Brain stimulation techniques for the study of fatigue induced by repeated movements

Técnicas de estimulación cerebral para el estudio de la fatiga producida por movimientos repetidos

Tesis doctoral defendida por D. Antonio Madrid López

2019

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Tesis doctoral con mención internacional UDC / 2019

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Programa Oficial de Doctorado en Neurociencias

Memoria presentada por Antonio Madrid López (alumno del Programa Interuniversitario de Neurociencias) en el Departamento de Fisioterapia, Medicina y Ciencias Biomédicas para optar al título de Doctor con Mención Internacional por la Universidade da Coruña.

A Coruña, abril 2019

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AUTORIZAN

La defensa de esta Tesis Doctoral titulada "Técnicas de estimulación cerebral para el estudio de la fatiga producida por movimientos repetidos", que D. Antonio Madrid López ha realizado bajo nuestra supervisión y que presenta las condiciones de originalidad, calidad y rigor científico para optar al título de Doctor con Mención Internacional por la Universidade da Coruña

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El doctorando ha participado en las siguientes publicaciones en revistas y comunicaciones en congresos:

- **Publicaciones en revistas científicas:**

1. Arias P., Corral-Bergantiños Y., Robles-García V., Madrid A., Oliviero A., and Cudeiro J. Bilateral tDCS on primary motor cortex: Effects on fast arm reaching tasks. PLOS ONE, 11(8):1 - 17, 08 2016.
2. Arias P., Robles-García V., Corral-Bergantiños Y., Madrid A., Espinosa N., Valls-Solé J., Grieve K. L., Oliviero A., and Cudeiro J. Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of silent periods evoked at spinal and supraspinal levels. Neuroscience, 305:316 - 327, 2015.
3. Madrid A., Valls-Solé J., Oliviero A., Cudeiro J., and Arias P. Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks. Neuroscience, 339:655 - 666, 2016.
4. Arias P., Adán-Arcay L., Puerta-Catoira B., Madrid A., and Cudeiro J. Transcranial static magnetic field stimulation of M1 reduces corticospinal excitability without distorting sensorimotor integration in humans. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation, 10:340 - 342, 03 2017.
5. Madrid A., Madinabeitia-Mancebo E., Cudeiro J., and Arias P. Effects of a finger tapping fatiguing task on m1-intracortical inhibition and central drive to the muscle. Scientific Reports, 8:9326, 2018.

- **Comunicaciones en congresos:**

1. Madrid A., Robles-García V., Corral-Bergantiños Y., Cudeiro J., Arias P. Efectos de la tDCS en la excitabilidad corticoespinal en sujetos jóvenes. **IX Jornadas para Jóvenes Investigadores en Neurociencia (ISBN: 1886-6786), 2013, España (Coruña, A).**
2. Corral-Bergantiños Y., Arias P., Madrid A., Robles-García V., Espinosa N., Cudeiro J.Efecto de la ECDt en la ejecución de una tarea moto-

ra funcional, el movimiento de alcance en humanos. **X Jornadas para Jóvenes Investigadores en Neurociencia (ISBN: 1886-6786), 2014, España (Santiago de Compostela).**

3. Madrid A., Arias P., Robles-García V., Corral-Bergantiños Y., Espinosa N., Valls-Solé J., Grieve K. L., Oliviero A., Cudeiro J. Differential contributions of spinal and supraspinal mechanisms of fatigue to the decrease of maximal motor output during short-lasting repetitive or isometric finger tasks. **XVI Congreso de la Sociedad Española de Neurociencia (SENC), 2015, España (Granada).**

4. Corral-Bergantiños Y., Robles-García V., Madrid A., Arias P., Cudeiro J. Improving Reaching Movements in Parkinson's Disease by Means of Virtual Reality Training. **XVI Congreso de la Sociedad Española de Neurociencia, 2015, (SENC) España (Granada).**

5. Arias P., Corral-Bergantiños Y., Robles-García V., Madrid A., Oliviero A., Cudeiro J. Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Tasks. **XVI Congreso de la Sociedad Española de Neurociencia (SENC), 2015, España (Granada).**

6. Arias P., Robles-García V., Corral-Bergantiños Y., Madrid A., Espinosa N., Valls-Solé J., Grieve K. L., Oliviero A., Cudeiro J. Differential contributions of spinal and supraspinal mechanisms of fatigue to the decrease of maximal motor output during short-lasting repetitive or isometric finger tasks. **XI Jornadas para Jóvenes Investigadores en Neurociencias, 2015, España (Vigo).**

7. Madrid A., Robles-García V., Corral-Bergantiños Y., Valls-Solé J., Oliviero A., Cudeiro J., Arias P. Response of Spinal Excitability to Different Short-Lasting Motor Tasks: Preliminary Results. **3rd International Conference on NeuroRehabilitation (ICNR 2016) (ISBN: 978-3-319-46668-2), 2016, España (Real Sitio de San Ildefonso).**

8. Cudeiro J., Madrid A., Madinabeitia-Mancebo E., Arias P. Changes in the level of voluntary activation after fatiguing finger tapping. **II International Brain Stimulation Conference, 2017, España (Barcelona).**

9. Arias P., Cudeiro J., Madrid A., Madinabeitia-Mancebo E. Primary motor cortex (M1) inhibition increases during fatiguing repetitive movements but force and central drive to the muscle are preserved. **47th Society for Neuroscience's Annual Meeting, 2017, Estados Unidos (Washington, D.C., USA).**

10. Arias P., Madrid A., Cudeiro J., Madinabeitia-Mancebo E. Force and central drive to the muscle are preservend when cortical inhibition increases during fatiguing repetitive movements. **XVII National Congress of the Spain Society of Neuroscience, 2017, España (Alicante/Alacant).**

11. Arias P. Adán-Arcay L., Madinabeitia-Mancebo E., Madrid A., Cudeiro J. Interaction of different inhibitory non-invasive brain stimulation techniques: preliminary results on human subjects. **XVII Congreso Nacional de la Sociedad Española de Neurociencia, 2017, España (Alicante/Alacant).**

12. Madinabeitia-Mancebo E., Madrid A., Cudeiro J., Arias P. M1 Inhibition Dependency on Slowing of Muscle Relaxation After Brief and Fast Fatiguing Repetitive Movements: Preliminary Results. **4th International conference on neurorehabilitation (ICNR), 2018, Italia (Pisa).**

A mi familia y amigos.

Agradecimientos

La realización de esta tesis doctoral no habría sido posible sin la inestimable ayuda y colaboración de las siguientes personas:

En primer lugar, a los doctores Pablo Arias y Javier Cudeiro, por la incontable cantidad de horas dedicadas tanto a la planificación, realización, análisis e interpretación de los experimentos, como a la ardua lectura y corrección de esta tesis. Por ello, me gustaría agradecerles sinceramente su sobresaliente labor en el papel de mentores, gracias.

También debo agradecer al doctor Casto Rivadulla, por acogerme, guiarme, y sacarme de algún que otro aprieto.

En general, a todo el grupo de NEUROcom, al Dr. Nelson Espinosa y sus programas de análisis que tanto me ayudaron, a la Dra. Verónica Robles y sus valiosos consejos (de los cuáles destaco mi favorito al poco de llegar: "Tú dale el follón a Pablo, que no te dé apuro"), al Dr. Jordi Aguilá, su acento y su humor, capaces de alegrar un día y a Sandra Yaneth, por sus palabras amables y su compresión.

A mis compañeras de laboratorio Yoanna Corral, Elena Madinabeitia, Lucía Adán, por su compañía.

Al personal de la UDC, a Manuel, Manuela, Laura e Iván, conserjes y amigos, a David de la cafetería, su energía inagotable y su memoria infalible.

A special mention to the people from the UCL that took me in my research stay, and taught me to have a different point of view, not only in science, but in life. Special mention to the *Effort Lab*, Anna, a special person, William, the fastest and most efficient person I met (and a true amigo) and Sasha and its ramblings, and to the other people I met there, Alex, the sun in the islands, Svenja, Jane, and Graziella.

A Joaquin Alcaraz Belzunces y sus preguntas en lugar de respuestas, a quienes culpo de esta aventura.

Y a mi familia y amigos, a quienes escribirles unos agradecimientos como se merecen, duplicarían el tiempo de escritura de esta tesis.

No obstante, no puedo dejar la oportunidad de agradecer a aquellos que han formado parte de mi vida, a Jose, Fer, Sergio, y Pau. A Sergio, Fran, Emmanuel, Lourdes y Elena. A Espejo, Clares, Pablo y Jorge.

Y por supuesto, a mi padre y a mi madre, quienes han sido mi ejemplo a seguir y mi apoyo para poder continuar; a mi hermano, por ser un ejemplo de superación, cuando quiere. Y a mi María, por hacer que todo merezca la pena.

Índice general

Abstract

Fatigue is a very limiting condition in many activities of the daily living and is present in different neurological pathologies. Although the underlying mechanisms are not well understood, it is considered to have a great impact on the different functional capacities of the people who suffer from it.

From a motor system perspective, fatigue is divided as central and peripheral. Central fatigue has been related to the inability to execute or maintain muscle force. Notwithstanding, there are many activities of the daily living, like rhythmic repetitive movements such as walking, typing, mouse-clicking, or movements in assembly lines, which can be executed with very low levels of muscle force and also induce fatigue, likely of central origin. For this reason, it seems paradoxical that central expressions of fatigue induced by repetitive movements have hardly been explored.

This PhD Thesis presents results from three different studies. We used non-invasive brain stimulation techniques to characterize central expressions of fatigue induced by movements performed repeatedly.

All studies enrolled young healthy participants. In the first study, we evaluated the effect of transcranial direct current stimulation on arm reaching movements, performed repeatedly as fast as possible in a reaction time protocol. In the sham stimulation session, we observed the development of fatigue with task progression, which was absent in the case of real stimulation sessions. Regretfully, the methodology used in this study did not permit us to understand physiological mechanism behind this observation. For the second and third studies, we used transcranial magnetic stimulation, stimulation at the level of the cervicomedullary junction (electric and magnetic) and percutaneous nerve stimulation to explore the central expressions of fatigue induced by repetitive movements. We compared its profile to the

central manifestations of fatigue during isometric contractions, which is considered the "gold-standard" to study fatigue. These non-invasive brain stimulation techniques allowed us to identify central cortical circuits as the loci of fatigue produced during un-resisted repetitive movements. Excitability of spinal motoneurons was not impaired. This profile is different from observations during isometric maximal voluntary contractions of same duration, for which an evident impact on the excitability of the spinal motoneurons is present.

Our results indicate that central expressions of fatigue induced by movements performed repeatedly are different from those generated during isometric contractions, despite a same task-duration and relative effort.

Our results are relevant because they permit dissociating central circuits and structures responsible for different types of motor system fatigue. This is important due to the impact of fatigue on activities of the daily living in physiological and clinical conditions, in sports, or ergonomics. Also, these results allow a better understanding of putative approaches to treat motor system fatigue based on neuromodulation techniques, either in physiological as pathological conditions.

Resumen

La fatiga en una condición limitante en múltiples actividades de la vida diaria y está presente en distintas patologías neurológicas. A pesar de no estar suficientemente definida se considera que afecta a distintos planos funcionales del individuo.

Desde el punto de vista motor, la fatiga se ha definido como fatiga central y periférica, y en el caso de la fatiga central la misma ha sido clásicamente vinculada a la merma experimentada por las personas a la hora de realizar fuerza muscular. Sin embargo, existen en las actividades de la vida diaria múltiples acciones, como movimientos repetitivos rítmicos ejecutados al caminar, durante el uso de teclados y ratones, o en cadenas de montaje, que pueden ser realizadas con bajos niveles de fuerza, y a pesar de ello ser susceptibles de generar fatiga, posiblemente de tipo central. Es por ello paradójico que las bases de la fatiga central producida por movimientos repetitivos apenas hayan sido exploradas.

En esta tesis doctoral presentamos los resultados de tres estudios en los que, mediante el uso de técnicas de estimulación cerebral no invasiva, hemos caracterizado las expresiones centrales de la fatiga producida por movimientos realizados repetidamente.

Todos los estudios se llevaron a cabo en participantes jóvenes sin patologías. En el primer estudio, hemos evaluado el efecto de la estimulación con corriente directa en las características de los movimientos de alcance realizados de manera repetida a la máxima velocidad en un protocolo de tiempo de reacción. Durante la sesión de estimulación placebo se observó un desarrollo de la fatiga conforme la tarea progresaba, que no se manifestó en el caso de las sesiones de estimulación real. Desafortunadamente, la metodología utilizada en el primer estudio no nos permitió explorar los posibles mecanismos fisiológicos que explicasen dicha observación. En el segundo y

tercer estudio, se utilizó la estimulación magnética transcraneal, la estimulación a la altura de la decusación piramidal, y la estimulación eléctrica del nervio (aplicada de modo percutáneo) para explorar las expresiones centrales de la fatiga inducida durante movimientos repetitivos. El perfil de dicha fatiga se comparó con la producida por contracciones isométricas, consideradas el estándar para el estudio de la fatiga. Mediante el uso de estas técnicas hemos identificado circuitos centrales de tipo cortical como loci de la fatiga producida durante movimientos repetitivos no resistidos, sin que la excitabilidad de las motoneuronas espinales se vea comprometida. Este perfil difiere de la fatiga producida por las contracciones isométricas máximas de la misma duración, que sí impactan de manera muy importante a la excitabilidad de la médula espinal.

Nuestros resultados indican, claramente, que las bases centrales de la fatiga producida por movimientos ejecutados repetidamente difieren de la fatiga generada por contracciones isométricas de la misma duración y esfuerzo relativo.

Estos resultados son muy relevantes debido a que permiten disgregar estructuras y circuitos centrales responsables de distintos tipos de fatiga del sistema motor; ello es importante dado el impacto de la fatiga en actividades de la vida diaria tanto en poblaciones fisiológicas como clínicas, en actividades deportivas o laborales. Permiten, asimismo, planificar más adecuadamente las posibles estrategias de intervención mediante técnicas de modulación de la excitabilidad del sistema nervioso central, las cuales son potencialmente útiles de cara a intervenir en distintas expresiones de la fatiga motora tanto en poblaciones fisiológicas como patológicas.

Resumo

A fatiga é una condición que limita múltiples actividades da vida diaria e está presente en distintas patoloxías neurolóxicas. Maila non estar suficientemente definida, coñécese que afecta a distintos planos funcionais da persoa.

Dende o punto de vista motor, a fatiga tense definido como fatiga central e periférica, e no caso da fatiga central a mesma ten sido clasicamente vencellada á diminución experimentada polas persoas na capacidade de facer forza muscular. Porén, existen nas actividades da vida diaria múltiples accións, como movementos repetitivos rítmicos executados ao camiñar, durante o uso de teclados e ratos, ou en cadeas de montaxe, que poden ser realizados con baixos niveis de forza, e malia elo ser susceptibles de xerar fatiga, posiblemente de tipo central. É por elo un paradoxo que as bases da fatiga central producida por movementos repetitivos apenas se teñan exploradas.

Nesta tese doutoral presentamos os resultados de tres estudos nos que, mediante o uso de técnicas de estimulación cerebral non invasivas, temos caracterizado as expresión centrais da fatiga producida por movementos realizados repetidamente.

Todos os estudos foron levados a cabo en persoas novas sen patoloxías. No primeiro estudo avaliamos o efecto da estimulación con corrente directa nas características dos movementos de alcance realizados de maneira repetida á máxima velocidade nun protocolo de tempo de reacción. Durante a sesión de estimulación placebo observouse un desenrolo da fatiga co progreso da tarefa, que non se manifestou no caso das sesións de estimulación real. Desafortunadamente a metodoloxía utilizada neste primeiro estudo non permitiu explorar os posibles mecanismos fisiolóxicos que explicasen dita observación. No segundo e terceiro estudo utilizouse a estimulación magnética transcraneal, a estimulación á altura da decusación piramidal, e

a estimulación eléctrica do nervio (aplicada de maneira percutánea) para explorar as expresións centrais da fatiga inducida durante movementos repetitivos. O perfil de dita fatiga comparouse co producido por contraccións isométricas, consideradas a referencia para o estudo da fatiga. Por medio do uso destas técnicas identificamos circuítos centrais de tipo cortical coma loci da fatiga producida durante movementos repetitivos non resistidos, sen que a excitabilidade das motoneuronas espiñais se comprometa. Este perfil é distinto da fatiga producida polas contracción isométricas máximas da mesma duración, que si impactan de maneira moi importante na excitabilidade da medula espiñal.

Os nosos resultados indican, claramente, que as bases centrais da fatiga producida por movementos executados repetidamente son distintas da fatiga xerada por contraccións isométricas da mesma duración e esforzo relativo.

Estes resultados son moi relevantes debido a que permiten disgregar estruturas e circuítos centrais responsables de distintos tipos de fatiga do sistema motor; elo é importante dado o impacto da fatiga nas actividades da vida diaria, tanto en poboación fisiolóxicas como clínicas, en actividades deportivas ou laborais. Permiten, asemade, planificar de maneira máis acaída as posibles estratexias de intervención mediante técnicas de modulación da excitabilidade do sistema nervioso central, as cales son potencialmente útiles para intervir en distintas expresión da fatiga motora, tanto en poboacións fisiolóxicas como patolóxicas.

Acrónimos

AL-CR Anode Left, Cathode Right

AMT Active Motor Threshold

AR-CL Anode Right, Cathode Left

CMAP Compound Muscle Action Potential

CMEP Cervico-medullary Motor Evoked Potential

CMS Cervico Medullary Stimulation

CV Coefficient of variation

EEG Electroencefalography

EMG Electromiography

FDI First Dorsal Interosseous

ft Finger Tapping

ICC Intra-class correlation coefficient

iMVC isometric Maximal Voluntary Contraction

iso Isometric Contraction

M1 Primary Motor Cortex

MT Motor Time

MVC Maximal Voluntary Contraction

PMT Pre-Motor Time

RMS Root mean square

RT Reaction Time

ROM Range of motion

SE Standard Error

SP Silent Period

tDCS transcranial Direct Current Stimulation

TMS Transcranial Magnetic Stimulation

V1 Primary Visual Cortex

Capítulo 1

Introducción

1.1. Definición de fatiga

Brenda Bigland-Ritchie definió la fatiga como: *"[...] a common experience, but the underling causes are complex and controversial."* [1]

La controversia que rodea a la definición de fatiga radica en su complejidad y en su dependencia del contexto en que se discuta. Su complejidad se debe a los muchos factores que la componen, a las causas y mecanismos, y a las múltiples manifestaciones asociadas que se pueden observar e.g.- apatía, dolor, debilidad, etc. Su dependencia del contexto, a la vez, cobra importancia por motivos más prácticos, ya que, generalmente, el estudio de la fatiga no se enfoca del mismo modo en clínica que en investigación, en condiciones fisiológicas que patológicas, e incluso en distintas patologías, por ejemplo en aquellas con origen cadio-pulmonar o neural.

Es por ello que se vuelve necesario aclarar el contexto en el que se han desarrollado los estudios experimentales que componen esta tesis doctoral [2] [3] y [4]. Los estudios desarrollados durante esta tesis se enmarcan en un contexto de investigación en condiciones fisiológicas, haciendo hincapié en los componentes neurales de la fatiga de tipo motriz; es decir, aquella que afecta principalmente al desempeño motor del ser humano.

A pesar de encontrarnos en un marco más definido, la fatiga continúa siendo un concepto esquivo y difícil de acotar. Destacamos, en primer lugar, el concepto de fatiga muscular, cuyas definiciones más destacables pertenecen a los profesores

Simon C. Gandevia [5] y *Roger M. Enoka* [6], siendo *"[La fatiga muscular es...] cualquier reducción inducida por el ejercicio en la habilidad para ejercer fuerza o generar potencia muscular, independientemente de si la tarea puede ser o no mantenida"* y *"[La fatiga muscular es...] una disminución aguda de la capacidad de ejecución que incluye tanto un aumento de la percepción del esfuerzo necesario para ejercer una fuerza y una imposibilidad eventual para producir la misma"*, respectivamente. Es importante señalar que, aunque terminológicamente el concepto de fatiga muscular parece focalizarse en el músculo, el origen de la merma de la capacidad muscular podría originarse proximalmente al mismo, por lo que el concepto de fatiga muscular es más amplio y abarca distintas expresiones en todo el sistema motor.

Como se puede observar, ambas definiciones de fatiga muscular hacen referencia a dos propiedades básicas, a saber, **debilidad** e **inducibilidad**, añadiendo la segunda definición la **reversibilidad** y la **astenia**, sin las cuales no podríamos estar hablando de fatiga. Es reseñable que estas propiedades se incluyen dentro de una conceptualización fisiológica de fatiga, ya que algunas propiedades, como la **reversibilidad** o la **inducibilidad** pueden verse comprometidas en patologías, ya sea porque es irreversible, o porque resulte imposible identificar el agente inductor si lo hubiere, como es el caso de la fatiga crónica.

Para entender la definición de fatiga, es necesario adentrarse un poco en su historia.

1.1.1. Aproximaciones históricas al estudio de la fatiga del sistema motor

El estudio de la fatiga se engloba dentro de la biología, concretamente en la fisiología humana, por lo que se benefició ampliamente de la aplicación del método científico a las ciencias biológicas, particularmente de la escuela italiana *Iatromecánica*.

Los trabajos del italiano *Giovanni Alfonso Borelli* iniciaron el cambio de paradigma en las ciencias biológicas, destacando su libro "*De Motu Animalium*", que estudia la motricidad animal y que sirvió a su vez de piedra angular para el primer tratado en fatiga, *"La fatica"*, o *"La fatiga"* [7], del hispano-italiano *Angelo Mosso*. En este libro, que data de 1904, se encuentra una recopilación del saber de la época sobre la fatiga y de las conclusiones extraídas por el propio *Mosso* de experimentos llevados a cabo en modelos tan dispares como la paloma, el humano, las anguilas o las abejas, y sobre factores tan variados como el color del músculo o del cerebro tras un esfuerzo extenuante, la disminución de la fuerza, el aumento de la frecuencia respiratoria, o los movimientos de la sangre dentro del cerebro.

Si bien *Mosso* no llega a definir en ningún momento la fatiga, sí que establece las primeras bases sobre cómo enfocar la investigación venidera. Consideró fenómenos como el cansancio físico tras un esfuerzo físico, el cansancio mental tras un esfuerzo mental, el cansancio sensorial, o incluso una interacción entre estos. Su prolífica mente le llevó a desarrollar diferentes artefactos en los que apoyar sus experimentos. Entre estos, destaca la *balanza de Mosso*, que mediante una estructura donde yacía el sujeto aspiraba a medir ligeros cambios en el volumen sanguíneo cerebral en respuesta a diferentes estímulos o tareas cognitivas que desplazaran el centro de gravedad. Si bien puede parece inverosímil, algunos estudios recientes, con métodos más precisos, han confirmado la efectividad de esta balanza [8] [9].

No obstante, en cuanto a esta tesis concierne, el principal invento de *Mosso* fue el ergógrafo, un aparato diseñado para evaluar el trabajo de un movimiento, originalmente la flexo-extensión contragravitatoria del tercer dedo de la mano. Para ello, se valía de una fijación de antebrazo y mano en supinación, permitiendo el movimiento del tercer dedo, que se encontraba unido a una estructura mecánica. Con el movimiento del dedo se generaba una marca sobre un tubo ahumado que

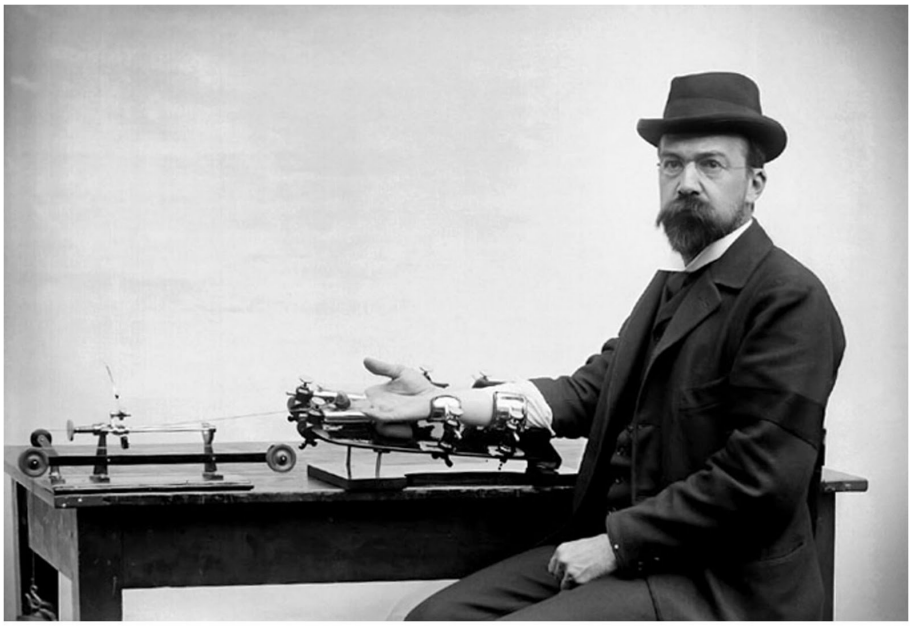

Figura 1.1: *Angelo Mosso* posa en su ergógrafo. Falta el tubo ahumado de registro.

permitía el registro del experimento y permitió a *Mosso* obtener la primera gráfica de fatiga tras numerosas repeticiones (ver Figura 1.1).

En sus muchos años de estudio, *Mosso* describió las *"Leyes de la extenuación muscular"*, que se sintetizaban en las siguientes:

- La fatiga muscular debería ser considerada como un fenómeno periférico que es independiente de la volición, pero a la vez hay una relación holística entre la energía en los centros nerviosos y la fatiga.
- La fatiga debería ser considerada una forma de *envenenamiento* debida a la producción de material de desecho que la irrigación sanguínea y la respiración tienden a eliminar.
- La presencia de ácido carbónico y ácido láctico impide y reduce la contracción muscular.
- La fatiga reduce la sensibilidad muscular y la sensibilidad general del cuerpo.
- La fatiga representa una *señal de alarma* para los músculos, el punto percutor para limpiar la *escoria* y recuperar la pérdida de energía inmediatamente durante contracciones extenuantes.

De estas leyes, podemos observar temáticas que permanecen en tiempos modernos, como la debilidad muscular como signo de fatiga, la afectación de la percepción, la inducibilidad por algún factor externo, la posibilidad de recuperación, y el debate entre mecanismos periféricos y centrales.

En ésta última línea, *Mosso* inauguró uno de los principales paradigmas de estudio en fatiga, inspirado por los experimentos del también italiano *Luigi Galvani* con estimulación eléctrica, e intentó utilizar esta misma electricidad para discernir si la disminución de fuerza podía evitarse. De esta manera, si la electricidad era capaz de estimular el músculo y superar la fuerza voluntaria, la disminución de fuerza se debería a una imposibilidad del sistema nervioso para obtener más fuerza del músculo.

Los trabajos de *Mosso* se mantienen como un referente en fatiga, y si el lector desea profundizar en su obra, se aconseja la lectura de *DiGiulio* [10].

Algo más tarde, *Charles Reid [11]* experimentó con distintos *loci* de estimulación, y diferentes protocolos donde variaba intensidad y frecuencia de estimulación. Así consiguió niveles de fuerza evocada comparables a los voluntarios, algo que *Mosso* y otros coetáneos no habían alcanzado. Desarrolló experimentos que incluían contracciones isotónicas usando el *ergógrafo de Mosso* [7], y contracciones isométricas tal y como había descrito *Adolf Fick* por primera vez, mediante el uso de un muelle de acero [12]. Encontró, en muchas ocasiones, que la estimulación eléctrica podía superar en fuerza evocada a la que podía obtenerse mediante esfuerzo voluntario, concluyendo que, en gran parte, la fatiga inducida tanto por movimientos isotónicos como isométricos era de origen central. El debate prosiguió con los trabajos de *Patrick Anthony Merton*, quien creía que el límite de la fuerza debía ser puramente muscular, lo que se conoce como fatiga periférica. Para demostrar esto, *Merton* desarrolló una nueva metodología que consistió en la superposición del estímulo eléctrico durante una contracción voluntaria. Según esta idea, el estímulo eléctrico sería capaz de activar aquellas fibras musculares que formarían parte de un *reservorio funcional* aumentando por tanto la fuerza o, por el contrario, demostrando que el total de las fibras musculares se encuentran ya activadas, por lo que el límite de la fuerza se encontraría en el propio músculo (*The principle of the method is that if all muscle fibres are fully activated an extra motor volley will not superimpose any twitch on the tension record* [13], Figura 1.2).

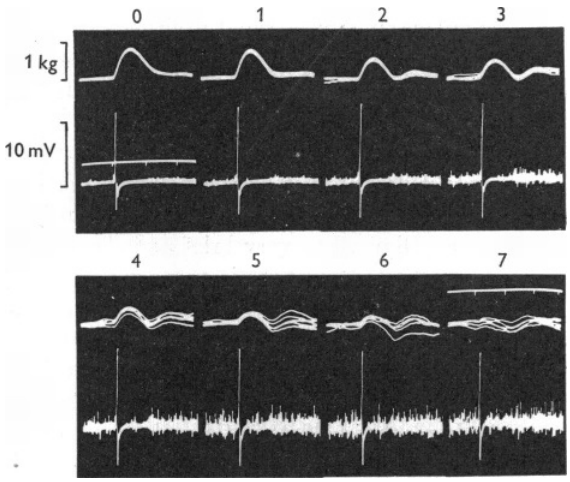

Figura 1.2: Ejemplo de estímulos eléctricos superpuestos a diferentes grados de fuerza voluntaria, siendo el 0 reposo absoluto, y el 7 contracción máxima voluntaria. Puede observarse cómo la ganancia de fuerza disminuye de manera inversamente proporcional al aumento de fuerza voluntaria, mientras que la actividad EMG aumenta de manera directamente proporcional, y la amplitud del potencial de acción muscular compuesto se mantiene. De esta manera se demuestra la relación entre el grado de activación voluntaria y la fuerza evocada de manera superpuesta.

A partir de la conceptualización básica de esta técnica, la modificación de los protocolos de estimulación eléctrica durante una activación muscular permite estimar el nivel de activación voluntaria por parte del sujeto, ello se debe a que el estímulo aplicado durante una contracción máxima voluntaria permite estimar (a partir de la fuerza superpuesta por el estímulo sobre la fuerza voluntaria) la cantidad de axones no reclutados durante la activación, y relativizarlos con respecto a los no reclutados durante el reposo (equivaliendo esta última al total de axones reclutables por el estímulo). De esta manera, se obtiene el porcentaje de axones que estaba en uso, y por tanto, el porcentaje de activación voluntaria en ese momento; todo ello a partir de registros de fuerza y el tamaño de estos.

Este concepto alcanzó tal nivel de importancia que en tiempos modernos, el Prof. *Simon C. Gandevia*, usó la disminución de la activación voluntaria como sinónimo de fatiga central [14] [5]. Sin embargo, como todo en la historia del estudio de la fatiga, no está exento de debate como se pudo observar en el año 2009 en la revista *Journal of Applied Physiology* [15], [16], [17], [18], [19], [20], [21], que lanzó un número específicamente destinado a la revisión de dicho concepto. El concepto de activación

voluntaria se basa en la **debilidad** o incapacidad de generar fuerza debido a motivos centrales. Sin embargo, la barrera entre fatiga periférica y fatiga central se difumina cuando las aferencias periféricas transmiten información del estado muscular permitiendo al sistema nervioso central adaptarse a este estado en un fenómeno conocido como **Muscle Wisdom**. Mediante este mecanismo la ralentización en la velocidad de contracción de las fibras musculares conduce a disminuir la frecuencia de disparo de la motoneurona, optimizando y preservando recursos y retrasando la aparición de fatiga [22] [23].

Los diferentes mecanismos que pueden desencadenar la disminución de la activación voluntaria dependen en primera instancia, de otra de las características atribuidas a la fatiga, la **inducibilidad**, y es que en función del factor inductor, es decir, del tipo de ejercicio, la fatiga se va a expresar de distinta manera. Este principio es conocido como *Task Dependency* y fue acuñado por el Prof. *Roger M. Enoka* [6] aunque ya puede entreverse en definiciones previas de fatiga, como la de *Brenda Bigland-Ritchie* al decir que es **compleja** [1].

1.2. Dependencia de la tarea

La *Task Dependency* es un concepto importante para el desarrollo de esta tesis doctoral. Existen múltiples factores que determinan la aparición de fatiga tanto a nivel periférico como central como pueden ser el tipo de contracción, su intensidad, el ciclo de trabajo o la complejidad de la tarea.

Por ejemplo, hace ya tiempo que se conoce que una contracción de tipo concéntrico no presenta las mismas características energéticas que una de tipo excéntrico, *Archibald V. Hill* lo evaluó en fibras musculares aisladas de *Xenopus* [24], y *Brenda Bigland-Ritchie* desarrolló un modelo completo basado en el uso de dos bicicletas contrapuestas y comunicadas, de manera que mientras un sujeto realizaba una contracción concéntrica, el otro realizaría la misma tarea a la misma velocidad y con la misma fuerza, pero en sentido opuesto, resultando en diferentes tasas de consumo de oxígeno [25].

La hipótesis más usual para este fenómeno se corresponde con una desproporción de la fuerza evocada para un mismo estímulo, siendo mayor durante una contracción excéntrica. De esta manera, cuando el músculo se estira, los puentes cruzados funcionan más arriba en su curva de estrés-tensión y así generan mayor tensión hasta el punto de ruptura mecánica, que cuando el músculo se acorta [26] [27]. Dado que las uniones de actina-miosina deben romperse mecánicamente principalmente en las contracciones excéntricas, el ciclo de interacción de los puentes cruzados ocurre con menor hidrólisis de *ATP* que las contracciones concéntricas [28].

También se encuentran diferencias en el comportamiento de estructuras centrales cuando una contracción es máxima o submáxima, cuando se enfrentan una tarea de posición y una de fuerza, y el punto que más nos atañe, cuando una tarea incluye una secuenciación de la actividad de músculos antagonistas entre sí [29] [30] [31] [32] [33].

El test de golpeo repetitivo del dedo se ha establecido como una forma sencilla de evaluar la capacidad central de generar movimientos repetitivos rítmicos [34] [35] [36]. El golpeo repetitivo del dedo es reconocido como una herramienta de evaluación clínica útil en diferentes patologías como la enfermedad de *Parkinson* [37] [38], Ataxia [39], enfermedad de *Alzheimer* [40] y accidente cerebro-vascular [41]. Su utilidad para evaluar fatiga ha quedado de manifiesto dado que diferentes autores

han remarcado que existe una rápida disminución de la frecuencia cuando el golpeo repetitivo del dedo se realiza a frecuencia de golpeo voluntaria máxima [29] [30] [42]. Es particularmente importante, en el contexto de esta tesis, que esta disminución en la eficiencia motriz podría no contemplarse como una aparición de debilidad o disminución en la activación voluntaria, sino que podría estar regulada por mecanismos centrales vinculados a la activación secuencial agonista-antagonista [29].

1.3. Impacto de la fatiga en las actividades de la vida diaria

Existen varios ámbitos en los que, cada vez más, se reconoce la importancia de la fatiga. De estos ámbitos destacan principalmente el ámbito clínico, el deportivo, y el ergonómico.

En el ámbito clínico, la fatiga se considera tanto como síntoma primario, en el síndrome de fatiga crónica [43], como síntoma secundario a otras enfermedades tales como esclerosis múltiple, accidente cerebro-vascular y enfermedad de *Parkinson*. En la esclerosis múltiple la fatiga puede ser un signo prodrómico y se describe como el síntoma más incapacitante, con una prevalencia del 70 % [44]. En el caso de supervivientes de accidente cerebro-vascular, una enfermedad con una incidencia [45] y una tasa de supervivencia en aumento [46], el síntoma de fatiga está presente en los mismos a pesar de una recuperación neurológica excelente [47], y es una de las secuelas más difíciles de gestionar [48] [49]. La fatiga es un síntoma asociado también a la enfermedad de *Parkinson*, controvertidamente relacionado con su gravedad y evolución [50] [51]. Está relacionada, al menos en parte, con la deficiencia dopaminérgica [52], ya que la levodopa normaliza la elevada excitabilidad cortico-espinal antes, durante y tras ejercicio fatigante [53].

Así, la fatiga se perfila como un síntoma común en múltiples patologías, con un origen distinto en ellas pero incierto en muchos casos, y con pocas opciones de tratamiento. A pesar de todo ello, la fatiga llega a ser identificada como uno de los síntomas más incapacitantes por un paciente con un desorden neuromuscular, como ya puso de manifiesto uno de sus pacientes de acuerdo a *Machiel J. Zwarts et al.*:

"Personally, I see fatigue as a greater problem than my quantifiable somatic symptoms and impairments. The fatigue makes me miss out on numerous things, whereas I could learn to live with my somatic disabilities. If the problem of my fatigue were to be solved, I would be able to function normally in society despite my somatic problems." [44]

En segundo lugar encontramos el ámbito deportivo, en el que los límites del cuerpo humano se han ido llevando cada vez más lejos con el paso de los años. Humanos cada vez más rápidos, más fuertes, más resistentes, o con mejor coordinación

o equilibrio han roto records que parecían imposibles hace años. A pesar de ello, los mecanismos centrales responsables de la fatiga han sido escasamente investigados, pese a compartir, probablemente, una fisiología común con las limitaciones impuestas por algunas patologías.

En el ámbito ergonómico, se ha reportado una prevalencia de fatiga ocupacional en más del 20 % de la población empleada de EEUU a fecha de 1993 [54], con un coste económico de 136400 millones de dólares estadounidenses [55]. Entre las causas más importantes de esta fatiga se encuentran la privación del sueño, el esfuerzo mental, el esfuerzo físico y la falta de recuperación [56]. Por desgracia, a pesar de sus implicaciones, se suele descubrir la fatiga en el entorno laboral a posteriori de algún accidente laboral. Por ejemplo, *Harrington* mostró cómo es posible que la fatiga jugara un papel importante en grandes tragedias como Chernobyl [57], ya que tuvo lugar durante las primeras horas de la mañana tras el turno de noche, cuando los niveles de fatiga entre trabajadores se encontraban en su máximo. El desarrollo de herramientas que permitan la detección temprana de la fatiga, o una intervención sobre esta, puede ser clave para la prevención de accidentes y enfermedades laborales.

1.4. Topografía de la fatiga del sistema motor

El binomio estructura/función mantiene su importancia en el contexto de la fatiga ya que, aunque el estudio de la fatiga es eminentemente el estudio de la función respecto al tiempo, toda está función está estructurada anatómicamente.

El sistema motor recibe la mayor parte de la respuesta efectora del sistema nervioso central [58], siendo este el último eslabón de una organización jerárquica que permite generar tensión intramuscular y por tanto aplicar fuerza a los elementos óseos que forman segmentos corporales para desarrollar una función vital, el movimiento.

El estudio del sistema motor puede abordarse por tanto de diferentes maneras en función del criterio utilizado. Por razones prácticas, en el estudio de la fatiga muscular se divide en los niveles periférico y central, a su vez la central se ha subdividido, clásicamente, en espinal y supraespinal [5].

La fatiga periférica sería aquella que se genera en la placa motora o el propio músculo, mientras que la fatiga central se desarrolla proximalmente a la unión neuromuscular [5]

1.4.1. Caracterización periférica de la fatiga del sistema motor

Los elementos anatómicos que destacan a nivel periférico son la fibra muscular y la placa motora, aunque se encuentran íntimamente relacionados.

La fibra muscular o miocito es la unidad citológica del músculo. Existen diferentes tipos de miocitos, cardíaco, liso y estriado, siendo éste último el de interés en esta tesis. Es una célula mesodérmica derivada del mioblasto, alargada, y con propiedades como la excitabilidad y la contractilidad. Un miocito es un tipo especializado de célula que consta de una membrana celular (sarcolema) que contiene el citosol (sarcoplasma). Presenta varios núcleos, abundante número de mitocondrias (sarcosomas), debido al gran uso energético; y un tipo especializado de citoesqueleto compuesto a base de largas cadenas contráctiles llamadas miofibrillas formadas por eslabones conocidos como sarcómeros.

Sus propiedades contráctiles se explican mediante el modelo de filamentos interdeslizantes propuesto simultáneamente por*Andrew F. Huxley* con *Rolf Niedergerke* y

Hugh Huxley con *Jean Hanson*[59] [60]. El sarcómero se comprende entre dos discos Z y está compuesto de distintos tipos de proteínas, entre las que se encuentran la actina (filamento fino) y la miosina (de filamento grueso), dispuestas en columnas o bandas. El evento contráctil va a depender de dos pasos secuenciales, uno relacionado con la actina y otro con la miosina. El aumento de los niveles sarcoplásmicos de Ca^{+2} produce la activación de la proteína sarcomérica troponina, que cambia su configuración desplazando la tropomiosina. Ello resulta en la liberación de los espacios activos de la actina, a la que se une la cabeza de la miosina permitiendo la formación del complejo acto-miosina con capacidad enzimática lo que produce la rotura del ATP liberando energía, adenosín difosfato y fósforo inorgánico. Esta energía se emplea para inducir un cambio conformacional en la molécula de miosina, de forma que el ángulo formado por la cabeza y la cola se acorta, lo que se traduce en un movimiento de los filamentos de actina de uno y otro lado del sarcómero, acercándose. Esto es lo que gráficamente se ha descrito como deslizamiento de los filamentos y es lo que genera la tensión mecánica al acortar los sarcómeros y, por extensión, toda la miofibrilla.

Una característica especial de los miocitos estriados es que son neurogénicos, es decir, su contracción está determinada por una neurona, a diferencia de los miocitos lisos o cardíacos, que son esencialmente miogénicos. La comunicación entre una neurona y un miocito se lleva a cabo a través de un tipo especial de sinápsis química conocida como placa motora. Elementos esenciales de la placa motora, y relevantes para esta tesis doctoral, son: el botón presináptico de la neurona, el neurotransmisor excitador, el espacio sináptico, y el miocito postsináptico, siendo el neurotransmisor la acetilcolina [61].

Por otro lado, sus propiedades excitables dependen de la placa motora, el sarcolema y el sistema de túbulos transversos (T). Los túbulos T son una red de invaginaciones membranosas anastomosadas de pequeño calibre que se prolongan desde el sarcolema hasta el retículo endoplasmático (sarcoplasmático) entretejiendo las miofibrillas, cuya luz da al exterior del miocito, y que permite una difusión iónica destacablemente rápida y homogénea comparable a la de la responsable de la propagación del potencial de acción a lo largo de la membrana neuronal. Todo ello ocurre de una manera secuenciada, muy similar a una transmisión sináptica neuronal. En

primer lugar, el potencial de acción proveniente de la motoneurona llega al extremo terminal del axón, el botón presináptico; esta despolarización abre canales dependientes de Ca^{+2}, lo que lleva a la movilización y posterior fusión de unas vesículas sinápticas que contienen el neurotransmisor excitatorio (Acetil-Colina) con la membrana mediante exocitosis, lo que lleva al neurotransmisor fuera de la célula, y en concreto, al espacio sináptico. Una vez liberado el neurotransmisor, este satura el espacio sináptico y se une a los receptores de Acetil-Colina (que en esta sinápsis son de tipo nicotínico, es decir ionotrópicos y siempre excitadores). Ello abre los canales pertinentes para causar una despolarización de la fibra muscular postsináptica que provoca un potencial de acción post-sináptico que se transmite al interior de las miofibrillas mediante el sistema de túbulos T, produciendo una liberación de calcio desde el retículo sarcoplásmico que inicia, como ya se ha explicado, la contracción muscular [62]. La placa motora es un locus potencial para la aparición de fatiga, habiéndose descrito al menos tres posibilidades, a nivel del botón presináptico, a nivel postsináptico, o en algún punto de la ramificación axonal [63]. Para evaluar la integridad de la transmisión neuromuscular, se utiliza una medida conocida como *factor de seguridad* [64], que involucra mediciones intracelulares del potencial de membrana junto con estimulación mediante microelectrodos (para obtener un potencial de placa motora), de manera que nos da un ratio entre la amplitud del potencial de placa motora y la diferencia entre el umbral de estimulación y el potencial de reposo [65]. En condiciones fisiológicas, este factor de seguridad es próximo a 1, por lo que la transmisión neuromuscular casi siempre ocurre [66]. Sin embargo, se ve afectado en condiciones adversas como algunas patologías (e.g.- Myasthenia Gravis, síndrome miasténico de Lambert-Eaton [67]), o fatiga inducida mediante estimulación [64] [68]. No obstante, en condiciones de fatiga, se ha estudiado utilizando otras técnicas, descritas a continuación.

Evaluación de la excitabilidad periférica del sistema motor

La señal electromiográfica de superficie representa una variación del potencial eléctrico debido a los cambios que acontecen dentro de la fibra muscular. Se evalúa mediante el uso de electrodos de registro colocados sobre la piel que recubre al músculo a evaluar. Es un potencial de campo dado que se corresponde con el su-

matorio de todos los cambios que ocurren en las fibras musculares, y por tanto, se enfrenta al problema del dipolo inverso, de manera similar a lo acontecido en la señal electroencefalográfica.

Debido a esto, es difícil conocer qué ocurre exactamente a nivel de fibras individuales, razón por la cual *Bigland-Ritchie y Lippold* desarrollaron una técnica de electromiografía con un electrodo en forma de aguja que permite explorar una fibra muscular individual, pero que se encuentra a su vez con la limitación de no poder evaluar el músculo al completo [69], junto con otras restricciones asociadas al tipo de contracción que con los mismos se puede registrar (i.e., contracciones isométricas).

La integridad funcional de la transmisión neuromuscular (i.e., transmisión de los potenciales del axón al músculo y propagación de los mismos por la membrana muscular) se evalúa mediante la onda M (M-wave en inglés) o el potencial de acción muscular compuesto (del ingles Compound Muscle Action Potential, y que se usa como sinónimo de la onda M cuando es máxima). La onda M es un potencial evocado motor que se registra en músculo determinado mediante EMG tras la estimulación del nervio correspondiente. Existen múltiples potenciales evocados motores cuyas características van a depender del locus de estimulación junto con el circuito que va a seguir el potencial de acción desencadenado mediante estimulación. En el caso de la onda M, la despolarización inducida por estimulación eléctrica se produce en algún lugar entre la placa motora y la raíz espinal. La onda M está determinada por el tamaño de los potenciales de cada fibra y el número de fibras que son activadas [70]. De esta manera, se pueden utilizar para evaluar la excitabilidad, en este caso, del conjunto de la placa motora y las fibras musculares.

Modificaciones funcionales en el sistema motor periférico producto de la fatiga

Existen al menos dos tipos de fenómenos reportados tras la fatiga que conciernen a la electromiografía y al potencial de acción muscular compuesto. El primero atañe al cambio del espectro de frecuencias de la señal electromiográfica durante la fatiga, principalmente debido al enlentecimiento de la velocidad de propagación de la fibra muscular [71]. De cualquier modo, las características espectrales pueden variar sin cambios en la velocidad de propagación de la fibra [72] [73]. Es destacable que el cambio en el espectro de frecuencias afecta de manera muy similar a la señal elec-

tromiográfica y al potencial de acción muscular compuesto [74] [75]. El segundo se refiere a la morfología del potencial de acción muscular compuesto; *Bigland-Ritchie et al.* demostraron que la actividad electromiográfica disminuía de manera paralela a la fuerza en una contracción isométrica máxima del *Adductor pollicis* sin que la onda M se viera afectada, por lo que esta disminución de la actividad eléctrica tenía que ser ocasionada por mecanismos proximales a la placa motora. Es decir, no existe un fallo de la transmisión del mensaje, sino de su orígen [70]. Es importante señalar, sin embargo, que la señal electromiográfica voluntaria aumenta durante la fatiga si el tipo de contracción isométrica fatigante no es máxima, sino sub-máxima y prolongada, y que además dicho patrón de crecimiento es más acentuado durante una tarea isométrica submáxima de *posición* que de *fuerza* [76], lo que sugiere un incremento en el número de unidades motoras o en su tasa de descarga conforme aumenta la fatiga.

Esto no significa que, para todas las tareas, la integridad del potencial de acción muscular compuesto esté garantizada. Por ejemplo, *Stephens et al.* encontraron una disminución tanto de la fuerza, como de la señal electromiográfica y el potencial de acción muscular compuesto, tras una tarea isométrica del primer dorsal interóseo de al menos 120 segundos [77], *Milner-Brown et al.* reportaron una mayor disminución del potencial de acción muscular compuesto en el primer dorsal interóseo en comparación con el *Adductor Pollicis* y el *Tibialis Anterior* tras una tarea isométrica máxima de entre 1 y 5 minutos [78], por lo que el fenómeno de la *Task-Dependency* también parece aplicarse a niveles periféricos. También se han encontrado aumentos en la excitabilidad del potencial de acción muscular compuesto empleando contracciones evocadas, *Fitch et al.* reportaron su aumento durante los primeros 30s de una estimulación a 20Hz en los dorsiflexores [79], mientras que *Cupido et al.* encontraron también un aumento tanto de la amplitud como del área a 10 y 20Hz en el *Biceps Brachii* [80].

Es particularmente importante destacar que gran parte de estos cambios se han estudiado en la fatiga inducida por tareas de tipo isométrico [13] [70] [81] [77], sin embargo, poco se ha explorado de los cambios en la señal electromiográfica dentro de contracciones repetidas. *Rodrigues et al.* evaluaron qué ocurre durante una tarea de golpeo repetitivo del dedo con la señal electromiográfica, descubriendo que conforme

disminuye la frecuencia de golpeo voluntaria máxima, el patrón de activación de la señal electromiográfica, entendido como la raíz de la media cuadrática, pasaba de un patrón claro de activación alternativa de los músculos flexores y extensores, a uno más similar al de co-contracción [29]; este fenómeno, a pesar de ser registrado a nivel periférico, tiene un claro origen central.

1.4.2. Caracterización central de la fatiga del sistema motor

Desde un punto de vista estructural y muy esquemático, el sistema nervioso central está compuesto por zonas (áreas, núcleos) donde se acumulan los somas neuronales, y tractos, que son proyecciones de axones que intercomunican las diferentes zonas. Atendiendo al sistema motor, y de forma restringida a las estructuras exploradas en esta tesis, destacan la médula espinal y la corteza motora primaria (**M1**).

Exploración funcional de la médula espinal

La médula espinal está organizada por niveles que se corresponden a la vértebra que los protege (e. g.- C1, T3 ó L1).). La vía neural que comunica **M1** con la médula espinal define el tracto descendente cortico-espinal y se asocia principalmente a movimientos voluntarios, y en ella las conexiones directas cortico-motoneuronales son especialmente numerosas en los conjuntos motoneuronales que controlan la musculatura de la mano [82]. Sin embargo, la médula espinal no sólo es un medio de comunicación entre núcleos supra-espinales y estructuras ajenas al sistema nervioso, sino que ella misma es un auténtico centro regulador [82]

La médula está compuesta de sustancia gris, que suele mostrar una forma de H en el plano transverso, y por la sustancia blanca, que rodea a la sustancia gris. Por una parte, en la sustancia gris se encuentran los somas de las neuronas que la componen, clasificadas por *Rexed* en diferentes zonas o láminas [83] [84], donde a grandes rasgos, las neuronas sensitivas se encuentran en las astas posteriores, las interneuronas repartidas por toda la sustancia gris, y las motoneuronas se organizan en columnas en las astas frontales. Por otra parte, dentro de la sustancia blanca se encuentran los axones que comunican el segmento espinal con otros núcleos, tanto

espinales del mismo u otro segmento (comunicación propioespinal), como supraespinales.

Las motoneuronas son aquellas neuronas espinales que se van a comunicar de manera efectora con el músculo y representan, tal y como lo definió *Sherrington*, "el punto último de salida del sistema motor"[85]. En función de qué tipo de órgano sea inervado, las motoneuronas se conocen como somáticas (si inervan musculatura estriada), simpáticas o parasimpáticas (si inervan musculatura o glándulas pertenecientes al sistema autónomo). Por el contexto de esta tesis nos centraremos en las motoneuronas somáticas, que a su vez pueden pertenecer a dos tipos, las extrafusales o motoneuronas-α y las intrafusales o motoneuronas-γ, asociadas con el movimiento voluntario y el mantenimiento del tono muscular, respectivamente.

Como ya hemos comentado previamente, las motoneuronas no se encuentran aisladas sino que se comunican bien enviando señales al músculo, bien recibiendo información de otras neuronas (principalmente interneuronas e incluso de otras motoneuronas).

El circuito neural más sencillo que se puede encontrar en el ser humano es de tipo monosináptico, como el caso del tipo de comunicación que da lugar al reflejo de estiramiento. Este reflejo monosináptico Ia involucra una neurona aferente que parte del huso neuromuscular, especializado en captar el estiramiento muscular, y que se comunica mediante una única sinapis, con una motoneurona-α que va a generar una respuesta en forma de potenciales de acción que inducirán una contracción muscular. Un ejemplo claro es el reflejo rotuliano (descrito por primera por *William Erb* en 1875), en el que un golpe en el tendón rotuliano desencadena una contracción del cuádriceps; este reflejo puede ser evaluado mediante electromiografía, lo que desencadenará en una señal con forma de onda conocida como onda H.

La onda H (H-wave en inglés) es conocida así por la inicial de su descubridor, el fisiólogo alemán *Johann Hoffman*. Cuando se estimula un nervio durante el reposo, y se incrementa la intensidad de estimulación progresivamente, se puede observar la aparición de esta onda (H-wave). La misma tiene una latencia mayor que la onda muscular (onda M), dado que la onda H viaja por la aferente desde el punto de estimulación y luego por la motoneurona, mientras que la onda M se registra en el músculo producto de la propagación ortodrómica del axón motor estimulado. La

dinámica de crecimiento de ambas ondas con la intensidad de estimulación se explica por dos elementos esenciales. El primero, es el distinto umbral de estimulación de la motoneurona y la aferente Ia; el segundo, el proceso de colisión entre la propagación ortodrómica y antidrómica por el axón de la motoneurona-α. Ello hace que a bajas intensidades de estimulación ambas ondas crezcan, pero que a partir de cierto nivel de estimulación ona onda H decrezca producto de las colisiones antidrómicas. La onda H es una buena opción de evaluación de la excitabilidad espinal si bien no refleja puramente la excitabilidad de la motoneurona al estar influenciada por distintos mecanismos presinápticos, como la inhibición presináptica [86].

La onda F es una alternativa a la evaluación de la excitabilidad mediante el reflejo H. Se llama así porque se observó por primera en músculos del pie (en inglés, *foot*) [87]. A nivel biológico es un artefacto ya que no se da en condiciones fisiológicas, sino que deriva de un potencial de acción antidrómico evocado a intensidad supramaximal en el axón de la motoneurona, que viaja en dirección inversa a la transmisión del potencial de acción convencional, y al alcanzar el cono axónico y soma celular desencadena una respuesta posterior ortodrómica que por tanto no está influenciada directamente por los inputs que le llegan a la motoneurona [88]. Debido a esto presenta características especiales con respecto a los otros potenciales evocados, esencialmente refleja la excitabilidad de unidades motoras grandes, y desde el pundo de vista metodológico su registro se basa en la evaluación de su persistencia, entendida como la cantidad de veces que se puede evocar con éxito [89].

La opción más directa para evaluar la excitabilidad motoneuronal se obtiene del uso de estimulación eléctrica o magnética en la decusación piramidal [90] [91] [92] [93] [86]. El estímulo evocado de esta forma recluta los axones del tracto cortico-espinal en la decusación de las piramides, de manera que el pulso ortodrómico activa de manera monosináptica a la motoneurona espinal, y de aquí sigue la trayectoria hasta el músculo, desencadenando una onda conocida como potencial evocado motor cervico-medular, debido al *locus* de estimulación [94]. El estímulo, tanto eléctrico como magnético puede reclutar otros tractos descendentes además del tracto cortico-espinal, sin embargo, hay pruebas de que el potencial evocado motor cervico-medular es principalmente evocado en el tracto cortico-espinal [95] [90] [94] [96] [86], ello se ha mostrado mediante registros a nivel epidural [97] [98] y experimentos de colisión

entre ondas [95] [99] [100] [90] [94] [96]. Dos características resaltan del potencial evocado motor cervico-medular, la primera es que es mayoritariamente monosináptico [101] [93]; y la segunda, que no está descrito que exista una inhibición presináptica de los axones cortico-espinales (que sí afecta a la onda H) [102] [103]. Es particularmente interesante en el contexto de esta tesis, que este tipo de estimulación genera un período de silencio electromiográfico si la estimulación se realiza durante una contracción voluntaria del participante.

Este período de silencio inducido por estimulación cérvico-medular es un segmento temporal de inactividad electromiográfica iniciado por un estímulo eléctrico o magnético a nivel del tracto cortico-espinal descendente y que aparece seguidamente al potencial evocado motor cervico-medular. De manera análoga al período de silencio inducido por estimulación cérvico-medular, existe un período de silencio evocado desde **M1**, que es de mayor duración y mediado por mecanismos diferentes [104]. El período de silencio presenta algunas características interesantes para el estudio de la fatiga; la primera característica es que es sensible a la misma. *Taylor et al.* demostraron que aumentando el *ciclo de trabajo*, aumentaba la duración del período de silencio (manteniendo la misma intensidad del estímulo evocador) [105]; la segunda es que no es sensible al grado de contracción, ya que al 30 % de la contracción máxima voluntaria puede ser más largo que al 100 % de la contracción máxima voluntaria [105], si bien sobre este fenómeno existe controversia. El mecanismo mediante el cual la motoneurona queda inhibida y silente es aún discutido a día de hoy; las dos principales propuestas abogan en primer lugar por una posthiperpolarización, propiedad intrínseca de la motoneurona y que tiene que ver con las propiedades excitatorias de membrana [106]; y en segundo lugar por una inhibición recurrente mediada por las interneuronas de *Renshaw* o por las interneuronas Ia [107] [108] [109] [104] [110] [111].

Exploración funcional de la corteza motora primaria y sus proyecciones cortico-espinales

La descripción de este punto conviene contextualizarla a partir de la temática de la tesis, y la consideración, clásica, de que la fatiga central se ha divido en espinal y supraespinal [5]. En este contexto, el concepto supraespinal de fatiga en la literatura se refiere a las cortezas motoras, esencialmente **M1**, y a la vía cortico-espinal[112]

[113] [114] [115]. Existen sin embargo otras vías descendentes con origen supraespinal que podrían estar relacionadas con la fatiga (e.g.- vías rubro-espinal, tecto-espinal, reticulo-espinal, etc.) pero que probablemente debido a la dificultad en su estudio con las técnicas disponibles, se las mantiene en un discreto segundo plano.

Desde el punto de vista anatómico, la corteza cerebral es una capa de materia gris que envuelve la parte más craneal del sistema nervioso central. Ésta se encuentra plegada sobre sí misma para aumentar su superficie dentro del espacio reducido del cráneo, y por tanto su conectividad y capacidad de procesamiento. En el *giro pre-central*, anterior al *surco central* y posterior al *surco pre-central*, se encuentra **M1**, íntimamente relacionada con otras dos áreas esenciales en la motricidad humana, el área *premotora* (que incluye una parte *dorsal* y una *ventral*) y el área motora suplementaria. Otro nombre que recibe **M1** es área 4 de *Brodmann*, debido a una clasificación citoarquitectónica que la distingue de otras áreas.

M1 pertenece al neocórtex, el tipo de corteza cerebral de aparición más reciente. Se caracteriza por la existencia de seis capas superpuestas, siendo la más profunda la capa VI y la más superficial la capa I [116], existiendo distintos subniveles en algunas de las mismas. La corteza motora primaria se distingue de otras partes del neocórtex por su densidad, ya que a pesar de tener una cantidad celular similar a otras áreas [116], es bastante más gruesa (hasta un 60 % más que la *corteza visual primaria*), y por tanto más densa en su sustancia blanca. Ello le permite disponer de más espacio para las conexiones intracorticales, confiriéndole mayor adaptabilidad [117]. Además, **M1** presenta otras características en su disposición por capas; por ejemplo, la capa IV (granular) de **M1** se encuentra ausente [116] o muy disminuida [118] [119].

La corteza motora está compuesta en gran parte por un tipo especial de neuronas de gran tamaño conocidas como neuronas piramidales, llamadas así por su descubridor, don *Santiago Ramón y Cajal*, debido a la forma cónica de su soma [120] [121]. Están presentes entre las capas II-VI, pero principalmente en las capas III y V [122] [123], siendo aproximadamente el 10-20 % de las neuronas piramidales de la capa V las que proyectarán su axón a lo largo del tracto cortico-espinal descendente para conectar monosinápticamente con las motoneuronas espinales. Este tipo de neuronas, con conexión directa, se conocen también como cortico-motoneuronas [58]

[124] [125]; además, las colaterales de estos axones se distribuyen sólo por las capas V y VI, mientras que las de otras piramidales de la capa III se distribuyen de una manera muchísimo más variada, con colaterales cortas y largas por todas las capas [126] [127] [128] [129]. Las células piramidales presentan además una dendrita apical, y dendritas basilares, estableciendo hasta 60000 conexiones dendríticas cada neurona piramidal [130].

Las neuronas piramidales se encuentran organizadas en columnas más o menos definidas a través de las capas [131] [123] [132] [133], y especialmente patentes en la capa III [134], llegando hasta ella las dendritas apicales de las células piramidales de mayor tamaño de M1, las células de Betz de capa V, formando distintos haces con las dendritas apicales de capa III. Los mismos recibirán input a través de conexiones horizontales principalmente en capa II y III de distintas neuronas no piramidales de pequeño y mediano tamaño [134]. Los distintos conjuntos de neuronas piramidales de las distintas capas están diferentemente representados en distintos tractos corticales, si bien el tracto corticoespinal, de especial interés en esta tesis, se origina del conjunto de neuronas piramidales, desde las más pequeñas a las gigantes, y por tanto desde distintas capas [134]. Este tipo de organización parece además no estar vinculada sólo al tipo de neuronas, sino también al área corporal controlada por los distintos conjuntos neuronales, y no ser puramente morfológica sino también funcional. Estudios de resonancia magnética funcional [135] indican que la representación somatotópica característica de la corteza motora no se explica (especialmente en zonas de la corteza con representación de la mano y el antebrazo) por agrupaciones perfectamente limitadas en su bordes para una determinada parte corporal, sino que presentan una importante superposición entre ellas, que se configura probablemente como el sustrato que permita una adecuada interconexión para la realización de los movimientos más complejos y finos.

El resto de neuronas presentes en la corteza motora se agrupan dentro de las "neuronas no piramidales" [122] [123]; destacando la ausencia de las células estrelladas espinosas pequeñas, responsables de la formación de la capa IV en el resto de la neocorteza, y la presencia de las células estrelladas espinosas grandes, también conocidas como células en cesto, en las capas III y V; estas células en cesto presentan una actividad inhibitoria gabaérgica sobre las neuronas piramidales, siendo sus

axones principalmente horizontales [82]. También son numerosas las células espinosas de variable tamaño distribuídas a través de distintas capas, pero especialmente numerosas en subcapas de la capa III [134].

La mayor parte de las aferentes a las neuronas corticales son locales [136], de las cuales, la mayoría son inhibitorias. Las aferencias recibidas por **M1** se enmarcan en tres grandes grupos, *tálamo-corticales*, *córtico-corticales* y *transcallosas*. Aunque las aferencias *tálamo-corticales* están estrechamente vinculadas a la capa IV, disminuida en tamaño en la **M1** de los humanos, se han encontrado en todas las capas de la corteza motora, más densamente en la parte más basal de la III y la V [137] [122] [123]. Estas aferentes parecen conectar en su mayoría de manera directa con las neuronas piramidales de manera excitadora [138] [139] [127] [140]. Por su parte, las aferencias *córtico-corticales* parecen distribuirse a través de las capas II y III, permitiendo el input desde distintos niveles de la corteza [141], predominantemente de las áreas 1 y 2 (i.e., corteza sensitiva primaria), 5 (i.e., corteza parietal), y las cortezas motora suplementaria y pre-motoras [141]. Por último, las aferencias *transcallosas* se originan en la capa III de la **M1** contralateral, conectando dendríticamente en las capas más superficiales de **M1** [142] [122] [123].

Las proyecciones descendentes del tracto cortico-espinal (que se originan principalmente en **M1** áreas premotoras dorsales y ventrales, área motora suplementaria y áreas cingulares motoras) alcanzan la médula espinal tanto contralateral como ipsilateralmente [143]. Regulan distintas funciones entre las que se encuentran el control de los generadores centrales de patrones (*central patterns generators*), esenciales en múltiples actividades motoras repetitivas [143], a través de conexiones no directas sobre las motoneuronas espinales, las cuales sí se producen de manera particularmente numerosa sobre motoneuronas espinales encargadas de controlar la musculatura digital, aunque también se pueden encontrar éstas últimas destinadas al control de músculos más proximales y en conexiones destinadas al control de los miembros inferiores [144] [145] [146] [147].

Las conexiones directas se encuentran en primates, pero no en otros mamíferos. Parecen de especial importancia para el control independiente de los movimientos de los distintos dedos. Se originan en la subcapa más profunda de la capa V, donde la proporción de neuronas piramidales gigantes es más marcada [143]. Por tanto,

las conexiones cortico-motoneuronales (directas) parecen circunscritas únicamente a la corteza motora primaria, de entre las distintas áreas motoras corticales. Pueden, sin embargo, ser moduladas desde cortezas motoras secundarias mediante conexiones cortico-corticales (muy abundantes en las capas II y III), que alcancen la célula gigante de la capa V de **M1** a través de su dendrita apical [143]. Al margen de estas conexiones motoneuronales, la excitación descendente desde las cortezas cerebrales también se lleva a cabo mediante conexiones indirectas, mediante interneuronas segmentarias e interneuronas propioespinales, especialmente en el caso de áreas motoras secundarias [148].

La exploración funcional de la corteza motora y del tracto cortico-espinal nace hace años. El primer potencial evocado motor fue obtenido mediante estimulación eléctrica de **M1** por *Merton* y *Morton* [149]. Esta técnica, conocida como estimulación eléctrica transcraneal resulta bastante molesta debido a la escasa conductancia del cráneo y a la elevada intensidad de estimulación necesaria para despolarizar los axones. Posiblemente impulsados por dichas limitaciones, *Barker et al.* desarrollaron un método mediante electromagnetismo que es capaz de solucionar este problema, conocida como estimulación magnética transcraneal [150], mediante la cual se consiguen despolarizar axones a través de un campo eléctrico inducido transcranealmente en el cerebro a partir de un campo magnético de alta intensidad y variable en el tiempo generado por el sistema de estimulación.

A pesar de que con la estimulación magnética transcraneal el potencial es inducido a nivel cortical, el tamaño del potencial evocado motor va a depender de un conjunto de propiedades de otras estructuras al margen de la corteza motora, y de la propia **M1**, y que de manera genérica y asumiendo la integridad en la propagación axónica son las siguientes:

- La excitabilidad de la corteza motora estimulada (dependiente de la excitabilidad de los distintos circuitos inhibidores y excitadores intracorticales).

- La excitabilidad de las motoneuronas espinales (y del equilibrio en las aferencias excitadoras e inhibidoras a nivel espinal).

- Las características de la transmisión neuromuscular.

Por otra parte, de manera análoga al período de silencio espinal inducido por estimulación cérvico-medular de los axones cortico-espinales, testado generalmente durante una contracción isométrica, la estimulación magnética transcraneal sobre **M1** también produce un periodo de silencio (cortical) en la actividad electromiográfica asociada a la contracción. El mismo es de importancia en el contexto de esta tesis debido a que con él se han evaluado mecanismos inhibidores corticales.

El período de silencio provocado por la estimulación magnética transcraneal de la corteza motora primaria va a tener un doble componente. La primera parte tiene un fuerte trasfondo espinal (cuyos mecanismos han sido previamente comentados [109] [111]) y la segunda va a ser cortical asociado a inhibición $GABA_B$ [110]. El mismo parece originarse a partir de la activación de interneuronas gabaérgicas en las capas II y III, especulándose que podrían ser interneuronas Golgi-II de largo axón las responsables de la inhibición de la célula piramidal [151] [152] [153] [154]. También ha sido descrito un aumento en su duración con posterioridad a la administración oral de inhibidores de la re-captación de $GABA$, o el uso de blacofeno intratecal (un agonista de $GABA_B$) [155] [156]. En condiciones de ausencia de fatiga, la duración del periodo de silencio inducido por estimulación magnética de M1 aumenta con el tamaño del potencial evocado motor, por lo que se ha sugerido que en su origen estén involucradas las conexiones recurrentes de las propias colaterales de las células piramidales [157]; sin embargo, y sin que se excluya dicha posibilidad, estudios previos sobre la fisiología del periodo de silencio cortical indican que el mismo aumenta con el incremento de la intensidad de estimulación magnética, y continúa su incremento a intensidades en los que el potencial evocado motor alcanza un plateau [104], por lo que los mecanismos recurrentes a partir de la colaterales de las propias neuronas piramidales no explicarían completamente la presencia del silencio. Lo mismo ocurre con la posibilidad de que el periodo de silencio esté mediado por mecanismos aferentes generados a partir de la contracción muscular, dado que la fuerza evocada por la contracción con el incremento de la intensidad de estimulación deja de aumentar cuando aún continúa haciéndolo el periodo de silencio [104].

Modificaciones en la excitabilidad en el sistema motor central producto de la fatiga

Si bien existen más técnicas de evaluación de la excitabilidad cortical, cortico-espinal y espinal, las introducidas hasta este momento han sido las más comúnmente utilizadas en el estudio de la fatiga del sistema motor. Las mismas (tal y como se refleja en las Tablas 1.1 y 1.2), han sido principalmente utilizadas para explorar la fatiga producida durante contracciones isométricas, hallándose un vacío importante en relación al conocimiento de las expresiones de la fatiga central cuando la misma se induce por movimientos repetitivos (tal y como se refleja en la Tabla 1.3), aún a pesar de su importancia en las actividades de la vida diaria.

Tabla 1.1: Fisiología de la fatiga inducida por contracciones máximas isométricas

Tarea	Variable/Momento de la evaluación	Comportamiento	Artículo
iCMV abducción de PDI durante 90 s	PAMC durante la tarea	Disiminuye, pero no significativamente	[158]
	PEM durante la tarea	Aumenta	
	EMT-PS	Aumenta poco pero no significativamente	
iCMV dorsiflexión durante 2 min	PEM durante la tarea	Crece pero no significativamente	[159]
	cPS durante la tarea	Crece hasta alcanzar un techo	
iCMV flexores de codo durante 2 min al 100 %	Área del PEM durante la tarea	Aumentó hasta alcanzar una meseta a los 45 s	[105]
	EMT-PS durante la tarea	Aumentó hasta alcanzar una meseta a los 30 s	
	PEMC durante la tarea	No cambió	
iCMV flexores de codo durante 2 min al 30 y al 64 %	Área del PEM durante la tarea	Aumentó menos que al 100 %CMV hasta alcanzar una meseta a los 45 s	
	EMT-PS durante la tarea	No aumentó al 30 % pero sí al 64 %, más lentamente que al 100 %	
	PEMC durante la tarea	No cambió	
iCMV flexores de codo durante 3 min	FSIEA durante la tarea	Fue creciendo a lo largo de la tarea	[14]
	FSIER tras la tarea	Disminuyó a lo largo de la tarea hasta el 33 %	
	EMT-FSIEA	Va creciendo conforme progresa la tarea	
iCMV flexores de codo durante 2 min	Área de la PAMC tras la tarea	Aumenta	[94]
	Área del PEMC tras la tarea	Disminuye	
	PEMC normalizado respecto a la PAMC tras la tarea	Disminuye	
iCMV flexores de codo durante 2 min	EMT-Superimpossed Twitch durante la tarea	Aumenta	[160]
	Área de la PAMC durante la tarea	Aumenta	
	Área del PEM durante la tarea	Aumenta hasta casi duplicarse	
	EMT-PS durante la tarea	Aumenta	
	ENP-PS durante la tarea	Aumenta	
iCMV flexores de codo durante 2 min	PEMC/PAMC tras la tarea	Disminuye a los 2 s de acabar la tarea, se recupera en 2 min	[161]
	PEM/PAMC tras la tarea	Tarda más en deprimirse y hasta 10 min en recuperarse	
iCMV flexores de codo durante 2 min	PAMC durante la tarea	Aumentó al principio de la contracción y luego empezó a disminuir (encima de niveles basales)	[162]
	PEMC durante la tarea	Aumentó pero luego disminuyó a niveles basales	
	PEMC/PAMC durante la tarea	Disminuyó desde el principio	
iCMV extensores de codo durante 2 min	Área de la PAMC durante la tarea	Se incrementó y empezó a disminuir al final de manera no significativa	[163]
	Área del PEMC normalizando a la PAMC	Disminuyó	
iCMV flexores de codo durante 2 min	Área del PEM durante la contracción	Se incrementó durante la contracción	[164]
	Área del PEMC durante la contracción	Empezó a crecer pero luego disminuyó	
Contracción isométrica superpuesta (18 Hz supramaximal) en músculos tenares durante 90 s	Persistencia de la onda F durante la tarea	Aumentó en los 10 primeros segundos (60-76 %) y luego disminuyó (76-33 %)	[165]

CMV: Contracción máxima voluntaria, PAMC: Potencial de acción muscular compuesto, PEM: Potencial Evocado Motor, EMT: Estimulación magnetica transcraneal, PS: Periodo de silencio, PEMC: Potencial evocado motor cervico-medular, FSIEA: Fuerza superimpuesta por la estimulación en activación, FSIER: Fuerza superimpuesta por la estimulación en reposo, ENP: Estimulación nerviosa percutánea, PDI: Primer dorsal interóseo

Tabla 1.2: Fisiología de la fatiga inducida por contracciones submáximas isométricas

Tarea	Variable/Momento de la evaluación	Comportamiento	Artículo
THFT abducción del primer dedo al 20, 35 y 60 %CMV	Amplitud y área de la PAMC tras la tarea	La amplitud disminuyó (más conforme menor era la intensidad, pero mayor el THFT), el área no	[166]
	FSIER tras la tarea	Disminuyó (más conforme menor era la intensidad, pero mayor el THFT)	
	EMG durante la tarea	Aumentó con la tarea, al 60 %CMV alcanzó una meseta	
	CMV tras la tarea	CMV disminuyó (más conforme menor era la intensidad, pero mayor el THFT)	
THFT flexión plantar al 30 %CMV	Onda-H/PAMC durante la contracción	Aumentó a lo largo de la tarea	[167]
	EMG durante la tarea	Aumentó a lo largo de la tarea	
THFT flexión de codo isométrica al 20 %CMV	EMG durante la tarea	Va creciendo, más conforme se acerca la claudicación	[168]
	PAMC durante la tarea	Disminuye muy levemente	
	EMT-PEM durante la tarea	Aumenta hasta duplicarse y se estabiliza en la segunda mitad de la tarea	
	EET-PEM durante la tarea	Aumenta hasta quintuplicarse	
	EMT-PS durante la tarea	Aumenta durante la segunda mitad de la tarea	
	EET-PS durante la tarea	No cambia	
THFT abducción del primer dedo al 25 y 50 %CMV, comparando con una contracción intermitente del mismo tiempo al 25 %CMV con 6 s de contracción 4 s de descanso	Onda H durante la tarea (Onda V)	Disminuye en las tareas sostenidas, no en la intermitente	[169]
	Onda H tras la tarea	Se recupera a los 5 min	
	PAMC durante la tarea	Disminuye en las tareas sostenidas, no en la intermitente	
	PAMC tras la tarea	Se recupera a los 5 min	
	EMG durante la tarea	Fue creciedno a partir de la mitad de la tarea	
THFT flexión de codo al 15 %CMV con CMV intercaladas (y ligeros reposos para evaluar FSIER)	EMG durante la tarea	Aumentó del 7 al 20 % del EMG máximo	[115]
	CMV durante la tarea	Disminuyó al 58 %	
	EMT-FSIER durante la tarea	Se duplicó en los primeros 20 minutos	
	FSIER durante breves reposos	Disminuyó del 97 al 77 %	
	EMT-AV durante CMV	Disminuyó al 58 %	
	AV durante CMV	Disminuyó del 98 al 71 %	
	EMT-PS durante la tarea y CMV	Aumentó consistentemente	
	EMT-PEM/PAMC durante la tarea y CMV	Aumentó para el bíceps pero no para el braquioradial	
THFT flexión de codo al 5 %CMV con CMV intercaladas (al 100, 75 y 50 %CMV) y ligeros reposos para evaluar FSIER	EMG durante la tarea	Aumentó al 60-80 %	[170]
	FSIEA durante CMV	Disminuyó al 72 % del control	
	FSIER durante reposo	Disminuyó	
	EMT-PEM/PAMC durante tarea	Muestra un aumento progresivo	
	AV	Disminuyó al 90 %	
	EMT-AV	Disminuyó al 90 %	

THFT: Tiempo hasta fallo en la tarea, CMV: Contracción Máxima voluntaria, PAMC: Potencial de acción muscular compuesto, FSIER: Fuerza superimpuesta por la estimulación en reposo, EMG: Electromiografía, PEM: Potencial Evocado Motor, EMT: Estimulación magnetica transcraneal, VA: Activación voluntaria, PS: Periodo de silencio, EET: Estimulación eléctrica transcraneal, FSIEA: Fuerza superimpuesta por la estimulación en activación

Tabla 1.3: Fisiología de la fatiga inducida por movimientos repetitivos

Tarea	Variable/Momento de la evaluación	Comportamiento	Artículo
Golpeo repetitivo del dedo a máxima frecuencia voluntaria durante 20 segundos (una serie)	Frecuencia de golpeo durante la tarea Amplitud de movimiento durante la tarea iMVC tras la tarea EMG durante la tarea	Disminuye hasta el 73 % Sin cambios Sin Cambios Cambio a un patrón de co-contracción	[29]
Golpeo repetitivo del dedo a máxima frecuencia voluntaria y a frecuencias submáximas durante 50 ciclos (3 series)	Frecuencia de golpeo durante la tarea EMT-PEM tras la tarea	Disminuyó durante las máximas Aumentó justo tras la tarea	[30]
Golpeo repetitivo del dedo a máxima frecuencia voluntaria y a frecuencias submáximas durante 10 segundos	Frecuencia de golpeo durante la tarea EMT-PEM tras la tarea	Disminuyó durante las máximas Disminuyó tras la tarea, y más cuanto menos máximo fuera	[31]
Golpeo repetitivo del dedo a máxima frecuencia voluntaria durante 10 segundos	Frecuencia de golpeo durante la tarea EMT-PEM tras la tarea	Disminuyó Disminuyó tras la tarea	[32]
Cicloergometría al 80 % de la potencia pico hasta la claudicación(fatigante) o durante 45 s (no fatigante)	PEM/PAMC durante la tarea PEMC/PAMC durante la tarea VA tras la tarea	Aumentaron cuando no era fatigante pero se redujeron a niveles basales cuando era fatigante Aumentaron cuando no era fatigante pero se redujeron a niveles basales cuando era fatigante Disminuyó tras la fatigante	[33]

CMVi: Contracción máxima voluntaria isométrica EMG: Electromiografía, EMT: Estimulación magnetica transcraneal, PEM: Potencial Evocado Motor, PAMC: Potencial de acción muscular compuesto, VA: Activación voluntaria

1.5. Técnicas de estimulación cerebral no invasiva para la intervención en la fatiga del sistema motor

La sociedad internacional de neuromodulación define la misma como: *"The alteration of nerve activity through targeted delivery of a stimulus, such as electrical stimulation or chemical agents, to specific neurological sites in the body."*. Dentro de las técnicas de neuromodulación, las que nos competen en esta tesis son las de estimulación cerebral no invasiva. Por estimulación cerebral no invasiva se entiende un conjunto de procedimientos que permiten intereactuar con el tejido nervioso, y que en función de las características de la técnica y del protocolo, posibilitan la evaluación funcional de estructuras, circuitos y redes neuronales, o la modificación de su excitabilidad. Algunas de estas técnicas ya han sido mencionadas en apartados anteriores, aquellas con un objetivo evaluador.

Entre las técnicas de estimulación cerebral no invasiva destinadas a modificar la excitabilidad destacan, principalmente debido al número de estudios realizados con las mismas, la estimulación magnética transcraneal aplicada de modo repetitivo, y la estimulación transcraneal por corriente directa. Existen más técnicas, algunas con una introducción mucho más reciente (como la estimulación con campos magnéticos estáticos [171] [172] [173] [174], la estimulación transcraneal con corriente alterna, o la estimulación con ultrasonidos focalizados), aunque han sido la estimulación transcraneal por corriente directa y la estimulación magnética transcraneal repetitiva las aplicadas para intervención sobre la fatiga. Si bien el efecto final "*global*" tanto de la estimulación magnética transcraneal repetitiva como de la estimulación transcraneal por corriente directa se expresa en un aumento o disminución de la excitabilidad cortico-espinal más allá del periodo de estimulación, y que revierte en el tiempo [175], los mecanismos por los que operan ambas técnicas son diferentes. La estimulación transcraneal por corriente directa ha demostrado modificar la excitabilidad cortical en humanos; este efecto se asocia principalmente con la modificación del potencial de membrana a nivel neuronal, acercándolo o alejándolo del umbral de disparo de las neuronas, pero no induciendo directamente potenciales de acción. Depende de varios factores, destacando en primer lugar la localización de los electrodos de estimula-

ción, la polaridad, la intensidad y el tiempo de estimulación [176] [177] [178] [179]. Además, la estimulación transcraneal por corriente directa presenta efectos posteriores a la estimulación; estos post-efectos están asociados, entre otros, con la síntesis protéica [180], los niveles de adenosín mono-fosfato cíclico [181] y calcio intracelular [182] de manera similar a lo acontecido en la potenciación y la depresión a largo plazo [183].

Inicialmente se describió que la polaridad sería determinante en sus efectos y que la estimulación transcraneal por corriente directa anódica aumentaría la excitabilidad mientras que la estimulación transcraneal por corriente directa catódica la disminuría [176] [183], aunque actualmente se conoce que el panorama es más complejo. Ya partiendo de estudios en rodajas aisladas de tejido cerebral en los que se encontró el efecto de la estimulación opuesto al inicialmente planteado [184], está actualmente establecido que las respuestas a la estimulación transcraneal por corriente directa anódica y catódica dependen no sólo de la polaridad sino también del tiempo y la intensidad de estimulación [185], y también (al igual que de otras técnicas de estimulación cerebral no invasiva) del estado de excitabilidad cerebral al recibir la estimulación (*stated-dependent response*) [186]. Por otra parte, incluso si es aplicada a nivel cortical, la estimulación transcraneal por corriente directa ha demostrado modular diferentes circuitos espinales tanto a nivel de miembros superiores como inferiores [187] [188] [189] [190], lo que la convierte en una técnica interesante dado que, como se ha presentado, los cambios de excitabilidad producto de la fatiga motora se localizan de manera importante en la médula espinal.

Los mecanismos de acción de la estimulación magnética transcraneal repetitiva, como se ha comentado, difieren de los de la estimulación transcraneal por corriente directa y parecen operar mediante cambios en la excitabilidad de la neurona post-sináptica mediados, en gran medida, por receptores *NMDA*, y que llevan a las neuronas al umbral de disparo, generando potenciales de acción [191] [192]. Al igual que en el caso de la técnica anterior, los efectos globales de la estimulación magnética transcraneal repetitiva se agrupan en aquellos protocolos que aumentan la excitabilidad cortical (cortico-espinal) y los que los disminuyen. Es importante sin embargo tener en cuenta que es posible que existan cambios en la excitabilidad de ciertos circuitos corticales producto de la estimulación cerebral no invasiva que podrían no

manifestarse en cambios en las características de los potenciales evocados motores, dado que el efecto en dichos circuitos podrían ser compensados por otros circuitos mediante procesos de metaplasticidad que garantizasen la homeostasis del sistema. Lo que parece evidente es que el uso de las técnicas de estimulación cerebral no invasiva es una posibilidad atractiva de cara a poder intervenir (revertir, o elentecer) en el desarrollo de la fatiga del sistema motor. La Tabla 1.4 muestra un resumen de los trabajos más destacados mediante el uso de la estimulación cerebral no invasiva sobre manifestaciones de la fatiga muscular inducida por contracciones isométricas máximas y submáximas, mientras que la Tabla 1.5 presenta los efectos de dichas técnicas sobre la fatiga producida por movimientos realizados repetitivamente.

Tabla 1.4: Efectos de las estimulación cerebral no invasiva en la fatiga inducida por contracciones isométricas máximas y submáximas (en cursiva aquellos estudios sobre los efectos en rendimiento motor en ausencia de fatiga)

Tarea	Protocolos de estimulación	Efecto	Artículo
CMVi pinza pulgar-índice	*EMTr* $1\,Hz$ *durante* $15\,min$ $115\,\%$*UMR sobre M1 derecha*	*Disminuyó PEM, no afectó CMVi*	[193]
CMVi pinza pulgar-índice (dedos pie)	EtCD-A sobre M1 y cátodo orbitofrontal y EtCD-P; $2\,mA$ $15\,min$	*Aumentó CMVi*	[194]
CMVi pinza pulgar-índice en supervivientes de ACV	*EtCD-A sobre M1 y cátodo orbitofrontal y EtCD-P*; $1\,mA$ $20\,min$	*Disminuyó TR, mejoró CMVi en supervivientes muy afectados de ACV*	[195]
CMVi pinza pulgar-índice	EMT pulso pareado (IEE $1,5\,ms$) $0{,}2\,Hz$ durante $15\,min$ sobre M1	Menor pérdida de fuerza en CMVi de $10\,s$	[175]
CMVi y THFT flexores de codo 35 %CMVi	EtCD-A, EtCD-C y EtCD-P; activo sobre M1 y referencia extracefálico; $1{,}5\,mA$ $10\,min$	EtCD-A Aumentó el THFT; no afectó CMVi	[196]
THFT flexores de codo 20 %CMVi	EtCD-A sobre M1 y cátodo orbitofrontal y EtCD-P; $1,5\,mA$ $20\,min$	Aumentó el THFT	[197]
THFT flexores de codo 30 %CMVi	EtCD-A sobre M1 y cátodo extracefálico y EtCD-P; $2\,mA$ $10\,min$	No aumentó el THFT ni la CMVi	[198]
THFT flexores de codo a 12,5 %, 25 %, 37,5 %, and 50 % CMVi	EtCD-A sobre M1 y cátodo orbitofrontal y EtCD-P; $2\,mA$ $10\,min$	EtCD-A al 37,5 y 50 % CMVi aumentó la relación EMG/torque y la CMVi	[199]
THFT extensores de rodilla 20 %CMVi	EtCD-A sobre M1 y cátodo orbitofrontal o extracefálico y EtCD-P; $2\,mA$ $10\,min$	Aumentó el THFT, más el extracefálico que el órbitofrontal	[200]
THFT flexores de codo 20 %CMVi	EtCD-A sobre M1 y cátodo orbitofrontal y EtCD-P; $1,5\,mA$ $20\,min$	Aumentó el THFT	[201]
THFT flexores de codo 35 %CMVi	EtCD-A sobre M1 y cátodo extracefálico y EtCD-P; $1{,}5\,mA$ $10\,min$	Aumentó el THFT	[202]

CMVi: Contracción máxima voluntaria isométrica, EMT: Estimulación magnética transcraneaL, EMTr: Estimulación magnética transcraneal repetitiva, UMR: Umbral motor de reposo, M1: Corteza motora primaria, PEM: Potencial Evocado Motor, EtCD-A: Estimulación transcraneal por corriente directa anódica, EtCD-P: Estimulación transcraneal por corriente directa placebo, TR: Tiempo de reacción, ACV: Accidente cerebro-vascular, EtCD-C: Estimulación transcraneal por corriente directa catódica, IEE: Intervalo entre estímulos, THFT: Tiempo hasta fallo en la tarea, EMG: Electromiografía

Tabla 1.5: Efectos de las estimulación cerebral no invasiva en la fatiga inducida por movimientos repetidos

Tarea	Variable/Momento de la evaluación	Comportamiento	Artículo
Golpeo repetitivo del dedo a máxima frecuencia	EMTr sobre M1 durante CMV (agarre) [6 Hz durante 5 s, repetidos cada 30 s hasta llegar a 10 min (80 % de la CMV)]	No aumentó la frecuencia de golpeo Disminuyó la excitabilidad del PEM	[203]
Tres series de golpeo repetitivo del dedo a máxima frecuencia durante 4 s separadas por 15 s de descanso	EMT pareada (1,5 ms entre el primer pulso y el segundo) sobre M1 a intervalos 5 $segundos$ durante 15 min (intensidad para generar un PEM de 1 mV)	La disminución de la frecuencia de golpeo fue menor cuando había estimulación La excitabilidad corticoespinal fue aumentando serie a serie más cuando había estimulación	[204]
Test de incremento de potencia en cicloergometría	EtCD-A orbitofrontal sobre Temporal izquierda durante 20 min a 2 mA	Aumentó el pico de potencia un 4 %; EEP se incrementó más lentamente; FC se redujo a cargas submáximas; EEP y FC máximas no cambiaron	[205]
THFT al 80 % W en Cicloergometría	EtCD-A, EtCD-C, EtCD-P bilateral durante 13 min a 2 mA	EtCD-A aumentó el THFT, sin cambios en el EMG, el EEP, o el FC	[206]

EMT: Estimulación magnetica transcraneal repetitiva, M1: Corteza motora primaria, CMV: Contracción máxima voluntaria, PEM: Potencial Evocado Motor, EtCD-A: Estimulación transcraneal por corriente directa anódica, EEP: Escala de esfuerzo percibido, FC: Frecuencia cardíaca, EtCD-C: Estimulación transcraneal por corriente directa catódica, EtCD-P: Estimulación transcraneal por corriente directa placebo, EMG: Electromiografía, THFT: Tiempo hasta fallo en la tarea

Como queda de manifiesto en la Tabla 1.4, el uso de las técnicas de estimulación cerebral no invasiva en el manejo o prevención de la fatiga (en poblaciones fisiológicas) se ha desarrollado principalmente utilizando modelos y tareas isométricas como inductoras de fatiga. Ello probablemente se debe al hecho de que las bases fisiológicas de la fatiga durante este tipo de contracciones musculares se comprenden mejor, mientras que el conocimiento de las bases centrales de la fatiga producida por movimientos repetidos/repetitivos es muy inferior, ver Tabla 1.5. El conjunto de experimentos y publicaciones que se presentan como parte de esta tesis doctoral aborda esta última problemática. El primer artículo del compendio, cuyos experimentos se llevaron a cabo en los años 2013-2014 describirá el efecto de la estimulación transcraneal por corriente directa aplicada bilateralmente sobre **M1** en la fatiga desarrollada durante la ejecución repetida de movimientos de alcance a máxima velocidad. Posteriormente se llevaron a cabo otros experimentos durante los años 2014 y 2015 donde se evaluaron, de una manera más precisa, las características de la fatiga sobre una tarea de golpeo repetitivo del segundo dedo de la mano dominante. Un elemento que es importante resaltar, de cara a la compresión de los procedimientos llevados a cabo en este periodo, es el hecho del cambio de tarea entre la primera publicación del compendio (movimientos de alcance realizados repetidamente) y las dos últimas (movimientos repetitivos del dedo). El motivo para ello fue la necesidad de acotar el estudio a las bases centrales de la fatiga del sistema motor durante las actividades repetidas. Hay que tener en cuenta que el movimiento de alcance tiene una cierta complejidad cognitiva [207] [208], por lo que el repetitivo del dedo nos permitió una mayor focalización en los aspectos de naturaleza motora de la actividad. Por motivos editoriales, las fechas de publicación no coinciden con la cronología de los experimentos.

Capítulo 2

Objetivos e hipótesis

Objetivo general de los estudios

- Conocer las bases centrales de la fatiga muscular cuando la misma es inducida por movimientos repetidos

Objetivo del primer estudio, titulado: Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Task

- Evaluar los efectos de la estimulación transcraneal por corriente directa aplicada bilateralmente sobre **M1** en el movimiento de alcance ejecutado repetidamente a máxima velocidad en un protocolo de tiempo de reacción.

Hipótesis

- La estimulación transcraneal por corriente directa modificará el comportamiento motor en función de la polaridad de estimulación, bien aumentándolo o evitando su merma a lo largo de la tarea (fatiga).
- La estimulación transcraneal por corriente directa anódica sobre la **M1** contralateral al brazo movilizado, como resultado de la excitabilidad aumentada, provocará respuestas más rápidas o evitará la aparición de la fatiga.
- La aplicación de la estimulación transcraneal por corriente directa catódica sobre la **M1** contralateral al brazo movilizado, al reducir su excitabilidad, inducirá resultados opuestos a la estimulación transcraneal por corriente directa anódica.

Objetivo del segundo estudio, titulado: Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of Silent Period evoked at spinal and supraspinal levels

- Evaluar las expresiones del decremento motor durante distintas tareas fatigantes de naturaleza repetitiva e isométrica, pero iguales en duración e intensidad del esfuerzo.
- Evaluar mecanismos inhibidores corticales y espinales subyacentes a la fatiga producida por movimientos repetitivos rítmicos y contracciones isométricas.

Hipótesis

- El rendimiento motor del golpeo repetitivo del dedo ejecutado a máxima frecuencia de golpeo disminuirá de manera distinta que el de una contracción isométrica máxima de la misma duración e intensidad del esfuerzo.
- Los cambios en la excitabilidad de los circuitos inhibidores espinales y de **M1** diferirán en tareas breves de máximo esfuerzo y naturaleza repetitiva (golpeo repetitivo del dedo) o isométricas.

Objetivo del tercer estudio, titulado: Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks

- Examinar el balance excitador-inhibidor a nivel espinal durante la fatiga generada por tareas de golpeo repetitivo del dedo a máxima frecuencia e isométricas máximas teniendo en cuenta los posibles cambios de la excitabilidad muscular.

Hipótesis

- El balance excitador-inhibidor de las motoneuronas espinales será distinto para las tareas de máximo esfuerzo de golpeo repetitivo del dedo e isométricas breves.
- La excitabilidad muscular se modificará de distinta manera para las tareas de máximo esfuerzo de golpeo repetitivo del dedo e isométricas breves.

Chapter 3

Experimental procedures

3.1. Paper 1 - Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Tasks

Ethical approval

All experimental subjects signed consent forms. The protocol conformed to the declaration of Helsinki and was approved by the Ethics Committee of the University of A Coruña (Spain). The individuals whose experimental data were included in this manuscript have given written informed consent allowing the use of photographs to illustrate the figures.

Subjects

Thirteen healthy subjects participated (seven male and six female, age range $20 - 37\,yrs$). None took medication or undertook hard physical work in the week prior the experimental sessions. Subjects were right-handed [209] and had normal or corrected-to-normal vision.

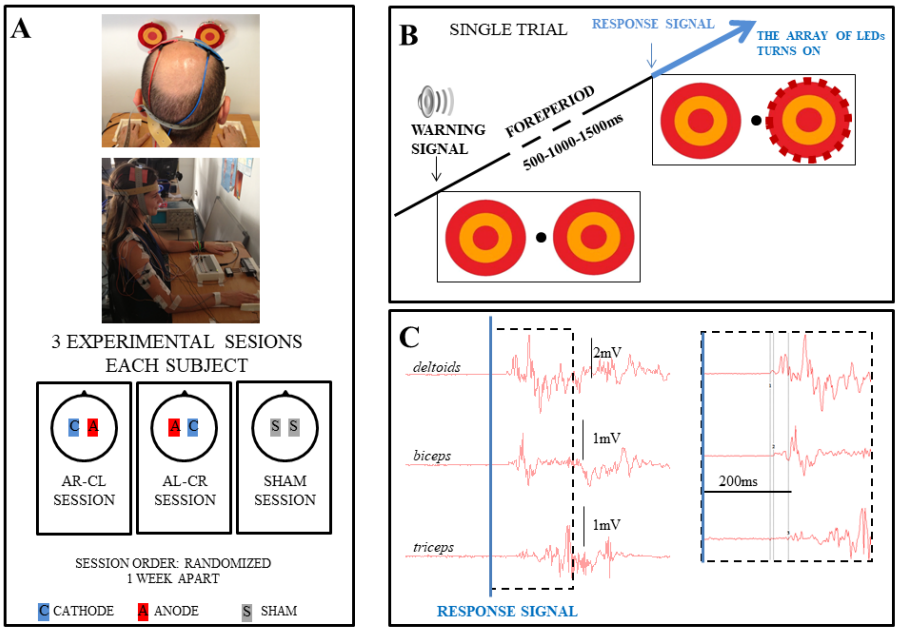

Figure 3.1: **(A)** Experimental setting and tDCS electrode montage in the 3 experimental sessions. The pictures show two subjects receiving tDCS at rest. **(B)** A single trial lasted 10 s; the response signal was presented 500, 1000 or 1500 ms after the warning cue. **(C)** Example of one recording reflecting the sequential activation of the three muscles evaluated. Recordings are synchronized to the response signal (marked as the blue vertical line). The dashed area is enlarged at the right to clarify the sequential muscle responses. The individuals in these pictures have given written informed consent (as outlined in PLOS consent form) to publish the images.

Procedure

Each subject performed three sessions, one week apart. In each session they executed the reaching tasks before and after the transcranial Direct Current Stimulation (tDCS) (*Pre* and *Post*, respectively). A different tDCS protocol was applied in each session; the order was randomized. Subjects reached to one of two round targets placed at gaze height in front of them (See Figure 3.1(A), top). Reaching was always performed as fast as possible with the right (dominant) hand on a frontoparallel plane (See Figure 3.1(A), middle). No instructions were provided on how to touch the target, apart from asking subjects to touch the centre of the target with the hand as fast as possible. All subjects chose to touch with finger joints extended.

Each of the three sessions comprised three different reaching tasks randomized in order, and each task included several trials. In all cases a low-tone audible *warning signal* was presented as a cue prior to the *response-signal*. The turning-on of an array of red LED's at the edge of the target was the *response-signal*. Without any other purpose than making the response-signal time unpredictable (with regards to the warning cue) we used three foreperiods (delays) between warning and response signals; 500, 1000, or 1500 ms, and their presentation order was randomized within trials. The sequence of events was controlled by Signal-4 software via a CED 1401mkII (Cambridge Electronic Design, UK). Subjects were asked to fixate on a central point between the two targets until the appearance of the response-signal (See Figure 3.1) after which they made the response.

Tasks

- T1: Subjects were informed they had to reach the target ipsilateral to their dominant (executing) hand (i.e., *single-ipsilateral response*).
- T2: Subjects were told that they had to reach the target in the contralateral space to their dominant arm (*single-contralateral*).
- T3: In this task (*choice*) subjects were informed they would reach either the ipsilateral or the contralateral target to the dominant arm, depending on what target was lit (in randomized order).

Each of the two single-tasks (*single-ipsilateral* and *single-contralateral*) included 12 reaching trials, plus 1 catch trial, all randomized. In the catch trial the response signal did not appear after the warning cue.

The *choice*-task included 12 trials to the ipsilateral target (*choice-ipsilateral*) and 12 trials to the contralateral target (choice-contralateral) and these also included a catch trial. The 25 trials were randomized in order.

In all cases the inter-trial interval was 10 s, and a one minute rest was given between the different tasks.

Pre and *Post* testing lasted 12 min 10 s each; Post started 1 min after the end of the tDCS . The order of presentation of the three tasks in *Pre* was randomized, and reproduced in *Post*.

Experimental Setting

The round targets were 15 cm diameter; their centers were 32 cm apart from each other, and halfway between them a small black spot (1 cm-diameter) served as the fixation spot. The subjects were seated on an adjustable chair, containing chest-straps to avoid trunk movements, but allowing unrestrained shoulder movements. The chair height was adapted to make eye level at fixation height; the distance from the subject to the targets was adjusted such that allowing the reaching to the contralateral target with nearly full-elbow extension, minimizing leg involvement in the task [210]. Subjects' hands were on contact-plates. After setting, subjects made three fast arm reaching movements to each target, as practice. The entire setup (See Figure 3.1) was reproduced in each of the different test days.

Motor Outcomes

Signals were acquired by means of Biometrics-Data-Link, Digitimer D-360 amplifiers and the CED 1401 ($3 - 3000\,Hz$; $10\,KHz$ sampling rate, $1000\,gain$). Electromiography EMG recordings determined the Pre-Motor Time (PMT) [211], as the time lags from the *response-signal* to the movement-related EMG-onset [212]. EMG recorded the activity of the *deltoid, biceps brachii* and *triceps brachii*. Surface electrodes were placed in a belly-tendon montage on the *anterior deltoid*; *large biceps head*; and *lateral triceps head* (*del; bic; tri*), always after skin preparation. EMG-onset was determined automatically (and visually checked [213]) by applying the doublethreshold method [214]. Thus the EMG signals were rectified and the EMG onset was considered at the first of ten consecutive samples (one threshold) above a given EMG amplitude (the other threshold); the latter threshold was equal to the mean EMG-background activity amplitude plus one standard deviation, which was calculated in the time-window just 50 ms prior the response signal. Such threshold was obtained for each of the three muscles independently. The time elapsing from the LEDs flash to lifting the hand and leaving the contact-plate was computed as the Reaction Time (RT); and the time from leaving the contact-plate to touch the target determined the Motor Time (MT). Customized MatLab programmes (The Mathworks, Ltd) were used to process the data.

Brain Stimulation

tDCS was applied bilaterally on Primary Motor Cortex (M1)'s with a Neuroconn DC-Stimulator connected to a pair of $5 \times 7\, cm$ saline-soaked electrodes. In all sessions, one electrode was placed on the left M1, and the other on the right M1; corresponding to C3 and C4 of the International 10–20 Electroencefalography (EEG) system.

In one session the anode was on the left M1, and the cathode on the right M1, and this is referred as *AL-CR* montage. In other session the anode was on the right M1, and the cathode on the left M1 *AR-CL*. Sham montage randomized electrode positions. For real stimulation $1\, mA$-intensity was applied for $10\, min$ (current fade-in and out was ramped and lasted the initial and final $8\, s$). The Sham protocol lasted the same time but the current was applied for $30\, s$ and then ceased [215]. Subject remained restful during the stimulation sessions.

Statistical Methods

The mean of the responses for each experimental condition and subject was the outcome-value introduced in the analysis. The mean was computed considering all events from each experimental condition, but those which PMT $< 80\, ms$ or $> 800\, ms$; thus those events with PMT $< 80\, ms$ were considered anticipations, and their proportion in the different stimulation protocols, conditions and testing time-points (*pre* and *post*) were evaluated with the Fisher Exact Probability Test. The threshold of $80\, ms$ was set based on the latencies of a visual-evoked potential to a flashing LED recorded in the primary visual cortex (V1), plus the minimum latency for an interaction between V1 and M1, plus the latency from M1 activation to EMG onset on the studied muscles ([216],[217],[218]). Events with PMT $> 800\, ms$ were discarded as sign of un-attentiveness [219], though this happened just once in all subjects and conditions. PMT, RT and MT from events within those thresholds were normalized to control variability related to daily differences in the experimental setting (see above), though care was taken to minimize it. Normalization was performed as follows: For each session the average for each variable was calculated from data including *Pre* outcome-values for all tasks and subjects (See Table 4.1). This normalizing value was used to divide all subjects' responses at *Pre* and *Post* for the corresponding day. This normalization procedure respects inter-subject variability,

while normalizing the responses to the daily pooled *Pre*-testing.

After checking the normality of the distributions with a Kolgomorov-Smirnov test for one sample, an ANOVA with repeated measures analyzed the effect of tDCS on the variables considering the 13 subjects.

We have used two different ANOVA designs: one for the PMT and one for RT and MT. The former is a five factors ANOVA with STIM (three levels: *AR-CL*, *AL-CR*, and *Sham*); Time (two levels: *Pre* and *Post*); LATERALITY (two levels: Target *Ipsilateral* or *Contralateral* to the dominant–executing- hand); OPTION (two levels: *single* and *choice* responses) and MUSCLE (three levels: *deltoid*, *biceps* and *triceps*). The ANOVA on RT and MT had the same design except for the factor MUSCLE which was not included since RT and MT derived from contact plates.

The W-Mauchly test checked the sphericity for ANOVA, if sphericity was violated the ANOVA degrees of freedom were corrected by means of the Greenhouse-Geisser coefficients. Effect sides were calculated by partial eta and eta squared (η_p^2, η^2). Significance was considered if $p < 0{,}05$.

3.2. Paper 2 - Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of Silent Period evoked at spinal and supraspinal levels

Ethical approval

Experimental protocols complied with the Helsinki declaration and were approved by the University of A Coruña Ethics Committee. Subjects were screened for incompatibility with brain stimulation techniques and were medication-free during the week preceding testing. All subjects consented to participate.

Subjects

The experiment included two groups of subjects: the Transcranial Magnetic Stimulation (TMS)-group composed of nine right-handed healthy subjects (eight males and one female; age range 22–38 years), and the Cervico Medullary Stimulation (CMS)-group composed of twelve righthanded healthy subjects (all males; age range 18–41 years), each group underwent both *ft* and *Isometric Contraction (iso)* fatigue protocols.

Protocol

Each subject underwent two experimental sessions, at least one week apart, in randomized order. Sessions were identical but for the type of *task* executed. In one session, subjects were requested to perform index *Finger Tapping (ft)*, and, in the other session, continuous index finger *iso* against a force sensor, with the direction of the force "toward" *flexion* of the first metacarpophalangeal joint of the index finger. Subjects always wore a small goniometer on the index finger metacarpophalangeal joint, and a metal ring at the distal phalanx of the same finger. Subjects pressed or tapped over a thin metal plate placed on the force sensor.

For both *ft* and *iso* sessions, subjects executed the tasks in three different modes, and each mode was executed four times (four *sets*). Therefore, the subjects performed four sets at comfort rate/effort (*comfort* mode) for 30 s; then four sets of 10 s at maximal rate/effort (*10max* mode); and finally four sets of 30 s at maximal rate/effort (*30max* mode), always in this order. In all cases there was an inter-set rest

period of $1\,min\ 40\,s$.

For the *comfort-ft* subjects were asked to *"tap at their most comfortable rate without feeling fatigued"* for as long as the set lasted. In a previous paper [30], we observed that this *ft* mode is reliable, and performed at a pace of about 1/3 of the maximal rate. Because *comfort-ft* is linked to lower metabolic activity in the sensorimotor cortex compared to faster ($> 3\,Hz$) and slower ($< 1\,Hz$) rates ([220], [221]), its use seems to be adequate as a control condition to evaluate the fatigue induced by maximum *ft*. For the *comfort-iso* participants were asked to press $\approx 1/3$ Maximal Voluntary Contraction (MVC) with visual feed-back provided by means of online isometric force display. For maximal modes subjects were requested to tap/press as fast/hard as they could from the very beginning to the end of the set.

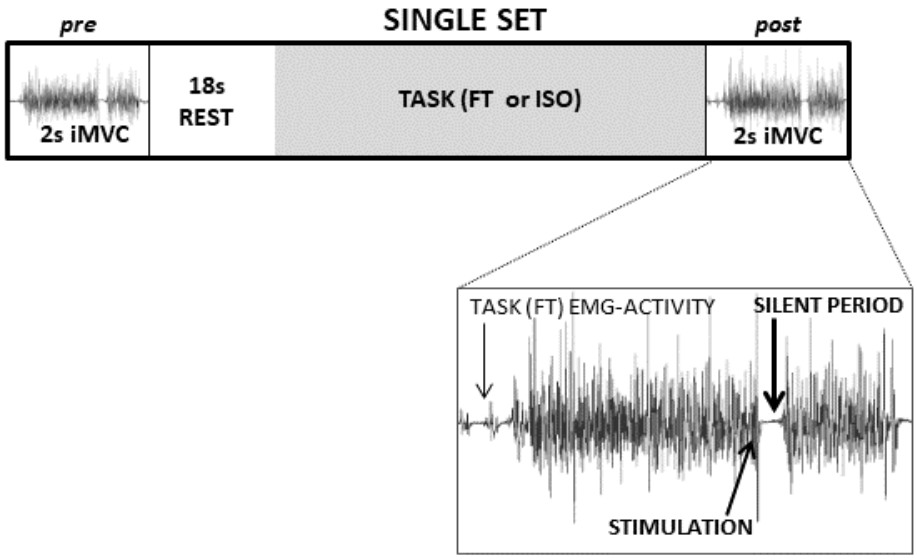

Figure 3.2: The set-structure: as soon as the LED was lit subjects performed an isometric Maximal Voluntary Contraction (iMVC) with their index against the dynamometer. The LED off ($2\,s$ after) served as a signal to stop the isometric Maximal Voluntary Contraction (iMVC). During the iMVC ($1{,}5\,s$ after LED on) subjects received an initial test stimulation (*pre*) (TMS in one group ($n = 9$); electric CMS in the other ($n = 12$). Stimulation induced subsequent silent periods, as shown in the enlarged area. After resting for $18\,s$, a LED flash indicated the start of the task (*ft* in one session; *iso* in the other). In continuation of the task, and with no resting time, the subjects performed another $2\,s$-iMVC in response to the LED turning-on, and received stimulation (*post*; in the same way as *pre*).

Fatigue was assessed as either the decrease in frequency or amplitude (for *ft*), or in force output (for *iso*). Central fatigue, either supraspinal or spinal, was evaluated by recording the changes in the SP duration in response to TMS or CMS [105]. The stimulation pulses were delivered during brief isometric MVCs (2 s-iMVC). The 2 s-iMVCs were performed before (*pre*) and immediately after (with no gap in between) task-execution (*post*), either for *ft* or *iso*, for all modes and sets (See Figure 3.2). The magnitude of the brief 2 s-iMVCs was also an analyzed variable for fatigue monitoring ([222]). An initial session was scheduled to allow some practice and answer all the subjects' questions about the experimental methods [5].

Setting, recording and stimulation protocols

The subjects were seated comfortably with the elbow flexed at 90–100º. The forearm, wrist, hand and all fingers except the index were firmly but comfortably fixed to a modified tablet-arm chair, allowing un-restrained degrees of freedom at the metacarpophalangeal joint of the index finger, permitting *ft*. During *ft*, a Biometrics DataLink system (Biometrics Ltd., Gwent, NP11 7HZ, UK) recorded the inter-tap intervals at $0{,}1\,KHz$ with a thin metal plate and a metal ring, the latter adapted to the distal phalanx of the index finger. It also recorded (at $500\,Hz$) the isometric force exerted during iMVC of the index flexion with a Pinch-Dynamometer (P200), which was placed flat and secured over the table, with the thin metal plate used to record tapping attached flat on its top. A single axis finger goniometer (F35) (sampled at $1\,kHz$) controlled the flexo-extension movement amplitudes of the index finger metacarpophalangeal joint. Electromyographic activity from the superficial head of the First Dorsal Interosseous (FDI) was monitored with surface electrodes in a belly-tendon arrangement, and acquired by means of D360 amplifiers (Digitimer, Welwyn Garden City, Herts), amplified (×250–1000) and band-width filtered between 3–3000 Hz. EMG were sampled at $10\,kHz$ and stored in a computer by means of a CED 1401 mkII Power A–D converter (Cambridge Electronic Design, Cambridge, UK). This device also controlled the on/off state of LED's (indicating the different phases of execution/rest within the sets) and timing of TMS/CMS pulses.

In separate sessions, in six subjects we recorded the level of *EMG* activation of the *FDI* and different intrinsic and extrinsic hand muscles during the *ft* and different *iso* tasks; i.e., index *iMVC*, and *iMVC* of the 3rd, 4th and 5th fingers working

together. Also, we recorded the *FDI* activation during different index-*iMVC* tasks applying force against immovable resistances in different directions, toward flexion (the same as above), abduction, adduction and extension. This was done using the same setting, hand position and fixation as during fatigue testing; and adapting immovable resistances to permit *iMVC* in the different planes. For this purpose the subjects executed two 3 *s*-trials/task, with task presentation order randomized. The inter-trial rest period was one minute.

The trial started with the recording of the Compound Muscle Action Potential (CMAP) of each muscle, which was acquired by supramaximal electrical stimulation at the Erb's point (1 *ms* electric pulses; EBNeuro Stimulator, Italy; cathode lateral, anode medial) with the muscle at rest; 10 *s* after stimulation the onset of a LED light indicated the start of 3 *s*-task. The Root mean square (RMS) of the CMAP was computed, which served as the divisor of RMS activity during task execution. For *ft*, we computed the *rms* within the tapping cycles (lasting about 150 *ms*, one per trial) with the highest frequency (shortest inter-tap interval). For the *iso* tasks, we identified the peaks (one per trial) in the force recordings, then the muscle activity was computed in the 150 *ms* around the peaks (75 *ms* before/after the corresponding peak in each trial).

TMS

During the sessions of fatigue evaluation, and for TMS-Silent Period (SP) generation, a Magstim 200^2 stimulator delivered monophasic wave-form pulses through a 70 *mm* figure of eight coil. The coil was positioned (and marked for reference) to induce currents in a posterioranterior direction, and placed over the hot-spot for the FDI muscle of the executing hand. The intensity was set to evoke a SP duration $\approx$ 150 *ms* during a 2 *s*-iMVC in the non-fatigued muscle (this produced TMS motor evoked potentials of about 50 % amplitude of the maximal CMAP); TMS intensity was expressed relative to the individual's active motor threshold, defined as the minimum intensity required to evoked five liminal responses (about 200 μV) in 10 consecutive pulses, in the activated muscle (5–10 % MVC) [223]).

CMS

CMS was applied using a Digitimer D180 stimulator connected to a pair of Ag–AgCl electrodes. Electrodes were placed behind the mastoid processes with the anode at the right and the cathode at the left. Active motor threshold was defined as described above for TMS [223]; then the stimulation intensity for the protocol was set (about 10 % above this threshold) to have a SP of $\approx 70\,ms$ in the un-fatigued muscle (this produced CMS motor evoked potentials of about 50 % amplitude of the CMAP). To make sure this intensity did not produce current spread to the spinal roots, the CMS motor-evoked potential latency was compared to that obtained at threshold intensity, such that the amplitude of the potentials increased in size with voluntary contraction with no or liminal latency shift when stimulated at the higher level [224].

Baseline unfatigued SP's durations for TMS and CMS setting were $\approx$ 150 and $70\,ms$ respectively, (CMS-SP is much shorter than TMS-SP; [104]. We set these values away from their ceiling to allow a potential modulation induced by fatigue. The mean TMS intensity applied was 17,9 % (Standard Error (SE) 1,0) above the Active Motor Threshold (AMT). The mean voltage used in the CMS experiments was $543{,}4\,V$ (SE 27,1).

Data reduction

The following dependent variables were analyzed:

- Level of muscle activation in intrinsic and extrinsic hand muscles:

  This is expressed as the percentage of the RMS of the CMAP, and defined as $100 \cdot \frac{TASK_{rms}}{CMAP_{rms}}$

In the fatigue sessions we studied the following variables:

- Motor output during tasks execution:

  We considered three measures of motor output for task execution: the tapping frequency, angular amplitude for *ft* and the force applied for *iso*. For each of the four Sets and three Modes (*comfort*; $10\,max$ and $30\,max$) we considered two time points which were embedded within task execution: the initial $3\,s$

(*pre*) and the final 3 *s* (*post*). To make data from *ft* frequency and *iso* torque comparable, we expressed the motor output at all evaluation time points as a function of the maximum obtained at any time point for each task and subject.

For normalizing the *ft* Range of motion (ROM) amplitude, we recorded the maximum (active) ROM of the index finger metacarpophalangeal joint for each subject before the protocol. The score served as divisor for the amplitude displayed at all the individual's evaluation time points during the *ft* task.

- MVC (before and following task execution):

  We evaluated the MVC at the time of stimulation (MVC in the $50-ms$ period before the stimulation) either for *ft* and *iso* task, normalized to the maximum obtained at any evaluation time-point for each task and subject.

- SP duration (recorded during the MVC explained just above):

  The SP duration was defined as the time-lag from the TMS/CMS pulse to the recovery of the EMG activity during the brief-iMVC, and determined visually by an experienced researcher blind to the conditions (in two separated sessions intra-rater reliability was checked on 124 random-chosen SPs, 62 each technique, TMS-CMS). Because we evaluated SP for both TMS and CMS, whose durations are not directly comparable [104], the SP durations were normalized. For each subject and task (*ft* or *iso*), we took as 100 % the average value from all the evaluation timepoints of the four sets executed in the *comfort* mode, which served as divisor for all the subject's values for all execution modes. Therefore in the figures representing the SP duration, the unit represents its average duration at *comfort* mode.

Statistical design

Statistical design for studying FDI activation during the tasks.

To study the level of the FDI activation in comparison with other muscles in each task, we first analyzed whether activation of each muscle was different in the two trials executed. After checking normality (one sample KS test) a paired Student t-test was used. Since activation in the two trials was never different ($p > 0{,}05$),

we averaged the values to compare the level of activation of the different muscles and tasks using different models of one-factor ANOVA (with repeated-measures). One model evaluated FDI activation compared to *opponens pollicis, abductor digiti minimi, extensor digitorum*, and *flexor digitorum supperficialis*. This model was applied independently to the tasks: *ft*, index *iso-flexion*, and *3–5th fingers* iso *flexion*. Another one-factor ANOVA model had four levels, and it was used to compare the activation of the FDI during iso in the four planes; "*toward flexion*" vs. "*extension*", "*abduction*" and "*adduction*".

Statistical design for studying intra-rater reliability in determining SP durations.

To study intra-rater reliability during *SP* duration determination (session 1 vs. 2, on the same random-chosen *SP*s) the intra-class correlation coefficient, and its 95 % confidence interval (95 % CI) were evaluated. For TMS-SP we obtained an $ICC = 0{,}97$ (0,94–0,98; 95 % CI); and for CMS-SP the $ICC = 0{,}97$ (0,96–0,98; 95 % CI).

Statistical design for studying behavior during fatigue tasks.

To study fatigue various repeated measures ANOVA's models were used, after checking the normality of distributions.

For the variables Motor Output (tapping *rate-ft*; or *force-iso*) decrement, SP duration, and MVC before and after task execution, we used an ANOVA with repeated measures. The ANOVA included one between-subject factor Group with two levels (the *TMS-group* and the *CMS-group*) and several within-subject factors. In the specific case of the SP, if the factor Group interacted significantly with any of the within-subject factors, it means that there was a significant different effect of within-subject factors on the response to spinal or corticospinal stimulation.

The within subject factors were Task (*ft, iso*), execution Mode (*comfort,* 10 max*,* 30 max), Set (the four sets for each execution mode), and evaluation Time points (*pre, post*). The levels of the latter factor were termed *i3* and *f3* when the variable analyzed was the motor output decrease in *ft* or *iso* tasks (as it included the initial and final 3 s embedded in task execution).

For the ANOVA of the ROM amplitude, only analyzed for *ft*, factor Task was excluded.

Results are expressed as the mean and the standard error of the mean (SE). During ANOVA execution the degrees of freedom were corrected with Greenhouse Coefficients (ε), if sphericity could not be assumed. Significance was set at $p < 0{,}05$. A Bonferroni correction was used for follow-up post hoc comparisons involving multiple levels within the factor.

3.3. Paper 3 - Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks

Ethical approval

Experimental protocols conformed to the Helsinki declaration and were approved by our institution Ethics Committee. All subjects were screened for incompatibility with brain stimulation protocols. All were medication free during the week preceding testing and signed a voluntary informed consent.

Subjects

The experiment included 15 healthy subjects (all men, age range 18–40 years). In all subjects the spinal excitability was evaluated with stimulation at the level of the cervicomedullary junction during several 2,5 s MVCs. Electrical stimulation was used in seven subjects, while magnetic stimulation using a double cone coil was used in the remaining eight subjects (who refused to participate if the stimulation was electrical due the produced discomfort). All subjects underwent both *ft* and *iso* fatigue testing sessions, 15 days apart.

Protocol

The two sessions were identical except for the type of task executed. In one of the session, participants were asked to perform index ft. In the other session they executed continuous index finger *iso* against a force sensor; the force direction was "toward" *flexion* of the first metacarpophalangeal joint. In all cases participants wore a small and light goniometer to monitor movements of the index finger metacarpophalangeal joint; we used also a metal ring attached at the distal phalanx of the index. Participants tapped or pressed over a thin metal plate located on the force sensor.

For both *ft* and *iso* sessions, subjects performed the tasks in three modes: comfort rate-effort (*comfort* mode) for 30 s; 10 s at maximal rate-effort (10 max mode); and finally 30 s at maximal rate-effort (30 max mode) in exactly the same way as in the previous protocol of Study 2.

In Study 2, MVC executed before and after the tasks lasted 2 seconds. Conversely, in this study participants executed 2,5 s MVCs before (*pre*) and right after (no

gap allowed) task-execution (post), either after *ft* or *iso*, for all modes and sets (See Figure 3.3). The magnitudes of 2,5 *s* MVCs were analyzed to monitor fatigue [222]. During the 2,5 *s* MVCs the CMS was applied (at 1,5 *s*), and we recorded the SP duration and Cervico-medullary Motor Evoked Potential (CMEP) amplitudes [105]. The peripheral transmission of the potentials during the same 2, 5 *s*-MVCs was also evaluated (at 2,2 *s*) with the amplitude of the CMAP [62]. We calculated the ratio CMEP/CMAP to evaluate spinal excitability accounting for the state of the periphery; this was always performed with the CMEP and CMAP acquired in the same MVC. Thus the stimulation pulses (CMS, and 700 *ms* later supramaximal to the ulnar nerve) were applied during the 2,5 *s* MVC. A practice sessions was scheduled [5].

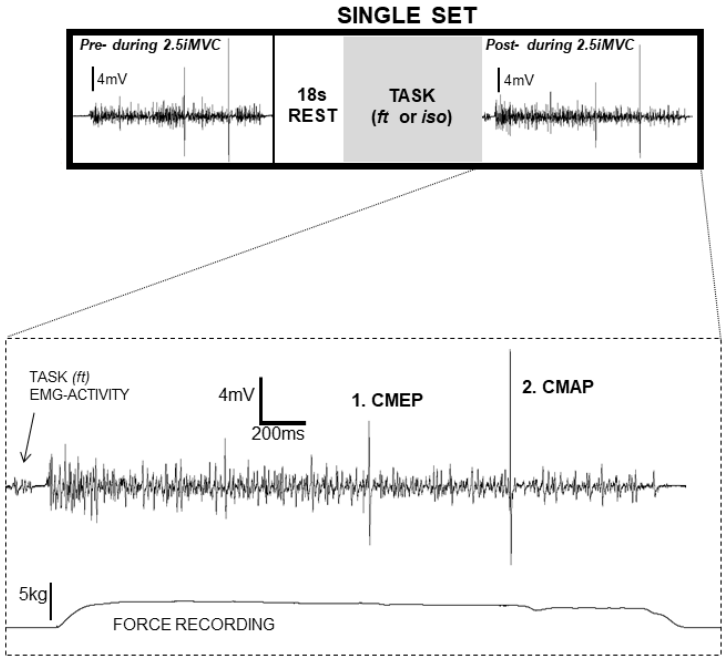

Figure 3.3: The set-structure: as soon as an LED was lit, subjects performed an isometric maximum voluntary contraction (MVC) with their index against a dynamometer. The LED off (2,5 *s* after) served as a signal to stop the MVC. During the MVC subjects received two different types of stimulation to get: 1st CMEP, at 1,5 *s* after LED on; and 2nd CMAP, at 2,2 *s* after LED on (*pre*). After this *paired*-stimulation subjects rested for 18 *s*. Then, a LED flash indicated the start of the task (*ft* in one session; *iso* in the other). In a continuation of the task, and with no resting time, the subjects performed another 2,5 *s* MVC in response to the LED turning on, and received stimulation (*post*; in the same way as *pre*).

Setting, recording and stimulation protocols

The set-up, and recording protocols and systems were the same as in Study 2.

- **Electric and magnetic CMS**

Electric CMS was applied using a Digitimer D180 stimulator by means of Ag–AgCl electrodes located behind the mastoid processes (anode at the right, cathode at the left).

Magnetic CMS was applied using a MagStim 200^2 (Whitland, Carmarthenshire, UK) stimulator connected to a double cone coil. The center of the coil was placed over the inion (in some subjects slightly lateral) and the direction of the current flowed down in the coil [224].

Active motor threshold (AMT) was defined as the minimum intensity required to evoke 5 liminal responses (approximately $200\,\mu V$) in 10 consecutive pulses in the activated muscle (5–10 % MVC) [223]. The stimulation intensity for the protocol was set to obtain a CMEP amplitude of approximately 50 % amplitude of the CMAP during MVC in the fresh muscle. To check the absence of current spread to the spinal roots, the CMEP latency was compared to that obtained at AMT intensity, such that the amplitude of the potentials increased in size with voluntary contraction with no or liminal latency shift when stimulated at the higher level [224].

We set 50 % of CMAP as the target for baseline values of the CMEPs to be more likely away from the ceiling, to monitor the potential modulation of the CMEPs induced by fatigue; previous reports have indicated that CMEPs may reach, at least, 70 % the CMAP when recorded in arm muscles [162].

- **Supramaximal electric stimulation of the ulnar nerve**

The FDI electrodes recorded the CMAP to ulnar nerve stimulation at the elbow (Digitimer DS7A stimulator). The anode was placed lateral to the medial epicondyle along the postcondylar groove and the cathode approximately $2\,cm$ distal to the anode, along the direction of the nerve. A $1000 - \mu s$ square pulse stimulus was used at an intensity 50 % above the supramaximal, and delivered during the 2,5 s-MVC.

Data reduction

The following dependent variables were analyzed:

- *CMEP and CMAP amplitudes:*

  These were defined as the peak-to-peak amplitude. CMEP was normalized to the CMAP amplitude acquired at the same time point (i.e., during the same 2,5 s-MVC). We analyzed the effect of fatigue on the CMEP and CMAP amplitudes.

- EMG Root Mean Square amplitude at the time of CMEP testing:

  Amplitude of the EMG activity in the 50 ms time window prior to CMEP ($\mathrm{EMG-RMS}_{PRIOR-CMEP}$). This value was normalized in relation to the RMS of the CMAP acquired at the same time point (i.e., during the same $2,5\,s$ MVC).

- *Motor output during task execution*:

  These were considered as in Study 2.

- *MVC (before and following task execution)*:

  These were considered as in Study 2.

- *SP duration:*

  These were considered as in Study 2

Statistical design

Statistical design to studying the effect of CMS on the amplitude of the CMAP

In our work, we recorded the CMAP 700 ms after the CMEP, during the same 2,5 s MVC. We examined if there was any influence of the preceding CMS on the amplitude of the CMAP as follows. Before each testing day (20 min before the fatigue protocol), subjects executed the 2,5 sMVC(with a 100 s rest interval) six times. In three MVCs both CMEPs and CMAPs were acquired (as in the protocol); in the other three we did not apply CMS and the order was randomized. The average of

the three CMAP amplitudes (potentially) conditioned by CMS was compared to the average obtained from the unconditioned CMAPs. An ANOVA was performed with factors Conditioning (two levels, *conditioned* and *unconditioned* CMAP amplitude) and Day (levels *day-1* and *day-2*, as this was evaluated for two days). We also evaluated the Intra-class correlation coefficient (ICC) considering the *Conditioned* vs. *Unconditioned* responses.

Statistical design for studying behavior during fatigue tasks

Fatigue induced by the *ft* and *iso* tasks was evaluated with repeated measures ANOVA. Previously, we checked the normality of distribution by means of Kolgomorov–Smirnov test for one sample. For each of the variables (normalized CMEP amplitude; EMG-Root mean squaresPRIOR-CMEP; motor output decrement -tapping rate in *ft* and force in *iso*; SP durations; MVC before and after task execution; and CMAP amplitude) we used an independent ANOVA with repeated measures. The ANOVA included several within-subject factors: The within-subject factors were Task (*ft, iso*), execution Mode (comfort, $10\,max$, $30\,max$), Set (the four sets for each execution mode), and evaluation Time points (*pre, post*). The levels of the latter factor were termed *i3* and *f3* when the variable analyzed was the motor output decrease in *ft* or *iso* tasks (as it included the initial and final $3\,s$ embedded in task execution).

For the ANOVA of the ROM amplitude, only analyzed for *ft*, factor Task was excluded. These procedures were the same as in Study 2.

Results are expressed as the mean and the standard error of the mean (SE). During ANOVA execution, the degrees of freedom were corrected with Greenhouse Coefficients (ε) if sphericity could not be assumed. Significance was set at ($\mathbf{p < 0{,}05}$). A Bonferroni correction was used for follow-up *post hoc* comparisons involving multiple levels within the factor.

Subsequent analyses were carried out to determine if the responses obtained with magnetic and electric stimulators were different; this analysis is justified since magnetic coil geometry might increase the risk of recruiting some cortical neurons. The model was the same as above but included a between-subjects factor (Group, with two levels: electric and magnetic).

Chapter 4

Results

4.1. Paper 1 - Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Tasks

Table 4.1 shows the mean PMT, RT, and MT at *Pre* under each experimental condition (values serving as normalizing factors and equivalent to the units in the y-axes of the corresponding graph). Subjects made no responses during the catch trials.

Table 4.1: PMT, RT and MT at *Pre* in the three days of the protocol; mean (Standard Error (SE)) considering all subjects.

	AL-CR	**AR-CL**	Sham
PMT (ms) at *Pre*	201,8 (11,5)	204,5 ms (12,3)	201,0 ms (10,5)
RT (ms) at *Pre*	243,3 (9,0)	245,1 ms (10,0)	245,1 ms (9,0)
MT (ms) at *Pre*	222,5 (12,5)	227,0 ms (10,3)	221,0 ms (12,0)

Effects of Brain Stimulation on PMT

Table 4.2 shows the mean values for the different levels of all factors in a *pre-post* basis.

Table 4.2: Mean values in ms (and SE) for the different factors in a *pre-post* basis.

	AL-CR		***AR-CL***		***Sham***	
	TIME		**TIME**		**TIME**	
PMT	***pre***	***post***	***pre***	***post***	***pre***	***post***
LATERALITY						
ipsi	198,7(12,9)	194,8(9,9)	203,8(11,1)	204,4(14,1)	198,5(11,3)	195,9(10,4)
contra	204,9(10,6)	197,7(9,3)	205,2(13,8)	204,3(14,5)	203,5(9,8)	208,1(10,0)
OPTION						
single	194,6(12,8)	190,7(9,7)	196,0(10,6)	196,2(14,1)	193,0(11,1)	195,5(10,3)
choice	209,0(11,2)	201,8(9,6)	213,0(14,4)	212,5(14,8)	209,0(10,5)	208,6(10,5)
MUSCLE						
del	191,4(9,9)	184,9(7,8)	190,0(10,2)	190,6(12,2)	192,2(8,7)	189,7(8,4)
bic	188,4(10,8)	183,9(9,0)	187,5(13,0)	186,0(14,0)	189,6(9,9)	187,1(9,5)
tri	225,6(16,0)	220,0(13,7)	236,0(16,9)	236,5(19,2)	221,1(14,4)	229,2(14,8)
RT	***pre***	***post***	***pre***	***post***	***pre***	***post***
LATERALITY						
ipsi	241,4(9,9)	241,2(6,9)	243,7(8,3)	243,1(11,2)	241,9(9,4)	241,5(9,3)
contra	245,2(8,4)	246,3(8,6)	246,5(12,0)	243,2(11,6)	248,3(8,8)	255,1(11,2)
OPTION						
single	237,0(9,8)	238,1(8,7)	237,2(9,3)	236,0(11,6)	238,6(10,9)	240,7(11,0)
choice	249,6(9,3)	249,3(6,7)	253,0(11,3)	250,3(11,5)	251,6(8,2)	255,8(9,2)
MT	***pre***	***post***	***pre***	***post***	***pre***	***post***
LATERALITY						
ipsi	205,4(11,0)	207,2(6,9)	206,8(8,7)	202,2(8,8)	203,3(10,8)	204,8(9,6)
contra	239,6(14,2)	242,4(16,9)	247,2(12,2)	242,6(12,5)	238,7(13,6)	236,8(13,0)
OPTION						
single	220,7(12,2)	222,8(14,1)	229,1(9,2)	221,7(10,0)	218,7(11,3)	221,1(11,4)
choice	224,3(12,9)	226,6(14,5)	224,9(11,6)	223,1(11,2)	223,3(12,8)	220,4(11,2)

Ipsi,contra: responses to the ipsilateral or contralateral targets to the dominant-executing hand.

Del(deltoids), *bic*(biceps), *tri*(triceps).

Table 4.3 summarizes the effects of tDCS on PMT (ANOVA factors, and significant interactions of the factors with TIME). If interactions TIME×STIM were significant (first column of results in the table), we followed-up with ANOVA's by pair of STIM modes and, if significance remained, by STIM mode in isolation (subsequent columns in Table 4.3).

Table 4.3: **ANOVA's for PMT**. Main effects and significant interactions with factor TIME. ANOVA's were executed considering the three STIM modes. If significant interactions indicate different responses to STIM in the testing TIMEs, the ANOVA's were followed-up by pairs and the by single STIM mode.

	AL-CR vs AR-CL vs Sham	***AR-CL vs Sham***	***AL-CR vs Sham***	***AL-CR vs AR-CL***	***Sham***
MAIN EFFECTS					
STIM	$F_{2,24} = 0{,}3$ $p = 0{,}3$	$F_{1,12} = 0{,}1$ $p = 0{,}9$	$F_{1,12} = 0{,}5$ $p = 0{,}5$	$F_{1,12} = 0{,}4$ $p = 0{,}5$	N.A
TIME	$F_{1,12} = 2{,}4$ $p = 0{,}15$	$F_{1,12} = 0{,}8$ $p = 0{,}8$	$F_{1,12} = 1{,}4$ $p = 0{,}3$	$F_{1,12} = 4{,}9$ **p = 0,048** $\eta_p^2 = 0{,}288$	$F_{1,12} = 0{,}2$ $p = 0{,}6$
OPTION	$F_{1,12} = 24{,}6$ **p < 0,001** $\eta_p^2 = 0{,}672$	$F_{1,12} = 30{,}3$ **p < 0,001** $\eta_p^2 = 0{,}716$	$F_{1,12} = 16{,}5$ $p = 0{,}002$ $\eta_p^2 = 0{,}579$	$F_{1,12} = 19{,}7$ **p < 0,001** $\eta_p^2 = 0{,}621$	$F_{1,12} = 16{,}0$ **p = 0,002** $\eta_p^2 = 0{,}572$
LATERALITY	$F_{1,12} = 7{,}0$ **p = 0,022** $\eta_p^2 = 0{,}368$	$F_{1,12} = 6{,}4$ **p < 0,027** $\eta_p^2 = 0{,}347$	$F_{1,12} = 6{,}6$ **p = 0,025** $\eta_p^2 = 0{,}355$	$F_{1,12} = 2{,}2$ **p = 0,2**	$F_{1,12} = 16{,}2$ **p = 0,002** $\eta_p^2 = 0{,}574$
MUSCLE	$F_{2,24} = 30{,}3$ ε=0,6 **p < 0,001** $\eta_p^2 = 0{,}876$	$F_{2,24} = 31{,}3$ ε=0,6 **p < 0,001** $\eta_p^2 = 0{,}723$	$F_{2,24} = 22{,}6$ ε=0,6 **p < 0,001** $\eta_p^2 = 0{,}653$	$F_{2,24} = 30{,}5$ ε=0,6 **p < 0,001** $\eta_p^2 = 0{,}717$	$F_{2,24} = 21{,}5$ ε=0,6 **p < 0,001** $\eta_p^2 = 0{,}642$
SIGNIFICANT INTERACTIONS	$F_{2,24} = 3{,}4$ $p = 0{,}052$ $STIM \times TIME \times LAT$ $F_{4,48} = 3{,}2$ **p = 0,020** $STIM \times TIME \times MUS$	$F_{1,12} = 5{,}6$ **p = 0,035** $STIM \times TIME \times LAT$ $F_{2,24} = 6{,}8$ **p = 0,042** $STIM \times TIME \times OPT$	$F_{1,12} = 6{,}1$ **p = 0,030** $STIM \times TIME \times LAT$ $F_{2,24} = 5{,}4$ ε=0,7 **p = 0,024** $STIM \times TIME \times MUS$	N.S	$F_{1,12} = 11{,}4$ **p = 0,005** $TIME \times LAT$ $F_{2,24} = 9{,}5$ ε=0,6 **p = 0,005** $TIME \times MUS$
FACTOR TIME			$F_{1,12} = 5{,}7$ **p = 0,035** $STIM \times TIME$		

N.S. = none was significant; N.A. = not applicable since such ANOVA had not that factor. Partial etha squared (η_p^2) is reported for significant main effects. Since significant interactions involving TIME and STIM (in the model with 3 STIM modes) do not inform whether the three STIM modes produced different responses compared to each other, or if there was just one STIM mode that produced different responses in TIME compared to the other two STIM modes, we followed-up ANOVA by pairs of STIM modes, and if needed, just with one STIM mode.

With the 3 STIM modes-ANOVA there were significant main effects showing that all PMT were faster in single compared to choice responses ($\eta_p^2 = 0{,}672$; $\eta^2 = 0{,}061$), in ipsilateral than contralteral responses ($\eta_p^2 = 0{,}368$; $\eta^2 = 0{,}006$); and presented a sequential muscle activation ($\eta_p^2 = 0{,}716$; $\eta^2 = 0{,}393$); the latter can be observed in a representative subject in Figure 3.1(C).

The significant interactions between TIME and STIM with other factors were observed in this model with 3 stimulation modes (also in the rest of models, except when the two active tDCS protocols were compared), Table 4.3. This means that the responses to Sham were different to the responses of the other to stimulation modes, and that the responses obtained with the two active stimulation modes were not significantly different of each other; for such reason their effects are shown pooled in figures (green tones). The individual's responses in *Pre* vs. *Post* basis are depicted in Figure 4.1.

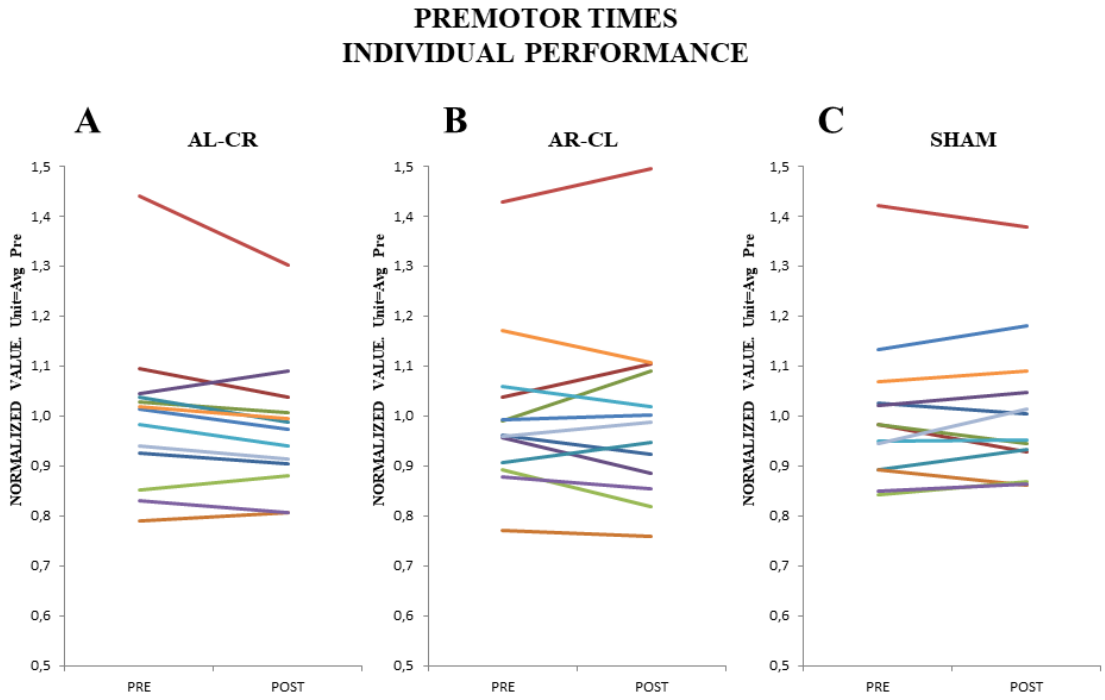

Figure 4.1: **Individuals' responses for PMT.** The y-axis unit indicates the mean response considering all subjects and conditions at *Pre*. It was equivalent to 201,8 ms (SE 11,5) for AL-CR (**A**); 204,5 ms (SE 12,3) for AR-CL (**B**); and 201,0 ms (SE 10,5) for Sham sessions (**C**).

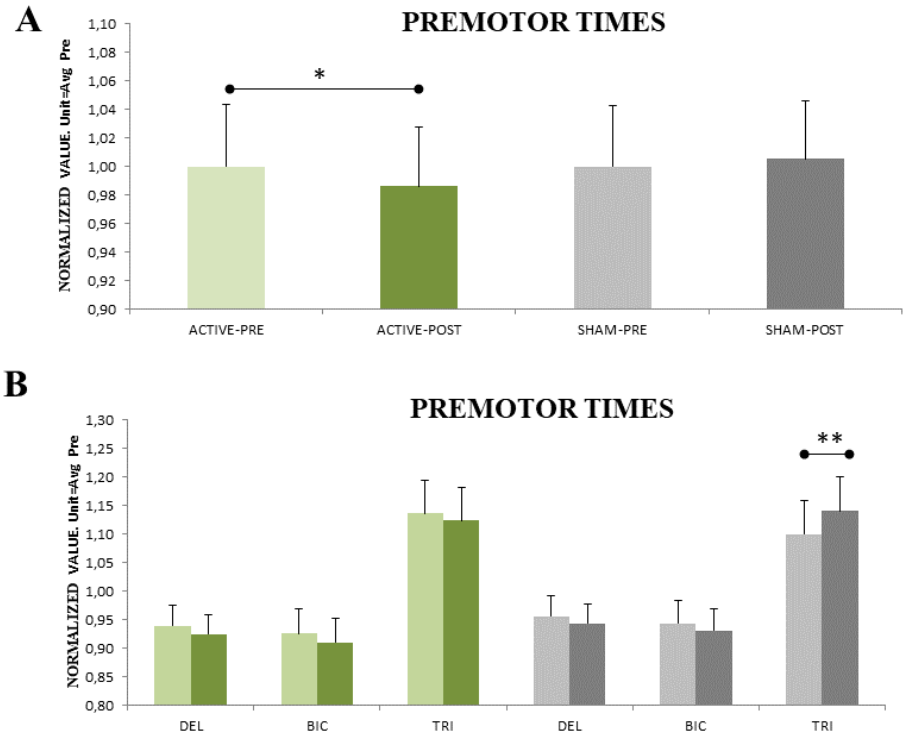

Figure 4.2: (**A**) PMT at *Post* were differently modulated by Sham-tDCS compared to the other two active protocols, which did not differ each other (shown pooled in green tones). There was a significant decrease at *Post* after both active protocols. (**B**) Sham stimulation increased *Post* PMT, specifically in the triceps muscle. The *y-axis* unit indicates the mean response across all subjects and conditions at *Pre*. $^{*}p < 0{,}05$; $^{**}p < 0{,}01$.

For the follow-up ANOVA including *AR-CL* and *AL-CR* stim modes the significant factor TIME, in absence of significant interactions with any other factor, indicates a small ($\eta_p^2 = 0{,}288$; $\eta^2 = 0{,}002$) but significant ($F_{1,12} = 4{,}9$ $\mathbf{p < 0{,}05}_{TIME}$) reduction of 1,5 % in the PMT at *Post* (Figure 4.2(A)). This effect was present in the three studied muscles and was independent on the laterality and options of the responses. Figure 4.1 indicates that in most of the subjects the reduction in the PMT was small, which explains the small (although significant) effect observed.

For the ANOVA with Sham stimulation, the effects were rather the opposite. The effect of Sham stimulation considering the three muscle together was a very mild (surely non significant) increase of PMT in the *Post* condition (Figure 4.2(A)). However the Sham effects where different for the three muscles ($F_{2,24} = 9{,}5_{\varepsilon=0{,}6}$; $p = 0{,}005_{TIME\times MUS}$ $\eta_p^2 = 0{,}443$ $\eta^2 = 0{,}009$), and PMT increased significantly (4 %) at *Post* in the *triceps* (*post-hoc* $p = 0{,}002$) (Fig 4.2(B)).

Effects of Brain Stimulation on RT and MT

Table 4.2 shows the mean values for the different levels of the different factors in a *pre-post* basis, in the RT and MT.

Table 4.4 shows significant main effects, indicating that the responses were faster in the case of the single than in choice tasks for RT ($\eta_p^2 = 0{,}659$;$\eta^2 = 0{,}069$). On the other hand, ipsilateral were faster than contralateral responses, this was shown by a significant main effect of factor "laterality" for RT ($\eta_p^2 = 0{,}291$; $\eta^2 = 0{,}010$) and also for MT ($\eta_p^2 = 0{,}873$; $\eta^2 = 0{,}495$).

Table 4.4 also shows that RT and MT were not modified by the different stimulation modes. For both variables factor TIME was never significant and it did not interact significantly with any other factor.

Table 4.4: ANOVA's for variables which response was not different for the three stimulation modes.

AL-CR vs AR-CL vs Sham	**MAIN EFFECTS**					**SIGNIFICANT INTERACTIONS FACTOR TIME**
	STIM MODE	**TIME**	**OPTION**	**LATERALITY**	**MUSCLE**	
RT	$F_{2,24} = 0{,}2$ $p = 0{,}9$	$F_{1,12} = 0{,}1$ $p = 0{,}8$	$F_{1,12} = 23{,}2$ $\mathbf{p < 0{,}001}$ $\eta_p^2 = 0{,}659$	$F_{1,12} = 4{,}9$ $\mathbf{p = 0{,}046}$ $\eta_p^2 = 0{,}291$	N.A	N.S
MT	$F_{2,24} = 0{,}1$ $p = 0{,}9$	$F_{1,12} = 0{,}3$ $p = 0{,}6$	$F_{1,12} = 1{,}3$ $p = 0{,}3$	$F_{1,12} = 82{,}2$ $\mathbf{p < 0{,}001}$ $\eta_p^2 = 0{,}873$	N.A	N.S
CV-PMT	$F_{2,24} = 0{,}7$ $p = 0{,}5$	$F_{1,12} = 25{,}1$ $\mathbf{p < 0{,}001}$ $\eta_p^2 = 0{,}677$	$F_{1,12} = 1{,}3$ $p = 0{,}3$	$F_{1,12} = 1{,}5$ $p = 0{,}2$	$F_{2,24} = 0{,}1$ $p = 0{,}4$	N.S
CV-RT	$F_{2,24} = 0{,}6$ $p = 0{,}6$	$F_{1,12} = 7{,}1$ $\mathbf{p = 0{,}020}$ $\eta_p^2 = 0{,}373$	$F_{1,12} = 0{,}3$ $p = 0{,}6$	$F_{1,12} = 1{,}9$ $p = 0{,}2$	N.A	N.S
CV-MT	$F_{2,24} = 1{,}4$ $p = 0{,}3$	$F_{1,12} = 0{,}7$ $p = 0{,}4$	$F_{1,12} = 0{,}1$ $p = 0{,}7$	$F_{1,12} = 3{,}1$ $p = 0{,}1$	N.A	N.S

Effects of Brain Stimulation on CV's of PMT, RT and MT

Table 4.4 indicates a change in the Coefficient of variation (CV)'s of PMT and RT at *post* compared to *pre*; but not in the CV of MT. Since there was a significant main effect of factor "time" for CV-PMT and for CV-RT (Figure 4.3(A) and 4.3(B) respectively), but there were not significant interactions with any other factor, this means that the significant reductions in the CV's after tDCS (from 18,3 to 17,0 % in PMT; and from 13,9 to 12,6 % in RT) were observed in all tasks (and muscles for PMT); and also in all stimulation protocols, including Sham ($\eta_p^2 = 0{,}677$; $\eta_p^2 = 0{,}01$ for PMT; and $\eta_p^2 = 0{,}373$; $\eta^2 = 0{,}014$ for RT).

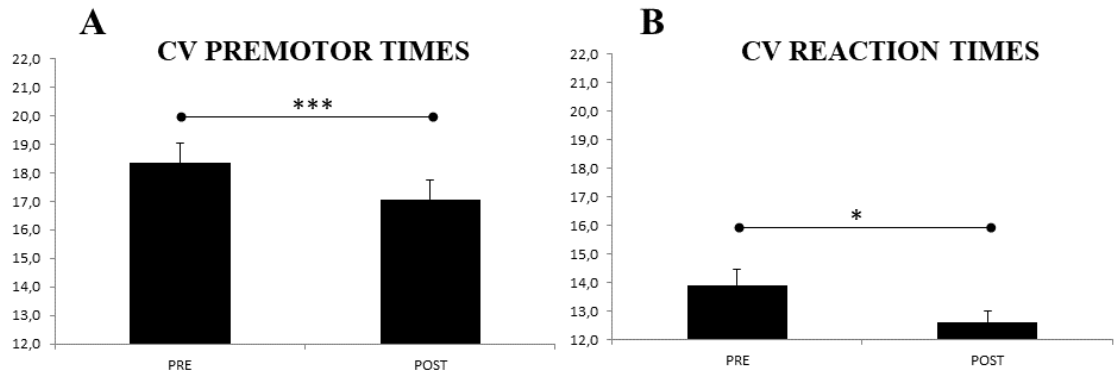

Figure 4.3: (**A**) The CV(%) of PMT was reduced significantly at *Post*, regardless stimulation modes, tasks or muscles (so that shown pooled). (**B**) A similar pattern was found for the CV(%) of RT. $*p < 0{,}05$; $***p < 0{,}001$.

Effects of Brain Stimulation on Anticipatory Responses

A total of 70 reaching movements were anticipatory responses, this is 5,6 % of the 1248 movements executed, in all subjects. The proportion of anticipations at Pre and Post was not significant different (Fisher Exact Probability Test $\mathbf{p > 0{,}05}$). Fragmentation of this analysis for the different tasks at Pre and Post was not considered due to the reduced number of anticipations.

4.2. Paper 2 - Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of Silent Period evoked at spinal and supraspinal levels

The Table 4.5 indicates the level of activation of the FDI obtained in *ft* and *iso* tasks in all subjects (Figure 4.4 shows activations in a representative participant). FDI had a main role in the tasks used in the fatigue protocols (index *ft* or *iso*).

Table 4.5: First dorsal introsseous activation in the tasks of the fatigue protocol (bold fonts) compared to other tasks and muscles.

FDI activation compared to activation of other muscles in three different tasks

Task	ANOVA task-effect	**FDI activation**	vs. opp (post hoc)	vs. adm (post hoc)	vs. ext (post hoc)	vs. fds (post hoc)
Index *ft* task	$F_{4,20} = 5{,}6$ $p < 0{,}05$	**6,7 %** **(SE 1,0)**	n.s.	n.s.	n.s.	n.s.
Index *iso*-MVC task	$F_{4,20} = 10{,}5$ $p < 0{,}001$	**13,3 %** **(SE 1,1)**	5,8 %* (SE 1,3)	4,4 %* (SE 0,5)	n.s.	n.s.
3–5th fingers *iso*-MVC task	$F_{4,20} = 21{,}9$ $p < 0{,}001$	**5,6 %** **(SE 1,1)**	n.s.	16,3 %* (SE 1,7)	n.s.	14,4 %** (SE 0,9)

FDI activation during different index *iso* MVC tasks

	ANOVA direction-effect	**toward flexion**	vs. abduction (post hoc)	vs. extension (post hoc)	vs. adduction (post hoc)
Index *iso*-MVC	$F_{3,15} = 37{,}1$ $p < 0{,}001$	**13,3 %** **(SE 1,1)**	n.s.	5,2 %* (SE 0,6)	4,0 %* (SE 0,9)

Units (%) = 100 (r.m.s.TASK/r.m.s.CMAP). *opp*: oponens pollicis; *adm*: abductor digiti minimi; *ext*: extensor digitorum; *fds*: flexor digitorum supercialis. Asterisks indicate Bonferroni post hoc *after ANOVA* evaluation of main eects. n.s.: not signicant.

* $p < 0{,}05$.

** $p < 0{,}01$.

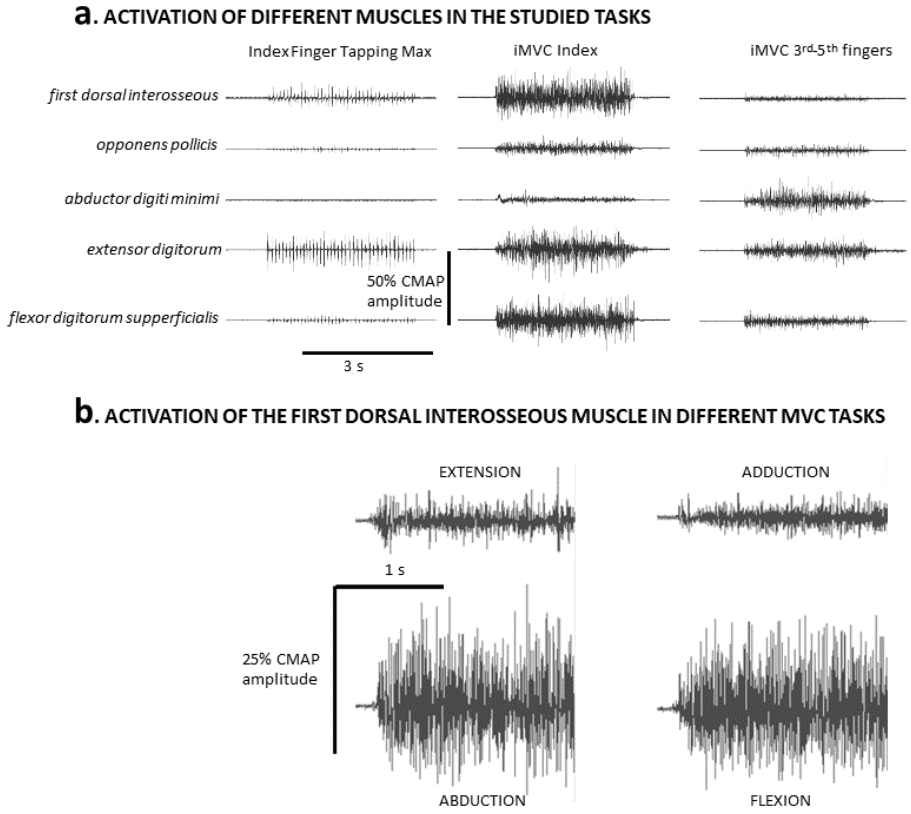

Figure 4.4: (A) Activation of the *first dorsal interosseous* in the *ft* and *iso* tasks, for comparison with another task (iMVC flexion of the 3rd, 4th and 5th fingers). Example of individual recordings in one subject (on a separate session) with the settings as describe in Figure 3.2. Execution of *ft* at maximal rate (left recordings); *iso* MVC with the index finger, as in the protocols (central recordings); and *iso* MVC flexion with 3–5th fingers together with no index finger pressing (right recordings). The EMG-recordings of the first dorsal interosseous, opponens pollicis, abductor digiti minimi, extensor digitorum and flexor digitorum superficialis are shown in the three tasks. The EMG-amplitudes are scaled to the vertical bar to allow raw-data representation, showing the 50 % of each muscle CMAP amplitude; statistical comparisons were applied for RMS activation; the involvement of the FDI compared to the rest of the muscles is clear and more specific in the task evaluated. (B) Similar maximal EMG-recruitment of the FDI during index finger flexion and abduction, larger than during adduction and extension, all iMVC against unmovable loads.

Table 4.6 shows the normalizing values equivalent to the unit in graphs during the fatigue testing sessions.

Table 4.6: Mean score including all subjects. The score is the y-axis unit in graphs.

Task	*Max output in task at any evaluation time-point	*Max active full ROM	Mean (%) *ft* amplitude along the task	*MVC at any stimulation time-point	Mean TMS-SP duration at comfort	Mean CMS-SP duration at comfort
ft	7,1 Hz (SE 0,1)	24, 7° (SE 2,0)	28,53 % (SE 3,9)	4,7 kg (SE 0,3)	145,1 ms (SE 4,1)	67,0 ms (SE 4,0)
iso	3,8 kg (SE 0,2)	n.a.	n.a.	4,5 kg (SE 0,2)	158,9 ms (SE 5,9)	71,5 ms (SE 4,6)

*The score utilizes the maximal value from each subject: it is the mean of the maximal scores including all subjects. n.a.: not applicable.

Frequency/force decrement during execution in *ft/iso* tasks

The motor output decrements during the task execution (comparing motor output in the initial and final 3 s of the tasks) were different for the tasks and execution modes ($F_{2,38} = 8{,}6;\ \mathbf{p < 0{,}01}_{TASK\times MODE\times TIME}$) Therefore, we split the analyses for each kind of task.

For *ft*, the frequency reduced over execution ($F_{1,19} = 86{,}4;\ \mathbf{p < 0{,}001}_{TIME}$), but differently on the three modes ($F_{2,38} = 43{,}7;\ \mathbf{p < 0{,}001}_{MODE\times TIME}$); this effect was not differently observed in the two groups (subjects stimulated with TMS or CMS; $F_{2,38} = 0{,}4;\ \mathbf{p > 0{,}05}_{MODE\times TIME\times GROUP}$ - note that the execution protocol was the same for the two groups of subjects). The frequency of *ft* at *comfort* was unchanged, conversely to 10 max and 30 max (both reduced in the last 3 s of task execution (i.e., *post*) compared to the initial 3 s of task execution (*pre*), *post* hoc $\mathbf{p < 0{,}001}$. Set progression had no effect ($F_{3,57} = 0{,}3;\ \mathbf{p > 0{,}05}_{SET}$), as clearly observed in Figure 4.5(A), and for this reason the effects are shown pooled at Figure 4.5(B).

Figure 4.5(C) illustrates the force developed during *iso* in the initial and final 3 s of the task in the different modes and sets. It was evident that the force dropped at the end of the task for the different modes ($F_{2,38} = 109{,}0_{\varepsilon=0{,}7};\ \mathbf{p < 0{,}001}_{MODE\times TIME}$); and also there was a set effect for some modes impacting the level of the dropping of force at the end of the *iso* task ($F_{6,114} = 11{,}0;\ \mathbf{p < 0{,}001}_{MODE\times SET}$). Again, the execution protocol was identical for the two groups of subjects, those stimulated with TMS and CMS; likely, this is the reason why the dropping of force was not sig-

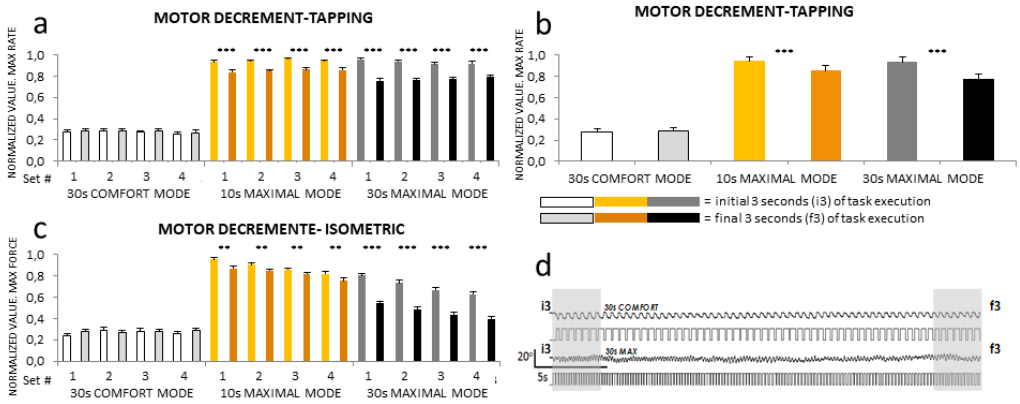

Figure 4.5: Motor decrement induced by the tasks. (A) The frequency of *ft* decreased significantly after the maximal modes; there was no set effect. (B) Same effect seen pooling sets as there was no set effect. (C) Set by set motor decrement along the *iso* tasks. The force reduced at post after maximal modes and set after set. (D) Representative example in one subject, *ft* amplitude for comfort and *30max*. The amplitude never decreased during the tasks. The unit in the y-axis represents the normalized value with respect to the maximal motor output (maximal *ft* rate, or maximal *iso* torque obtained at any evaluation time of the corresponding session). In all figures, light colors are the initial $3\,s$ of execution (*pre*), dark colors the final $3\,s$ (*post*); asterisks denote statistical significance between *pre* and *post*, $^{*}p < 0{,}05$; $^{**}p < 0{,}01$; $^{***}p < 0{,}001$.

nificantly different for the two groups of subjects ($\mathbf{p > 0{,}05}$ for $_{MODE\times SET\times GROUP}$ or $_{MODE\times TIME\times GROUP}$ interactions). Then, we followed up ANOVAs by modes of execution, this was done to understand the before-mentioned differential effects of the modes of execution on the dropping of force at the end of the *iso*-tasks. We observed no effects of *comfort-iso* on force drop at *post* ($\mathbf{p > 0{,}05}$ for any main effect or interaction). For *10max-iso*, the execution dropped significantly at *post* ($F_{1,19} = 13{,}4$; $\mathbf{p < 0{,}01}_{TIME}$), and set after set ($F_{3,57} = 13{,}7$; $\mathbf{p < 0{,}001}_{SET}$). A similar pattern of force-drop was observed for *30max-iso* ($F_{1,19} = 174{,}5$; $\mathbf{p < 0{,}001}_{TIME}$ and $F_{3,57} = 39{,}6$ $_{\varepsilon=0{,}7}$; $\mathbf{p < 0{,}001}_{SET}$).

Finger angular amplitude during tapping

During *ft*, the expressions of fatigue might affect not only the tapping-frequency, but also the tapping amplitude. For this reason, we analyzed the ROM amplitude at the first (*i3* = pre) and last (*f3* = post) $3\,s$ of the task. Remarkably, during *ft* the ROM amplitude was not significantly different at *pre* vs. *post* (*i3* vs. *f3*; $F_{1,19} = 1{,}7$; $\mathbf{p > 0{,}05}_{TIME}$; Table 4.6). Likewise, it is worth mentioning, that in all

sets, the mean ROM amplitude was not different for the three tapping modes either ($F_{2,38} = 2{,}3_{\varepsilon=0{,}7}$; $\mathbf{p > 0{,}05}_{MODE}$; Figure 4.5(D) shows an example of the ROM amplitude in one subject). These effects on motor execution were not differently expressed for the subjects stimulated with TMS or CMS (identical task for both groups).

MVC at the time of stimulation

The MVC at the time of stimulation (Figure 4.6(A)) was reduced at *post* differently for the *ft* and *iso* tasks ($F_{1,19} = 30{,}1$; $\mathbf{p < 0{,}001}_{TASK\times TIME}$; Figure 4.6(B–D)). We followed by splitting the analyses by task-type.

For *ft* tasks, MVC force was reduced at *post* (i.e., in the MVC performed right at the end of tapping; $F_{1,13} = 12{,}3$; $\mathbf{p < 0{,}01}_{TIME}$) and differed also for the three modes of tapping ($F_{2,38} = 18{,}7$; $\mathbf{p < 0{,}001}_{MODE}$). Thus, *post* MVC was weaker after tapping (vs. *pre*), at all modes and sets (Figure 4.6), and it also was reduced from mode to mode.

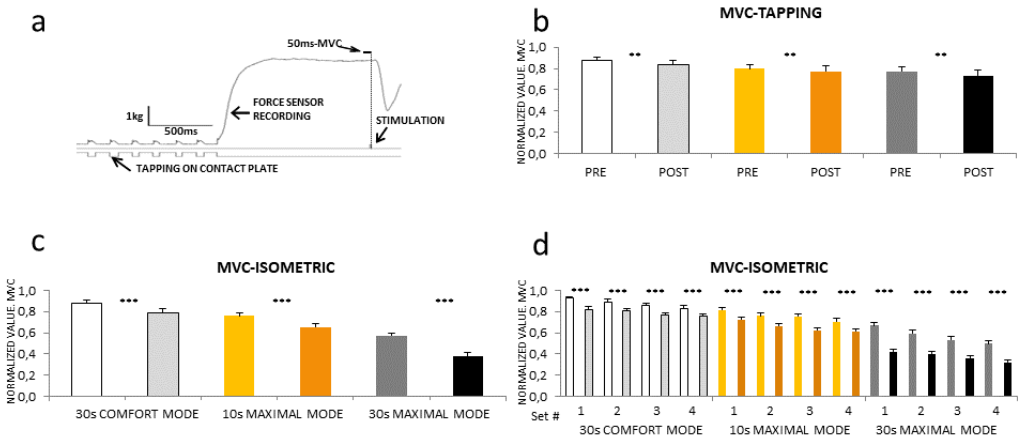

Figure 4.6: (A) Example of force recording at *post-ft*. MVCs shown in 4.6(b–d) sections are acquired in the 50 *ms* prior to stimulation. (B) MVC decreased after *ft* (*post*) in all modes and from mode to mode. (C–D) MVC decrease after *iso* (*post*) in all modes and also from mode to mode and set to set.

A different matter happened for *iso*; we initially observed that the MVC decrease at *post* was different for the three modes ($F_{2,38} = 15{,}9$; $\mathbf{p < 0{,}001}_{TIME\times MODE}$; Figure 4.6(D)). Then, we followed up the analyses by modes. The analyses indicated

that for *comfort* and *10max* the MVC dropped at *post* and set after set (i.e., factors TIME and SET for these to modes were always significant ($\mathbf{p < 0{,}001}$). This was also the case of *30max*, but in addition, the responses at *pre* and *post* also differed for the different sets ($F_{3,57} = 4{,}9_{\varepsilon=0,7};\ \mathbf{p < 0{,}01}_{SET\times TIME}$) notwithstanding the fact that post hoc *pre* vs. *post* were always significant ($\mathbf{p < 0{,}001}$; see Figure 4.6(C-D)).

Again, in agreement with the other variables described thus far, these responses were not significantly different for the subjects stimulated with TMS or CMS (i.e., factor group did not interact significantly in any case), and comparable levels of fatigue were shown in the two groups for each task.

SP adaptation to maximal *ft* and *iso* tasks

As described in the introduction, the SP duration increases with central fatigue. Therefore, we analyzed if SP duration changed after execution (at *post*); and also if the putative change was different for *ft* and *iso*. In one group of subjects the SP was induced by TMS in the two different task-sessions (*ft* and *iso*); in the other it was induced by CMS in the two sessions. Therefore, in all cases, the task-dependency (*ft* vs. *iso*) changes in SP were evaluated for the two kinds of stimulation (i.e., in the two groups). As mentioned above, fatigue did not affect differently the motor execution in the two groups and tasks; thus we compared SP's duration evoked by TMS and CMS in the two tasks; to make their baseline duration comparable the durations were normalized. For this purpose the SP duration at *comfort* was considered the reference for each kind of stimulation.

The normalized SP durations increased after execution (at *post*), in a significant different manner for the two tasks, and for TMS and CMS groups ($F_{1,19} = 9{,}4;\ \mathbf{p < 0{,}01}_{GROUP\times TASK\times TIME}$). See Figure 4.7. (individual's raw-examples), and Figure 4.8. We split the analyses to evaluate the task-dependency effect on the two groups.

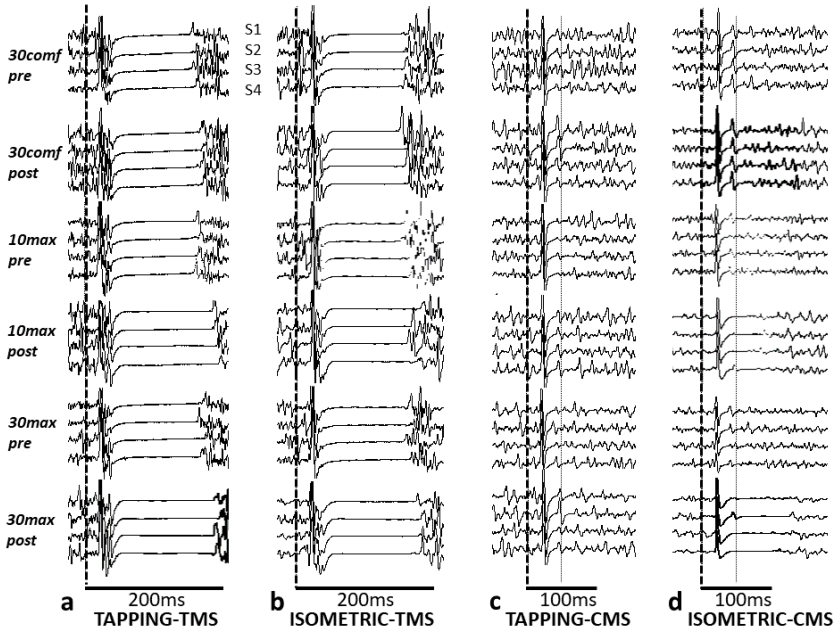

Figure 4.7: Individual's recordings of the SP *pre* and *post* execution. S1. . .S4 are the sequential recordings of the four sets for a given time of evaluation. TMS-SP increased at post after *ft* (A) and after *iso* (B), only at maximal modes. Conversely CMS-SP was unchanged after *ft* in all modes (C), but increased after the maximal modes for the *iso* task, see (D) section. The vertical thick dashed lines indicate the time of stimulation. The vertical thin dashed line lies at the end of the transcortical reflex potential, evoked by CMS. The representation occludes part of the motor evoked potentials, in order to optimize the representation of the SPs.

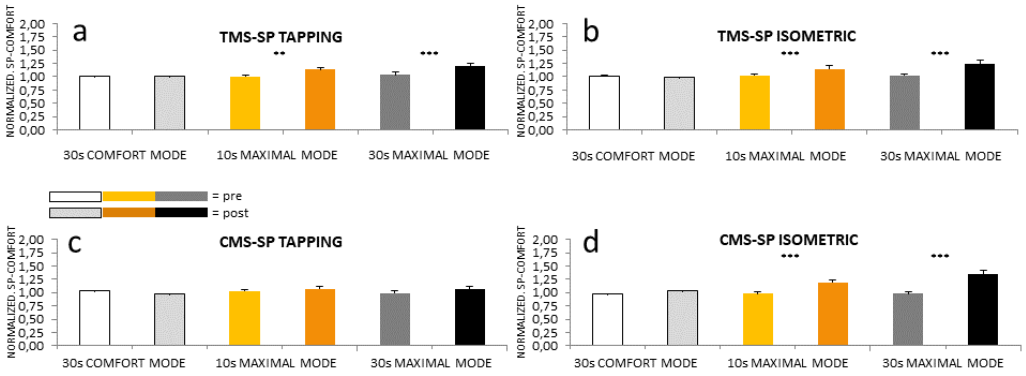

Figure 4.8: SPs in response to TMS or CMS during fatiguing motor activities in all subjects. (A) and (B) show that the TMS-SP significantly increased after executing at maximal modes, either 10 or 30 s for *ft* and *iso*. (C) However in no case did the CMS-SP increase after *ft*. (D) CMS-SP significantly increased after *10max* and *30max iso*. A significant effect of set never appeared for SP – durations, therefore the four sets are shown pooled. The y-axis expresses the mean duration of the SP in comfort mode (pooling all sets and *pre* and *post* values).

For *ft*-task, the SP increase at *post* was significantly different for the CMS and TMS groups ($F_{1,19} = 11{,}0$; $\mathbf{p < 0{,}01}_{GROUP \times TIME}$). Subsequently, the analyses of the TMS-SPs showed a significant increase at the end of execution, which was also different for the execution modes ($F_{2,16} = 9{,}1$; $\mathbf{p < 0{,}01}_{MODE \times TIME}$). We observed that the increase of the TMS-SP was only significant after *10max* ($\mathbf{p < 0{,}01}$ *post* hoc) and *30max* ($\mathbf{p < 0{,}001}$ post hoc), not for *comfort*. Conversely the CMS-SP never increased at the end of the *ft* ($F_{1,11} = 0{,}7$; $\mathbf{p > 0{,}05}_{TIME}$).

For *iso*-tasks the responses to fatigue of the SP induced by TMS and CMS were never significantly different. For both types of stimulation, the normalized SP increase at the end of the execution was different in the three modes ($F_{2,38} = 25{,}0$; $\mathbf{p < 0{,}001}_{MODE \times TIME}$); and the analyses by modes of execution indicated that for both the TMS and CMS groups, the SP increased significantly only after *10max* and *30max* (Post-hoc; $\mathbf{p < 0{,}001}$; in two cases) (Figure 4.7(B), (D)). Set sequence never had a significant effect on SP ($\mathbf{p > 0{,}05}$ for main effects or interaction; i.e., in all modes and task).

4.3. Paper 3 - Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks

The mean output intensity used for magnetic CMS was 89,8 % (*SE* 4,3) (in 9 of the 16 sessions it was at 100 %); for electric CMS was 551,0 *V* (*SE* 30,9). Before the fatigue sessions, we observed that the CMS (magnetic and electric) did not condition CMAP amplitudes acquired at the same 2,5 *s* MVC ($F_{1,14} = 0{,}7376; \mathbf{p > 0{,}05}_{CONDITIONING}$); this happened the two testing days ($F_{1,14} = 0{,}7;\ \mathbf{p > 0{,}05}_{DAY}$; and $F_{1,14} = 0{,}1;\ p > 0{,}05_{DAY \times CONDITIONING}$). The ICC were excellent when comparing the conditioned vs. the unconditioned CMAP amplitudes (0,98 [0,96–0,99, *CI*95 %]) mean of the two days pooling electric and magnetic CMS, as well as in isolation: 0,98 [0,96–0,99; *CI*95 %] for electric and 0,98 [0,95–0,99; *CI*95 %] for magnetic stimulation. Therefore, CMS did not influence the CMAP amplitude acquired at the same MVC. The Table 4.7 shows the normalizing values for the fatigue protocol, equivalent to the units depicted in the graphs.

Table 4.7: Mean score including all subjects. The score is the *y*-axis unit in graphs.

Task	*Max output in task at any evaluation time-point	*Max active full ROM	*MVC at any stimulation time-point	Mean CMS-SP duration at comfort
ft	7,4 *Hz* (SE 0,3)	33, 0º (SE 19)	4,2 *kg* (SE 0,2)	61,1 *ms* (SE 2,7)
iso	3,7 *kg* (SE 0,1)	n.a.	4,1 *kg* (SE 0,2)	67,0 *ms* (SE 4,0)

n.a.: not applicable.

*The score utilizes the maximal value from each subject: it is the mean of the maximal scores including all subjects.

Table 4.8 indicates the main effects and significant interactions for all variables, one column for each of the evaluated variables. For each variable (column), the first row corresponds to the ANOVA model with the two tasks (*ft* and *iso*), the three execution modes, the four sets and the two testing time points. In some variables, the interactions of Task with other factors were significant and indicated a different response for *ft* and *iso*; in those cases a follow-up ANOVA was executed by task type (indicated in subsequent rows). We proceeded likewise if the responses differed for execution mode (i.e., ANOVA by pair of modes and, eventually, with just one mode). *Post-hoc* analyses are shown in the figures.

Table 4.8: Summary of main effects and interactions for the different variables.

CMEP amplitude	EMG – RMS $PRIOR-CMEP$	Task motor execution	MVC force at CMS	SP duration	CMAP amplitude	Row
$F_{1,14}$=12,4 p<0,01 $TASK\times MODE$ $F_{2,28}$=3,9 p<0,05 $MODE\times TIME$	$F_{2,28}$=9,6**p=0,001** $TASK\times MODE\times TIME$	$F_{2,28}$=90,9**p<0,001** $MODE\times TIME$ $F_{2,28}$=8,6**p=0,001** $TASK\times MODE\times TIME$	$F_{1,14}$=12,8**p<0,01** $TASK\times TIME$	$F_{1,14}$=14,7**p<0,01** $TIME\times TASK$	$F_{2,28}$=5,4**p<0,05** $TASK\times MODE$	1st
FINGER TAPPING						
$F_{2,28}$=6,8 p < 0,01 $MODE\times TIME$	$F_{2,28}$=9,7$_{\varepsilon=0,6}$ **p<0,001** $MODE\times TIME$	$F_{1,14}$=101,1 **p<0,001**$_{TIME}$ $F_{2,28}$=45,4**p<0,001** $MODE\times TIME$	$F_{1,14}$=5,8 **p<0,05**$_{TIME}$ $F_{2,28}$=10,2$_{\varepsilon=0,6}$ **p<0,01**$_{MODE}$ $F_{3,42}$=4,0 **p<0,05**$_{SET}$	p>0,05 for factors and interactions	p>0,05 for factors and interactions	2nd
Comfort	**Comfort**	**Comfort**	N.A	N.A	N.A	
$F_{1,14}$=2,6 p>0,05$_{TIME}$	$F_{1,14}$=5,6 **p<0,05**$_{TIME}$	p>0,05				3rd
10max & 30max	***10max***	***10max***	N.A	N.A	N.A	
$_{1,14}$ = 5,5 p<0,05$_{TIME}$; p>0,05 for interactions	$F_{1,14}$=1,1 p>0,05$_{TIME}$	$F_{1,14}$=39,3 **p>0,001**$_{TIME}$				4th
N.A	***30max***	***30max***	N.A	N.A	N.A	
	$F_{1,14}$=5,6 **p<0,01**$_{TIME}$	$F_{1,14}$=81,8 **p<0,001**$_{TIME}$ $F_{3,42}$=3,6 **p<0,05**$_{SET}$				5th
ISOMETRIC						
$F_{1,14}$=4,8 **p<0,05**$_{TIME}$ $F_{2,28}$=5,4 **p<0,05**$_{MODE}$	p>0,05 for factors and interactions	$F_{2,28}$=90,9 **p<0,001**$_{MODE\times TIME}$ $F_{6,84}$=5,4$_{\varepsilon=0,5}$ **p<0,001**$_{MODE\times SET}$	$F_{2,28}$=25,8 **p<0,001**$_{TIME\times MODE}$	$F_{1,14}$=13,9 **p<0,01**$_{TIME}$ $F_{2,28}$=3,6 **p<0,005**$_{TIME\times MODE}$	$F_{2,28}$=3,8 **p=0,051**$_{MODE}$ $F_{2,28}$=3,8 **p<0,05**$_{TIME\times MODE}$	6th
Comfort						
p>0,05 for factors and interactions		$F_{1,14}$=17,2 **p<0,001**$_{TIME}$	$F_{1,14}$=15,9 **p<0,01**$_{TIME}$ $F_{3,42}$=4,6 **p<0,05**$_{SET}$	$F_{1,14}$=12,4 **p<0,01**$_{TIME}$	p>0,05 for factors and interactions	7th
10max						
$F_{1,14}$=6,4 **p>0,05**$_{TIME}$		$F_{1,14}$=23,8 **p<0,001**$_{TIME}$ $F_{3,42}$=5,2$_{\varepsilon=0,6}$ **p<0,05**$_{SET}$	$F_{1,14}$=17,6 **p<0,001**$_{TIME}$ $F_{3,42}$=6,0 **p<0,01**$_{SET}$	$F_{1,14}$=7,6 **p<0,05**$_{TIME}$	p>0,05 for factors and interactions	8th
30max						
p>0,05 for factors and interactions		$F_{1,14}$=119,9 **p<0,001**$_{TIME}$ $F_{3,42}$=25,3 **p<0,001**$_{SET}$	$F_{1,14}$=114,0 **p<0,001**$_{TIME}$ $F_{3,42}$=25,5 **p<0,01**$_{SET}$	$F_{1,14}$=10,7 **p<0,01**$_{TIME}$	p>0,05 (p=0,09)	9th

The first (un-shaded) row of statistical values corresponds to the ANOVA model with the two tasks (ft & iso), the three execution modes, the four sets and the two testing time points. For some variables, the interactions of Task with otherfactor/s were significant and indicated a different response of those variables for ft and iso; in these cases follow-up ANOVA were executed by task type (in subsequent rows). We proceeded likewise with subsequent ANOVA if the responses differed for execution modes (i.e., ANOVA were executed by pair of modes and, eventually, with just one mode). Those spaces left empty are shown when subsequent ANOVA were not applicable (N.A), because interactions were not significant. The last column (Row) links statistics to main text.

CMEP amplitude modulation during fatiguing *ft* and *iso* tasks

CMEP amplitudes (relative to CMAP) behaved differently in the three modes of execution for *ft* and *iso* tasks (ANOVA $\mathbf{p < 0{,}05}$), and also differently at *pre/post* in each of the different modes (ANOVA $\mathbf{p < 0{,}01}$). The responses can be observed in Figure 4.9 (representative individual examples during isometric maximal modes) and Figure 4.10, means for all subjects in all modes and tasks.

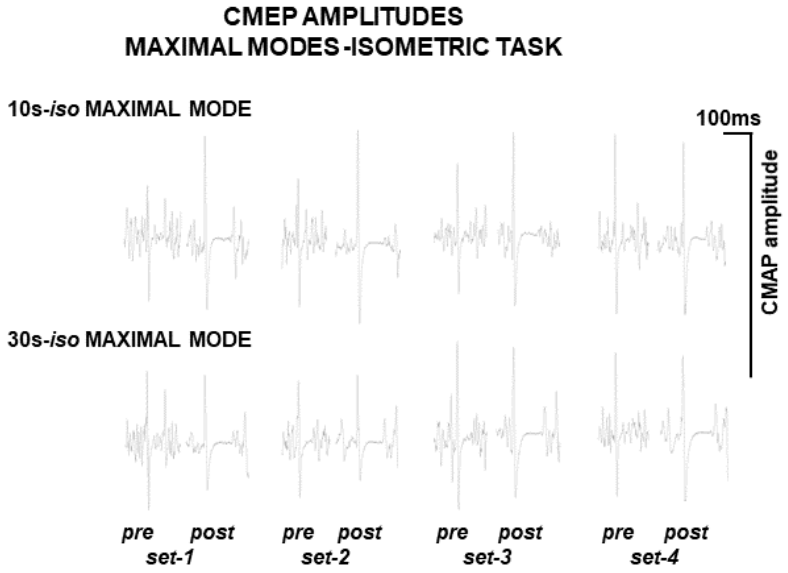

Figure 4.9: Examples taken from a representative subject for *iso* maximal modes. The amplitudes are scaled so that the amplitude of the vertical line at the right corresponds to the size of the corresponding CMAP

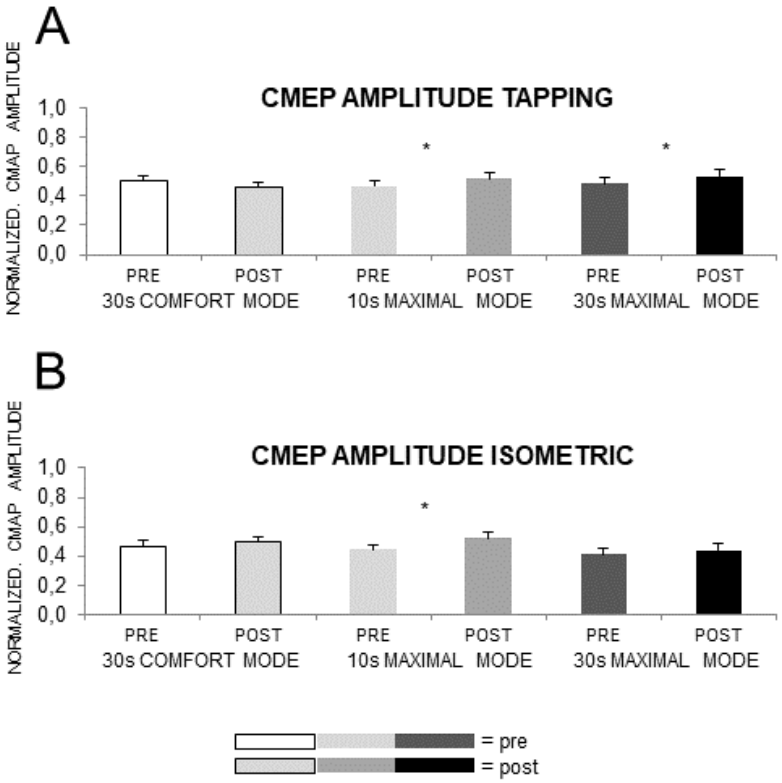

Figure 4.10: CMEP amplitude changes in both tasks. CMEP amplitudes are normalized to the amplitude of the corresponding CMAP. (A) For ft the amplitudes were significantly increased at the end of both maximal execution modes. (B) For iso the amplitudes were significantly increased only at the end of *10max*, but not for *30max*. $^*p < 0{,}05$.

CMEP amplitude after fatiguing *ft* tasks

During *ft*, CMEP at pre and post also behaved differently for the three execution modes (ANOVA $\mathbf{p < 0{,}01}$); thus we performed follow-up ANOVA by pair of modes and modes in isolation. For comfort-ft the CMEP did not change after tapping (ANOVA $\mathbf{p > 0{,}05}$). Conversely, ANOVA including both maximal modes indicated that the amplitude of the potentials increased right at the end of maximal *ft* (ANOVA $\mathbf{p < 0{,}05}$); the effect of TIME did not interact significantly with any other factor (i.e., mode *10 max* and *30 max*; or set), thus a small (5 %) but significant increase in the spinal excitability was shown after maximal *ft*.

CMEP amplitude after fatiguing iso tasks

For the *iso* task, the CMEP amplitudes changed with the three execution modes (ANOVA $\mathbf{p < 0{,}05}$), and also at the end of the tasks (ANOVA $\mathbf{p < 0{,}05}$); a similar pattern was observed if the analysis was performed by pairs of modes. When analyzing the effects mode by mode and in the case of *comfort*, main effects of TIME or SET were not significant, nor their interaction ($\mathbf{p > 0{,}05}$ in all cases). For *10 max* CMEP increased significantly at the end of execution (ANOVA $\mathbf{p < 0{,}05}$), from pre-values of 44,2 % to post-values of 52,1 % of the CMAP-amplitude (increment of 17,9 %); the effect was observed in all sets. For *30 max*, main effects and interactions were always $\mathbf{p > 0{,}15}$; thus CMEP post *30 max* were similar to those observed at pre.

$\mathbf{EMG - RMS_{PRIOR-CMEP}}$

The $\mathrm{EMG - RMS}_{PRIOR-CMEP}$ differed for the two tasks and modes at *pre* and *post* (Figure 4.11, ANOVA $\mathbf{p < 0{,}001}$). This was observed since it remained stable at all times for *iso* ($\mathbf{p = 0{,}115}$ was the smallest p-value for main effects and interactions), but not for *ft*. For *ft*, $\mathrm{EMG - RMS}_{PRIOR-CMEP}$ changed from *pre* to *post*, differently for the three tapping modes (ANOVA $\mathbf{p < 0{,}01}$). It was reduced after comfort tapping (ANOVA $\mathbf{p < 0{,}05}$) and increased after *30max* (ANOVA $\mathbf{p < 0{,}01}$), with no set effects. Before and after *10max* the $\mathrm{EMG - RMS}_{PRIOR-CMEP}$ remained unchanged.

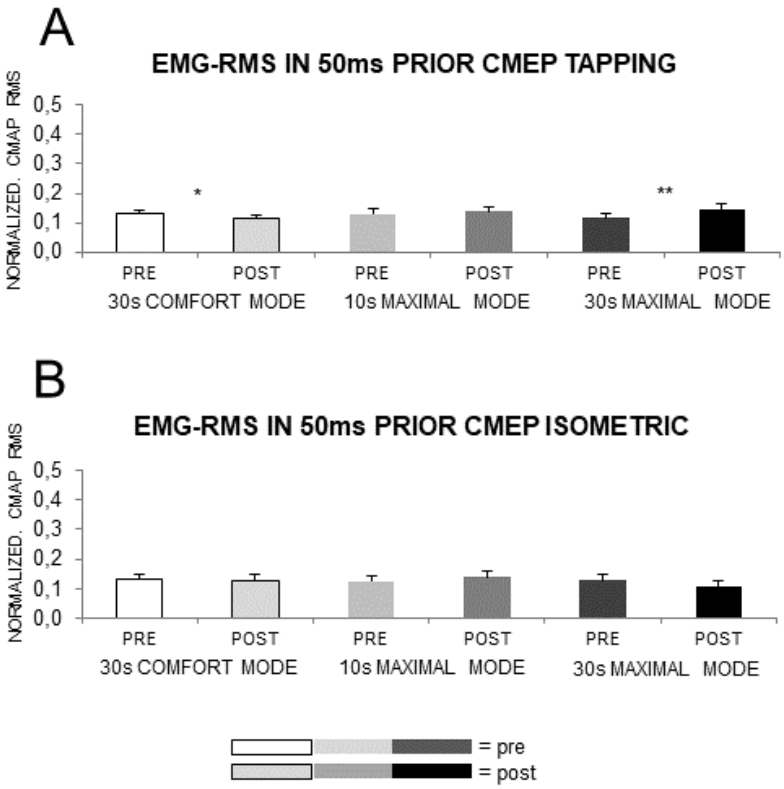

Figure 4.11: EMG-RMS during MVCs at the time of CMS. EMG-RMS are normalized to the RMS of the corresponding CMAP. (A) Right after *ft* the EMG-RMS decreased for the *30 comfort*, and increased for the *30 max* modes. (B) For *iso* EMG-RMS remained unchanged after execution for all modes. $^{*}p < 0{,}05$; $^{**}p < 0{,}01$.

Task motor execution. Frequency/force decrement during execution in *ft/iso* tasks

The observed motor output decrements along the studied tasks (initial vs. final 3 *s* of each set) differed for *ft* and *iso*, and three execution modes (comfort, *10 max* and *30 max*) (ANOVA $\mathbf{p = 0{,}001}$; Figure 4.12). Subsequently, we performed the analyses for each type of task.

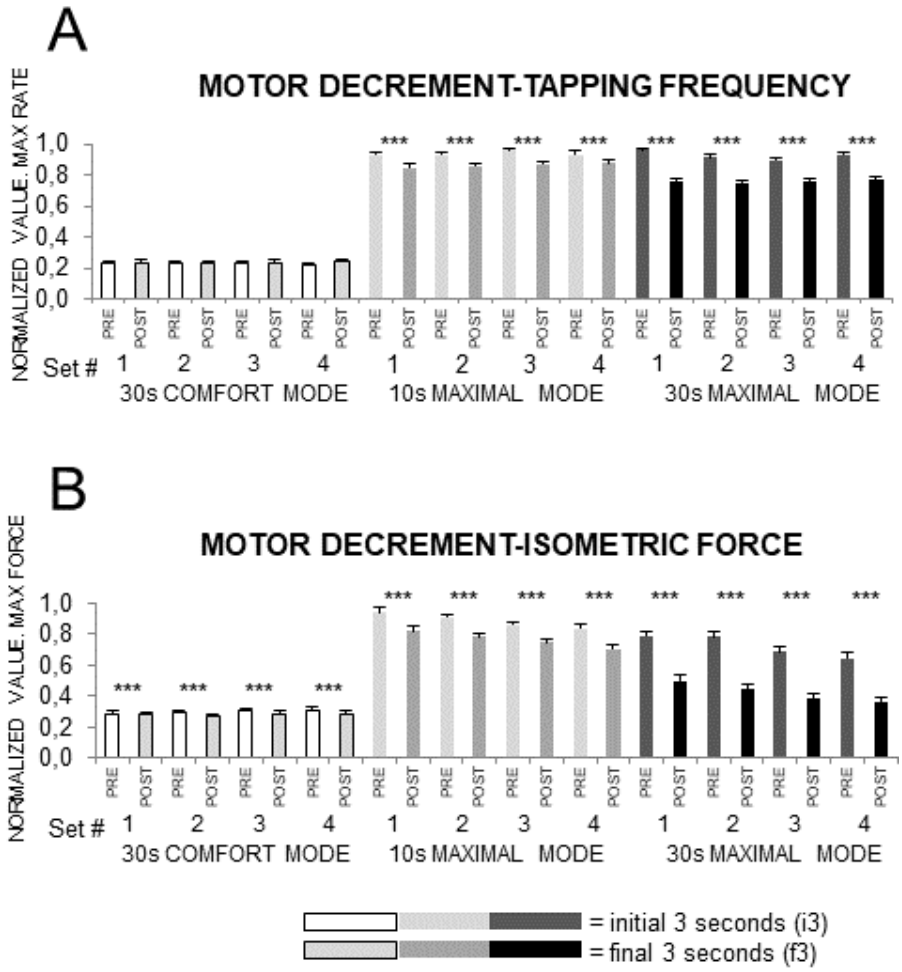

Figure 4.12: Motor execution decrement induced by the tasks. (A) The frequency of ft decreased significantly after the maximal modes. For 30 max a set effect was present. (B) Set by set motor decrement along the *max iso* tasks was more evident. The force reduced at post after maximal modes and also after *comfort*. $^{***}p < 0{,}001$

Task motor execution during finger tapping

For *ft*, the frequency of tapping dropped along execution, but the three modes of execution were affected differently (ANOVA $\mathbf{p < 0{,}001}$). *ft* frequency at comfort was unchanged, but it dropped after *10 max* and *30 max* (ANOVA $\mathbf{p < 0{,}001}$; Figure 4.12(A)); in this latter case there was also an effect on set progression (ANOVA $\mathbf{p < 0{,}05}$), which affected similarly the initial and final seconds of execution.

Task motor execution during isometric contraction

The Figure 4.12(B) shows the force drop in the *i3* and *f3 s* periods of the *iso*-task. Force dropped differently at the end of the iso-task for the three different modes (ANOVA $\mathbf{p < 0{,}001}$). Additionally, a set effect was present with different expressions in the three execution modes (ANOVA $\mathbf{p < 0{,}001}$). Follow-up ANOVA by modes indicated that for *10 max-iso* post the force dropped significantly (ANOVA $\mathbf{p < 0{,}001}$), and set after set (ANOVA $\mathbf{p < 0{,}05}$). A similar pattern was observed for *30 max-iso*, but more significantly expressed (ANOVA $\mathbf{p < 0{,}001}$ for TIME and SET). Conversely, for comfort there was no set effect ($\mathbf{p > 0{,}05}$), though the force at the end of the 30 *s* of *comfort* was significantly lower that at the beginning (ANOVA $\mathbf{p < 0{,}001}$; Figure 4.13), the effect was small. This means that, at the end of execution, subjects remained slightly below (1,5 %) pre, when trying to maintained the target proposed (i.e., 30 % of their MVC).

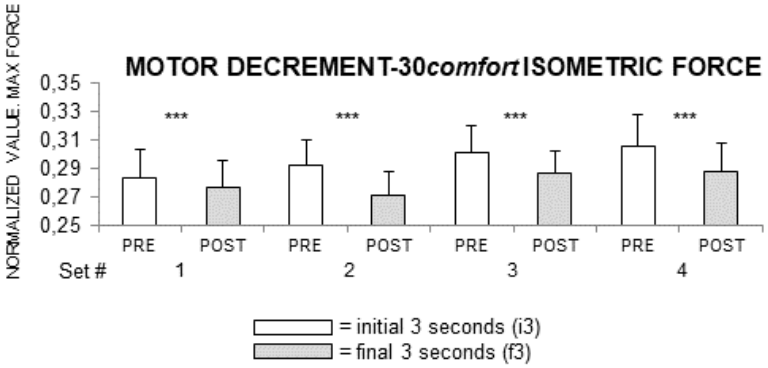

Figure 4.13: Enlarged view to observe the size of the motor decrement for *comfort-iso*. $^{***}p < 0{,}001$.

Angular amplitude during *ft*

ROM amplitudes remained constant from *pre* to *post* ($F_{1,14} = 0{,}2; \mathbf{p > 0{,}05}_{TIME}$) but were different in the three modes ($F_{2,28} = 13{,}2_{\varepsilon=0{,}6}; \mathbf{p < 0{,}01}_{MODE}$). For *10max* it was 13,1 % (SE 2,9) of the maximal active ROM, and 12,6 % (SE 2,3) for *30max maximal*. In both cases smaller than *comfort* (23,8 %, SE 2,9); *post hoc* $\mathbf{p < 0{,}01}$.

MVC force at the time of CMS

The MVC force at the time of CMS waned at post; this was expressed differently in the two tasks, *ft* and *iso* (ANOVA $\mathbf{p < 0{,}01}$; Figure 4.14).

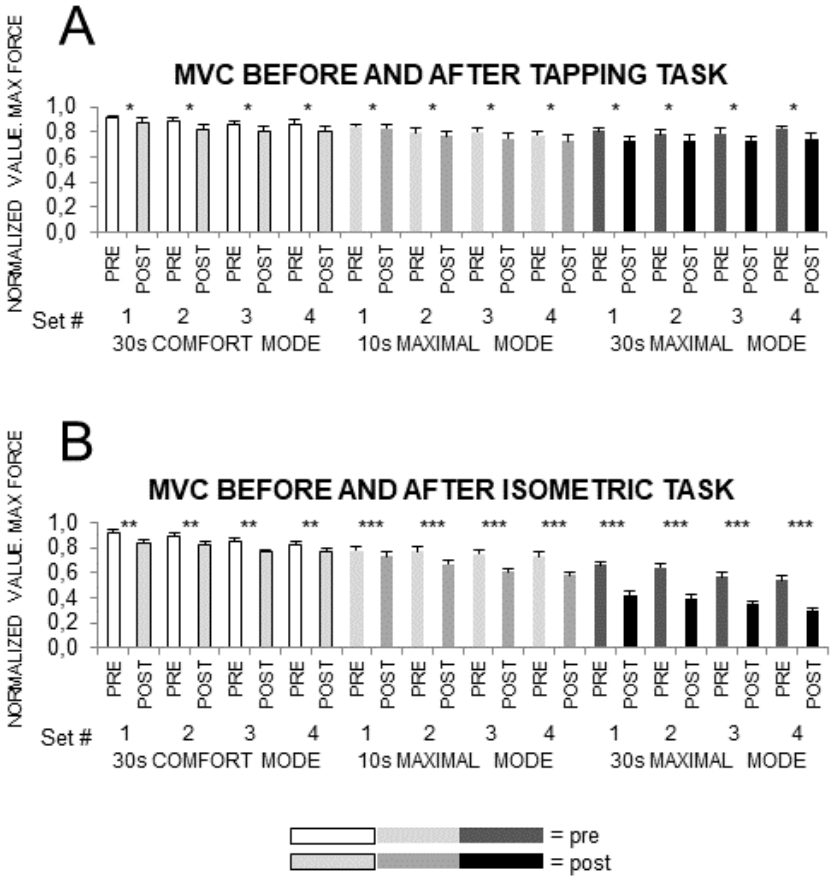

Figure 4.14: MVC force at the time of CMS in both tasks. (A) MVC force was reduced at *post-ft* compared to *pre* in all cases, and also from mode to mode and set to set. (B) MVC force reduction with iso task resembled the pattern of ft but the effect was larger and more significantly expressed. $^{*}p < 0{,}05$; $^{**}p < 0{,}01$; $^{***}p < 0{,}001$.

MVC at the time of stimulation after finger tapping

For the ft task, MVC force reduced at post (ANOVA $\mathbf{p < 0{,}05}$), from mode to mode (ANOVA $\mathbf{p < 0{,}01}$), and set to set (ANOVA $\mathbf{p < 0{,}05}$). Thus, *post* MVC waned always after tapping (compared to *pre*). A reduction in MVC with the progression of the testing protocol was also observed in all cases (modes and sets) (Figure 4.14(A)).

MVC at the time of stimulation after isometric contraction

The case of *iso* was different, as the MVC dropping from *pre* to *post* was differently expressed for the three modes (ANOVA $\mathbf{p < 0{,}001}$; Figure 4.14(B)).

Analyses by modes indicated, however, that in all modes MVC dropped from pre to post (ANOVA $\mathbf{p < 0{,}01}$ for *comfort*; and $\mathbf{p < 0{,}001}$ for *10max* and *30max*), and set after set (ANOVA $\mathbf{p < 0{,}05}$ for *comfort*; and $\mathbf{p < 0{,}01}$ for *10max* and *30max*).

SP after *ft* and *iso* tasks

At post the SP change was significantly different for the two tasks (ANOVA $\mathbf{p < 0{,}01}$), this is clearly observable in the representative individual recordings shown in Figure 4.15.

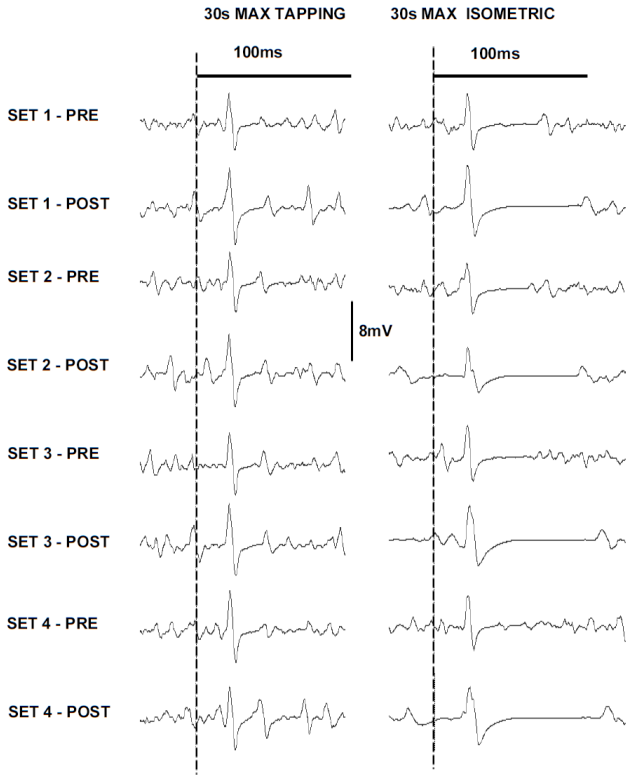

Figure 4.15: Examples of silent periods acquired during the 30 *s maximal mode* before and after each set for *ft* and *iso* tasks (for the same representative subject). The increase in the SP duration *post* activity in the case of *iso* is very clear, with no set effect.

SP after *ft* tasks

The SPs were not modified after *ft*, regardless of the mode of execution or set progression ($\mathbf{p > 0{,}05}$ for all main effects and interactions). Thus, clearly, the fatiguing *ft* activity had no reflection in SPs, Figure 4.16(A).

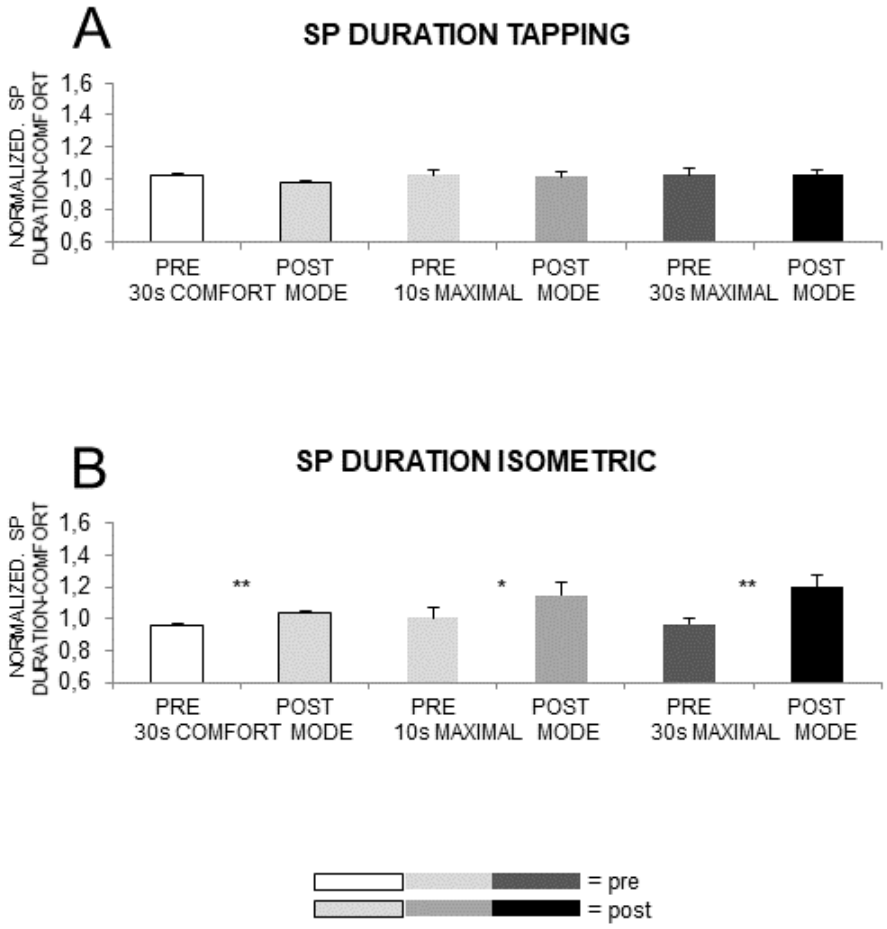

Figure 4.16: Silent period changes observed in both tasks in all subjects. (A) for *ft*, SP were never modulated by the execution. (B) SP increased after *iso* tasks for all modes. $*p < 0{,}05$; $**p < 0{,}01$

SP after *iso* tasks

SP increased after *iso* execution, and such an increment was different in magnitude for the three execution modes (ANOVA $\mathbf{p < 0{,}05}$). It turned out to be significant in all the cases (Figure 4.16(B)). For *comfort-iso* SP increased by 7 % (ANOVA $\mathbf{p < 0{,}01}$), for *10 max-iso* the increment reached 15 % (ANOVA $\mathbf{p < 0{,}01}$), and for *30 max-iso* the increase in SP at post was 23 % (ANOVA $\mathbf{p < 0{,}01}$); such effects were present in all sets.

CMAP-amplitude modulation during fatiguing *ft* and *iso* tasks

The CMAP-amplitude responses were modified differently in the different tasks for the different modes (ANOVA $\mathbf{p < 0{,}05}$; Figure 4.17).

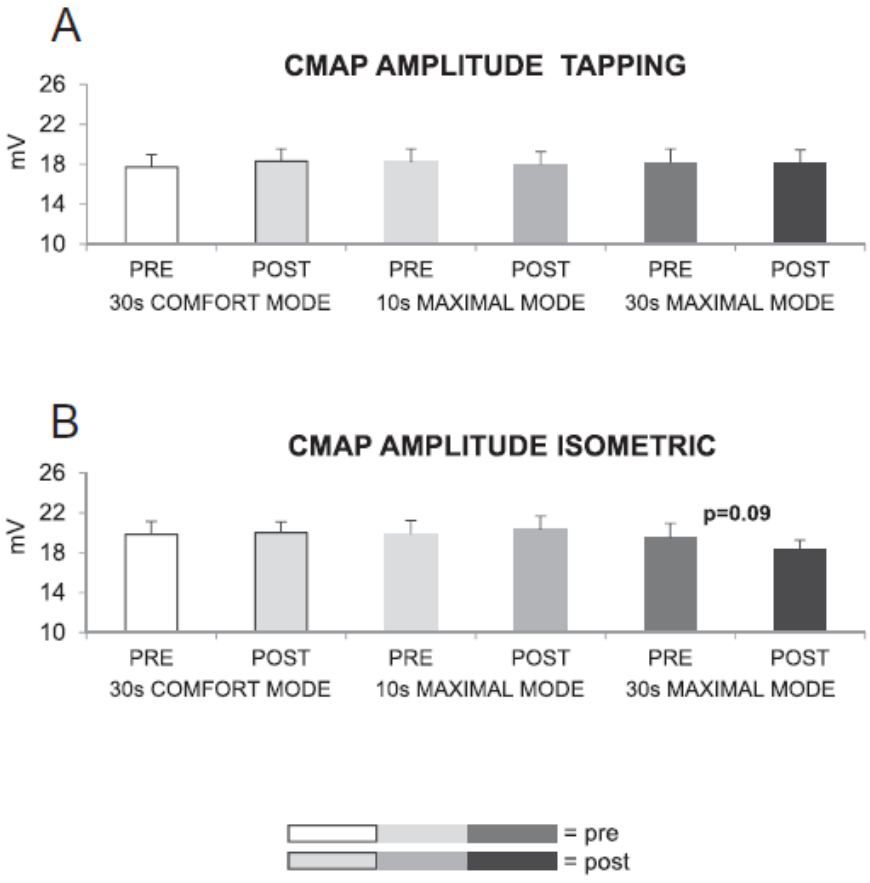

Figure 4.17: CMAP amplitude changes achieved in both tasks were different. (A) the *ft* did not change the CMAP amplitudes. (B) The change in the CMAP amplitudes at the *post-iso-30 max* did not reach significance (see main text por changes other than those reported in *pre-post* conditions.

CMAP-amplitude modulation during fatiguing *ft* tasks

For *ft*, all main effects and interactions between factors were non-significant ($\mathbf{p > 0{,}8}$ was the smallest p value; Figure 4.17(A)). Thus, CMAPs were not modified at any moment of testing in the ft tasks.

CMAP-amplitude modulation during fatiguing iso tasks

For the *iso* tasks (Figure 4.17(B)), the CMAP amplitude changed from mode to mode (ANOVA $\mathbf{p = 0{,}051}$); the amplitude was always smaller at *30 max* than *10 max* (both *pre* and *post*; *post hoc* $\mathbf{p < 0{,}05}$). It was also observed that the change from pre to post was significantly different for the three modes (ANOVA $\mathbf{p < 0{,}05}$), although post hoc analysis indicated that the smallest *p*-value was $\mathbf{p = 0{,}09}$; for *30 max*.

Responses induced by electric vs. magnetic CMS

Finally, we reproduced the above-mentioned analyses modifying the statistical design; the within-subjects factors were the same as above (*task, mode, set and time*), but a between-subjects factor (*group*) with two levels (electric-CMS, and magnetic-CMS) was added to check if the responses induced by both types of stimulation differed. This was never the case.

Capítulo 5

Discusión

5.1. Resumen general

En esta tesis doctoral por compendio se presentan los resultados obtenidos en experimentos que han sido aceptados en revistas científicas pertenecientes al "Journal Citations Report", y que forman parte de una línea de estudio enfocada a conocer las bases de la fatiga de tipo central inducida por movimientos repetidos. El objetivo de esta línea de investigación es conocer las manifestaciones de la fatiga en este tipo de movimientos para, en un futuro, poder intervenir en ellas y disminuir el impacto de la fatiga inducida por contracciones musculares de este tipo, las cuales son fundamentales para distintas actividades de la vida diaria, algunas simples, como el uso de ratones o teclados, pero también otras más complejas, como la marcha. Este tipo de actividad muscular, además de lo comentado, se encuentra presente en deportes de alto nivel o actividades ocupacionales (cadenas de montaje, conducción, etc.). Asimismo, las expresiones de fatiga producidas por este tipo de movimientos podrían verse aumentadas por el envejecimiento, patologías neurológicas y/o neurodegenerativas, o incluso, de ser objeto de neuromejora [225] [226]. Para poder abordar estos objetivos en el futuro, es esencial comprender las manifestaciones centrales de la fatiga producida por este tipo de movimientos.

Cronológicamente, este compendio se inicia con experimentos realizados en el año 2013 que mostraron el efecto de la estimulación transcraneal por corriente directa en la prevención de la fatiga en un movimiento de alcance, ejecutado repetidamente a

máxima velocidad [2]. Posteriormente se llevaron a cabo otros experimentos durante los años 2014 y 2015 donde se evaluaron, de una manera más precisa, los efectos de la fatiga sobre una tarea de golpeo repetitivo del segundo dedo de la mano dominante [3], [4].

El primer artículo científico del compendio consiste en un estudio del efecto de la estimulación transcraneal por corriente directa aplicada sobre **M1** de manera bilateral, en un movimiento de alcance consistente en la proyección de extremidad superior hacia una diana situada en frente, dentro del espacio peripersonal. El movimiento fue realizado de manera repetida, a la máxima velocidad, y enmarcado dentro de una tarea de tiempo de reacción simple y con doble opción (*choice*).

Por las características de la estimulación transcraneal por corriente directa, una de las sesiones realizadas consistió en una aplicación placebo, como control a las sesiones de intervención [215]. En esta sesión placebo, idéntica a las otras sesiones y aleatorizada en orden de realización, se pudo observar el efecto inducido por la repetición del movimiento de la ejecución del sujeto a lo largo del experimento, sin la posible interferencia producida por la estimulación cortical. Así, el primer efecto observado fue el aumento del tiempo pre-motor en el músculo tríceps braquial, principal músculo responsable de la proyección del miembro superior, al comparar la primera parte de la sesión (antes de la estimulación transcraneal por corriente directa de tipo placebo) con la segunda (después de la misma) [227].

Sin embargo, se observó que la aplicación de estimulación transcraneal por corriente directa de manera bilateral en la corteza motora primaria evita la aparición de este efecto inducido por la repetición de la tarea (i.e., fatiga), independientemente de la polaridad del montaje.

Comprobamos además, un efecto de la tarea de movimientos repetidos a máxima velocidad sobre el coeficiente de variación (del tiempo pre-motor y del tiempo de reacción pero no en la duración del movimiento), en la que disminuyó la variabilidad con la progresión de la tarea. La disminución de la variabilidad implica que los movimientos se vuelven más estables. Se ha reportado previamente que la estimulación transcraneal por corriente directa aplicada sobre el hemisferio izquierdo puede disminuir la variabilidad [228], sin embargo, y dado que el efecto se presenta, tanto si hubo neuromodulación como si no la hubo, es posible que se deba tanto a un factor

de fatiga [229] como a un factor de aprendizaje [230]. No obstante, el hecho de que se presente para el tiempo pre-motor de los tres músculos evaluados y no sólo para el músculo tríceps braquial, que es el que resulta fatigado, junto con el hecho de que no se previene con la neuromodulación, apunta a que no es un efecto de la fatiga. Algunas teorías clásicas de aprendizaje motor hipotetizan que el aprendizaje se debe a mecanismos de compensación tendentes a reducir la variabilidad en la ejecución [231]; de esta manera, tiene sentido que sea un efecto de aprendizaje de la tarea, aunque el experimento no fuera diseñado para ello.

Para el desarrollo del segundo y del tercer artículo que componen esta tesis doctoral, se cambió el modelo experimental a uno que permitiera la evaluación de los mecanismos neuromusculares subyacentes a la fatiga de tipo repetitiva; tratando de minimizar el posible efecto que los procesos implicados en la planificación y las bases cognitivas de la ejecución motriz. Para esto, se escogió el modelo del golpeo repetitivo del segundo dedo en un movimiento de flexo-extensión, que ya había sido empleado en el estudio de la fatiga [29] [30]. Además, y dado que las bases centrales de la fatiga han sido estudiadas principalmente durante contracciones isométricas [14] [105] [5] [169] [162], incluímos una condición "*control*"de tipo isométrica. De tal modo, los resultados del estudio de las bases centrales de la fatiga muscular producida por los movimientos repetitivos se compararon con los producidos por tareas isométricas ejecutadas con los mismos segmentos corporales.

5.2. Elección de las variables de estudio de la fatiga y resumen general de los resultados

Debido precisamente a que la investigación en fatiga neuromuscular se ha centrado en la evaluación de la fuerza durante una contracción isométrica, muchas de las variables comúnmente utilizadas en su estudio son principalmente evaluables durante la propia ejecución de una contracción máxima voluntaria isométrica (el período de silencio, la ganancia de torque superimpuesta o el potencial de acción muscular compuesto durante la contracción). Por dicho motivo, y para poder realizar una comparación directa entre expresiones de la fatiga producida por movimientos repetitivos rítmicos y contracciones isométricas, decidimos realizar una contracción máxima voluntaria previa a la ejecución y otra posterior a la ejecución de la tarea objeto de estudio de la fatiga (el golpeo repetitivo del dedo), independientemente de si se trataba de estudiar la fatiga generada por el movimiento repetitivo o por la de isometría control; de tal manera testamos variables asociadas a la fatiga que se obtienen durante dicha contracción máxima voluntaria. Esta metodología ha sido utilizada comúnmente en protocolos de evaluación de la fatiga producida por contracciones no isométricas [222] [105] [162] [232].

5.2.1. Evaluación de variables comportamentales

En nuestros estudios el rendimiento motor disminuye cuando la ejecución es máxima y prolongada en el tiempo. Ello se observa independientemente del tipo de ejecución realizada, un aspecto totalmente compatible con una de las definiciones propuestas de fatiga definida como *Un decremento del rendimiento motor máximo con el mantenimiento de la tarea*. Ello se vió reflejado tanto en la disminución de la frecuencia de golpeo voluntaria máxima, como en la fuerza máxima en los artículos segundo [3] y tercero [4] de esta tesis, así como en un incremento del tiempo pre-motor en el primero [2]. Cuando la ejecución es submáxima o confortable en los experimentos de golpeo repetitivo del dedo, el rendimiento motor no expresó fatiga, si bien hay que considerar la reducida duración de la tarea (30s), que es ampliamente menor a los clásicos experimentos en los que la contracción submáxima se mantiene hasta el fallo muscular (e.g.- en los experimentos de *Duchateau et al.*, al 25 % de la

contracción máxima voluntaria, el tiempo de contracción hasta el fallo se prolongó entre 360 y 660 s; mientras que en los de *Cogiamanian et al.*, al 35 % de la contracción máxima voluntaria, se prolongaron hasta 260 s aproximadamente) [169] [196].

Por otra parte, en la ejecución de la contracción isométrica submáxima los resultados del artículo segundo y tercero presentan diferencias en cómo fue el comportamiento de los sujetos al principio y final de los 30 s de ejecución (en uno se mantiene mientras que en otro disminuye al final de los 30 s; si bien muy levemente). Creemos que ello no se debe a que en unos sujetos y otros las expresiones de la fatiga fuesen diferentes, sino a una distinta capacidad (o estrategia) de los participantes a ajustarse a la retroalimentación proporcionada durante la tarea; a los participantes de ambos estudios se les requirió que ajustasen su fuerza al 30 % de su MVC durante los 30 s de la tarea, mientras que en el primer experimento lo lograron, en el segundo experimento el rendimiento fue significativamente menor del solicitado al final de la tarea (si bien tan sólo disminuyeron su ejecución en menos del 1 %). Ambos grupos fueron capaces de aumentar su fuerza a niveles muy elevados inmediatamente después de la contracción submáxima, por lo que es probable que el efecto previamente indicado no se atribuya a ninguna expresión de fatiga, si no a la estrategia general de ejecución del sujeto o a una distinta capacidad de ajustarse a un feed-back visual en ambos grupos (algo que no fue evaluado en ausencia de fatiga).

Sin embargo, cuando la ejecución fue máxima, la caída en el rendimiento motor de la ejecución isométrica mantenida (por 10 o 30 segundos) es más marcada conforme se repiten el número de ejecuciones (4 series de cada duración), algo que, curiosamente no ocurrió con la ejecución del golpeo repetitivo del dedo, en la que la caída en el rendimiento motor del inicio al final de la tarea se mantuvo serie tras serie. Este comportamiento distinto, en el que una tarea es capaz de recuperarse en el tiempo de descanso establecido (ejecución de golpeo repetitivo del dedo) mientras que la otra no (ejecución isométrica), a pesar de la intensidad máxima de ambas tareas, refuerza el principio del *Task Dependency* en el desarrollo de la fatiga [6] [233].

No hay que olvidar que estamos hablando de rendimiento motor, es decir, del desempeño de cada tarea respecto a su capacidad máxima (de generar fuerza para la ejecución isométrica y frecuencia de golpeo para la ejecución de golpeo repetitivo del dedo). Sin embargo, debido a que las contracciones máximas voluntarias de eva-

luación se realizan antes e inmediatamente después (sin descanso) en ambos tipos de tarea (isométrica y golpeo repetitivo del dedo), fue posible comparar cómo cada tarea afecta a la capacidad de generación de fuerza sin permitir la recuperación del sistema motor respecto a la tarea evaluada.

En cuanto a la capacidad de generar fuerza, tanto inmediatamente después de la ejecución isométrica como tras el golpeo repetitivo del dedo, a mayor demanda (máxima vs. confortable) encontramos mayor detrimento de la capacidad de generar fuerza. Además, cuando la ejecución es isométrica máxima, se produce una acumulación de la fatiga de una serie a otra (con 100s de recuperación inter-serie) que fue mayor en la ejecución de 30 s, pero presente también en la de 10 s, algo que no se observó en la frecuencia de golpeo del movimiento repetitivo del dedo. A este respecto, un elemento pendiente de definir en nuestros estudios y en otros muchos [105] [162] es el coste, en términos de generación de fatiga, producto de los propios protocolos de evaluación de la fatiga. Ello es una posibilidad debido al uso de contracciones máximas voluntarias realizadas varias veces a lo largo de un protocolo de evaluación. En experimentos que han continuado los presentes hemos acotado dicho elemento [234], por lo que incluiremos dichas evidencias una vez finalizado este resumen de nuestros datos del compendio de publicaciones de esta tesis, para establecer una adecuada discusión.

En el segundo experimento de esta tesis es destacable el papel identificado de los mecanismos inhibitorios corticales y espinales evaluados a través del período de silencio en ambas tareas. De esta manera, observamos que el período de silencio inducido por estimulación magnética transcraneal se incrementó inmediatamente después de la ejecución, tanto si esta era isométrica como si era golpeo repetitivo del dedo, pero sólo sí la ejecución es máxima (de 10 o 30 s). Por tanto la duración del período de silencio inducido por estimulación magnética transcraneal se prolonga al final de la tarea fatigante, al comparárlo con su duración previa a la tarea, en estado no fatigado. Es tremendamente importante la observación de que el período de silencio inducido por estimulación cérvico-medular no se comporta de manera diferente al período de silencio inducido por estimulación magnética transcraneal si la ejecución de la tarea es la isométrica fatigante (tanto de 10 como de 30 s, ya que ambos casos se elonga); caso contrario para las tareas fatigantes de golpeo repetitivo del dedo,

en las que el período de silencio inducido por estimulación cérvico-medular evaluado inmediatamente después de la ejecución de golpeo repetitivo del dedo (y en las mismas condiciones que el período de silencio inducido por estimulación magnética transcraneal) no se vio modificado respecto a sus valores antes de la tarea fatigante de 10 y 30 s máximos, mientras que sí se observaba un incremento en su duración en el período de silencio inducido por estimulación magnética transcraneal de **M1**. Esto nos permite concluir (parcialmente) que la excitabilidad de las motoneuronas espinales no se ve comprometida por tareas fatigantes repetitivas de corta duración (hasta 30 s), mientras que los circuitos inhibitorios corticales sí que aumentan su excitabilidad durante los movimientos repetitivos rítmicos: este es un dato relevante y novedoso. Además, este estudio apoya resultados previos que indican un incremento de la inhibición (es posible que también exista una desfacilitación) a nivel espinal producida por contracciones isométricas máximas fatigantes [105] [162] [235], sin poder discernir si el efecto en el período de silencio inducido por estimulación magnética transcraneal observado en esta tarea es parcialmente cortical en origen, lo cual también ha sido defendido por estudios previos [236]. Otros autores como *Maruyama et al.*, utilizando otras técnicas de evaluación fisiológica cortical tras contracciones isométricas máximas, encontraron una disminución de la inhibición cortical de corta latencia[237]. La disminución de la inhibición intracortical de corta latencia en **M1** parece contradecirse con la inhibición asociada al período de silencio inducido por estimulación magnética transcraneal, sin embargo, ambas variables evalúan poblaciones inhibidoras distintas [110]. A su vez, *Hunter et al.*, encontraron una disminución de la facilitación intra-cortical en este tipo de tareas [238]. Además de lo comentado, recientemente *Otieno et al.* [239], evaluaron el efecto sobre el potencial *N100* de la fatiga producida por una contracción máxima voluntaria de 30 s y encontraron una disminución del *N100* con la fatiga. Dado que la disminución del N100 se asocia con una disminución de la inhibición $GABA_B$ [240] parece que el aumento del período de silencio inducido por estimulación magnética transcraneal tras una tarea isométrica puede estar más asociado a mecanismos espinales, que a cambios ocurrentes a nivel cortical.

En el tercer experimento de esta tesis [4], el foco se puso en el papel de la excitabilidad espinal evaluada a través del potencial evocado motor cervico-medular.

Observamos un aumento de la excitabilidad (amplitud del potencial evocado motor cervico-medular) durante la ejecución máxima del golpeo repetitivo del dedo; por otra parte, en el caso de la tarea isométrica observamos un fenómeno curioso: La excitabilidad aumenta cuando la ejecución es máxima y breve (10s), pero al final de una ejecución máxima y prolongada ($30\,s$) no difiere de la observada antes de la tarea fatigante. La explicación a dicho fenómeno parece simple, y se argumenta en que la excitabilidad espinal aumentada a los 10s de actividad isométrica máxima, que es probablemente necesaria para dicha ejecución máxima, no se puede mantener en niveles tan elevados si la tarea se prolonga hasta los 30s. Es decir, durante el transcurso de los 30s de actividad máxima isométrica la excitabilidad espinal aumenta, lo cual se observa a los $10\,s$, y comienza a decaer desde dichos niveles aumentados de forma que a los $30\,s$ está a niveles similares a los observados antes de comenzar la tarea. Con elevada probabilidad, si aumentáramos la duración de la tarea la excitabilidad continuaría decayendo [162]. En esta misma línea, es importante decir que dichos resultados se observaron haciendo relativo el tamaño del potencial evocado motor cervico-medular al tamaño del potencial de acción muscular compuesto, controlando de esta manera el posible efecto de los cambios de la excitabilidad periférica durante la evaluación de la excitabilidad espinal [5] [162]. La excitabilidad periférica, expresada a través del potencial de acción muscular compuesto muestra una tendencia a la disminución, sólo con la ejecución isométrica, máxima y prolongada (30s). Por lo tanto, la longitud del período de silencio inducido por estimulación cérvico-medular y la amplitud del potencial evocado motor cervico-medular parecen expresar estados de excitabilidad de distintas poblaciones neuronales, siendo unas inhibidoras (responsables del período de silencio). La amplitud del potencial evocado motor cervico-medular evalúa la excitabilidad motoneuronal reflejando, probablemente, la influencia de inputs de diferente naturaleza (tanto inhibidores como excitadores sobre la motoneurona espinal) siendo el potencial evocado motor cervico-medular el resultado de dicho balance. La complejidad de dicho equilibrio a nivel de redes, se hace especialmente palpable en el caso de la respuesta observada tras 10s de contracción máxima isométrica, donde el potencial evocado motor cervico-medular (evaluado considerando posibles cambios en la transmisión y propagación neuromuscular) aumenta, mientras que también lo hace el período de silencio evocado a nivel cérvico-

medular (fenómeno de inhibición espinal mediado, probablemente por mecanismos de inhibición recurrente y mecanismos de posthiperpolarización [111]).

5.2.2. Fatiga producida por las contracciones isométricas en el contexto científico

La fatiga central ha sido ampliamente estudiada cuando es inducida por contracciones isométricas ([241], [13], [69] [6][14] [105] [94] [242] [5] [162] [199]). Dentro de este contexto, y como ya se ha expuesto previamente, se han utilizado diferentes modelos, tareas y grupos musculares, lo que afecta al desarrollo de la fatiga [6]. En los experimentos llevados a cabo durante el segundo y el tercer artículos, y refiriéndonos a la modalidad de ejecución isométrica, hemos podido contrastar en las variables comportamentales (rendimiento motor y contracción máxima voluntaria) que la ejecución isométrica induce efectivamente una pérdida de fuerza al comparar la fuerza máxima voluntaria previa a la tarea con la fuerza máxima voluntaria inmediatamente posterior a la tarea. Esta merma de fuerza se produce de manera más rápida de lo previamente descrito [105] [162], y tan sólo 10 segundos de ejecución son suficientes para producir una merma significativa y pronunciada de la fuerza muscular. Esta fatiga presenta una serie de características que conviene destacar en el contexto de la literatura científica.

En un artículo publicado en 1984 [222], *Bigland-Ritchie et al.*, defienden la progresividad de la fatiga, propiedad por la cual las manifestaciones de fatiga tales como la pérdida de fuerza escapan a una contracción submáxima voluntaria pero no a una contracción máxima voluntaria. Este fenómeno lo encontramos en el segundo artículo del compendio [3] [4], especialmente en las ejecuciones isométricas no fatigantes, donde las contracciones máximas voluntarias de evaluación sufren una disminución a pesar de no verse afectado el rendimiento submáximo (al menos en lo referente a ser capaces de mantener el rendimiento propuesto, i.e. 30 % de la contracción máxima voluntaria, ya que quizás en otros aspectos del rendimiento motor sí podría haberse visto afectado, como el ratio entre la señal electromiográfica y el torque [238]).

Asimismo, y de acuerdo a los trabajos de Rohmert [243], existe una relación entre la intensidad de contracción isométrica y el tiempo de contracción en la inducción de fatiga, conocida como relación fuerza-intensidad. Para las ejecuciones isométricas,

es evidente una disminución mayor de la contracción máxima voluntaria conforme aumenta la demanda de la tarea, algo observado tanto en el segundo como en el tercer artículo de nuestros estudios.

De la misma manera, *Bellemare* y *Grassino* [244], con un modelo basado en la contracción diafragmática, introdujeron el concepto de *Ciclo de Trabajo*. Si bien el objetivo de nuestros estudios no era evaluar esta relación, se desvela que los tiempos de descanso pautados resultan insuficientes para la recuperación total entre una serie y la siguiente para las ejecuciones isométricas tanto del segundo como del tercer artículo. Por este hecho, observamos un fenómeno entre series para las contracciones isométricas máximas, en el que se va acumulando la pérdida de fuerza por un descanso insuficiente.

Atendiendo a los mecanismos neurofisiológicos que subyacen a la fatiga producida por contracciones isométricas, *Brasil-Neto et al.* [245], fueron pioneros en aprovechar las técnicas de evaluación de la excitabilidad cortico-espinal para el estudio de la fatiga. Encontraron y definieron un fenómeno denominado *Post-exercise Depression* de la excitabilidad cortico-espinal producida por contracciones repetidas. Por otra parte, *Taylor et al.* [105], describieron que la fatiga, producto de contracciones isométricas sostenidas máximas (en flexión de codo supinado), producen un incremento de la excitabilidad de circuitos inhibidores corticales y espinales, algo que en nuestros estudios hemos encontrado en el caso de las contracciones isométricas, pero con la novedad mostrada en nuestros estudios de que el efecto se manifestaba incluso si la tarea fatigante máxima era, tan sólo, de 10s de duración. Este es un dato relevante en el contexto de la literatura, que clásicamente había explorado duraciones de tareas mayores [105] [111].

Ante una contracción sostenida fatigante, el período de silencio tanto cortical como espinal muestran una prolongación para una contracción del 100 % de la contracción máxima voluntaria pero no para una contracción del 30 % de la contracción máxima voluntaria [105]. Además, durante contracciones isométricas repetidas con breve descanso, la duración del período de silencio cortical demostró ser dependiente del ciclo de trabajo (i.e. ratio esfuerzo/descanso), prolongándose más conforme aumenta el tiempo de contracción y disminuye el descanso dentro del ciclo [246].

Nuestros resultados coinciden tanto con el aumento del SP cortical y espinal tras

una contracción isométrica sostenida cuando la contracción es al 100 % de la contracción máxima voluntaria como con su invariabilidad ante una contracción submáxima del 30 % de la contracción máxima voluntaria [105].

Como se ha comentado, el aumento del período de silencio, tanto cortical como espinal, es dependiente de fatiga, de tal modo que a mayores niveles de fatiga, mayor es el aumento en la duración del período de silencio, si bien llega un momento en el que se puede producir un efecto techo, en el que el período de silencio deja de aumentar [105]. Es importante entender, para contextualizar esta respuesta, el comportamiendo del período de silencio en ausencia de fatiga; en tales condiciones, durante contracciones isométricas breves (2-3 s), algunos estudios indican que el período de silencio disminuye conforme aumenta la fuerza [247] [105], si bien algunos otros indican que el mismo no es dependiente del nivel de fuerza en ausencia de fatiga [104]. En consonancia con lo previamente indicado, en nuestros estudios hemos encontrado una mayor duración del período de silencio (tanto cortical como espinal) para el modo 30max que para el modo 10max. También constatamos que la caída de la fuerza no sigue la misma dinámica que el período de silencio, ya que la pérdida de fuerza se acumula serie a serie (como se comentó previamente), mientras que para el período de silencio dicha acumulación no se manifiesta. Esta discordancia entre el comportamiento del período de silencio y el comportamiento de la fuerza podría tener varios orígenes: i.) la existencia de un efecto techo, ii.) la distinta dinámica en la recuperación del período de silencio y los niveles de fuerza [105].

El primer mecanismo se explica debido a que durante la ejecución de una contracción máxima voluntaria de dos minutos, la longitud del período de silencio tanto cortical como espinal aumenta conforme la fuerza máxima disminuye, pero termina alcanzando una longitud máxima y estable [105]. Sin embargo, aunque el período de silencio espinal aumenta, no llega a alcanzar la longitud del período de silencio cortico-espinal. Los mecanismos responsables de estos resultados hallados en contracciones máximas de larga duración parecen operar también en el caso de que las contracciones máximas sean de corta duración, de acuerdo a nuestros datos.

Sin embargo, de acuerdo al segundo mecanismo, el aumento del período de silencio va asociado a fatiga, y por tanto, su retorno a la normalidad se asocia al descanso. Con períodos breves de descanso, incluso muy breves de hasta 5 segundos [246], el período

de silencio es capaz de recuperarse completamente a niveles basales. Por dicho motivo es esencial, de capital importancia, evaluar la fatiga sin permitir la recuperación del sistema motor. Esta propiedad ha sido observada en nuestros resultados, dado que los períodos de silencio, independientemente del modo de estimulación, siempre se recuperaron entre una serie y la siguiente, mientras que esto no ocurrió en el caso de la fuerza.

Ello necesariamente implica que han de existir otros mecanismos responsables de la fatiga producida por las contracciones isométricas, más allá de lo que se pueda atribuir al incremento de los niveles de inhibición a nivel cortical y espinal, responsables de que la fatiga se acumule serie tras serie en el caso de las contracciones isométricas, estos mecanismos podrían ser de origen periférico. Es más posible que en nuestros estudios los mismos se localicen a nivel contráctil, ya que los mecanismos excitatorios musculares han sido controlados mediante la evaluación de la *onda M* máxima, que presentan tendencia (disminución leve y casi significativa) a verse afectada. De esta manera, mientras que el período de silencio se recupera rápidamente, el tiempo de descanso presente en nuestro protocolo sería insuficiente para la recuperación de dichos mecanismos periféricos, que acabarían acumulándose serie a serie impactando en la merma de la fuerza muscular.

Con el objetivo de ahondar más en los mecanismos implicados en la fatiga isométrica, el tercer artículo de este compendio se centró en explorar elementos de la excitabilidad espinal, no acotados en la segunda publicación, ya conocida como "punto último del sistema neuromotor". De las distintas posibilidades existentes para la exploración de la excitabilidad motoneuronal, escogimos el uso de la estimulación cérvico-medular tanto eléctrica como magnética; ambas variedades de este tipo de estimulación son válidas para la evaluación de la excitabilidad espinal [224], si bien la eléctrica es más molesta y menos tolerada por algunos participantes. Las respuestas a la fatiga evaluada con ambas técnicas no fueron distintas.

Como ya se ha comentado, en nuestros estudios, la amplitud del potencial evocado motor cervico-medular se encuentra relativizada a la del potencial de acción muscular compuesto, ya que se ha reportado que no hacerlo puede condicionar la adecuada evaluación de los cambios en la amplitud del potencial evocado motor cervico-medular [105] [5] [162]. Observamos un aumento de la amplitud del poten-

cial evocado motor cervico-medular durante la ejecución isométrica máxima breve ($10\,s$) respecto a la ejecución no fatigante, y una vuelta a valores basales durante la ejecución máxima prolongada ($30\,s$). Esta disminución de la excitabilidad motoneuronal ya había sido descrita por *Butler et al.*, tras una tarea isométrica máxima de $2\,min$ de duración [162], por lo que es de suponer que el comportamiento en fatiga se compone de dos fases, en la primera, la excitabilidad aumenta, y conforme aumenta la fatiga, existe la imposibilidad de mantener esta excitabilidad aumentada, por lo que regresa a niveles basales, y, si hubiéramos prolongado la tarea, probablemente habría disminuido por debajo de éstos. Dicho comportamiento es claramente distinto al observado para las actividades repetitivas máximas en nuestro estudio, en los que la excitabilidad espinal se mantuvo aumentada al final de la ejecución máxima, tanto fuese esta de 10 o 30 segundos.

Teniendo en cuenta los factores que pueden influir en la excitabilidad espinal (i. e.- inputs aferentes, propiedades motoneuronales intrínsecas, inputs propioespinales e inputs supraespinales), algunos autores han prestado una especial atención a alguno de ellos, en especial a los efectos de los inputs aferentes durante la fatiga generada por contracciones isométricas. *Taylor et al.*, y *Butler et al.*, descartaron un papel determinante para las aferentes tipo III y IV, que son sensibles a estímulos como la acumulación de metabolitos producto de una contracción isométrica sostenida o el dolor. El efecto de estas aferentes se evaluó causando una isquemia transitoria durante la contracción para impedir la limpieza de estos metabolitos, lo que en teoría, mantendría la influencia (i.e.- descarga) de las aferentes tipo III y IV durante más tiempo, que mediante mecanismos polisinápticos reducirían la excitabilidad motoneuronal. Como la excitabilidad espinal se recuperó independientemente de esta isquemia, parece que el papel de las aferentes tipo III y IV en la generación de la fatiga "motoneuronal" es improbable [161] [162]. También *Taylor et al.*, hablan de fatiga supraespinal, haciendo referencia al aumento de fuerza existente tras un estímulo con estimulación magnética transcraneal sobre **M1** durante una contracción isométrica máxima fatigante, evidenciando una comunicación sub-óptima entre la corteza y el músculo; este aumento de fuerza inducida por estimulación magnética transcraneal se agranda con la pérdida de fuerza, evidenciando de manera similar a lo acontecido con la activación voluntaria [5], una disminución de la capacidad cortical para ejercer

fuerza.

En cuanto a las alteraciones en la excitabilidad intrínseca de la motoneurona. *Butler et al.*, mediante *ondas F* demostró una disminución en la excitabilidad motoneuronal durante una contracción isométrica de 90 s generada mediante estimulación eléctrica [89]. Parece, por tanto, que los mecanismos que actúan podrían ser similares a los que hemos encontrado en nuestros estudios, ya que las motoneuronas tienen tendencia a disminuir su excitabilidad debido a la ejecución máxima sin que dicha respuesta sea una adaptación al estado de la periferia.

Modificación de la excitabilidad periférica durante la fatiga muscular

El primer efecto de la actividad muscular sobre la excitabilidad periférica es un aumento del tamaño de la onda M. *Cupido et al.*, [80] revela un aumento dependiente de la frecuencia de estimulación eléctrica y del tiempo de aplicación, resultando en un ligero incremento en el área de la *onda M* máxima tras una estimulación a 3 Hz, y un aumento del 100 % a 10 y 20 Hz durante 3 min. Otros estudios que usaron contracciones voluntarias también reportan un aumento del área o de la amplitud del potencial de acción muscular compuesto [160] [94] [162]. Este aumento no tiene por qué ser instantáneo sino que puede demorarse hasta 20 o 30 s [80] [160]. Además, este aumento es sensible a fatiga y puede disminuir tras haber alcanzado un máximo. De tal modo, con estimulación a 20 Hz la amplitud comienza a decrecer tras un minuto de estimulación, lo cual también parece ser el caso cuando se dan contracciones musculares máximas voluntarias [80] [162].

A diferencia de la contracción isométrica continuada de los trabajos de *Cupido et al.*, o *Butler et al.*, en nuestro trabajo no aparecen contracciones que superen los 30-33 segundos de duración, incluyendo además descansos entre contracciones. Es por esto que quizás no encontramos una disminución significativa de la *onda M* máxima asociada a fatiga, aunque sí que aparece una tendencia a la disminución cuando la demanda es máxima, que roza la significación estadística. Algunos experimentos han evidenciado cambios en la excitabilidad espinal tras tareas isométricas máximas de dos minutos de duración [94] o negado cambios durante estas [160] [248]. Sin embargo, estos estudios no contemplaban el factor periférico evaluado mediante la *onda M* máxima, que una vez controlado, permite desvelar los efectos de una contracción isométrica máxima de dos minutos de duración sobre la excitabilidad espinal [162].

5.3. Uso de las técnicas de estimulación cerebral no invasiva en el estudio de la fatiga del sistema motor

La estimulación cerebral no invasiva agrupa un conjunto de técnicas que han demostrado modificar la excitabilidad neuronal, ya sea la estimulación transcraneal por corriente directa [176] [249] [250] [251], la estimulación magnética transcraneal repetitiva [252] [253] [254] [255] [191] u otras menos conocidas como el uso de campos magnéticos estáticos [171] [172] [173]. Si bien cada una tiene sus particularidades, en general, sus efectos duran más allá del período de estimulación, siendo reversibles si no se aplican nuevamente [256] [257] [173]. Dado que algunos estudios han vinculado la aparición de fatiga con cambios en la excitabilidad cortical, parece posible utilizar la estimulación cerebral no invasiva para contribuir al conocimiento de las bases neurales de la fatiga, y, al mismo tiempo, imaginar que influyendo sobre la excitabilidad cortical en el sentido opuesto a los cambios generados por la fatiga, se podría intervenir sobre ésta. Sin embargo, algunos autores consideran que estos cambios en la excitabilidad cortical inducidos por la fatiga no son causales, si no que son epifenómenos, por lo que los cambios inducidos por la estimulación cerebral no invasiva podrían no traducirse necesariamente en mejoras comportamentales [14] [5]. También es posible que los cambios acontecidos durante la fatiga, puedan interpretarse como adaptaciones que optimicen la ejecución en presencia de la fatiga (e.g.- Muscle wisdom). Por todo esto, la estimulación cerebral no invasiva se convierte en una herramienta interesante para discernir posibles relaciones causa-efecto y las adaptaciones de las expresiones de fatiga.

La estimulación transcraneal por corriente directa presenta la particularidad de que aplicada sobre la corteza motora modula la excitabilidad cortical en función de la polaridad [258] [176] [256] [249] [250] [259]. Esta modulación de la excitabilidad cortical ha demostrado tener un efecto sobre la ejecución motriz, particularmente en tareas fatigantes [196] [202], aunque algunos autores no hayan encontrado este efecto [260], estas diferencias puede deberse a distintos protocolos de estimulación o a que el efecto de la estimulación transcraneal por corriente directa puede variar entre individuos [261].

Otra particularidad específica de la estimulación transcraneal por corriente directa es que la estimulación anódica es capaz de afectar a la excitabilidad de circuitos espinales tanto a nivel de miembro superior como a nivel de miembro inferior [187] [188] [189] [190]. De esta manera, y conociendo que la fatiga inducida por contracciones isométricas máximas se expresa a nivel central principalmente mediante una disminución de la excitabilidad de las motoneuronas espinales, es importante considerar este efecto en aquellos experimentos que ambicionen el estudio de la fatiga mediante el empleo de la estimulación transcraneal por corriente directa.

En lo que respecta al primer artículo de esta tesis [2], el efecto de la estimulación cerebral no invasiva se vuelve complejo de dilucidar debido a la complejidad de la tarea. Nuestra tarea se compone al menos de dos partes, en primer lugar, el componente del tiempo de reacción, simple o choice; y en segundo lugar, el movimiento balístico de alcance.

Los efectos de la estimulación transcraneal por corriente directa en tareas de tiempo de reacción es controvertido [262]. Ello se debe, posiblemente, a que pocos estudios abordan directamente la temática, sino que se focalizan en el uso del tiempo de reacción (seriado) principalmente en tareas de aprendizaje implícito [263] [264] [265] [266].

Al margen de los estudios de tiempo de reacción para la evalaución del aprendizaje implícito, *Devanathan et al.*, llevaron a cabo el estudio más comparable a nuestro protocolo. Evaluaron el efecto de la estimulación transcraneal por corriente directa anódica sobre **M1** del miembro inferior en el tiempo de reacción simple y choice tanto en movimientos del miembro inferior como superior. Sus resultados destacan un aumento del tiempo de reacción choice en el miembro inferior tras la estimulación transcraneal por corriente directa anódica, sin una mejoría notable del tiempo de reacción simple en miembro inferior, ni simple o choice en miembro superior. Sin embargo, la ejecución tras la intervención placebo arrojó unos resultados destacables al resaltar un aumento del tiempo de reacción para todas las condiciones. Teniendo en cuenta que el tiempo de reacción fue definido como el tiempo transcurrido desde el estímulo-respuesta hasta el inicio de la actividad electromiográfica, lo que concuerda con nuestra definición de tiempo pre-motor, podemos destacar un efecto de fatiga en el tiempo pre-motor que se ve atenuado tras una estimulación anódica [267].

A pesar de las limitaciones para comparar los estudios que emplean estimulación transcraneal por corriente directa en experimentos de tiempo de reacción, parece existir tanto un aumento del tiempo pre-motor tras la ejecución de movimientos repetidos, como una prevención de dicho retraso tras una neuromodulación. No obstante, aunque se ha estudiado ampliamente la evolución de la excitabilidad cortico-espinal desde la señal de aviso hasta la señal de respuesta, y desde esta hasta el inicio del movimiento [268] [269] [270], la misma no se ha investigado en condiciones de fatiga, lo que dificulta ampliamente la discusión sobre qué efectos puede causar la neuromodulación en nuestro estudio.

Se conoce además que a partir de la aparición del estímulo en una tarea de reacción y hasta la aparición de un movimiento voluntario (e.g.- reaching) se produce una disminución de la inhibición intracortical siendo esta mínima durante los 80ms previos al inicio del tiempo pre-motor [271], así como durante la contracción muscular [152]; que la inhibición intracortical pueda ser modulada por la estimulación transcraneal por corriente directa [272] y que esta además pueda verse afectada por la fatiga [238], podría explicar que el tiempo pre-motor se ve afectado por la estimulación transcraneal por corriente directa en nuestros experimentos mientras que el tiempo de reacción no. Otra posibilidad, no excluyente con la anterior, es que la estimulación transcraneal por corriente directa influya en lo relativo al *timing* de la descarga de las estructuras centrales hacia el músculo, pero no tanto en su magnitud o en la actividad sinérgica de varios grupos musculares implicados en el movimiento; ello sería también compatible con un efecto de la estimulación transcraneal por corriente directa en el tiempo pre-motor pero no en el tiempo de reacción, dado que este último es identificado en el momento del inicio del movimiento.

En el estudio de los efectos de las técnicas de estimulación cerebral no invasiva en los tiempos de reacción, otro factor importante a tener en cuenta, es la posibilidad de la existencia de un efecto techo en la ejecución del tiempo de reacción. De encontrarnos con una ejecución óptima, quizás no sería hasta que esta comienza a deteriorarse (debido a la fatiga), que se volviese susceptible de verse afectada por la estimulación transcraneal por corriente directa. Esto ocurre por ejemplo en el experimento llevado a cabo por *Hummel et al.*, en supervivientes de accidente cerebro-vascular [273].

5.3.1. Efectos de la estimulación cerebral no invasiva en tareas isométricas fatigantes

Los efectos de las distintas técnicas de estimulación cerebral no invasiva sobre la fatiga han sido principalmente estudiada en el contexto de contracciones isométricas [5].

Básicamente existen dos elementos fundamentales que determinan las características de las contracciones isométricas fatigantes que han sido estudiadas. Estos son la intensidad de contracción (máxima vs. submáxima) y la duración de la tarea (durante un tiempo limitado vs. hasta el fallo en la tarea). Además, dentro de las contracciones isométricas existen distintos tipos (tareas de fuerza o de posición, tal y como hemos presentado en la introducción), cuyos mecanismos fisiológicos asociados a la fatiga son diferentes de acuerdo al principio de *Task Dependency* [235].

Tanaka et al., han reportado que la neuromodulación mediante estimulación transcraneal por corriente directa anódica es capaz de aumentar la contracción máxima voluntaria de la pinza entre el primer y segundo dedo del pie no dominante en individuos sanos no fatigados [194]; asímismo, *Krishnan et al.*, reportaron un aumento de la fuerza pico y de la relación entre la señal electromiográfica y el torque tras la estimulación transcraneal por corriente directa anódica en comparación con un grupo control [199]; mientras que otros estudios han reportado la ausencia de efecto de la estimulación transcraneal por corriente directa sobre la contracción máxima voluntaria en estado fresco [196] [198] [274] [202] [200]. Entre estos últimos, *Kan et al.*, achacaron la falta de efecto de la estimulación a la dificultad de mejorar algo que ya funciona de manera óptima [198], esto podría explicar por qué *Tanaka et al.*, sí obtuvieron un aumento de la fuerza, dado que es posible que la contracción máxima voluntaria de la pinza de los dos primeros dedos del pie, y más aún, de la pierna no dominante, sea subóptima. En esta línea, *Hummel et al.*, encontraron un aumento de la contracción máxima voluntaria tras una estimulación transcraneal por corriente directa anódica en supervivientes de accidente cerebro-vascular [195], así como *Tanaka et al.*, encontraron también un aumento de la fuerza de extensión de cuádriceps tras una sesión única de estimulación transcraneal por corriente directa anódica en supervivientes de accidente cerebro-vascular en comparación con una sesión de estimulación transcraneal por corriente directa catódica y placebo. De esta manera, parecería ser

que sólo cuando la ejecución es subóptima la estimulación transcraneal por corriente directa es capaz de mejorarla, aunque *Benwell et al.*, encontraron tras un protocolo facilitador de estimulación magnética transcraneal repetitiva que la pérdida de fuerza en la pinza en la mano dominante se evitaba [175]. Resulta interesante destacar que *Oki et al.*, no encontraron un efecto de la estimulación transcraneal por corriente directa anódica, en personas muy mayores, a la hora de aumentar la contracción máxima voluntaria en flexores de codo, pero también hallaron que enfrentando la fuerza máxima voluntaria con la evocada eléctricamente, no había diferencia, por lo que no deberían considerarse en un estado subóptimo [275]. Por otra parte, *Muellbacher et al.* encontraron que la aplicación de estimulación magnética transcraneal repetitiva a baja frecuencia durante el reposo, que resulta inhibitoria, no afecta a la generación de fuerza durante esfuerzos máximos [193].

Curiosamente, durante contracciones máximas repetidas que reducen la activación voluntaria evaluada mediante estimulación magnética transcraneal independientemente del *Ciclo de trabajo* [246], se observa un efecto pernicioso de la estimulación, dado que reduce la contracción máxima voluntaria durante la aplicación de estimulación transcraneal por corriente directa en **M1** tanto anódica como catódica, de acuerdo al trabajo de *Giboin et al.*, [276]. Por otra parte, la activación voluntaria se reduce con la estimulación transcraneal por corriente directa catódica comparada con la placebo, observándose también un incremento de la fatiga periférica (torque en reposo inducido por estimulación) tras la estimulación transcraneal por corriente directa anódica [276]. De esta manera, aunque la estimulación tiene un efecto, éste parece contrario a la hipótesis original donde la estimulación transcraneal por corriente directa anódica, al aumentar la excitabilidad, facilitaría la ejecución. El efecto de la estimulación transcraneal por corriente directa anódica puede explicarse mediante la *Muscle Wisdom*, donde un incremento en la frecuencia de disparo de las motoneuronas espinales y la contractilidad periférica del músculo puede acelerar el desarrollo de fatiga periférica [23].

Otros autores han evaluado el efecto de la estimulación transcraneal por corriente directa en tareas isométricas submáximas. *Cogiamanian et al.*, expusieron el positivo efecto de la estimulación transcraneal por corriente directa anódica sobre la corteza motora primaria en el tiempo hasta el fallo en la tarea en una contracción

al 35 % de la contracción máxima voluntaria en el brazo no dominante [196]; a su vez, *Williams et al.*, aplicaron estimulación transcraneal por corriente directa anódica durante 20 minutos a la vez que el sujeto realizaba una contracción isométrica submáxima al 20 % de la contracción máxima voluntaria, encontrando un aumento del tiempo hasta el fallo en la tarea sólo en aquellos sujetos que claudicaron antes de los 20 minutos de estimulación [197]. Por otra parte, *Angius et al.*, refuerzan la idea de que la estimulación transcraneal por corriente directa anódica aumenta el tiempo hasta la extenuación, aún más si se compara un montaje extracefálico con uno órbitofrontal [200]. En contra del efecto positivo de la estimulación transcraneal por corriente directa anódica sobre el tiempo hasta el fallo en la tarea, *Muthalib et al.* no encontraron efecto cuando la estimulación transcraneal por corriente directa anódica era aplicada sobre la corteza prefrontal dorsolateral con una contracción al 30 % de la contracción máxima voluntaria [260]; tampoco *Kan et al.*, contraron efecto de la estimulación transcraneal por corriente directa anódica en ninguna variable con un montaje extracefálico [198], ni *Abdelmoula et al.* aplicando estimulación transcraneal por corriente directa anódica sobre el tiempo hasta el fallo en la tarea [277]. Todos los investigadores previamente mencionados desarollaron una única sesión de estimulación, por lo que dedicamos atención especial al experimento llevado a cabo por *Frazer et al.*, que incluye cuatro sesiones consecutivas de estimulación transcraneal por corriente directa anódica, encontrando una mejora en la activación voluntaria aunque sólo explorada en condiciones de no fatiga [278].

5.3.2. Efectos de la estimulación cerebral no invasiva en la fatiga producida por movimientos repetitivos

Si a pesar del amplio conocimiento de la fisiología subyacente a la fatiga inducida por contracciones isométricas, discernir el efecto de la estimulación cerebral no invasiva en la misma es una tarea complicada, tanto o más lo es cuando se trata de una fatiga inducida por movimientos repetidos, ya que de acuerdo al principio de *Task Dependency* [6] el trasfondo fisiológico es distinto, y el mismo está mucho menos investigado. A pesar de esto, existen algunos estudios que evalúan el efecto de la estimulación cerebral no invasiva en tareas inductoras de fatiga basadas en movimientos repetidos.

Okano et al., evaluaron el efecto de la estimulación transcraneal por corriente directa aplicada sobre la corteza temporal en una tarea de cicloergometría, encontrando un aumento del pico de potencia ejercida, junto con una disminución del ritmo cardíaco en cargas submáximas; además indicaron un retraso en el aumento de fatiga percibida tras una estimulación transcraneal por corriente directa anódica. Ello parece indicar que el papel de la estimulación cerebral no invasiva en el comportamiento del sistema nervioso autónomo es importante a pesar de que no se le haya prestado mucha atención en la literatura [205]. También en tareas de cicloergometría *Vitor-Costa et al.*, apreciaron un aumento del tiempo hasta el fallo en la tarea tras la estimulación transcraneal por corriente directa anódica de **M1** pero no tras la catódica o la placebo; curiosamente, ni la fatiga percibida ni la frecuencia cardíaca se vieron afectadas en contraposición a lo encontrado por *Okano et al.*, [206]. También merece la pena comentar que algunos trabajos han aplicado la estimulación transcraneal por corriente directa transespinalmente en una tarea de cicloergometría máxima de 30 segundos, aumentando la potencia promedio tras la estimulación espinal catódica, pero disminuyendo tras la estimulación cortical catódica [279].

Finalmente, existen algunos estudios que combinan golpeo repetitivo del dedo y estimulación transcraneal por corriente directa o estimulación magnética transcraneal repetitiva. *Boehringer et al.*, no encontraron ningún efecto de la estimulación transcraneal por corriente directa catódica sobre el cerebelo en una tarea de golpeo repetitivo del dedo a frecuencia de golpeo voluntaria máxima durante 10 s, tiempo más que suficiente para desarrollar fatiga [29] [280]. *Todd et al.*, tras protocolos inhibidores de estimulación magnética transcraneal repetitiva, encontraron que la frecuencia de golpeo voluntaria máxima no se veía afectada [203]. Por otra parte, *Teo et al.* concluyen que tanto la combinación de un protocolo de estimulación magnética transcraneal repetitiva excitador como el entrenamiento de la tarea, aumentan la frecuencia de golpeo y reducen la aparición de fatiga en el golpeo repetitivo del dedo, sin investigar los posibles mecanismos que subyacen a los mismos [204].

En resumen, parece claro que el rol de las técnicas de estimulación cerebral no invasiva en la fatiga producida por movimientos repetitivos está todavía por definir. Probablemente una limitación en el uso de dichas técnicas para intervenir en la fatiga producida por este tipo de movimientos es el amplio desconocimiento que existe de

las bases neurofisiológicas de este tipo de fatiga. Dado que la fatiga es dependiente de la tarea, asumir los mecanismos conocidos durante tareas fatigantes isométricas como limitantes en las tareas repetitivas parece poco fundamentado. Por dicho motivo la descripción de los mecanismos neurofisiológicos y las expresiones de fatiga central durante los movimientos repetitivos es esencial, y por dicho motivo hemos desarrollado los experimentos de los artículos segundo y tercero de este compendio.

5.4. Perfil neurofisiológico de la fatiga producida por movimientos repetitivos

La principal aportación de las publicaciones de este compendio consiste en la acotación de los fenómenos electrofisiológicos asociados a la fatiga inducida mediante movimientos repetidos, con el añadido de su comparación con contracciones isométricas. Esta contraposición de las manifestaciones inducidas por diferentes tareas respalda el principio de la *Task Dependency*.

El golpeo repetitivo del dedo como test de evaluación de los movimientos repetitivos rítmicos se utiliza con asiduidad en estudios de control motor [34][281] [36] [282] [283] [284] [30]. Se ha usado como herramienta para la evaluación de diferentes trastornos del sistema motor [285] [286] [287][284], y se ha constatado su validez y fiabilidad para dicho fin [284] [30]. Se ha descrito que cuando se ejecuta a máxima frecuencia voluntaria, la frecuencia de golpeo disminuye en los primeros segundos [288] [29] [30]; sin embargo, las bases neurales dicha merma en la ejecución no son suficientemente conocidas.

Rodrigues et al., demostraron que la frecuencia de golpeo máxima comienza a disminuir a los 7-9 s de haber comenzado la tarea, y que a los 20s se ha reducido hasta el 73 % sin que la amplitud de golpeo se vea afectada. Además, observaron que tanto la contracción máxima voluntaria como la velocidad pico de un movimiento balístico permanecían inalteradas tras la tarea fatigante de 20s. Esto parece indicar que los mecanismos responsables de la disminución en la frecuencia de golpeo voluntaria máxima no afectan a la generación de fuerza ni a la velocidad de contracción pico en un movimiento balístico, viéndose posiblemente afectada la ritmicidad del patrón de movimiento a nivel central. Además, la actividad electromiográfica evolucionó desde un patrón definido de flexo-extensión a uno de co-contracción durante la tarea, lo que señala a mecanismos centrales vinculados con la capacidad de alternar la activación de grupos antagonistas entre sí como responsables de la fatiga, al margen de posibles sucesos periféricos [29].

Por otro lado, *Arias et al.*, evaluaron la excitabilidad corticoespinal asociada a una tarea de golpeo repetitivo del dedo a frecuencia de golpeo voluntaria máxima y submáxima [30]. Para ello realizaron una evaluación de la excitabilidad cortico-

espinal tras una tarea de golpeo repetitivo del dedo durante 50 ciclos, encontrando un fenómeno de facilitación similar a la *Post-exercise facilitation*, pero no *Post-exercise depression* [245] [289], si bien la duración de la exploración tras la tarea pudo ser insuficientemente duradera para permitir dicha expresión.

En el mismo año, *Teo et al.*, en un protocolo similar al desarrollado por *Arias et al.*, y *Rodrigues et al.*, encontraron también *Post-exercise facilitation* inmediatamente después del golpeo repetitivo del dedo y *Post-exercise depression* (al igual que *Zanette et al.*, [290]) que fue más marcada tras una tarea submáxima que tras una máxima, lo que fue explicado por una mayor *Post-exercise facilitation* en la ejecución máxima en comparación con las submáximas [31][32] que no permitía una expresión tan marcada de la posterior *Post-exercise depression*. Paralelamente este estudio sugirió, además, la ausencia de signos de fatiga periférica de acuerdo a los mismos parámetros que *Rodrigues et al.* [29]. Es importante señalar, sin embargo, que los protocolos de evaluación de la fatiga periférica utilizados en dichos estudios no se encuentran entre los estándares metodológicos reconocidos para dicho fin, dado que incluían la contracción voluntaria del sujeto durante su evaluación. El *gold-standard* para la evaluación de expresiones periféricas de la fatiga es la estimulación eléctrica percutánea del músculo o nervio, y el registro de variables observadas (fuerza evocada, velocidad de relajación muscular y/o amplitud del potencial de acción muscular compuesto [13] [14] [291] [28] [80]). Utilizando algunas de dichas técnicas, recientemente, y en un estudio que continúa la línea de investigación de este compendio de publicaciones, hemos observado claros signos de fatiga periférica inducida por el golpeo repetitivo del dedo a máxima frecuencia durante $30\,s$, en presencia de un aumento en la inhibición de la corteza motora [234]. Que el golpeo repetitivo del dedo de $30\,s$ provoque claros signos de fatiga periférica está en consonancia con las evidentes mermas funcionales a nivel muscular producido por contracciones dinámicas, que han sido clásicamente reportados en estudios con estimulación eléctrica en humanos y en fibras aisladas [22] [292] [293].

Hasta donde alcanza nuestro conocimiento, el segundo artículo de este compendio es el primer trabajo en evaluar los circuitos inhibitorios durante el desarrollo de fatiga inducida por golpeo repetitivo del dedo y no a posteriori [290], [31], [32], [204] [30]. Esto es importante debido a la rápida recuperación de los circuitos inhibitorios [105],

y a que respeta uno de los principales axiomas descritos para el estudio de la fatiga, que es la necesidad de la evaluación de la misma en el momento de su desarrollo y sin permitir recuperación [105] [5].

La ejecución del golpeo repetitivo del dedo a frecuencia de golpeo voluntaria máxima provoca un aumento de la excitabilidad de los circuitos inhibitorios corticales de **M1**, expresada mediante un alargamiento del período de silencio inducido por estimulación magnética transcraneal, de manera similar a la que se expresa tras la ejecución de una contracción máxima voluntaria isométrica. Sin embargo, cuando se evalúan los circuitos inhibitorios a nivel espinal, se manifiesta una diferencia en función de la tarea. Tras una ejecución máxima voluntaria isométrica el período de silencio inducido por estimulación cérvico-medular aumenta significativamente, mientras que tras una ejecución frecuencia de golpeo voluntaria máxima el período de silencio inducido por estimulación cérvico-medular permanece inalterado; ello quiere decir que la fatiga inducida por la frecuencia de golpeo voluntaria máxima parece no tener expresiones a nivel de las motoneuronas espinales, pero sí a nivel cortical, al menos en las duraciones de las tareas evaluadas en estos estudios (hasta $30\,s$ de actividad máxima).

El aumento del período de silencio inducido por estimulación magnética transcraneal pero no del período de silencio inducido por estimulación cérvico-medular localiza una alteración muy clara de los circuitos inhibitorios gabaérgicos corticales asociados a receptores $GABA_B$ [294]. De todos modos es importante señalar que, hasta la fecha, no podemos saber si este aumento de la inhibición se corresponde con un mecanismo compensatorio a un deficitario estado muscular o si refleja la imposibilidad del sistema nervioso para mantener la máxima frecuencia de golpeo. Existen experimentos que se están llevando a cabo en nuestro laboratorio para tratar de acotar estos posibles mecanismos.

Observamos, tras la ejecución del golpeo repetitivo del dedo, que el período de silencio inducido por estimulación magnética transcraneal aumenta de acuerdo a la merma en la frecuencia de golpeo voluntaria máxima, y que cuando mayor era la merma mayor duración presentaba el período de silencio inducido por estimulación magnética transcraneal. De esta manera, queda pendiente la comprobación de la existencia de un *ceiling effect* en el incremento del periodo de silencio asociado a

fatiga producida por los movimientos repetitivos, que es conocido en el caso de las contracciones isométricas [105] [295]

Asimismo, el tercer artículo de este compendio, además de reevaluar los circuitos inhibitorios espinales, prestó especial atención al balance excitador-inhibidor a nivel de las motoneruonas espinales evaluado mediante el uso del potencial evocado motor cervico-medular. En *Madrid et al.* [4] encontramos un aumento de la excitabilidad espinal asociado a la ejecución a máxima frecuencia de golpeo voluntaria [4]. El significado de dicho aumento en la excitabilidad no está plenamente definido, hipotéticamente un aumento de la excitabilidad espinal podría dificultar la contracción selectiva y alternativa de flexores y extensores, desembocando en mayores niveles de co-contracción agonista antagonista durante la tarea de golpeo repetitivo del dedo [29] [31]; por otra parte, dicho aumento en la excitabilidad espinal podría ser necesario para facilitar un esfuerzo máximo y compensar una excitabilidad cortical en declive.

Los datos obtenidos de los experimentos en movimientos repetidos parecen arrojar que, cuanto más se complica la secuencia de movimientos, más peso recae sobre el componente central supraespinal [3] [4]. En línea con ello, creemos que la demanda en un movimiento alternante repetido a máxima frecuencia no recae tanto sobre la potencia muscular contráctil, sino sobre la precisión y la adaptación de las secuencias de activación agonista-antagonista a alta frecuencia.

En resumen, los artículos segundo y tercero del compendio han definido un papel de los circuitos inhibitorios corticales en el desarrollo de la fatiga producida por movimientos repetitivos. Por otra parte, se describe una facilitación de los circuitos medulares (incremento de la excitabilidad de las motoneuronas espinales) que podrían ser un sustrato necesario para la ejecución de movimientos a frecuencia de golpeo voluntaria máxima.

5.5. Limitaciones de los estudios y futuras líneas de actuación

Existen varias limitaciones en los estudios presentados que es necesario presentar para una correcta interpretación de los resultados y para tratar de atajarlas en futuros experimentos.

Como ha sido recurrente a lo largo de la discusión, el primer artículo de esta tesis no evaluó ningún tipo de correspondencia entre fenómenos neurofisiológicos y conductuales. Un problema importante dentro de los estudios que utilizan la estimulación transcraneal por corriente directa en el estudio de la fatiga es precisamente esta falta de descripción de los mecanismos fisiológicos, ya destacado en el trabajo de *Horvath et al.*, en el que se revisan los efectos de la estimulación transcraneal por corriente directa en distintos tipos de funciones y comportamientos motores [296].

Por otra parte, en nuestro trabajo sólo se evaluó el efecto sobre la mano dominante. De hecho, la estimulación transcraneal por corriente directa ha demostrado tener un efecto dependiente de la dominancia evaluada, ya que una ejecución subóptima (con la mano no dominante) podría ser más susceptible de mejora que la realizada con la mano dominante [297].

También hay que considerar en la interpretación de los resultados del primer estudio, que los efectos de las técnicas de estimulación cerebral no invasiva presentan una amplia y reconocida variabilidad entre sujetos, por lo que es posible que algunos de los sujetos fueran no respondedores, reduciendo de esta manera el tamaño del efecto de la estimulación [215] [261].

Retroalimentación y motivación verbal

Otro elemento potencialmente limitante del primer estudio, es que los sujetos ejecutaban las tareas sin una retroalimentación constante de sus ejecuciones y sin ánimos verbales que les alentasen a mantener una ejecución máxima [5]. Por ello es posible que un sistema de retroalimentación (i.e. una puntuación...) pudiese haber ayudado a mantener la motivación y quizás a paliar la merma en el rendimiento motor.

Papel de la fatiga periférica en el rendimiento motor

En cuanto a las expresiones de la fatiga evaluadas, reconocemos que el conjunto

de trabajos aquí presentados han centrado sus esfuerzos en la comprensión de las formas centrales de la fatiga, si bien las periféricas no han sido tan minuciosamente atendidas. Por ejemplo, en el trabajo publicado como *Madrid et al.*, [4] tan sólo hemos controlado expresiones periféricas de la fatiga mediante la evaluación de la transmisión y propagación neuromuscular, a través del potencial de acción muscular compuesto. Hay sin embargo que tener en cuenta que dicho potencial no evalúa la capacidad contráctil del músculo [80]. Es posible que el uso de otras variables, como la velocidad de relajación muscular podrían haber ayudado a acotar la aparición de fatiga periférica en nuestros estudios [298] [28]. A este respecto existen experimentos llevados a cabo en nuestro grupo de investigación que, siendo continuación de los presentados en este compendio, indican una evidente merma de la capacidad contráctil muscular al finalizar 30s de golpeo repetitivo del dedo a frecuencia de golpeo voluntaria máxima (ver *Madrid et al.* [234]).

Diferencias de género

Algunos autores han demostrado que poblaciones de diferente sexo expresan distintas manifestaciones de fatiga para las mismas tareas [299] [300] [114]. En el primer artículo de esta tesis ambos géneros estuvieron representados, con un 46 % y un 54 % de mujeres y hombres respectivamente. Sin embargo, en los experimentos pertenecientes al segundo y tercer artículo de esta tesis la población femenina está profundamente infrarepresentada, con apenas un 5 % de mujeres en el segundo y ninguna en el tercero. Los motivos de dicha distribución no tienen que ver con ningún criterio de inclusión, si bien tiene que ser considerado a la hora de interpretar los resultados. Por ello, también sería interesante, en el futuro, comprender si los efectos obtenidos en segundo y tercer artículos de esta tesis se observan de la misma manera en mujeres.

Activación voluntaria y fuerza muscular durante movimientos repetitivos rítmicos fatigantes

El Prof. *Simon C. Gandevia* definió la fatiga central como *"A progressive reduction in voluntary activation of muscle during exercise"* [5], por lo que hablar de fatiga central sin haber evaluado la activación voluntaria puede considerarse una limitación de nuestros estudios, aún a pesar de haber descrito pormenorizadamente múltiples respuestas neurales a la fatiga producida por los movimientos ejecutados

repetidamente. De cualquier modo, la naturaleza de la evaluación de la activación voluntaria está intrínsecamente relacionada con la capacidad de contraer el músculo hasta el máximo, de manera similar al desempeño motor en la tarea isométrica del segundo y tercer artículos.

En experimentos posteriores a los presentados en este compendio de publicaciones, llevados a cabo en la línea de investigación de fatiga de nuestro grupo de investigación, hemos intentado explicar varias limitaciones de los estudios de este compendio. Por ejemplo, en los estudios incluídos en esta tesis no hemos evaluado el nivel de activación voluntaria tras una tarea de golpeo repetitivo del dedo fatigante. Tampoco se ha definido el posible coste que tienen los protocolos de evaluación de la fatiga por medio de contracciones máximas repetidas en el propio desarrollo de la fatiga, tal y como hemos hecho en los experimentos segundo y tercero de este compendio. Los resultados de estudios de nuestro laboratorio recientemente publicados indicaron, por una parte, que efectivamente la realización de contracciones máximas repetidas (con períodos de descanso superiores incluso a los permitidos durante los artículos segundo y tercero de este compendio) generan una merma funcional expresada en una pérdida de fuerza, muy leve en magnitud, pero suficientemente consistente en los sujetos como para alcanzar significación estadística [234]. Es importante señalar, por otra parte, que los parámetros de excitabilidad vinculados a los períodos de silencio no se vieron comprometidos por la mera realización de las contracciones máximas voluntarias, por lo que se refrenda que los efectos observados en los experimentos segundo y tercero de este compendio son inducidos por el golpeo repetitivo del dedo. Otro dato fundamental fue observar que la realización del golpeo repetitivo del dedo a frecuencia de golpeo máxima durante $30\,s$ (que como hemos indicado aumenta la excitabilidad espinal en el tercer experimento) evita la pérdida de fuerza y también la pérdida de activación voluntaria [234], lo cual atribuimos, precisamente, al aumento de la excitabilidad espinal observado en el tercer artículo de este compendio.

Chapter 6

Conclusions

Conclusions for the first study "Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Task"

- Bilateral tDCS on M1 reduces pre-motor times for fast reaching movements executed repeatedly in Reaction Time protocols.
- The bilateral-M1 tDCS aftereffects prevent the fatigue development during fast reaching movements.
- These aftereffects are similarly expressed if the anode is over the M1 contralateral to the executing arm and the cathode on the ipsilateral M1, as with the opposite electrode montage.

Conclusions for the second study "Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of Silent Periods evoked at spinal and supraspinal levels"

- Fatigue induced by short-lasting un-resisted repetitive movements at the maximal rate is not expressed by spinal mechanisms dependent on recurrent inhibition or neuronal after-hyperpolarization. This was tested by means of EMG silent periods to cervicomedullary stimulation.
- Such spinal mechanisms are clearly expressed during fatiguing isometric maximal voluntary contractions.

- Fatigue induced by short-lasting un-resisted repetitive movements at the maximal rate involves M1-intracortical inhibitory circuits, *GABA-B* dependent. As the tapping rate is decreased they increase their excitability. This work does not prove if these mechanisms are also operating during fatiguing isometric maximal voluntary contractions.

Conclusions for the third study "Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks"

- Fatigue induced by short-lasting un-resisted repetitive movements at the maximal rate does not impair inhibitory-excitatory balance at spinal motoneurons. This finding supports conclusions from study 2 and binds fatigue induced by these movements to supraspinal *loci*.

- Short-lasting un-resisted repetitive movements at the maximal rate (for 30 seconds) does not impair neuromuscular transmission or muscle excitability.

- Fatigue task-dependency is supported by our work. Short-lasting isometric maximal voluntary contractions performed with the same body segment as finger tapping clearly impacts spinal excitability.

- Likewise, short-lasting isometric maximal voluntary contractions (for 30 seconds) performed with the same body segment as finger tapping impairs neuromuscular transmission or sarcolemmal excitability.

Bibliografía

[1] Bigland-Ritchie B. EMG/force relations and fatigue of human voluntary contractions. *Exercise and sport sciences reviews*, 9:75–117, 1981.

[2] Arias P., Corral-Bergantiños Y., Robles-García V., Madrid A., Oliviero A., and Cudeiro J. Bilateral tDCS on primary motor cortex: Effects on fast arm reaching tasks. *PLoS One*, 11(8):1–17, 2016.

[3] Arias P., Robles-García V., Corral-Bergantiños Y., Madrid A., Espinosa N., Valls-Solé J., Grieve K. L., Oliviero A., and Cudeiro J. Central fatigue induced by short-lasting finger tapping and isometric tasks: A study of silent periods evoked at spinal and supraspinal levels. *Neuroscience*, 305:316–327, 2015.

[4] Madrid A., Valls-Solé J., Oliviero A., Cudeiro J., and Arias P. Differential responses of spinal motoneurons to fatigue induced by short-lasting repetitive and isometric tasks. *Neuroscience*, 339:655–666, 2016.

[5] Gandevia S. C. Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews*, 81(4):1725–1789, 2001.

[6] Enoka R. M. and Stuart D. G. Neurobiology of muscle fatigue. *Journal of Applied Physiology*, 72(5):1631–1648, 1992.

[7] Angelo Mosso. *La fatica*. Treves, 1904.

[8] Sandrone S., Bacigaluppi M., Galloni M.R., Cappa S.F., Moro A., Catani M., Filippi M., Monti M.M., Perani D., and Martino G. Weighing brain activity with the balance: Angelo Mosso's original manuscripts come to light. *Brain*, 137(2):621–633, 2014.

[9] Field D. T. and Inman L. A. Weighing brain activity with the balance: a contemporary replication of Angelo Mosso's historical experiment. *Brain*, 137(2):634–639, 2014.

[10] DiGiulio Casandro. Angelo Mosso: a holistic approach to muscular fatigue. In *Archives Italiennes de Biologie*, 2012.

[11] Charles Reid. Voluntary muscle strength and endurance: "the mechanism of voluntary muscle fatigue". *Experimental Physiology*, 93(9):1030–1033, 1928.

[12] Adolf Fick. Myographische versuche am lebenden menschen. *Archiv für die gesamte Physiologie des Menschen und der Tiere*, 60:578 – 588, 1887.

[13] Merton P.A. Voluntary strength and fatigue. *The Journal of Physiology*, 123(3):553–564, 1954.

[14] Gandevia S. C., Allen G. M., Butler J. E., and Taylor J. L. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *The Journal of Physiology*, 490:529 – 536, 1996.

[15] Taylor J. L. Point:counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of Applied Physiology*, 107(1):354–355, 2009.

[16] de Haan A., Gerrits K. H. L., and de Ruiter C. J. Counterpoint: The interpolated twitch does not provide a valid measure of the voluntary activation of muscle. *Journal of Applied Physiology*, 107(1):355–357, 2009.

[17] Taylor J. Rebuttal from taylor. *Journal of Applied Physiology*, 107(1):357–358, 2009.

[18] de Haan A., Gerrits K. H. L., and de Ruiter C. J. Rebuttal from de haan, gerrits, and de ruiter. *Journal of Applied Physiology*, 107(1):358–358, 2009.

[19] Taylor J. L. Last word on point:counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of Applied Physiology*, 107(1):367–367, 2009.

[20] de Haan A., Gerrits K. H. L., and de Ruiter C. J. Last word on point:counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of Applied Physiology*, 107(1):368–368, 2009.

[21] Horstman A. M. Comments on point:counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *Journal of Applied Physiology*, 107(1):359–366, 2009.

[22] Jones D. A., Bigland-Ritchie B., and Edwards R. H. T. Excitation frequency and muscle fatigue: Mechanical responses during voluntary and stimulated contractions. *Experimental Neurology*, 64(2):401–413, 1979.

[23] Binder-Macleod S. A and Guerin T. Preservation of force output through progressive reduction of stimulation frequency in human quadriceps femoris muscle. *Physical Therapy*, 70(10):619–625, 1990.

[24] Hill A. V. The heat of shortening and the dynamic constants of muscle. proc r soc lond ser b biol sci. *Proceedings of The Royal Society of London*, 126:136–195, 1938.

[25] Abbott B. C., Bigland B., and Ritchie J. M. The physiological cost of negative work. *The Journal of Physiology*, 117:380–390, 1952.

[26] Edman K. A., Elzinga G., and Noble M. I. Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *The Journal of Physiology*, 281:139–155, 1978.

[27] Huxley A.F. and Simmons R.M. Proposed mechanism of force generation in striated muscle. *Nature*, 233:533–538, 1971.

[28] Pasquet B., Carpentier A., Duchateau J., and Hainaut K. Muscle fatigue during concentric and eccentric contractions. *Muscle & Nerve*, 23(11):1727–1735, 2000.

[29] Rodrigues J. P., Mastaglia F. L., and Thickbroom G. W. Rapid slowing of maximal finger movement rate: fatigue of central motor control? *Experimental Brain Research*, 196(4):557–563, 2009.

[30] Arias P., Robles-García V., Espinosa N., Corral Y., and Cudeiro J. Validity of the finger tapping test in Parkinson's disease, elderly and young healthy subjects: Is there a role for central fatigue? *Clinical Neurophysiology*, 123(10):2034–2041, 2012.

[31] Teo W. P., Rodrigues J. P., Mastaglia F. L., and Thickbroom G. W. Post-exercise depression in corticomotor excitability after dynamic movement: a general property of fatiguing and non-fatiguing exercise. *Experimental Brain Research*, 216(1):41–49, 2012.

[32] Teo W.P., Rodrigues J.P., Mastaglia F.L., and Thickbroom G.W. Changes in corticomotor excitability and inhibition after exercise are influenced by hand dominance and motor demand. *Neuroscience*, 210:110–117, 2012.

[33] Weavil J., Sidhu S., Mangum T., Richardson R. S., and Markus A. Fatigue diminishes motoneuronal excitability during cycling exercise. *Journal of Neurophysiology*, 116(4):1743–1751, 2016.

[34] Astolfi L., Babiloni F., Babiloni C., Carducci F., Cincotti F., Basilisco A., Rossini P., Salinari S., Ni Y., he B., and Ding L. Time-varying cortical connectivity by high resolution eeg and directed transfer function: simulations and application to finger tapping data. *Proceedings of the Annual International Conference of the I.E.E.E. Engineering in Medicine and Biology Society*, 6:4405–4408, 2004.

[35] Jobbágy A., Harcos P., Karoly R., and Fazekas G. Analysis of finger-tapping movement. *Journal of Neuroscience Methods*, 141(1):29–39, 2005.

[36] Leijnse J., Campbell-Kyureghyan N., Spektor D., and Quesada P. Assessment of individual finger muscle activity in the extensor digitorum communis by surface EMG. *Journal of Neurophysiology*, 100:3225–3235, 2008.

[37] Yokoe M., Okuno R., Hamasaki T., Kurachi Y., Akazawa K., and Sakoda S. Opening velocity, a novel parameter, for finger tapping test in patients with parkinson's disease. *Parkinsonism & Related Disorders*, 15:440–444, 2009.

[38] Barut C., Kiziltan E., Ethem G., and Köktürk F. Advanced analysis of finger-tapping performance: A preliminary study. *Balkan Medical Journal*, 30:167–171, 2013.

[39] Notermans N. C., van Dijk G. W., van der Graaf Y., van Gijn J., and Wokke J. H. Measuring ataxia: quantification based on the standard neurological examination. *Journal of Neurology, Neurosurgery, and Psychiatry*, 57:22–26, 1994.

[40] Ott B., Ellias S., and Lannon M. C. Quantitative assessment of movement in alzheimer's disease. *Journal of Geriatric Psychiatry and Neurology*, 8:71–75, 1995.

[41] Heller A., Wade D., Wood V.A., Sunderland A., Hewer R. L., and Ward E. Arm function after stroke-measurement and recovery over the 1st 3 months. *Journal of Neurology, Neurosurgery, and Psychiatry*, 50:714–719, 1987.

[42] Aydin L., Kiziltan E., and Gündogan N. Polyphasic temporal behavior of finger-tapping performance: A measure of motor skills and fatigue. *Journal of Motor Behavior*, 48:1–7, 2015.

[43] Fukuda K., Straus S. E., Hickie I., Sharpe M. C., Dobbins J. G., and Komaroff A. The chronic fatigue syndrome: A comprehensive approach to its definition and study. *Annals of Internal Medicine*, 121(12):953–959, 1994.

[44] Zwarts M. J., Bleijenberg G., and van Engelen B. G. M. Clinical neurophysiology of fatigue. *Clinical Neurophysiology*, 119:2–10, 2008.

[45] Feigin V. L., Mohammad H. F., Krishnamurthi R., Mensah G. A., Connor M., and Bennett D. A. Global and regional burden of stroke during 1990–2010: findings from the global burden of disease study 2010. *The Lancet*, 383:245–255, 2014.

[46] Lackland D. T., Roccella E. J., Deutsch A. F., Fornage M., George M.G., Howard G., Kissela B. M., Kittner S. J., Lichtman J. H., Lisabeth L. D., Schwamm L. H., Smith E. E., and Towfighi A. Factors influencing the decline in stroke mortality a statement from the american heart association/american stroke association. *Stroke*, 45:315–353, 2013.

[47] de Groot M. H., Phillips S. J., and Eskes G. A. Fatigue associated with stroke and other neurologic conditions: implications for stroke rehabilitation. *Archives of Physical Medicine and Rehabilitation*, 84:1714–1720, 2003.

[48] Ward N. S. Restoring brain function after stroke — bridging the gap between animals and humans. *Nature Reviews Neurology*, 13:244–255, 2017.

[49] DeDoncker W., Dantzer R., Ormstad H., and Kuppuswamy A. Mechanisms of poststroke fatigue. *Journal of Neurology, Neurosurgery & Psychiatry*, 89:287–293, 2017.

[50] Alves G., Wentzel-Larsen T., and Larsen J. P. Is fatigue an independent and persistent symptom in patients with parkinson disease? *Neurology*, 63:1908–1911, 2004.

[51] Friedman J.H. and Friedman H. Fatigue in parkinson's disease: a nine-year follow-up. *Movement Disorders*, 16(6):1120–1122, 2001.

[52] Lou J., Kearns G., Benice T., Oken B., Sexton G., and Nutt J. Levodopa improves physical fatigue in parkinson's disease: A double-blind, placebo-controlled, crossover study. *Movement disorders*, 18:1108–1114, 2003.

[53] Lou J., Benice T., Kearns G., Sexton G., and Nutt J. Levodopa normalizes exercise related cortico-motoneuron excitability abnormalities in parkinson's disease. *Clinical Neurophysiology*, 114:930–937, 2003.

[54] Kroenke K. and Price R.K. Symptoms in the community: Prevalence, classification, and psychiatric comorbidity. *Archives of Internal Medicine*, 153(21):2474–2480, 1993.

[55] Ricci A. J., Chee E., Lorandeau L. A., and Berger J. Fatigue in the u.s. workforce: Prevalence and implications for lost productive work time. *Journal of Occupational and Environmental Medicine*, 49:1–10, 2007.

[56] Techera U., Hallowell M., Stambaugh N., and Littlejohn R. Causes and consequences of occupational fatigue: Meta-analysis and systems model. *Journal of Occupational and Enviromental Medicine*, 58, 2016.

[57] Harrington J.M. Health effects of shift work and extended hours of work. *Occupational and Environmental Medicine*, 58:68–72, 2001.

[58] Kandel E. R., Schwartz J. H., and Jessell T. M. *Principles of Neural Science*. McGraw-Hill Medical, 4th edition, 2000.

[59] Huxley A. F. and Niedergerke R. Structural changes in muscle during contraction: Interference microscopy of living muscle fibres. *Nature*, 173:971–973, 1954.

[60] Huxley H. and Hanson J. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature*, 173:973–976, 1954.

[61] Del Castillo J. and Katz B. Action, and spontaneous release, of acetylcholine at an inexcitable nerve-muscle junction. *The Journal of Physiology*, 126:27P, 1954.

[62] Rich M. M. The control of neuromuscular transmission in health and disease. *The Neuroscientist*, 12(2):134–142, 2006.

[63] Pierce P. A. *Fatigue: neural and molecular mechanisms*, volume 384. Gandevia S. C. and Enoka R. M. and McComas A. J. and Stuart D. G. and Thomas C. K., 1995.

[64] Aldrich T., Shander A., Chaudhry I., and Nagashima H. Fatigue of isolated rat diaphragm: Role of impaired neuromuscular transmission. *Journal of Applied Physiology*, 61:1077–1083, 1986.

[65] Ruff R. and Lennon V. A. End-plate voltage-gated sodium channels are lost in clinical and experimental myasthenia gravis. *Annals of Neurology*, 43:370–379, 1998.

[66] A. Serra, Ruff R., and Leigh R. Neuromuscular transmission failure in myasthenia gravis: Decrement of safety factor and susceptibility of extraocular muscles. *Annals of the New York Academy of Sciences*, 1275:129–135, 2012.

[67] Ruff R. and V. A Lennon. How myasthenia gravis alters the safety factor for neuromuscular transmission. *Journal of Neuroimmunology*, 201-202:13–20, 2008.

[68] Johnson B. D. and Sieck G. Differential susceptibility of diaphragm muscle fibers to neuromuscular transmission failure. *Journal of Applied Physiology*, 75:341–348, 1993.

[69] Bigland B. and Lippold O. C. J. Motor unit activity in the voluntary contraction of human muscle. *The Journal of Physiology*, 125(2):322–335, 1954.

[70] Bigland-Ritchie B. EMG and fatigue of human voluntary and stimulated contractions. *Ciba Foundation Symposium*, 82:130–156, 1981.

[71] Gerdle B., Larsson B., and Karlsson S. Criterion validation of surface EMG variables as fatigue indicators using peak torque. *Journal of Electromyography and Kinesiology*, 10:225–232, 2000.

[72] Linssen W. H. J. P., Jacobs M., Stegeman D., Joosten E. M. G., and Moleman J. Muscle fatigue in mcardle's disease: Muscle fibre conduction velocity and surface EMG frequency spectrum during ischaemic exercise. *Brain*, 113:1779–1793, 1991.

[73] Naeije M. and Zorn H. Relation between EMG power spectrum shifts and muscle fibre action potential conduction velocity changes during local muscular fatigue in man. *European Journal of Applied Physiology*, 50:23–33, 1982.

[74] Mills K. Power spectral analysis of electromyogram and compound muscle action potential during muscle fatigue and recovery. *The Journal of Physiology*, 326:401–409, 1982.

[75] Kranz H., Williams A. M., Cassell J., Caddy D. J., and Silberstein R. Factor determining the frequency content of the electromyogram. *Journal of Applied Physiology*, 55:392–399, 1983.

[76] Maluf K. S. and Enoka R. Task failure during fatiguing contractions performed by humans. *Journal of Applied Physiology*, 99:389–396, 2005.

[77] Stephens J. A. and Taylor A. Fatigue of maintained voluntary contraction in man. *The Journal of Physiology*, 220:1–18, 1972.

[78] Milner-Brown H. S. and G. Miller R. Muscle membrane excitation and impulse propagation velocity are reduced during fatigue. *Muscle & Nerve*, 9:367–374, 1986.

[79] Fitch S. and McComas A. Influence of human muscle length on fatigue. *The Journal of Physiology*, 362:205–213, 1985.

[80] Cupido C. M., Galea V., and McComas A. J. Potentiation and depression of the m wave in human biceps brachii. *The Journal of Physiology*, 491(2):541–550, 1996.

[81] Bigland-Ritchie B., Donovan E. F., and Roussos C. S. Conduction velocity and EMG power spectrum changes in fatigue of sustained maximal efforts. *Journal of Applied Physiology*, 51:1300–1305, 1981.

[82] Porter R. and Lemon R. *Corticospinal Function Voluntary Movement*. 1993.

[83] Rexed B. The cytoarchitectonic organization of the spinal cord in the cat. *Journal of Comparative Neurology*, 96(3):415–495, 1952.

[84] Rexed B. A cytoarchitectonic atlas of the spinal coed in the cat. *Journal of Comparative Neurology*, 100(2):297–379, 1954.

[85] Sherrington C. *The integrative action of the nervous system*. 1911.

[86] McNeil C. J., Butler J., Taylor J. L., and Gandevia S. C. Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, 7:152–152, 2013.

[87] Magladery J. W. and McDougal D.B. Electrophysiological studies of nerve and reflex activity in normal man. i. identification of certain reflexes in the electromyogram and the conduction velocity of peripheral nerve fibers. *Bulletin of the Johns Hopkins Hospital*, 86(5):265 — 290, 1950.

[88] Kimura J., Yanagisawa H., Yamada T., Mitsudome A., Sasaki H., and Kimura A. Is the F wave elicited in a select group of motoneurons? *Muscle & Nerve*, 7:392–399, 1984.

[89] Butler J. E. and Thomas C. K. Effects of sustained stimulation on the excitability of motoneurons innervating paralyzed and control muscles. *Journal of Applied Physiology*, 94(2):567–575, 2003.

[90] Ugawa Y., Rothwell J. C., Day B. L., Thompson P. D., and Marsden C. D. Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. *Annals of Neurology*, 29(4):418–427, 1991.

[91] Ugawa Y., Uesaka Y., Terao Y., Hanajima R., and Kanazawa I. Magnetic stimulation of corticospinal pathways at the foramen magnum level in humans. *Annals of Neurology*, 36(4):618–624, 1994.

[92] Ugawa Y., Uesaka Y., Terao Y., Hanajima R., and Kanazawa I. Magnetic stimulation of the descending and ascending tracts at the foramen magnum level. *Electroencephalography and Clinical Neurophysiology*, 105:128–131, 1997.

[93] Martin P. G., Butler J. E., Gandevia S. C., and Taylor J. L. Noninvasive stimulation of human corticospinal axons innervating leg muscles. *Journal of Neurophysiology*, 100:1080–1086, 2008.

[94] Gandevia S. C., Petersen N., Butler J. E., and Taylor J. L. Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *The Journal of Physiology*, 521(3):749–759, 1999.

[95] Berardelli A., Inghilleri M., Cruccu G., and Manfredi M. Descending volley after electrical and magnetic transcranial stimulation in man. *Neuroscience Letters*, 112:54–58, 1990.

[96] Taylor J. L., Petersen N., Butler J., and Gandevia S. C. Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *The Journal of Physiology*, 541:949–958, 2002.

[97] Rothwell J. C., Burke D., Hicks R., Stephen J., Woodforth I., and Crawford M. Transcranial electrical stimulation of the motor cortex in man: further evidence for the site of activation. *The Journal of Physiology*, 481:243–250, 1994.

[98] Di Lazzaro V., Oliviero A., Profice P., Insola A., Mazzone P., Tonali P., and Rothwell J. C. Effects of voluntary contraction on descending volleys evoked by transcranial electrical stimulation over the motor cortex hand area in conscious humans. *Experimental Brain Research*, 124(4):525–528, 1999.

[99] Day B.L., Rothwell J., Thompson P.D., Dick J. P. R., Cowan J., Berardelli A., and Marsden C. D. Motor cortex stimulation in intact man. 2. multiple descending volleys. *Brain*, 110:1191–1209, 1987.

[100] Hess C., Mills K., and Murray N. M. F. Responses in small hand muscles form magnetic stimulation of the human brain. *The Journal of Physiology*, 388:397–419, 1987.

[101] Petersen N., Taylor J. L., and Gandevia S. C. The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. *The Journal of Physiology*, 544:277–284, 2002.

[102] Nielsen J. and Petersen N. Is presynaptic inhibition distributed to corticospinal fibres in man? j physiol. *The Journal of Physiology*, 477:47–58, 1994.

[103] Jackson A., Baker S. N., and Fetz E. E. Tests for presynaptic modulation of corticospinal terminals from peripheral afferents and pyramidal tract in the macaque. *The Journal of Physiology*, 573:107–120, 2006.

[104] Inghilleri M., Berardelli A., Cruccu G., and Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *The Journal of Physiology*, 466(1):521–534, 1993.

[105] Taylor J. L., Jane E Butler J. E., Gabrielle Allen M., and Gandevia S. C. Changes in motor cortical excitability during human muscle fatigue. *The Journal of Physiology*, 490:519–528, 1996.

[106] Hodgkin A.L. and Huxley A.F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of Physiology*, 117:500–544, 1952.

[107] Renshaw B. Central effects of centripetal impulses in axons of spinal ventral roots. *Journal of Neurophysiology*, 9(3):191–204, 1946.

[108] Eccles J. C., Fatt P., and Koketsu K. Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *The Journal of Physiology*, 126:524–562, 1954.

[109] Fuhr P., Agostino R., and Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 81(4):257–262, 1991.

[110] Ziemann U., Netz J., Szelényi A., and Hömberg V. Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neuroscience Letters*, 156(1):167–171, 1993.

[111] Yacyshyn A. F., Woo E. J., Price M. C., and McNeil C. J. Motoneuron responsiveness to corticospinal tract stimulation during the silent period induced by transcranial magnetic stimulation. *Experimental Brain Research*, 234(12):3457–3463, 2016.

[112] Taylor J., Butler J., and Gandevia S. Changes in muscle afferents, motoneurons and motor drive during muscle fatigue. *European Journal of Applied Physiology*, 83:106–115, 2000.

[113] Taylor J. L., Todd G., and Gandevia S. C. Evidence for a supraspinal contribution to human muscle fatigue. *Clinical and Experimental Pharmacology & Physiology*, 33:400–405, 2006.

[114] Hunter S. K., Butler J. E., Todd G., Gandevia S. C., and Taylor J. L. Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions. *Journal of Applied Physiology*, 101(4):1036–1044, 2006.

[115] Søgaard K., Gandevia S. C., Todd G., Petersen N., and Taylor J. L. The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *The Journal of Physiology*, 573:511–523, 2006.

[116] Rockel A. J., Hiorns R., and Powell T. P. S. The basic uniformity in structure of the neocortex. *Brain*, 103:221–244, 1980.

[117] Phillips C. Microarchitecture of the motor cortex of primates. *Progress in Anatomy*, 1:61–94, 1981.

[118] Barbas H. and García-Cabezas M. Á. Motor cortex layer 4: Less is more. *Trends in Neurosciences*, 38:259–261, 2015.

[119] Herculano-Houzel S., Collins C. E., Wong P., Kaas J. H., and Lent R. The basic nonuniformity of the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*, 105:12593–12598, 2008.

[120] Ramón y Cajal S. *Histologie du Systeme Nerveux de I'Homme et des Vertebres*, volume 1. 1909.

[121] Ramón y Cajal S. *Histologie du Systeme Nerveux de I'Homme et des Vertebres*, volume 2. 1911.

[122] Sloper J. J. and Powell T. P. S. An experimental electron microscopic study of afferent connections to the primate motor and somatic sensory cortices. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, 285:199–226, 1979.

[123] Sloper J. J., Hiorns R., and Powell T. P. S. A qualitative and quantitative electron microscopic study of the neurons in the primate motor and somatic sensory cortices. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, 285:141–171, 1979.

[124] Landgren S., Phillips C. G., and Porter R. Minimal synaptic actions of pyrimidal impulses on some alpha motoneurones of the baboon's hand and forearm. *The Journal of Physiology*, 161:91–111, 1962.

[125] Landgren S., Phillips C. G., and Porter R. Cortical fields of origin of the monosynaptic pyramidal pathways to some alpha motoneurons of the baboon's hand and forearm. *The Journal of Physiology*, 161:112–125, 1962.

[126] Landry P., Labelle A., and Deschenes M. A. Intracortical distribution of axonal collaterals of pyramidal tract cells in the cat motor cortex. *Brain Research*, 191:327–336, 1980.

[127] Ghosh S., Fyffe R. E. W., and Porter R. Morphology of neurons in area 4? of the cat's cortex studied with intracellular injection of hrp. *The Journal of Comparative Neurology*, 269:290–312, 1988.

[128] Ghosh S. and Porter R. Morphology of pyramidal neurons in monkey motor cortex and the synaptic actions of their intracortical axon collaterals. *The Journal of Physiology*, 400:593–615, 1988.

[129] Defelipe J., Conley M., and Jones E.G. Long-range focal collateralization of axons arising from corticocortical cells in monkey sensory-motor cortex. *The Journal of Neuroscience*, 6:3749–3766, 1987.

[130] Cragg B. G. The density of synapses and neurons in normal, mentally defective and ageing human brains. *Brain*, 98:81–90, 1975.

[131] Sloper J. J. An electron microscope study of the termination of afferent connections to the primate motor cortex. *Journal of Neurocytology*, 2:361–368, 1974.

[132] Jones E. G. and Wise S. Laminar and columnar distribution of efferent cells in the sensory-motor cortex of monkeys. *The Journal of Comparative Neurology*, 175:391–438, 1977.

[133] Murray E. A. and Coulter J. Organization of corticospinal neurons in monkey. *The Journal of Comparative Neurology*, 195:339–365, 1981.

[134] Meyer G. Forms and spatial arrangement of neurons in the primary motor cortex of man. *Journal of Comparative Neurology*, 262(3):402–428, 1987.

[135] Strother L., Medendorp W. P., Coros A. M., and Vilis T. Double representation of the wrist and elbow in human motor cortex. *European Journal of Neuroscience*, 36(9):3291–3298, 2012.

[136] Gatter K. C., Sloper J. J., and Powell T. P. S. The intrinsic connections of the cortex of area 4 of the monkey. *Brain*, 101:513–541, 1978.

[137] Strick P. L. and Sterling P. Synaptic termination of afferents from the ventrolateral nucleus of the thalamus in the cat motor cortex. a light and electron microscope study. *The Journal of Comparative Neurology*, 153:77–106, 1974.

[138] Amassian V. E. and Weiner H. Monosynaptic and polysynaptic activation of pyramidal tract neurons by thalamic stimulation. *The Thalamus*, 1:255–282, 1966.

[139] Deschenes M. A., Labelle A., and Landry P. A comparative study of ventrolateral and recurrent excitatory postsynaptic potentials in large pyramidal tract cells in the cat. *Brain Research*, 160:37–46, 1979.

[140] Yamamoto T., Samejima A., and Oka H. The mode of synaptic activation of pyramidal neurons in the cat primary somatosensory cortex: An intracellular hrp study. *Experimental Brain Research*, 80:12–22, 1990.

[141] Jones E. G., Burton H. B., and Porter R. Commissural and corticocortical "columns" in the somatic sensory cortex of primates. *Science (New York, N.Y.)*, 190:572–574, 1975.

[142] Lund J. S. and Lund R. D. The termination of callosal fibers in the paravisual cortex of rat. *Brain Research*, 17:25–45, 1970.

[143] Lemon R. N. Descending pathways in motor control. *Annual Review of Neuroscience*, 31(1):195–218, 2008.

[144] Day B. L., Marsden C. D., Rothwell J. C., Colebatch J. G., and Thompson P. D. Cortical outflow to proximal arm muscles in man. *Brain*, 113(6):1843–1856, 1990.

[145] Brouwer B. and Ashby P. Corticospinal projections to upper and lower limb spinal motoneurons in man. *Electroencephalography and Clinical Neurophysiology*, 76:509–519, 1991.

[146] Rothwell J.C., Thompson P.D., Day B.L., Boyd S., and Marsden C.D. Stimulation of the human motor cortex through the scalp. *Experimental Physiology*, 76(2):159–200, 1991.

[147] Nielsen J. B., Petersen N., and Ballegaard M. Latency of effects evoked by electrical and magnetic brain stimulation in lower limb motoneurones in man. *The Journal of Physiology*, 484:791–802, 1995.

[148] Shimazu H., Maier M.A., Cerri G., Kirkwood P., and Lemon R. Macaque ventral premotor cortex exerts powerful facilitation of motor cortex outputs to upper limb motoneurons. *The Journal of Neuroscience*, 24:1200–1211, 2004.

[149] Merton P. A. and Morton H. B. Stimulation of the cerebral cortex in the intact human subject. *Nature*, 285:227–227, 1980.

[150] Barker A. T., Jalinous R., and Freeston I. L. Non-invasive magnetic stimulation of human motor cortex. *The Lancet*, 325:1106–1107, 1985.

[151] Cracco R. Q., Amassian V. E., Maccabee P., and Cracco J. Comparison of human transcallosal responses evoked by magnetic coil and electrical stimulation. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 74:417–424, 1989.

[152] Reis J., Swayne O. B., Vandermeeren Y., Camus M., Dimyan M. A., Harris-Love M., Perez M. A., Ragert P., Rothwell J., and Cohen L. Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. *The Journal of Physiology*, 586:325–351, 2008.

[153] Krnjević K., Randić M., and Straughan D.W. Cortical inhibition. *Nature*, 201:1294–1296, 1964.

[154] Krnjević K., Randić M., and Straughan D.W. An inhibitory process in the cerebral cortex. *The Journal of Physiology*, 184(1):16–48, 1966.

[155] Siebner H.R., Dressnandt J., Auer C., and Conrad B. Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle & Nerve*, 21(9):1209–1212, 1998.

[156] Werhahn K.J., Kunesch E., Noachtar S., Benecke R., and Classen J. Differential effects on motorcortical inhibition induced by blockade of gaba uptake in humans. *The Journal of Physiology*, 517(2):591–597, 1999.

[157] Orth M. and Rothwell J.C. The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. *Clinical Neurophysiology*, 115(5):1076–1082, 2004.

[158] Mills K. R. and Thomson C. C. B. Human muscle fatigue investigated by transcranial magnetic stimulation. *NeuroReport*, 6:1966–1968, 1995.

[159] Mckay W., Stokic D., Sherwood A., Vrbová G., and Dimitrijevic M. Effect of fatiguing maximal voluntary contraction on excitatory and inhibitory responses elicited by transcranial magnetic moto cortex stimulation. *Muscle & Nerve*, 19:1017–1024, 1996.

[160] Taylor J. L., Butler J. E., and Gandevia S. C. Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. *Experimental Brain Research*, 127(1):108–115, 1999.

[161] Taylor J. L., Petersen N., Butler J. E., and Gandevia S. C. Ischaemia after exercise does not reduce responses of human motoneurons to cortical or corticospinal tract stimulation. *The Journal of Physiology*, 525:793–801, 2000.

[162] Butler J. E., Taylor J. L., and Gandevia S. C. Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *Journal of Neuroscience*, 23(32):10224–10230, 2003.

[163] Martin P. G., Smith J., Butler J. E., Gandevia S. C., and Taylor J. L. Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans. *The Journal of Neuroscience*, 26:4796–4802, 2006.

[164] McNeil C. J., Martin P. G., Gandevia S. C., and Taylor J. L. The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. *The Journal of Physiology*, 587:5601–5612, 2009.

[165] Eichelberger T. D. and Bilodeau M. Central fatigue of the first dorsal interosseous muscle during low-force and high-force sustained submaximal contractions. *Clinical Physiology and Functional Imaging*, 27:298–304, 2007.

[166] Fuglevand A., Zackowski K., Huey K.A., and Enoka R. Impairment of neuromuscular propagation during human fatiguing contraction at submaximal forces. *The Journal of Physiology*, 460:549–572, 1993.

[167] Löscher W.N., Cresswell A., and Thorstensson A. Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. *The Journal of Physiology*, 491:271–280, 1996.

[168] Sacco P., Thickbroom G.W., Thompson M.L., and Mastaglia F. Changes in corticomotor excitation and inhibition during prolonged submaximal muscle contractions. *Muscle & Nerve*, 20:1158–1166, 1997.

[169] Duchateau J., Balestra C., Carpentier A., and Hainaut K. Reflex regulation during sustained and intermittent submaximal contractions in humans. *The Journal of Physiology*, 541:959–967, 2002.

[170] Smith J., Martin P. G., Gandevia S. C., and Taylor J. L. Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *Journal of Applied Physiology*, 103:560–568, 2007.

[171] Oliviero A., Mordillo-Mateos L., Arias P., Panyavin I., Foffani G., and Aguilar J. Transcranial static magnetic field stimulation of the human motor cortex. *The Journal of Physiology*, 589(20):4949–4958, 2011.

[172] Oliviero A., Carrasco-López M. C., Campolo M., Perez-Borrego Y. A., Soto-León V., Gonzalez-Rosa J. J., Higuero A. M., Strange B. A., Abad-Rodriguez J., and Foffani G. Safety study of transcranial static magnetic field stimulation (tsms) of the human cortex. *Brain Stimulation*, 8:481–485, 2015.

[173] Dileone M., Mordillo-Mateos L., Oliviero A., and Foffani G. Long-lasting effects of transcranial static magnetic field stimulation on motor cortex excitability. *Brain Stimulation*, 11:676–688, 2018.

[174] Arias P., Adán-Arcay L., Puerta-Catoira B., Madrid A., and Cudeiro J. Transcranial static magnetic field stimulation of m1 reduces corticospinal excitability without distorting sensorimotor integration in humans. *Brain Stimulation*, 10(2):340–342, 2017.

[175] Benwell N. M., Mastaglia F. L., and Thickbroom G. W. Paired-pulse rtms at trans-synaptic intervals increases corticomotor excitability and reduces the rate of force loss during a fatiguing exercise of the hand. *Experimental Brain Research*, 175(4):626–632, 2006.

[176] Nitsche M. A. and Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology*, 527(3):633–639, 2000.

[177] Lang N., Nitsche M. A., Paulus W., Rothwell J. C., and Lemon R. N. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Experimental Brain Research*, 156(4):439–443, 2004.

[178] Power H. A., Norton J. A., Porter C. L., Doyle Z., Hui I., and Chan K. M. Transcranial direct current stimulation of the primary motor cortex affects cortical drive to human musculature as assessed by intermuscular coherence. *The Journal of Physiology*, 577:795–803, 2006.

[179] Furubayashi T., Terao Y., Arai N., Okabe S., Mochizuki H., Hanajima R., Hamada Masashi, Yugeta A., Inomata-Terada S., and Ugawa Y. Short and long duration transcranial direct current stimulation (tDCS) over the human motor area. *Experimental Brain Research*, 185:279–86, 2007.

[180] Gartside I. B. Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: Reverberating circuits or modification of synaptic conductance? *Nature*, 220:382–383, 1968.

[181] Hattori Y., Moriwaki A., and Hori Y. Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic amp in rat cerebral cortex. *Neuroscience Letters*, 116:320–324, 1990.

[182] Islam N., Aftabuddin M., Moriwaki A., Hattori Y., and Hori Y. Increase in the calcium level following anodal polarization in the rat brain. *Brain Research*, 684:206–208, 1995.

[183] Nitsche M., Cohen L., Wassermann E. M., Priori A., Lang N., Antal A., Paulus W., Hummel F., Boggio P., Fregni F., and Pascual-Leone A. Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*, 1:206–23, 2008.

[184] Purpura D. P. and McMurtry J. G. Intracellular activities and evoked potential changes during polarization of motor cortex. *Journal of Neurophysiology*, 28(1):166–185, 1965.

[185] Nitsche M. A., Liebetanz D., Lang N., Antal A., Tergau F., and Paulus W. Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clinical Neurophysiology*, 114:2220–2222, 2003.

[186] Hsu T.-Y., Juan C.-., and Tseng P. Individual differences and state-dependent responses in transcranial direct current stimulation. *Frontiers in Human Neuroscience*, 10:643–643, 2016.

[187] Roche N., Lackmy A., Achache V., Bussel B., and Katz R. Impact of transcranial direct current stimulation on spinal network excitability in humans. *The Journal of Physiology*, 587(23):5653–5664, 2009.

[188] Roche N., Lackmy A., Achache V., Bussel B., and Katz R. Effects of anodal transcranial direct current stimulation over the leg motor area on lumbar spinal network excitability in healthy subjects. *The Journal of Physiology*, 589(11):2813–2826, 2011.

[189] Roche N., Lackmy A., Achache V., Bussel B., and Katz R. Effects of anodal tDCS on lumbar propriospinal system in healthy subjects. *Clinical Neurophysiology*, 123(5):1027–1034, 2012.

[190] Lackmy-Vallée A., Klomjai W., Bussel B., Katz R., and Roche N. Anodal transcranial direct current stimulation of the motor cortex induces opposite modulation of reciprocal inhibition in wrist extensor and flexor. *Journal of Neurophysiology*, 112(6):1505–1515, 2014.

[191] Huang Y.-Z., Edwards M. J., Rounis E., Bhatia K. P., and Rothwell J. C. Theta burst stimulation of the human motor cortex. *Neuron*, 45(2):201–206, 2005.

[192] Huang Y.-Z., Rothwell J. C., Edwards M., and Chen R.-S. Effect of physiological activity on an nmda-dependent form of cortical plasticity in human. *Cerebral Cortex (New York, N.Y. : 1991)*, 18:563–570, 2008.

[193] Muellbacher W., Ziemann U., Boroojerdi B., and Hallett M. Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. *Clinical Neurophysiology*, 111(6):1002–1007, 2000.

[194] Tanaka S., Hanakawa T., Honda M., and Watanabe Katsumi. Enhancement of pinch force in the lower leg by anodal transcranial direct current stimulation. *Experimental Brain Research*, 196(3):459–465, 2009.

[195] Hummel F., Voller, B., Celnik, P., Floel A., Giraux P., Gerloff C., and Cohen L. Effects of brain polarization on reaction times and pinch force in chronic stroke. *BMC Neuroscience*, 7:73–73, 2006.

[196] Cogiamanian F., Marceglia S., Ardolino G., Barbieri S., and Priori A. Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. *European Journal of Neuroscience*, 26(1):242–249, 2007.

[197] Williams P. S., Hoffman R. L., and Clark B. C. Preliminary evidence that anodal transcranial direct current stimulation enhances time to task failure of a sustained submaximal contraction. *PLoS One*, 8(12):1–11, 2013.

[198] Kan B., Dundas J. E., and Nosaka K. Effect of transcranial direct current stimulation on elbow flexor maximal voluntary isometric strength and endurance. *Applied Physiology, Nutrition, and Metabolism*, 38:734–739, 2013.

[199] Krishnan C., Ranganathan R., Kantak S. S., Dhaher Y. Y., and Rymer W. Z. Anodal transcranial direct current stimulation alters elbow flexor muscle recruitment strategies. *Brain Stimulation*, 7(3):443–450, 2014.

[200] Angius L., Pageaux B., Hopker J., Marcora S. M., and Mauger A. R. Transcranial direct current stimulation improves isometric time to exhaustion of the knee extensors. *Neuroscience*, 339:363–375, 2016.

[201] Oki K., Mahato N. K., Nakazawa M., Amano S., France C., Russ D. W., and Clark B. Preliminary evidence that excitatory transcranial direct current stimulation extends time to task failure of a sustained, submaximal muscular contraction in older adults. *The Journals of Gerontology*, 71:1109–1112, 2016.

[202] Abdelmoula A., Baudry S., and Duchateau J. Anodal transcranial direct current stimulation enhances time to task failure of a submaximal contraction of elbow flexors without changing corticospinal excitability. *Neuroscience*, 322:94–103, 2016.

[203] Todd G., Rogasch N. C., Flavel S., and Ridding M. Voluntary movement and repetitive transcranial magnetic stimulation over human motor cortex. *Journal of Applied Physiology*, 106:1593–1603, 2009.

[204] Teo W.P., Rodrigues J.P., Mastaglia F.L., and Thickbroom G.W. Breakdown in central motor control can be attenuated by motor practice and neuromodulation of the primary motor cortex. *Neuroscience*, 220:11–18, 2012.

[205] Okano A. H., Fontes E. B., Montenegro R. A., Farinatti P, Cyrino E. S., Min L. L., Bikson M., and Noakes T. D. Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise. *British Journal of Sports Medicine*, 49:1213–1218, 2013.

[206] Vitor-Costa M., Okuno N. L., Bortolotti H., Bertollo M., Boggio P., Fregni F., and Altimari L. Improving cycling performance: Transcranial direct current stimulation increases time to exhaustion in cycling. *PLoS One*, 10:1–15, 2015.

[207] Cisek P. and Kalaska J. F. Neural correlates of reaching decisions in dorsal premotor cortex: Specification of multiple direction choices and final selection of action. *Neuron*, 45(5):801–814, 2005.

[208] Cisek P. and Kalaska J. F. Neural mechanisms for interacting with a world full of action choices. *Annual Review of Neuroscience*, 33(1):269–298, 2010.

[209] Oldfield R.C. The assessment and analysis of handedness: The edinburgh inventory. *Neuropsychologia*, 9(1):97–113, 1971.

[210] Dean C., Shepherd R., and Adams R. Sitting balance i, trunk-arm coordination and the contribution of the lower limbs during self-paced reaching in sitting. *Gait & Posture*, 10(2):135–146, 1999.

[211] Riedo R. and Rüegg D.G. Origin of the specific h reflex facilitation preceding a voluntary movement in man. *The Journal of Physiology*, 397:371–388, 1988.

[212] Eichenberger A. and Rüegg D. G. Relation between the specific h reflex facilitation preceding a voluntary movement and movement parameters in man. *The Journal of Physiology*, 347:545 – 559, 1984.

[213] Hodges P. W. and Bui B. H. A comparison of computer-based methods for the determination of onset of muscle contraction using electromyography. *Elec-*

troencephalography and Clinical Neurophysiology/Electromyography and Motor Control, 101(6):511–519, 1996.

[214] Andrews R. K., Schabrun S. M., Ridding M. C., Galea M. P., Hodges P. W., and Chipchase L. S. The effect of electrical stimulation on corticospinal excitability is dependent on application duration: a same subject pre-post test design. *Journal of Neuroengineering and Rehabilitation*, 10(1):51–51, 2013.

[215] Mordillo-Mateos L., Turpin-Fenoll L., Millán-Pascual J., Núñez-Pérez N., Panyavin I., Gómez-Argüelles J.M., Botia-Paniagua E., Foffani G., Lang N., and Oliviero A. Effects of simultaneous bilateral tDCS of the human motor cortex. *Brain Stimulation*, 5(3):214–222, 2012.

[216] Strigaro G., Ruge D., Chen J.-C., Marshall L., Desikan M., Cantello R., and Rothwell J.C. Interaction between visual and motor cortex: a transcranial magnetic stimulation study. *The Journal of Physiology*, 593(10):2365–2377, 2015.

[217] Cantello R., Strigaro G., Prandi P., Varrasi C., Mula M., and Monaco F. Paired-pulse flash-visual evoked potentials: New methods revive an old test. *Clinical Neurophysiology*, 122(8):1622–1628, 2011.

[218] Schieppati M., Trompetto C., and Abbruzzese G. Selective facilitation of responses to cortical stimulation of proximal and distal arm muscles by precision tasks in man. *The Journal of Physiology*, 491(2):551–562, 1996.

[219] Brass M., Bekkering H., and Prinz W. Movement observation affects movement execution in a simple response task. *Acta Psychologica*, 106(1):3–22, 2001.

[220] Jäncke L., Specht K., Mirzazade S., Loose R., Himmelbach M., Lutz K., and Shah N. J. A parametric analysis of the "rate effect" in the sensorimotor cortex: a functional magnetic resonance imaging analysis in human subjects. *Neuroscience Letters*, 252(1):37–40, 1998.

[221] Lutz K., Koeneke S., Wüstenberg T., and Jäncke L. Asymmetry of cortical activation during maximum and convenient tapping speed. *Neuroscience Letters*, 373(1):61–66, 2004.

[222] Bigland-Ritchie B. and Woods J. J. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle & Nerve*, 7(9):691–699, 1984.

[223] Rossini P. M., Burke B., Chen R., Cohen L. G., Daskalakis Z., Di Iorio R., Di Lazzaro V., Ferreri F., Fitzgerald P.B., George M.S., Hallett M., Lefaucheur J.P., Langguth B., Matsumoto H., Miniussi C., Nitsche M.A., Pascual-Leone A., Paulus W., Rossi S., Rothwell J.C., Siebner H.R., Ugawa Y., Walsh V., and Ziemann U. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. an updated report from an I.F.C.N. committee. *Clinical Neurophysiology*, 126(6):1071–1107, 1994.

[224] Taylor J. L. and Gandevia S. C. Noninvasive stimulation of the human corticospinal tract. *Journal of Applied Physiology*, 96(4):1496–1503, 2004.

[225] Banissy M. and Muggleton N. Transcranial direct current stimulation in sports training: Potential approaches. *Frontiers in Human Neuroscience*, 7:129–129, 2013.

[226] Edwards D. J., Cortes M., Wortman-Jutt S., Putrino D., Bikson M., Thickbroom G., and Pascual-Leone A. Transcranial direct current stimulation and sports performance. *Frontiers in Human Neuroscience*, 11:243–243, 2017.

[227] Delis I., Berret B., Pozzo T., and Panzeri S. Quantitative evaluation of muscle synergy models: a single-trial task decoding approach. *Frontiers in Computational Neuroscience*, 7:8–8, 2013.

[228] Vicario C. M., Martino D., and Koch G. Temporal accuracy and variability in the left and right posterior parietal cortex. *Neuroscience*, 245:121–128, 2013.

[229] Lomond K. V. and Côté J. N. Movement timing and reach to reach variability during a repetitive reaching task in persons with chronic neck/shoulder pain and healthy subjects. *Experimental Brain Research*, 206(3):271–282, 2010.

[230] Kornatz K. W., Christou E. A., and Enoka R. M. Practice reduces motor unit discharge variability in a hand muscle and improves manual dexterity in old adults. *Journal of Applied Physiology*, 98:2072–2080, 2005.

[231] Pienciak-Siewert A., Horan D. P., and Ahmed A. A. Trial-to-trial adaptation in control of arm reaching and standing posture. *Journal of Neurophysiology*, 116:2936–2949, 2016.

[232] Giboin L. S., Thumm P., Bertschinger R., and Gruber M. Intermittent theta burst over m1 may increase peak power of a wingate anaerobic test and prevent the reduction of voluntary activation measured with transcranial magnetic stimulation. *Frontiers in Behavioral Neuroscience*, 10:150–150, 2016.

[233] Barry B. K. and Enoka R. M. The neurobiology of muscle fatigue: 15 years later. *Integrative and Comparative Biology*, 47(4):465–473, 2007.

[234] Madrid A., Madinabeitia-Mancebo E., Cudeiro J., and Arias P. Effects of a finger tapping fatiguing task on m1-intracortical inhibition and central drive to the muscle. *Scientific Reports*, 8:9326–9326, 2018.

[235] Klass M., Lévénez M., Enoka R. M., and Duchateau J. Spinal mechanisms contribute to differences in the time to failure of submaximal fatiguing contractions performed with different loads. *Journal of Neurophysiology*, 99:1096–1104, 2008.

[236] Di Lazzaro V., Oliviero A., Tonali P. A., Mazzone P., Insola A., Pilato F., Saturno E., Dileone M., and Rothwell J. C. Direct demonstration of reduction of the output of the human motor cortex induced by a fatiguing muscle contraction. *Experimental Brain Research*, 149(4):535–538, 2003.

[237] Maruyama A., Matsunaga K., Tanaka N., and Rothwell J. Muscle fatigue decreases short-interval intracortical inhibition after exhaustive intermittent tasks. *Clinical Neurophysiology*, 117:864–870, 2006.

[238] Hunter S. K., McNeil C. J., Butler J. E., Gandevia S. C., and Taylor J. L. Short-interval cortical inhibition and intracortical facilitation during submaxi-

mal voluntary contractions changes with fatigue. *Experimental Brain Research*, 234(9):2541–2551, 2016.

[239] Otieno L. A., Opie G., Semmler J., Ridding M., and Sidhu S. Intermittent single-joint fatiguing exercise reduces tms-eeg measures of cortical inhibition. *Journal of Neurophysiology*, pages 471–479, 2018.

[240] Premoli I., Rivolta D., Espenhahn S., Castellanos N., Belardinelli P., Ziemann U., and Müller-Dahlhaus F. Characterization of gabab-receptor mediated neurotransmission in the human cortex by paired-pulse tms–eeg. *NeuroImage*, 103:152–162, 2014.

[241] Jones D.A., Bigland-Ritchie B., and Edwards R.H.T. .excitation frequency and muscle fatigue: Mechanical responses during voluntary and stimulated contractions". *Experimental Neurology*, 64(2):401–413, 1979.

[242] Taylor J. L., Butler J. E., and Gandevia S. C. Changes in muscle afferents, motoneurons and motor drive during muscle fatigue. *European Journal of Applied Physiology*, 83:106–115, 2000.

[243] Rohmert W. *Ermittlung von Erholungspausen für statische Arbeit des Menschen*. Springer, 1960.

[244] Bellemare F. and Grassino A. Effect of pressure and timing of contraction on human diaphragm fatigue. *Journal of Applied Physiology*, 53(5):1190–1195, 1982.

[245] Brasil-Neto J. P., Pascual-Leone A., Valls-Solé J., Cammarota A., Cohen L. G., and Hallett M. Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. *Experimental Brain Research*, 93(1):181–184, 1993.

[246] Taylor J. L., Allen G. M., Butler J. E., and Gandevia S. C. Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. *Journal of Applied Physiology*, 89(1):305–313, 2000.

[247] Wilson S. A., Lockwood R. J., Thickbroom G. W., and Mastaglia F. L. The muscle silent period following transcranial magnetic cortical stimulation. *Journal of the Neurological Sciences*, 114:216–222, 1993.

[248] Petersen N. T., Taylor J. L., Butler J. E., and Gandevia S. C. Depression of activity in the corticospinal pathway during human motor behavior after strong voluntary contractions. *Journal of Neuroscience*, 23(22):7974–7980, 2003.

[249] Priori A. Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clinical Neurophysiology*, 114(4):589–595, 2003.

[250] Paulus W. *Outlasting excitability shifts induced by direct current stimulation of the human brain*, volume 57 of *Supplements to Clinical Neurophysiology*. Elsevier, 2004.

[251] Wassermann E. M. and Grafman J. Recharging cognition with dc brain polarization. *Trends in Cognitive Sciences*, 9(11):503–505, 2005.

[252] Pascual-Leone A., Valls-Solé J., Wassermann E. M., and Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain*, 117(4):847–858, 1994.

[253] Chen R., Classen J., Gerloff C., Celnik P., Wassermann E. M., Hallett M., and Cohen L. G. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, 48(5):1398–1403, 1997.

[254] Stefan K., Kunesch E., Leonardo G. C., Benecke R., and Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain*, 123:572–584, 2000.

[255] Khedr E. M., Gilio F., and Rothwell J. C. Effects of low frequency and low intensity repetitive paired pulse stimulation of the primary motor cortex. *Clinical Neurophysiology*, 115(6):1259–1263, 2004.

[256] Nitsche M. A. and Paulus W. Sustained excitability elevations induced by transcranial dc motor cortex stimulation in humans. *Neurology*, 57(10):1899–1901, 2001.

[257] Nitsche M. A., Fricke K., Henschke U., Schlitterlau A., Liebetanz D., Lang N., Henning S., Tergau F., and Paulus W. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of Physiology*, 553(1):293–301, 2004.

[258] Priori A., Berardelli A., Rona S., Accornero N., and Manfredi M. Polarization of the human motor cortex through the scalp. *NeuroReport*, 9:2257 – 2260, 1998.

[259] Ardolino G., Bossi B., Barbieri S., and Priori A. Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *The Journal of Physiology*, 568(2):653–663, 2005.

[260] Muthalib M., Kan B., Nosaka K., and Perrey S. *Effects of Transcranial Direct Current Stimulation of the Motor Cortex on Prefrontal Cortex Activation During a Neuromuscular Fatigue Task: An fNIRS Study*. 2013.

[261] Wiethoff W., Hamada M., and Rothwell J. C. Variability in response to transcranial direct current stimulation of the motor cortex. *Brain Stimulation*, 7(3):468–475, 2014.

[262] Horvath J. C., Carter O., and Forte F. D. No significant effect of transcranial direct current stimulation (tDCS) found on simple motor reaction time comparing 15 different simulation protocols. *Neuropsychologia*, 91:544–552, 2016.

[263] Nitsche M. A., Schauenburg A., Lang N., Liebetanz D., Exner C., Paulus W., and Tergau F. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *Journal of Cognitive Neuroscience*, 15(4):619–626, 2003.

[264] Leite J., Carvalho S., Fregni F., and Gonçalves Ó. F. Task-specific effects of tDCS-induced cortical excitability changes on cognitive and motor sequence set shifting performance. *PLoS One*, 6(9):1–9, 2011.

[265] López-Alonso V., Cheeran B., and Fernández del Olmo M. Relationship between non-invasive brain stimulation-induced plasticity and capacity for motor learning. *Brain Stimulation*, 8(6):1209–1219, 2015.

[266] Hupfeld K., Ketcham C., and Schneider H. Transcranial direct current stimulation (tDCS) to the supplementary motor area (SMA) influences performance on motor tasks. *Experimental Brain Research*, 235:1–9, 2016.

[267] Devanathan D. and Madhavan S. Effects of anodal tDCS of the lower limb m1 on ankle reaction time in young adults. *Experimental Brain Research*, 234(2):377–385, 2016.

[268] Pascual-Leone A., Brasil-Neto J., Valls-Solé J., Cohen L. G., and Hallett M. Simple reaction time to focal transcranial magnetic stimulation: Comparison with reaction time to acoustic, visual and somatosensory stimuli. *Brain*, 115:109–122, 1992.

[269] Hashimoto T., Inaba D., Matsumura M., and Naito E. Two different effects of transcranial magnetic stimulation to the human motor cortex during the pre-movement period. *Neuroscience Research*, 50:427–436, 2005.

[270] Kennefick M., Maslovat D., and Carlsen A. N. The time course of corticospinal excitability during a simple reaction time task. *PLoS One*, 9(11):1–7, 2014.

[271] Reynolds C. and Ashby P. Inhibition in the human motor cortex is reduced just before a voluntary contraction. *Neurology*, 53:730–735, 1999.

[272] Cengiz B., Murase N., and Rothwell J. C. Opposite effects of weak transcranial direct current stimulation on different phases of short interval intracortical inhibition (sici). *Experimental Brain Research*, 225(3):321–331, 2013.

[273] Hummel F. and Cohen L. G. Improvement of motor function with noninvasive cortical stimulation in a patient with chronic stroke. *Neurorehabilitation and Neural Repair*, 19(1):14–19, 2005.

[274] Lampropoulou S. and Nowicky A. The effect of transcranial direct current stimulation on perception of effort in an isolated isometric elbow flexion task. *Motor Control*, 17:412–426, 2013.

[275] Oki K., Mahato N. K., Nakazawa M., Amano S., France C. R., Russ D. W., and Clark B. C. Preliminary evidence that excitatory transcranial direct current stimulation extends time to task failure of a sustained, submaximal muscular contraction in older adults. *The Journals of Gerontology*, 71(8):1109–1112, 2016.

[276] Giboin L.-S. and Gruber M. Anodal and cathodal transcranial direct current stimulation can decrease force output of knee extensors during an intermittent mvc fatiguing task in young healthy male participants. *Journal of Neuroscience Research*, 96(9):1600–1609, 2018.

[277] Abdelmoula A., Baudry S., and Duchateau J. Anodal transcranial direct current stimulation does not influence the neural adjustments associated with fatiguing contractions in a hand muscle. *European Journal of Applied Physiology*, pages 597–609, 2018.

[278] Frazer A., Williams J., Spittle M., Rantalainen T., and Kidgell D. Anodal transcranial direct current stimulation of the motor cortex increases cortical voluntary activation and neural plasticity. *Muscle & Nerve*, 54:903–913, 2016.

[279] Sasada S., Endoh T., Ishii T., and Komiyama T. Polarity-dependent improvement of maximal-effort sprint cycling performance by direct current stimulation of the central nervous system. *Neuroscience Letters*, 657:97–101, 2017.

[280] Boehringer A., Macher K., Dukart J., Villringer A., and Pleger B. Cerebellar transcranial direct current stimulation modulates verbal working memory. *Brain Stimulation*, 6:649–653, 2012.

[281] Stavrinou M., Moraru L., Cimponeriu L., Della Penna S., and Bezerianos A. Evaluation of cortical connectivity during real and imagined rhythmic finger tapping. *Brain Topography*, 19:137–145, 2007.

[282] Stoodley C., Valera E., and Schmahmann J. An fmri study of intra-individual functional topography in the human cerebellum. *Behavioural Neurology*, 23:65–79, 2010.

[283] Foki T., Pirker W., Klinger N., Geissler A., Rath J., Steinkellner T., Höllinger I., Gruber S., Haubenberger D., Lehrner J., Pusswald G., Trattnig S., Auff E., and Beisteiner R. Fmri correlates of apraxia in parkinson's disease patients off medication. *Experimental Neurology*, 225:416–422, 2010.

[284] Shimoyama I., Ninchoji T., and Uemura K. The finger-tapping test: A quantitative analysis. *Archives of Neurology*, 47(6):681–684, 1990.

[285] Fleming G. W. T. H. A new sign of cerebellar disease. *Journal of Mental Science*, 76(312):150–150, 1930.

[286] Nakamura R., Nagasaki H., and Narabayashi H. Disturbances of rhythm formation in patients with parkinson's disease: Part I. Characteristics of tapping response to the periodic signals. *Perceptual and Motor Skills*, 46:63–75, 1978.

[287] Nagasaki H., Nakamura R., and Taniguchi R. Disturbances of rhythm formation in patients with parkinson's disease: Part II. A forced oscillation model. *Perceptual and Motor Skills*, 46(1):79–87, 1978.

[288] Aoki T. and Kinoshita H. Temporal and force characteristics of fast double-finger, single-finger and hand tapping. *Ergonomics*, 44(15):1368–1383, 2001.

[289] Brasil-Neto J. P., Cohen L. G., and Hallett M. Central fatigue as revealed by postexercise decrement of motor evoked potentials. *Muscle & Nerve*, 17(7):713–719, 1994.

[290] Zanette G., Bonato C., Polo A., Tinazzi M., Manganotti P., and Fiaschi A. Long-lasting depression of motor-evoked potentials to transcranial magnetic stimulation following exercise. *Experimental Brain Research*, 107(1):80–86, 1995.

[291] de Ruiter C. J., Jones D. A., Sargeant A. J., and De Haan A. The measurement of force/velocity relationships of fresh and fatigued human adductor pollicis

muscle. *European Journal of Applied Physiology and Occupational Physiology*, 80(4):386–393, 1999.

[292] Bigland-Ritchie B., Jones D.A., and Woods J.J. Excitation frequency and muscle fatigue: Electrical responses during human voluntary and stimulated contractions. *Experimental Neurology*, 64(2):414–427, 1979.

[293] Simpson M., Burke J. M. R., and David J. M. Cumulative effects of intermittent maximal contractions on voluntary activation deficits. *International Journal of Neuroscience*, 114:671–692, 2004.

[294] Ziemann U. *Pharmaco-transcranial magnetic stimulation studies of motor excitability*, volume 116 of *Handbook of Clinical Neurology*. Elsevier, 2013.

[295] Taylor J. L. and Gandevia S. C. Transcranial magnetic stimulation and human muscle fatigue. *Muscle & Nerve*, 24(1):18–29, 2001.

[296] Horvath J., Carter O., and Forte J. Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). *Frontiers in Systems Neuroscience*, 8:2–2, 2014.

[297] Marquez J., Conley A., Karayanidis F., Lagopoulos J., and Parsons M. Anodal direct current stimulation in the healthy aged: Effects determined by the hemisphere stimulated. *Restorative Neurology and Neuroscience*, 33:509–519, 2015.

[298] Fitts R. H. Cellular mechanisms of muscle fatigue. *Physiological Reviews*, 74(1):49–94, 1994.

[299] Hunter S. K. and Enoka R. M. Sex differences in the fatigability of arm muscles depends on absolute force during isometric contractions. *Journal of Applied Physiology*, 91(6):2686–2694, 2001.

[300] Hunter S. K., Critchlow A., and Enoka R. M. Muscle endurance is greater for old men compared with strength-matched young men. *Journal of Applied Physiology*, 99(3):890–897, 2005.

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RESEARCH ARTICLE

Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Tasks

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Citation: Arias P, Corral-Bergantiños Y, Robles-García V, Madrid A, Oliviero A, Cudeiro J (2016) Bilateral tDCS on Primary Motor Cortex: Effects on Fast Arm Reaching Tasks. PLoS ONE 11(8): e0160063. doi:10.1371/journal.pone.0160063

Editor: Alessio Avenanti, University of Bologna, ITALY

Received: May 5, 2016

Accepted: July 13, 2016

Published: August 4, 2016

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: This work was supported by Xunta de Galicia, Ayudas Grupos Consolidados (Consellería de Educación, 2014), Spain. VRG and YCB are granted by FPU-MECD AP2010-2774 and AP2010-2775 Spain.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Background

The effects produced by transcranial direct current stimulation (tDCS) applied to the motor system have been widely studied in the past, chiefly focused on primary motor cortex (M1) excitability. However, the effects on functional tasks are less well documented.

Objective

This study aims to evaluate the effect of tDCS-M1 on goal-oriented actions (i.e., arm-reaching movements; ARM), in a reaction-time protocol.

Methods

13 healthy subjects executed dominant ARM as fast as possible to one of two targets in front of them while surface EMG was recorded. Participants performed three different sessions. In each session they first executed ARM (*Pre)*, then received tDCS, and finally executed *Post*, similar to *Pre*. Subjects received three different types of tDCS, one per session: In one session the anode was on right-M1 (AR), and the cathode on the left-M1 (CL), thus termed *AR-CL*; *AL-CR* reversed the montage; and *Sham* session was applied likewise. Real stimulation was 1mA-10min while subjects at rest. Three different variables and their coefficients of variation (CV) were analyzed: Premotor times (PMT), reaction-times (RT) and movement-times (MT).

Results

triceps-PMT were significantly increased at *Post-Sham*, suggesting fatigue. Results obtained with real tDCS were not different depending on the montage used, in both cases PMT were significantly reduced in all recorded muscles. RT and MT did not change for real or sham stimulation. RT-CV and PMT-CV were reduced after all stimulation protocols.

Conclusion

tDCS reduces premotor time and fatigability during the execution of fast motor tasks. Possible underlying mechanisms are discussed.

Introduction

Transcranial direct current stimulation (tDCS) is a promising tool for neurorehabilitation purposes. Over the past few years a solid background has been built on the capacity of tDCS to modulate functional brain networks and guidelines have been formulated for its safe use on humans [1, 2]. tDCS permits a transient modulation of cortical excitability by the application of currents in a non-invasive way. tDCS is able to produce long lasting depolarization or hyperpolarization of cell membranes depending on stimulation polarity [3]; these aftereffects are thought to be mediated by calcium-dependent plasticity of glutamatergic neurons [4]. As a result, anodal tDCS applied to the primary motor cortex (M1) increases cortico-spinal excitability and cathodal stimulation produces the opposite effect [3]. These effects depend on the intensity and duration of the stimulation [3], but just ten minutes at 1mA induces consistent aftereffects, lasting for a period of minutes [5, 6].

The classic electrode montage to modulate the excitability of the motor cortex places one electrode over M1 and the other at the contralateral supraorbital forehead [3]. However, simultaneous bilateral M1 stimulation induces a similar effect in terms of excitability modulation, weaker in magnitude than unilateral montages [7, 8], but with smaller inter-subject variability [7]. The use of bilateral tDCS-M1 is appealing in pathologies like stroke, where the interhemispheric imbalance might be controlled by up-regulating the excitability of the ipsilesional motor cortex, while down-regulating the contralesional M1 [9]. Also, it is believed that bilateral tDCS-M1 produces better effects than uni-hemispheric tDCS on behavioral motor tasks [10].

Despite what has been mentioned above, tDCS effects on functional motor tasks have received less attention than effects related to cortical excitability; however, its applicability seems suitable for rehabilitation and motor learning [11, 12]. Remarkably, tDCS of M1 modulates excitability at sites distant from the cortex [13], such as the propriospinal circuits within the spinal cord [14, 15]. This particular point should be considered when evaluating the effect of tDCS on goal-oriented motor tasks (including movement preparation and execution), because of a inter-play between M1 and spinal cord excitability precedes the execution of movements [16–18]. In fact, cathodal tDCS-M1 reduces cortical excitability but does not change spinal excitability [3, 5, 14], whereas anodal tDCS increases excitability in M1 [3, 6] but also modulates the excitability of propriospinal circuits [14, 15]. Therefore, it seems that the understanding of bilateral tDCS effects on motor functional activities is of relevance in order to design new strategies aimed to improve function in certain pathologies [19–21]. In this sense, one essential task in daily living activities is arm reaching.

A classical view of the parieto-frontal network considers several loci to study the effect of tDCS on arm reaching towards a target in the peri-personal space. The posterior parietal cortex receives input from visual areas through the dorsal stream [22] and generates representations of eye, head, body and surrounding frameworks for early visuomotor planning [23, 24] of reaching and grasping [22]; some of these functions are shared by M1 [25] and other areas [26]. The prefrontal cortex (PFC) has a role in the control of reaching, especially for choice reaching tasks [27, 28] in intimate relation with the dorsal premotor cortex (d)PMC [23, 29]; while the ventral premotor cortex (vPMC) may contribute to transformations from extrinsic to intrinsic coordinates to guide movement directed to objects in peri-personal space [23].

However, a modern view of this network is much wider, challenging the "serial" assumption that selection (decision making) occurs before specification (movement planning), and rather advocates that these processes operate simultaneously and in an integrated manner [30]. It is now suggested that potential reaching actions are specified by the dorsal stream, while their selection involves cortical (like PFC [30] or dPMC[31, 32]) and subcortical structures (like the Basal Ganglia [33, 34]) engaged in the evaluation of the opportunity of executing a reaching movement while minimizing costs and maximizing profits [35]. In this sense the network predicts future outcomes and cancels not sufficiently valuable actions [30]. Within the whole network M1 is a crucial area to study the effect of tDCS on reaching tasks for its role in planning and releasing the movement [23, 25, 35–38], as well on cancelling those that are on-going [32, 35]. M1 (amongst other areas) is also involved in choosing between alternative actions to get the goal and the generation of their motor commands [35, 39].

The effect of tDCS-M1 on the planning and execution of goal-oriented actions can be inferred from the reaction times (RT) and the movement times (MT), respectively [40]. In this study we have evaluated the effects of bilateral tDCS-M1 on arm reaching movements. We used RT tasks of different complexity while controlling the effect of polarity, and placebo.

Our research hypothesis is that tDCS modulates motor behavior in a polarity dependent manner. We predict faster responses after bilateral tDCS, when anodal stimulation is applied to the M1 contralateral to the dominant (executing) arm, as a result of the increased excitability of M1 circuits. Conversely, cathodal stimulation shall render opposite effects.

Experimental Procedures

All experimental subjects signed consent forms. The protocol conformed to the declaration of Helsinki and was approved by the Ethics Committee of the University of A Coruña (Spain). The individuals whose experimental data were included in this manuscript have given written informed consent (as outlined in PLOS consent form) allowing the use of photographs to illustrate the figures.

Subjects

Thirteen healthy subjects participated (six women, age range 20-37yrs). None took medication or undertook hard physical work in the week prior the experimental sessions. Subjects were right-handed [41] and had normal or corrected-to-normal vision.

Procedure

Each subject performed three sessions, one week apart. In each session they executed the reaching tasks before and after the tDCS (*Pre* and *Post*, respectively). A different tDCS protocol was applied in each session; the order was randomized. Subjects reached to one of two round targets placed at gaze height in front of them (see below). Reaching was always performed as fast as possible with the right (dominant) hand on a frontoparallel plane (Fig 1). No instructions were provided on how to touch the target, apart from asking subjects to touch the centre of the target with the hand as fast as possible. All subjects chose to touch with finger joints extended.

Each of the three sessions comprised three different reaching tasks randomized in order, and each task included several trials. In all cases a low-tone audible *warning signal* was presented as a cue prior to the *response-signal*. The turning-on of an array of red LED's at the edge of the target was the *response-signal*. Without any other purpose than making the response-signal time unpredictable (with regards to the warning cue) we used three foreperiods (delays) between warning and response signals; 500, 1000, or 1500ms, and their presentation order was randomized within trials. The sequence of events was controlled by Signal-4 software via a

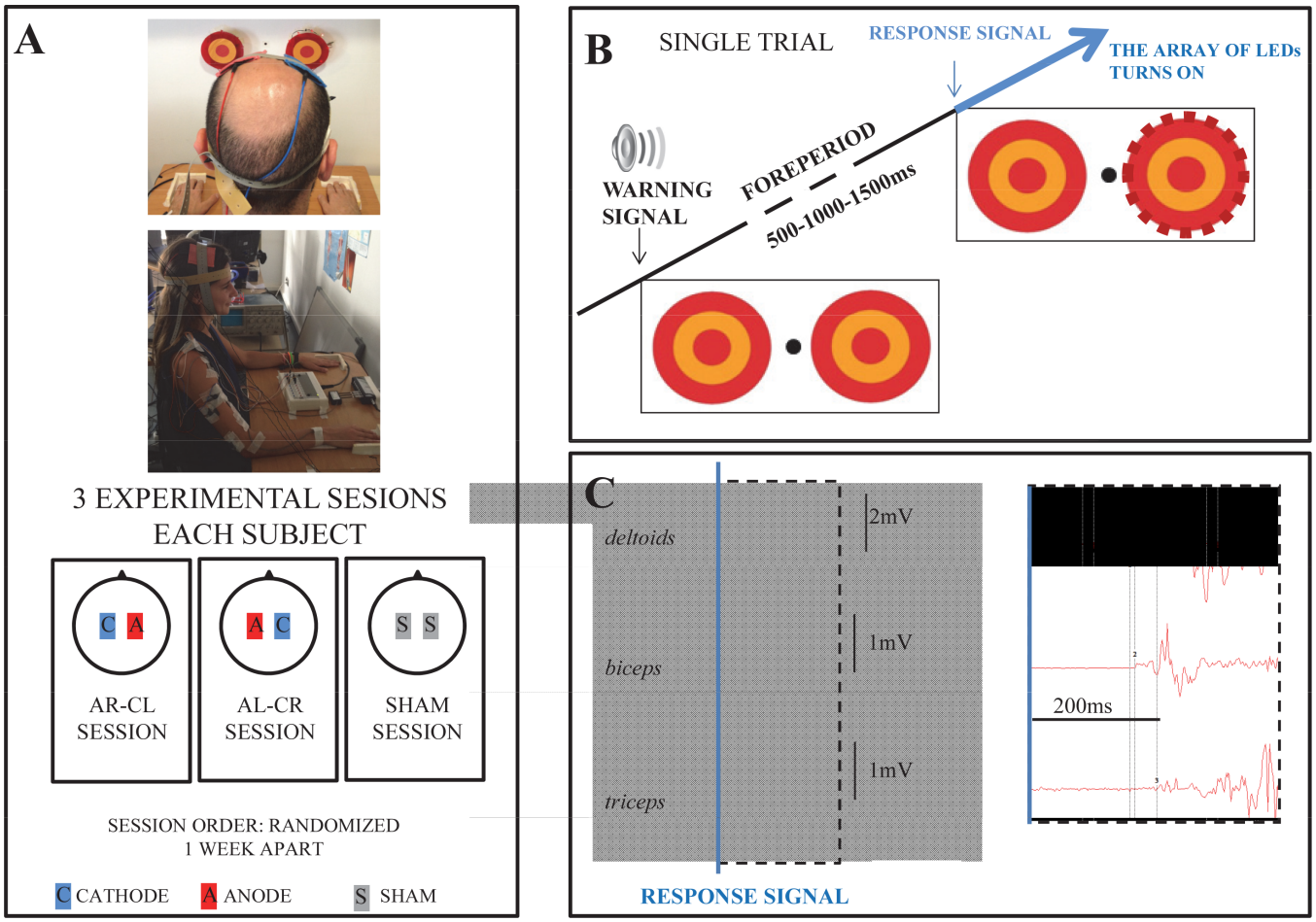

Fig 1. **(A)** Experimental setting and tDCS electrode montage in the 3 experimental sessions. The pictures show two subjects receiving tDCS at rest. **(B)** A single trial lasted 10s; the response signal was presented 500, 1000 or 1500ms after the warning cue. **(C)** Example of one recording reflecting the sequential activation of the three muscles evaluated. Recordings are synchronized to the response signal (marked as the blue vertical line). The dashed area is enlarged at the right to clarify the sequential muscle responses. The individuals in these pictures have given written informed consent (as outlined in PLOS consent form) to publish the images.

doi:10.1371/journal.pone.0160063.g001

CED 1401mkII (Cambridge Electronic Design, UK). Subjects were asked to fixate on a central point between the two targets until the appearance of the response-signal (Fig 1) after which they made the response.

Tasks. T1: Subjects were informed they had to reach the target ipsilateral to their dominant (executing) hand (i.e., *single-ipsilateral* response).

T2: Subjects were told that they had to reach the target in the contralateral space to their dominant arm (*single-contralateral*).

T3: In this task (*choice)* subjects were informed they would reach either the ipsilateral or the contralateral target to the dominant arm, depending on what target was lit (in randomized order).

Each of the two *single*-tasks (*single-ipsilateral* and *single-contralateral*) included 12 reaching trials, plus 1 catch trial, all randomized. In the catch trial the response signal did not appear after the warning cue.

The *choice*-task included 12 trials to the ipsilateral target (*choice-ipsilateral*) and 12 trials to the contralateral target (*choice-contralateral*) and these also included a catch trial. The 25 trials were randomized in order.

In all cases the inter-trial interval was 10s, and a one minute rest was given between the different tasks.

Pre and *Post* testing lasted 12min 10s each; *Post* started 1min after the end of the tDCS. The order of presentation of the three tasks in *Pre* was randomized, and reproduced in *Post*.

Experimental Setting

The round targets were 15cm diameter; their centers were 32cm apart from each other, and halfway between them a small black spot (1 cm-diameter) served as the fixation spot. The subjects were seated on an adjustable chair, containing chest-straps to avoid trunk movements, but allowing unrestrained shoulder movements. The chair height was adapted to make eye level at fixation height; the distance from the subject to the targets was adjusted such that allowing the reaching to the contralateral target with nearly full-elbow extension, minimizing leg involvement in the task [42]. Subjects' hands were on contact-plates. After setting, subjects made three fast ARM's to each target, as practice. The entire setup (Fig 1) was reproduced in each of the different test days.

Motor Outcomes

Signals were acquired by means of Biometrics-Data-Link, Digitimer D-360 amplifiers and the CED 1401 (3-3000Hz; 10KHz sampling rate, 1000 gain). Electromyographic (EMG) recordings determined the pre-motor time (PMT) [17], as the time lags from the *response-signal* to the movement-related EMG-onset [43]. EMG recorded the activity of the *deltoid*, *biceps brachii* and *triceps brachii*. Surface electrodes were placed in a belly-tendon montage on the *anterior deltoid*; *large biceps head*; and *lateral triceps head* (*del; bic; tri*), always after skin preparation. EMG-onset was determined automatically (and visually checked [44]) by applying the double-threshold method [45]. Thus the EMG signals were rectified and the EMG onset was considered at the first of ten consecutive samples (one threshold) above a given EMG amplitude (the other threshold); the latter threshold was equal to the mean EMG-background activity amplitude plus one standard deviation, which was calculated in the time-window just 50ms prior the response signal. Such threshold was obtained for each of the three muscles independently. The time elapsing from the LEDs flash to lifting the hand and leaving the contact-plate was computed as the RT; and the time from leaving the contact-plate to touch the target determined the MT. Customized MatLab programmes (The Mathworks, Ltd) were used to process the data.

Brain Stimulation

tDCS was applied bilaterally on M1's with a Neuroconn DC-Stimulator connected to a pair of 5x7cm saline-soaked electrodes. In all sessions, one electrode was placed on the left M1, and the other on the right M1; corresponding to C3 and C4 of the International 10–20 EEG system.

In one session the anode was on the left M1, and the cathode on the right M1, and this is referred as *AL-CR* montage. In other session the anode was on the right M1, and the cathode on the left M1 (*AR-CL*). *Sham* montage randomized electrode positions. For real stimulation 1mA-intensity was applied for 10min (current fade-in and out was ramped and lasted the initial and final 8s). The *Sham* protocol lasted the same time but the current was applied for 30s and then ceased [7]. Subject remained restful during the stimulation sessions.

Statistical Methods

The mean of the responses for each experimental condition and subject was the outcome-value introduced in the analysis. The mean was computed considering all events from each experimental condition, but those which PMT <80ms or >800ms; thus those events with PMT <80ms were considered anticipations, and their proportion in the different stimulation protocols, conditions and testing time-points (*pre* and *post*) were evaluated with the Fisher Exact Probability Test. The threshold of 80ms was set based on the latencies of a visual-evoked potential to a flashing LED recorded in the primary visual cortex (V1), plus the minimum latency for an interaction between V1 and M1, plus the latency from M1 activation to EMG onset on the studied muscles [46–48]. Events with PMT >800ms were discarded as sign of un-attentiveness [49], though this happened just once in all subjects and conditions. PMT, RT and MT from events within those thresholds were normalized to control variability related to daily differences in the experimental setting (see above), though care was taken to minimize it. Normalization was performed as follows: For each session the average for each variable was calculated from data including *Pre* outcome-values for all tasks and subjects (Table 1). This normalizing value was used to divide all subjects' responses at *Pre* and *Post* for the corresponding day. This normalization procedure respects inter-subject variability, while normalizing the responses to the daily pooled *Pre*-testing.

After checking the normality of the distributions with a Kolgomorov-Smirnov test for one sample, an ANOVA with repeated measures analyzed the effect of tDCS on the variables considering the 13 subjects.

We have used two different ANOVA designs: one for the PMT and one for RT and MT. The former is a five factors ANOVA with STIM (three levels: *AR-CL*, *AL-CR*, and *Sham*); Time (two levels: *Pre* and *Post*); LATERALITY (two levels: Target *Ipsilateral* or *Contralateral* to the dominant–executing- hand); OPTION (two levels: *single* and *choice* responses) and MUSCLE (three levels: *deltoid*, *biceps* and *triceps*). The ANOVA on RT and MT had the same design except for the factor MUSCLE which was not included since RT and MT derived from contact plates.

The W-Mauchly test checked the sphericity for ANOVA, if sphericity was violated the ANOVA degrees of freedom were corrected by means of the Greenhouse-Geisser coefficients. Effect sides were calculated by partial etha and etha squared (η_p^2, η^2). Significance was considered if $p<0.05$.

Results

Table 1 shows the mean PMT, RT, and MT at *Pre* under each experimental condition (values serving as normalizing factors and equivalent to the units in the y-axes of the corresponding graph). Subjects made no responses during the catch trials. See also S1 Data.

Effects of Brain Stimulation on PMT

Table 2 shows the mean values for the different levels of all factors in a *pre-post* basis.

Table 1. PMT, RT and MT at *Pre* in the three days of the protocol; mean (SE) considering all subjects.

	AL-CR	*AR-CL*	*Sham*
PMT (ms) at *Pre*	201.8 (11.5)	204.5ms (12.3)	201.0ms (10.5)
RT (ms) at *Pre*	243.3 (9.0)	245.1ms (10.0)	245.1ms (9.0)
MT(ms) at *Pre*	222.5 (12.5)	227.0ms (10.3)	221.0ms (12.0)

doi:10.1371/journal.pone.0160063.t001

Table 2. Mean values in ms (and SE) for the different levels of the different factors in a *pre-post* basis. PMT: premotor times; RT: reaction times; MT: movement times.

	AL-CR		*AR-CL*		*Sham*	
	TIME		TIME		TIME	
PMT	***pre***	***post***	***pre***	***post***	***pre***	***post***
LATERALITY						
ipsi	198.7 (12.9)	194.8 (9.9)	203.8 (11.1)	204.4 (14.1)	198.5 (11.3)	195.9 (10.4)
contra	204.9 (10.6)	197.7 (9.3)	205.2 (13.8)	204.3 (14.5)	203.5 (9.8)	208.1 (10.0)
OPTION						
single	194.6 (12.8)	190.7 (9.7)	196.0 (10.6)	196.2 (14.1)	193.0 (11.1)	195.5 (10.3)
choice	209.0 (11.2)	201.8 (9.6)	213.0 (14.4)	212.5 (14.8)	209.0 (10.5)	208.6 (10.5)
MUSCLE						
del	191.4 (9.9)	184.9 (7.8)	190.0 (10.2)	190.6 (12.2)	192.2 (8.7)	189.7 (8.4)
bic	188.4 (10.8)	183.9 (9.0)	187.5 (13.0)	186.0 (14.0)	189.6 (9.9)	187.1 (9.5)
tri	225.6 (16.0)	220.0 (13.7)	236.0 (16.9)	236.5 (19.2)	221.1 (14.4)	229.2 (14.8)
RT	***Pre***	***post***	***pre***	***post***	***pre***	***post***
LATERALITY						
ipsi	241.4 (9.9)	241.2 (6.9)	243.7 (8.3)	243.1 (11.2)	241.9 (9.4)	241.5 (9.3)
contra	245.2 (8.4)	246.3 (8.6)	246.5 (12.0)	243.2 (11.6)	248.3 (8.8)	255.1 (11.2)
OPTION						
single	237.0 (9.8)	238.1 (8.7)	237.2 (9.3)	236.0 (11.6)	238.6 (10.9)	240.7 (11.0)
choice	249.6 (9.3)	249.3 (6.7)	253.0 (11.3)	250.3 (11.5)	251.6 (8.2)	255.8 (9.2)
MT	***pre***	***post***	***pre***	***post***	***pre***	***post***
LATERALITY						
ipsi	205.4 (11.0)	207.0 (11.9)	206.8 (8.7)	202.2 (8.8)	203.3 (10.8)	204.8 (9.6)
contra	239.6 (14.2)	242.4 (16.9)	247.2 (12.2)	242.6 (12.5)	238.7 (13.6)	236.8 (13.0)
OPTION						
single	220.7 (12.2)	222.8 (14.1)	229.1 (9.2)	221.7 (10.0)	218.7 (11.3)	221.1 (11.4)
choice	224.3 (12.9)	226.6 (14.5)	224.9 (11.6)	223.1 (11.2)	223.3 (12.8)	220.4 (11.2)

Ipsi, *contra*: responses to the ipsilateral or contralateral targets to the dominant-executing hand. *Del* (deltoids), *bic* (biceps), *tri* (triceps).

doi:10.1371/journal.pone.0160063.t002

Table 3 summarizes the effects of tDCS on PMT (ANOVA factors, and significant interactions of the factors with TIME). If interactions TIME x STIM were significant (first column of results in the table), we followed-up with ANOVA's by pair of STIM modes and, if significance remained, by STIM mode in isolation (subsequent columns in Table 3).

With the 3 STIM modes-ANOVA there were significant main effects showing that all PMT were faster in single compared to choice responses ($\eta_p^2 = 0.672$; $\eta^2 = 0.061$), in ipsilateral than contralteral responses ($\eta_p^2 = 0.368$; $\eta^2 = 0.006$); and presented a sequential muscle activation ($\eta_p^2 = 0.716$ $\eta^2 = 0.393$; the latter can be observed in a representative subject in Fig 1C).

The significant interactions between TIME and STIM with other factors were observed in this model with 3 stimulation modes (also in the rest of models, except when the two active tDCS protocols were compared), Table 3. This means that the responses to Sham were different to the responses of the other to stimulation modes, and that the responses obtained with the two active stimulation modes were not significantly different of each other; for such reason their effects are shown pooled in figures (green tones). The individual's responses in *Pre* vs. *Post* basis are depicted in Fig 2.

For the follow-up ANOVA including *AR-CL* and *AL-CR* stim modes the significant factor TIME, in absence of significant interactions with any other factor, indicates a small ($\eta_p^2 =$

Table 3. ANOVA's for PMT. Main effects and significant interactions with factor TIME. ANOVA's were executed considering the three STIM modes. If significant interactions indicate different responses to STIM in the testing TIMEs, the ANOVA's were followed-up by pairs and the by single STIM mode.

	AR-CL vs AL-CR vs Sham	*AR-CL vs Sham*	*AL-CR vs Sham*	*AR-CL vs AL-CR*	*Sham*
MAIN EFFECTS					
STIM	$F_{2,24} = 0.3$ p = 0.3	$F_{1,12} = 0.1$ p = 0.9	$F_{1,12} = 0.5$ p = 0.5	$F_{1,12} = 0.4$ p = 0.5	N.A
TIME	$F_{1,12} = 2.4$ p = 0.15	$F_{1,12} = 0.8$ p = 0.8	$F_{1,12} = 1.4$ p = 0.3	$F_{1,12} = 4.9$ **p = 0.048**	$F_{1,12} = 0.2$ p = 0.6
				$\eta_p^2 = 0.288$	
OPTION	$F_{1,12} = 24.6$ **p<0.001**	$F_{1,12} = 30.3$ **p<0.001**	$F_{1,12} = 16.5$ **p = 0.002**	$F_{1,12} = 19.7$ **p<0.001**	$F_{1,12} = 16.0$ **p = 0.002**
	$\eta_p^2 = 0.672$	$\eta_p^2 = 0.716$	$\eta_p^2 = 0.579$	$\eta_p^2 = 0.621$	$\eta_p^2 = 0.572$
LATERALITY	$F_{1,12} = 7.0$ **p = 0.022**	$F_{1,12} = 6.4$ **p = 0.027**	$F_{1,12} = 6.6$ **p = 0.025**	$F_{1,12} = 2.2$ p = 0.2	$F_{1,12} = 16.2$ **p = 0.002**
	$\eta_p^2 = 0.368$	$\eta_p^2 = 0.347$	$\eta_p^2 = 0.355$		$\eta_p^2 = 0.574$
MUSCLE	$F_{2,24} = 30.3_{\varepsilon = 0.6}$ **p<0.001**	$F_{2,24} = 31.3_{\varepsilon = 0.6}$ **p<0.001**	$F_{2,24} = 22.6_{\varepsilon = 0.6}$ **p<0.001**	$F_{2,24} = 30.5_{\varepsilon = 0.6}$ **p<0.001**	$F_{2,24} = 21.5_{\varepsilon = 0.6}$ **p<0.001**
	$\eta_p^2 = 0.876$	$\eta_p^2 = 0.723$	$\eta_p^2 = 0.653$	$\eta_p^2 = 0.717$	$\eta_p^2 = 0.642$
SIGNIFICANT	$F_{2,24} = 3.4$ p = $0.052_{STIMxTIMExLAT}$	$F_{1,12} = 5.6$ **p = 0.035**$_{STIMxTIMExLAT}$	$F_{1,12} = 6.1$ **p = 0.030**$_{STIMxTIMExLAT}$	N.S	$F_{1,12} = 11.4$ **p = 0.005**$_{TIMExLAT}$
INTERACTIONS	$F_{4,48} = 3.2$ **p = 0.020**$_{STIMxTIMExMUS}$	$F_{2,24} = 6.8$ **p = 0.042**$_{STIMxTIMExOPT}$	$F_{2,24} = 5.4_{\varepsilon = 0.7}$ **p = 0.024**$_{STIMxTIMExMUS}$		$F_{2,24} = 9.5_{\varepsilon = 0.6}$ **p = 0.005**$_{TIMExMUS}$
FACTOR TIME			$F_{1,12} = 5.7$ **p = 0.035**$_{STIMxTIME}$		

N.S. = none was significant; N.A. = not applicable since such ANOVA had not that factor. Partial etha squared (η_p^2) is reported for significant main effects. Since significant interactions involving TIME and STIM (in the model with 3 STIM modes) do not inform whether the three STIM modes produced different responses compared to each other, or if there was just one STIM mode that produced different responses in TIME compared to the other two STIM modes, we followed-up ANOVA by pairs of STIM modes, and if needed, just with one STIM mode.

doi:10.1371/journal.pone.0160063.t003

0.288 $\eta^2 = 0.002$) but significant ($F_{1,12} = 4.9$ $p<0.05_{TIME}$) reduction of 1.5% in the PMT at *Post* (Fig 3A). This effect was present in the three studied muscles and was independent on the laterality and options of the responses. Fig 2 indicates that in most of the subjects the reduction in the PMT was small, which explains the small (although significant) effect observed.

For the ANOVA with Sham stimulation, the effects were rather the opposite. The effect of Sham stimulation considering the three muscle together was a very mild (surely non significant) increase of PMT in the Post condition (Fig 3A). However the Sham effects where different for the three muscles ($F_{2,24} = 9.5_{\varepsilon = 0.6}$ $p = 0.005_{TIMExMUS}$ $\eta_p^2 = 0.443$ $\eta^2 = 0.009$), and PMT increased significantly (4%) at *Post* in the *triceps* (*post-hoc* p = 0.002) (Fig 3B).

Effects of Brain Stimulation on RT and MT

Table 2 shows the mean values for the different levels of the different factors in a *pre-post* basis, in the RT and MT.

Table 4 shows significant main effects, indicating that the responses were faster in the case of the single than in choice tasks for RT ($\eta_p^2 = 0.659$; $\eta^2 = 0.069$). On the other hand, ipsilateral were faster than contralateral responses, this was shown by a significant main effect of factor "laterality" for RT ($\eta_p^2 = 0.291$; $\eta^2 = 0.010$) and also for MT ($\eta_p^2 = 0.873$; $\eta^2 = 0.495$).

Table 4 also shows that RT and MT were not modified by the different stimulation modes. For both variables factor TIME was never significant and it did not interact significantly with any other factor.

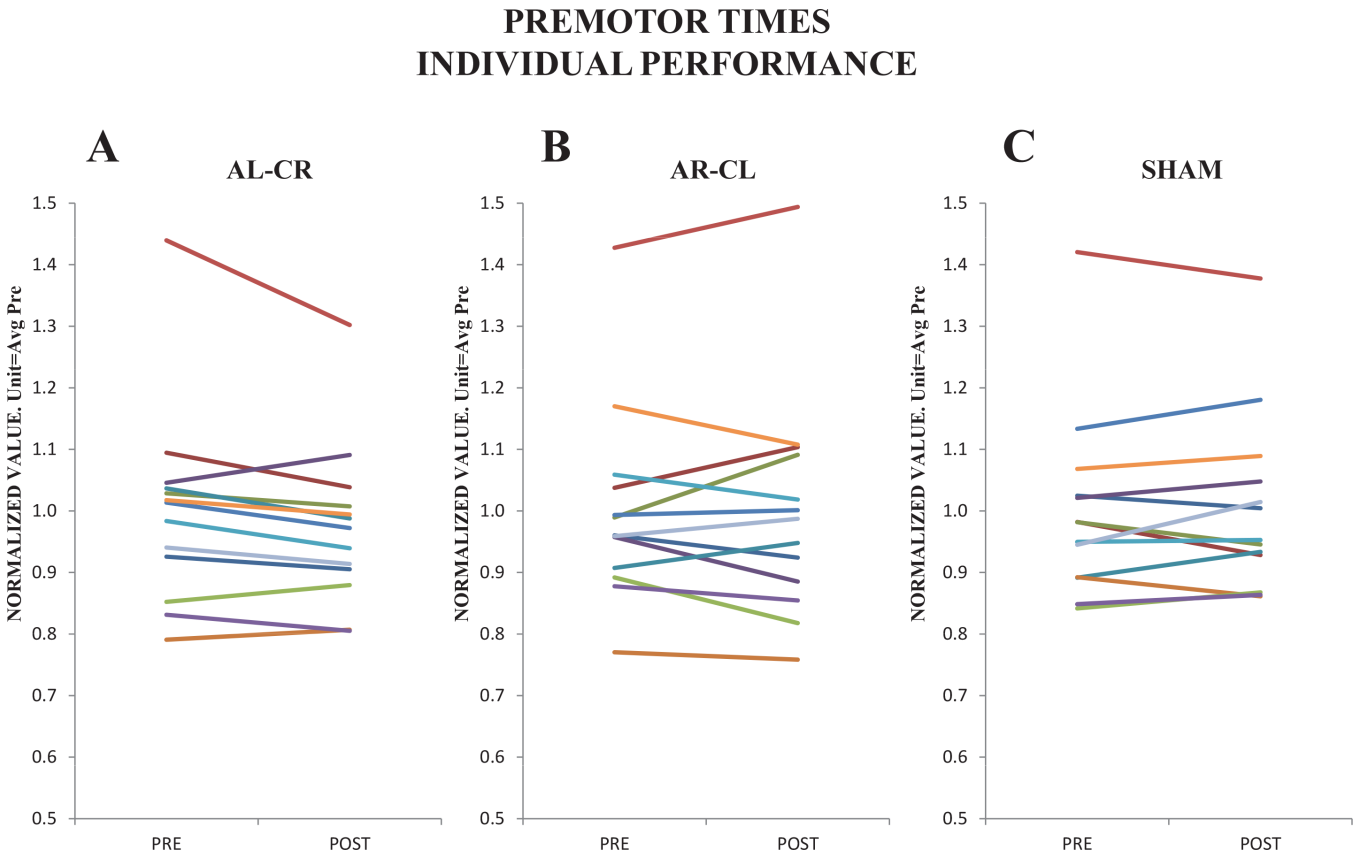

Fig 2. Individuals' responses for PMT. The *y*-axis unit indicates the mean response considering all subjects and conditions at *Pre*. It was equivalent to 201.8ms (*sem* 11.5) for AL-CR **(A)**; 204.5ms (*sem* 12.3) for AR-CL **(B)**; and 201.0ms (*sem* 10.5) for Sham sessions **(C)**.

doi:10.1371/journal.pone.0160063.g002

Effects of Brain Stimulation on CV's of PMT, RT and MT

Table 4 indicates a change in the CV's of PMT and RT at *post* compared to *pre*; but not in the CV of MT. Since there was a significant main effect of factor "time" for CV-PMT and for CV-RT (Fig 4A and 4B respectively), but there were not significant interactions with any other factor, this means that the significant reductions in the CV's after tDCS (from 18.3 to 17.0% in PMT; and from 13.9 to 12.6% in RT) were observed in all tasks (and muscles for PMT); and also in all stimulation protocols, including Sham ($\eta_p^2 = 0.677$, $\eta^2 = 0.01$ for PMT; and $\eta_p^2 = 0.373$, $\eta^2 = 0.014$ for RT).

Effects of Brain Stimulation on Anticipatory Responses

A total of 70 reaching movements were anticipatory responses, this is 5.6% of the 1,248 movements executed, in all subjects. The proportion of anticipations at Pre and Post was not significant different (Fisher Exact Probability Test $p>0.05$). Fragmentation of this analysis for the different tasks at Pre and Post was not considered due to the reduced number of anticipations.

Discussion

In the tasks performed in our experiments we have observed some well known features of reaction responses: i.) faster reactions to single than to choice options [50, 51]; ii.) faster reactions with ipsilateral than contralateral movements [52] and iii.) sequential muscle activation during

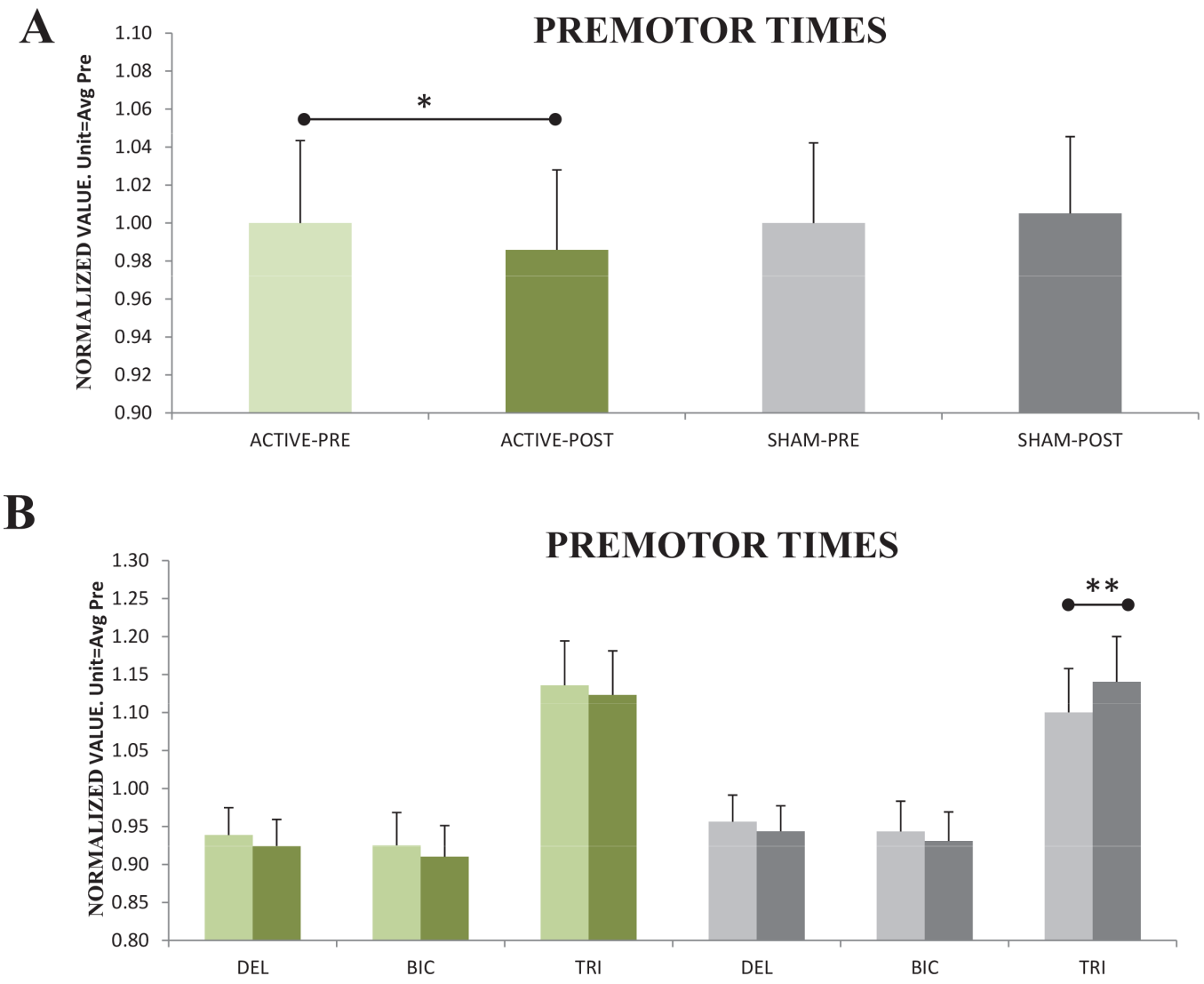

Fig 3. (A) PMT at *Post* were differently modulated by *Sham*-tDCS compared to the other two active protocols, which did not differ each other (shown pooled in green tones). There was a significant decrease at *Post* after both active protocols. **(B)** Sham stimulation increased *Post* PMT, specifically in the *triceps* muscle. The *y*-axis unit indicates the mean response across all subjects and conditions at *Pre*. *$p<0.05$; **$p<0.01$.

doi:10.1371/journal.pone.0160063.g003

reaching tasks [53–55]. However, the main finding of this work is that real tDCS of M1 reduces the PMT of reaching movements. Remarkably, the effect of tDCS was not different for the two active electrode montages. The significant increase in triceps PMT *Post-Sham* might be explained by fatigability, since the muscle has a main role in the fast projection of the hand towards the target. Fatigability (i.e., reduction of performance at *Post*) was not only avoided by real stimulation, but the net effect was a step further and reduced PMT (in all muscles). Admittedly the reduction of PMT after active tDCS was small but statistically significant.

Sham Effects on PMT and RT

The increment in PMT after *Sham* stimulation was localized at the level of the *triceps* muscle, without affecting the *deltoids* and *biceps*. These results might provide some insights on the operational mechanisms at *post-Sham*. The role of the *triceps* is of capital importance in the

Table 4. ANOVA's for variables which response was not different for the three stimulation modes.

						SIGNIFICANT
AR-CL vs AL-CR vs Sham	**MAIN EFFECTS**					**INTERACTIONS**
						FACTOR TIME
	STIM MODE	**TIME**	**OPTION**	**LATERALITY**	**MUSCLE**	
RT	$F_{2,24} = 0.2$ p = 0.9	$F_{1,12} = 0.1$ p = 0.8	$F_{1,12} = 23.2$ **p<0.001**	$F_{1,12} = 4.9$ **p = 0.046**	N.A	N.S
			$\eta_p^2 = 0.659$	$\eta_p^2 = 0.291$		
MT	$F_{2,24} = 0.1$ p = 0.9	$F_{1,12} = 0.3$ p = 0.6	$F_{1,12} = 1.3$ p = 0.3	$F_{1,12} = 82.2$ **p<0.001**	N.A	N.S
				$\eta_p^2 = 0.873$		
CV-PMT	$F_{2,24} = 0.7$ p = 0.5	$F_{1,12} = 25.1$ **p<0.001**	$F_{1,12} = 1.3$ p = 0.3	$F_{1,12} = 1.5$ p = 0.2	$F_{2,24} = 0.1$ p = 0.4	N.S
		$\eta_p^2 = 0.677$				
CV-RT	$F_{2,24} = 0.6$ p = 0.6	$F_{1,12} = 7.1$ **p = 0.020**	$F_{1,12} = 0.3$ p = 0.6	$F_{1,12} = 1.9$ p = 0.2	N.A	N.S
		$\eta_p^2 = 0.373$				
CV-MT	$F_{2,24} = 1.4$ p = 0.3	$F_{1,12} = 0.7$ p = 0.4	$F_{1,12} = 0.1$ p = 0.7	$F_{1,12} = 3.1$ p = 0.1	N.A	N.S

N.A = not applicable since RT, MT and their CV's were not obtained from EMG but from contact plates. N.S = none was significant. Partial etha squared (η_p^2) is reported for significant main effects.

doi:10.1371/journal.pone.0160063.t004

projection of the hand to the target, as an elbow extensor, while, on the other hand, the *biceps* is an elbow and shoulder flexor, this latter function shared with *anterior head of deltoids*. Thus, *deltoids* and *biceps* main involvement in the tasks is to lift the hand from the plates; later on the *triceps* is activated to project the hand towards the target [54]. Such a sequential activation is reflected in all our EMG recordings. It is tempting to speculate that, in our protocol, fatigue only affects the more demanding muscular activity and, consequently, only the triceps was affected post *Sham* (active stimulation affected all muscles, see discussion below). These results matched the fact that RT (recorded at plate lift) were unaffected by the *Sham* stimulation. Therefore task progression might induce fatigue which is reflected in an alteration in muscle recruitment patterns.

Remarkably fatigue might arise from different sources. Muscle fatigue is defined as a progressive failure of muscle-output generating capacity during and after the tasks. Its origin appears to lie in a deficit of the neural motor system to generate or propagate the action

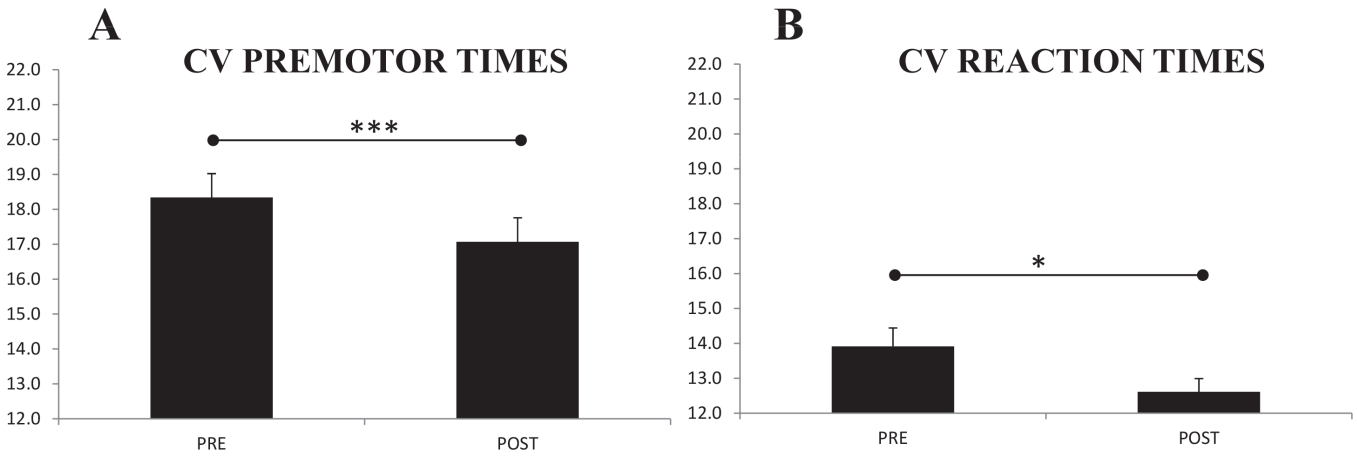

Fig 4. (A) The CV of PMT was reduced significantly at *Post*, regardless stimulation modes, tasks or muscles (so that shown pooled). **(B)** A similar pattern was found for the CV of RT. *$p<0.05$; ***$p<0.001$.

doi:10.1371/journal.pone.0160063.g004

potentials to the muscle in an efficient manner [56]—this is a form of central fatigue (CF). However, CF might also emerge from other (non motor) factors (i.e., mental fatigue) [57, 58] where the increase in PMT during a prolonged period of attention might be a marker of cognitive functionality waning [57, 58]. Bilateral tDCS of prefrontal areas (regardless polarity) has been shown to improve cognitive processes like attention [59], and it seems that tDCS improved attention might be a key element to reduce high order expressions of fatigability (such as mental fatigue) [57].

Despite that with our protocol we cannot discard that the reduction in PMT after bilateral tDCS could be related to modulations of cognitive processing, we believe that mental fatigue expression on PMT should have been shown in all the three muscles recorded, and not only in the *triceps*. Note also that the anticipatory responses (likely to be related with mental fatigue) were not modified by the protocols, and that very delayed PMT reflecting loss of attention (longer than 800ms) were present only once across subjects, which weakens the possibility that the effects of our tDCS protocol are related to cognitive processing.

Active tDCS Reduces PMT Regardless Polarity

Contrary to our initial hypothesis, the effects observed with real stimulation were polarity independent. Our hypothesis followed the "*contralaterality of motor control*"[60]; this considers the presence of inter-hemispheric interactions between both motor areas, with a stronger inhibition on the non-dominant M1 emerging from the dominant motor cortex [60–64]. Considering such asymmetry, it should never be expected that both active tDCS montages produce a same effect. However, it should also be considered that there are a number of ipsilateral projections from the motor areas to the spinal cord [65]. These ipsilateral projections likely target a great proportion of motoneuron pools controlling proximal limb muscles (like those evaluated in our study *deltoids*, *biceps* or *triceps*) [66], which might contribute to our polarity independent results. In fact, there is a growing amount of data showing that when we move a hand or an arm the activity in both the contralateral and the ipsilateral hemispheres are simultaneously activated. The neurophysiological significance of the bilateral activation of the motor cortices remains unclear. Kobayashi et al., [64] suggested that "ipsilateral activation during non-dominant hand movements could reflect an increased inhibition exerted by the right over the left hemisphere through callosal fibers". Others support the idea that all movements are initiated in the dominant hemisphere with the non-dominant would be responsible just for the execution of the command issued by the dominant hemisphere [60]. Interestingly, we started with an hypothesis which would sustain in principle the theory of contralaterality of motor control (i.e. "we predict a shortening of the MT and RT after bilateral tDCS, when anodal stimulation is applied to the M1 contralateral to the executing arm, as a result of the increased excitability of M1 circuits"), but we ended up with something completely different which seem to follow the new stream of thought.

Effects of tDCS on RT and MT

Our results indicate that active tDCS reduces PMT but not RT. At a first glance these results seems incompatible. Reynolds and Ashby [67] have shown that PMT are periods characterized by a progressive reduction of intracortical inhibition. This is to say that the level of intracortical inhibition is minimal from the last ≈80ms preceding EMG-onset to end of the PMT [67], as well as during muscle contraction [68]. As mentioned before, this inhibitory process is likely to be modulated by tDCS [69]. However, RT (once finished PMT) corresponds with periods where inhibition is not altered much and, perhaps not affected by tDCS.

Likewise, MT were unaffected by tDCS. Since the reaction-time protocols reflect both, cognitive and motor components, the lack of changes in MT seems to favor a specific effect of tDCS on the cognitive component of the response. However, some evidences might not support this possibility. Firstly, PMT was differently affected in the three muscles after Sham. This suggests an alteration in muscle recruitment rather than in cognitive processing. Second, some previous work has indicated that MT variations depend on the speed of reactions; faster reactions produce increments in MT and vice versa [33, 52]; thus, perhaps the option that tDCS prevents the increment of MT should be taken into account.

Effects of tDCS on Variability of Responses

Some previous work indicates that tDCS on parietal cortex reduces the variability of time estimation if applied to the left hemisphere, or impacts the accuracy of time estimation after right hemisphere stimulation [70]. However, in our case, the reduction of the variability (in PMT and RT, but not of MT) occurred after all stimulation protocols, either active or *Sham*. The reduction of variability during repetitive reaching movements is not a new finding and has been related to progressive appearance of fatigue along the task. In this situation the kinematics of reaching movements becomes more stereotyped [71] and movements less variable. However, we believe that a reduction of CV's of PMT and RT simply due to motor practice is also possible. The protocol was not conceived to test this hypothesis but this is a possibility to be considered since the reduction in CV was observed in all muscles (regardless they expressed fatigue -i.e., *triceps*- post Sham or not -i.e., *deltoids* and *biceps*-) and in all stimulation conditions. Though this possibility does not explain why the CV of MT was not reduced with practice, it is plausible that different expressions of a motor act might need different levels of practice to reduce their variability.

Limitations of the Study

Our study only explored the effects of tDCS on the dominant limb. It is possible that effect sizes would have been greater in the case of studying the non-dominant hand, because in healthy subjects motor execution with the dominant arm is likely to experience ceiling-effects. In fact, some reports indicate greater effects of tDCS for the non-dominant limb [72]. In addition our sample size is modest, therefore future studies should clarify the effect of tDCS on reaching movements executed with dominant/non-dominant arms, on greater samples.

Conclusions

In our hands, bilateral tDCS-M1 reduced PMT and avoided fatigability in a functional reaching task; the effects were polarity independent. Futures studies might include different reaction time protocols to disentangle the cognitive and motor effects produced by tDCS on these kinds of tasks.

Supporting Information

S1 Data. Data Set.
(RAR)

Acknowledgments

We thank Dr. Kenneth Grieve for his useful comments on this work.

Author Contributions

Conceived and designed the experiments: PA AO JC.

Performed the experiments: PA YC VR AM.

Analyzed the data: PA.

Contributed reagents/materials/analysis tools: AO JC.

Wrote the paper: PA YC VR AM AO JC.

References

1. Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W. Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clin Neurophysiol. 2003; 114(11):2220–2; author reply 2–3. PMID: 14580622
2. Poreisz C, Boros K, Antal A, Paulus W. Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Res Bull. 2007; 72(4–6):208–14. PMID: 17452283
3. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000; 527 Pt 3:633–9. PMID: 10990547
4. Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol. 2003; 553(Pt 1):293–301. PMID: 12949224
5. 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; 114(4):600–4. PMID: 12686268
6. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001; 57(10):1899–901. PMID: 11723286
7. Mordillo-Mateos L, Turpin-Fenoll L, Millan-Pascual J, Nunez-Perez N, Panyavin I, Gomez-Arguelles JM, et al. Effects of simultaneous bilateral tDCS of the human motor cortex. Brain Stimul. 2012; 5 (3):214–22. doi: 10.1016/j.brs.2011.05.001 PMID: 21782545
8. Kidgell DJ, Goodwill AM, Frazer AK, Daly RM. Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex. BMC Neurosci. 2013; 14:64. doi: 10.1186/1471-2202-14-64 PMID: 23815634
9. Schlaug G, Renga V, Nair D. Transcranial direct current stimulation in stroke recovery. Arch Neurol. 2008; 65(12):1571–6. doi: 10.1001/archneur.65.12.1571 PMID: 19064743
10. Vines BW, Cerruti C, Schlaug G. Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. BMC Neurosci. 2008; 9:103. doi: 10.1186/1471-2202-9-103 PMID: 18957075
11. Lindenberg R, Renga V, Zhu LL, Nair D, Schlaug G. Bihemispheric brain stimulation facilitates motor recovery in chronic stroke patients. Neurology. 2010; 75(24):2176–84. doi: 10.1212/WNL.0b013e318202013a PMID: 21068427
12. Waters-Metenier S, Husain M, Wiestler T, Diedrichsen J. Bihemispheric transcranial direct current stimulation enhances effector-independent representations of motor synergy and sequence learning. J Neurosci. 2014; 34(3):1037–50. doi: 10.1523/JNEUROSCI.2282-13.2014 PMID: 24431461
13. Stagg CJ, O'Shea J, Kincses ZT, Woolrich M, Matthews PM, Johansen-Berg H. Modulation of movement-associated cortical activation by transcranial direct current stimulation. Eur J Neurosci. 2009; 30 (7):1412–23. doi: 10.1111/j.1460-9568.2009.06937.x PMID: 19788568
14. Roche N, Lackmy A, Achache V, Bussel B, Katz R. Impact of transcranial direct current stimulation on spinal network excitability in humans. J Physiol. 2009; 587(Pt 23):5653–64. doi: 10.1113/jphysiol.2009.177550 PMID: 19805746
15. Roche N, Lackmy A, Achache V, Bussel B, Katz R. Effects of anodal transcranial direct current stimulation over the leg motor area on lumbar spinal network excitability in healthy subjects. J Physiol. 2011; 589(Pt 11):2813–26. doi: 10.1113/jphysiol.2011.205161 PMID: 21502292
16. Mellah S, Rispal-Padel L, Riviere G. Changes in excitability of motor units during preparation for movement. Exp Brain Res. 1990; 82(1):178–86. PMID: 2257902
17. Riedo R, Ruegg DG. Origin of the specific H reflex facilitation preceding a voluntary movement in man. J Physiol. 1988; 397:371–88. PMID: 3411511

18. Frank JS. Spinal motor preparation in humans. Electroencephalogr Clin Neurophysiol. 1986; 63 (4):361–70. PMID: 2419094

19. Hummel F, Celnik P, Giraux P, Floel A, Wu WH, Gerloff C, et al. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. Brain. 2005; 128(Pt 3):490–9. PMID: 15634731

20. Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol. 2006; 5(8):708–12. PMID: 16857577

21. Saiote C, Goldschmidt T, Timaus C, Steenwijk MD, Opitz A, Antal A, et al. Impact of transcranial direct current stimulation on fatigue in multiple sclerosis. Restor Neurol Neurosci. 2014; 32(3):423–36. doi: 10.3233/RNN-130372 PMID: 24531295

22. Galletti C, Kutz DF, Gamberini M, Breveglieri R, Fattori P. Role of the medial parieto-occipital cortex in the control of reaching and grasping movements. Exp Brain Res. 2003; 153(2):158–70. PMID: 14517595

23. Kalaska JF, Scott SH, Cisek P, Sergio LE. Cortical control of reaching movements. Curr Opin Neurobiol. 1997; 7(6):849–59. PMID: 9464979

24. Kalaska JF, Caminiti R, Georgopoulos AP. Cortical mechanisms related to the direction of two-dimensional arm movements: relations in parietal area 5 and comparison with motor cortex. Exp Brain Res. 1983; 51(2):247–60. PMID: 6617794

25. Georgopoulos AP, Schwartz AB, Kettner RE. Neuronal population coding of movement direction. Science. 1986; 233(4771):1416–9. PMID: 3749885

26. Battaglia-Mayer A, Caminiti R, Lacquaniti F, Zago M. Multiple levels of representation of reaching in the parieto-frontal network. Cereb Cortex. 2003; 13(10):1009–22. PMID: 12967918

27. Goto K, Hoshi Y, Sata M, Kawahara M, Takahashi M, Murohashi H. Role of the prefrontal cortex in the cognitive control of reaching movements: near-infrared spectroscopy study. J Biomed Opt. 2011; 16 (12):127003. doi: 10.1117/1.3658757 PMID: 22191933

28. Katsuki F, Saito M, Constantinidis C. Influence of monkey dorsolateral prefrontal and posterior parietal activity on behavioral choice during attention tasks. Eur J Neurosci. 2014.

29. Hanakawa T. Rostral premotor cortex as a gateway between motor and cognitive networks. Neurosci Res. 2011; 70(2):144–54. doi: 10.1016/j.neures.2011.02.010 PMID: 21382425

30. Cisek P, Kalaska JF. Neural mechanisms for interacting with a world full of action choices. Annu Rev Neurosci. 2010; 33:269–98. doi: 10.1146/annurev.neuro.051508.135409 PMID: 20345247

31. Mattia M, Pani P, Mirabella G, Costa S, Del Giudice P, Ferraina S. Heterogeneous attractor cell assemblies for motor planning in premotor cortex. J Neurosci. 2013; 33(27):11155–68. doi: 10.1523/JNEUROSCI.4664-12.2013 PMID: 23825419

32. Mattia M, Spadacenta S, Pavone L, Quarato P, Esposito V, Sparano A, et al. Stop-event-related potentials from intracranial electrodes reveal a key role of premotor and motor cortices in stopping ongoing movements. Front Neuroeng. 2012; 5:12. doi: 10.3389/fneng.2012.00012 PMID: 22754525

33. Mirabella G, Iaconelli S, Modugno N, Giannini G, Lena F, Cantore G. Stimulation of subthalamic nuclei restores a near normal planning strategy in Parkinson's patients. PLoS One. 2013; 8(5):e62793. doi: 10.1371/journal.pone.0062793 PMID: 23658775

34. Mirabella G, Iaconelli S, Romanelli P, Modugno N, Lena F, Manfredi M, et al. Deep brain stimulation of subthalamic nuclei affects arm response inhibition in Parkinson's patients. Cereb Cortex. 2012; 22 (5):1124–32. doi: 10.1093/cercor/bhr187 PMID: 21810782

35. Mirabella G. Should I stay or should I go? Conceptual underpinnings of goal-directed actions. Front Syst Neurosci. 2014; 8:206. doi: 10.3389/fnsys.2014.00206 PMID: 25404898

36. Georgopoulos AP. Current issues in directional motor control. Trends Neurosci. 1995; 18(11):506–10. PMID: 8592761

37. Glover S, Wall MB, Smith AT. Distinct cortical networks support the planning and online control of reaching-to-grasp in humans. Eur J Neurosci. 2012; 35(6):909–15. doi: 10.1111/j.1460-9568.2012.08018.x PMID: 22429244

38. Fu QG, Suarez JI, Ebner TJ. Neuronal specification of direction and distance during reaching movements in the superior precentral premotor area and primary motor cortex of monkeys. J Neurophysiol. 1993; 70(5):2097–116. PMID: 8294972

39. Tanji J, Evarts EV. Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. J Neurophysiol. 1976; 39(5):1062–8. PMID: 824409

40. Zhang J, Riehle A, Requin J, Kornblum S. Dynamics of single neuron activity in monkey primary motor cortex related to sensorimotor transformation. J Neurosci. 1997; 17(6):2227–46. PMID: 9045746

41. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 1971; 9(1):97–113. PMID: 5146491

42. Dean C, Shepherd R, Adams R. Sitting balance I: trunk-arm coordination and the contribution of the lower limbs during self-paced reaching in sitting. Gait Posture. 1999; 10(2):135–46. PMID: 10502647

43. Eichenberger A, Ruegg DG. Relation between the specific H reflex facilitation preceding a voluntary movement and movement parameters in man. J Physiol. 1984; 347:545–59. PMID: 6707967

44. Hodges PW, Bui BH. A comparison of computer-based methods for the determination of onset of muscle contraction using electromyography. Electroencephalogr Clin Neurophysiol. 1996; 101(6):511–9. PMID: 9020824

45. Andrews RK, Schabrun SM, Ridding MC, Galea MP, Hodges PW, Chipchase LS. The effect of electrical stimulation on corticospinal excitability is dependent on application duration: a same subject pre-post test design. J Neuroeng Rehabil. 2013; 10:51. doi: 10.1186/1743-0003-10-51 PMID: 23758902

46. Strigaro G, Ruge D, Chen JC, Marshall L, Desikan M, Cantello R, et al. Interaction between visual and motor cortex: a transcranial magnetic stimulation study. J Physiol. 2015; 593(10):2365–77. doi: 10.1113/JP270135 PMID: 25762215

47. Cantello R, Strigaro G, Prandi P, Varrasi C, Mula M, Monaco F. Paired-pulse flash-visual evoked potentials: new methods revive an old test. Clin Neurophysiol. 2011; 122(8):1622–8. doi: 10.1016/j.clinph.2011.01.008 PMID: 21306943

48. Schieppati M, Trompetto C, Abbruzzese G. Selective facilitation of responses to cortical stimulation of proximal and distal arm muscles by precision tasks in man. J Physiol. 1996; 491 (Pt 2):551–62. PMID: 8866878

49. Brass M, Bekkering H, Prinz W. Movement observation affects movement execution in a simple response task. Acta Psychol (Amst). 2001; 106(1–2):3–22.

50. Gottsdanker R, Shragg GP. Verification of Donders' subtraction method. J Exp Psychol Hum Percept Perform. 1985; 11(6):765–76. PMID: 2934507

51. Donders FC. On the speed of mental processes. Acta Psychol (Amst). 1969; 30:412–31.

52. Mirabella G, Pani P, Ferraina S. Context influences on the preparation and execution of reaching movements. Cogn Neuropsychol. 2008; 25(7–8):996–1010. PMID: 19378414

53. Flanders M. Temporal patterns of muscle activation for arm movements in three-dimensional space. J Neurosci. 1991; 11(9):2680–93. PMID: 1880544

54. Flanders M, Pellegrini JJ, Geisler SD. Basic features of phasic activation for reaching in vertical planes. Exp Brain Res. 1996; 110(1):67–79. PMID: 8817258

55. d'Avella A, Lacquaniti F. Control of reaching movements by muscle synergy combinations. Front Comput Neurosci. 2013; 7:42. doi: 10.3389/fncom.2013.00042 PMID: 23626534

56. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001; 81(4):1725–89. PMID: 11581501

57. Tanaka M, Ishii A, Watanabe Y. Neural effects of mental fatigue caused by continuous attention load: a magnetoencephalography study. Brain Res. 2014; 1561:60–6. doi: 10.1016/j.brainres.2014.03.009 PMID: 24642273

58. Faber LG, Maurits NM, Lorist MM. Mental fatigue affects visual selective attention. PLoS One. 2012; 7(10):e48073. doi: 10.1371/journal.pone.0048073 PMID: 23118927

59. Nelson JT, McKinley RA, Golob EJ, Warm JS, Parasuraman R. Enhancing vigilance in operators with prefrontal cortex transcranial direct current stimulation (tDCS). Neuroimage. 2014; 85 Pt 3:909–17. doi: 10.1016/j.neuroimage.2012.11.061 PMID: 23235272

60. Derakhshan I. Callosum and movement control: case reports. Neurol Res. 2003; 25(5):538–42. PMID: 12866205

61. Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. J Physiol. 1992; 453:525–46. PMID: 1464843

62. Gorsler A, Baumer T, Weiller C, Munchau A, Liepert J. Interhemispheric effects of high and low frequency rTMS in healthy humans. Clin Neurophysiol. 2003; 114(10):1800–7. PMID: 14499741

63. Netz J, Ziemann U, Homberg V. Hemispheric asymmetry of transcallosal inhibition in man. Exp Brain Res. 1995; 104(3):527–33. PMID: 7589304

64. Kobayashi M, Hutchinson S, Schlaug G, Pascual-Leone A. Ipsilateral motor cortex activation on functional magnetic resonance imaging during unilateral hand movements is related to interhemispheric interactions. Neuroimage. 2003; 20(4):2259–70. PMID: 14683727

65. Ziemann U, Ishii K, Borgheresi A, Yaseen Z, Battaglia F, Hallett M, et al. Dissociation of the pathways mediating ipsilateral and contralateral motor-evoked potentials in human hand and arm muscles. J Physiol. 1999;518 (Pt 3):895–906.

66. Colebatch JG, Gandevia SC. The distribution of muscular weakness in upper motor neuron lesions affecting the arm. Brain. 1989; 112 (Pt 3):749–63. PMID: 2731028

67. Reynolds C, Ashby P. Inhibition in the human motor cortex is reduced just before a voluntary contraction. Neurology. 1999; 53(4):730–5. PMID: 10489033

68. 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; 586(2):325–51. PMID: 17974592

69. Cengiz B, Murase N, Rothwell JC. Opposite effects of weak transcranial direct current stimulation on different phases of short interval intracortical inhibition (SICI). Exp Brain Res. 2013; 225(3):321–31. doi: 10.1007/s00221-012-3369-0 PMID: 23292100

70. Vicario CM, Martino D, Koch G. Temporal accuracy and variability in the left and right posterior parietal cortex. Neuroscience. 2013; 245:121–8. doi: 10.1016/j.neuroscience.2013.04.041 PMID: 23628777

71. Lomond KV, Cote JN. Movement timing and reach to reach variability during a repetitive reaching task in persons with chronic neck/shoulder pain and healthy subjects. Exp Brain Res. 2010; 206(3):271–82. doi: 10.1007/s00221-010-2405-1 PMID: 20848276

72. Marquez J, Conley A, Karayanidis F, Lagopoulos J, Parsons M. Anodal direct current stimulation in the healthy aged: Effects determined by the hemisphere stimulated. Restor Neurol Neurosci. 2015; 33 (4):509–19. doi: 10.3233/RNN-140490 PMID: 26409409

Neuroscience 305 (2015) 316–327

CENTRAL FATIGUE INDUCED BY SHORT-LASTING FINGER TAPPING AND ISOMETRIC TASKS: A STUDY OF SILENT PERIODS EVOKED AT SPINAL AND SUPRASPINAL LEVELS

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Abstract—The neural substrates of fatigue induced by muscular activity have been addressed in depth in relation to isometric tasks. For these activities, when fatigue develops, it has been noted that the duration of the silent periods (SPs) increases in response to both transcranial magnetic stimulation (TMS) of primary motor cortex or electric cervicomedullary stimulation (CMS). However, fatigue is known to be task-dependent and the mechanisms giving rise to a decrease in motor performance during brief, fast repetitive tasks have been less studied. We hypothesized that fatigue induced by repetitive fast finger tapping may have physiological mechanisms different from those accounting for fatigue during an isometric contraction, even in cases of matched effort durations. In these tasks, we examined the contribution of spinal and supraspinal motor circuits to the production of fatigue. The tapping rate and maximal voluntary contractions (MVC), and TMS- and CMS-evoked SPs were obtained at the time of fatigue, and while subjects maintained maximal muscle activation after fast finger-tapping (or isometric activity) of different durations (10 or 30 s). Results showed different mechanisms of fatigue triggered by isometric contraction and repetitive movements, even of short duration. Short-lasting repetitive movements induce fatigue within intracortical inhibitory circuits. They increased TMS-SPs, but not CMS-SPs. On the other hand, isometric contraction had a clear impact on spinal circuits. The consideration of these differences might help to optimize the study of fatigue in physiological conditions and neurological disorders. © **2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: central fatigue, repetitive movements, human.

INTRODUCTION

The finger tapping (*ft*) test is a reliable procedure used world-wide to evaluate physiological and pathological mechanisms of repetitive movements (Shimoyama et al., 1990; Arias et al., 2012). However, when *ft* is performed at the fastest rate its frequency drops in very few seconds, suggesting the development of muscle fatigue during the task (Arias et al., 2012; Teo et al., 2012). Remarkably, muscle fatigue has been chiefly studied when induced by isometric tasks, but the underlying neurophysiological mechanisms of fatigue during fast repetitive movements have been hardly explored.

Muscle fatigue is characterized by a reduction in maximal voluntary contraction (MVC) muscle force (Bigland-Ritchie and Woods, 1984). The progressive failure of muscle-force generating capacity, and failure of impulse conduction through the neuromuscular junction, are known peripheral causes of fatigue (Allen et al., 2008). However, fatigue also involves circuits of the spinal cord, cerebral cortex and subcortical structures (i.e. central fatigue, CF) (Gandevia, 2001), which is of interest in neurological conditions (Zwarts et al., 2008; Kluger et al., 2013).

In order to evaluate the contribution of supra-spinal and spinal motor circuits to CF, transcranial magnetic stimulation (TMS) permits the study of the motor-evoked potentials resulting from activation of M1 and the corticospinal tract. Likewise, electrical cervicomedullary stimulation (CMS) induces motor responses by depolarization of the axons in the corticospinal tract at the cervicomedullary junction (Ugawa et al., 1991).

When a single TMS pulse is applied to the motor cortex during an active contralateral muscle contraction, the electromyographic activity is arrested for a few hundred milliseconds after the motor-evoked potential. This period of electromyographic suppression is referred to as a silent period (SP) and can be induced by either

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Abbreviations: 10*max*-30*max*, 10 or 30 s of maximal mode execution; CF, central fatigue; CMAP, compound muscle action potential; CMS, cervicomedullary stimulation; *comfort*, 30 s of comfort mode execution; FDI, first dorsal interossesous muscle; *ft*, finger tapping task; *iso*, isometric task; MVC, (isometric) maximal voluntary contraction; ROM, range of motion; SP, silent period; TMS, transcranial magnetic stimulation.

http://dx.doi.org/10.1016/j.neuroscience.2015.07.081

TMS or CMS. Therefore, the SP is usually studied while the target muscle maintains a certain level of isometric (*iso*) contraction. TMS-SP duration increases with stimulus intensity and can reach durations of 200–300 ms in hand muscles (Cantello et al., 1992; Inghilleri et al., 1993). However, TMS-SP has two components: the early part corresponds to the first ≈75 ms and is due to spinal inhibition (Fuhr et al., 1991; Cantello et al., 1992; Inghilleri et al., 1993; Brasil-Neto et al., 1995; Butler et al., 2012) and involves mechanisms following on from motoneuron excitation, like recurrent (Renshaw) inhibition and axonal after-hyperpolarization. These processes are known to be dependent on the level of preceding motoneuron activity (Hultborn et al., 1979; Ziemann et al., 1993).

The second, late part is supraspinal; it is cortical in origin (Fuhr et al., 1991; Cantello et al., 1992; Inghilleri et al., 1993; Ziemann et al., 1993; Brasil-Neto et al., 1995; Butler et al., 2012; Ziemann, 2013) and is linked to intracortical inhibitory circuits operating via GABAb receptors (Ziemann et al., 1993; Ziemann, 2013). Thus, it is considered a marker of motor cortical inhibition.

On the other hand, the CMS-SP is induced by spinal mechanisms exclusively (Fuhr et al., 1991; Inghilleri et al., 1993; Brasil-Neto et al., 1995), sharing the mechanisms of the early part of the TMS-SP.

The development of CF is task-dependent and each task may involve different circuits and mechanisms within the central nervous system (Enoka and Stuart, 1992; Enoka and Duchateau, 2008). For instance, fatiguing isometric activity (i.e., during a 2 min *iso*-MVC) increases SP duration in response to TMS and CMS (Taylor et al., 1996). This suggests that fatigue induced by *iso*-MVC is generated both at the spinal cord level (Butler et al., 2003), and, potentially, at a supraspinal level (Taylor et al., 1996; Di Lazzaro et al., 2003; Hilty et al., 2011a). Interestingly, repetitive isometric activity has been shown to increase TMS-SP (Taylor et al., 2000; Hilty et al., 2011b), and part of this increase seems to be caused by processing of afferent (opioid) signaling from the fatigued muscle to the brain (Hilty et al., 2011b). Conversely, CF induced by non-isometric tasks (1 Hz resisted concentric-eccentric contractions to reaching task-failure) has been attributed to supraspinal mechanisms (Brasil-Neto et al., 1993) after testing the motor-evoked potentials and H-reflex at rest; however a possible contribution of fast-recovery forms of spinal fatigue could not be totally ruled out. This was also the case with CF evaluation after short-lasting and maximal-rate *ft* (Arias et al., 2012; Teo et al., 2012). To avoid this drawback, it is recommended that evaluation of CF be made during maximal effort, without allowing recovery of the nervous system after the fatiguing activity (Taylor et al., 2000).

In this study, we have evaluated the SPs evoked by TMS and CMS to explore the origin of CF underlying the reduction in motor-output that is expected during a *ft* task, performed at the fastest possible rate for 10 and 30 s. We compared neurophysiological signs of fatigue induced by *ft* with those induced by *iso*-MVC, both of the highest intensity and matched in durations of the effort. In all cases, we evaluated fatigue with no gap in muscle activation to avoid any possibility of recovery of the motor system. Based on the ''task-dependency'' of fatigue signs (Enoka and Stuart, 1992; Enoka and Duchateau, 2008; Enoka et al., 2011), our hypothesis is twofold: Firstly, maximal *ft* compared to the same duration of maximal *iso* effort would produce a different reduction of motor output; secondly, changes in excitability of the cortical and spinal circuits under investigation would be different for short-lasting maximal *ft* and *iso* tasks.

EXPERIMENTAL PROCEDURES

Experimental protocols complied with the Helsinki declaration and were approved by the University of A Coruña Ethics Committee. Subjects were screened for incompatibility with brain stimulation techniques and were medication-free during the week preceding testing. All subjects consented to participate.

Subjects

The experiment included two groups of subjects: the TMS-group composed of nine right-handed healthy subjects (eight males and one female; age range 22–38 years), and the CMS-group composed of 12 right-handed healthy subjects (all males; age range 18–41 years), each group underwent both *ft* and *iso* fatigue protocols.

Protocol

Each subject underwent two experimental sessions, at least one week apart, in randomized order. Sessions were identical but for the type of *task* executed. In one session, subjects were requested to perform index *ft*, and, in the other session, continuous index finger *iso* against a force sensor, with the direction of the force ''toward'' *flexion* of the first metacarpophalangeal joint of the index finger. Subjects always wore a small goniometer on the index finger metacarpophalangeal joint, and a metal ring at the distal phalanx of the same finger. Subjects pressed or tapped over a thin metal plate placed on the force sensor.

For both *ft* and *iso* sessions, subjects executed the tasks in three different modes, and each mode was executed four times (four *sets*). Therefore, the subjects performed four sets at comfort rate/effort (*comfort* mode) for 30 s; then four sets of 10 s at maximal rate/effort (*10max* mode); and finally four sets of 30 s at maximal rate/effort (*30max* mode), always in this order. In all cases there was an inter-set rest period of 1 min 40 s.

For the *comfort-ft* subjects were asked to ''*tap at their most comfortable rate without feeling fatigued*'' for as long as the set lasted. In a previous paper (Arias et al., 2012), we observed that this *ft* mode is reliable, and performed at a pace of about 1/3 of the maximal rate. Because *comfort ft* is linked to lower metabolic activity in the sensorimotor cortex compared to faster (> 3 Hz) and slower (< 1 Hz) rates (Jancke et al., 1998; Lutz et al., 2005), its use seems to be adequate as a control condition to evaluate the fatigue induced by maximum *ft*. For the *comfort-iso*

participants were asked to press ≈1/3 MVC with visual feed-back provided by means of online isometric force display. For maximal modes subjects were requested to tap/press as fast/hard as they could from the very beginning to the end of the set.

Fatigue was assessed as either the decrease in frequency or amplitude (for *ft*), or in force output (for *iso*). CF, either supraspinal or spinal, was evaluated by recording the changes in the SP duration in response to TMS or CMS (Taylor et al., 1996). The stimulation pulses were delivered during brief isometric MVCs (2s-iMVC). The 2s-iMVCs were performed before (*pre*) and immediately after (with no gap inbetween) task-execution (*post*), either for *ft* or *iso*, for all modes and sets (Fig. 1). The magnitude of the brief 2s-iMVCs was also an analyzed variable for fatigue monitoring (Bigland-Ritchie and Woods, 1984). An initial session was scheduled to allow some practice and answer all the subjects' questions about the experimental methods (Gandevia, 2001).

Setting, recording and stimulation protocols. The subjects were seated comfortably with the elbow flexed at 90–100°. The forearm, wrist, hand and all fingers except the index were firmly but comfortably fixed to a modified tablet-arm chair, allowing un-restrained degrees of freedom at the metacarpophalangeal joint of the index finger, permitting *ft*. During *ft*, a Biometrics DataLink system (Biometrics Ltd., Gwent, NP11 7HZ, UK) recorded the inter-tap intervals at 0.1 KHz with a thin metal plate and a metal ring, the latter adapted to the distal phalanx of the index finger. It also recorded (at 500 Hz) the isometric force exerted during iMVC of the index flexion with a Pinch-Dynamometer (P200), which was placed flat and secured over the table, with the thin metal plate used to record tapping attached flat on its top. A single axis finger goniometer (F35) (sampled at 1 kHz) controlled the flexo-extension movement amplitudes of the index finger metacarpophalangeal joint. Electromyographic activity from the superficial head of the first dorsal interosseous muscle (FDI) was monitored with surface electrodes in a belly-tendon arrangement, and acquired by means of D360 amplifiers (Digitimer, Welwyn Garden City, Herts), amplified (×250–1000) and band-width filtered between 3–3000 Hz.

EMG were sampled at 10 kHz and stored in a computer by means of a CED 1401 mkII Power A–D converter (Cambridge Electronic Design, Cambridge, UK). This device also controlled the on/off state of LED's (indicating the different phases of execution/rest within the sets) and timing of TMS/CMS pulses.

In separate sessions, in six subjects we recorded the level of EMG activation of the FDI and different intrinsic and extrinsic hand muscles during the *ft* and different *iso* tasks; i.e., index iMVC, and iMVC of the 3rd, 4th and 5th fingers working together. Also, we recorded the FDI activation during different index-iMVC tasks applying force against immovable resistances in different directions, toward flexion (the same as above), abduction, adduction and extension. This was done using the same setting, hand position and fixation as during fatigue testing; and adapting immovable resistances to permit iMVC in the different planes. For this purpose the subjects executed two 3s-trials/task, with task presentation order randomized. The inter-trial rest period was one minute.

The trial started with the recording of the compound muscle action potential (CMAP) of each muscle, which was acquired by supramaximal electrical stimulation at

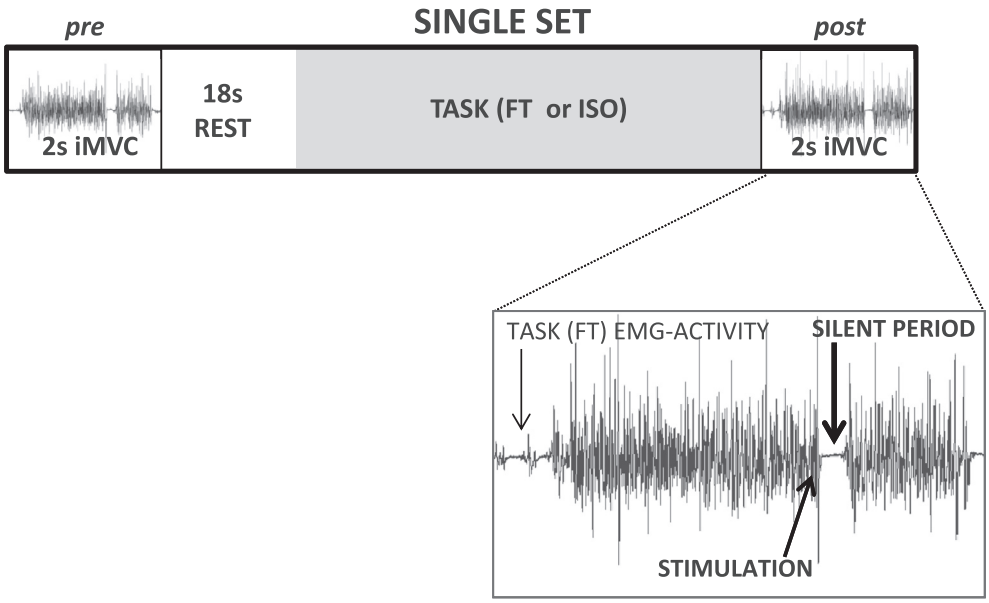

Fig. 1. The set-structure: as soon as the LED was lit subjects performed an isometric maximum voluntary contraction (iMVC) with their index against the dynamometer. The LED off (2 s after) served as a signal to stop the iMVC. During the iMVC (1.5 s after LED on) subjects received an initial test stimulation (*pre*) (TMS in one group (n = 9); electric CMS in the other (n = 12). Stimulation induced subsequent silent periods, as shown in the enlarged area. After resting for 18 s, a LED flash indicated the start of the task (*ft* in one session; *iso* in the other). In continuation of the task, and with no resting time, the subjects performed another 2s-iMVC in response to the LED turning-on, and received stimulation (*post*; in the same way as *pre*).

the Erb's point (1 ms electric pulses; EBNeuro Stimulator, Italy; cathode lateral, anode medial) with the muscle at rest; 10 s after stimulation the onset of a LED light indicated the start of 3s-task. The root mean square (*r.m.s.*) of the CMAP was computed, which served as the divisor of *r.m.s.* activity during task execution.

For *ft*, we computed the *r.m.s.* within the tapping cycles (lasting about 150 ms, one per trial) with the highest frequency (shortest inter-tap interval). For the *iso* tasks, we identified the peaks (one per trial) in the force recordings, then the muscle activity was computed in the 150 ms around the peaks (75 ms before/after the corresponding peak in each trial).

TMS. During the sessions of fatigue evaluation, and for TMS-SP generation, a Magstim 200[2] stimulator delivered monophasic wave-form pulses through a 70 mm figure of eight coil. The coil was positioned (and marked for reference) to induce currents in a posterior-anterior direction, and placed over the hot-spot for the FDI muscle of the executing hand.

The intensity was set to evoke a SP duration ≈150 ms during a 2s-iMVC in the non-fatigued muscle (this produced TMS motor evoked potentials of about 50% amplitude of the maximal CMAP); TMS intensity was expressed relative to the individual's active motor threshold, defined as the minimum intensity required to evoked five liminal responses (about 200 μV) in 10 consecutive pulses, in the activated muscle (5–10% MVC) (Rossini et al., 1994).

CMS. CMS was applied using a Digitimer D180 stimulator connected to a pair of Ag–AgCl electrodes. Electrodes were placed behind the mastoid processes with the anode at the right and the cathode at the left. Active motor threshold was defined as described above for TMS (Rossini et al., 1994); then the stimulation intensity for the protocol was set (about 10% above this threshold) to have a SP of ≈70 ms in the un-fatigued muscle (this produced CMS motor evoked potentials of about 50% amplitude of the CMAP). To make sure this intensity did not produce current spread to the spinal roots, the CMS motor-evoked potential latency was compared to that obtained at threshold intensity, such that the amplitude of the potentials increased in size with voluntary contraction with no or liminal latency shift when stimulated at the higher level (Taylor and Gandevia, 2004).

Baseline unfatigued SP's durations for TMS and CMS setting were ≈150 and 70 ms respectively, (CMS-SP is much shorter than TMS-SP; (Inghilleri et al., 1993). We set these values away from their ceiling to allow a potential modulation induced by fatigue. The mean TMS intensity applied was 17.9% (*s.e.m.* 1.0) above the AMT. The mean voltage used in the CMS experiments was 543.4V (*s.e.m.* 27.1).

Data reduction

The following dependent variables were analyzed:

Level of muscle activation in intrinsic and extrinsic hand muscles: This is expressed as the percentage of the *r.m.s.* of the CMAP, and defined as 100 ($TASK_{r.m.s}/CMAP_{r.m.s}$).

In the fatigue sessions we studied the following variables:

Motor output during tasks execution: We considered three measures of motor output for task execution: the tapping frequency, angular amplitude for *ft* and the force applied for *iso*. For each of the four Sets and three Modes (*comfort; 10max* and *30max)* we considered two time points which were embedded within task execution: the initial 3 s *(pre*) and the final 3 s (*post*). To make data from *ft* frequency and *iso* torque comparable, we expressed the motor output at all evaluation time points as a function of the maximum obtained at any time point for each task and subject.

For normalizing the *ft* range of motion (ROM) amplitude, we recorded the maximum (active) ROM of the index finger metacarpophalangeal joint for each subject before the protocol. The score served as divisor for the amplitude displayed at all the individual's evaluation time points during the *ft* task.

MVC (before and following task execution): We evaluated the MVC at the time of stimulation (MVC in the 50-ms period before the stimulation) either for *ft* and *iso* task, normalized to the maximum obtained at any evaluation time-point for each task and subject.

SP duration (recorded during the MVC explained just above): The SP duration was defined as the time-lag from the TMS/CMS pulse to the recovery of the EMG activity during the brief-iMVC, and determined visually by an experienced researcher blind to the conditions (in two separated sessions intra-rater reliability was checked on 124 random-chosen SPs, 62 each technique, TMS-CMS). Because we evaluated SP for both TMS and CMS, whose durations are not directly comparable (Inghilleri et al., 1993), the SP durations were normalized. For each subject and task (*ft* or *iso*), we took as 100% the average value from all the evaluation time-points of the four sets executed in the *comfort* mode, which served as divisor for all the subject's values for all execution modes. Therefore in the figures representing the SP duration, the unit represents its average duration at *comfort* mode.

Statistical design

Statistical design for studying FDI activation during the tasks. To study the level of the FDI activation in comparison with other muscles in each task, we first analyzed whether activation of each muscle was different in the two trials executed. After checking normality (one sample KS test) a paired Student *t*-test was used. Since activation in the two trials was never different ($p > 0.05$), we averaged the values to compare the level of activation of the different muscles and tasks using different models of one-factor ANOVA (with repeated-measures). One model evaluated FDI activation compared to *opponens pollicis*, *abductor digiti minimi*, *extensor digitorum*, and *flexor digitorum supperficialis*. This model was applied independently to the tasks: *ft, index iso-flexion, and 3–5th fingers iso-*

flexion. Another one-factor ANOVA model had four levels, and it was used to compare the activation of the FDI during *iso* in the four planes; "toward *flexion*" vs. "*extension*", "*abduction*" and "*adduction*".

Statistical design for studying intra-rater reliability in determining SP durations. To study intra-rater reliability during SP duration determination (session 1 vs. 2, on the same random-chosen SPs) the intra-class correlation coefficient, and its 95% confidence interval (95% CI) were evaluated. For TMS-SP we obtained an ICC = 0.97 (0.94–0.98; 95% CI); and for CMS-SP the ICC = 0.97 (0.96–0.98; 95% CI).

Statistical design for studying behavior during fatigue tasks. To study fatigue various repeated measures ANOVA's models were used, after checking the normality of distributions.

For the variables Motor Output (tapping rate-*ft*; or force-*iso*) decrement, SP duration, and MVC before and after task execution, we used an ANOVA with repeated measures. The ANOVA included one between-subject factor Group with two levels (the *TMS-group* and the *CMS-group*) and several within-subject factors. In the specific case of the SP, if the factor Group interacted significantly with any of the within-subject factors, it means that there was a significant different effect of within-subject factors on the response to spinal or corticospinal stimulation.

The within subject factors were Task (*ft, iso*), execution Mode (*comfort, 10max, 30max*), Set (the four sets for each execution mode), and evaluation Time points (*pre, post*). The levels of the latter factor were termed *i3* and *f3* when the variable analyzed was the motor output decrease in *ft* or *iso* tasks (as it included the initial and final 3 s embedded in task execution).

For the ANOVA of the ROM amplitude, only analyzed for *ft*, factor Task was excluded.

Results are expressed as the mean and the standard error of the mean (*s.e.m*). During ANOVA execution the degrees of freedom were corrected with Greenhouse Coefficients (ε), if sphericity could not be assumed. Significance was set at $p < 0.05$. A Bonferroni correction was used for follow-up post hoc comparisons involving multiple levels within the factor.

RESULTS

The Table 1 indicates the level of activation of the FDI obtained in *ft* and *iso* tasks in all subjects (Fig. 2 shows activations in a representative participant). FDI had a main role in the tasks used in the fatigue protocols (index *ft* or *iso*). Table 2 shows the normalizing values equivalent to the unit in graphs during the fatigue testing sessions.

Frequency/force decrement during execution in *ft/iso* tasks

The motor output decrements during the task execution (comparing motor output in the initial and final 3 s of the tasks) were different for the tasks and execution modes ($F_{2,38} = 8.6$, $\boldsymbol{p < 0.01}_{\text{TASK} \times \text{MODE} \times \text{TIME.}}$) Therefore, we split the analyses for each kind of task.

For *ft*, the frequency reduced over execution ($F_{1,19} = 86.4$, $\boldsymbol{p < 0.001}_{\text{TIME}}$), but differently on the three modes ($F_{2,38} = 43.7$, $\boldsymbol{p < 0.001}_{\text{MODE} \times \text{TIME}}$); this effect was not differently observed in the two groups (subjects stimulated with TMS or CMS; $F_{2,38} = 0.4$, $\boldsymbol{p > 0.05}_{\text{MODE} \times \text{TIME} \times \text{GROUP}}$ - note that the execution protocol was the same for the two groups of subjects). The frequency of *ft* at *comfort* was unchanged, conversely to *10max* and *30max* (both reduced in the last 3 s of task execution (i.e., *post*) compared to the initial 3 s of task execution (*pre*), post hoc $\boldsymbol{p < 0.001}$. Set progression had no effect ($F_{3,57} = 0.3$, $\boldsymbol{p > 0.05}_{\text{SET}}$), as clearly observed in Fig. 3a, and for this reason the effects are shown pooled at Fig. 3b.

Fig. 3c illustrates the force developed during *iso* in the initial and final 3s of the task in the different modes and sets. It was evident that the force dropped at the end of

Table 1. First dorsal interosseous activation in the tasks of the fatigue protocol (bold fonts) compared to other tasks and muscles

FDI activation compared to activation of other muscles in three different tasks						
Task	ANOVA task-effect	**FDI *activation***	*vs. opp* (*post hoc*)	*vs. adm* (*post hoc*)	*vs. ext* (*post hoc*)	*vs. fds* (*post hoc*)
Index ft task	$F_{4,20} = 5.6$ $p < 0.05$	**6.7% (*s.e.m.* 1.0)**	n.s.	n.s.	n.s.	n.s.
Index iso-MVC task	$F_{4,20} = 10.5$ $p < 0.001$	**13.3% (*s.e.m.* 1.1)**	5.8%* (*s.e.m.* 1.3)	4.4%* (*s.e.m.* 0.5)	n.s.	n.s.
3–5th fingers iso-MVC task	$F_{4,20} = 21.9$ $p < 0.001$	**5.6% (*s.e.m.* 1.1)**	n.s.	16.3%* (*s.e.m.* 1.7)	n.s.	14.4%** (*s.e.m.* 0.9)
FDI activation during different index *iso* MVC tasks						
	ANOVA direction-effect	***toward flexion***	*vs. abduction* (*post hoc*)	*vs. extension* (*post hoc*)	*vs. adduction* (*post hoc*)	
Index iso-MVC	$F_{3,15} = 37.1$ $p < 0.001$	**13.3% (*s.e.m.* 1.1)**	n.s.	5.2%* (*s.e.m.* 0.6)	4.0%* (*s.e.m.* 0.9)	

Units (%) = 100 ($r.m.s._{TASK}/r.m.s._{CMAP}$). *opp*: oponens pollicis; *adm*: abductor digiti minimi; *ext*: extensor digitorum; *fds*: flexor digitorum supperficialis. Asterisks indicate Bonferroni post hoc *after ANOVA* evaluation of main effects. n.s.: not significant.

* $p < 0.05$.

** $p < 0.01$.

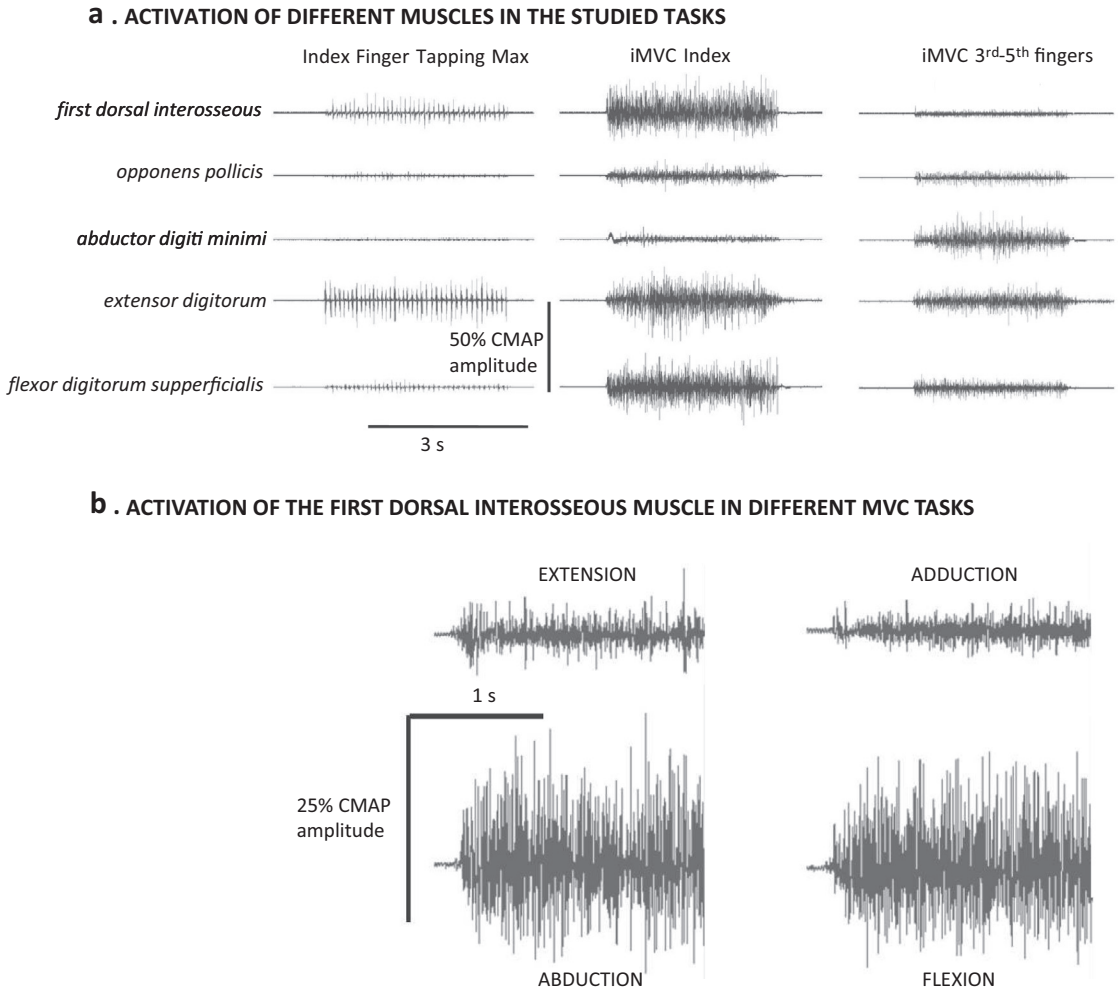

Fig. 2. (a) Activation of the *first dorsal interosseous* in the *ft* and *iso* tasks, for comparison with another task (iMVC flexion of the 3rd, 4th and 5th fingers). Example of individual recordings in one subject (on a separate session) with the settings as describe in Fig. 1. Execution of *ft* at maximal rate (left recordings); *iso* MVC with the index finger, as in the protocols (central recordings); and *iso* MVC flexion with 3–5th fingers together with no index finger pressing (right recordings). The EMG-recordings of the *first dorsal interosseous*, *opponens pollicis*, *abductor digiti minimi*, *extensor digitorum* and *flexor digitorum superficialis* are shown in the three tasks. The EMG-amplitudes are scaled to the vertical bar to allow raw-data representation, showing the 50% of each muscle CMAP amplitude; statistical comparisons were applied for *r.m.s.* activation; the involvement of the FDI compared to the rest of the muscles is clear and more specific in the task evaluated. (b) Similar maximal EMG-recruitment of the FDI during index finger flexion and abduction, larger than during adduction and extension, all iMVC against unmovable loads.

Table 2. Mean score including all subjects. The score is the *y*-axis unit in graphs

Task	*Max output in task at any evaluation time-point	*Max active full ROM[1]	Mean (%[1]) *ft* amplitude along the task	*MVC at any stimulation time-point	Mean TMS-SP duration at comfort	Mean CMS-SP duration at comfort
ft	7.1 Hz (*s.e.m.* 0.1)	24.7 ° (*s.e.m.* 2.0)	28.3% (*s.e.m.* 3.9)	4.7 kg (*s.e.m.* 0.3)	145.1 ms (*s.e.m.* 4.1)	67.0 ms (*s.e.m.* 4.0)
iso	3.8 kg (*s.e.m.* 0.2)	n.a.	n.a.	4.5 kg (*s.e.m.* 0.2)	158.9 ms (*s.e.m.* 5.9)	71.5 ms (*s.e.m.* 4.6)

* The score utilizes the maximal value from each subject: it is the mean of the maximal scores including all subjects. n.a.: not applicable.

the task for the different modes ($F_{2,38}$ = 109.0 $_{\varepsilon = 0.7,}$ $\boldsymbol{p < 0.001}_{\text{MODE} \times \text{TIME}}$); and also there was a set effect for some modes impacting the level of the dropping of force at the end of the *iso* task ($F_{6,114}$ = 11.0, $\boldsymbol{p < 0.001}_{\text{MODE} \times \text{SET}}$). Again, the execution protocol was identical for the two groups of subjects, those stimulated with TMS and CMS; likely, this is the reason why the dropping of force was not significantly different for the two groups of subjects ($\boldsymbol{p > 0.05}$ for $_{\text{MODE} \times \text{SET} \times \text{GROUP}}$ or $_{\text{MODE} \times \text{TIME} \times \text{GROUP}}$ interactions). Then, we followed-up ANOVAs by modes of execution, this was done to understand the before-mentioned differential effects of

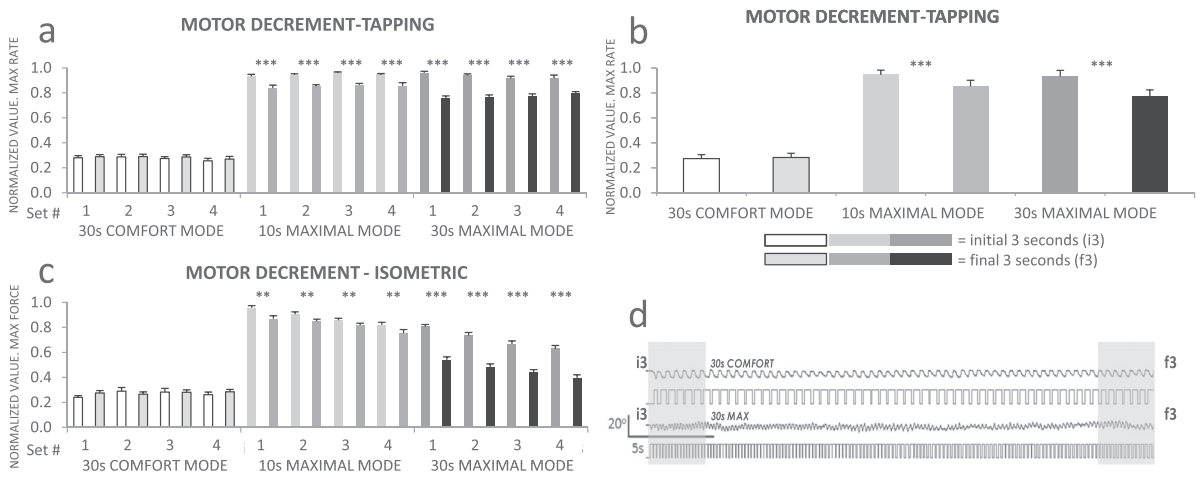

Fig. 3. Motor decrement induced by the tasks. (a) The frequency of *ft* decreased significantly after the maximal modes; there was no set effect. (b) Same effect seen pooling sets as there was no set effect. (c) Set by set motor decrement along the *iso* tasks. The force reduced at *post* after maximal modes and set after set. (d) Representative example in one subject, *ft* amplitude for *comfort* and *30max*. The amplitude never decreased during the tasks. The unit in the *y*-axis represents the normalized value with respect to the maximal motor output (maximal *ft* rate, or maximal *iso* torque obtained at any evaluation time of the corresponding session). In all figures, light colors are the initial 3s of execution (*pre*), dark colors the final 3 s (*post*); asterisks denote statistical significance between *pre* and *post*, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

the modes of execution on the dropping of force at the end of the *iso-tasks*. We observed no effects of *comfort-iso* on force drop at *post* ($\boldsymbol{p > 0.05}$ for any main effect or interaction). For *10max-iso*, the execution dropped significantly at *post* ($F_{1,19} = 13.4$, $\boldsymbol{p < 0.01}_{\text{TIME}}$), and set after set ($F_{3,57} = 13.7$, $\boldsymbol{p < 0.001}_{\text{SET}}$). A similar pattern of force-drop was observed for *30max-iso* ($F_{1,19} = 174.5$, $\boldsymbol{p < 0.001}_{\text{TIME}}$ and $F_{3,57} = 39.6$ $_{\varepsilon = 0.7,}$ $\boldsymbol{p < 0.001}_{\text{SET}}$).

Finger angular amplitude during tapping

During *ft*, the expressions of fatigue might affect not only the tapping-frequency, but also the tapping amplitude. For this reason, we analyzed the ROM amplitude at the first (*i3* = pre) and last (*f3* = post) 3 s of the task. Remarkably, during *ft* the ROM amplitude was not significantly different at *pre* vs. *post* (*i3* vs. *f3*; $F_{1,19} = 1.7$, $\boldsymbol{p > 0.05}_{\text{TIME}}$; Table 2). Likewise, it is worth mentioning, that in all sets, the mean ROM amplitude was not different for the three tapping modes either ($F_{2,38} = 2.3$ $_{\varepsilon = 0.7,}$ $\boldsymbol{p > 0.05}_{\text{MODE}}$; Fig. 3d shows an example of the ROM amplitude in one subject). These effects on motor execution were not differently expressed for the subjects stimulated with TMS or CMS (identical task for both groups).

MVC at the time of stimulation

The MVC at the time of stimulation (Fig. 4a) was reduced at *post* differently for the *ft* and *iso* tasks ($F_{1,19} = 30.1$, $\boldsymbol{p < 0.001}_{\text{TASK} \times \text{TIME}}$; Fig. 4b–d). We followed by splitting the analyses by task-type.

For *ft* tasks, MVC force was reduced at *post* (i.e., in the MVC performed right at the end of tapping; $F_{1,13} = 12.3$, $\boldsymbol{p < 0.01}_{\text{TIME}}$) and differed also for the three modes of tapping ($F_{2,38} = 18.7$, $\boldsymbol{p < 0.001}_{\text{MODE}}$). Thus, *post* MVC was weaker after tapping (*vs. pre*), at all modes and sets (Fig. 4b), and it also was reduced from mode to mode.

A different matter happened for *iso*; we initially observed that the MVC decrease at *post* was different for the three modes ($F_{2,38} = 15.9$, $\boldsymbol{p < 0.001}_{\text{TIME} \times \text{MODE}}$; Fig. 4c, d). Then, we followed-up the analyses by modes. The analyses indicated that for *comfort* and *10max* the MVC dropped at *post* and set after set (i.e., factors TIME and SET for these to modes were always significant ($\boldsymbol{p < 0.001}$). This was also the case of *30max*, but in addition, the responses at *pre* and *post* also differed for the different sets ($F_{3,57} = 4.9$ $_{\varepsilon = 0.7,}$ $\boldsymbol{p < 0.01}_{\text{SET} \times \text{TIME}}$) notwithstanding the fact that post hoc *pre* vs. *post* were always significant ($\boldsymbol{p < 0.001}$, see Fig. 4c, d).

Again, in agreement with the other variables described thus far, these responses were not significantly different for the subjects stimulated with TMS or CMS (i.e., factor group did not interact significantly in any case), and comparable levels of fatigue were shown in the two groups for each task.

SP adaptation to maximal *ft* and *iso* tasks

As described in the introduction, the SP duration increases with CF. Therefore, we analyzed if SP duration changed after execution (at *post*); and also if the putative change was different for *ft* and *iso*. In one group of subjects the SP was induced by TMS in the two different task-sessions (*ft* and *iso*); in the other it was induced by CMS in the two sessions. Therefore, in all cases, the task-dependency (*ft* vs. *iso*) changes in SP were evaluated for the two kinds of stimulation (i.e., in the two groups). As mentioned above, fatigue did not affect differently the motor execution in the two groups and tasks; thus we compared SP's duration evoked by TMS and CMS in the two tasks; to make their baseline duration comparable the durations were normalized. For

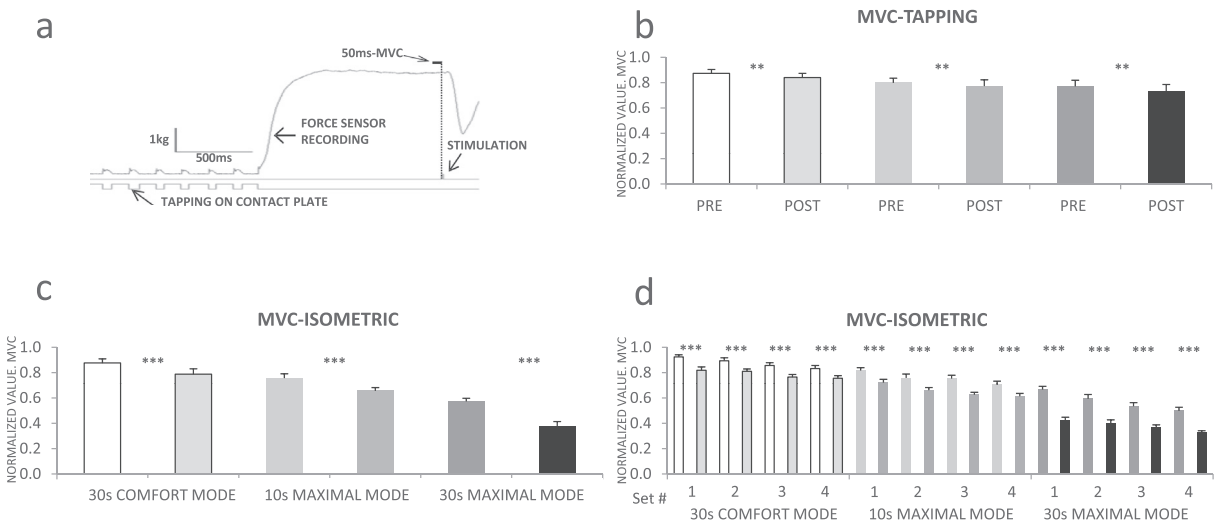

Fig. 4. (a) Example of force recording at *post-ft*. MVCs shown in 4(b–d) sections are acquired in the 50 ms prior to stimulation. (b) MVC decreased after *ft* (*post*) in all modes and from mode to mode. (c–d) MVC decrease after *iso* (*post*) in all modes and also from mode to mode and set to set.

this purpose the SP duration at *comfort* was considered the reference for each kind of stimulation.

The normalized SP durations increased after execution (at *post*), in a significant different manner for the two task, and for TMS and CMS groups ($F_{1,19}$ = 9.4, $\boldsymbol{p < 0.01}_{\text{GROUP} \times \text{TASK} \times \text{TIME}}$). See Fig. 5 (individual's raw-examples), and Fig. 6. We split the analyses to evaluate the task-dependency effect on the two groups.

For *ft*-task, the SP increase at *post* was significantly different for the CMS and TMS groups ($F_{1,19}$ = 11.0, $\boldsymbol{p < 0.01}_{\text{GROUP} \times \text{TIME}}$). Subsequently, the analyses of the TMS-SPs showed a significant increase at the end of execution, which was also different for the execution modes ($F_{2,16}$ = 9.1, $\boldsymbol{p < 0.01}_{\text{MODE} \times \text{TIME}}$). We observed that the increase of the TMS-SP was only significant after *10max* ($\boldsymbol{p < 0.01}$ post hoc) and *30max* ($\boldsymbol{p < 0.001}$ post hoc), not for *comfort*. Conversely the CMS-SP never increased at the end of the *ft* ($F_{1,11}$ = 0.7, $\boldsymbol{p > 0.05}_{\text{TIME}}$).

For *iso*-tasks the responses to fatigue of the SP induced by TMS and CMS were never significantly different. For both types of stimulation, the normalized SP increase at the end of the execution was different in the three modes ($F_{2,38}$ = 25.0, $\boldsymbol{p < 0.001}_{\text{MODE} \times \text{TIME}}$); and the analyses by modes of execution indicated that for both the TMS and CMS groups, the SP increased significantly only after *10max* and *30max* (Post-hoc; $\boldsymbol{p < 0.001}$, in two cases) (Fig. 5b, d).

Set sequence never had a significant effect on SP ($\boldsymbol{p > 0.05}$ for main effects or interaction; i.e., in all modes and task).

DISCUSSION

The main goal of this study was to determine the contribution of some spinal and supraspinal motor circuits to the generation of fatigue during short-lasting repetitive movements (finger tapping), if performed at the fastest possible rate. The site of CF was assessed by means of SPs evoked by TMS and electrical CMS at the time of fatigue within the FDI, a muscle with a more specific role in the tasks of our protocol than the other muscles explored in this work. Then during fatigue protocols, we evaluate SPs allowing no recovery of the neural system after the fatiguing activity (Taylor et al., 2000). While the SP generated by TMS has cortical and spinal components, the one generated by CMS has a purely spinal origin (Fuhr et al., 1991; Inghilleri et al., 1993; Brasil-Neto et al., 1995). To our knowledge, this is the first study attributing fatigue induced by short-lasting repetitive *ft* activity at the maximal rate to supraspinal structures, while controlling fast forms of fatigue-recovery from the task. Moreover, our results are consistent with a prominent contribution of spinal circuits to CF during isometric MVC (Butler et al., 2003; Klass et al., 2008), which does not rule out the additional contribution of a supraspinal component (Gandevia et al., 1996; Di Lazzaro et al., 2003; Maruyama et al., 2006; Taylor and Gandevia, 2008; McNeil et al., 2009).

The decrement in motor output was expressed as a reduction in *ft* frequency, and in force for *iso,* in both cases at the end of *10max* and *30max*; this effect was observed in the two groups of subjects (one group stimulated with TMS, and the other with CMS), without significant differences in their task executions. We consider these changes in motor output as signs of fatigue, as they paralleled known fatigue behavioral markers like the reduction in MVC output right at the end (in continuation) of the fatiguing-tasks. There was also an increase in SP-durations evoked by TMS in both tasks after maximal modes (in a same group of subjects) (Gandevia, 2001; Taylor and Gandevia, 2001). Remarkably, CMS-SP (tested in a same group of subjects during *ft* and *iso* tasks) increased only after maximal *iso* tasks, and not after *ft* tasks; this is a clear sign of different contribution of spinal cord mechanisms to the development of fatigue during maximal *iso* and *ft*.

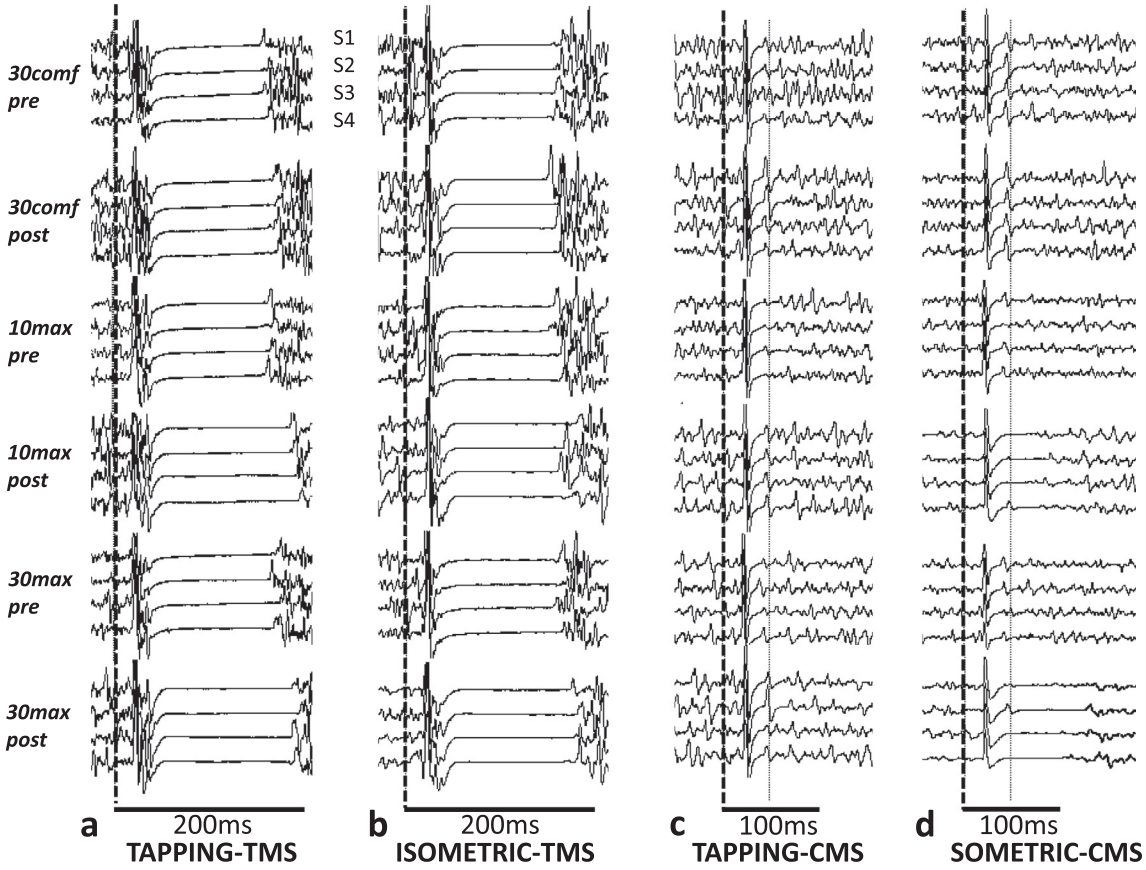

Fig. 5. Individual's recordings of the SP *pre* and *post* execution. S1. . .S4 are the sequential recordings of the four sets for a given time of evaluation. TMS-SP increased at *post* after *ft* (a) and after *iso* (b), only at maximal modes. Conversely CMS-SP was unchanged after *ft* in all modes (c), but increased after the maximal modes for the *iso* task, see (d) section. The vertical thick dashed lines indicate the time of stimulation. The vertical thin dashed line lies at the end of the transcortical reflex potential, evoked by CMS. The representation occludes part of the motor evoked potentials, in order to optimize the representation of the SPs.

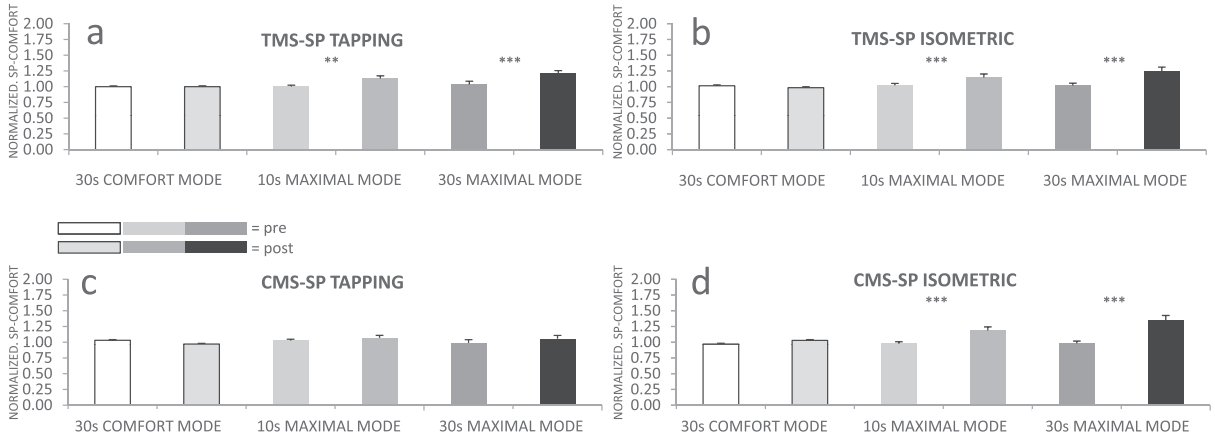

Fig. 6. SPs in response to TMS or CMS during fatiguing motor activities in all subjects. (a) and (b) show that the TMS-SP significantly increased after executing at maximal modes, either 10 or 30 s for *ft* and *iso*. (c) However in no case did the CMS-SP increase after *ft*. (d) CMS-SP significantly increased after *10max* and *30max iso*. A significant effect of set never appeared for SP – durations, therefore the four sets are shown pooled. The *y*-axis expresses the mean duration of the SP in *comfort* mode (pooling all sets and *pre* and *post* values).

For fatigue-induced SP increase, there seems to be a ceiling effect at about 30 s (Taylor et al., 1996; Taylor and Gandevia, 2001). In line with this, we never detected significant effects of set on SP, although it was present in behavior (MVC) in the case of *iso*.

The analyses of the SP-durations contribute to the understanding of the different origin of fatigue and task dependency (Enoka and Stuart, 1992) for *ft* and *iso* at *maximal* modes. SP to TMS increased immediately at the end of both maximal *ft* and *iso*. However, when in a

same group of subjects we evaluated the task-dependency response of SP to CMS, it only increased after *maximal iso* activity, and not after maximal *ft activity*, despite the fact that in these subjects maximal *ft* induced reduction of tapping rate and MVC at the end of the task. CMS-SP emerges from spinal inhibitory mechanisms following motoneuronal excitation, such as after-hyperpolarization and recurrent inhibition (Inghilleri et al., 1993; Brasil-Neto et al., 1995), whereas the late part of the TMS-SP arises from the activation of inhibitory cortical interneurons projecting to the pyramidal cortico-spinal neurons (Inghilleri et al., 1993), acting via GABAb receptors (Ziemann et al., 1993; Ziemann, 2013). This suggests that fatigue induced by *ft* in our study is not induced by the spinal mechanisms mentioned above, but takes place at supraspinal levels. On the other hand a spinal origin of fatigue seems to be clear for *iso* maximal (Duchateau and Hainaut, 1993; Gandevia et al., 1999; Taylor et al., 1999; Butler et al., 2003; McNeil et al., 2009), but likely to be present also at a supraspinal level (Di Lazzaro et al., 2003).

Maximal voluntary contractions at fatigue time

At the time of testing, our subjects exhibited a reduction of MVC as an expression of fatigue (Bigland-Ritchie and Woods, 1984; Gandevia, 2001). The *ft* and *iso* tasks were matched for efforts (*comfort* or *maximal*) and durations (10–30 s). However, it was observed that the different combinations of efforts, durations, and tasks had different impacts on the MVC at the time of fatigue testing, reflecting the fatigue tasks dependencies and suggesting different mechanisms of fatigue. For this reason, it is work for the future to design a protocol to match the drop of MVC after the different tasks (note that this was present in some sets of our protocol).

Fatigue accumulation after iso maximal tasks

For *iso* tasks we observed an accumulation of fatigue set after set expressed in the MVC dropping of force, which was not different for subjects belonging to TMS or CMS groups. However, this effect was not reflected in a set effect for SPs (regardless TMS or CMS); this is in agreement with a previously described ceiling-effect for this variable (Taylor et al., 1996; Taylor and Gandevia, 2001). It is possible that some other circuits different to the ones explored in this study, or even peripheral expressions of fatigue, might explain this effect. A sign of peripheral fatigue, such the reduction on the CMAP (a marker of action potential sarcoplasmic propagation), should not be expectable for 30 s of maximal *iso* accordingly to previous studies (Butler et al., 2003); but the effect of set after set accumulation on the CMAP response is unknown. Admittedly, the objective of this study (SP exploration) does not permit the recording of CMAP at the same time of testing. Notwithstanding that, although the CMAP is essential to normalized motor evoked potential amplitudes in fatiguing conditions, it does not condition the SP durations explained in this study.

MVC drop during comfort mode evaluation at the time of fatigue

MVC testing right at the end of the tasks (*post*) showed that the force dropped in all cases, including *comfort* modes for *ft* and *iso* tasks. This suggests fatigue development even after *comfort* execution. However, the SP durations were not modified at *post* for *comfort* modes. The presence of fatigue after *ft comfort* execution has been suggested previously (Teo et al., 2012), but the evaluation was done with the fatigued muscle at rest and using TMS paired-pulse protocols. It is possible that fatigue after *ft* and *iso comfort* develops first in cortical circuits un-explored in our protocol. For instance, intracortical inhibitory gabaergic mechanisms acting via GABAa receptors (Werhahn et al., 1999; Di Lazzaro et al., 2000; Hallett, 2007; Ziemann, 2013), or excitatory glutamatergic circuits responsible for motor evoked potential amplitude. In fact, an involvement of intracortical circuits (GABAa receptor mediated) has been suggested previously for the development of fatigue in *ft* tasks (Teo et al., 2012).

Fatigue expressions on rate but not on amplitude for maximal *ft* tasks

The subjects were asked to tap ‘‘as fast as possible’’, with no instructions about angular movement amplitude. In our study, fatigue never induced changes in angular amplitude during the task, and the movement amplitude did not change across execution modes (Rodrigues et al., 2009). This supports the view that the expression of supraspinal fatigue observed during maximal *ft* was directly related to the tapping-rate, i.e. to the number of changes in the finger directions (sequences) per unit of time, and not to their amplitude. Interestingly, we observed that the level of activation of the FDI during maximal rate *ft* is much lower than during *iso* MVC. This reinforces the view that the control of alternating fast sequences is demanding for supraspinal centers even when the level of muscle activation is not very high.

Conclusion

In conclusion, early forms of fatigue during short-lasting repetitive movements at the fastest rate do not originate at the circuits responsible for CMS-SP generation at spinal level. In contrast, short-lasting isometric MVC tasks induce spinal inhibitory mechanisms following motoneuronal excitation, likely explained by recurrent inhibition and after-hyperpolarization in response to the sustained maximal contraction. The fatigue induced by fast-rate repetitive movements possibly involves intracortical inhibitory circuits operating via GABAb interneurons, and its main expression is the decrease in tapping frequency.

Fatigue is one of the main signs in diseases of the spinal cord and supraspinal centers. We have provided neurophysiological evidence for different physiological mechanisms in *ft* and *iso*. Our results, if replicated in the future with larger samples, may help to improve the

design of the clinical tests aiming at the evaluation of CF in pathological and physiological conditions.

DISCLOSURE

The authors declare no conflict of interests.

AUTHORS CONTRIBUTIONS

1. Conception and design of the experiments: P.A., V.R.G., J.C.
2. Collection, analysis and interpretation of data: P.A., V.R.G., Y.C.B., A.M., N.E., J.V., K.G., A.O., J.C.
3. Drafting the article or revising it critically for important intellectual content: P.A., V.R.G., Y.C.B., A.M., N.E., J.V., K.G., A.O., J.C.

Acknowledgments—**Grants:** *This work was supported by Xunta de Galicia (Consellería de Educación 2007/000140-0; Ayudas Grupos Consolidados, Consellería de Educación, 2014 and Dirección Xeral de I+D+i; 2010-2012), Spain. V.R.G. and Y.C.B. are granted by the FPU-MECD AP2010-2774 and AP2010-2775 Spain.*

REFERENCES

Allen DG, Lamb GD, Westerblad H (2008) Skeletal muscle fatigue: cellular mechanisms. Physiol Rev 88:287–332.

Arias P, Robles-Garcia V, Espinosa N, Corral Y, Cudeiro J (2012) Validity of the finger tapping test in Parkinson's disease, elderly and young healthy subjects: is there a role for central fatigue? Clin Neurophysiol 123:2034–2041.

Bigland-Ritchie B, Woods JJ (1984) Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle Nerve 7:691–699.

Brasil-Neto JP, Pascual-Leone A, Valls-Sole J, Cammarota A, Cohen LG, Hallett M (1993) Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. Exp Brain Res 93:181–184.

Brasil-Neto JP, Cammarota A, Valls-Sole J, Pascual-Leone A, Hallett M, Cohen LG (1995) Role of intracortical mechanisms in the late part of the silent period to transcranial stimulation of the human motor cortex. Acta Neurol Scand 92:383–386.

Butler JE, Taylor JL, Gandevia SC (2003) Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. J Neurosci 23:10224–10230.

Butler JE, Petersen NC, Herbert RD, Gandevia SC, Taylor JL (2012) Origin of the low-level EMG during the silent period following transcranial magnetic stimulation. Clin Neurophysiol 123:1409–1414.

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

Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, Rothwell JC (2000) Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. Clin Neurophysiol 111:794–799.

Di Lazzaro V, Oliviero A, Tonali PA, Mazzone P, Insola A, Pilato F, Saturno E, Dileone M, Rothwell JC (2003) Direct demonstration of reduction of the output of the human motor cortex induced by a fatiguing muscle contraction. Exp Brain Res 149:535–538.

Duchateau J, Hainaut K (1993) Behaviour of short and long latency reflexes in fatigued human muscles. J Physiol 471:787–799.

Enoka RM, Stuart DG (1992) Neurobiology of muscle fatigue. J Appl Physiol 72:1631–1648.

Enoka RM, Duchateau J (2008) Muscle fatigue: what, why and how it influences muscle function. J Physiol 586:11–23.

Enoka RM, Baudry S, Rudroff T, Farina D, Klass M, Duchateau J (2011) Unraveling the neurophysiology of muscle fatigue. J Electromyogr Kinesiol 21:208–219.

Fuhr P, Agostino R, Hallett M (1991) Spinal motor neuron excitability during the silent period after cortical stimulation. Electroencephalogr Clin Neurophysiol 81:257–262.

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725–1789.

Gandevia SC, Allen GM, Butler JE, Taylor JL (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 490(Pt 2):529–536.

Gandevia SC, Petersen N, Butler JE, Taylor JL (1999) Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. J Physiol 521(Pt 3):749–759.

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

Hilty L, Jancke L, Luechinger R, Boutellier U, Lutz K (2011a) Limitation of physical performance in a muscle fatiguing handgrip exercise is mediated by thalamo-insular activity. Hum Brain Mapp 32:2151–2160.

Hilty L, Lutz K, Maurer K, Rodenkirch T, Spengler CM, Boutellier U, Jancke L, Amann M (2011b) Spinal opioid receptor-sensitive muscle afferents contribute to the fatigue-induced increase in intracortical inhibition in healthy humans. Exp Physiol 96:505–517.

Hultborn H, Pierrot-Deseilligny E, Wigstrom H (1979) Recurrent inhibition and afterhyperpolarization following motoneuronal discharge in the cat. J Physiol 297:253–266.

Inghilleri M, Berardelli A, Cruccu G, Manfredi M (1993) Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. J Physiol 466:521–534.

Jancke L, Specht K, Mirzazade S, Loose R, Himmelbach M, Lutz K, Shah NJ (1998) A parametric analysis of the 'rate effect' in the sensorimotor cortex: a functional magnetic resonance imaging analysis in human subjects. Neurosci Lett 252:37–40.

Klass M, Levenez M, Enoka RM, Duchateau J (2008) Spinal mechanisms contribute to differences in the time to failure of submaximal. J Neurophysiol 99:1096–1104.

Kluger BM, Krupp LB, Enoka RM (2013) Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology 80:409–416.

Lutz K, Koeneke S, Wustenberg T, Jancke L (2005) Asymmetry of cortical activation during maximum and convenient tapping speed. Neurosci Lett 373:61–66.

Maruyama A, Matsunaga K, Tanaka N, Rothwell JC (2006) Muscle fatigue decreases short-interval intracortical inhibition after exhaustive intermittent tasks. Clin Neurophysiol 117:864–870.

McNeil CJ, Martin PG, Gandevia SC, Taylor JL (2009) The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. J Physiol 587:5601–5612.

Rodrigues JP, Mastaglia FL, Thickbroom GW (2009) Rapid slowing of maximal finger movement rate: fatigue of central motor control? Exp Brain Res 196:557–563.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH, et al. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 91:79–92.

Shimoyama I, Ninchoji T, Uemura K (1990) The finger-tapping test. A quantitative analysis. Arch Neurol 47:681–684.

Taylor JL, Gandevia SC (2004) Noninvasive stimulation of the human corticospinal tract. J Appl Physiol (1985) 96:1496–1503.

Taylor JL, Gandevia SC (2001) Transcranial magnetic stimulation and human muscle fatigue. Muscle Nerve 24:18–29.

Taylor JL, Gandevia SC (2008) A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. J Appl Physiol 104:542–550.

Taylor JL, Butler JE, Allen GM, Gandevia SC (1996) Changes in motor cortical excitability during human muscle fatigue. J Physiol 490(Pt 2):519–528.

Taylor JL, Butler JE, Gandevia SC (1999) Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. Exp Brain Res 127:108–115.

Taylor JL, Allen GM, Butler JE, Gandevia SC (2000) Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. J Appl Physiol 89:305–313.

Teo WP, Rodrigues JP, Mastaglia FL, Thickbroom GW (2012) Post-exercise depression in corticomotor excitability after dynamic movement: a general property of fatiguing and non-fatiguing exercise. Exp Brain Res 216:41–49.

Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD (1991) Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol 29:418–427.

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

Ziemann U (2013) Pharmaco-transcranial magnetic stimulation studies of motor excitability. Handb Clin Neurol 116:387–397.

Ziemann U, Netz J, Szelenyi A, Homberg V (1993) Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. Neurosci Lett 156:167–171.

Zwarts MJ, Bleijenberg G, van Engelen BG (2008) Clinical neurophysiology of fatigue. Clin Neurophysiol 119:2–10.

(Accepted 30 July 2015)
(Available online 1 August 2015)

Neuroscience 339 (2016) 655–666

DIFFERENTIAL RESPONSES OF SPINAL MOTONEURONS TO FATIGUE INDUCED BY SHORT-LASTING REPETITIVE AND ISOMETRIC TASKS

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Abstract—Compared to isometric activities, the neural basis of fatigue induced by repetitive tasks has been scarcely studied. Recently, we showed that during short-lasting repetitive tasks at the maximal possible rate (finger tapping for 10 and 30 s), tapping rate and maximal voluntary contraction (MVC) force decrease at the end of finger tapping. We also observed larger silent periods (SP) induced by transcranial magnetic stimulation during MVC *post* finger tapping. However, if SP were induced by cervicomedullary stimulation (CMS) they remained unchanged. This suggested a supraspinal origin of fatigue for repetitive tasks. Nevertheless, CMS SP only partially explore spinal excitability; therefore, to evaluate a spinal origin of fatigue it is essential to know the features of the CMS-evoked potentials (CMEP). Herein, we evaluated (n = 15) the amplitude of the CMEP during MVC executed immediately (no gap) after a short-lasting finger tapping task; we also evaluated the compound muscle action potential (CMAP) so that the amplitude of the CMEP was expressed as a function of the CMAP amplitude. Indices of fatigue obtained during finger tapping were compared with those obtained during short-lasting maximal isometric tasks. While indices of excitability increased initially in both tasks, they decreased with the isometric task only when the task was prolonged to 30 s. We suggest that the inability to maintain increased levels of spinal excitability during task execution is a neurophysiological mark of fatigue. Our results suggest that the origin of fatigue induced by brief and fast repetitive tasks is not spinal.

Key words: fatigue, repetitive movements, human, spinal cord.

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Abbreviations: AMT, active motor threshold; ANOVA, analysis of variance; CMAP, compound muscle action potential; CMS, cervicomedullary stimulation; MVC, maximal voluntary contraction; SP, silent periods.

http://dx.doi.org/10.1016/j.neuroscience.2016.10.038

INTRODUCTION

The understanding of fatigue of the human motor system is of paramount importance in the fields of ergonomics, sport and neurology. The neural basis of fatigue has been studied extensively in the case of isometric contractions, either maximal or submaximal (Gandevia, 2001; Duchateau et al., 2002; Maluf and Enoka, 2005; Klass et al., 2008; Taylor and Gandevia, 2008; Williams et al., 2014), and there is strong evidence that isometric fatiguing tasks induce a reduction in the excitability of circuitry in both the spinal cord (Taylor et al., 1996; Duchateau et al., 2002; Butler et al., 2003; Klass et al., 2008) and motor cortex (Gandevia et al., 1996; Taylor et al., 1996; Di Lazzaro et al., 2003). The evaluation of evoked potentials in response to transcranial magnetic stimulation (TMS) and electric or magnetic cervicomedullary stimulation (CMS) has permitted the localization of the sites where excitability of the motor system has been modified during fatigue. While TMS is used to evaluate intracortical and corticospinal excitability (Hallett, 2000, 2007), the potentials induced by CMS (i.e. CMEP) are adequate to explore the excitability of the spinal cord circuits (Ugawa et al., 1991; Taylor and Gandevia, 2004; McNeil et al., 2013).

Fatiguing isometric contractions of maximal effort increase the duration of the silent periods (SP) induced by CMS and reduce muscle force during maximal voluntary contraction (MVC) (Taylor et al., 1996), an effect already present after 10 s of MVC in the small muscles of the hand (Arias et al., 2015). This is an indication of longer lasting decreased spinal motoneuronal excitability, which is partly due to recurrent inhibition and afterhyperpolarization (Inghilleri et al., 1993; Brasil-Neto et al., 1995). Fatigue also induces neurophysiological changes that reveal adaptations of intracortical motor circuits: an increase of TMS-SP, mediated by GABA receptors (Taylor et al., 1996; Arias et al., 2015); a reduction in the descending volleys in the corticospinal tract (Di Lazzaro et al., 2003); and a increase in cortico-cortical inhibition to paired-pulse TMS (McNeil et al., 2009).

However, cortical and spinal adaptations to fatigue are known to be task dependent (Enoka and Stuart, 1992; Barry and Enoka, 2007; Enoka and Duchateau, 2008; Enoka et al., 2011). While neural mechanisms related to fatigue during isometric tasks have been thoroughly studied, those related to fatigue during repetitive movements have been much less studied. One fundamental point during the study of the neural basis of fatigue is to control (or to avoid) the recovery of the system at the time of testing

(Taylor et al., 2000). This is relatively simple in the case of isometric activities where the stimulation of the brain can be performed during the task without stopping the activity. On the other hand, the study of fatigue induced by repetitive movements has been traditionally performed at rest, after the fatiguing activity (Brasil-Neto et al., 1993; Arias et al., 2012; Teo et al., 2012), which would not allow for the assessment of fast recovering forms of fatigue. This could be the case with fatigue developed during short-lasting repetitive movements executed at maximal possible rate. Recently, we have developed a protocol that allowed us to evaluate cortical and spinal adaptations to muscle fatigue when performing a repetitive finger tapping task (*ft*) with no time for recovery (Arias et al., 2015). The study involved the evaluation of TMS and CMS SPs during brief (2 s) episodes of MVC, which were executed immediately after maximal rate *ft* (10 or 30 s). When fatigued, subjects were unable to maintain the maximal tapping rate and their force was decreased during MVC, this was accompanied by an increase SP induced by TMS but not by CMS (Arias et al., 2015); those results advocate for a cortical locus of fatigue for fast rate *ft*. On the contrary, the reduction in force after short isometric MVC was accompanied by an increase in both CMS-SP and TMS-SP (Arias et al., 2015).

Therefore, the findings only partially ruled out the development of fatigue at spinal circuitry during maximal rate *ft* because we only evaluated CMS-SP duration but not the CMEP amplitude. A meaningful interpretation of the CMEP amplitude can only be achieved if considered in relation to the amplitude of the compound muscle action potential (CMAP) in response to supramaximal stimulation of the corresponding nerve (at the same time of testing the CMEP). This is fundamental because the CMAP reflects the efficiency of transmission in the periphery (Rich, 2006).

In the present work, we have modified our previous protocol to examine the spinal mechanisms of fatigue during maximal rate *ft* by mean of CMEP amplitude evaluation. We have been careful in exploring spinal and neuromuscular transmission at the time of fatigue, without allowing time for recovery. For comparison, we also explored the responses induced by a fatiguing isometric (*iso*) task. In all cases the tasks were short lasting, 10 or 30 s. We predict that spinal motoneurons will behave differently depending on the task employed.

EXPERIMENTAL PROCEDURES

Experimental protocols conformed to the Helsinki declaration and were approved by our institution Ethics Committee. All subjects were screened for incompatibility with brain stimulation protocols. All were medication free during the week preceding testing and signed a voluntary informed consent.

Subjects

The experiment included 15 healthy subjects (all men, age range 18–40 years). In all subjects the spinal excitability was evaluated with stimulation at the level of the cervicomedullary junction during several 2.5-s MVCs. Electrical stimulation was used in seven subjects, while magnetic stimulation using a double cone coil was used in the remaining eight subjects (who refused to participate if the stimulation was electrical due the produced discomfort). All subjects underwent both *ft* and *iso* fatigue testing sessions, 15 days apart.

Protocol

The two sessions were identical except for the type of *task* executed. In one of the session, participants were asked to perform index *ft*. In the other session they executed continuous index finger *iso* against a force sensor; the force direction was ‘‘toward” *flexion* of the first metacarpophalangeal joint. In all cases participants wore a small and light goniometer to monitor movements of the index finger metacarpophalangeal joint; we used also a metal ring attached at the distal phalanx of the index. Participants tapped or pressed over a thin metal plate located on the force sensor.

For both *ft* and *iso* sessions, subjects performed the tasks in three modes: comfort rate-effort (*comfort* mode) for 30 s; 10 s at maximal rate-effort (*10 max* mode); and finally 30 s at maximal rate-effort (*30 max* mode). Each mode was repeated four consecutive times (i.e., sets); rest periods between sets lasted 1 min 40 s. The decrease in frequency or amplitude (for *ft*), or in force output (for *iso*) defined the presence of fatigue.

For the *comfort-ft*, subjects were asked to ‘‘*tap at their most comfortable rate without feeling fatigued*” for as long as the set lasted. *Comfort-ft* is reliable (Arias et al., 2012) and paced about 1/3 of *ft* maximal rate. During *comfort-ft* the metabolic activity in the sensorimotor cortex is lower than at faster (> 3 Hz) or slower (< 1 Hz) rates (Jancke et al., 1998; Lutz et al., 2005), showing its suitability as control condition in our protocol. Participants were asked to press ≈1/3 MVC for *comfort-iso*; and visual feedback was provided. For maximal modes, subjects were encouraged to tap/press as fast/hard as they could from the very beginning to the end of the set.

The participants also executed 2.5-s MVCs before (*pre*) and right after (no gap allowed) task-execution (*post*), either after *ft* or *iso*, for all modes and sets (Fig. 1). The magnitudes of 2.5-s MVCs were analyzed to monitor fatigue (Bigland-Ritchie and Woods, 1984). During the 2.5-s MVCs the CMS was applied (at 1.5 s), and we recorded the SP duration and CMEP amplitudes (Taylor et al., 1996). The peripheral transmission of the potentials during the same 2.5-s-MVCs was also evaluated (at 2.2 s) with the amplitude of the CMAP (Rich, 2006). We calculated the ratio CMEP/CMAP to evaluate spinal excitability accounting for the state of the periphery; this was always performed with the CMEP and CMAP acquired in the same MVC. Thus the stimulation pulses (CMS, and 700 ms later supramaximal to the ulnar nerve) were applied during the 2.5-s MVC. A practice sessions was scheduled (Gandevia, 2001).

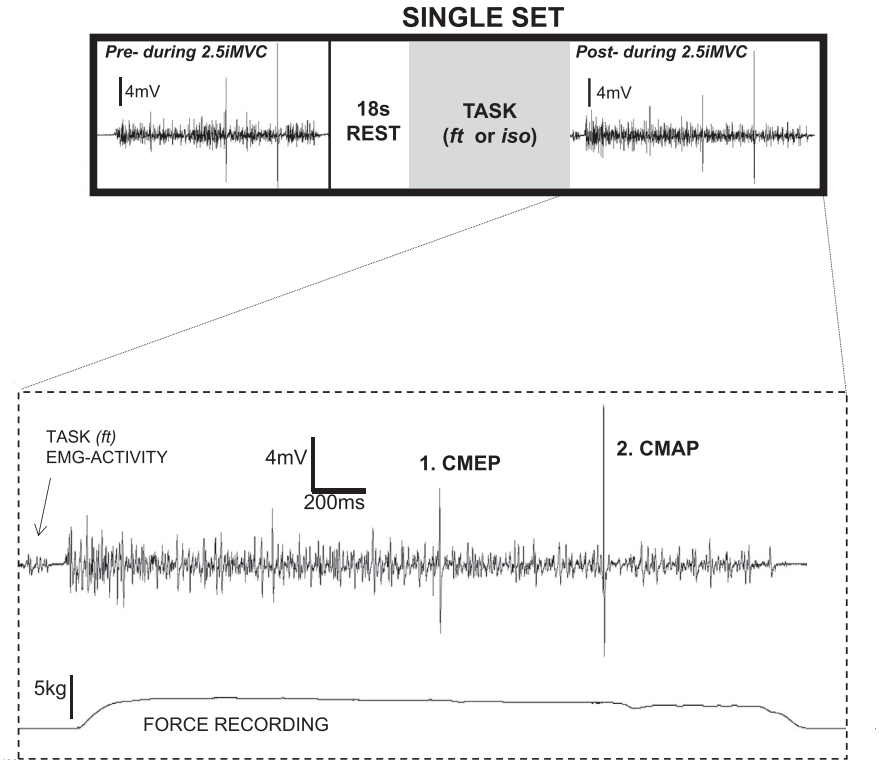

Fig. 1. The set-structure: as soon as an LED was lit, subjects performed an isometric maximum voluntary contraction (MVC) with their index against a dynamometer. The LED off (2.5 s after) served as a signal to stop the MVC. During the MVC subjects received two different types of stimulation to get: 1st CMEP, at 1.5 s after LED on; and 2nd CMAP, at 2.2 s after LED on (*pre*). After this *paired*-stimulation subjects rested for 18 s. Then, an LED flash indicated the start of the task (*ft* in one session; *iso* in the other). In a continuation of the task, and with no resting time, the subjects performed another 2.5-s MVC in response to the LED turning on, and received stimulation (*post*; in the same way as *pre*).

Setting, recording and stimulation protocols

The subjects were sitting comfortably with the elbow flexed at 90–100°. The forearm, wrist, hand and all fingers except the index were firmly but comfortably immobilized, and fixed to a modified tablet-arm chair. The setup allowed unrestrained degrees of freedom at the metacarpophalangeal joint of the index finger, permitting *ft*. During *ft*, a Biometrics DataLink (Biometrics Ltd, Gwent, NP11 7HZ, UK) system recorded the inter-tap intervals at 0.1 kHz with a thin metal plate and a metal ring, the latter adapted to the distal phalanx of the index finger. It also recorded (at 500 Hz) the isometric force exerted during the MVC of the index flexion with a Pinch-Dynamometer (P200), which was placed flat and secured over the tablet, with the thin metal plate used to record tapping attached flat on its top. A single axis finger goniometer (F35) (sampled at 1 kHz) controlled the flexo-extension movement amplitudes of the index finger metacarpophalangeal joint. Electromyographic activity from the superficial head of the first dorsal interosseous muscle (FDI) was monitored with surface electrodes in a belly tendon arrangement, and acquired by means of D360 amplifiers (Digitimer, Welwyn Garden City, Herts), amplified (250–1000×) and bandwidth filtered between 3 and 3000 Hz. The involvement of the muscle in the tasks has been shown elsewhere (Arias et al., 2015).

A CED 1401 mkII Power A-D converter (Cambridge Electronic Design, Cambridge, UK) sampled EMG at 10 kHz; it also controlled a red LED (indicating the task execution/rest phases) and the timing of stimulation pulses.

Electric and magnetic CMS

Electric CMS was applied using a Digitimer D180 stimulator by means of Ag–AgCl electrodes located behind the mastoid processes (anode at the right, cathode at the left).

Magnetic CMS was applied using a MagStim 200^2 (Whitland, Carmarthenshire, UK) stimulator connected to a double cone coil. The center of the coil was placed over the inion (in some subjects slightly lateral) and the direction of the current flowed down in the coil (Taylor and Gandevia, 2004).

Active motor threshold (AMT) was defined as the minimum intensity required to evoke 5 liminal responses (approximately 200 μV) in 10 consecutive pulses in the activated muscle (5–10% MVC) (Rossini et al., 1994). The stimulation intensity for the protocol was set to obtain a CMEP amplitude of approximately 50% amplitude of the CMAP during MVC in the fresh muscle. To check the absence of current spread to the spinal roots, the CMEP latency was compared to that obtained at AMT intensity, such that the amplitude of the potentials increased in size with voluntary contraction with no or liminal latency shift when stimulated at the higher level (Taylor and Gandevia, 2004).

We set 50% of CMAP as the target for baseline values of the CMEPs to be more likely away from the ceiling, to monitor the potential modulation of the CMEPs induced by fatigue; previous reports have indicated that CMEPs may reach, at least, 70% the CMAP when recorded in arm muscles (Butler et al., 2003).

Supramaximal electric stimulation of the ulnar nerve

The FDI electrodes recorded the CMAP to ulnar nerve stimulation at the elbow (Digitimer DS7A stimulator). The anode was placed lateral to the medial epicondyle along the postcondylar groove and the cathode approximately 2 cm distal to the anode, along the direction of the nerve. A 1000-μs square pulse stimulus was used at an intensity 50% above the supramaximal, and delivered during the 2.5-s-MVC.

Data reduction

The following dependent variables were analyzed:

CMEP and CMAP amplitudes: These were defined as the peak-to-peak amplitude. CMEP was normalized to the CMAP amplitude acquired at the same time point (i.e., during the same 2.5 s MVC). We analyzed the effect of fatigue on the CMEP and CMAP amplitudes.

EMG Root Mean Square amplitude at the time of CMEP testing: Amplitude of the EMG activity in the 50-ms time window prior to CMEP (EMG-$RMS_{PRIOR\text{-}CMEP}$). This value was normalized in relation to the RMS of the CMAP acquired at the same time point (i.e., during the same 2.5 s MVC).

Motor output during task execution: We considered three measures of motor output for task execution: the tapping frequency and angular amplitude for *ft*, and the force applied for *iso*. For each of the 4 Sets and 3 Modes (*comfort; 10 max* and *30 max)* we considered two time points that were embedded within task execution: the initial 3 s *(i3; pre*) and the final 3 s (*f3*; *post*). To compare data from *ft* frequency and *iso* torque, we expressed the motor output at all evaluation time points as a function of the maximum obtained at any time point for each task and subject.

For normalizing the *ft* range of motion (ROM) amplitude, we recorded the maximum (active) ROM of the index finger metacarpophalangeal joint for each subject before the protocol. The score served as divisor for the amplitude displayed at all the individual's evaluation time points during the *ft* task.

MVC (before and following task execution): We evaluated the MVC force at the time of CMS (MVC in the 50-ms period before the stimulation) either for *ft* and *iso* task, normalized to the maximum MVC obtained at any of the CMS evaluation time points for each task and subject.

SP duration (recorded during the MVC explained just above): The SP duration was defined as the time lag from the CMS pulse to the recovery of the EMG activity during the brief MVC, and determined visually by an experienced researcher blind to the conditions; under these conditions the test–retest reliability (on 100 randomly chosen SP) has been shown to be excellent for this methodology of SP determination in our previous work (Arias et al., 2015). The SP durations were normalized for their analyses. For each subject and task (*ft* or *iso*), we took as 100% the average value from all the evaluation time points of the four sets executed in the *comfort* mode, which served as divisor for all the subject's values for all execution modes. Therefore, in the figures representing the SP duration, the unit represents its average duration at *comfort* mode.

Statistical design

Statistical design to studying the effect of CMS on the amplitude of the CMAP. In our work, we recorded the CMAP 700 ms after the CMEP, during the same 2.5-s MVC. We examined if there was any influence of the preceding CMS on the amplitude of the CMAP as follows. Before each testing day (20 min before the fatigue protocol), subjects executed the 2.5-s MVC (with a 100-s rest interval) six times. In three MVCs both CMEPs and CMAPs were acquired (as in the protocol); in the other three we did not apply CMS and the order was randomized. The average of the three CMAP amplitudes (potentially) conditioned by CMS was compared to the average obtained from the unconditioned CMAPs. An ANOVA was performed with factors Conditioning (two levels, *conditioned* and *unconditioned* CMAP amplitude) and Day (levels *day-1* and *day-2*, as this was evaluated for two days). We also evaluated the intra-class correlation coefficients (ICC) considering the Conditioned vs. Unconditioned responses.

Statistical design for studying behavior during fatigue tasks. Fatigue induced by the *ft* and *iso* tasks was evaluated with repeated measures ANOVA. Previously, we checked the normality of distribution by means of Kolgomorov–Smirnov test for one sample.

For each of the variables (normalized CMEP amplitude; EMG-$RMS_{PRIOR\text{-}CMEP}$; motor output decrement -tapping rate in *ft* and force in *iso*-; SP durations; MVC before and after task execution; and CMAP amplitude) we used an independent ANOVA with repeated measures. The ANOVA included several within-subject factors: The within-subject factors were Task (*ft, iso*), execution Mode (*comfort, 10 max, 30 max*), Set (the four sets for each execution mode), and evaluation Time points (*pre, post*). The levels of the latter factor were termed *i3* and *f3* when the variable analyzed was the motor output decrease in *ft* or *iso* tasks (as it included the initial and final 3 s embedded in task execution).

For the ANOVA of the ROM amplitude, only analyzed for *ft*, factor Task was excluded.

Results are expressed as the mean and the standard error of the mean (*s.e.m*). During ANOVA execution, the degrees of freedom were corrected with Greenhouse Coefficients (ε) if sphericity could not be assumed. Significance was set at $p < 0.05$. A Bonferroni correction was used for follow-up *post hoc* comparisons involving multiple levels within the factor.

Subsequent analyses were carried out to determine if the responses obtained with magnetic and electric stimulators were different; this analysis is justified since magnetic coil geometry might increase the risk of recruiting some cortical neurons. The model was the same as above but included a between-subjects factor (Group, with two levels: electric and magnetic).

RESULTS

The mean output intensity used for magnetic CMS was 89.8% (*s.e.m* 4.3) (in 9 of the 16 sessions it was at 100%); for electric CMS was 551.0 V (*s.e.m* 30.9). Before the fatigue sessions, we observed that the CMS (magnetic and electric) did not condition CMAP amplitudes acquired at the same 2.5-s MVC ($F_{1,14} = 0.7$ $p > 0.05_{CONDITIONING}$); this happened the two testing days ($F_{1,14} = 0.7$ $p > 0.05_{DAY}$; and $F_{1,14} = 0.1$

$p > 0.05_{DAY \times CONDITIONING}$). The ICC were excellent when comparing the conditioned vs. the unconditioned CMAP amplitudes (0.98 [0.96–0.99, CI95%]) mean of the two days pooling electric and magnetic CMS, as well as in isolation: 0.98 [0.96–0.99, CI95%] for electric and 0.98 [0.95–0.99, CI95%] for magnetic stimulation. Therefore, CMS did not influence the CMAP amplitude acquired at the same MVC.

The Table 1 shows the normalizing values for the fatigue protocol, equivalent to the units depicted in the graphs.

The Table 2 indicates the main effects and significant interactions for all variables, one column for each of the evaluated variables. For each variable (column), the first row corresponds to the ANOVA model with the two tasks (*ft* and *iso*), the three execution modes, the four sets and the two testing time points. In some variables, the interactions of Task with other factors were significant and indicated a different response for *ft* and *iso*; in those cases a follow-up ANOVA was executed by task type (indicated in subsequent rows). We proceeded likewise if the responses differed for execution mode (i.e., ANOVA by pair of modes and, eventually, with just one mode). *Post-hoc* analyses are shown in the figures.

CMEP amplitude modulation during fatiguing *ft* and *iso* tasks

CMEP amplitudes (relative to CMAP) behaved differently in the three modes of execution for *ft* and *iso* tasks (ANOVA $p < 0.05$), and also differently at *pre*/*post* in each of the different modes (ANOVA $p < 0.01$). The responses can be observed in Fig. 2 (representative individual examples during isometric maximal modes) and Fig. 3, means for all subjects in all modes and tasks..

CMEP amplitude after fatiguing *ft* tasks

During *ft*, CMEP at *pre* and *post* also behaved differently for the three execution modes (ANOVA $p < 0.01$); thus we performed follow-up ANOVA by pair of modes and modes in isolation. For *comfort-ft* the CMEP did not change after tapping (ANOVA $p > 0.05$). Conversely, ANOVA including both maximal modes indicated that the amplitude of the potentials increased right at the end of maximal *ft* (ANOVA $p < 0.05$); the effect of TIME did not interact significantly with any other factor (i.e., mode *–10 max* and *30 max*; or set), thus a small (5%) but significant increase in the spinal excitability was shown after maximal *ft*.

CMEP amplitude after fatiguing *iso tasks*

For the *iso* task, the CMEP amplitudes changed with the three execution modes (ANOVA $p < 0.05$), and also at the end of the tasks (ANOVA $p < 0.05$); a similar pattern was observed if the analysis was performed by pairs of modes. When analyzing the effects mode by mode and in the case of *comfort*, main effects of TIME or SET were not significant, nor their interaction ($p > 0.05$ in all cases). For *10 max* CMEP increased significantly at the end of execution (ANOVA $p < 0.05$), from *pre*-values of 44.2% to *post*-values of 52.1% of the CMAP-amplitude (increment of 17.9%); the effect was observed in all sets. For *30 max,* main effects and interactions were always $p > 0.15$; thus CMEP *post 30 max* were similar to those observed at *pre*.

EMG-RMS$_{PRIOR\text{-}CMEP}$

The EMG-RMS$_{PRIOR\text{-}CMEP}$ differed for the two tasks and modes at *pre* and *post* (Fig. 4, ANOVA $p < 0.001$). This was observed since it remained stable at all times for *iso* ($p = 0.115$ was the smallest *p*-value for main effects and interactions), but not for *ft*. For *ft*, EMG-RMS$_{PRIOR\text{-}CMEP}$ changed from *pre* to *post*, differently for the three tapping modes (ANOVA $p < 0.01$). It was reduced after *comfort* tapping (ANOVA $p < 0.05$) and increased after *30max* (ANOVA $p < 0.01$), with no set effects. Before and after *10max* the EMG-RMS$_{PRIOR\text{-}CMEP}$ remained unchanged.

Task motor execution. Frequency/force decrement during execution in *ft*/*iso* tasks

The observed motor output decrements along the studied tasks (initial vs. final 3 s of each set) differed for *ft* and *iso*, and three execution modes (*comfort*, *10 max* and *30 max*) (ANOVA $p = 0.001$). Subsequently, we performed the analyses for each type of task.

Task motor execution during finger tapping

For *ft*, the frequency of tapping dropped along execution, but the three modes of execution were affected differently (ANOVA $p < 0.001$). *Ft* frequency at *comfort* was unchanged, but it dropped after *10 max* and *30 max* (ANOVA $p < 0.001$, Fig. 5A); in this latter case there was also an effect on set progression (ANOVA $p < 0.05$), which affected similarly the initial and final seconds of execution.

Task motor execution during isometric contraction

The Fig. 5B shows the force drop in the *i3* and *f3 s* periods of the *iso*-task. Force dropped differently at the end of the *iso*-task for the three different modes (ANOVA $p < 0.001$). Additionally, a set effect was present with different expressions in the three execution modes

Table 1. Mean score including all subjects. The score corresponds with the *y*-axis unit in graphs.

Task	*Max output in task at any evaluation time-point	*Max active full ROM	*MVC at any stimulation time-point	Mean CMS-SP duration at comfort
ft	7.4 Hz (*s.e.m* 0.3)	33.0° (*s.e.m* 1.9)	4.2 kg (*s.e.m* 0.2)	61.1 ms (*s.e.m* 2.7)
iso	3.7 kg (*s.e.m* 0.1)	n.a.	4.1 kg (*s.e.m* 0.2)	67.0 ms (*s.e.m* 4.0)

n.a: not applicable.

* The score utilizes the maximal value from each subject: it is the mean of the maximal scores including all subjects.

Table 2. Summary of main effects and interactions for the different variables

CMEP amplitude	EMG-RMS$_{PRIOR-CMEP}$	Task motor execution	MVC force at CMS	SP duration	CMAP amplitude	Row
$F_{1,14}$ = 12.4 **$p < 0.01$**$_{TASK\ X\ MODE}$ $F_{2,28}$ = 3.9**$p < 0.05$**$_{MODE\ X\ TIME}$	$F_{2,28}$ = 9.6 **$p = 0.001$**$_{TASK\ X\ MODE\ X\ TIME}$	$F_{2,28}$ = 90.9 **$p < 0.001$**$_{MODE\ X\ TIME}$ $F_{2,28}$ = 8.6 **$p = 0.001$**$_{TASK\ X\ MODE\ X\ TIME}$	$F_{1,14}$ = 12.8 **$p < 0.01$**$_{TASK\ X\ TIME}$	$F_{1,14}$ = 14.7 **$p < 0.01$**$_{TIME\ X\ TASK}$	$F_{2,28}$ = 5.4 **$p < 0.05$**$_{TASK\ X\ MODE}$	1st
FINGER TAPPING		***FINGER TAPPING***	***FINGER TAPPING***	***FINGER TAPPING***	***FINGER TAPPING***	
$F_{2,28}$ = 6.8**$p < 0.01$**$_{MODE\ X\ TIME}$	$F_{2,28}$ = 9.7$_{\varepsilon=0.6}$ **$p < 0.01$**$_{MODE\ X\ TIME}$	$F_{1,14}$ = 101.1 **$p < 0.001$**$_{TIME}$ $F_{2,28}$ = 45.4 **$p < 0.001$**$_{MODE\ X\ TIME}$	$F_{1,14}$ = 5.8 **$p < 0.05$**$_{TIME}$ $F_{2,28}$ = 10.2 $_{\varepsilon=0.6}$ **$p < 0.01$**$_{MODE}$ $F_{3,42}$ = 4.0 **$p < 0.05$**$_{SET}$	$p > 0.05$ for factors and interactions	$p > 0.05$ for factors and interactions	2nd
Comfort	***Comfort***	***Comfort***	N.A	N.A	N.A	
$F_{1,14}$ = 2.6 $p > 0.05$$_{TIME}$	$F_{1,14}$ = 5.6 **$p < 0.05$**$_{TIME}$	$p > 0.05$				3rd
10max & 30max	***10max***	***10max***	N.A	N.A	N.A	
$F_{1,14}$ = 5.5**$p < 0.05$**$_{TIME}$ $p > 0.05$ for interactions	$F_{1,14}$ = 1.1 $p > 0.05$$_{TIME}$	$F_{1,14}$ = 39.3 **$p < 0.001$**$_{TIME}$				4th
N.A	***30max***	***30max***	N.A	N.A	N.A	
	$F_{1,14}$ = 5.6 **$p < 0.01$**$_{TIME}$	$F_{1,14}$ = 81.8 **$p < 0.001$**$_{TIME}$ $F_{3,42}$ = 3.6 **$p < 0.05$**$_{SET}$				5th
ISOMETRIC		***ISOMETRIC***	***ISOMETRIC***	***ISOMETRIC***	***ISOMETRIC***	
$F_{1,14}$ = 4.8**$p < 0.05$**$_{TIME}$ $F_{2,28}$ = 5.4 **$p < 0.05$**$_{MODE}$	$p > 0.05$ for factors and interactions	$F_{2,28}$ = 90.9 **$p < 0.001$**$_{MODE\ X\ TIME}$ $F_{6,84}$ = 5.4 $_{\varepsilon=0.5}$ **$p < 0.01$**$_{MODE\ X\ SET}$	$F_{2,28}$ = 25.8 **$p < 0.001$**$_{TIME\ X\ MODE}$	$F_{1,14}$ = 13.9 **$p < 0.01$**$_{TIME}$ $F_{2,28}$ = 3.6 **$p < 0.05$**$_{TIME\ X\ MODE}$	$F_{2,28}$ = 3.8 **$p = 0.051$**$_{MODE}$ $F_{2,28}$ = 3.8 **$p < 0.05$**$_{TIME\ X\ MODE}$	6th
Comfort		***Comfort***	***Comfort***	***Comfort***	***Comfort***	
$p > 0.05$ for factors and interactions		$F_{1,14}$ = 17.2 **$p < 0.001$**$_{TIME}$	$F_{1,14}$ = 15.9 **$p < 0.01$**$_{TIME}$ $F_{3,42}$ = 4.6 **$p < 0.05$** $_{SET}$	$F_{1,14}$ = 12.4 **$p < 0.01$**$_{TIME}$	p > 0.05 for factors and interactions	7th
10max		***10max***	***10max***	***10max***	***10max***	
$F_{1,14}$ = 6.4 **$p < 0.05$**$_{TIME}$		$F_{1,14}$ = 23.8 **p < 0.001**$_{TIME}$ $F_{3,42}$ = 5.2 $_{\varepsilon=0.6}$ **$p < 0.05$**$_{SET}$	$F_{1,14}$ = 17.6 **$p < 0.001$**$_{TIME}$ $F_{3,42}$ = 6.0 **p < 0.01** $_{SET}$	$F_{1,14}$ = 7.6 **$p < 0.05$**$_{TIME}$	$p > 0.05$ for factors and interactions	8th
30max		***30max***	***30max***	***30max***	***30max***	
$p > 0.05$ for factors and interactions		$F_{1,14}$ = 119.9 **$p < 0.001$**$_{TIME}$ $F_{3,42}$ = 25.3 **$p < 0.001$**$_{SET}$	$F_{1,14}$ = 114.0 **$p < 0.001$**$_{TIME}$ $F_{3,42}$ = 25.5 **$p < 0.01$** $_{SET}$	$F_{1,14}$ = 10.7 **$p < 0.01$**$_{TIME}$	$p > 0.05$ ($p = 0.09$)	9th

The first (un-shaded) row of statistical values corresponds to the ANOVA model with the two tasks (ft & iso), the three execution modes, the four sets and the two testing time points. For some variables, the interactions of Task with other factor/s were significant and indicated a different response of those variables for *ft* and *iso*; in these cases follow-up ANOVA were executed by task type (in subsequent rows). We proceeded likewise with subsequent ANOVA if the responses differed for execution modes (i.e., ANOVA were executed by pair of modes and, eventually, with just one mode). Those spaces left empty are shown when subsequent ANOVA were not applicable (N.A), because interactions were not significant.

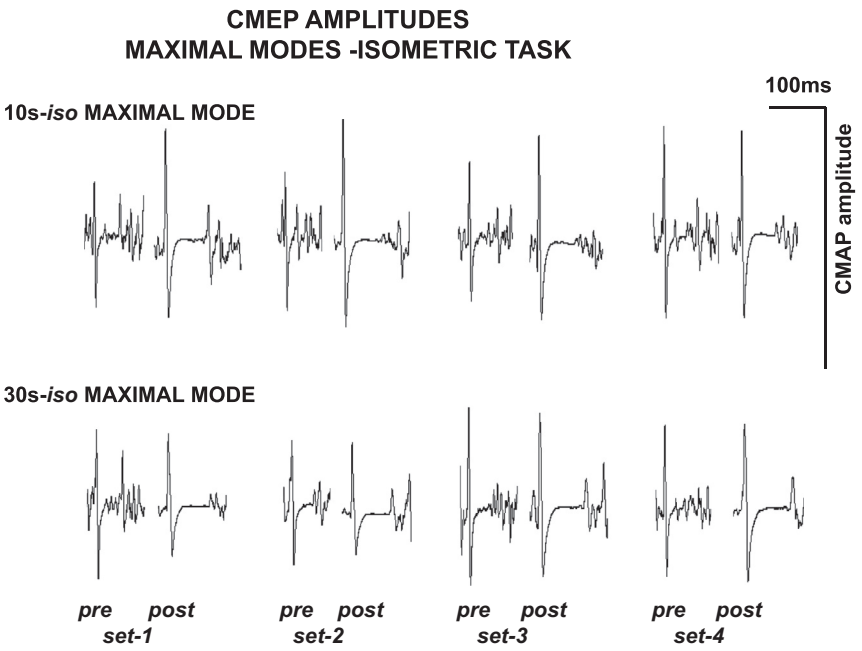

Fig. 2. Examples taken from a representative subject for *iso* maximal modes. The amplitudes are scaled so that the amplitude of the vertical line at the right corresponds to the size of the corresponding CMAP.

(ANOVA $p < 0.001$). Follow-up ANOVA by modes indicated that for *10 max-iso post* the force dropped significantly (ANOVA $p < 0.001$), and set after set (ANOVA $p < 0.05$). A similar pattern was observed for *30 max-iso,* but more significantly expressed (ANOVA $p < 0.001$ for TIME and SET). Conversely, for *comfort* there was no set effect ($p > 0.05$), though the force at the end of the 30 s of *comfort* was significantly lower that at the beginning (ANOVA $p < 0.001$, Fig. 6), the effect was small. This means that, at the end of execution, subjects remained slightly below (1.5%) *pre*, when trying to maintained the target proposed (i.e., 30% of their MVC).

Angular amplitude during *ft*

ROM amplitudes remained constant from *pre* to *post* ($F_{1,14} = 0.2$ $p > 0.05_{TIME}$) but were different in the three modes ($F_{2,28} = 13.2$ $_{\varepsilon=0.6}$ $p < 0.01_{MODE}$). For *10max* it was 13.1% (*s.e.m* 2.9) of the maximal active ROM, and 12.6% (*s.e.m* 2.3) for *30max*. In both cases smaller than *comfort* (23.8%, *s.e.m* 2.9); *post hoc* $p < 0.01$.

MVC force at the time of CMS

The MVC force at the time of CMS waned at post; this was expressed differently in the two tasks, ft and iso (ANOVA $p < 0.01$).

MVC at the time of stimulation after finger tapping

For the *ft* task, MVC force reduced at *post* (ANOVA $p < 0.05$), from mode to mode (ANOVA $p < 0.01$), and

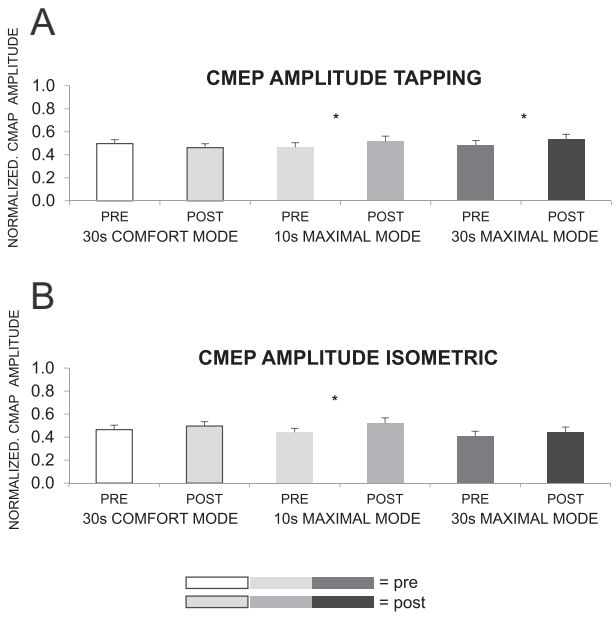

Fig. 3. CMEP amplitude changes in both tasks. CMEP amplitudes are normalized to the amplitude of the corresponding CMAP. (A) For *ft* the amplitudes were significantly increased at the end of both maximal execution modes. (B) For *iso* the amplitudes were significantly increased only at the end of *10 max*, but not for *30 max.* $^{*}p < 0.05$.

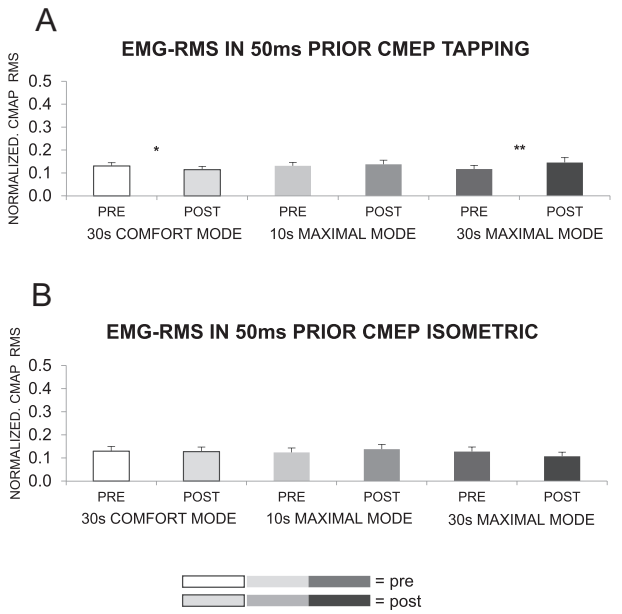

Fig. 4. EMG-RMS during MVCs at the time of CMS. EMG-RMS are normalized to the RMS of the corresponding CMAP. (A) Right after *ft* the EMG-RMS decreased for the *30 comfort*, and increased for the *30 max* modes. (B) For *iso* EMG-RMS remained unchanged after execution for all modes. $^{*}p < 0.05$; $^{**}p < 0.01$.

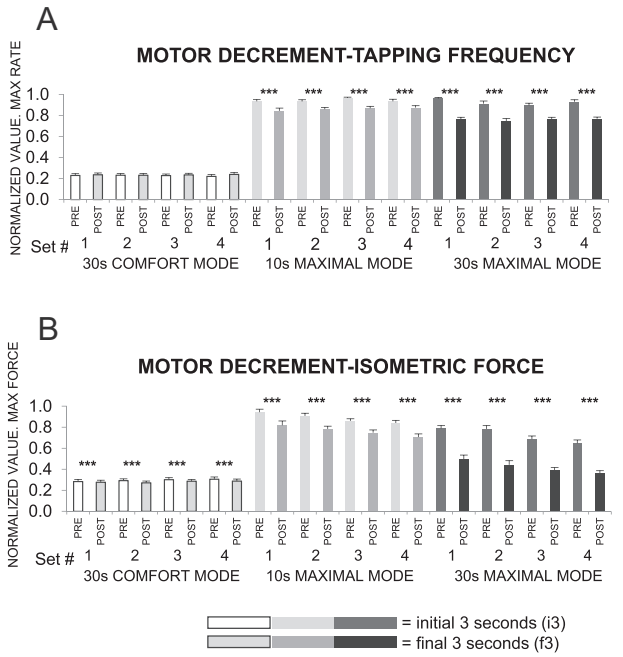

Fig. 5. Motor execution decrement induced by the tasks. (A) The frequency of *ft* decreased significantly after the maximal modes. For *30 max* a set effect was present. (B) Set by set motor decrement along the *max iso* tasks was more evident. The force reduced at *post* after *maximal* modes and also after *comfort*. $^{***}p < 0.001$.

set to set (ANOVA $p < 0.05$). Thus, *post* MVC waned always after tapping (compared to *pre*). A reduction in MVC with the progression of the testing protocol was also observed in all cases (modes and sets) (Fig. 7A).

MVC at the time of stimulation after isometric contraction

The case of *iso* was different, as the MVC dropping from *pre* to *post* was differently expressed for the three modes (ANOVA $p < 0.001$; Fig. 7B).

Analyses by modes indicated, however, that in all modes MVC dropped from *pre* to *post* (ANOVA $p < 0.01$ for *Comfort*; and $p < 0.001$ for *10max* and *30max*), and set after set (ANOVA $p < 0.05$ for *Comfort*; and $p < 0.01$ for *10max* and *30max*).

SP after *ft* and *iso* tasks

At *post* the SP change was significantly different for the two tasks (ANOVA $p < 0.01$), this is clearly observable in the representative individual recordings shown in Fig. 8.

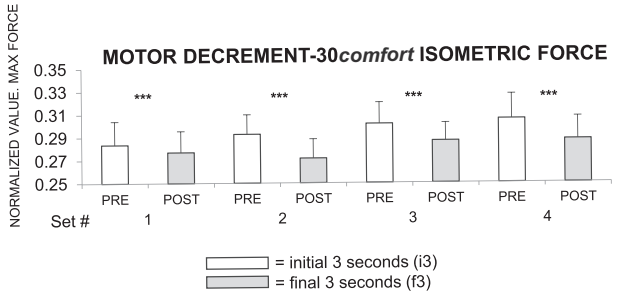

Fig. 6. Enlarged view to observe the size of the motor decrement for *comfort-iso*. $^{***}p < 0.001$.

SP after *ft tasks*

The SPs were not modified after *ft*, regardless of the mode of execution or set progression ($p > 0.05$ for all main effects and interactions). Thus, clearly, the fatiguing *ft* activity had no reflection in SPs, Fig. 9A.

SP after *iso tasks*

SP increased after *iso* execution, and such an increment was different in magnitude for the three execution modes (ANOVA $p < 0.05$). It turned out to be significant in all the cases (Fig. 9B). For *comfort-iso* SP increased by 7% (ANOVA $p < 0.01$), for *10 max-iso* the increment reached 15% (ANOVA $p < 0.01$), and for *30 max-iso* the increase in SP at *post was* 23% (ANOVA $p < 0.01$; such effects were present in all sets.

CMAP-amplitude modulation during fatiguing *ft* and *iso* tasks

The CMAP-amplitude responses were modified differently in the different tasks for the different modes (ANOVA $p < 0.05$).

CMAP-amplitude modulation during fatiguing *ft* tasks

For *ft,* all main effects and interactions between factors were non-significant ($p > 0.8$ was the smallest p value; Fig. 10A). Thus, CMAPs were not modified at any moment of testing in the *ft* tasks.

CMAP-amplitude modulation during fatiguing *iso* tasks

For the *iso* tasks (Fig. 10B), the CMAP amplitude changed from mode to mode (ANOVA $p = 0.051$); the

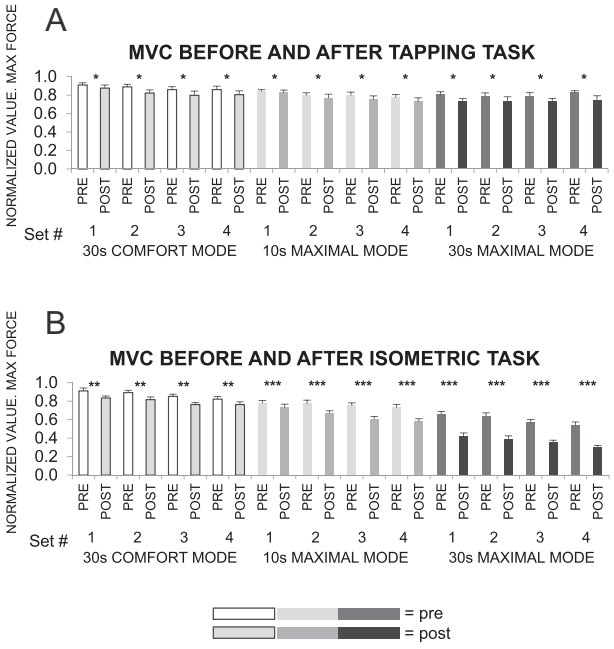

Fig. 7. MVC force at the time of CMS in both tasks. (A) MVC force was reduced at *post-ft* compared to *pre* in all cases, and also from mode to mode and set to set. (B) MVC force reduction with *iso* task resembled the pattern of *ft* but the effect was larger and more significantly expressed. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

amplitude was always smaller at *30 max* than *10 max* (both *pre* and *post; post hoc* $p < 0.05$). It was also observed that the change from *pre* to *post* was significantly different for the three modes (ANOVA $p < 0.05$), although *post hoc* analysis indicated that the smallest *p*-value was $p = 0.09$, for *30 max*.

Responses induced by electric vs. magnetic CMS

Finally, we reproduced the above-mentioned analyses modifying the statistical design; the within-subjects factors were the same as above (*task, mode, set and time*), but a between-subjects factor (*group*) with two levels (electric-CMS, and magnetic-CMS) was added to check if the responses induced by both types of stimulation differed. This was never the case.

DISCUSSION

In a previous study, we suggested a supraspinal but not spinal origin of fatigue induced by short-lasting maximal *ft* tasks. This was based on the waning of *ft* frequency with task progression, a reduction of the MVC executed right after *ft*, and the increasing of SP if induced by TMS but not by CMS during such MVC *post* task execution (Arias et al., 2015). The present work reinforces the idea that the origin of the fatigue leading to *ft* frequency reduction (with no effect on amplitude) is not located at the spinal cord because the CMS-SPs were not modulated by fatigue and the amplitude of the CMEP did not wane with motor output reduction. Different effects were observed for maximal *iso* tasks. Because CMEPs obtained in this study were analyzed relative to the amplitude of the CMAP, the possible confounding factors affecting the neuromuscular junction, which can bias the interpretation of CMEP amplitude values, were controlled for. CMS (either magnetic or electric) is a technique that generates large short-latency responses with a main

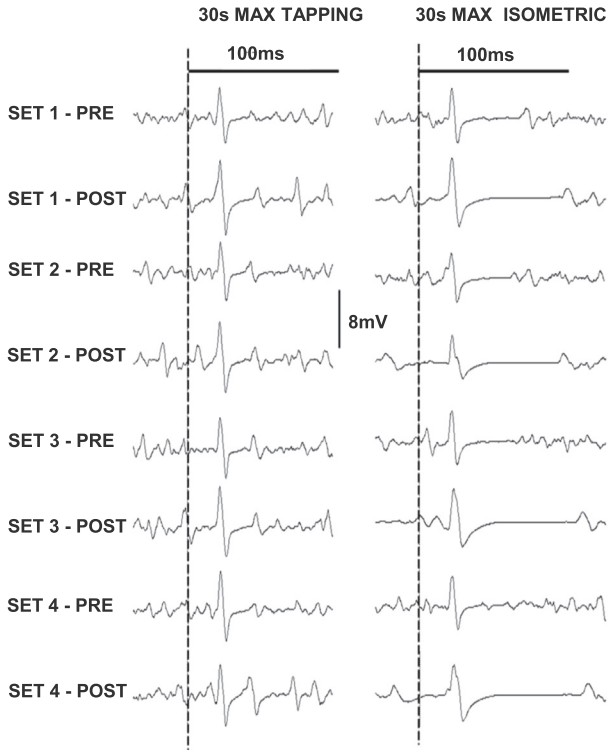

Fig. 8. Examples of silent periods acquired during the 30-s *maximal mode* before and after each set for *ft* and *iso* tasks (for the same representative subject). The increase in the SP duration *post* activity in the case of *iso* is very clear, with no set effect.

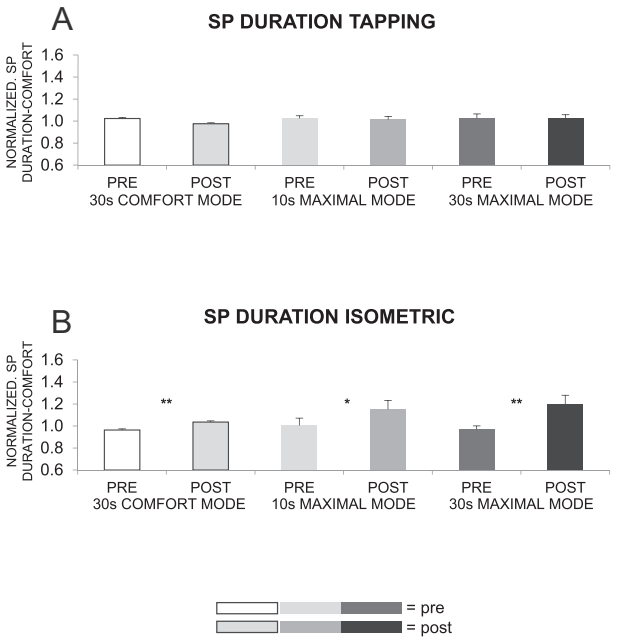

Fig. 9. Silent period changes observed in both tasks in all subjects. (A) For *ft*, SP were never modulated by the execution. (B) SP increased after *iso* tasks for all modes. $^{*}p < 0.05$; $^{**}p < 0.01$.

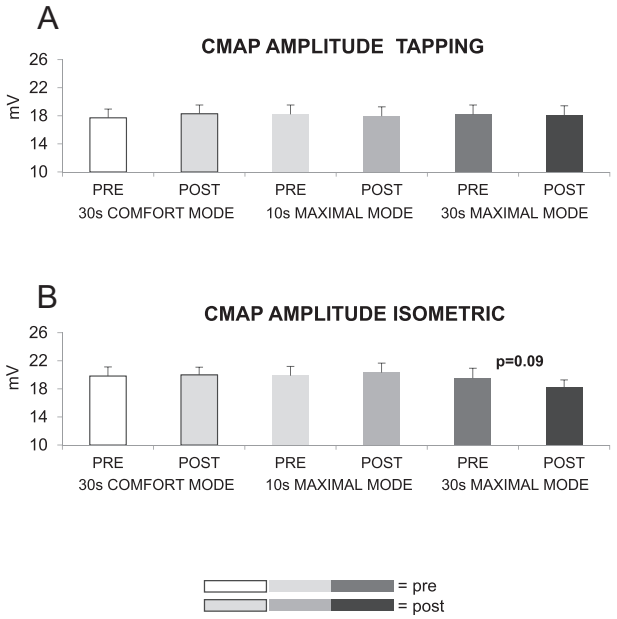

Fig. 10. CMAP amplitude changes achieved in both tasks were different. (A) The *ft* did not change the CMAP amplitudes. (B) The change in the CMAP amplitudes at the *post iso-30 max* did not reach significance (see main text for changes other than those reported in *pre-post* conditions).

motoneuronal monosynaptic component by the stimulation of the axons at the corticospinal tract level (Taylor and Gandevia, 2004). For such reasons it provides a direct way to evaluate the behavior of spinal motoneurons at rest and in different tasks in human participants. Because presynaptic inhibition has not been described in the corticospinal axons (Nielsen and Petersen, 1994), the use of CMS is optimum for testing spinal motoneuronal excitability compared to other techniques (McNeil et al., 2013).

Fatigue task-dependency was manifested also in the EMG just preceding CMS, during the MVC. Stephen & Taylor reported (1972) that EMG activity decreases with MVC of long duration; a drop that in our 30 max *iso* did not reach significance, perhaps due to the shorter effort inherent to our task in comparison with the one used by Stephen & Taylor. Conversely, we have observed that 30 s of tapping at maximal rate increased the RMS. The profile of the RMS resembles, somehow, the responses of the CMEPs. This was expectable since the CMEPs amplitude depends, in part, on the preceding motoneuronal activity just prior stimulation. However, CMEPs are optimal to evaluate motoneuronal spinal excitability because they arise from a monosynaptic response (Ugawa et al., 1991; McNeil et al., 2013), while the origin of ongoing EMG activity is heterogeneous and includes: The supra-spinal descending drive; the afferent input to the spinal cord resulting from the stimulation of mechanoreceptors or nociceptors of hand fingers (Leis et al., 2000; McNulty and Macefield, 2001; Pierrot-Deseilligny and Burke, 2005); or the pre-synaptic inhibition of Ia fibers arising from tendon afferents (Priori et al., 1994), a form of inhibition not reflected in CMEPs (Nielsen and Petersen, 1994). Ongoing EMG is also influenced by the transmission in the periphery (Stephens and Taylor, 1972), for which we normalized the ongoing EMG-RMS relative to the CMAP-RMS, both acquired at a same time. Some of these mechanisms may account for different responses of CMEPs and its immediately preceding EMG-RMS activity obtained in our study.

Responses during fatiguing *ft* tasks

Earlier, we showed that 10 and 30 s of *ft* at maximal rate induced an effect compatible with an excitability increment of inhibitory GABAb interneurons located at the motor cortex, in parallel with the inability to maintain maximal rates of *ft* frequency and a loss in muscle force. In addition, Renshaw recurrent inhibition and axonal after hyperpolarization, which are known to be responsible for SP induced by CMS at spinal levels (Inghilleri et al., 1993), remained unchanged (Arias et al., 2015). We now extend our previous results by showing that motoneuron excitability, directly evaluated by CMEP amplitudes, seems not to be responsible for the tapping rate reduction during maximal tapping rates, up to 30 s. The spinal excitability increased at the end of both maximal activities (10 and 30 s), but *ft* rate decreased (15% at *10 max post*, and 25% *30 max post*). Therefore, the increment of excitability can hardly be considered a sign of motoneuronal fatigue but perhaps a neuronal substrate to facilitate maximal effort tasks.

Responses during fatiguing *iso* tasks

The pattern of fatigue observed for *iso* tasks is in agreement with a good deal of scientific research showing a reduction of spinal excitability during fatiguing isometric muscular activities (Taylor et al., 1996; Duchateau et al., 2002; Butler et al., 2003; Klass et al., 2008; McNeil et al., 2009). A characteristic finding was the increase in the SP induced by CMS during the MVC after maximal *iso* modes (Taylor et al., 1996; McNeil et al., 2009), which was absent for the *ft* task (Arias et al., 2015).

Considering the amplitudes of the responses, some previous work has reported that during 2-min MVC the CMAP area wanes in the last ≈30 s of the task, compared to its maximum value at the initial part of the task (Butler et al., 2003). In our work the *iso* protocol modified the CMAP amplitudes significantly, not in the comparison of *pre* vs. *post* within sets, but in how the modification of CMAP from *pre* to *post* progressed along the modes, perhaps showing that the changes were cumulative and slowly developed over time. Such effects on CMAP during *iso* might condition the interpretation of CMEP amplitudes, and the evaluation of task-dependent effects (Enoka and Stuart, 1992; Barry and Enoka, 2007). However, when analyzed relative to the CMAP amplitude at the same time of testing, we observed that the CMEP amplitude presented a different profile for *10 s* and *30 s iso-max*. In both cases, set after set effects were absent, but CMEP amplitudes *post 10 s iso-max* were larger than *pre* values (in agreement with both maximal *ft* modes). We suggest that the increased excitability is an adaptation of the motoneurons that contributes and permits the execution during maximal activity. However, at the end of the *30 s iso-max* the increased level of spinal excitability (compared to *pre*) could not be maintained; for this reason we suggest that the inability to maintain an increased level of spinal excitability is a hallmark of spinal fatigue. Our results complement those by Butler et al. (2003) obtained in elbow flexors. We observed increased levels of spinal excitability 10 s after task initiation, an effect possibly not observable with different testing time courses, or differentially expressed in small hand muscles (responsible for fine movements and perhaps less adapted to sustained MVC) compared to larger muscles (Butler et al., 2003). In agreement with this idea, it has been shown that motor units of intrinsic hand muscles, and those of larger muscles in the arm, present different firing rates and recruitment patterns. Small muscles increase recruitment of units up to a relatively small level of force (≈50% of MVC), with a need to increase firing rate to produce larger forces; conversely, larger muscles increase recruitment up to higher force levels (≈90% of MVC) and their maximum firing rates are lower (Masakado, 1994). This might account for a different modulatory time course of spinal excitability in our study com-

pared to other works evaluating some other muscles (Butler et al., 2003).

Study limitations

In this study the fatigue induced by *ft* was tested during the course of a MVC. We adopted this methodology because it permits the evaluation of spinal excitability and a direct comparison on how it was influenced by immediately preceding activities of different nature (*ft* and *iso*). It also makes possible the recording of standard variables which are commonly used in this field. We recognize, however, that powerful activations during MVC might overwhelm weak expressions of spinal fatigue induced by *ft*.

CONCLUSIONS

Our works suggest that the development of fatigue induced by short-lasting repetitive tasks emerges in sites other than the spinal cord. The supraspinal origin of fatigue for this type of task (Arias et al., 2015) gains support with the present study. We have provided neurophysiological evidence for different fatigue mechanisms in *ft* and *iso*; however the mechanisms behind the changes in excitability remain unknown, for which further studies are required. Fatigue is one of the main signs in diseases affecting the spinal cord and supraspinal centers, our results may help to improve the design of the clinical tests aimed at the evaluation of fatigue in physiological and neurological conditions.

DISCLOSURE

The authors declare no conflict of interests.

Acknowledgments—Grants: This work was supported by Xunta de Galicia (Conselleria de Educación 2007/000140-0; Ayudas Grupos Consolidados, Consellería de Educación, 2014 and Dirección Xeral de I+D+i; 2010-2012), Spain. VRG & YCB are granted by the FPU-MECD AP2010-2774 & AP2010-2775 Spain.

REFERENCES

Arias P, Robles-Garcia V, Espinosa N, Corral Y, Cudeiro J (2012) Validity of the finger tapping test in Parkinson's disease, elderly and young healthy subjects: is there a role for central fatigue? Clin Neurophysiol 123:2034–2041.

Arias P, Robles-Garcia V, Corral-Bergantinos Y, Madrid A, Espinosa N, Valls-Sole J, Grieve KL, Oliviero A, Cudeiro J (2015) Central fatigue induced by short-lasting finger tapping and isometric tasks: a study of silent periods evoked at spinal and supraspinal levels. Neuroscience 305:316–327.

Barry BK, Enoka RM (2007) The neurobiology of muscle fatigue: 15 years later. Integr Comp Biol 47:465–473.

Bigland-Ritchie B, Woods JJ (1984) Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle Nerve 7:691–699.

Brasil-Neto JP, Pascual-Leone A, Valls-Sole J, Cammarota A, Cohen LG, Hallett M (1993) Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. Exp Brain Res 93:181–184.

Brasil-Neto JP, Cammarota A, Valls-Sole J, Pascual-Leone A, Hallett M, Cohen LG (1995) Role of intracortical mechanisms in the late part of the silent period to transcranial stimulation of the human motor cortex. Acta Neurol Scand 92:383–386.

Butler JE, Taylor JL, Gandevia SC (2003) Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. J Neurosci 23:10224–10230.

Di Lazzaro V, Oliviero A, Tonali PA, Mazzone P, Insola A, Pilato F, Saturno E, Dileone M, Rothwell JC (2003) Direct demonstration of reduction of the output of the human motor cortex induced by a fatiguing muscle contraction. Exp Brain Res 149:535–538.

Duchateau J, Balestra C, Carpentier A, Hainaut K (2002) Reflex regulation during sustained and intermittent submaximal contractions in humans. J Physiol 541:959–967.

Enoka RM, Duchateau J (2008) Muscle fatigue: what, why and how it influences muscle function. J Physiol 586:11–23.

Enoka RM, Stuart DG (1992) Neurobiology of muscle fatigue. J Appl Physiol 72:1631–1648.

Enoka RM, Baudry S, Rudroff T, Farina D, Klass M, Duchateau J (2011) Unraveling the neurophysiology of muscle fatigue. J Electromyogr Kinesiol 21:208–219.

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725–1789.

Gandevia SC, Allen GM, Butler JE, Taylor JL (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 490(Pt 2):529–536.

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

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

Inghilleri M, Berardelli A, Cruccu G, Manfredi M (1993) Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. J Physiol 466:521–534.

Jancke L, Specht K, Mirzazade S, Loose R, Himmelbach M, Lutz K, Shah NJ (1998) A parametric analysis of the 'rate effect' in the sensorimotor cortex: a functional magnetic resonance imaging analysis in human subjects. Neurosci Lett 252:37–40.

Klass M, Levenez M, Enoka RM, Duchateau J (2008) Spinal mechanisms contribute to differences in the time to failure of submaximal. J Neurophysiol 99:1096–1104.

Leis AA, Stokic DS, Fuhr P, Kofler M, Kronenberg MF, Wissel J, Glocker FX, Seifert C, Stetkarova I (2000) Nociceptive fingertip stimulation inhibits synergistic motoneuron pools in the human upper limb. Neurology 55:1305–1309.

Lutz K, Koeneke S, Wustenberg T, Jancke L (2005) Asymmetry of cortical activation during maximum and convenient tapping speed. Neurosci Lett 373:61–66.

Maluf KS, Enoka RM (2005) Task failure during fatiguing contractions performed by humans. J Appl Physiol 99:389–396.

Masakado Y (1994) Motor unit firing behavior in man. Keio J Med 43:137–142.

McNeil CJ, Martin PG, Gandevia SC, Taylor JL (2009) The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. J Physiol 587:5601–5612.

McNeil CJ, Butler JE, Taylor JL, Gandevia SC (2013) Testing the excitability of human motoneurons. Front Hum Neurosci 7:152.

McNulty PA, Macefield VG (2001) Modulation of ongoing EMG by different classes of low-threshold mechanoreceptors in the human hand. J Physiol 537:1021–1032.

Nielsen J, Petersen N (1994) Is presynaptic inhibition distributed to corticospinal fibres in man? J Physiol 477(Pt 1):47–58.

Pierrot-Deseilligny E, Burke D (2005) The circuitry of the human spinal cord. UK: Cambridge University Press.

Priori A, Berardelli A, Inghilleri M, Accornero N, Manfredi M (1994) Motor cortical inhibition and the dopaminergic system. Pharmacological changes in the silent period after transcranial brain stimulation in normal subjects, patients with Parkinson's disease and drug-induced Parkinsonism. Brain 117(Pt 2):317–323.

Rich MM (2006) The control of neuromuscular transmission in health and disease. Neuroscientist 12:134–142.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH, et al. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 91:79–92.

Stephens JA, Taylor A (1972) Fatigue of maintained voluntary muscle contraction in man. J Physiol 220:1–18.

Taylor JL, Gandevia SC (2004) Noninvasive stimulation of the human corticospinal tract. J Appl Physiol 96:1496–1503.

Taylor JL, Gandevia SC (2008) A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. J Appl Physiol 104:542–550.

Taylor JL, Butler JE, Allen GM, Gandevia SC (1996) Changes in motor cortical excitability during human muscle fatigue. J Physiol 490(Pt 2):519–528.

Taylor JL, Allen GM, Butler JE, Gandevia SC (2000) Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. J Appl Physiol 89:305–313.

Teo WP, Rodrigues JP, Mastaglia FL, Thickbroom GW (2012) Post-exercise depression in corticomotor excitability after dynamic movement: a general property of fatiguing and non-fatiguing exercise. Exp Brain Res 216:41–49.

Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD (1991) Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol 29:418–427.

Williams PS, Hoffman RL, Clark BC (2014) Cortical and spinal mechanisms of task failure of sustained submaximal fatiguing contractions. PLoS One 9:e93284.

(Accepted 14 October 2016)
(Available online 24 October 2016)