al., 2012) subjects were seated in an armless chair 80 cm in front of a 46 x 29 cm size computer monitor, on which the task was presented. Subjects were asked to hold a joystick with their right hand, regardless of hand-dominance. An opaque shield covered the joystick so that the subjects could not see their hand or the joystick. Movement of the joystick controlled a green cursor (1 x 1 cm) on the computer screen, and was recorded at a sampling rate of 60 Hz. The goal of the task was to follow a red target (1 x 1 cm) initially presented at the centre of the screen that quickly jumped to one of eight points equidistantly located at the perimeter of a (13 x 13 cm) visible circle once every 2 seconds. The sequence in which the peripheral targets were presented was random. Subjects were instructed to move toward the target and back in a single, straight, striking motion without correcting for initial errors, and were reminded to move as quickly as possible in response to the cue.

The task began with a baseline test (b) consisting of 48 trials in which the movement of the joystick matched the movement of the green cursor on the screen (~1.6 minutes in duration). After a one-minute break, a learning session (l; learning) began. During this period the relationship between the movement of the joystick and the cursor was altered so that the cursor moved with a +60° rotation relative to the joystick (152 trials; ~5 minutes). During the learning session, there were large initial errors (~60°) that decreased over the course of

the session. Subjects were told that a rotation would occur, and were also instructed not to allow the rotation to disrupt their response profile and to continue to make straight, striking motions as in the baseline session. After the completion of the learning session, the participants took a 45-minute break. Participants returned and were retested (c1; consolidation1) with the same 60° rotation (152 trials; ~5 minutes). There was then a second break lasting 24 hours, in which the subjects engaged in their normal activities, including sleep. Another set of 152 trials with 60° rotation (c2; consolidation2) was performed after the 24-hour break. Finally, a de-adaptation (d) session was conducted in which the veridical relationship between cursor and target was restored (152 trials). Here, participants were initially perturbed from the target as a result of their previous motor remapping and returned to baseline through de-adaptation (Figure 19).



DAY 1					DAY 2	
Baseline	Learning		Consolidation_1		Consolidation_2	De-adaptation
48 trial 0º deviation	152 trials 60º deviation	45'	152 trials 60º deviation	24 h	152 trials 60º deviation	152 trials 0º deviation

Figure 19. Visuomotor adaptation task (VAT). The goal of the task was to follow the target that quickly jumped to one of the eight points marked with dashed circles. The table on the bottom shows the different tests with the corresponding number of trials and deviation of the cursor with respect to the joystick movement.

4.3.3.9. Sequential visual isometric pinch task (SVIPT)

This task was an adaptation of the sequential visual isometric pinch task described by Reis et al. (2009) (Reis, Schambra, et al., 2009). Subjects were seated in an armchair 80 cm from a 46 x 29 cm size screen. On the table in front of the subject there was a force transducer. Subjects pinched the transducer between the thumb and the second phalange of index finger. Squeezing the force transducer moved a screen cursor up vertically in the screen. The goal of the task was to move the cursor following a fixed sequence of four numbered targets corresponded to four levels of force. Subjects were instructed to perform the task as fast and accurately as possible. Between each target, the cursor should return to the baseline position (Figure 20). To increase the difficultly of the task, we adjusted the vertical scale on the screen in a way that the maximum upward movement was set to 35%-45% of maximum force of each subject.

Figure 20. Sequential visual isometric pinch task (SVIPT). Squeezing the force transducer moved the red screen cursor up vertically. The goal of the task was to move the cursor following the numbered targets marked on both sides of the screen.

Subjects were encouraged to perform motor tasks by giving a reward of 500 euros for the subject who get the highest score in the three tasks (Abe, Schambra, et al., 2011).

4.3.3.10. Data analysis

4.3.3.10.1. Non-invasive brain stimulation

For each of the NIBS protocols, MEP amplitudes of each block (0, 5,… 60), normalized to the baseline, were considered as the measure of cortical excitability (see (Lopez-Alonso, Cheeran, et al., 2014) for a more detailed information).

4.3.3.10.2. Motor tasks

For the *serial reaction time task,* reaction time (RT) (measured from the appearance of the stimulus (asterisk) until any key was pressed) and the name of the key pressed (correct or incorrect answer) were recorded in each trial. For each block of trials, mean RT was calculated for each subject separately. Incorrect responses and response times of more than 3000 ms or those that were above three standard deviations of the individual subject's mean response time were discarded.

Two variables, learning rate (LR) and implicit learning (IL), were computed as specific measures of procedural learning. Learning rate is defined as the reduction of RT in the repeating sequence blocks (S1–S6) and is a measure of both the ability in execution of the reaction time task (reaction-time task learning) and sequence-specific learning. Implicit learning, defined as the decrease in RT between blocks R2 (last random block) and S6 (the last repeating sequence block), reflects only the sequence-specific learning. A greater difference in RTs between random and sequence blocks corresponds to better sequence-specific learning (Muslimovic, Post, et al., 2007).

In addition, RT of the first random block (R1) was used as a measure of reaction time.

For the *visuomotor adaptation task*, data were analyzed trial-by-trial using semi-automated-in-house code written in MATLAB (Mathworks Inc, Natick, USA) following the procedure described by Joundi et al. (2012) (Joundi, Lopez-

Alonso, et al., 2012). The square root of the sum of squared x- and y-coordinates was taken to determine the trajectory of the joystick movement. The trajectory was then filtered with a 150-ms moving average. The start point of the movement was defined as the point at which velocity reached 25% of its maximum after a minimum of 50 ms from the start of the trial (target jump). The end point was defined as the point at which the same threshold velocity was crossed on the downslope. Our main measure was the absolute angular error (AE) between the initial outward movement of the cursor and the target angle. This was calculated as the angle of the point of maximum velocity relative to the origin.

Data from each session were first divided into contiguous blocks of eight trials. Individual trials that exceeded an angular error of two standard deviations of the mean from each block of eight trials were rejected.

We also adopted a similar approach to previous studies in which motor adaptation data have been analyzed by fitting individual learning sessions with exponential curves (Huang, Haith, et al., 2011; Krakauer, Ghez, et al., 2005). Thus, all the remaining individual trials (rather than blocks) in each adaptation session for every subject were fitted with a single exponential function:

$y=C_1*\exp(-rate*x)+C_0$,

where C_1 and C_0 are constants, x is the trial number and y is the error. The "rate" variable provided an index for the rate of error reduction.

For *sequential visual isometric pinch task* we measured the average movement time per block, defined as the time between start of the cursor movement and the return to baseline position after reaching the fourth and last target, and the error rate, calculated as the proportion of trials with at least one over- or undershooting movement. Skill was defined as the combination of both variables, using the same mathematical model fitting the speed-accuracy trade-off function curve for the SVIPT in Reis et al., 2009 (Reis, Schambra, et al., 2009), after validated it for our own data:

$$skill = \frac{1 - error\_rate}{error\_rate(\ln\,(duration)^{3.6747})}$$

4.3.3.11. Statistical analysis

4.3.3.11.1. Non-invasive brain stimulation

In order to categorize the subjects by their response to the NIBS protocols, TwoStep cluster analyses were conducted with the normalized-to-baseline MEP amplitude of the 13 time-points (from minute 0 to minute 60) post-stimulation for each protocol (this analysis has been described in (Lopez-Alonso, Cheeran, et al., 2014)).

We also calculated the mean of the normalized-to-the-baseline MEP amplitude at three different time bins or points for each NIBS protocol. 1) Time bin from baseline to minute 30 (0-30), 2) time bin between minute 5 and minute 15 (5-15) and 3) the time point with maximum MEP increase from baseline between minute 0 and minute 30 post-stimulation (max response) (minute 15 for AtDCS, minute 30 for PAS and minute 25 for iTBS). We have chosen these points based previous findings on effects and reliability of NIBS paradigms published by our group (Lopez-Alonso, Cheeran, et al., 2014; Lopez-Alonso, Fernandez-Del-Olmo, et al., 2015).

4.3.3.11.2. NIBS-induced plasticity and motor learning

Independent t-tests were used to determine differences between clusters (responders and non-responders) for learning rate (LR) and implicit learning (IL) during SRTT, angular error (AE) (in the last block of learning, consolidation1, consolidation2 and in the first de-adaptation blocks) and learning rate (LR) of each block for VAT and finally for SVIPT.

Stepwise linear regression was conducted for each summary variable of motor learning (LR and IL for SRTT, LR and AE for VAT and skill for SVIPT) as the dependent variable and a number of summary measures of the change in MEPs (0-30 minutes, 5-15 minutes and max change 0-30 minutes) to each NIBS protocol as independent variables. So, three stepwise linear regressions were used for each variable of learning (one per summary measure of response to NIBS).

4.3.3.11.3. NIBS-induced plasticity and reaction time

Independent t-tests were used to determine differences between clusters for reaction time during the first block of the SRTT.

When differences were found, Pearson product–moment correlation coefficients were used to calculate the association between the response to NIBS protocol (whole average response after each stimulation protocol, normalized to baseline) and the variable of the learning.

All statistical analyses were performed using SPSS (SPSS, Chicago, IL). Significant main effect was set at a P value < 0.05.

4.3.4. Results

No adverse effects were reported during the NIBS sessions.

HenzeeZirkler test confirmed normality in the set of data for NIBS (p=0.2092).

4.3.4.1. Non-invasive brain stimulation

The results for the TwoStep analysis have been reported previously (see (Lopez-Alonso, Cheeran, et al., 2014) for a more detailed results explanations). Briefly, TwoStep analysis resulted in a two-cluster distribution for each of the three paradigms, a cluster of “responders” (showing an increase in the normalized MEP amplitudes) and “non-responders” (those who do not show an increase).

4.3.4.2. NIBS-induced plasticity and motor learning

No significant differences were observed between clusters in any NIBS protocol and any learning variable tested for the three tasks.

Table 6 summarizes the motor learning performance for each cluster and task.

Stepwise linear regression between summary VARIABLE OF motor LEARNING and AVERAGED MEP RESPONSE 0-30, revealed that only tDCS significantly predicted AE in the consolidation 1 block (R^2 = 0.162; P = 0.002) and AE in the de-adaptation block of the VAT (R^2 = -0.088; P = 0.027).

Stepwise linear regression between VARIABLE OF LEARNING and AVERAGED MEP RESPONSE 5-15, revealed that tDCS significantly predicted AE in the consolidation 1 block (R^2 = 0.185; P = 0.001) and AE in the de-adaptation block of the VAT (R^2 = -0.166; P = 0.025).

Stepwise linear regression between VARIABLE OF LEARNING and POINT OF MAXIMUM MEP INCREASE 0-30, revealed that only tDCS max significantly predicted AE in the learning (R^2 = 0.071; P = 0.047), AE in the consolidation 1 (R^2 = 0.272; P < 0.001) and AE in the de-adaptation (R^2 = -0.172; P = 0.001) blocks of the VAT.

Table 6. Motor learning variables for responders and non-responders. This table shows the descriptive values (mean±SD) for each variable of motor learning tested for the cluster of responders and non-responders.

*** The n for SVIPT is R=19 and NR=25 for tDCS; R=15 and NR=29 for PAS; and R=20 and NR=24 for iTBS.**

		AtDCS		PAS		iTBS	
		R (n=25)	NR (n=31)	R (n=22)	NR (n=34)	R (n=24)	NR (n=32)
SRTT	Learning rate	34.6±36.9	42.6±50.3	34.0±45.2	42.3±44.6	40.7±49.6	37.7±41.3
	Implicit learning	87.3±10.0	86.7±12.2	85.8±10.6	87.3±11.7	85.3±13.0	87.7±9.7
VAT	Angular error_l	24.5±16.6	21.1±11.0	20.1±14.3	24.3±13.3	19.9±14.3	24.7±13.2
	Angular error_c1	22.9±15.8	16.5±10.1	18.4±14.6	20.0±12.5	18.2±12.5	20.2±13.9
	Angular error_c2	21.6±15.6	23.3±14.7	17.5±11.9	24.8±15.2	19.9±15.6	24.5±14.5
	Angular error_d	28.9±12.3	34.7±8.3	31.5±11.0	32.2±10.5	31.0±10.8	32.9±10.5
	Learning rate_l	-0.03±0.05	-0.05±0.06	-0.04±0.07	-0.05±0.06	-0.05±0.07	-0.04±0.06
	Learning rate_c1	-0.12±0.29	-0.09±0.09	-0.09±0.09	-0.12±0.25	-0.08±0.08	-0.12±0.26
	Learning rate_c2	-0.07±0.13	-0.03±0.03	-0.07±0.13	-0.04±0.05	-0.07±0.13	-0.03±0.04
	Learning rate_d	-0.03±0.02	-0.03±0.02	-0.03±0.03	-0.02±0.01	-0.03±0.03	-0.03±0.02
SVIPT *	Skill	-0.13±0.3	-0.06±0.07	-0.07±0.09	-0.10±0.24	-0.13±0.30	-0.06±0.08

Linear regression did not revealed any predictors for SRTT or for SVIPT.

Our results reflect that an increase in MEPs post-stimulation was related with higher angular errors in last blocks of both learning and consolidation, whilst the opposite relation was found with the first block of de-adaptation. High angular

error values at the end of learning or consolidation blocks mean less adaptation, whilst low angular error values at the beginning of the de-adaptation block mean that poor adaptation was achieved in previous blocks.

4.3.4.3. NIBS-induced plasticity and reaction time

The reaction time for the first random block of the SRTT was significant different between "responders" and "non-responders" to the tDCS (t=-2.546; p=0.014). Similar results were obtained when the clusters were categorized by their response to iTBS (t=2.876; p=0.006). For both the tDCS and iTBS the "responders" showed a lower reaction time than "non-responders". No significant differences were found between PAS-clusters.

tDCS and iTBS-induced plasticity correlated negatively with the reaction time during the first random block of the SRTT (R= -0.286; p=0.032 and R= -0.307; p=0.021, respectively) (Figure 21).

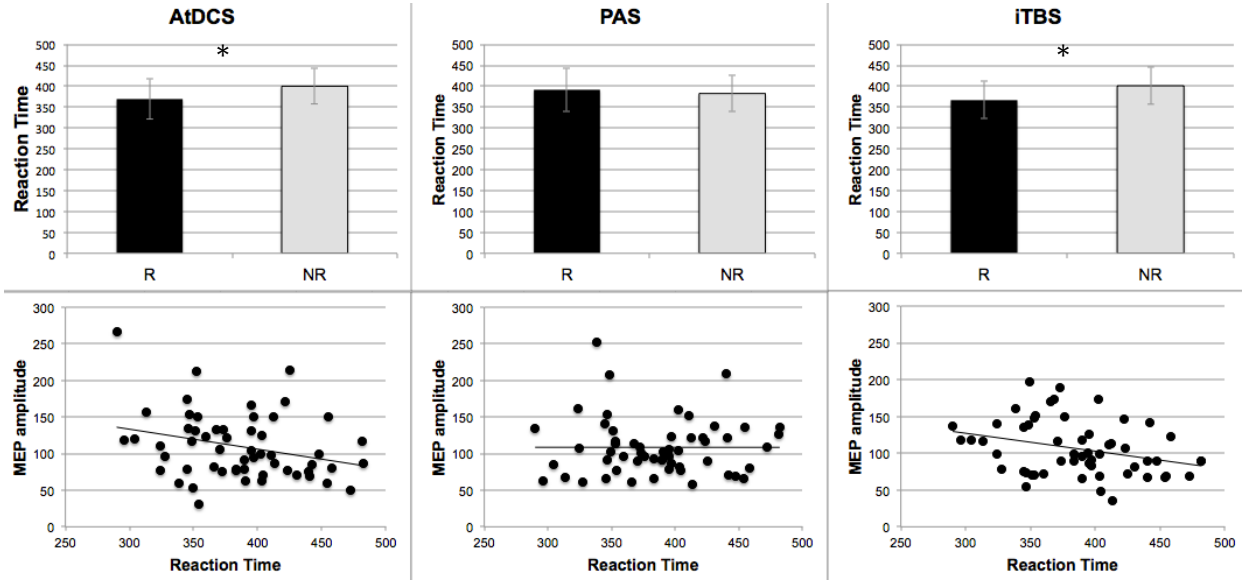


Figure 21. NIBS-induced changes and reaction time. In the upper plots are shown the RT (ms) of the random block for the SRTT of "responders" (black) and "non-responders" (grey) for each NIBS protocol. On the bottom, scatterplots of the individual data with RT (ms) in the "x axis" and M1 modulation after each NIBS protocol (averaged MEP amplitude normalized-to-baseline during the whole hour post-stimulation, in %) after each stimulation protocol in the "y axis".

4.3.5. Discussion

The purpose of our study was to explore whether the cortical plasticity induced by tDCS, PAS and iTBS protocols is related with the subject's motor learning capacity as assessed by 3 lab-based motor learning tasks. To our knowledge this is the first study correlating the neuroplastic responses to three different NIBS-induced plasticity protocols with three different paradigms of learning with a reasonable sample size (n=56).

Our results suggest that the cortical plasticity induced by those protocols is largely not associated with the motor learning capacity. There may be some correlation between tDCS-induced plasticity and some measures of VAT, but the results suggest that the relationship is inverse. However, the cortical plasticity induced by AtDCS and iTBS seems to relate with reaction time performance.

4.3.5.1. NIBS-induced plasticity and motor learning

As reported in our previous study, and in line with other authors, we found two patterns of MEP modulation in response to the three facilitatory NIBS protocols. Approximately half of the sample responded as expected to each protocol by increasing the MEP amplitudes at least during the 30-minutes post-stimulation while the other half did not show such facilitation (Hamada, Murase, et al., 2013; Lopez-Alonso, Cheeran, et al., 2014; Muller-Dahlhaus, Orekhov, et al., 2008).

The comparative analysis between responders and not responders for the motor learning tasks did not reveal any significant differences. The variables related with the motor learning were similar for both groups. We did not find a relation between the M1 modulation induced by NIBS and performance on 3 established motor learning tasks.

To look more closely into the possible relationship between NIBS-induced changes in cortical excitability and motor learning, we tested averages of MEPs at different time points after NIBS, for each of the three NIBS paradigms tested (Lopez-Alonso, Cheeran, et al., 2014). Using this more exploratory approach, we found a relationship between tDCS-induced changes in cortical excitability and certain phases in the visuomotor adaptation task. However, the results are not in keeping with our a priori hypothesis or commonly held views on the

relationship between NIBS-induced cortical plasticity and motor learning. Subjects with higher increases in cortical excitability after tDCS show less adaptation than those subjects where tDCS is ineffective at producing changes in cortical excitability.

This lack of correlation between plasticity and motor learning is consistent with results from some previous studies that also evaluated, in separate sessions, the relationship between modulation of MEPs and motor learning. Li Voti et al. (2011) (Li Voti, Conte, et al., 2011) and Vallence et al. (2013) (Vallence, Kurylowicz, et al., 2013) did not find a significant correlation between MEP modulation induced by TBS or PAS and motor learning using a finger movement task. The performance in a rotor pursuit-learning task did also not correlate with the response to iTBS and PAS (Player, Taylor, et al., 2012; Player, Taylor, et al., 2013). However, Witte et al (2012) (Witte, Kurten, et al., 2012) reported a correlation between PAS and implicit learning during a SRTT. Our study did not find a correlation between PAS and SRTT. Differences in SRTT protocol used in both studies could explain this discrepancy. Witte et al. used a modified version of SRTT, introducing probabilistic sequences, which could involve different neural networks in comparison with the more conventional SRTT protocol used in our study. Therefore, both tasks would place different demands on M1, and the role of this cortical area may vary with the complexity of the task (Smyth, Summers, et al., 2010). Moreover, Witte's study was conducted only in females and it has been reported that gender influences NIBS-induced plasticity since hormone changes during menstrual cycle alter cortical excitability (Inghilleri, Conte, et al., 2004). So far, no data has been reported about the relation between tDCS-induced plasticity and motor learning capacity measured in different sessions. In our study, the modulation in MEPs induced by tDCS did not correlate with the motor learning capacity on the SRTT or sequential visual isometric pinch task. The results show a link between the effectiveness of aTDCS and certain phases of the VAT. However, the link may be tenuous, and the physiological significance of effects in the consolidation and de-adaptation phases is uncertain and open to interpretation.

The lack of correlation between the MEP modulation induced by NIBS on the M1 and motor learning is also supported for studies that have explored the

behavioural short-term effect of NIBS. Agostino et al. (Agostino, Iezzi, et al., 2007; Agostino, Iezzi, et al., 2008) did not find a correlation between MEP increments in response to NIBS and the effects on a motor learning task (practice-related changes in fast finger movements). The authors argued that since motor behaviour engages a distributed cortical and subcortical neuronal network, excitatory conditioning of the primary motor cortex is probably not sufficient to influence or predict the behavioural output. Therefore, it is possible that the learning tasks used in our study involved a far more complex neural network and thus, may mask any possible relationship between motor learning and the modulation induced by NIBS on M1.

The contradictory results over the effect of the NIBS protocols on the performance of motor tasks could also be related with the high inter-individual variability in response to the NIBS protocols (Hamada, Murase, et al., 2013; Lopez-Alonso, Cheeran, et al., 2014; Muller-Dahlhaus, Orekhov, et al., 2008). However, our results do not support this hypothesis since no differences were found in the variables related with motor learning between “responders” and “non-responders”. Therefore, the inter-individual variability in response to the NIBS may be of relevance from a physiological point of view rather than from a motor learning perspective.

4.3.5.2. NIBS-induced plasticity and reaction time

An important finding from our study is that both tDCS- and iTBS-induced plasticity correlates with the reaction time during the randomized block of the SRTT. However, there were no correlations during the non-randomized blocks (the sequence blocks), which involved an implicit learning process. The randomized block is a form of choice reaction time task that involves a spatial compatibility between the stimuli and the finger movements. The “responders” to tDCS and iTBS protocols showed significantly faster reaction times than the “non-responders”. The relationship found in our study between M1 modulation and RT is in line with those studies showing that single pulse of TMS over M1 in a simple RT task influences the RT of the subjects (Hashimoto, Inaba, et al., 2004; Pascual-Leone, Brasil-Neto, et al., 1992). Interestingly, Nitsche et al. (2003) (Nitsche, Schauenburg, et al., 2003) has shown that anodal tDCS applied over M1 during the performance of a choice reaction time task reduces

the RT. They used a similar task to the SRTT, with the difference that all blocks were random blocks (and not interspersed with sequence blocks). Anodal tDCS shortened RT compared to sham constantly in each block.

The lack of correlation between RT and PAS protocol can be explained by the mechanisms underlying the MEP modulation by the PAS protocol. All the three protocols used in our study (tDCS, PAS and iTBS) induce changes in cortical excitability. However, tDCS and iTBS are thought to induce its effect by direct changes to M1 (Huang, Edwards, et al., 2005; Nitsche, Nitsche, et al., 2003; Ziemann, Paulus, et al., 2008), whilst PAS-induced changes rely on sensorimotor integration. PAS-induced plasticity is associative-dependent, whilst tDCS and TBS do not induce associative plasticity (Nathan, Cobb, et al., 2011). Furthermore, although the three techniques seem to induce these LTP-like effects in an NMDA-dependent manner (Huang, Chen, et al., 2007; Nitsche, Jaussi, et al., 2004; Stefan, Kunesch, et al., 2002), pharmacological studies have shown that blockade of D2 receptors abolishes the plasticity induced by AtDCS and iTBS only. D2 blockade has no effect in the MEP facilitation by excitatory PAS (Monte-Silva, Ruge, et al., 2011). D2 receptors may be responsible of the plasticity modulation by dopamine, an important neurotransmissor for memory and learning (for a review see (Iversen and Iversen, 2007). Furthermore, dopamine has shown to have effect on reaction time in choice reaction time tasks. Pullman et. al (Pullman, Watts, et al., 1988) have demonstrated longer reaction times in those tasks in Parkinson disease patients treated with dopamine medication (L-dopa) replacement, suggesting a role of dopamine in choice reaction time tasks (Gauntlett-Gilbert and Brown, 1998), for a review). As PAS has been suggested not to be D2 receptor dependent, this could explain the lack of correlation between reaction time and the MEP modulation induced by PAS protocol. Therefore, it is plausible that the modulation induced by the NIBS protocols could predict, at least to some extent, *performance* in a motor task.

In summary, our study supports and expands previous findings that there may be little or no significant relationships between modulation of cortical excitability by NIBS and motor learning capacity tested using a number of lab-

based motor learning tasks. Furthermore, our results suggest that the modulation of cortical excitability by certain NIBS pardigms could correlate with motor performance as suggested by correlation with RT in a choice reaction time task.

Chapter 5

Conclusions

5. CONCLUSIONS

- There is ***no significant effect*** of iTBS, PAS or AtDCS on MEP amplitude or SICI over a one-hour time period for a sample of 56 subjects.

- There is ***significant effect*** of AtDCS on MEP amplitude over 30 minutes in a sample of 45 subjects.

- There is considerable ***inter-individual variability*** in the response to all the three protocols (iTBS, PAS and AtDCS), both in MEP and SICI measures.

- ***Cluster analysis*** based on changes in MEP amplitude revealed distinct groups of responders and non-responders to each protocol.

- There is a fair ***intra-individual reliability*** of NIBS (tested with anodal tDCS during the half-hour post-stimulation) in two separate sessions.

- Intra-individual variability in response to two separate sessions of anodal is lower than inter-individual variability.

- Baseline SICI partially ***predicts*** the response to the PAS_{25} protocol.

- The ***build-up of excitability*** for anodal tDCS peaked around minute 15.

- Cortical plasticity induced in M1 by iTBS, PAS and AtDCS is not associated with the ***motor learning capacity*** tested with SRTT, VAT and SVIPT.

- Cortical plasticity induced in M1 by iTBS and AtDCS seems to be related with ***reaction time performance***.

Chapter 6

Limitations

6. LIMITATIONS

- We have only tested three different NIBS protocols and all of them were expected to induce “facilitatory” effects. Therefore, it remains to replicate the studies included in the current thesis to a wider number of NIBS protocols including that ones that induce inhibitory effects.

- The sample used in the current thesis were young and healthy subjects and thus, cautions must be taken in apply our findings to other populations.

- Only one cortical area was stimulated. Although, primary motor cortex has been the most often target for NIBS, it remains to be tested whether our findings are also valid for other cortical areas.

- The effects of each NIBS used in our studies was constraint to a single session. Therefore, it should be tested the accumulative effects of successive sessions of stimulation. In addition, the reliability of the effects induced by the NIBS protocols must be evaluated in more than two sessions.

- The motor tasks used in our study only represent a small number of the wide motor task paradigms.

Chapter 7

References

7. REFERENCES

Abe M, Schambra H, Wassermann EM, Luckenbaugh D, Schweighofer N, Cohen LG. Reward improves long-term retention of a motor memory through induction of offline memory gains. Curr Biol. 2011 Apr 12;21(7):557-62.

Agostino R, Iezzi E, Dinapoli L, Gilio F, Conte A, Mari F, et al. Effects of 5 Hz subthreshold magnetic stimulation of primary motor cortex on fast finger movements in normal subjects. Exp Brain Res. 2007 Jun;180(1):105-11.

Agostino R, Iezzi E, Dinapoli L, Suppa A, Conte A, Berardelli A. Effects of intermittent theta-burst stimulation on practice-related changes in fast finger movements in healthy subjects. Eur J Neurosci. 2008 Aug;28(4):822-8.

Amassian VE, Stewart M, Quirk GJ, Rosenthal JL. Physiological basis of motor effects of a transient stimulus to cerebral cortex. Neurosurgery. 1987 Jan;20(1):74-93.

Antal A, Nitsche MA, Kincses TZ, Kruse W, Hoffmann KP, Paulus W. Facilitation of visuo-motor learning by transcranial direct current stimulation of the motor and extrastriate visual areas in humans. Eur J Neurosci. 2004 May;19(10):2888-92.

Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet. 1985 May 11;1(8437):1106-7.

Barrionuevo G, Brown TH. Associative long-term potentiation in hippocampal slices. Proc Natl Acad Sci U S A. 1983 Dec;80(23):7347-51.

Bear MF. A synaptic basis for memory storage in the cerebral cortex. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):13453-9.

Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993 Jan 7;361(6407):31-9.

Bliss TV, Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol. 1973 Jul;232(2):357-74.

Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol. 1973 Jul;232(2):331-56.

Boggio PS, Castro LO, Savagim EA, Braite R, Cruz VC, Rocha RR, et al. Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation. Neurosci Lett. 2006 Aug 14;404(1-2):232-6.

Boggio PS, Rigonatti SP, Ribeiro RB, Myczkowski ML, Nitsche MA, Pascual-Leone A, et al. A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. Int J Neuropsychopharmacol. 2008 Mar;11(2):249-54.

Boggio PS, Zaghi S, Villani AB, Fecteau S, Pascual-Leone A, Fregni F. Modulation of risk-taking in marijuana users by transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (DLPFC). Drug Alcohol Depend. 2010 Dec 1;112(3):220-5.

Buch ER, Young S, Contreras-Vidal JL. Visuomotor adaptation in normal aging. Learn Mem. 2003 Jan-Feb;10(1):55-63.

Butefisch CM, Khurana V, Kopylev L, Cohen LG. Enhancing encoding of a motor memory in the primary motor cortex by cortical stimulation. J Neurophysiol. 2004 May;91(5):2110-6.

Cajal S. Textura del sistema nervioso del hombre y de los vertebrados. Madrid: Moya; 1899.

Carson RG, Kennedy NC. Modulation of human corticospinal excitability by paired associative stimulation. Frontiers in human neuroscience. 2013;7:823.

Cheeran B, Koch G, Stagg CJ, Baig F, Teo J. Transcranial magnetic stimulation: from neurophysiology to pharmacology, molecular biology and genomics. Neuroscientist. 2010 Jun;16(3):210-21.

Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, et al. A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. The Journal of Physiology. 2008;586(Pt 23):5717-25.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology. 1997 May;48(5):1398-403.

Cicchetti DV, Sparrow SA. Developing criteria for establishing interrater reliability of specific items: applications to assessment of adaptive behavior. American journal of mental deficiency. 1981 Sep;86(2):127-37.

Cirillo J, Lavender AP, Ridding MC, Semmler JG. Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. J Physiol. 2009 Dec 15;587(Pt 24):5831-42.

Citri A, Malenka RC. Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology. 2008 Jan;33(1):18-41.

Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol. 1998 Feb;79(2):1117-23.

Classen J, Stefan K. Changes in TMS measures induced by repetitive TMS. In: Wassermann E, Epstein CM, Ziemann U, editors. The Oxford handbook of transcranial stimulation. New York: Oxford University Press; 2008.

Cohen LG, Roth BJ, Nilsson J, Dang N, Panizza M, Bandinelli S, et al. Effects of coil design on delivery of focal magnetic stimulation. Technical considerations. Electroencephalogr Clin Neurophysiol. 1990 Apr;75(4):350-7.

Collingridge G. Synaptic plasticity. The role of NMDA receptors in learning and memory. Nature. 1987 Dec 17-23;330(6149):604-5.

Collingridge GL, Kehl SJ, McLennan H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol. 1983 Jan;334:33-46.

Conde V, Vollmann H, Sehm B, Taubert M, Villringer A, Ragert P. Cortical thickness in primary sensorimotor cortex influences the effectiveness of paired associative stimulation. Neuroimage. 2012 Apr 2;60(2):864-70.

Cooke SF, Bliss TV. Plasticity in the human central nervous system. Brain. 2006 Jul;129(7):1659-73.

Dayan E, Censor N, Buch ER, Sandrini M, Cohen LG. Noninvasive brain stimulation: from physiology to network dynamics and back. Nat Neurosci. 2013 Jul;16(7):838-44.

De Beaumont L, Tremblay S, Poirier J, Lassonde M, Théoret H. Altered Bidirectional Plasticity and Reduced Implicit Motor Learning in Concussed Athletes. Cerebral Cortex. 2012;22:112-21.

Dekeyser R. Implicit and explicit learning. In: Doughty CJ, Long MH, editors. The Handbook of second language acquisition: Blackwell Publishing; 2008.

Demirtas-Tatlidede A, Vahabzadeh-Hagh AM, Pascual-Leone A. Can noninvasive brain stimulation enhance cognition in neuropsychiatric disorders? Neuropharmacology. 2013 Jan;64:566-78.

Di Lazzaro V, Dileone M, Pilato F, Capone F, Musumeci G, Ranieri F, et al. Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. J Neurophysiol. 2011 May;105(5):2150-6.

Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, et al. Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. Clin Neurophysiol. 2000 May;111(5):794-9.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. Clin Neurophysiol. 2004 Feb;115(2):255-66.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, et al. Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol. 1998 Oct;109(5):397-401.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol. 2008 Aug 15;586(16):3871-9.

Di Lazzaro V, Profice P, Pilato F, Capone F, Ranieri F, Pasqualetti P, et al. Motor cortex plasticity predicts recovery in acute stroke. Cereb Cortex. 2010 Jul;20(7):1523-8.

Di Lazzaro V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero A, et al. I-wave origin and modulation. Brain Stimul. 2012 Oct;5(4):512-25.

Di Lazzaro V, Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Frontiers in neural circuits. 2013;7:18.

Di Lazzaro V, Ziemann U, Lemon RN. State of the art: Physiology of transcranial motor cortex stimulation. Brain Stimul. 2008 Oct;1(4):345-62.

Dileone M, Profice P, Pilato F, Alfieri P, Cesarini L, Mercuri E, et al. Enhanced human brain associative plasticity in Costello syndrome. J Physiol. 2010 Sep 15;588(Pt 18):3445-56.

Dudek SM, Bear MF. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci U S A. 1992 May 15;89(10):4363-7.

Dudek SM, Bear MF. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J Neurosci. 1993 Jul;13(7):2910-8.

Duffau H. Brain plasticity: from pathophysiological mechanisms to therapeutic applications. J Clin Neurosci. 2006 Nov;13(9):885-97.

Ellaway PH, Davey NJ, Maskill DW, Rawlinson SR, Lewis HS, Anissimova NP. Variability in the amplitude of skeletal muscle responses to magnetic stimulation of the motor cortex in man. Electroencephalogr Clin Neurophysiol. 1998 Apr;109(2):104-13.

Epstein CM. The stimulation coils. In: Wassermann E, Epstein CM, Ziemann U, editors. The Oxford handbook of transcranial stimulation. New York: Oxford University Press; 2008.

Fisher RJ, Nakamura Y, Bestmann S, Rothwell JC, Bostock H. Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking. Exp Brain Res. 2002 Mar;143(2):240-8.

Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley1981.

Fratello F, Veniero D, Curcio G, Ferrara M, Marzano C, Moroni F, et al. Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. Clin Neurophysiol. 2006 Dec;117(12):2667-74.

Fregni F, Thome-Souza S, Nitsche MA, Freedman SD, Valente KD, Pascual-Leone A. A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy. Epilepsia. 2006 Feb;47(2):335-42.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, et al. Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron. 2010 Apr 29;66(2):198-204.

Galea JM, Vazquez A, Pasricha N, Orban de Xivry J-J, Celnik P. Dissociating the Roles of the Cerebellum and Motor Cortex during Adaptive Learning: The Motor Cortex Retains What the Cerebellum Learns. Cerebral Cortex (New York, NY: 1991). 2011;21(8):1761-70.

Gandiga PC, Hummel FC, Cohen LG. Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology. 2006;117(4):845-50.

Gangitano M, Valero-Cabre A, Tormos JM, Mottaghy FM, Romero JR, Pascual-Leone A. Modulation of input-output curves by low and high frequency repetitive transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol. 2002 Aug;113(8):1249-57.

Gauntlett-Gilbert J, Brown VJ. Reaction time deficits and Parkinson's disease. Neurosci Biobehav Rev. 1998 Oct;22(6):865-81.

Gilio F, Rizzo V, Siebner HR, Rothwell JC. Effects on the right motor hand-area excitability produced by low-frequency rTMS over human contralateral homologous cortex. J Physiol. 2003 Sep 1;551(Pt 2):563-73.

Gualtierotti T, Paterson AS. Electric stimulation of the unexpected cerebral cortex. J Physiol. 1954;125:278-91.

Hadipour-Niktarash A, Lee CK, Desmond JE, Shadmehr R. Impairment of retention but not acquisition of a visuomotor skill through time-dependent disruption of primary motor cortex. J Neurosci. 2007 Dec 5;27(49):13413-9.

Hallett M. Transcranial magnetic stimulation: a tool for mapping the central nervous system. Electroencephalogr Clin Neurophysiol Suppl. 1996;46:43-51.

Hallett M. Transcranial magnetic stimulation: a useful tool for clinical neurophysiology. Ann Neurol. 1996 Sep;40(3):344-5.

Hallett M. Transcranial magnetic stimulation and the human brain. Nature. 2000 Jul 13;406(6792):147-50.

Hallett M. Transcranial magnetic stimulation: a primer. Neuron. 2007 Jul 19;55(2):187-99.

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The Role of Interneuron Networks in Driving Human Motor Cortical Plasticity. Cereb Cortex. 2013 Jun 1;23:1593-605.

Hashimoto T, Inaba D, Matsumura M, Naito E. Two different effects of transcranial magnetic stimulation to the human motor cortex during the pre-movement period. Neurosci Res. 2004 Dec;50(4):427-36.

Hebb D. The Organization of Behavior. New York: Wiley; 1949.

Hess G, Aizenman CD, Donoghue JP. Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. J Neurophysiol. 1996 May;75(5):1765-78.

Hinder MR, Goss EL, Fujiyama H, Canty AJ, Garry MI, Rodger J, et al. Inter- and Intra-individual Variability Following Intermittent Theta Burst

Stimulation: Implications for Rehabilitation and Recovery. Brain Stimul. 2014 Jan 9.

Hoogendam JM, Ramakers GM, Di Lazzaro V. Physiology of repetitive transcranial magnetic stimulation of the human brain. Brain Stimul. 2010 Apr;3(2):95-118.

Horvath JC, Carter O, Forte JD. Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). Frontiers in systems neuroscience. 2014;8:2.

Horvath JC, Forte JD, Carter O. Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. Neuropsychologia. 2015;66:213-36.

Huang VS, Haith A, Mazzoni P, Krakauer JW. Rethinking motor learning and savings in adaptation paradigms: model-free memory for successful actions combines with internal models. Neuron. 2011 May 26;70(4):787-801.

Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol. 2007 May;118(5):1028-32.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron. 2005 Jan 20;45(2):201-6.

Huang YZ, Rothwell JC, Edwards MJ, Chen RS. Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. Cereb Cortex. 2008 Mar;18(3):563-70.

Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol. 2006 Aug;5(8):708-12.

Iezzi E, Suppa A, Conte A, Agostino R, Nardella A, Berardelli A. Theta-burst stimulation over primary motor cortex degrades early motor learning. Eur J Neurosci. 2010 Feb;31(3):585-92.

Inghilleri M, Conte A, Curra A, Frasca V, Lorenzano C, Berardelli A. Ovarian hormones and cortical excitability. An rTMS study in humans. Clin Neurophysiol. 2004 May;115(5):1063-8.

Iversen SD, Iversen LL. Dopamine: 50 years in perspective. Trends in neurosciences. 2007 May;30(5):188-93.

Joundi RA, Lopez-Alonso V, Lago A, Brittain JS, Fernandez-Del-Olmo M, Gomez-Garre P, et al. The effect of BDNF val66met polymorphism on visuomotor adaptation. Exp Brain Res. 2012 Sep 1;223(1):43-50.

Jung P, Ziemann U. Homeostatic and nonhomeostatic modulation of learning in human motor cortex. J Neurosci. 2009 Apr 29;29(17):5597-604.

Kandel ER, Schwartz JH. Molecular biology of learning: modulation of transmitter release. Science. 1982 Oct 29;218(4571):433-43.

Kandel ER, Schwartz JH, Jessell TH. Principles of neural science. Fourth Edition ed. New York: McGraw-Hill; 2000.

Kantak SS, Mummidisetty CK, Stinear JW. Primary motor and premotor cortex in implicit sequence learning - evidence for competition between implicit and explicit human motor memory systems. Eur J Neurosci. 2012 Jul 4.

Karni A, Meyer G, Rey-Hipolito C, Jezzard P, Adams MM, Turner R, et al. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proceedings of the National Academy of Sciences of the United States of America. 1998;95(3):861-8.

Khaleel SH, Bayoumy IM, El-Nabil LM, Moustafa RR. Differential hemodynamic response to repetitive transcranial magnetic stimulation in acute stroke patients with cortical versus subcortical infarcts. European neurology. 2010;63(6):337-42.

Kidgell DJ, Daly RM, Young K, Lum J, Tooley G, Jaberzadeh S, et al. Different current intensities of anodal transcranial direct current stimulation do not differentially modulate motor cortex plasticity. Neural plasticity. 2013;2013:Epub 2013 Mar 18.

Kiers L, Cros D, Chiappa KH, Fang J. Variability of motor potentials evoked by transcranial magnetic stimulation. Electroencephalogr Clin Neurophysiol. 1993 Dec;89(6):415-23.

Kobayashi M, Hutchinson S, Theoret H, Schlaug G, Pascual-Leone A. Repetitive TMS of the motor cortex improves ipsilateral sequential simple finger movements. Neurology. 2004 Jan 13;62(1):91-8.

Krakauer JW, Ghez C, Ghilardi MF. Adaptation to visuomotor transformations: consolidation, interference, and forgetting. J Neurosci. 2005 Jan 12;25(2):473-8.

Krakauer JW, Ghilardi MF, Ghez C. Independent learning of internal models for kinematic and dynamic control of reaching. Nat Neurosci. 1999 Nov;2(11):1026-31.

Krakauer JW, Mazzoni P. Human sensorimotor learning: adaptation, skill, and beyond. Curr Opin Neurobiol. 2011 Aug;21(4):636-44.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol. 1993 Nov;471:501-19.

Lahey M, Downey RG, Saal FE. Intraclass correlations: There's more there than meets the eye. Phychological Bulletin. 1983;93:586-95.

Lefaucheur JP, Antal A, Ahdab R, Ciampi de Andrade D, Fregni F, Khedr EM, et al. The use of repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) to relieve pain. Brain Stimul. 2008 Oct;1(4):337-44.

Levy WB, Steward O. Synapses as associative memory elements in the hippocampal formation. Brain Res. 1979 Oct 19;175(2):233-45.

Levy WB, Steward O. Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. Neuroscience. 1983 Apr;8(4):791-7.

Li Voti P, Conte A, Suppa A, Iezzi E, Bologna M, Aniello MS, et al. Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. Experimental Brain Research. 2011;212:91-9.

Liew SL, Santarnecchi E, Buch ER, Cohen LG. Non-invasive brain stimulation in neurorehabilitation: local and distant effects for motor recovery. Frontiers in human neuroscience. 2014;8:378.

Lopez-Alonso V, Cheeran B, Rio-Rodriguez D, Fernandez-Del-Olmo M. Inter-individual Variability in Response to Non-invasive Brain Stimulation Paradigms. Brain Stimul. 2014 Feb 15;7(3):372-80.

Lopez-Alonso V, Fernandez-Del-Olmo M, Costantini A, Gonzalez-Henriquez J, Cheeran B. Intra-individual variability in the response to anodal Transcranial Direct Current Stimulation. Clinical Neurophysiology. 2015;Under review.

Lynch G, Baudry M. The biochemistry of memory: a new and specific hypothesis. Science. 1984 Jun 8;224(4653):1057-63.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. Exp Brain Res. 2000 Aug;133(4):425-30.

Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004 Sep 30;44(1):5-21.

Martin SJ, Grimwood PD, Morris RG. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci. 2000;23:649-711.

McAllister SM, Rothwell JC, Ridding MC. Selective modulation of intracortical inhibition by low-intensity Theta Burst Stimulation. Clin Neurophysiol. 2009 Apr;120(4):820-6.

McEachern JC, Shaw CA. An alternative to the LTP orthodoxy: a plasticity-pathology continuum model. Brain Res Brain Res Rev. 1996 Jun;22(1):51-92.

McKinley RA, Bridges N, Walters CM, Nelson J. Modulating the brain at work using noninvasive transcranial stimulation. Neuroimage. 2012 Jan 2;59(1):129-37.

McNaughton BL, Morris RGM. Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends in neurosciences. 1987;10(10):408-15.

Meesen RL, Thijs H, Leenus DJ, Cuypers K. A single session of 1 mA anodal tDCS-supported motor training does not improve motor performance in patients with multiple sclerosis. Restor Neurol Neurosci. 2014;32(2):293-300.

Merton PA, Morton HB. Stimulation of the cerebral cortex in the intact human subject. Nature. 1980 May 22;285(5762):227.

Monte-Silva K, Ruge D, Teo JT, Paulus W, Rothwell JC, Nitsche MA. D2 receptor block abolishes theta burst stimulation-induced neuroplasticity in the human motor cortex. Neuropsychopharmacology. 2011 Sep;36(10):2097-102.

Moser EI, Krobert KA, Moser MB, Morris RG. Impaired spatial learning after saturation of long-term potentiation. Science. 1998 Sep 25;281(5385):2038-42.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. Exp Brain Res. 2001 Feb;136(4):431-8.

Muellbacher W, Ziemann U, Boroojerdi B, Hallett M. Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. Clin Neurophysiol. 2000 Jun;111(6):1002-7.

Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res. 2008 May;187(3):467-75.

Muslimovic D, Post B, Speelman JD, Schmand B. Motor procedural learning in Parkinson's disease. Brain. 2007 Nov;130(Pt 11):2887-97.

Nathan PJ, Cobb SR, Lu B, Bullmore ET, Davies CH. Studying synaptic plasticity in the human brain and opportunities for drug discovery. Current opinion in pharmacology. 2011 Oct;11(5):540-8.

Nissen MJ, Bullemer P. Attentional requirements of learning: Evidence from performance measures. Cognitive Psychology. 1987;19(1):1-32.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: State of the art 2008. Brain Stimul. 2008 Jul;1(3):206-23.

Nitsche MA, Jaussi W, Liebetanz D, Lang N, Tergau F, Paulus W. Consolidation of human motor cortical neuroplasticity by D-cycloserine. Neuropsychopharmacology. 2004 Aug;29(8):1573-8.

Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC, Paulus W. Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin Neurophysiol. 2003 Apr;114(4):600-4.

Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000 Sep 15;527 Pt 3:633-9.

Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001 Nov 27;57(10):1899-901.

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, et al. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. J Cogn Neurosci. 2003 May 15;15(4):619-26.

O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH. Non-invasive brain stimulation techniques for chronic pain. The Cochrane database of systematic reviews. 2014;4.

Orth M, Snijders AH, Rothwell JC. The variability of intracortical inhibition and facilitation. Clin Neurophysiol. 2003 Dec;114(12):2362-9.

Pascual-Leone A, Brasil-Neto JP, Valls-Sole J, Cohen LG, Hallett M. Simple reaction time to focal transcranial magnetic stimulation. Comparison with reaction time to acoustic, visual and somatosensory stimuli. Brain. 1992 Feb;115 Pt 1:109-22.

Pascual-Leone A, Tarazona F, Keenan J, Tormos JM, Hamilton R, Catala MD. Transcranial magnetic stimulation and neuroplasticity. Neuropsychologia. 1999 Feb;37(2):207-17.

Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain. 1994 Aug;117 (Pt 4):847-58.

Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. Storage of spatial information by the maintenance mechanism of LTP. Science. 2006 Aug 25;313(5790):1141-4.

Paulus W, Antal A, Nitsche M. Physiological Basis and Methodological Aspects of Transcranial Electric Stimulation (tDCS, tACS, and tRNS). In: Miniussi C, Paulus W, Rossini M, editors. Transcranial Brain Stimulation: CRC Press; 2012.

Penhune VB, Steele CJ. Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. Behav Brain Res. 2012 Jan 15;226(2):579-91.

Player MJ, Taylor JL, Alonzo A, Loo CK. Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. Clin Neurophysiol. 2012 May 17;123(11):2220-6.

Player MJ, Taylor JL, Weickert CS, Alonzo A, Sachdev P, Martin D, et al. Neuroplasticity in Depressed Individuals Compared with Healthy Controls. Neuropsychopharmacology. 2013 May 16.

Polanowska KE, Lesniak M, Seniow JB, Czlonkowska A. No effects of anodal transcranial direct stimulation on language abilities in early rehabilitation of post-stroke aphasic patients. Neurol Neurochir Pol. 2013 Sep-Oct;47(5):414-22.

Priori A, Berardelli A, Rona S, Accornero N, Manfredi M. Polarization of the human motor cortex through the scalp. Neuroreport. 1998 Jul 13;9(10):2257-60.

Pullman SL, Watts RL, Juncos JL, Chase TN, Sanes JN. Dopaminergic effects on simple and choice reaction time performance in Parkinson's disease. Neurology. 1988 Feb;38(2):249-54.

Quartarone A, Bagnato S, Rizzo V, Morgante F, Sant'angelo A, Battaglia F, et al. Distinct changes in cortical and spinal excitability following high-frequency repetitive TMS to the human motor cortex. Exp Brain Res. 2005 Feb;161(1):114-24.

Rajji TK, Liu SK, Frantseva MV, Mulsant BH, Thoma J, Chen R, et al. Exploring the effect of inducing long-term potentiation in the human motor cortex on motor learning. Brain Stimul. 2011 Jul;4(3):137-44.

Reis J, Fritsch B. Modulation of motor performance and motor learning by transcranial direct current stimulation. Current opinion in neurology. 2011 Dec;24(6):590-6.

Reis J, Robertson E, Krakauer JW, Rothwell J, Marshall L, Gerloff C, et al. Consensus: "Can tDCS and TMS enhance motor learning and memory formation?". Brain Stimul. 2008 Oct;1(4):363-9.

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, et al. Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(5):1590-5.

Reis J, Swayne OB, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, et al. Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. J Physiol. 2008 Jan 15;586(2):325-51.

Ridding MC, Ziemann U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. J Physiol. 2010 Jul 1;588(Pt 13):2291-304.

Riek S, Hinder MR, Carson RG. Primary motor cortex involvement in initial learning during visuomotor adaptation. Neuropsychologia. 2012 Aug;50(10):2515-23.

Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. Science. 2000 Oct 20;290(5491):533-6.

Robertson EM, Press DZ, Pascual-Leone A. Off-line learning and the primary motor cortex. J Neurosci. 2005 Jul 6;25(27):6372-8.

Roshan L, Paradiso GO, Chen R. Two phases of short-interval intracortical inhibition. Exp Brain Res. 2003 Aug;151(3):330-7.

Rosler KM. Transcranial magnetic brain stimulation: a tool to investigate central motor pathways. News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society. 2001 Dec;16:297-302.

Rosler KM, Magistris MR. The Size of Motor-evoked Potentials: Influencing Parameters and Quantification. In: Wassermann E, Epstein CM, Ziemann U, editors. The Oxford handbook of transcranial stimulation. New York: Oxford University Press; 2008.

Rothwell JC. Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. Journal of Neuroscience Methods. 1997;74(2):113-22.

Rothwell JC, Thompson PD, Day BL, Boyd S, Marsden CD. Stimulation of the human motor cortex through the scalp. Experimental Physiology. 1991;76(2):159-200.

Sale MV, Ridding MC, Nordstrom MA. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res. 2007 Aug;181(4):615-26.

Sanes JN, Donoghue JP. Plasticity and primary motor cortex. Annu Rev Neurosci. 2000;23:393-415.

Sarkar A, Dowker A, Cohen Kadosh R. Cognitive Enhancement or Cognitive Cost: Trait-Specific Outcomes of Brain Stimulation in the Case of Mathematics Anxiety. The Journal of Neuroscience. 2014;34:16605-10.

Schmidt RA, Lee TD. Motor control and learning: a behavioral emphasis. Fifth Edition ed: Human Kinetics; 2011.

Seber AS, Allen R, Reber PJ. Implicit versus explicit learning. In: Sternberg RJ, editor. The nature of cognition: MIT Press; 1999.

Shors TJ, Matzel LD. Long-term potentiation: what's learning got to do with it? The Behavioral and brain sciences. 1997 Dec;20(4):597-614; discussion -55.

Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychological bulletin. 1979 Mar;86(2):420-8.

Siebner HR, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res. 2003 Jan;148(1):1-16.

Smyth C, Summers JJ, Garry MI. Differences in motor learning success are associated with differences in M1 excitability. Human movement science. 2010 Oct;29(5):618-30.

Sommer M, Wu T, Tergau F, Paulus W. Intra- and interindividual variability of motor responses to repetitive transcranial magnetic stimulation. Clin Neurophysiol. 2002 Feb;113(2):265-9.

Stagg CJ, Best JG, Stephenson MC, O'Shea J, Wylezinska M, Kincses ZT, et al. Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. J Neurosci. 2009 Apr 22;29(16):5202-6.

Stagg CJ, Nitsche MA. Physiological basis of transcranial direct current stimulation. Neuroscientist. 2011 Feb;17(1):37-53.

Stanton PK, Sejnowski TJ. Associative long-term depression in the hippocampus induced by hebbian covariance. Nature. 1989 May 18;339(6221):215-8.

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol. 2002 Sep 1;543(Pt 2):699-708.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain. 2000 Mar;123 Pt 3:572-84.

Stefan K, Wycislo M, Gentner R, Schramm A, Naumann M, Reiners K, et al. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb Cortex. 2006 Mar;16(3):376-85.

Talelli P, Cheeran BJ, Teo JT, Rothwell JC. Pattern-specific role of the current orientation used to deliver Theta Burst Stimulation. Clin Neurophysiol. 2007 Aug;118(8):1815-23.

Teo JT, Swayne OB, Cheeran B, Greenwood RJ, Rothwell JC. Human theta burst stimulation enhances subsequent motor learning and increases performance variability. Cereb Cortex. 2011 Jul;21(7):1627-38.

Terao Y, Ugawa Y. Basic mechanisms of TMS. J Clin Neurophysiol. 2002 Aug;19(4):322-43.

Thomson RH, Maller JJ, Daskalakis ZJ, Fitzgerald PB. Blood oxygenation changes resulting from trains of low frequency transcranial magnetic stimulation. Cortex. 2012 Apr;48(4):487-91.

Ungerleider LG, Doyon J, Karni A. Imaging brain plasticity during motor skill learning. Neurobiol Learn Mem. 2002 Nov;78(3):553-64.

Vallence AM, Kurylowicz L, Ridding MC. A comparison of neuroplastic responses to non-invasive brain stimulation protocols and motor learning in healthy adults. Neurosci Lett. 2013 Jun 10;549:151-6.

van der Kamp W, Zwinderman AH, Ferrari MD, van Dijk JG. Cortical excitability and response variability of transcranial magnetic stimulation. J Clin Neurophysiol. 1996 Mar;13(2):164-71.

Vernet M, Bashir S, Yoo WK, Oberman L, Mizrahi I, Ifert-Miller F, et al. Reproducibility of the effects of theta burst stimulation on motor cortical plasticity in healthy participants. Clin Neurophysiol. 2014 Aug 7;125(2):320-6.

Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol. 1998 Jan;108(1):1-16.

Wiethoff S, Hamada M, Rothwell JC. Variability in Response to Transcranial Direct Current Stimulation of the Motor Cortex. Brain Stimul. 2014 Feb 15.

Wilkinson L, Teo JT, Obeso I, Rothwell JC, Jahanshahi M. The contribution of primary motor cortex is essential for probabilistic implicit sequence learning: evidence from theta burst magnetic stimulation. J Cogn Neurosci. 2010 Mar;22(3):427-36.

Witte AV, Kurten J, Jansen S, Schirmacher A, Brand E, Sommer J, et al. Interaction of BDNF and COMT Polymorphisms on Paired-Associative Stimulation-Induced Cortical Plasticity. J Neurosci. 2012 Mar 28;32(13):4553-61.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, et al. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol. 2003 May;89(5):2339-45.

Wrigley PJ, Gustin SM, McIndoe LN, Chakiath RJ, Henderson LA, Siddall PJ. Longstanding neuropathic pain after spinal cord injury is refractory to transcranial direct current stimulation: a randomized controlled trial. Pain. 2013 Oct;154(10):2178-84.

Ziemann U, Ilic TV, Alle H, Meintzschel F. Cortico-motoneuronal excitation of three hand muscles determined by a novel penta-stimulation technique. Brain. 2004 Aug;127(Pt 8):1887-98.

Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J Neurosci. 2004 Feb 18;24(7):1666-72.

Ziemann U, Paulus W, Nitsche MA, Pascual-Leone A, Byblow WD, Berardelli A, et al. Consensus: Motor cortex plasticity protocols. Brain Stimul. 2008 Jul;1(3):164-82.

Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and Drugs revisited 2014. Clin Neurophysiol. 2014;In press, accepted manuscript.

Ziemann U, Rothwell J, Ridding M. Interaction between intracortical inhibition and facilitation in human motor cortex. The Journal of Physiology. 1996;196(Pt 3):873-81.

Ziemann U, Rothwell JC. I-waves in motor cortex. J Clin Neurophysiol. 2000 Jul;17(4):397-405.

Zimerman M, Heise KF, Hoppe J, Cohen LG, Gerloff C, Hummel FC. Modulation of training by single-session transcranial direct current stimulation to the intact motor cortex enhances motor skill acquisition of the paretic hand. Stroke. 2012 Aug;43(8):2185-91.

Chapter 8

Appendix

8. APPENDIX

8.1. Resumen

Históricamente se pensaba que el papel de las sinapsis se limitaba a la transferencia de información de una neurona a otra, o de una neurona a un músculo. Además, se creía que estas conexiones eran relativamente fijas. Sin embargo, gracias a trabajos pioneros como los de Santiago Ramón y Cajal o Donald Hebb, estas ideas han ido evolucionando hasta llegar a aceptarse del concepto de plasticidad.

En general, la plasticidad se define como la capacidad del sistema nervioso para producir modificaciones en su estructura o función. Estas modificaciones se dan a lo largo de la vida del individuo y tendrían lugar en varios niveles de la organización cerebral, desde el nivel ultraestructural hasta el nivel sináptico.

En el año 1973 se publicó el primer trabajo en el que se describía detalladamente la potenciación a largo plazo (LTP) en conejos. Esta potenciación a largo plazo se refiere al incremento en la eficiencia de la transmisión sináptica que perdura en el tiempo. Dicha potenciación se logró por medio de la aplicación de breves trenes de estimulación eléctrica a alta frecuencia sobre las vías excitatorias monosinápticas en el hipocampo. Si esta estimulación es aplicada a baja frecuencia se obtiene el fenómeno inverso, la depresión a largo plazo (LTD) o disminución en la eficiencia de la transmisión sináptica que se mantiene en el tiempo.

En los años 1950 se realizaron varios intentos de estimular la corteza cerebral humana por medio de estimulación eléctrica aplicada en el cuero cabelludo, sin embargo, esta estimulación era muy dolorosa e ineficiente. Fue 30 años después se logró la primera aplicación clínica de la estimulación eléctrica transcraneal (TES), aunque el dolor asociado a la estimulación continuaba siendo una limitación para su aplicación. Cinco años más tarde se demostró que era posible estimular el cerebro, así como nervios periféricos, con estimulación indolora, gracias a la estimulación magnética transcraneal (TMS).

La estimulación magnética transcraneal mostró tener diversas aplicaciones:

- Demostrar cambios plásticos.
- Tratar de explicar los mecanismos subyacentes a la plasticidad.
- Modular la plasticidad (aumentando o disminuyendo la excitabilidad cortical), pudiendo influir en las consecuencias del comportamiento.

La medida (o *output*) más utilizada de la TMS es el potencial motor evocado (MEP). En este caso, el MEP es la señal electromiográfica que llega al músculo tras ser estimulada con TMS la correspondiente área motora.

La TMS puede ser aplicada con pulsos simples, pareados o de manera repetitiva. Los pulsos simples se utilizan fundamentalmente para conocer el estado de la excitabilidad cortico-espinal. Los pulsos pareados sirven para estudiar la excitabilidad intra-cortical, así como el nivel de actividad de diferentes conexiones cortico-corticales y sistemas de neurotransmisión. Dos de las medidas más comunes obtenidas por estimulación pareada son la inhibición intra-cortical corta (SICI) y la facilitación intra-cortical (ICF). La estimulación magnética repetitiva se utiliza para inducir cambios (aumento o disminución de la excitabilidad cortical) que perduran más allá del periodo de estimulación. La duración de sus efectos depende del protocolo utilizado.

Una de las técnicas de estimulación magnética transcraneal repetitiva más utilizada para producir modificaciones en la excitabilidad cortical es la estimulación transcraneal por ráfagas theta (TBS). Esta técnica se basa en reproducir el ritmo theta natural del hipocampo durante el aprendizaje.

Además de la TMS repetitiva, existen otros protocolos de estimulación capaces de producir cambios de relativa duración en la excitabilidad cortical. Unos de estos protocolos es la estimulación pareada asociativa (PAS), en la que una estimulación eléctrica periférica es pareada con estimulación magnética sobre la corteza motora contralateral. Esta técnica se basa en la teoría Hebbiana que propone que la “fuerza” de una conexión sináptica se ve incrementada si las neuronas de ambos lados de la sinapsis se activan de forma simultánea repetidamente.

Por otro lado, en las últimas décadas se ha incrementado el uso de la estimulación transcraneal por corriente directa (tDCS), la cual permite también la modulación de la excitabilidad sináptica, por medio de débiles corrientes

liberadas a través de dos pequeños electrodos situados en el cuero cabelludo. Dependiendo de si se aplica anodal o catodalmente, incrementará o disminuirá la excitabilidad cortical. Los estudios científicos sugieren que la tDCS actúa modificando el potencial de la membrana de reposo.

Todas estas técnicas, al inducir modificaciones duraderas en la excitabilidad cortical, han sido utilizadas también para influir sobre el aprendizaje motor, ya que éste requiere de modificaciones sinápticas para producirse. Sin embargo, hasta el momento, no está demostrada totalmente la relación entre plasticidad cortical y aprendizaje motor.

Por lo tanto, pese a la abundante investigación en torno a la estimulación magnética transcraneal, a la plasticidad cortical y al aprendizaje motor, existen todavía varias cuestiones sin resolver. Con este trabajo se ha pretendido responder a las siguientes:

1) ¿Responden todos los sujetos de la misma manera a cada uno de los protocolos de estimulación cerebral no invasiva?

2) ¿Tienen todas las técnicas de estimulación cerebral no invasiva la misma eficacia?

3) ¿Existe algún predictor para la respuesta a la estimulación cerebral no invasiva?

4) ¿Responden los sujetos de la misma manera al mismo protocolo en diferentes sesiones?

5) ¿Existe relación entre la plasticidad cortical inducida por la estimulación cerebral no invasiva y la capacidad de aprendizaje motor?

Para responder a estas preguntas se han llevado a cabo tres estudios.

Estudio 1: Variabilidad inter-individual en la respuesta a los paradigmas de estimulación cerebral no invasiva.

A pesar de que son numerosos los estudios que han demostrado el efecto de las diferentes técnicas de estimulación cerebral no invasiva sobre la excitabilidad cortical, un número importante de investigaciones han reportado resultados contradictorios, sugiriendo que no todos los sujetos responden como es esperado a la estimulación.

En este primer estudio de la tesis se pretendió abordar la cuestión de la variabilidad inter-individual en la respuesta a la estimulación cerebral no invasiva, en concreto, a los tres protocolos de facilitación más utilizados en la actualidad, la estimulación pareada asociativa (PAS), la estimulación transcraneal por corriente directa (tDCS) y la estimulación intermitente por ráfagas theta (iTBS). Un segundo objetivo del estudio fue establecer posibles predictores a dicha respuesta. Por último, se testó si todos los protocolos eran igual de eficaces.

56 sujetos fueron sometidos a los tres protocolos de facilitación más utilizados (tDCS, PAS y iTBS). Los participantes eran sujetos sanos jóvenes, de entre 19 y 24 años, 50 hombres y 6 mujeres. Cada sujeto participó en tres sesiones separadas, al menos, una semana para evitar efectos acumulativos de la estimulación. Las tres sesiones para cada sujeto fueron realizadas aproximadamente a la misma hora del día y el orden de las mismas fue contrabalanceado. Todas ellas siguieron exactamente la misma metodología, con la única diferencia del protocolo de estimulación utilizado (PAS, tDCS o iTBS). Cada sesión comenzó con la colocación de los electrodos en el músculo primer dorsal interóseo de la mano derecha. A continuación, se localizó el *hotspot* (lugar de la corteza sobre la que, aplicando un pulso de TMS supra-umbral, se produce la mayor respuesta) del área motora izquierda, que fue marcada para asegurarse la colocación de la bobina en el mismo lugar a lo largo de toda la sesión. Se realizó una primera medición (*baseline*) de la excitabilidad cortical, evaluando tanto la amplitud del potencial motor evocado (MEP), como la inhibición intracortical (SICI). A continuación, se aplicó el

correspondiente protocolo de estimulación (con los parámetros descritos en la tabla A1). Tras la estimulación se evaluó la amplitud del MEP cada 5 minutos hasta el minuto 60, a la misma intensidad que en el *baseline*. Además, en el minuto 6 y 26 tras la estimulación, se evaluó el SICI, de la misma manera que en el *baseline*.

Tabla A1.- Parámetros de estimulación de los protocolos de estimulación cerebral no invasiva utilizados.

	PAS	**tDCS**	**iTBS**
Intensidad	TMS – SI_{1mV} ES – 300% PT	1 mA	80% AMT
Duración	~13 min	13 min	3 min
Otros parámetros	ES – nervio cubital ISI – 25 ms Frecuencia – 0,25 Hz	Electrodos – 35 cm^2 Electrodo referencia – supraorbital Polaridad – anodal	

SI_{1mV} intensidad de estimulación que provoca un potencial motor avocado de 1mV, ***ES*** estímulo eléctrico, ***PT*** umbral perceptivo, ***AMT*** umbral motor activo, ***ISI*** intervalo entre estímulos.

El ANOVA de medidas repetidas para los valores absolutos de la amplitud del MEP medidos en los 13 momentos tras la estimulación (0, 5, 10,…, 60), reveló que no había diferencias significativas entre el *baseline* y ninguno de los momentos medidos. Indicando que ninguno de los protocolos de estimulación producía una modificación significativa en la excitabilidad cortical en el total de la muestra. Tampoco se encontraron modificaciones en el SICI.

El análisis de clusters reveló que existen dos patrones de respuesta a cada uno de los protocolos evaluados. Un grupo de sujetos respondió como es esperado a la estimulación (incrementando su excitabilidad cortical tras la estimulación), mientras que el otro grupo no mostró esta respuesta. Todos los protocolos eran similares en cuanto al número de sujetos "respondedores", así como a la intensidad del incremento producido en la excitabilidad cortical de este grupo. Además, los resultados mostraron que el que un sujeto sea

"respondedor" a un protocolo no es indicativo de que vaya a responder a los dos protocolos restantes.

En cuanto a los predictores testados (momento del día, edad, intensidad de estimulación/umbral motor en reposo, SICI pre- estimulación, umbral perceptivo y umbral motor activo), solamente el SICI pre-estimulación parece predecir parcialmente (~10%) la respuesta al PAS. Sujetos con menor SICI antes de la estimulación son los que muestran un mayor aumento de la excitabilidad cortical tras la estimulación.

Estudio 2: Variabilidad intra-individual en la respuesta a la estimulación transcraneal anodal por corriente directa.

Sabiendo que existe una gran variabilidad en respuesta a los diferentes protocolos de estimulación, la siguiente cuestión que nos ocupó fue conocer la variabilidad intra-individual en la respuesta a uno de los protocolos. En otras palabras, establecer si una persona que responde positivamente en una sesión de estimulación, responderá de la misma manera en sesiones sucesivas con el mismo protocolo. Este estudio se centró en la tDCS dado a su amplio uso en el ámbito clínico, además de facilidad para inducir estimulación *sham* (placebo) con eficacia y de la posibilidad de poder ser aplicada simultáneamente con diferentes tipos de tareas (motoras, cognitivas…).

Para este estudio se reclutaron 45 sujetos sanos, jóvenes (entre 19 y 24 años), de los cuales 39 eran hombres. Todos ellos participaron en dos sesiones completamente iguales separadas, al menos, una semana para evitar efectos acumulativos de la estimulación.

La metodología utilizada en cada una de las sesiones fue la misma que en la sesión de estimulación con tDCS del primer estudio de la tesis. Tras localizar el área motora izquierda correspondiente al primer dorsal interóseo de la mano derecha, se registró un primer bloque *baseline* en el que se evaluaron tanto la amplitud del MEP como el SICI. A continuación, se aplicó la tDCS con los parámetros descritos en la tabla A1. Una vez finalizada la estimulación, se

registró la amplitud del MEP cada 5 minutos hasta el minuto 60. También se evaluó el SICI tanto en el minuto 6 como en el 46 tras la estimulación.

Los resultados de este estudio no han mostrado cambios en la excitabilidad cortical al evaluar el efecto de la tDCS en toda la muestra durante la hora completa tras la estimulación (al igual que había ocurrido en el estudio 1). Sin embargo, testando solamente la primera media hora post-estimulación se observa un aumento en la excitabilidad de M1.

Respecto a la fiabilidad intra-sujeto, se ha visto que es moderada durante este periodo de tiempo (media hora tras la estimulación). Casi el 70% de los sujetos testados respondió de la misma manera en ambas sesiones.

La variabilidad intra-sujeto en respuesta a la tDCS es menor que la variabilidad inter-sujeto.

Estudio 3: Relación entre la plasticidad inducida por estimulación cerebral no invasiva y la capacidad de aprendizaje motor.

En el tercer y último estudio del presente trabajo quisimos conocer la relación entre la plasticidad inducida en el área motora primaria (M1) por diferentes protocolos de estimulación cerebral no invasiva y la capacidad de aprendizaje motor. Hasta el momento han sido muchos los estudios que han utilizado la estimulación cerebral no invasiva tanto para mejorar los programas de rehabilitación en poblaciones de pacientes, como para potenciar el aprendizaje en sujetos sanos. Se ha demostrado que la estimulación previa o simultánea a diferentes tareas, incrementa la eficacia de las mismas. Sin embargo, poco se sabe en cuanto a la relación entre los cambios producidos en la excitabilidad cortical y la capacidad de aprendizaje de los sujetos, testados en sesiones diferentes. Es decir, ¿aquellos sujetos que son más susceptibles de modificar su excitabilidad cortical por medio de protocolos de estimulación, son aquéllos con una mayor capacidad de aprendizaje motor? La literatura existente que propone la plasticidad como clave para el aprendizaje, sugiere que éste es el caso. En este último estudio hemos querido testar la hipótesis de que aquellos sujetos con mayor excitabilidad cortical inducida son los que muestran una mayor capacidad de aprendizaje motor.

Debido a que existen diferentes protocolos de estimulación y multitud de tareas de aprendizaje, hemos decidido elegir aquellos protocolos de facilitación más utilizados y tres tareas de aprendizaje que testan tres tipos diferentes de aprendizaje motor.

En este tercer estudio han participado los 56 sujetos del estudio 1, dado que ya se disponía de sus datos de los tres protocolos de estimulación (tDCS, PAS y iTBS). El estudio consistió en la realización de tres sesiones correspondientes a tres tareas de aprendizaje motor. Las tres sesiones se realizaron de manera contrabalanceada y separadas, al menos, una semana para evitar interferencias entre los aprendizajes.

Una de las tareas de aprendizaje utilizadas es la tarea de tiempo de reacción seriada (SRTT), que permite evaluar el aprendizaje implícito (aquél que tiene lugar sin el sujeto ser consciente de que lo está adquiriendo). En ella, se situaba al sujeto delante de una pantalla de ordenador, con un teclado en el que estaban coloreadas las teclas necesarias para la tarea (letras “j”, “k”, “l” y “ñ”, a partir de ahora “1”, “2”, “3” y “4”, respectivamente). Los sujetos debían pulsar cada tecla con un dedo diferente de la mano derecha (índice, corazón, anular y meñique, para “1”, “2”, “3” y “4”, respectivamente). Un asterisco aparecía en una de cuatro posiciones, situadas horizontalmente y marcadas permanentemente en la pantalla con un cuadrado negro sobre un fondo blanco. Cada uno de los cuadrados correspondía a una tecla. La configuración espacial de las teclas era totalmente compatible con la posición de los cuadrados en la pantalla. Los sujetos debían responder lo más rápidamente posible, y con la mayor precisión, a la aparición de cada asterisco. En el experimento se intercalaban bloques en los que la aparición del asterisco era totalmente aleatoria, con bloques en los cuales la aparición de los asteriscos seguía siempre la misma secuencia de 12 estímulos (sin que el sujeto fuese informado). Con ello, comparando el tiempo de reacción entre los bloques aleatorios y de secuencia, se obtiene una medida del aprendizaje implícito.

Otra de las tareas de aprendizaje es una tarea de adaptación visuomotora (VAT). En ella, los sujetos manejaban un joystick con su mano derecha. Una cubierta opaca impedía a los sujetos ver el joystick y su mano. El joystick

controlaba un cursor verde en la pantalla. El objetivo de la tarea era seguir un cursor rojo, inicialmente presentado en el centro de la pantalla y que saltaba rápidamente a una de ocho posiciones localizadas equidistalmente en el perímetro de un círculo visible permanentemente. La tarea comenzaba con un primer bloque en el que el movimiento del joystick se correspondía con el movimiento del cursor verde en la pantalla. Tras un breve descanso, se realizaba un segundo bloque en el que la relación entre el movimiento del joystick con respecto al movimiento del cursor verde en la pantalla se veía alterado. El cursor verde se movía con una rotación de ~60º respecto al joystick. Durante este bloque, los grandes errores iniciales iban disminuyendo a medida que avanzaban los intentos, ya que se iba produciendo una adaptación. Se registraron un tercer bloque a los 45 minutos tras el segundo para testar la consolidación, y un cuarto bloque a las 24 horas para testar la retención. Ambos bloques fueron iguales al segundo (con ~60º de desviación). Por último, se testó un quinto bloque, sin desviación de ningún tipo para testar la re-adaptación.

Otra de las tareas utilizadas consistía en el aprendizaje secuencial de fuerza de pinza isométrica (SVIPT), con el objetivo de evaluar la precisión motora o coordinación fina. En ella, los sujetos debían apretar un trasductor de fuerza que movía verticalmente un cursor en la pantalla. Al apretar el trasductor el cursor subía, al soltarlo el cursor bajaba. Los sujetos debían alcanzar con el cursor, lo más rápidamente posible, cuatro objetivos marcados en la pantalla, en el orden indicado y sin sobrepasar o no alcanzar dichos objetivos.

Dado que la motivación juega un papel imprescindible en el aprendizaje, se ofreció una recompensa económica al sujeto que mejor ejecutase las tres pruebas.

Los resultados del estudio indican que no existe relación en las modificaciones inducidas en la excitabilidad del área motora con ninguna de las técnicas de estimulación utilizadas (tDCS, PAS ó iTBS) con ninguna de las variables del aprendizaje motor. Sin embargo, se ha observado que el tiempo de reacción sí está relacionado con las modificaciones inducidas tanto por la tDCS como por la iTBS. Es decir, aquéllos sujetos que responden positivamente a la tDCS o a la iTBS, muestran tiempo de reacción menores en

el primer bloque aleatorio de la SRTT. Este tiempo de reacción no es ninguna medida de aprendizaje, si no de ejecución motora.

Conclusiones finales:

- No se han encontrado efectos significativos de la tDCS, PAS o iTBS sobre la amplitud del MEP ni sobre el SICI, cuando se tiene en cuenta los 60 minutos post-estimulación en una muestra de 56 sujetos.

- La tDCS anodal ha mostrado un efecto significativo en la amplitud del MEP cuando se evalúa a la media hora post-estimulación en una muestra de 45 sujetos.

- Existe una alta variabilidad inter-individual en respuesta a los tres protocolos de estimulación testados (tDCS, PAS y iTBS), tanto en la amplitud del MEP como en el SICI.

- El análisis de clusters basado en los cambios en la amplitud del MEP, revela dos patrones diferentes de respuesta a los protocolos de estimulación. Un grupo responde como es esperado, mientras que otro no muestra dicha respuesta.

- Existe una fiabilidad intra-sujeto moderada de los protocolos de estimulación (testada con tDCS durante la media hora post-estimulación) en dos sesiones diferentes.

- La variabilidad intra-individual en respuesta a dos sesiones de estimulación, es menor que la variabilidad inter-individual.

- El SICI pre-estimulación predice parcialmente la respuesta al PAS.

- El pico de incremento de excitabilidad cortical tras tDCS anodal es alrededor del minuto 15 post-estimulación.

- La plasticidad cortical inducida por tDCS, PAS y iTBS no está relacionada con la capacidad de aprendizaje motor testada con SRTT, VAT y SVIPT.

- La plasticidad cortical inducida por tDCS y iTBS en el área motora está relacionada con el tiempo de reacción.

Limitaciones:

- Hemos testado únicamente tres protocolos de estimulación cerebral no invasiva y de todos ellos se esperaba una respuesta facilitadora. Por lo tanto, deberían replicarse los estudios incluidos en esta tesis con otros tipos de estimulación, incluyendo aquéllos que inducen efectos inhibitorios.

- La muestra utilizada para esta tesis fue de sujetos jóvenes y sanos. Por lo tanto se ha de ser cauto a la hora de aplicar nuestros hallazgos a otro tipo de poblaciones.

- Solamente se ha estimulado un área cortical. Aunque el área motora primaria ha sido el objetivo más común de los estudios de estimulación cerebral no invasiva, todavía se ha de comprobar que nuestros resultados sean válidos para otras áreas corticales.

- Los efectos de las técnicas de estimulación utilizadas en nuestros estudios se han limitado a una sesión. Por lo tanto, se debería testar también el efecto acumulativo de sucesivas sesiones de estimulación. Además, la fiabilidad de los efectos inducidos por los protocolos de estimulación cerebral no invasiva, deben ser evaluados en más de dos sesiones.

- Las tareas motoras utilizadas en nuestro estudio representan un pequeño número de la amplia variedad de paradigmas del aprendizaje motor.