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A treatment of precision Electroceuticals: fatigue relief in Multiple Sclerosis with personalized home-neuromodulation

Eugenia Gianni

Coordinatore

Prof. Raffaele Antonelli Incalzi

Tutori

Prof.ssa Franca Tecchio

Prof. Giovanni Di Pino

Ing. PhD Luca Paulon

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"The brain is a tissue. It is a complicated, intricately woven tissue, like nothing else we know of in the universe, but it is composed of cells, as any tissue is. They are, to be sure, highly specialized cells, but they function according to the laws that govern any other cells. Their electrical and chemical signals can be detected, recorded, and interpreted and their chemicals can be identified; the connections that constitute the brain's woven feltwork can be mapped. In short, the brain can be studied, just as the kidney can."

David H. Hubel (Neurophysiologist 1926-2013)

Acknowledgments

I thank my Professor Franca Tecchio who has followed me with dedication and who, with her humility combined with a deep passion for her work as a neuroscientist, is an inspiration to us all. I thank my secondary supervisor Luca Paulon, who, with his brilliant ideas is a reference point for us. I thank Prof.Vincenzo Di Lazzaro and my secondary supervisor Prof. Giovanni Di Pino as well for making my pathway possible. I thank my colleagues Massimo Bertoli, Teresa L'Abbate and Joy Grifoni for joining me on this journey. I also would like to thank Dr Rosanna Squitti for indicating me – during this journey - the right pathways.

Summary

Introduction Summary

The present work is the result of a three years Phd project matured in the context of a collaboration between CNR, the University Campus Bio-Medico of Rome (UCBM) and Igea Clinical Biophysics, an enterprise that develops electroceutical treatments and devices for multiple pathologies[1]. The fellowship was an industrial and research one, therefore the work reflects both the effort of developing basic research for advancing our product and the understanding of its mechanisms of action as well as relating to industrial actors to the aim of its commercialization and to the aim of opening a therapeutic service available to patients.

Precisely, the project concerned the development of a precision electroceuticals home treatment for curing fatigue in multiple sclerosis with a personalized neuromodulation.

The project has its roots in the project FaReMuS that stands for "**F**atigue **R**elief in **Mu**ltiple **S**clerosis". FaReMuS is a treatment against fatigue in multiple sclerosis (MS) that my Lab (Laboratory of Electrophysiology for Translational Neuroscience – LET'S) developed over the years. Faremus is a treatment of transcranial direct current stimulation of 5 days, 15 min per day. The target area of the stimulation is the bilateral whole body representation somatosensory cortex (S1). Indeed, we learnt from previous literature this area to be hypoexcitable in MS patients suffering from fatigue, together with an hyperexcitability of the motor cortex (M1). For the target's area stimulation my Lab developed a personalized anodal electrode that is shaped and positioned to fit the central sulcus cortical folding as derived by the cerebral MRI of each single patient against an occipital cathode. The electrode was designed to specifically target S1 of each single patient carefully avoiding M1. Two randomized, crossover

[1] https://i-one.igeamedical.com/terapie-ortopediche/?gclid=Cj0KCQjw48OaBhDWARIsAMd966CNiG2xyiQ2N8U5H2BEMGJYc2E8IE_L4EoM-FLymK4pm8aZOvBZfZMaAon8EALw_wcB

and sham-controlled trials (RCTs) verified the efficacy of the treatment on MS patients, observing a reduction in the modified fatigue impact scale values (mFIS).

Treasuring the recent advances in the field of electroceutical, but also of precision medicine and telemedicine, my project regards the effort of delivering FaReMuS at home and creating an electroceutical domiciliary therapeutic service.

The present work aims at illustrating the pathway we have done during the three years of my grant under the supervision of Prof. Franca Tecchio.

The work divides itself into three parts.

The first part is aimed at presenting the theoretical background to the work that has been conducted over the past three years: the reader will become familiar with the concept of electroceuticals (Chapter 1), fatigue in multiple sclerosis (Chapter 2) and with the theory of functioning of the body-brain system (Chapter 3) that frames our electroceutical approach.

The Second Part will be dedicated to the work done for setting up the electroceutical service both in terms of assessing the clinical validity of the device (Chapter 4) and in terms of meetings with industrial actors (Chapter 5).

Finally, the third part will be dedicated to the research we carried out for the understanding of the mechanisms behind the FaReMuS treatment (Chapter 6) as well as for a better comprehension of the functioning of the motor and sensorimotor system (Chapter 7) with the aim of optimizing the personalized electroceutical tools for relieving neurological and neuropsychiatric disorders.

Part I: Personalized Electroceuticals, how and why

The first Chapter will be dedicated at introducing the reader to the broad concept of electroceuticals (Section 1.1). What do we mean by electroceuticals? How and why electroceuticals raised and acquired relevance into the medicine framework? How to combine electroceuticals and personalized medicine? What has been the strategy of our Lab in these and past years?

Moreover, the last studies and progresses of the use of NIBS – with a special focus on tDCS – applied to neuropsychiatric disorders will be illustrated throughout a book review (Section 1.2: Gianni & Tecchio 2022).

The second Chapter is dedicated at exploring the pathogenesis of fatigue as it emerges from the literature. The relevant aim of this literature review is to answer the question: what is the advantage of applying electroceuticals to fatigue in MS (by a personalized montage) (Section 2.1)? We show that, where the functional damage prevails, electroceuticals might be a relevant option.

The third Chapter is devoted to justifying the use of electroceuticals on the background of our vision of the principle governing the body-brain functioning: the triadic principle "Feedback-Synchrony-Plasticity" (Sections 3.1, 3.2: Tecchio et al. 2020). Here we show such a principle to lay at the ground of the possibility to detect the neurodynamic of each specific region of the brain (Section 3.3: Armonaite et al. 2022). In turn, the ability to decipher "neuronal language" lays the foundation for intervening on specific areas with electroceuticals. This motivated the setting up of an innovative tool designed and pioneered by our Lab: the tIDS, e.g., the brain stimulation with personalized current. Around this idea we designed a research project to be implemented in the next years (Section 3.4).

Part II: An electroceutical service

The fourth and fifth Chapters will be dedicated to the description of the path we made these years for setting up the domiciliary electroceuticals therapeutic service. Premising that the service against fatigue hasn't been finalized yet, we describe preparatory steps to the proper commercialization project we developed during these years (Section 4.1).

Namely in the Fourth Chapter we will describe how we have been able to assess the clinical validity of our instrument FaReMuS (Section 4.2: et al. 2021) throughout a quantitative review, also divulgated by a press release (Section 4.3), and to test its possible domiciliary use presenting a phase II medical device study (Section 4.4: Tecchio et al. 2022). Both studies are intended to constitute a very

relevant milestone for the aim of setting up a clinical electroceutical domiciliary service.

The Fifth Chapter will be dedicated to the unfolding of meetings with the relevant enterprise Igea (Section 5.1) to the aim of paving the way for the engineering of our device and commercialization of our treatment. To optimizing the communication among our Lab and industrial actors we developed commercial tools for presenting our project, like the pitch (Section 5.2).

Part III: Deepening the mechanisms of Faremus

Finally, the sixth and seventh Chapters will illustrate current works and publications by our Lab. What gathers these relevant works has been the double aim of better comprehending the dynamics of the functioning of Faremus and its beneficial effects on parietal connectivity (Chapter 6, sections 6.1: Tecchio et al. 2021) and motor control (Section 6.2: Padalino et al. 2021) and of the sensorimotor and motor system in search of the best indexes to measure synchronization phenomena (Chapter 7, sections 7.1: L'Abbate et al. 2022 and 7.2: Pascarella et al. 2022). Overall, we consider these works and current publications very relevant for continuously ameliorating and personalizing our current treatment FaReMuS as well as for developing further personalized treatments aimed at relieving unbalances in the motor system.

Conclusions

In conclusion, the common thread framing the multiple articles we present in this work - spanning from electroceuticals to personalized medicine approaches, network physiology and mathematical approaches for indexing complex systems – is the continuous effort of our Lab to find optimal strategies to sustain psychophysical wellbeing (Persichilli et al. 2022). To this aim we think the collaborations among multiple professional skills to be essential, on the relevant background of a synergy between research, media, enterprise and education (Figure 11, Chapter 1).

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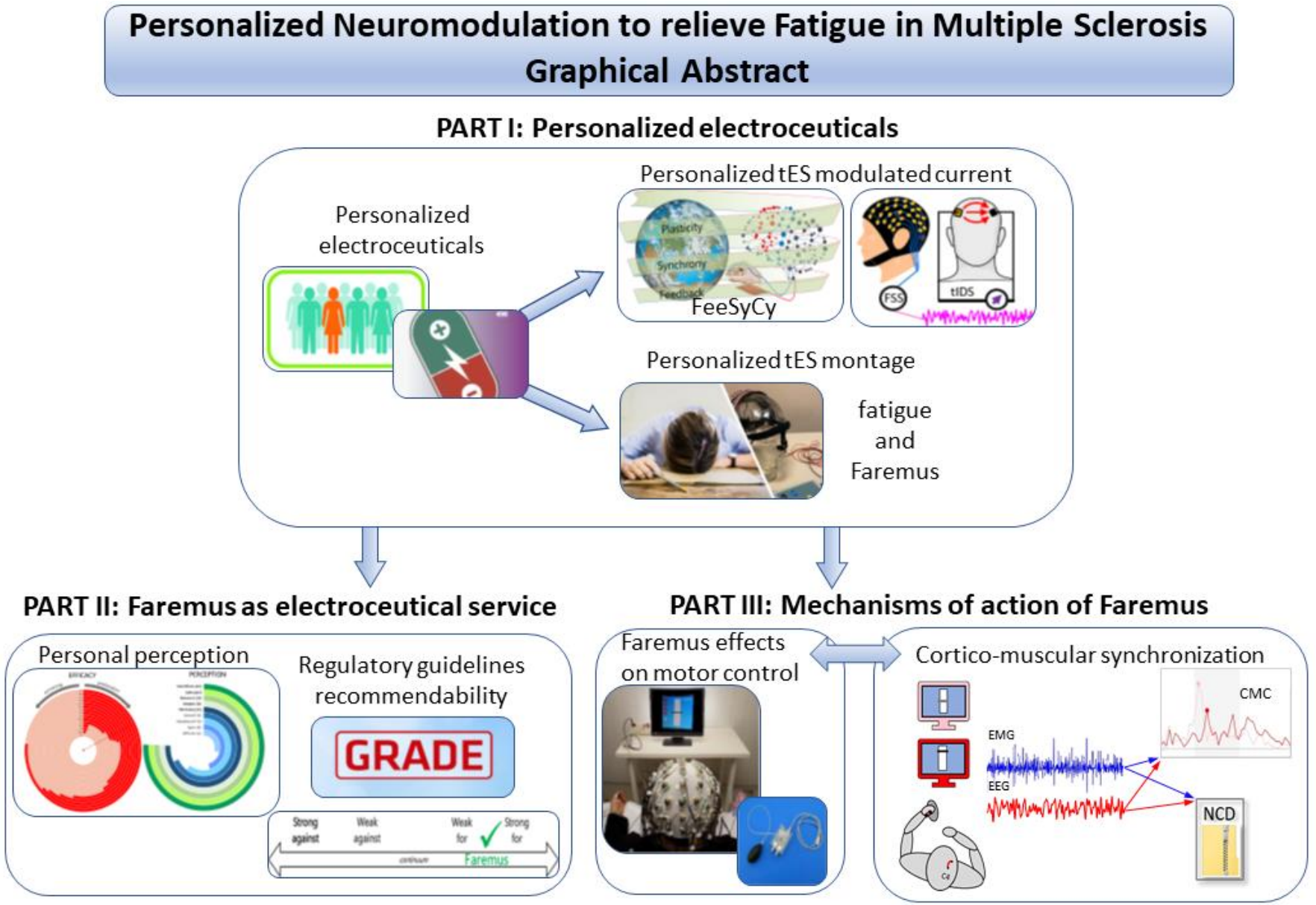
Graphical Abstract



Top left: this work starts with a general overview of the concept of Electroceuticals, e.g., the treatment of ailments bye electrical signals. To then turn to personalized Electroceuticals, e.g. electroceuticals tailored on the individual characteristics of each patient.

Top right: Our approach as a Lab includes personalization in two ways. One one side we have the Faremus treatment that is a transcranial direct current stimulation treatment (tDCS) for fatigue in multiple sclerosis with a personalized electrode designed on the shape of the whole-body somatosensory cortex (S1) of each individual subject derived by MRI. On the other side (top) we have personalized current. Thanks to the Feedback-synchrony-plasticity (FeeSyCY) principle it is possible to "listen" to the brain and intervene on it for compensating its alterations. This led us to the innovation of what we called "tIDS": transcranial individual neurodynamics stimulation. Indeed, we found out that a current that mimics the andogenous activity of a target neuronal pool is effective in altering the excitability of that neuronal pool.

Bottom left: On the strength of our findings, we matured the idea of developing a home electroceutical service against fatigue. Along these years, we have developed essential steps in this direction. On one side, we realized a quantitative review of randomized, controlled trials of tDCS in nonstructural diseases which purpose was to assess whether it would be possible to include tDCS treatment within the framework of medical therapies according to the indications of international competent authorities. The results of the meta-analysis indicated that tDCS treatment for fatigue in MS ranked between moderately and highly recommendable and that the recommended montage was the one of Faremus (right). A second important step toward establishing an electroceutical service was to test a home version of our Faremus. Our objective was to evaluate the feasibility, efficacy, and patient acceptance of FaReMuS home treatment. All patients reported excellent values of safety, acceptance, and absence of side effects of home treatment, and efficacy was above 30% as in previous studies (Left).

Bottom right: To further ameliorating and personalizing our treatment and for improving our knowledge of fatigue's origin mechanisms and functioning of the motor system we engaged in carrying out research exploring the Faremus effects. For example, we tested whether the treatment modifies the cortico-muscular-coherence – CMC - (left), an index subtending motor control that was found to be altered in MS fatigued patients. As a result, we found out that Faremus reverts the cortico-muscular frequency in MS fatigued patients to physiological parameters. The Faremus effects on cortico-muscular coherence measure, led us to go deeper into this parameter to test its sensitivity to physiological features. Therefore, in healthy volunteers, we decided to test CMC sensitivity to visual feedback and handedness. We found out that, tough CMC was sensitive to visual feedback, was not sensitive to manual dominance. Given this limitation we were interested to find out more sensitive measures of cortico-muscular synchronization. Therefore, in the same subject we measured cortico-muscular synchronization using a novel index: the Normalized Compression Distance (NCD). We found out this measure to be sensitive to both visual feedback and handedness and concluded this is a tool that can enrich the measurement of synchronization phenomena in the brain (right).

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Abstract

In my thesis, we start with a general overview of electroceuticals, the treatment of diseases using electrical signals (PART I, Chapter 1). We then turn to the concept of personalized electroceuticals introducing Faremus, a tDCS treatment against fatigue in multiple sclerosis (MS). Faremus is first personalized by targeting whole-body primary somatosensory area (S1) which is hypoexcitable in MS fatigued patients (PART I, Chapter 2). Further personalization is at individual level with the S1 electrode shaped on the MRI derived individual cortical folding. An option to enhance electroceuticals tools emerges exploiting the electrical patterns of our brain (i.e., the neurodynamics), sustained by the recursive feedback-synchrony-plasticity principle. By studying the EEG patterns through the fractal dimension, we suggest that each area has its own specific neurodymanics. From this idea derives the intervention we called transcranial individual neurodynamics current stimulation (tIDS). Indeed, we realized that if we deliver through the transcranial stimulator electrodes a current that mimics the electrical pattern generated by a target region, we can more effectively modify its activity (Part I, Chapter 3).

On the strength of our findings, we matured further steps in the direction of the realization of a therapeutical service. We realized a quantitative review of randomized controlled trials with tDCS in no-structural diseases. The results of the meta-analysis indicated that tDCS treatment for fatigue in MS ranked between moderately and highly recommendable under the guidelines of international authorities and that the recommended montage was the one of Faremus. A second important step towards establishing an electroceutical service was to test a home version of our Faremus. All patients reported excellent values of safety, acceptance and absence of side effects of home treatment which efficacy was above 30% as observed in clinical settings (PART II, Chapter 4). In the direction of the device engineering and commercialization, we set up devoted meetings with the enterprise Igea (PART II, Chapter 5).

To further ameliorating and personalizing our treatment, we improved knowledge on fatigue's mechanisms and Faremus effects. We tested whether the treatment modifies the cortico-muscular-coherence – CMC - (PART III, Chapter 6), which was previously observed to be altered in MS fatigued patients. We confirmed the hypothesis observing normalization by Faremus. These interactions led us to deepen understanding of the phenomena underlying cortico-muscular synchronization. In healthy volunteers, we tested CMC sensitivity to visual feedback and handedness of two synchronization measures: CMC and Normalized compression distance (NCD). Whereas CMC resulted to be only sensitive to visual feedback manipulation, NCD was sensitive also to handedness (PART III, Chapter 7)

From this work we can conclude that i) fatigue in MS emerges as a functional alteration of sensorimotor and motor pathways that can surely benefit from electroceutical intervention ii) the treatment Faremus is between moderately and highly recommendable under the indications of the international authorities and confirms its efficacy also in home environment iii) the study of the nervous system and its communication with the muscle effectors, can be enriched by measures that take into account the complexity and non-stationarity of the neural activity.

PART I: PERSONALIZED ELECTROCEUTICALS, HOW AND WHY

Chapter 1: Personalized electroceuticals, current and future perspectives

1.1 The Let's Lab[2] electroceutical approach

It was about 3 years ago that the World Economic Forum (WEF) of 2018 in Davos recognized among the top-10 emerging technologies - as most promising for the world economic and social development - the Electroceuticals, together with Personalized Medicine and Digital Helpers.

"Could we cut down our reliance on drugs to treat most health conditions? Some say yes, with electroceuticals offering the ability to treat ailments using electrical impulses. One approach, targeting the vagus nerve - the system that sends signals from the brain to most organs - is poised to transform care for many conditions, since it has the potential to regulate the immune system. This has been used to treat epilepsy and depression for more than a decade, and now looks set to aid sufferers of migraines, obesity, and rheumatoid arthritis"[3].

In this review article we will explore the meaning, the use, and the potential of Electroceuticals, i.e., the treatment of ailments by electrical signals, and we will illustrate how, step by step, our LET's Lab implemented these relevant tools up to project of the realization of an electroceutical therapeutic service.

[2] Laboratory of Translational Electrophysiology. LET's Lab is active from about 20 years in Rome under the direction of the Director of Research of CNR Franca Tecchio. First located at Fatebenefratelli Hospital; it is now located in ISTC-CNR, Via Palestro 32, Rome (https://www.istc.cnr.it/it/group/lets).

[3] Quotation from: https://www.weforum.org/agenda/2018/09/top-10-emerging-technologies-of-2018/. We believe that we can treasure of the top-10 lists provided annually by the WEF as a reference for our everyday work, indicating, thanks to the expertise of multidisciplinary international experts, what is promising, and indicating in which specific fields it is relevant and advantageous to move steps forward.

The innovation of Electroceuticals

Electroceuticals is a term recently coined (Mishra 2017); generally referred to the encompassing fields of bioelectrical and bioelectronics medicine. Precisely it was first coined in 2013 by the pharmaceutical company GlaxoSmithKline which decided to relevantly invest in this new field of technology (Sinha 2013). It is based on the use, either enhancing or therapeutic, of electric impulses for modifying biological functions or pathological processes (Majid 2017).

Famm et al. (2013) remind us - basing on Kandel's teaching (Kandel et al. 2012) how is it possible to modify biological functions simply by electrical impulses. "Electrical impulses — action potentials — are the language of the body's nervous system. Virtually all organs and functions are regulated through circuits of neurons communicating through such impulses."

We can conclude that by sending appropriate messages to neurons, i.e., sending electrical impulses, it is possible to modify their signals and signals themselves can exert their control an all-body function. Thereby, by modifying signals we can modify the functioning of specific cells, areas, or organs of our body.

Electroceuticals includes a bulk of highly sophisticated technology that we think can constitute a very promising medical treatment for the present and for the future (Brunoni et al. 2016; Brunoni et al. 2021; see also Section 1,2). But why directly targeting nerves may be advantageous over traditional approaches?

Famm et al. (2013) continue: "Two features make these circuits excellent targets for therapeutic intervention. First, they comprise discrete components — interconnected cells, fibre tracts and nerve bundles — allowing for pinpoint intervention. Second, they are controlled by patterns of action potentials, which can be altered for treatment.". Indeed, rather than targeting cells by a drug, electroceuticals treatments can alter the control by the nerves on a specific organ critically influencing its function, gaining in precision and efficacy with respect to traditional pharmaceutical treatments (Reardon 2014). This reveals to be really

advantageous for example in autoimmune diseases, where it is possible to place the electroceutical device on a specific nerve bundle rather than to target the entire immune system with a drug (Reardon 2014) which can weaken the system itself in its entirety. Temporal and spatial precision are very important features of the electroceutical devices as long as they ar able to target specific sets of cells by altering their specific action pontetials that last milliseconds. As an example, the authors (Famm et al. 2013) illustrate us the great precision and efficacy of electroceuticals treatments by showing that stimulation and/or ablation of a specific, tiny group of cells can finely modulate the level of food intake by the mice up to let them switch from voracity to anorexia (Aponte et al. 2011).

As well as food intake, electroceuticals can regulate cardiac activity, pancreatic activity, liver, kidney, or spleen functions (Mishra 2017).

The immune and metabolic systems are but only two of the possible targets of the electroceuticals treatments, that spans from cochlear implants for hearing disorders (Carr & Ray 2017), deep brain stimulation for movement disorders (Silverdale 2017; Chou et al. 2017), vagal nerve stimulation for epilepsy (Wright et al. 2017) and for gait disorders (Bonaz et al. 2017), neuromodulation for migraine and headaches (Miller & Matharau 2017), transcranial magnetic stimulation and direct current stimulation for psychiatric disorders (Brunoni 2016; Kim et al. 2020). But these are only few examples of the myriad of possible applications of electroceuticals (Kambouris et al. 2014; Mishra et al. 2017; Majid et al. 2017) and some of them will be potentially available in the future since they are currently under experimentation like applications of electroceuticals for enhancing and/or retrieving functions such as memory or consciousness (Ciurea et al. 2017; Slater et al. 2021). According to the estimates, electroceuticals is supposed to widely spread as a medical treatment over the next two decades, up to reach 2 billion of people who are suffering from chronic diseases (Mishra 2017).

Other advantages are that electroceuticals make possible to personalize and optimize the therapy and at the same time produce far less side-effects than drugs (Mishra 2017) and they can help reducing the therapeutic costs.

Building electroceutical systems

Building up Electroceuticals therapeutic interventions involves three relevant steps. First it is due to map the neural circuits associated with the disease both at the anatomical level and at the functional level, i.e., by studying their electrical activity patterns[4] both in disease and healthy conditions. Then it is due to identify the electrical patterns that can be sent to elicit a therapeutic response. Finally, it is possible to develop the electroceuticals device to be attached either to the sculp, to nerve bundles, or to specific organs (Mishra 2017). Electroceuticals devices can assume multiple shapes, but the last frontier is to get electroceuticals instruments in the form of nano-devices (Mishra 2016).

Indeed, the increasing development of electroceuticals field nowadays is made possible by two substantial factors: the increasing refinement and shrinking of technology giving rise to the field of precision technology, and the effort by the scientists to examine and trace electrical pathways in the body-brain system (Majid 2017). Regarding this, it is sufficient to think about the *Human Connectome Project* funded by the NIH and launched in 2009, which aims to shed light on functional and anatomical connectivity in the human brain, both at an individual and at a population level (https://www.humanconnectome.org/).

Finally, the increased interest in electroceuticals born about ten years ago, led important funding bodies such as the NIH (National Institute of Health) in the USA to establish a US $248 million fund to map the electrical wiring of the body and advance the development of new electroceuticals devices (Reardon 2014). Similarly, as we mentioned above, the pharmaceutical company GlaxoSmithKline

[4] We will refer to the electrical patterns in the next Chapters as “the neurodynamics”. See Chapter 3.

(GSK) offered a $1 million prize for funding up to 40 researchers to stimulate innovation in this novel field (Blau 2013).

The field of Electroceuticals and its branches

The one of electroceuticals is a complex technological field encompassing multiple branches. These can be divided into two main sectors: invasive and non-invasive techniques.

Among the multiple invasive applications of electroceuticals it is worth that we mention the cardiac pacemakers, targeting the heart, that from the ’60 constitute a crucial therapeutic approach for heart dysfunctions (Barold et. al 2008). As well as the retinal (Chuang 2014) and cochlear implants (Zeng 2008), that respectively electrically stimulate the retinal neurons for restoring visual information or electrically stimulate the cochlear nerve for restoring the auditory function. Targeting the brain we mention deep brain stimulation - functioning by implanted electrodes for treating movement disorders - which was recently approved by FDA as a treatment for Parkinson’s disease (Lozano 2019). Targeting peripheral nerves, we have vagal nerve stimulation. First used for drug-resistant epilepsy and then for depression (FDA approved for these two pathologies), vagal nerve stimulation (VNS) is under experimental examination for the treatment of immune disorders like rheumatoid artritis, cardiovascular disease and obesity (Groves 2005; Johnson & Wilson 2018). Non-invasive forms of VNS are currently under development (Ben-Menachem 2015).

The present work aims to focus on non-invasive techniques also called NIBS (Non-invasive brain stimulation) (Polania 2018). Non-invasive techniques divide into tES (transcranial electrical stimulation) (Yavari et al. 2018) and TMS or rTMS (transcranial magnetic stimulation and repetitive TMS) (Leufaucheur et al. 2014). tES divides itself in turn into tACS (transcranial alternating current stimulation, Hermann et al. 2013) tDCS (transcranial direct current stimulation) and tRNS (transcranial random noise stimulation) (Paulus 2011). However, the most used

techniques, we will describe below (that are also in use by our Lab) are TMS (and rTMS) and tDCS.

a) TMS

Transcranial magnetic stimulation (TMS) (Figure 2) is a non-invasive form of brain stimulation creating a magnetic field upon the brain that induces an electric current into a specific area of the nervous system through electromagnetic induction. This electric field induces a change in neurons' membrane potentials resulting in depolarization or hyperpolarization, causing neurons to be more or less excitable, respectively.

A TMS device consists of a transducing plastic-enclosed coil of wire which is attached to a high-voltage (400 V– 3 kV), high-current (4 kA–20 kA) pulse generator (Jalinous, 1991). The coil is held next to the scalp or attached to the scalp. The stimulator generates a time-varying current into the coil which in turn generates a magnetic field orthogonal to the plain of the coil, over the scalp. The magnetic field can induce an inverted electric current into the brain that can influence neuronal excitability. The induced magnetic field reaches peak strengths of 1–2.5 Tesla and is very short lasting ($\leqslant$1 ms) (Groppa et al. 2012).

The extent of action of the current density generated into the brain may vary according to several parameters, such as the type and orientation of coil, the distance between the coil and the brain, the magnetic pulse waveform, the intensity, frequency and pattern of stimulation (Paulus et al. 2013).

Therapeutic treatments typically involve delivering repetitive magnetic pulses; therefore, the technique is called repetitive TMS or rTMS.

So far; there is high and robust evidence for the beneficial therapeutic effect of rTMS for example on neuropathic pain and depression; but multiple therapeutic uses are currently under experimentation (Leufaucheur et al. 2014). Several studies show that the effects can last several weeks after the treatment (Klomjai et al. 2015).

Single- or repetitive-pulse stimulation of the brain causes the spinal cord and peripheral muscles to produce neuroelectrical signals known as motor evoked potentials (MEPs). MEPs can be recorded from the surface of the skin of the involved muscles by electro-miography (EMG). The properties of MEPs – like latency, amplitude, and size - can be studied make inferences about the functional properties and status of the central and peripheral motor pathway.

In clinical neuroscience MEPs are widely used as a diagnostic (Di Lazzaro 1999) and monitoring tools for motor disease progression (Rossini & Rossi 1998). But they can also be used to study cortico-spinal excitability in healthy subjects and patients for research purposes.

Figure 2. TMS Device

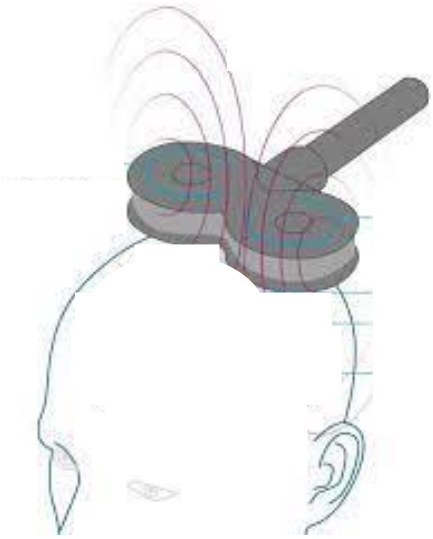

Fig.2: The picture presents the montage of TMS. The coil is positioned just above the scalp without touching it. The magnetic field created by the coil generates in turn an electric field that modifies the excitability of the neurons.

b) tDCS

Transcranial direct current stimulation (Figure 3), also called tDCS, is a non-invasive technique that applies mild (1-2mA) direct currents to the scalp. Creating a low voltage (0.3 to 1.6 V/m) electrical field into the nervous system tDCS can either enhance or decrease cortical excitability.

The tDCS device is quite simple and is made up of typically two electrodes (or more) placed in contact with the scalp and connected to a stimulator. The typically used electrodes range from 25 cm^2 and 35 cm^2 and are one positive (anode) and one negative (cathode). Thereby the current is direct (in contrast to alternating currents used for example in tACS) flowing from the anode to the cathode and the voltage difference is always positive. The electrodes consist of conductive material, such as conductive rubber and are typically soaked in a conductive saline solution.

Excitability as well as inhibition are possible since the generated electric field can cause the neurons' resting membrane potential to either depolarize or hyperpolarize. When positive stimulation (anodal tDCS) is delivered, the current induces a depolarization of the membrane potential, which increases neuronal excitability and causes spontaneous cell firing. When negative stimulation (cathodal tDCS) is delivered, the current induces a hyperpolarization of the membrane potential which results in a decreasing of the excitability.

Typical protocols involve a constant long-lasting (1-30 minutes) weak current (1-2 mA). The current is kept constant throughout the stimulation period, except at the beginning and at the end when there are rump-up/rump-down periods lasting 10 s were the current either increases or decreases (Ktnotkova et al. 2019).

Figure 3. tDCS Device

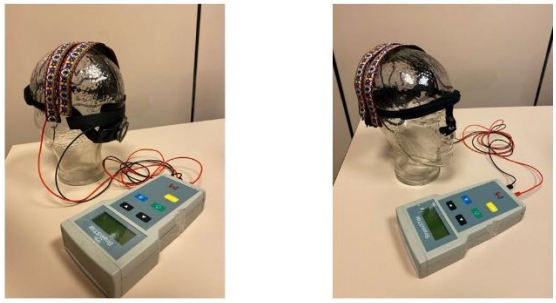

Fig. 3: Montage of tDCS as in Faremus device: Anode (colored band) and cathode (black band left) are positioned over the scalp and kept by a band. They are attached to a stimulator delivering a week current over the scalp.

The therapeutic uses of tDCS are promising and will be broadly discussed in the Section 2.1 of this work and in the fourth Chapter as well.

FaReMuS – a personalized electroceuticals approach

Our approach aims at integrating electroceuticals and personalized medicine. Where personalized medicine (Jain 2002) divides itself in two big branches: personalized diagnosis and personalized therapy. Our work focuses on personalized therapy, which itself can be divided in three branches: personalized prognosis, personalized treatment, and communication. We will focalize here on the personalized treatment by presenting our ideas: FaReMuS (**Fa**tigue **Re**lief in **Mu**ltiple **S**clerosis) and tIDS (**T**ranscranial **I**ndividual Neuro**D**ynamics **S**timulation).

Both treatments originate from the strategy that our laboratory LET'S developed along the years. Indeed, we believe that is crucial to listen to what we call "the body-brain system"[5] searching for altered indexes or parameters of the

[5] By referring to the body-brain system we aim at underlying how the brain and the body, in its multiplicity of organs and systems, deeply interact and influence each other via hemodynamic,

symptom that we want to treat and develop the compensatory intervention. These are, in other words, the main steps we showed in the previous Paragraph to be relevant[6] for setting up electroceutical devices and treatments: mapping the functional properties related to the disease and establishing the best intervention. These are as well the key-passages of what my Lab did against the symptom of fatigue in Multiple Sclerosis (MS): they learnt how fatigue affects the electrical patterns of the brain and developed a tailored intervention.

The symptom of fatigue[7] is considered by half of MS people the most invalidating and impairing their quality of life (Giovannoni 2006). There are no effective pharmacological treatments for this symptom and all of them create big side effects (Kesselring & Beer 2005; Zielinska-Nowak 2020; Murray 1985; Nourbakhsh 2021; see also Chapter 4); while non-pharmacological treatments like physical exercise are not always applicable due to patients' degree of disability (Donzè et al. 2021; Raziazian et al. 2020).

In the emergence of the symptom, my Lab found out that the alteration of the electrical activity is crucial. In particular, the literature indicates a reduction of the parietal – with a special focus on the primary somatosensory areas (Dell'Acqua et al. 2010; Tecchio et al. 2008; Vecchio et al. 2017) - excitability and increased excitability of the frontal and in particular the primary motor areas (Bisecco et al. 2018; Lipert et al. 2005; Tecchio & Bertoli 2020 for a review).

Starting from these assumptions, my Lab re-adapted a neuromodulation intervention that was able in healthy subjects to increase endurance to fatigue (Cogiamanian 2007). In the study by Cogiamanian et al. 2007, transcranial current stimulation was delivered over the right (contralateral) motor cortex and the endurance time (ET) for a sub-maximal isometric contraction of the elbow flexors

metabolic, hormonal, and neurological functions. This aspect will find a broader conceptualization in Chapter 3.

[6] Chapter 1, pp. 15.

[7] The pathophysiology of fatigue in MS will be broadly discussed in Chapter 2. Here we only mention the mechanisms we think are the basis of it and can explain the specific design of our FaReMuS device.

with or without stimulation was evaluated. The authors found out that, after 1 hour from the fatiguing task, the ET of the group who received anodal tDCS was increased by 15%. Thus, my Lab decided to apply a similar protocol while targeting the whole-body somatosensory representation area bilaterally; strictly avoiding giving the excitatory stimulation to the contiguous M1 area that shows opposite alteration to S1. To this aim, my Lab created an anode that was modelled on the specific shape of the central sulcus of individual subjects developed by an automated procedure (Tecchio et al. 2013). The procedure of electrode shaping employed the three-dimensional reconstruction of the brain MRI of each subject and matching the projection on the scalp of a determined cortical area (Figure 4, Tecchio et al. 2013). In the FaReMuS intervention, a 5-day anodal transcranial Direct Current Stimulation (tDCS, 1.5 mA, 15 min per day) bilaterally targets the primary somatosensory areas (S1) through a personalized electrode (Figure 5 and 6) (area 35 cm2), with the cathode placed on bilateral occipital sites (70 cm2).

Figure 4. Regional Personalized Electrode shaping.

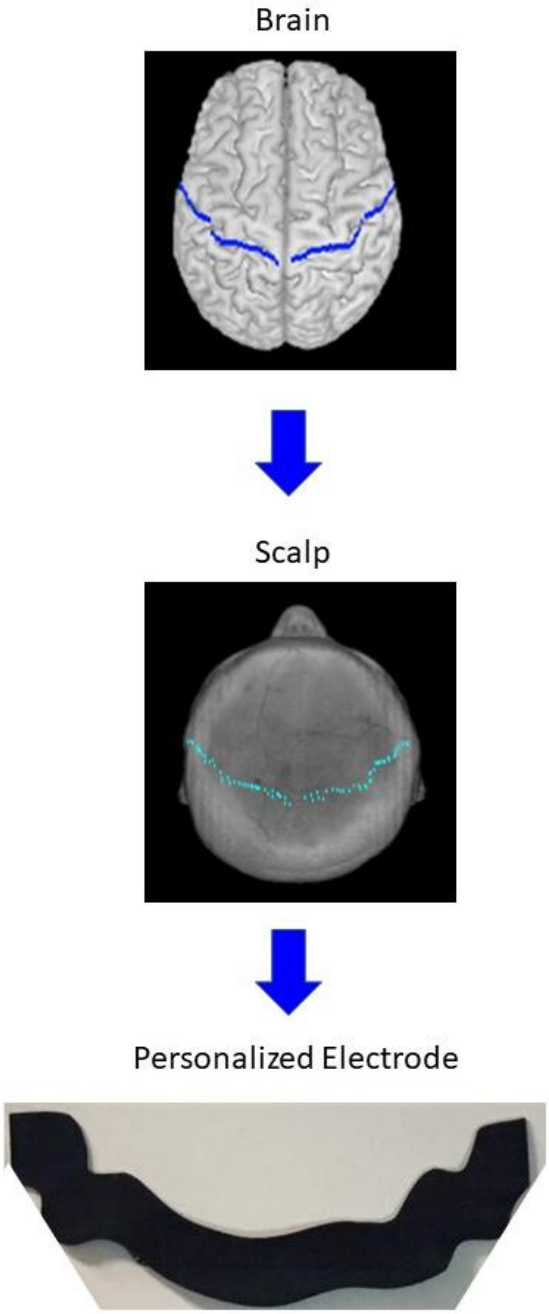

FIG.4. The left and right central sulci are drawn on a piece of paper using softtaxic software from a volumetric mri, and then parallelograms with a 2 cm width are fitted into the central sulcus for each participant. for each s1 and m1 electrode, the shape is then drawn on, cut out, and sewn onto two sponge sheets to allow for the introduction of conductive material.

Figure 5. Faremus device

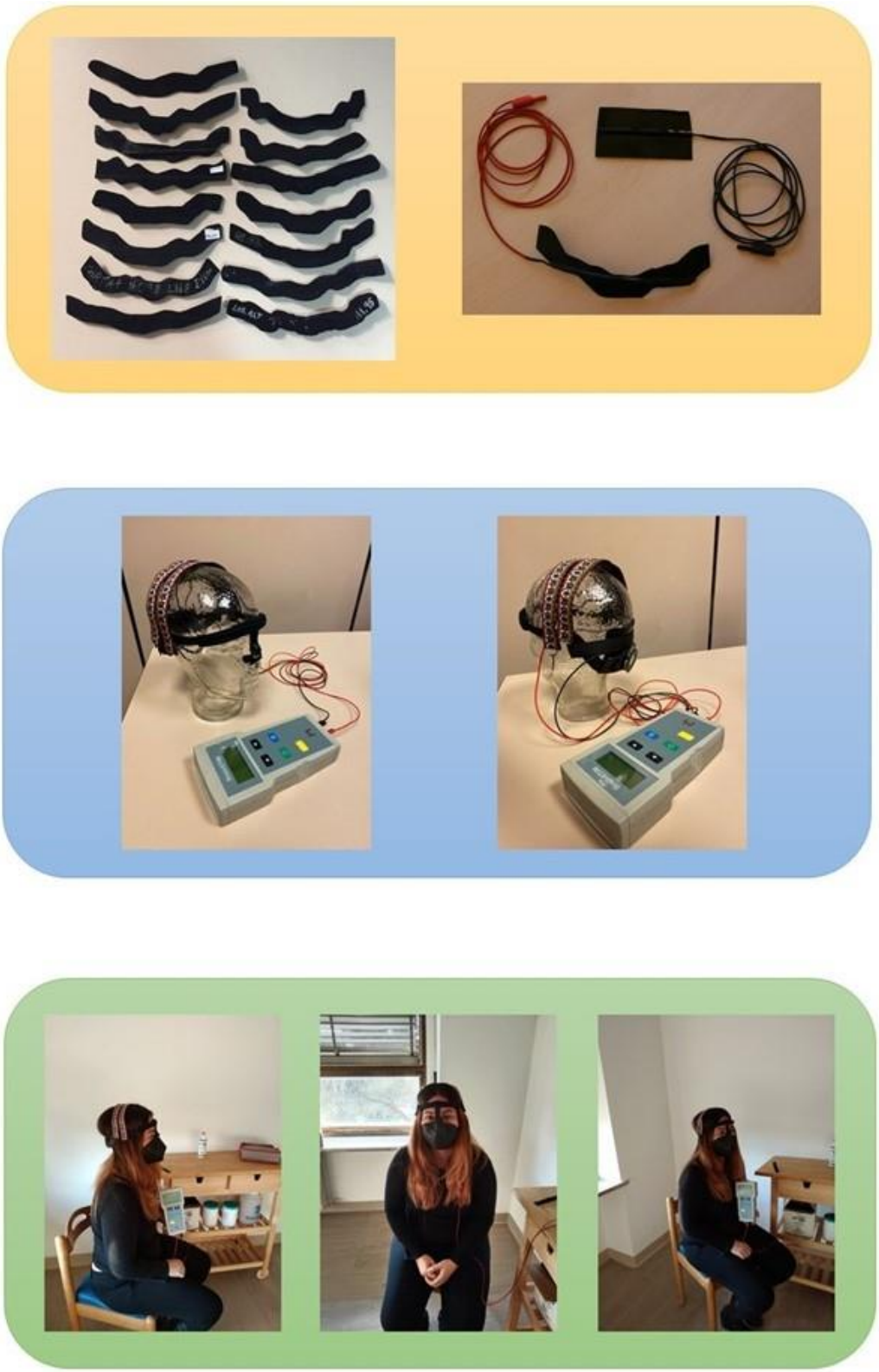

Fig.5 TOP: Left, multiple personalized electrodes. Right, Anode (red) and cathode (black) of the Faremus. **MIDDLE:** Faremus Helmet, with mounted electrodes attached to the stimulator. **BOTTOM:** A subject wearing Faremus during Pandemic.

In a first seminal study (Cancelli et al. 2015), my Lab decided to test whether this novel personalization showed to be efficacious by verifying in 12 healthy subjects what occured in the primary somatosensory cortex if the tACS neuromodulation effects of the personalized electrode were compared with the effects of a standard one[8].

In this study they compared the amplitude of the EMG signal induced by MEPs[9] while neuromodulating the cortex with either the non-personalized or personalized electrode and observed the effects in the lower and upper limbs M1 representation of the right and left hemi body. Here they based our investigation on an already published protocol, which showed that transcranial Alternating Current Stimulation (tACS) at 20 Hz induces M1 cortical excitability changes (Feurra et al. 2011).

As a first result, both the personalized and non-personalized electrodes showed an increased amplitude of the EMG signal both of left and right sides in the lower limb representation. In this case the authors presumably observed no difference because the lower limb representation stands near the inter-hemispheric commissure where we centred both electrodes, so that both electrodes could have exerted their influence on the contralateral area. Indeed, what they noticed thereafter was that in the area corresponding to the upper limb representation only the personalized electrode was able to produce a statistically significant enhancement of excitability while the non-personalized electrode was not able to produce variations of excitability of the area.

The efficacy of the personalization was confirmed by further recent modelling studies (Parazzini et al. 2015; Cancelli et al. 2018). Based on high quality MRI scans of healthy volunteer subjects, Parazzini et al. (2015) employed two

[8] The standard electrode consisted of a 2 cm wide strip size-matched with the personalized electrode but shaped on a standard model fitting the curve passing through C3-CZ-C4 sites of the electroencephalographic (EEG) 10-20 International System (Homan et al. 1987). (See Figure 4 and 5).

[9] For definition of MEPs see previous paragraph.

realistic human models from the Virtual Family (a 26-years-old female and a 34-years-old male). They targeted bilateral primary motor (M1) and somatosensory cortex (S1) alternatively with the personalized and non-personalized electrode, with the reference on the occipital area in both cases, using a virtual reproduction of the ad-hoc neuro-navigation procedure to shape and place the personalized electrode on the basis of individual brain anatomy. Next, using a computational electromagnetic technique, the authors approximated the distribution of the electric field throughout the structures of the brain. The customized electrode was able to modulate the region of the central sulcus more profoundly and forcefully than the nonpersonalized electrode, according to the results. Furthermore, while the personalized electrode used to target M1 expanded its effects throughout both the pre and postcentral gyrus, the personalized electrode used to target S1 more specifically affected the postcentral gyrus.

Cancelli et al. 2018 is a work dedicated to the automatization procedure of the development of the electrode but contains an estimate of the relevance of RePE shape and position in terms of the tDCS differential efficacy on S1 with respect to primary motor cortex (M1) using a personalized vs. a non-personalized electrode. Computational modeling exploits high resolution MRI and accurate brain models based on Finite Element Method (FEM) to predict the electric field induced in each voxel of the brain during tDCS. The results indicated that a non-RePE electrode cannot reach the same efficiency in terms of local specificity of the induced currents, regardless of its position on the scalp.

Thus, we concluded that the personalization seems to be necessary if we wish to target the whole region of the primary somatosensory representation; what led us to test the proper Faremus treatment on MS fatigued patients.

The FaReMuS treatment against fatigue in MS was tested in two subsequent RCTs (Tecchio et al. 2014; Cancelli et al. 2018). In both RCTs were

enrolled 10 patients[10] with MS in a mild state (EDSS ≤3.5)[11] but suffering from fatigue (mFIS>38[12]). In the randomized, double-blind, sham-controlled, cross-over studies the MS patients, after being prepared individualized electrodes for each of them, were submitted to a bilateral whole-body S1 anodal tDCS for 5 consecutive days, 15 minutes a day. mFIS scores were collected before (t0); 4 hours after the treatment (t1) and four (t4) and eight weeks (t8) after the treatment was done (Figure 6).

Figure 6. Experimental procedure and study design.

Fig. 6 Main steps of the experimental procedure: personalized electrode shaped (day 1). Day 2: collection

What was observed was a consistent reduction of fatigue both at t1 and t4 of about 30% for the Real Stimulation in both RCTs (Figure 8). Cohen's d coefficient resulted 1.6 (between large and huge) in the first RCT and 1.1 in the second RCT (near to large effect – 1.2). The total effect of the 20 patients together was 1.3[13].

[10] The number of patients enrolled was relatively low, but sufficient according to our sample size estimates.

[11] We decided to enroll low EDSS patients to avoid confoundings factors.

[12] mFIS is one of the several scale to clinically measure fatigue (see Chapter 2). It consists of 21 items enquiring how fatigue impacts patient's lives. This instrument is based on patients' interviews and provides an assessment of the impact of fatigue on physical, cognitive, and psychosocial functioning.

[13] Reference values indicate that a coefcient of 0.2 indicates a small efect size (ES), 0.5 a medium ES and higher than 0.8 large ESs, thus clearly evidencing a large efect, consistent with the further classifcation by Sawilowsky13, who indicated as very large efects those corresponding to Cohen's d above 1.2.

Figure 7. **FaReMuS effects on MS fatigue.**

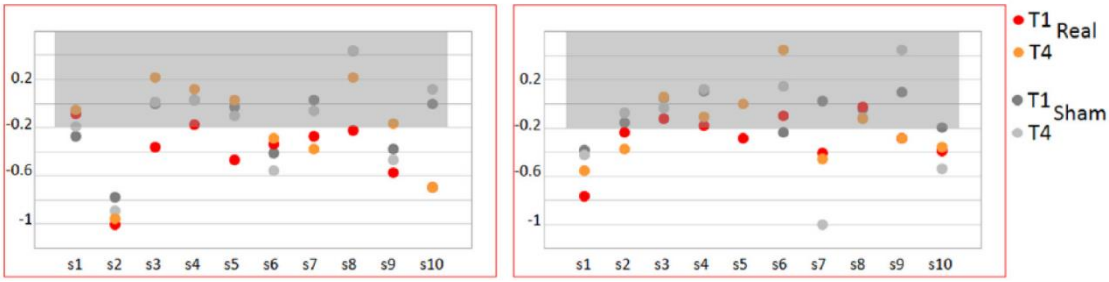

Fig. 7 In the two independent groups, mFIS percentage changes (post–pre/pre) in single subjects at T1 (main outcome) and at T4.

Figure and caption readapted from Cancelli et al. 2018

These two RCTs results verified both the efficacy (Figure 7) of the treatment FaReMuS and the importance of the electrode personalization.

In 2020 we decided to go a step further in assessing the clinical validity of FaReMuS (Gianni et al. 2021, Section 4.2). To this aim we investigated, throughout a quantitative review, whether the treatment, as well as other treatments employing tDCS and targeting also other pathologies, could gain the definition of proper medical therapies according to the official indications of the international authorities. Thus, we applied to the two previously described RCTs the GRADE (Grading of Recommendation, assessment, development, and evaluation) guidelines that quantify the recommendability of a treatment and allow to position an intervention within a continuum that goes from "strong against" to "strong for" recommendation to applying the therapy (Gianni et al. 2021[14]) (Figure 8).

Our meta-analysis results, integrated with extensive evidence of negligible side effects and low-cost, easy-to-use procedures, indicated that tDCS treatments

[14] Chapter 4, section 4.2.

for depression and fatigue in Multiple Sclerosis (by a montage like the one we used for Faremus) ranked between moderately and highly recommendable.

We concluded that high-quality indications support tDCS as a promising tool to build electroceutical treatments against diseases involving neurodynamics alterations and that the evidence we gathered around Faremus paved the way to classify it as a clinically valid treatment.

Figure 8. Treatment recommendation strength.

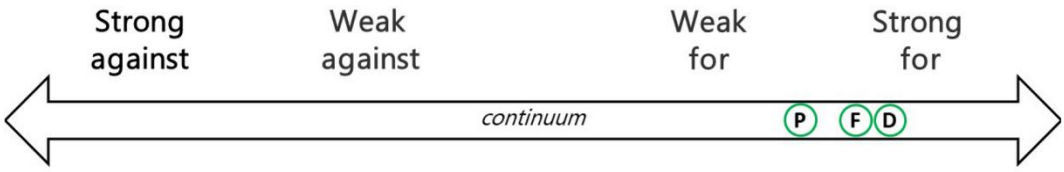

Fig. 8 The strength of a recommendation of a clinical procedure ranges in a continuum divided into categories and reflects the extent to which a guideline panel is confident that desirable effects outweigh undesirable effects. The results of the meta-analysis indicate FaReMuS ranges between moderate and strong.

Figure and caption from Gianni et al. 2021

Along the same period, we decided to publish a study that was done to test a home version of the treatment (Tecchio et al. 2022, Section 4.4). Three main factors were conving for developing a home-treatment was necessary: traveling to the hospital or other treatment facilities on a daily basis causes itself fatigue, especially in congested areas, during severe weather, and during pandemic situations; the dedicated device-setup is straightforward and can be operated by the patient without special assistance; and finally, the efficiency and practicality of home treatments promise to achieve sustainable repetitions over time.

The same protocol and eligibility criteria of the two previous RCTs were applied; but differently from the RCTs that were carried out in clinical settings the 15 MS fatigue patients, were first instructed in the clinics on how to use the device and then could bring it at home and apply it with the help of a familiar or a

caregiver thanks to an ad-hoc adaptable helmet frame (AHF) that allowed precise repositioning. Telephone assistance was offered based on need.

The feasibility of the treatment was evaluated by the mFIS, as in the previous RCTs protocol while Individual ad-hoc questionnaires quantified the acceptance, safety and side effects during the treatment. As a result, all 15 patients completed the treatment, reporting optimal acceptance and safety on using Faremus at their home without side-effects (Figure 10). The treatment ameliorated fatigue symptoms more than 20% of baseline in 10 out of the 15 patients and of 37% on average, with a corresponding effect size 1.21.

Possible limitations in our pathway towards testing the efficacy of Faremus is that the ancillary studies executed for assessing the brain reorganizations induced by Faremus and their association with fatigue symptom amelioration were executed and focused only on the upper limb. The effect of personalized electrode induced neuromodulation, as assessed in M1, was tested in healthy subjects also for the lower limb (Cancelli et al. 2015). They are to be investigated in the future the Faremus effects induced on the lower limb central representation and possible relationship with symptom amelioration.

Figure 9: FaremusH efficacy and perception

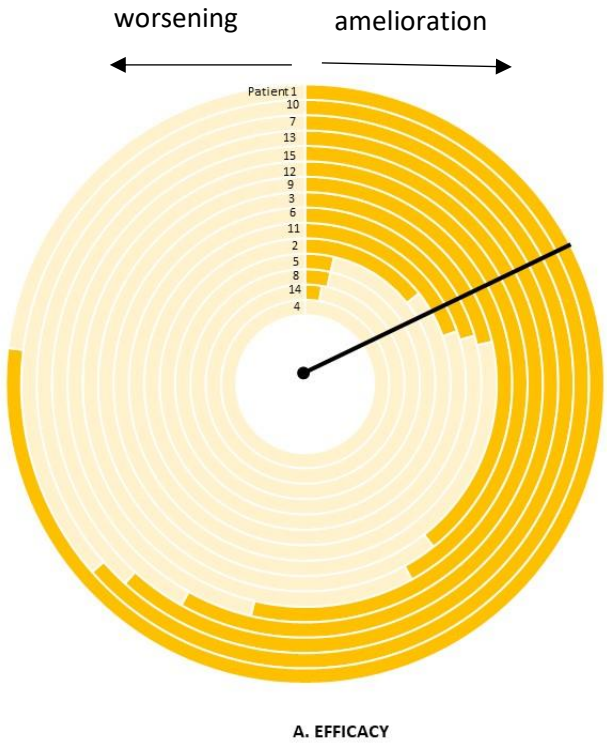

Fig 9 A. Faremus-H efficacy quantified by the percentual change with respect to the baseline mFIS value. The dashed radius indicates the 20% threshold of responsiveness. Note that none of the patients worsened the fatigue status after Faremus-H. Patients are ordered by their responsiveness to Faremus-H.

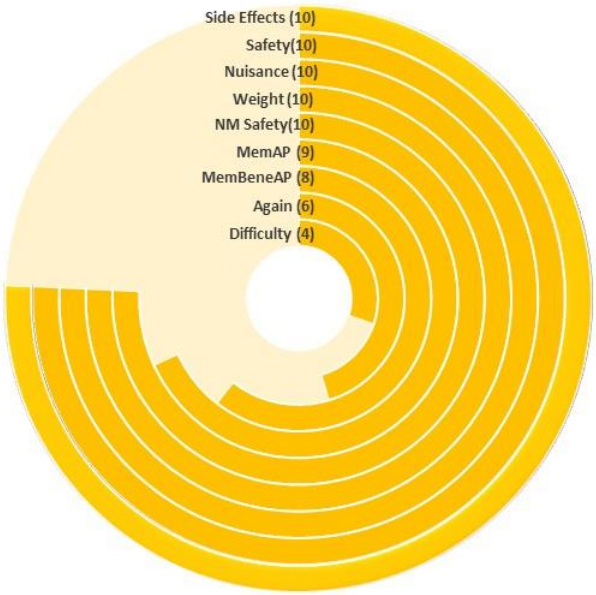

Fig. 9 B. Subjects' scoring for treatment while the treatment was ongoing: Side Effects, Safety, Niusance, Weight, Safety and inquired by the technician 3 years later (MN Safety), Memory of the treatment after 3 years (MemAP); Fine memory after 3 years (MemBeneAP); wish to repeat it (Again); difficulty in applying the treatment (difficulty).

Figure and caption re-adapted from L'Abbate et al. 2022

Exploring FaReMuS mechanisms and effects

So far, we spoke about the procedures for personalizing the treatment, testing it, assessing its clinical validity, and delivering it at home. Hovewer, observing Faremus efficacy on fatigue in MS patients brought my Lab team to further questions. What are the exact mechanisms that explain the beneficial effects of Faremus and how can we make inferences from these to the mechanisms subtending fatigue? In other words: how can neuromodulation teach us about the origin of fatigue as an imbalance of the motor system?

In a study of 2013 (Tomasevich et al. 2013) was observed that the cortico-muscular coherence[15] (CMC), evaluated in fatigued MS patients while executing as isometric contraction, showed a particular alteration that didn't occur in the non fatigued group. The observed frequency was higher than the physiological level.

In a recently published work (Padalino et al. 2021), the working hypothesis that the personalized neuromodulation FaReMuS reverts CMC to lower physiological frequency (see Figure 11 for the study design) was tested. In 11 fatigued patients, were recorded EEG and MEG simultaneously for studying CMC before and after Faremus.

What was found out was that before FaReMuS, the CMC was observed at a high frequency of 31.5 ± 1.6 Hz (gammaband) and positively correlated with the level of fatigue. After FaReMuS the rate of fatigue reduction was 28% ± 33% and the CMC frequency reduced, thus forthcoming the physiological beta band as observed in healthy people. On the side of what we learnt about Faremus effects; the study showed that Faremus normalized the central-peripheral communication that subtends everyday movements. On the side of what we learnt about fatigue's mechanisms; the CMC and fatigue patterns relationship suggests more a central than a peripheral origin of fatigue.

In a 2019 study (Porcaro et al. 2019) was investigated what occurs in terms of electrical activity patterns (i.e., in terms of neurodynamics) in fatigued patients and from this point of view by which mechanisms does Faremus induce amelioration. In the two RCTs I mentioned before, in a total of 18 patients, were

[15] Cortico-muscular coherence is the spectral coherence (CMC) - between the EEG/MEG signals from the contralateral sensorimotor cortex and the electromyography (EMG) from the prime mover muscle recorded simultaneously (Mima & Hallet 2003). We will investigate this measure in Chapter 7.

detected with EEG equipped by FSS[16], in addition to the fatigue levels, data about the functional organization of the sensorimotor network both before and after the treatment (Porcaro et al. 2019). The first step was to identify M1 and S1 sources through the FSS. The procedure to obtain the field distribution and the time-course of M1 activity involved asking the subject executing an isometric handgrip with either the left or the right hand while recording the brain signals with the EEG. Then the authors searched for those neurons that expressed maximal coherence with the contracted muscles and detected M1 source. Similarly, for identifying S1 source, the medial nerve of either the left or the right hand was electrically stimulated, obtaining the somatosensory representation within the brain devoted to the hand. Once identified M1 and S1 activity, the authors were able to study their electrical activity at rest.

To analyze the neurodynamics the authors used the Fractal Dimension because it already proved to be a good candidate for assessing the brain networks state and functionality (Di Leva et al. 2015) and for typifying cortical districts at rest (Cottone et al. 2017) (Figure 10). Moreover, we believe that complex-system measures are proper to describe the physiology of neuronal electric activity, whose dynamics display hugely complex temporal structures. The functional connectivity between S1 and M1 homologs and hemi-bodies was studied through the mutual information (Pereda et al. 2005).

[16] The FSS (Functional Source Separation) is an AI tool that our lab developed 15 years ago and exploited for multiple investigations (Tecchio et al. 2007; Porcaro et al. 2009; Porcaro et al. 2008; Cottone et al. 2017; Barbati et al. 2006). It enables to reconstructs the activity of specific regions measured with either MEG or EEG starting from functional specific fingerprints; thus, exploiting the dynamic information emerging from those methods of assessment of brain electrical activity. The FSS, like blind and semi-blind source identification methods, as for example ICA, produces the time evolution of the activity and the field distribution of the scalp. So that it is possible to exploit the field distribution measure to solve the inverse problem to know where the structures whose dynamics are under study rely into the brain. Moreover, by applying FSS it is possible to detect the activity of the regions of interest under many different conditions and – what is important for our purposes - we can study the activity at rest. Indeed, the potential of FSS is that once identified the distribution related to one specific source it is possible to consider the activity in other conditions of interest and extract it. This is crucial if we want to study the characteristics of our brain associated with a chronic condition like fatigue, because since this is a symptom appears to be not dependent on specific tasks, its dynamics should for sure emerge in the activity of our brain at rest.

First, it was observed that there was a higher resting state pre-treatment impairment of S1 with respect to M1, as compared to control values, for what concerns the dominant hemisphere. Secondly, analyzing post-treatment data, it was observed that FaReMuS normalized. The difference in FD between M1 and S1 that was huge in the pre-treatment phase, disappeared after the treatment.

Concerning intra and inter hemispheric functional connectivity between S1 and M1 areas, it was observed that, overall, the Mutual Information values improved after the treatment mostly between homologs M1 areas. In the pre-treatment phase indeed, compared to healthy controls, lower values were displayed in fatigued patients, while after the treatment mutual information values improved significantly reaching the levels of healthy controls.

What seems to be interesting is that both aspects (FD and mutual information) as modulated by FaReMuS, correlated with the mFIS such that they explained about half of its amelioration. This witnesses the fatigue's profile to be strongly correlated with electrical activity patterns.

Our pathway for better comprehending FaReMuS mechanisms didn't stop at the 2019 study we described before. In Tecchio & Bertoli 2020 the authors aimed at better clarifying why a treatment that focuses on the functional properties of the brain – cortico-muscular coherence, neurodynamics and functional connectivity - is beneficial against fatigue. The underlying hypothesis was that the treatment works as long as fatigue is related to functional markers (over anatomical ones). Thereby modifying functional properties, we can modify fatigue levels.

A review was created just selecting the small group of papers where the structural and functional counterparts generating symptoms of fatigue, were studied exactly in the same patients. It was observed that in all cases whereas the symptom of fatigue increased together with functional alterations, it was completely uncorrelated with the structural features. Indeed, from these papers

emerged that, in absence of a relationship with MS disability status, functional alterations of the sensorimotor and motor networks and defects of connectivity between parietal homologs in M1 and S1 can explain the symptom of fatigue even in absence of gross anatomical damage.

On a very interesting note, the same profile of functional M1 and S1 alterations emerges in other model of fatigue as a symptom of different pathologies like stroke. Here again the most consolidated hypothesis Is “sensory attenuation” (Kuppuswamy et al. 2015; Kuppuswamy 2017; Kuppuswamy 2022).

The functional with respect to structural prevalence supports the use of electroceuticals and the sensory parietal origin of the symptom supports the FaReMuS approach (see Chapter 2).

Moreover, the literature seems to suggest that tDCS can be a solution to fatigue even in condition different from MS and even in healthy subjects (Workman et al. 2021). If we take into account that the world health organization indicated the pandemic fatigue as one of the most severe effects of the current Covid pandemic, the relevance of a treatment like Faremus clearly emerges.

tIDS - Electroceuticals by personalizing the current

Until now, we spoke about personalization of the transcranial electrical stimulation related to the montage, but there is a completely orthogonal aspect of personalization that is related to the delivered current. Indeed, the international community is looking with much and much interest at the effects currents that are modulated in time (Inukai et al. 2016). As for example the transcranial alternate current stimulation (tACS) or the transcranial random noise stimulation (tRNS) (Antal & Paulus 2013; Moret 2019). The evidence showed that neuromodulations with time-varying transcranial current (tRNS) support cognitive domains more effectively than direct current (Fertonani et al. 2011). In vitro studies have shown that both oscillatory (Fröhlich et al. 2010) and 'scale-free'

stimulation (Gal 2013) can induce 'entrainment', defined as the effective change in target excitability induced through stimulations with modulated currents. Thus, the effectiveness of neuromodulation induced by modulated currents depends on the dynamic characteristics of the current modulation.

Within this scenario my Lab set up for building up an individual current stimulation - that they called tIDS (transcranial individual neurodynamics stimulation).

Here again (Cottone et al. 2018) the FSS was exploited to extract the activity at rest of the primary motor area of each individual subject and then those fluctuations were provided to single subjects. It is to be underlined that even in this group of 18 subjects that was involved in the experiment the time course of the M1 activity during 1 and half minute, was very different from one subject to the other such that we can say the neurodynamics has a great inter-subject variability. This is very important to consider if we want to properly, individually tune neuromodulation treatments.

After determining the individual neurodynamics it was provided to a typical tES stimulator while posing above M1 electrode the TMS Coil to the aim of collecting the motor evoked potentials both when tES was on and when it was off. By this way the authors had both baseline values (tES off) and values revealing the level of excitability that tES produced. Subjects were tested in 4 different conditions: Sham, i.e., control condition; tACS at 20 Hertz[17]; tRNS at same range frequencies of tIDS that is between 1 and 250 Hertz and tIDS itself (Figure 11).

[17] This exactly because tACS at 20 Hertz was proved by the paper appeared on Journal of Neuroscience by Feurra and colleagues on 2011 to be the frequency most effective for M1 at rest.

Figure 10. tIDS Protocol

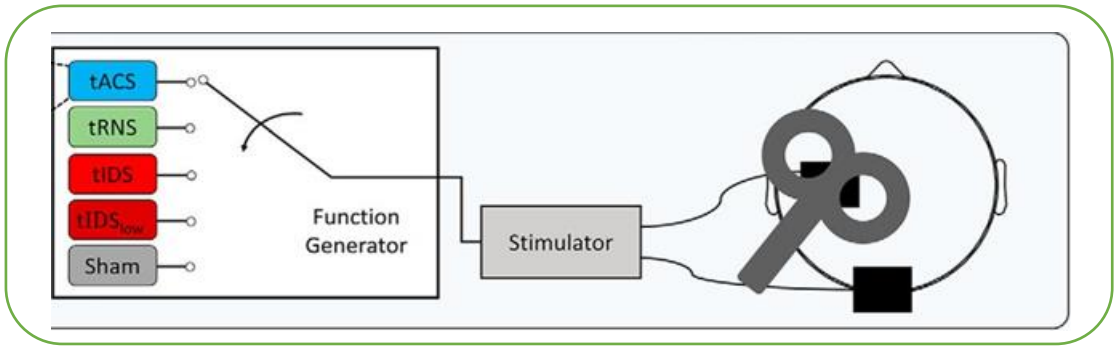

Fig.11. Experimental protocol for testing tIDS neuromodulation efficacy.

Figure re-adapted from Cottone et al. 2018.

What was observed was that the transcranial individual stimulation was able to modify the excitability of M1, and that the effect was much bigger than that obtained through tACS while tRNS stimulation and sham did not produce any effect. In future studies we aim at better exploring the effects of tIDS by not only enlarging the sample, but also investigating its inhibitory effects and effects on the behavioral performance (see Section 3.4 for the research protocol).

By this way, my Lab team hope to have opened the way through a new electroceutical strategy that exploits the neurodynamics of the target. If confirmed to be effective this could be used both invasively and non-invasively, on diverse areas and for diverse pathologies or with the aim of enhancing cognitive functions.

Notably in a paper of 2022 (Armonaite et al. 2022a) we demonstrated how is it possible to decipher the neurodynamics of specific regions of the brain throughout the fractal dimension; in other words, we showed how to every specific region of the brain correspond its own “electrical signature”. This relevant finding paves the way for exploiting such exchange patterns to enhance the efficacy of neuromodulation interventions aimed at curing the electrical activity imbalances featuring multiple pathologies by targeting specific areas.

We like to end up this review by underlining that my Lab's ideas exposed in a TEDx[18] obtained a great wealth of visualizations and solicited many patients to take contact with the Lab, witnessing the growing interest of the scientific community but also of pantients and ordinary people in the topics of electroceuticals.

Conclusions

In this review article which aims somehow at scientifically and historically framing the experimental approach of our LET's Lab we faced multiple aspects and issues: from electroceuticals to personalized medicine to Artificial Intelligence applications to mathematical approaches for indexing complex systems. All those aspects are complementarily at the basis of our approach. Therefore, we think that working in synergy with mathematicians, neurologists, physicians, psychologists, physics and informatics constitutes an added value for reaching the very objective of unravelling the complexity of our body-brain system and project therapeutical solutions.

On the strength of our achievements and the many contacts we have received since the dissemination of our ideas, our plan today is to open a therapeutic electroceuticals service in IC Technology[19] (i.e., supported by the aid the aid of telemedicine and digital helpers - LetsElectrIC).

The electroceutical approach, conceived both as a way of thinking – to know the laws and the language of the body brain system to intervene on it – and as a biomedical therapy develop itself into three branches. The service, the personalization, and the technologies (Figure 12). Where the actual device is complementary to the digital tools that that keep the thread of communication between patient and clinician alive and are at the heart of any modern personalised medicine approach.

[18] https://www.youtube.com/watch?v=WckYnQCZ1qs&t=49s
[19] IC Technology: Information and communication technology.

Figure 11. LetsElectrIC

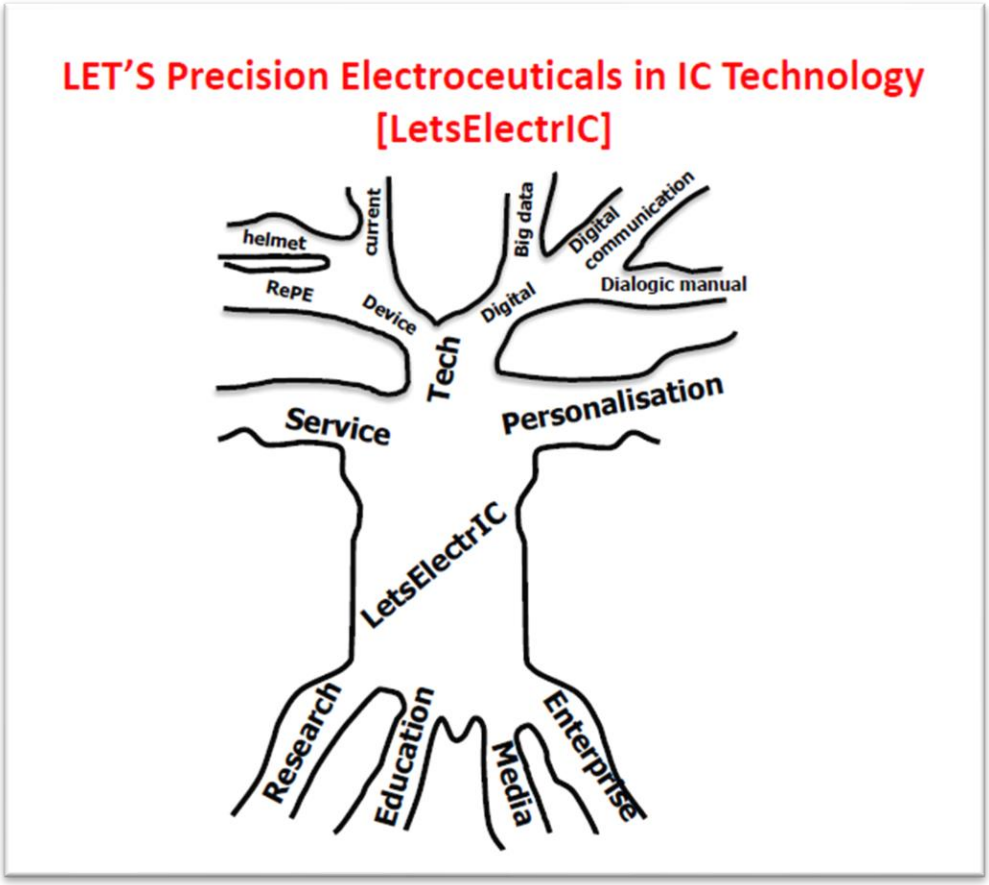

Fig. 12 The picture presents the Let's Lab way of conceiving its pathway to the electroceutical service. Like a tree, the pathway has its roots in a synergy between Research, Education, Media and Enterprise aimed at developing the service (LetsElectric – that stands for Let's electroceutical approach in IC technology). Where the service has multiple components: Service, Technology, and personalization. The technology arm divides itself into the digital aspect of developing tools for optimizing communication between patients and clinicians (Dialogic manual and digital communication) and for processing data (Big Data); and into the aspect of the device with its personalized electrode (Repe, that stands for regional personalized electrode), the helmet and the current.

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1.2 “Transcranial Direct Current Stimulation in Neuropsychiatric Disorders. Clinical Principles and Management” (2016), by André R. Brunoni, Michael A. Nitsche, Colleen K. Loo: a book review*

The following authors contributed to the final and published version of this manuscript:
For details see “author contribution statement” at the end of the manuscript

Eugenia Gianni [1-2]**,** Franca Tecchio [1-3].

1. Laboratory of Electrophysiology for Translational NeuroScience (LET’S), Institute of Cognitive Sciences and Technologies - Consiglio Nazionale delle Ricerche, Rome 00185, Italy

2. Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome 00128, Italy

3. Faculty of Psychology, Uninettuno University, Rome 00186, Italy

Here we propose the review of the Edition of 2016 of the book. The review of the 2021 Edition was published in Frontiers of Neuroscience in 2022.

Gianni, E., & Tecchio, F. (2022). Book review: Transcranial direct current stimulation in neuropsychiatric disorders. Clinical principles and management. *Frontiers in Neuroscience*, *16*.

Introduction

A relevant part of our work during my Phd was dedicated to find out and examine evidence of the use of tDCS in neurological disorders (see also Chapter 4; paragraph 4.2). This work was aimed at streghthening our knowledge in the field for consequentially ameliorating our Faremus system and widen its field of application. Therefore, we decided to examine and review the relevant book "Transcranial Direct Current Stimulation in Neuropsychiatric Disorders. Clinical Principles and Management" by André R. Brunoni, Michael A. Nitsche, Colleen K. Loo. In 2022 we published in *Frontiers of Neroscience* the review of the 2021 Edition (Brunoni et al. 2021) for which I contributed as a First Author. Here we propose the review of the 2016 Edition (Brunoni et al. 2016a) that we prepliminarly read to have a general overview of the entire work.

Our work was part of the innovative and urgent strand of personalised medicine and the development of tools for the protection and development of psychophysical well-being. By reviewing this book, we emphasised the availability of a broad and comprehensive overview that helps to highlight the great potential and novelty of transcranial electrical stimulation (tES) technologies belonging to the broader field of electroceuticals. By describing the mechanisms of action of tES, the procedural framework of tES in research and clinical perspectives in neuropsychiatric disorders, and its availability throughout the lifespan, the book contributes to the understanding that tES can be an alternative approach to pharmacological therapies, providing interventions that can safely alleviate troublesome symptoms secondary to alterations in brain activity while reducing costs and side effects.

The review

Why writing a book about transcranial direct current stimulation (tDCS), one could ask. F. Padberg, Professor of Psychiatry and Psychotherapy, poses this question in the forward to the First Edition, noting that a wide range of open-

access scientific data and papers are also available on this subject. He contends that in light of the tendency for the majority of people to be readily persuaded to utilize tDCS on their own, it is more crucial than ever to present a critical point of view on a scientific approach with practical applications. On the other hand, we believe it is crucial to emphasize the significant originality and promise of NIBS (non-invasive brain stimulation) technology, which is a subset of the larger area of electroceuticals (Reardon, 2014); and to emphasize how they might serve as substitutes for pharmaceutical treatments by offering interventions that can safely relieve bothersome symptoms while lowering costs and side-effects (Lefaucheur et al., 2017). Notably, in a recent quantitative review, we demonstrated how tDCS treatments may be included into the context of medical therapies in accordance with the international authorities' guidelines for classification of clinical trials (Gianni et al., 2021).

The book, first released in 2016 and then re-issued in 2021, is well structured and divides itself into three parts. The first part aims at illustrating the neurophysiology of tDCS and framing it into the broader field of non-invasive brain stimulation techniques (NIBS). This part is devoted to introducing the mechanisms of the relevant NIBS techniques and to give a first approach to their fields of application and possible uses both in patients and healthy brains: from animal studies to neuropsychiatric, cognitive, social and emotional research. The second part is properly focused on the use of tDCS in the field of neuropsychiatric disorders: from mood disorders, to epilepsy, to the so-called disorders of consciousness. The third part is dedicated to various aspects of the clinical use of tDCS: from safety and tolerability to ethical, regulatory, and relevant methodological aspects. To sum up, the book is aimed at offering a 360-degree view of physiological, computational, effects-related and ethical aspects of tDCS without avoiding speaking of its most proximal techniques such as transcranial alternating current stimulation (tACS), transcranial random noise stimulation (tRNS), and repetitive transcranial magnetic stimulation (rTMS). In doing so, it

indicates both the innovative and progressive aspects of current studies involving the application of these techniques as well as their current limitations.

By the very beginning the reader is immersed into an exciting historical overview of the use of electrical stimulation to treat ailments (Zago et al., 2016). From electric fish to the most modern applications of tDCS the reader may learn that the use of electricity to treat illness has a long tradition and deep roots in the history of medicine. Further, the reader has the possibility to be acquainted not only with the physiology, neural mechanisms and computational models of tDCS, but also with its closest relatives – tACS and tRNS – so to have a picture of the multiple modalities of the application of electric stimulation (Antal et al., 2016; Kuo et al., 2016; Truong et al., 2016). Not only we can get in touch with the mechanisms of the tDCS underlying human research, but we are also offered to investigate this throughout the perspective of animal models (Ling et al., 2016): how animal models can teach us about the biomarkers of tDCS and other electric stimulation techniques?

Further, the reader know that TMS and tDCS respectively can contribute to unravel underpinnings of cognitive and behavioural processes both in patients' and healthy subjects' brains in two relevant fields such as neuropsychiatric disorders (Radhu et al., 2016) and socio-emotional research (Boggio et al., 2016); in the meantime he can get acquainted with tDCS beneficial effects on multiple cognitive functions in healthy subjects (Harty et al., 2016). To properly study the effects of tDCS it is possible to combine it with two other essential techniques: EEG (Bolognini and Miniussi, 2016) and MRI (Johnstone et al., 2016). Not only we can closely study tES mechanisms and effects when applied to the scalp (Fröhlich et al., 2016) but also when applied to the cerebellum and spinal cord (Ferrucci et al., 2016).

The heart of the book is devoted to the promising applications of tDCS to psychiatric illnesses. In the field of Major depressive disorders (Brunoni and Loo, 2016), for example, where the drug refractoriness is high, the reader learns that

tDCS can be a valid alternative given its “tolerability, portability and easy of use”. While in the field of schizophrenia, tough tDCS studies are giving promising results there is a lack of randomized controlled studies (Mondino et al., 2016) as well as for diseases like OCD, Anxiety Disorders and PTSD, where the research on tDCS applications is still in its “infancy” (D’Urso et al., 2016). Novel and interesting research applies also to neurodegenerative disorders like Alzheimer, but current studies present many limitations like smallness of sample size (Rajji, 2016).

On the other side, the overview of the promising studies of tDCS’ applications to substance-abuse disorders lays the foundation – for the authors – for a potential explanation of the neural mechanisms behind its effectiveness (Labbe and Fecteau, 2016). Research in vivo and in vitro investigating mechanic insights is also on the rise given the effectiveness of tDCS applications to drug-resistant epileptic patients (Dhamne et al., 2016).

For pain as well, the effectiveness of tDCS is given for granted: the results of several studies suggest that tDCS can produce long-lasting pain relief in different chronic pain syndromes, including migraine, fibromyalgia, and neuropathic pain, though its mechanisms are still to unfold. (DaSilva and DosSantos, 2016). Stroke is also a field where tDCS may serve as an adjuvant therapy for rehabilitation (Paik and Kim, 2016). We finally get into the complex field of Consciousness disorder, where pioneers applications of tDCS seem promising (Aurore et al., 2016).

Beyond these promising results, what is it possible to say in terms of safety and tolerability of tDCS? The issue is debated but no serious adverse effects have been shown so far (Brunoni et al., 2016b). Safety, accessibility, and convenience in terms of costs makes tDCS an optimal treatment also for home- environment (Alonzo and Charvet, 2016). However, it is properly the extreme accessibility of tDCS that makes important to consider ethical aspects related to misuse and autonomy (Wurzman and Hamilton, 2016) and to reflect upon defining regulatory aspects (Vasquez and Fregni, 2016) and invite caution. Moreover, much attention

must be devoted to developing proper study designs and methods in order for the researchers to obtain robust and reliable results (Woods and Martin, 2016).

Author contributions

EG wrote the commentary with the supervision of FT. Both authors contributed to the article and approved the submitted version.

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Chapter 2: Personalized Montage, curing fatigue in multiple sclerosis

2.1 The origin of fatigue in MS: why curing fatigue with electroceuticals?

Multiple Sclerosis and fatigue

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory disease of the central nervous system (CNS). It attacks the myelin that lines the axons causing axonal damage and deteriorating axonal functions (Goldenberg 2012). It is considered the most common non-traumatic disease affecting the CNS and leading to permanent disability in young adults (Dutta & Trapp 2011). Depending on the lesions' location and their severity and spread, it causes mild to severe neurological symptoms including loss or alteration of sensation, motor function, vision, bowel dysfunction and cognitive impairment (Induruwa et al. 2012). According to the disease progression, MS can be divided into three subtypes: 1. a primary progressive type (PPMS) in which symptoms develop linearly through time, 2. a relapsing-remitting type (RRMS) during which symptoms are intermitted with periods of recovery, and 3. a secondary progressive type (SPMS) in which RRMS develops into PPMS without periods of recovery (Lublin et al. 2014).

The main measure of disability and severity is the expanded disability status scale (EDSS) (Kurtzke 1983). It consists of ordinal rating system ranging from 0 (normal neurological status) to 10 (death due to MS) in 0.5 increments interval (Kurtzke 1983).

The estimated number of people with MS is 2.000.000 million of people worldwide. 2 million of people of which 750.000 in Europe and 122.000 of them in Italy (AISM, barometro 2019). Women are more affected than men (2-3:1) (Gilmoure et al. 2018).

The aetiology of the disease remains largely not understood but there is general agreement on a complex interaction of genes and the environment at the

basis of MS emergence (Dobson & Giovannoni 2019). The unknown pathogenesis makes – together with the unpredictability and individual particularity of its course - prevention, treatment and management of the disease particularly challenging. There's no existing treatment curing the illness approved by FDA (Goldenberg 2012), but only delaying or modifying the disease' progression (Ponzio et al. 2015; Goldenberg 2012). Treatments are currently based on pharmacological interventions plus multidisciplinary rehabilitation (Vosoughi & Freedman 2010). The lifelong need for healthcare and rehabilitation makes the socio-economic impact of the disease very high (Ponzio et al. 2015).

Among the above-mentioned symptoms, fatigue is highly common: almost 80% of MS patients report fatigue. For half of these patients, fatigue is the most boredom and disabling symptom (Giovannoni 2006). It is reported to significantly impair quality of life and be one of the primary causes for departure from work (Roessler et al. 2003). It can be also the only perceived symptom at the onset or even before the onset of the disease (Bergamaschi et al. 1997), significantly interfering with everyday activities.

Primary fatigue in MS, which the present work focuses on, must be distinguished from non-primary fatigue. Primary fatigue indeed is a sympthom directly related to the disease while non-primary or secondary fatigue is the result of other synpthoms like depression, sleep disorders, mobility inefficiency, respiratory problems, or even side effects of the therapy (Forwell et al. 2008). Primary fatigue can be diagnosed when non primary fatigue sympthoms may be excluded and it should last more than 6 months (Forwell et al. 2008). Despite its definition is still largely debated (Induruwa et al.2012; Brailey et al. 2010) the Council for Clinical Practice Guidelines reached in 1998 a consensus on such a definition "a subjective lack of physical or mental energy that is perceived by the individual or the caregiver to interfere with usual and desired activities" (Multiple Sclerosis Council for Clinical Practice Guidelines 1998).

Since this symptom became a focus for the researchers, multiple scales have been drawn for measuring fatigue over the past 15 years (summarized in the table below). One of the reasons for the renewal of older scales has been that they didn't comprehend mental fatigue (Penner 2016). One scale which embraces the two main components of fatigue (cognitive and motor fatigue) is the Modified Fatigue Impact Scale (MFIS) derived from the older 40-items FIS (Fisk et al. 1994) and consisting of 21 items concerning how fatigue impacts patient's life. This instrument is based on patients' interviews and provides an assessment of the impact of fatigue on physical, cognitive, and psychosocial functioning (Larson 2013; Tellez 2005). mFIS is the rating scale of fatigue my Lab decided to use in past and current research. The most used scales are FSS and mFIS.

Table I. Some of the principal instruments available to measure self-reported fatigue in MS patients

Fatigue Severity Scale (FSS) *Lerdal 2021*
Fatigue Impact Scale (FIS) *Fisk 1994*
Modified Fatigue Impact Scale (mFIS) *Larson 2013*
Multidimensional Assessment of Fatigue (MAF) *Belza et al. 2018*
Checklist of Individual Strength (CIS) *Worm-Smeitink et al. 2017*
Multidimensional Fatigue Inventory (MFI) *Smets et al. 1995*

Fatigue Assessment Instrument (FAI) *Schwartz et al. 1993*
Fatigue Rating Scale (FRS) *Chalder et al. 1993*
Fatigue Descriptive Scale (FDS) *Iriarte et al. 1999*
Functional Assessment of Multiple Sclerosis (FAMS) *Yorke & Cohen 2015*

(Table re-adapted from Krupp 2003).

Despite its clinical significance, the origin of fatigue is poorly understood and still largely debated (Bethoux 2006; Krupp 2003; Rudroff et al. 2016). Furthermore, drug therapies provide only partial improvements in fatigue treatment and there is none specifically indicated for this symptom (Kesselring & Beer 2005). In fact, currently available medications such as amantadine, acetyl L-carnitine, and amino-pyridines (3-4-diaminopyridine, 4-aminopyridine) showed relatively small efficacy and presented various degrees of non-marginal side-effects (De Luca et al. 2011).

Our aim here is to review existing literature on the origin of fatigue in MS to then pose the hypothesis of a functional damage involving specific brain areas (sensorimotor and motor cortex) at the basis the sympthom (Tecchio Bertoli 2020).

For conducting this mini review, we first selected 5 relevant reviews (Ayache 2017; Brailey et al. 2010; Comi 2001; Kos et al. 2008; Induruwa et al. 2012) on the origin of fatigue in multiple sclerosis. The research of these reviews was conducted by searching for ORIGIN OR PATHOGENESIS OR PATHOPHYSIOLOGY OR CAUSE AND FATIGUE AND MULTIPLE SCLEROSIS on PubMed. From these reviews we extracted the most relevant factors generating fatigue in multiple sclerosis:

citokynes, HPA dysregulation, lesion load, brain atrophy, axonal damage, cortico-striatal-thalamo cortical loop, excitability imbalances. To be able to get the most updated articles on each topic, we did research on PubMed by typing every single factor + FATIGUE + MULTIPLE SCLEROSIS. (Es. Cytokines + fatigue + multiple sclerosis).

Concerning the last part (paragraphs d, e, f), in which we reviewed those articles presenting functional imbalances at the basis of fatigue in MS and those analysing the structural and functional counterparts of fatigue in MS we followed and expanded the analysis conducted by Bertoli and Tecchio (2020).

a) *Bio-molecular markers: the role of citokines*

Pro-inflammatory cytokines are small non-structural proteins promoting inflammation (Dinarello 2000) in the body[20]. Triggered by complex - T-cells mediated - bio-molecular mechanisms they are thought to be likely involved in the pathogenesis of multiple sclerosis (Navikas & Link 1996) and some authors investigated whether their levels could be correlated with the levels of fatigue in MS patients (Heesen et al. 2006; Flachenecker et al. 2004; Malekzadeh et al. 2014; Ackali et al. 2017).

Heseen et al. 2006 investigated the correlation between levels of proinflammatory cytokines IFNɣ e TNFα and anti-inflammatory cytokine IL-10 in the serum and m-FIS values and found out significant results for the first two ones, while no significant results were found for IL-10; indicating a substantial role for pro-inflammatory cytokines in the pathogenesis of fatigue.

Flachenecker et al. 2004 found that high levels of MS fatigue measured by FSS correlated with the levels of TNFα mRNA expression in the peripheral blood but not with the levels of IFN and IL-10 concluding for a relevant role of solely TNFα in the pathogenesis of fatigue.

[20] Examples of pro-inflammatory cytokines are Interleukin (IL)-1 and tumor necrosis factor (TNF), see Dinarello 2000 for a comprehensive review on the identification and role of pro-inflammatory and anti-inflammatory cytokines.

Malekzadeh et al. 2014 investigated the role of pro-inflammatory (IL-1β, IL-2, IL-6, IL-8, IL-12p70, IL-17, TNFα, and IFN-γ) and anti-inflammatory cytokines (IL-4, IL-5, IL-10, and IL-13) in the pathogenesis of fatigue by determining correlations between fatigue assessed through the self-reported Checklist Individual Strength (CIS20r) and blood serum concentrations of cytokines. They found a single significant relationship between the pro-inflammatory cytokine interleukin-6 (IL-6) and fatigue levels, concluding that this cytokine may play a role in the pathophysiology of primary fatigue in patients with MS.

Finally; Ackali et al. 2017 who investigated both the role of cytokines and HPA[21] axis, investigated the relationship between fatigue measured by FSS and serum IL-1β, TNF-α, IL-35, IL-2, IL-10; they found out that while IL-1β, IL-10 and TNF-α levels did not differ between patient and control groups; while IL-35 and IL-2 levels were significantly higher among MS patients but there were no significant differences between fatigued and non-fatigued patients.

Despite a significant role of Cytokines in the pathogenesis of MS and more in particular a role of cytokines in the etiopathogenesis of fatigue seem given for granted by the literature; the above-mentioned studies gave discrepant results, probably due to the use of different fatigue-scales and focus on different kinds of cytokines. More homogeneous studies are needed to determine whether the cytokines have a role in the pathophysiology of fatigue.

Interestingly, in support of the hypothesis of a significant role of cytokine in the pathophysiology of fatigue, Hanken et al. 2014 hypothesized, in a review, that the subjective feeling of MS-related fatigue may be a variant of inflammation-induced sickness behavior, resulting from cytokine-mediated activity changes within specific brain areas. On this basis they deduced that elevated levels of pro-inflammatory cytokines should also cause fatigue in healthy individuals. In support of this hypothesis, they presented studies demonstrating a relationship between

[21] The role of HPA axis in the aethipathogenesis of fatigue in MS will be treated in the next paragraph (b). HPA= Hypothalamic-Pituitary-Adrenal axis.

pro-inflammatory cytokines and subjective fatigue in healthy individuals (Vollmer-Conna et al. 2004; Dantzer et al. 2008; Kerr et al. 2001; Konsman et al. 2002; Dantzer et al. 2014) indicating that insisting on investigating the relationship between fatigue and immune-mediated phenomenon might be a promising pathway.

b) *Endocrinal markers: the role Hypothalamo–pituitary–adrenal axis*

The hypothalamic–pituitary–adrenal axis (HPA axis) is a complex set of direct influences and feedback interactions among three components: the hypothalamus, the pituitary gland, and the adrenal glands (Spencer & Deak 2017). These organs constitute a major neuroendocrine pathway that modulates the body's response to stress - and other body's functions as well such as digestion - through complex interactions and feedback mechanisms. Cortisol, adrenocorticotropic hormone (ACTH) and other melanocortins are involved in the HPA axis.

The putative role of HPA in the pathogenesis of chronic fatigue syndrome (Papadopoulous 2012; Patarca et al. 2001; Yang et al. 2019; Blundell e t al. 2015; Montoya et al. 2017) let some researchers hypothesize a connection between HPA axis dysfunction and fatigue in MS.

Gotthslak et al. (2005) measured the correlation between fatigue assessed by both FSS and mFIS and HPA axis regulation assessed trough the combined dexamethasone–corticotropin releasing hormone (DexCRH) test. Interestingly, first, they found out that about 48% of MS patients experienced fatigue and that fatigue was not correlated with EDSS score reflecting many observations (Baskhi 2000) also shared by us that fatigue occurs at the early onset of the disease and may be one of the leading sympthom. Moreover, in their study, the MS patients with fatigue showed a dysregulation of the HPA axis, as demonstrated by significantly elevated serum ACTH levels with respect to non-fatigued patients. These results are in contrast with results showing a dysregulation of HPA axis in fatigue chronic syndrome which showed a hypo reactivity of the HPA (Tanriverdi

et al. 2007; Tomas 2013; Van Den Eede 2007). However, they postulated the hypothesis of HPA axis dysregulation to be connected to elevated levels of proinflammatory cytokines highlighting the deep interconnection between the immune and endocrinal system.

However, Ackali et al. 2017 who investigated both the role of cytokines and HPA axis in fatigued patients were fatigue was assesessd by multiple scales (FSS included) found no correlation between ACTH levels and fatigue, despite ACTH levels were higher in MS patients in general.

To conclude, results from clinical trials in chronic fatigue syndrome may indicate a strong interrelation of dysregulation of endocrine pathways and fatigue. Therefore, investigating this relation in MS fatigued patients seem promising. However, the results are still seminal and conflicting, more homogeneous studies are to be done to deeply investigate endocrinal factors on the pathogenesis of fatigue in MS.

c) *Anatomical markers: cortical athrophy, lesion load and axonal damage*

Cortical atrophy and lesion load

Several studies have measured the relationship between MS fatigue and brain atrophy and/or lesion load.

In a study of 2010 Pellicano et al. measured, in 24 MS patients and 24 MS volunteers, the macrostructural damage (volume loss) to the thalamus, basal ganglia, frontal, and parietal lobes using high-resolution structural MR imaging and an automated reconstruction of cortical surface and subcortical structures. The relationship between fatigue severity (measured by mFIS) and atrophy of the cortical and subcortical structures in MS was then researched. The only significant correlation was found between posterior parietal cortex volume loss and fatigue severity. However, as the same authors admit, the study was limited in power by the smallness of the sample size.

In a study of 2005, in a group of 134 individuals, Marrie et al. examined the association between fatigue and brain atrophy longitudinally over an 8-year period. The brain parenchymal fraction (BPF) was used to quantify brain atrophy, and the Sickness Impact Profile's Sleep and Rest Scale (SIPSR)[22] was used to assess fatigue. Fatigue's measurements were taken at baseline, year 2 and year 8. The correlation between variations in fatigue and the development of atrophy was examined using linear regression analysis. Increasing fatigue was strongly linked to progressive brain shrinkage over the six years. This study gave promising results however, the authors as they themselves admit used a non-validated scale of fatigue for MS patients.

In a 2007 study, Tedeschi et al. assessed normal and abnormal White Matter and Grey Matter fractions and lesion load in 222 RRMS patients with low disability. Fatigue was assessed using the FSS and subjects were divided into low fatigued and high fatigued groups. High-fatigue patients' group showed significantly higher abnormal white matter fraction, lesion load, and significant lower WM-f (white matter fraction), and GM-f (grey matter fraction). Moreover, high FSS was significantly associated with lower WM-f, and GM-f. However, as athe authors themselves refere a possible limitation of this study was the lack of a formal measurement for depression, in order to exclude significant association between fatigue and depression.

In a study of 2010, Calabrese et al. investigated the association between deep and cortical grey matter atrophy and fatigue assessed both by the FSS and mFIS. In 152 RRMS patients, they measured thalamic and basal ganglia volumes and regional cortical thickness. After dividing patients into fatigued and non-fatigued groups, they observed significant atrophy of striatum, thalamus, superior frontal gyrus and inferior parietal gyrus was observed in fatigued patients compared with non-fatigued patients.

[22] The Sickness Impact Profile Sleep and Rest Scale (SIPSR) consists of seven items, and total scores range from 0 to 100, with higher scores indicating more dysfunction.

In a 2013 study, Gomez et al. evaluated a group of 60 RRMS patients and 18 healthy controls with FSS to determine their level of fatigue and divided them into non fatigued and fatigued groups. They then investigated Grey-matter (GM) and White-matter (WM) atrophy using voxel-based morphometry. Fatigued patients showed extended GM and WM atrophy focused on areas related to the sensorymotor network. In particular, involving the sensori-motor area, the cerebellum, the posterior motor cortex and the brainstem. Interstingly while investigating Resting state FC (resting-state functional connectivity) they found out diminished FC levels in the sensorymotor pathways.

To sum up, these studies indicated a significant relationship between cortical ahrophy and the genesis of fatigue, but facing disohomogeneous results, more research is required to shed light on this stream of studies (Constantinescu et al. 2012).

Axonal Damage

The study which explored the hypothesis of an association of diffuse cerebral axonal damage with fatigue was the one by Tartaglia et al. (2004) who assessed the relationship between fatigue measured with FSS and the level of N-Acetyl Aspartate[23] measured through non-invasive proton magnetic resonance spectroscopy. The results of this study suggest that diffuse periventricular axonal injury is associated with increased fatigue in patients with MS. Indeed, independently of EDSS score, T2 lesion volume, age, and disease duration, the NAA/Cr ratio was significantly lower in the high-fatigue group as compared with the low-fatigue group. These results suggest an interesting research pathway, but more research is needed to go deeper into this suggestion.

[23] N-Acetyl Aspartate is a marker of neuronal integrity that is localized to neurons and neuronal processes in the mature brain. Clark, J. B. (1998). N-acetyl aspartate: a marker for neuronal loss or mitochondrial dysfunction. *Developmental neuroscience*, *20*(4-5), 271.

Cortico-subcortical pathways

Among the several studies focusing on brain abnormalities of the cortico-striato-thalamo cortical loop (Bisecco et al. 2016; Gomez et al. 2013 as an example) it worth to speack about studies focusing on the role of thalamus in the pathophysiology of fatigue in MS (reviewed in Capone et al. 2019).

Indeed, several investigations indicated both structural and functional alterations of the thalamus in relation of fatigue. Thalamic atrophy, basal ganglia shrinkage, and fronto-parietal brain volume decrease were observed (Calabrese et al. 2010; Bernitsas et al. 2017). Furthermore, the thalamus of fatigued MS patients had evidence of demyelination and axonal loss (Niepel et al. 2006; Wilting et al. 2016). However, the authors (Capone et al. 2019) observed that, although it is now widely acknowledged that a dysfunction in a cortico-subcortical pathway centered on the thalamus contributes to fatigue in MS, different types and degrees of this dysfunction have been studied. Some studies have found a decrease in the activity (Filippi 2002; Cruz-Gomez et al. 2013) and connectivity of the thalamic network, while others have found an increase (Rocca et al. 2007; Zhou et al. 2016). Further research employing multiple techniques and longitudinal studies is to be done to properly unravel the role of thalamus in the pathophysiology of fatigue (Capone et al. 2019)

d) *Functional damage: excitability imbalances and functional connectivity alterations*

By registering the brain signals by techniques such as electroencephalography (EEG), magnetoencephalography (MEG) functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) while the subject is performing a fatiguing task it is possible to study functional profile and functional connectivity patterns related to fatigue in MS (Capone et al. 2020; Santernecchi et al. 2015). Thanks to these studies a framework of neural

excitability imbalances and functional connectivity alterations is emerging strongly as a marker of fatigue in MS.

Excitability imbalances

Given the nature of fatigue as a disturbance of the motor system, most of studies investigating its underpinnings focused on the motor and sensorimotor systems. From these studies a pattern of excitability imbalances and dynsfunctional connectivity is emerging at the level of these systems.

Specogna et al. (2012) measured trough functional MRI in 24 RRMS patients with and without fatigue and 15 healthy controls cortical activation while patients were fingertapping with the right hand. Compared with controls, patients without fatigue showed greater activation of the primary sensorimotor cortex bilaterally, of the right supplementary motor cortex, of the left premotor cortex, of the left cerebellum and of the superior parietal lobule bilaterally. Compared with patients without fatigue, patients with fatigue demonstrated greater activation of the right premotor area, of the putamen and the dorsolateral prefrontal cortex. They concluded that patients with fatigue have greater activation of the motor-attentional network when performing a simple motor task.

Liepert et al. 2005 investigated electrophysiological correlates of fatigue in MS. In two groups of RRMS patients (fatigued, non-fatigued and healthy controls) they explored motor excitability trough TMS and measuring MEPs prior and after a fatiguing exercise. They found out that prior to the motor task, MS fatigued patients had less inhibition of the motor cortex compared to both other groups and post-exercise as well. Moreover, the post exercise time interval for normalization of the motor threshold was correlated with fatigue severity in fatigued patients.

In an EEG study, Leocani et al. 2001 tested the pattern of cortical activation prior, during and after self-paced movement in a group of 15 RRMS patients, 18

non fatigued patients and 14 healthy controls. They found out a pattern of dysfunction of cortical activation in fatigued patients compared to non fatigued patients and healthy controls. This pattern resulted in hyperactivity during movement execution and failure of inhibition after termination indicating cortical dysfunction even during a simple motor task associated with fatigue and a supposed central origin of fatigue.

Overall, these studies indicated that M1 of MS fatigued patients is more excitable at rest, it hyperactivates during exercise and its inhibition impairs after exercise.

Paralleling signs of hyperexcitability of M1, a framework of hypoexcitability of the sensorimotor cortex S1 emerged.

MS patients demonstrated poor primary sensorimotor system activation patterns (Dell'Acqua 2010) as well as a lack of functional specialization in the cortical representation of distinct fingers (Tecchio et al. 2008). Since the somatosensory cortex's dysfunctional functioning was unrelated to central sensory conduction, sensory abnormalities in MS patients are likely due to this dysfunction rather than a problem with the signaling of sensory input to the brain. Reduced S1 excitability was also observed (Dell'Acqua et al. 2010).

The balance between local connection and global integration in the left and right frontal (motor) and parieto-occipito-temporal (sensory) brain networks was evaluated using the small-world index in an EEG-derived graph theory study (Vecchio et al. 2017). The modification of the dominant-hemisphere sensory network's small-world index was accompanied by a rise in fatigue sensations. Additionally, during a cognitively fatiguing task, perceived levels of fatigue in MS patients were associated with altered posterior parietal cortex activation as well as decreased functional connectivity between cortical (the posterior parietal and frontal regions) and subcortical (the striatum and the thalamus) structures (Engstrom 2013).

To sum up, as fatigue levels rise, primary and non-primary motor regions exhibit altered functioning and become overexcited, whereas sensorimotor areas exhibit depleted excitability and connection.

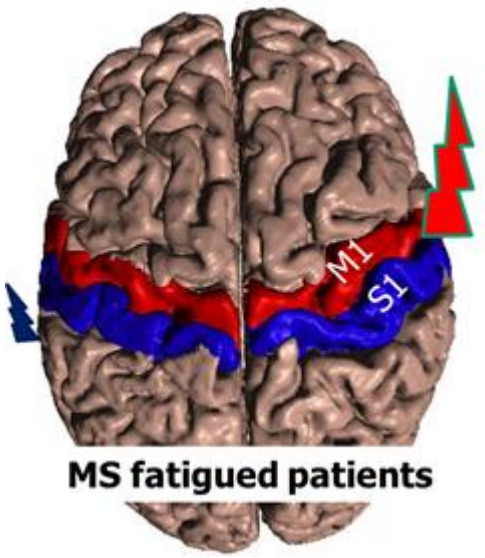

Figure 1. the picture presents the two main areas involved in the pathogenesis of fatigued: the somatosensory cortex S1 (in blue) and the motor cortex (in red). It highlights the fact that in MS fatigued patients S1 shows to be hypoexcitable while M1 shows to be hyperexcitable.

Functional connectivity alterations

MS fatigue appears to be a result of the interhemispheric communication channels being damaged.

At rest (Bisecco e tal. 2018) and during movement execution (Zito et al. 2014; Cogliati Dezza et al. 2015) in connection to fatigue increase, MS patients showed imbalanced interhemispheric functional connectivity between homologous sensorimotor regions. Increased degrees of fatigue were also associated with changes in the functional connection between the temporo-parietal hemispheric homologs at rest (Buyukturkoglu 2017).

In MS fatigue, it was shown to be poor communication between S1 and M1 or impaired responsiveness of M1 to S1 projection. S1-M1 communication can be predicted to have a significant role in MS fatigue given the crucial role of ascending

pathways in cortico-muscular synchronization and the fact that cortico-muscular coherence changes in MS patients explained 67% of subjective fatigue (Tomasevic et al. 2013). Additionally, it was shown that MS patients had an imbalanced recruitment of the S1 inhibitory and M1 excitatory pathways (Dell'Acqua et al. 2010).

Overall, the results show that parietal regions play a particular role in MS fatigue in terms of functional connectivity between the two hemispheres and in communication with frontal areas.

e) *Functional vs anatomical damage*

Bertoli and Tecchio (2020) reflected upon studies that investigated both the structural and functional profiles in MS fatigued patients not differing for disability status[24] to check whether the functional damage could be correlated to the structural damage or not.

Tomasevich et al. (2013) used both structural and functional measurements in 20 mildly disabled MS patients divided into two groups according to their mFIS score. While they measured by MRI thalamic volume and cortical thickness of the primary sensorimotor areas, they acquired measurements of CMC from simultaneous electroencephalo- and surface electromyographic recordings during a weak handgrip task. They found out that while higher level of CMC characterized more fatigued patients, abnormalities in cortical thickness and thalamic volume didn't correlate with fatigue levels.

Cogliati Dezza et al. (2015) investigated functional connectivity between homologous regions in the sensorimotor network in 27 mildly disabled MS patients. While recording trough the EEG fcuntional connectivity measures at rest and during a simple motor task, they measured trough MRI interhemispheric asymmetries in the thickness of Rolandic regions and volume of thalami, after

[24] Indeed, it is very important to primarily exclude fatigue to be correlated to the level of disability because disability may be misinterpreted by the patient as a level of fatigue. This is why in our studies we selected patients with the same level of disability (low, EDSS<3).

measuring levels of fatigue with the mFIS scale. They found out that fatigue increased along with functional imbalances of the two homologous areas both at rest and during movement while structural asymmetries and alterations didn't correlate with fatigue levels.

Finke et al. (2015) analyzed the association of fatigue severity, assessed with FSS, with basal ganglia functional connectivity, basal ganglia volumes, white matter integrity and grey matter density in 44 patients with RRMS and 20 healthy controls. To this aim they performed resting-state fMRI, diffusion tensor imaging and voxel-based morphometry. They found out that in comparison with healthy controls, patients showed alteration of grey matter density, white matter integrity, basal ganglia volumes and basal ganglia functional connectivity. No association of fatigue severity with grey matter density, white matter integrity and basal ganglia volumes was observed within patients.

Bisecco et al. (2018) investigated through fMRI resting-state functional connectivity of default mode network and sensorimotor network in 59 RRMS patients (divided into two groups: fatigued and non-fatigued – fatigue levels assessed with FSS) and 29 healthy controls. Moreover, they measured regional gray matter athrophy by voxel-based morphometry. While no significant structural changes were found, functional connectivity changed in both networks in fatigued patients compared to healthy controls. Indeed, fatigued patients showed altered resting state functional connectivity in the posterior parietal cortex, in the anterior cingulate cortex as well as an increased functional connectivity in the motor cortex and supplementary motor cortices of both networks.

Jaeger et al. (2019) investigated trough fMRI resting state functional connectivity alterations of the striatum and dorso-lateral prefrontal cortex in 77 RR MS patients (fatigued and non fatigued, while fatigue levels were measured by FSS) and 41 healthy controls. Results showed an altered functional connectivity in fatigued patients between the striatal areas and sensorimotor cortex, frontal

parietal and temporal cortex regions. Notably, the volume of none of these structures correlated with fatigue levels.

f) *Functional rather than anatomical damages: the option of the electroceuticals to relieve fatigue*

Multiple sclerosis induces recruitment alterations which can be assessed in the central peripheral pathway via clinically relevant experimental paradigms evidencing central component of the cumulative cortico-muscular latency variability with a minimally invasive approach (Caliandro et al. 2014).

Overall, research comparing structural and functional aspects of MS fatigue clearly suggests that functional rather than structural brain changes are more likely to be involved in the origin of fatigue. These findings were made without regard to any association with MS-related disability status. The brain-body cortico-muscular synchronization, the dynamic connectivity between hemispheric sensorimotor and parietal homologs, the resting-state connectivity within the cingulate non-primary motor cortices, the striatum, and its projections are specifically included in the primarily functional neural origin of MS fatigue.

Most of these studies include MS patients who exhibit little clinical symptoms suggesting that sensorimotor communication dysfunction was involved in the fatigue symptom early on (Dell'Acqua 2010; Cogliati Dezza 2015).

These relevant observations led my Lab team to hypothesize that MS fatigued patients may benefit from interventions aimed at compensating excitability imbalance using techniques like repetitive TMS and tDCS.

Indeed, we are currently preparing a review summarizing the results found so far by applying tDCS to fatigued patients[25].

Basing on previous evidence (see paragraphs d and e) indicating a specific involvement of the sensorimotor network, with an hyperexcitability of the motor

[25] 'What can neuromodulation effective against symptoms teach us about fatigue in multiple sclerosis? 'to be submitted in *Biomedicines Journal*

cortex M1 counterbalanced by an hypoexcitability of the sensorimotor cortex S1, my Lab decided to personalize a treatment which has already been proven to enhance endurance to fatigue when applied to healthy subjects (Cogiamanian et al. 2007).

Indeed, my Lab team decided to selectively target S1 by carefully avoiding M1 by a personalized electrode. They thus exploited an ad hoc procedure to properly shape and position the customized S1 electrode using individual brain MRI data (Cancelli 2015; Tecchio 2013) and tested the treatment in two consecutive randomized control trials.

By this time, the system of neuromodulation consists in a protoptype, in the future, we aim at improving its engineering and explore market's possibilities.

Conclusions

MS fatigue appears to have a multifaceted origin involving immune, hormonal, structural and functional factors. More research is to be done to disentangle the specific contribution of each factor in the pathogenesis of fatigue. However, we demonstrated that functional alterations prevail on structural changes in the complex aetiology of fatigue. Therefore, modulating neuronal excitability using electroceuticals seems to be an effective strategy against MS fatigue. The bilateral somatosensory cortex appears to be the ideal neuromodulation target. The encouraging findings of transcranial electric neuromodulation approaches on MS fatigue open the door for novel therapies that are more affordable, simple to administer at home, and individualized to increase efficacy.

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Chapter 3: Personalized Current, from the FeeSyCy principle to the innovation of tIDS

3.1 Introduction: the body-brain system and the electroceutical approach

The human organism's functioning is possible thanks to the refined interaction among organs which finely interplay - each one with its own regulatory mechanisms - among each other. So, the immune, neurological, metabolic, hemodynamic, and hormonal systems result to be deeply intertwined and to influence each other; this is what emerges from the very recent studies on the human body's complexity (Zmora et al. 2017; Wrona 2006; Dal Lin et al. 2015; Dal Lin et al. 2019; Dal Lin et al. 2021; Dal Lin 2018). The interaction occurs at multiple scales: from the level of molecules to the level of entire organs or systems (Wrona et al. 2006). Disrupting organ communications can lead to dysfunction of individual systems or to collapse of the entire organism as observed under clinical conditions such as sepsis, coma, and multiple organ failure (Ivanov 2021).

The nature of this interaction is very well caught by the interdisciplinary field of Network Physiology (Bashan et al., 2012; Ivanov and Bartsch, 2014;Bartsch et al., 2015; Ivanov et al., 2016; Lin et al., 2016) where computational, statistical and non-linear dynamics approaches are merged to unravel the dynamic complexity of the human organism conceived as a macroscopic multimodal network of interacting nodes/organs (Ivanov 2021).

But the interaction can occur also at the level of the sub-system: the brain is a striking example of this interaction (Bullmore & Sporns 2012). Indeed, the brain is made up of elements that are at the same time nodes (soma of the neuron) and connectors (axons). Neurons can be of the same type (like a couple of cortical neurons) or of different types (like glia and pyramidal neurons – Fields et al. 2002) but what we aim at stressing here is their intrinsic communicative nature (Laughlin 2003) creating hierarchies as well as coordinated sets among multiple neurons that makes brain functions possible.

Not only the brain displays interaction among its parts at multiple levels (from the levels of neurons to the level of neural assemblies to the level of brain areas), but its functioning also requires continuous dynamic interaction with the environment. This flow of information is organized into a definite spatio-temporal pattern, resulting in a local course of brain activity, the local "neurodynamics" (Marino et al. 2019; Cottone 2018; Armonaite et al. 2022a-b; Porcaro et al. 2019).

Going deeper into the modes of interaction among multiple neuronal assemblies in the brain and into the modes of exchange of the brain with the environment we think that a general principle governs these complex mechanisms; a principle that we called "feedback-synchrony-plasticity". Since this principle presents itself at multiple scales, we think it reflects the fractal properties of the neurodynamics that emerge from our studies (Zappasodi et al., 2015; Smits et al., 2016, Croce et al., 2018, Marino et al., 2019, Zappasodi et al., 2014; Porcaro et al., 2019, Armonaite et al. 2022a-b; Porcaro et al. 2019).

In other words, we think that the neurodynamics contains a principle that is repeated at different magnitudes as a sort of signature of our body functioning and that manifests itself not only in the interaction between neurons, but also in the exchange between entire parts of our body-brain system (Tecchio et al. 2020).

The fractal dimension appears as a means to decipher the neuronal language expressed by the neurodynamics; but to decipher we must be able to listen. In this sense we can use the techniques of electrophysiology (electro- and magnetoencephalography and electromyography) to study the features of the electrical activity of the brain.

We said above that the absence of a coordination among multiple nodes can result in an imbalance or a proper disease involving the entire organism. Dysfunctions of the neurodynamics as well could be signs of such imbalances and restore the proper neurodynamics become important for treating the disease.

In this case we are helped by NIBS (non-invasive brain stimulation techniques) allowing us to intervene - after "listening" to the brain rhythm - where we identify a dysfunction by exploiting the same language of neuronal networks.

In other words, by sending electrical signals to the scalp, that is, the electroceutical approach (Reardon 2014).

Section 3.2: The FeeSycy principle and tIDS

In the first article we present, which I contributed to writing and editing, our aim was to go deeper into the triadic principle FeeSyCy for arriving to introduce the reader to a novel and original adaptation of the tES approach that my Lab realized and that was called tIDS (Cottone et al. 2018). Behind this novel approach, lies the paradigm of "listening to the brain for intervening"; indeed, by tIDS, after studying the local neurodynamics of a neuronal pool, we target the neuronal assembly by a current that mimics its endogenous neurodynamics.

Section 3.3: Electrical signatures in the brain

In the second article which I contributed to writing we present a study that lays the foundation for expanding the application of personalized current to different areas of the brain. In fact, we show how it is possible, through the fractal dimension, to decipher the specific neurodynamics of different areas of the brain as a proper "cortical signature".

Section 3.4: DopBytIDS, a research protocol

Finally, we will present a research project we designed during the first year of my Phd aimed at testing the excitatory and inhibitory effects of the personalized current tIDS.

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3.2 Fractal neurodynamics of the BodyBrain's control system*

The following author contributed to the final and published version of this work*:
For details see author contribution statement at the end of the manuscript:

Franca Tecchio*[1], Massimo Bertoli[1-2], **Eugenia Gianni** [1-3], Teresa L'Abbate[1], Luca Paulon[1], Filippo Zappasodi[2]

[1] LET'S - Laboratory of Electrophysiology for Translational neuroscience, ISTC - CNR, Rome, Italy

[2] Department of Imaging and Neuroscience and Clinical Sciences, University 'G. D'Annunzio' of Chieti-Pescara, Chieti, Italy

[3] University Campus Biomedico of Rome, Rome, Italy.

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Introduction

The new scenario recognized to the Electroceuticals (Famm *et al.*, 2013; Reardon, 2014) increases the interest in understanding the phenomena of electrical transmission in our Body & Brain system. Through non-invasive investigations, we have observed that the fractal dimension (FD) is a measure of neuronal dynamics (neurodynamics) sensitive to physiologically relevant phenomena: aging (Smits et al., 2016; Zappasodi et al., 2015), damage (acute stroke (Zappasodi et al., 2014), Alzheimer disease (Babiloni et al., 2004), circadian cycle (Croce *et al.*, 2018), behavioural state (Cottone *et al.*, 2017; Marino et al. 2019). We would like to introduce here a model with a triadic principle - (Feedback, Syncrony, Plasticity, e.g., FeeSyCy) presenting itself at different scales of the system - that we think is the origin of the adequacy of FD in quantifying the state of neurodynamics. We will close by indicating the first signs that, by using the same neuronal language, the neurodynamics of the target region, we can make a neuromodulation intervention more effective.

What do we mean by the fractal dimension of the neurodynamics? If we observe a system where the whole structure is made up of single blocks, which are similar to the whole, and are in turn made of smaller blocks, which mirror the intermediate and the whole structure (like broccoli for example), we are actually observing a fractal structure. Mandelbrot also exploited this example to explain these systems, which he baptized 'fractals' as their dimensions can be expressed by a non-integer number. The paradigmatic example of a linear fractal structure is the coastline: Lewis Fry Richardson (Mandelbrot, 1967) used as an example the Great Britain's coast, whose length increases as we magnify it looking closer and closer, until our eyes see the boundary between the sea and the earth. Instead of the distance between two successive points of the cost in relationship with the spatial sampling scale, in our case the fractal dimension estimates the distance between two successive neuronal electric amplitudes in relationship with the time sampling.

FD of neuronal dynamics is sensitive to relevant physiological features

The brain shows a wonderful hierarchical morphology (Kandel & Schwartz, 1985) based on a huge number of neurons, which form a self-organizing complex system (Turvey, 1990; Graziano & Aflalo, 2007; Tognoli & Kelso, 2014). From an electrical point of view, neurons' only function is to communicate, via action potentials. As a result, a cortical area, for example, expresses a time course of its neuronal electric activity, i.e., its neurodynamics, which is a 'single' descriptor of its entire functionality, resulting from the tens of thousands of receiving projections from diverse brain structures and the tens of thousands of its projections impinging other brain structures. The neurodynamics can be studied by free-scale measures like for example the fractal dimension (Freyer *et al.*, 2009; Van De Ville *et al.*, 2010; Buzsaki & Mizuseki, 2014; Kopell *et al.*, 2014; Roberts *et al.*, 2014).

I will try here to communicate the concepts that I got from my experience on the brain activity, as derived from magneto- and electro-encephalography (MEG and EEG), in favour of the fractal nature of the neurodynamics, which derives from the multi-scale representation of a triadic principle made of feedback, synchrony and plasticity that we called FeeSyCy.

Some years ago, we moved from the knowledge that the power as a function of frequency of the neuronal population activity displays the so-called 'power law' dependence (Buzsaki and Mizuseki, 2014; He, 2011; Ramon and Holmes, 2015; Roberts et al., 2014). Since in this case, the signal's fractal dimension corresponds to the exponent of this exponential function, we considered evaluating and measuring the scale-free organization of the neuroelectric activity via its fractal dimension. Our experience developed using Higuchi's estimate.

In healthy people, the Higuchi fractal dimension (HFD) of the electroencephalographic signal (EEG) was correlated with age (Smits et al., 2016;

Zappasodi et al., 2015), thus indicating a sensitivity to the structuring of the neuroelectric organization parallel to human development in young, mature and elderly subjects. In particular, FD with age fitted a parabola with the vertex located around 50 years of age, increasing from 20 and decreasing from 50 to 90. The FD age-related changes were topographically specific, with inter-hemispheric FD asymmetry emerging in elderly individuals in the frontal and central regions. This reveals a FD reduction with age faster in the left primary motor and premotor areas than in the right hemisphere. A slower age-related FD decrease in the right hemisphere could be a sign of compensating phenomena occurring in the older brain. Indeed, the network including the parietal and frontal areas of the right hemisphere has been proposed to contribute to cognitive reserve, protecting the brain activity from dysfunction due to age-related changes or disease (Robertson, 2014).

In brain network damage pathological situations, we observed FD to be sensitive to relevant functionality impairment. The FD was sensitive to the neuronal dysfunction secondary to the brain lesion induced by a stroke in the middle cerebral artery of one hemisphere (Zappasodi et al., 2014). We investigated it in the sub-acute phase in the period between 3-10 days from symptoms onset, after stabilization of the vital parameters. In this phase we focused on investigating whether FD could assess the clinical impairment and the clinical recovery prognosis. FD reduced more as the acute clinical status worsened. Interestingly, the asymmetry between FDs in contra-lesional and ipsi-lesional hemispheres did not associate with acute phase clinical state; instead, it increased in correlation with the worsening of clinical recovery at six months. Overall, while whole head FD captured the neurodynamics alteration secondary to the structural damage after a stroke, its inter-hemispheric asymmetry highlighted the functional relevance of the balance between homologous brain structures' activities in functional abilities and clinical recovery, with a prognostic potential.

In people diagnosed with Alzheimer's disease (AD), enhancing previous findings with other measures of EEG complexity (Stam et al., 2007), we found that FD reduced compared to healthy elderly individuals. FD reduced in a correlated manner with the decrease in cognitive capacity as assessed by Mini-Mental State Examination (MMSE) score. These findings indicate that FD assists detecting the loss of neural efficiency and the reduction of the cortical communication in AD. Consistent with this hypothesis, region-specific FD decline in AD-affected individuals as compared to healthy elderly patients occurred only in temporal and occipital regions, cortical districts with more acute involvement in the disease (Babiloni et al., 2004).

We recently discovered that the cortex can be parcelled based on its local neurodynamics (Cottone et al. 2017; Marino et al. 2019), integrating the classical parcelling based on the cytoarchitecture and connectivity of the neuronal cells (Brodmann, 2006). Notably, contrary to multiple spectral features differentiating cortical neurodynamics, the fractal dimension of the electrical activity of the neuronal pool is a single number enabling the local characterization (Figure 1). Furthermore, the fractal dimension of the neurodynamics of the primary motor counterpart of the hand representation, correlates with the quality of the hand-fine motor control as scored by the 9 Hole Peg Test.

Figure 1. Fractal dimension of the neurodynamics

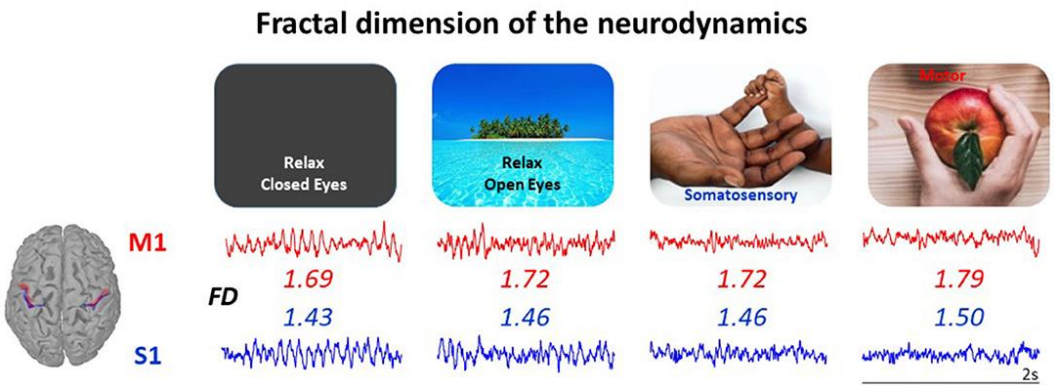

Figure 1. A neural network node's state may be described, even while at rest, using the neurodynamic complexity as measured by its fractal dimension (FD). When transitioning from relaxed in the absence of any stimuli (LEFT) to selective sensory awareness, then to active sensorimotor control, the FD of the neurodynamics (2 s in each stage), rises (RIGHT). The FD of a node reflects the structural specialization of that node; in this case, in all network states, the principal motor hand region (M1, red) has a smaller FD than the primary somatosensory hand area (S1, blue). According to Cottone et al. (2017), where the data are from, both the state-dependency and the cortical district-dependency are statistically significant in the population of the 20 healthy volunteers.

Figure and caption readapted from Tecchio et al. 2020.

Triadic principle: Feedback-synchrony-plasticity [FeeSyCy]

I am going to explain now why we consider the fractal dimension particularly suitable to describe the dynamics of the nervous system's neuronal pools: FD is a proper measure of the neuronal state since a triadic principle holds on different scales of the Body&Brain system.

The motor actions produce feedback from the environment to our brain via somatic, proprioceptive (Fink *et al.*, 2014; Scott *et al.*, 2015), visual and auditory sensory receptors. This feedback stimulates the brain neurons and induces synchronizations among the nodes of dedicated functional networks (Tecchio *et al.*, 2008; Pittaccio *et al.*, 2011; Gandolla *et al.*, 2014). These induced synchronizations engage the system in plastic adaptations either sustaining the execution as planned or enabling proper corrections (Fink *et al.*, 2014). In this process, our neurons implement plastic changes following a key rule (Kandel & Schwartz, 1985): if two input signals reach the neuron simultaneously, the neuron increases its probability to fire (Hebb, 1949), i.e., to produce an action potential transmitting a message. Changing the firing probability means changing the entire neuronal functionality. This continuous adaptation capability constitutes the brain plasticity, i.e., the ability of neurons to change their output according to what is required, quantified depending on the distance between the expected outcome and the current one. When the distance is small, plasticity produces functional adaptations (plastic adaptation). When the distance is big, plasticity engages huge

structural changes to fulfil the functional need (plastic learning). Strikingly, plasticity mechanisms occurring at synapses' level are integrated by changes in activity-dependent myelin multilaminar sheath to optimize the timing of information transmission between relay points through neural circuits. A high degree of precision is required for appropriate spike-time arrival. Thus, modulating conduction velocities critically changes the arrival simultaneity of electrical signal fundamental for synaptic plasticity (Gibson *et al.*, 2014; Fields, 2015). Overall, feedback-synchrony-plasticity, FeeSyCy, the triadic principle governing motor control manifests itself recursively at different scales, from neuronal network node made by single neurons (Turrigiano *et al.*, 1998; Sjöström & Nelson, 2002), to small networks (Shadlen & Newsome, 1998; Barsalou, 1999), to complex networks (Hopfield, 1982; Roskies, 1999), to the entire BodyBrain system (Edwards *et al.*, 2007; Kello *et al.*, 2010; Wolpert *et al.*, 2011; Fink *et al.*, 2014; Gandolla *et al.*, 2014).

At the Body&Brain system level, we can recognize paradigmatic examples of the breakup of one link of this chain, which generates the breakup of the whole process.

Example - Feedback link breakup: Deaf people were deaf mutes for centuries: although in the presence of proper organization of the motor executive functions, the lack of auditory stimuli feedback lead to the total absence of development of linguistic production up until the early 20th century (Sacks, 1989).

Example - Synchrony link breakup: In the presence of proper sensory feedback stimuli, which come from preserved sensory systems, the lack of intracerebral synchronization occurs in people affected by dystonia (Abbruzzese and Berardelli, 2003), where the absence of sensorimotor integration induces alteration of the motor control.

Example - Plasticity link breakup: A breakup in the third link of the chain is considered crucial in schizophrenia, where people are able to move and receive

proper sensory feedback stimuli, these induce proper communication within the brain, but cannot engage proper adaptation due to neuronal inability to involve the metabolic chains to adapt the cells via plasticity (Ramocki and Zoghbi, 2008).

Speaking the neurodynamics language to enhance neuromodulation efficacy

In a seminal study of 2017 (Cottone et al. 2017), we posed the following working hypothesis: in humans, transcranial electric stimulation (tES) with a time course that mimics the endogenous activity of its target is capable of altering the target's excitability. In our case, the target was the primary motor cortex (M1). We identified the endogenous neurodynamics of hand M1's subgroups of pyramidal neuronal pools in each of our subjects by applying Functional Source Separation (FSS) to their electroencephalographic (EEG) recordings. We then tested whether the cortico-spinal excitability of the hand representation under the above-described stimulation, which we named transcranial Individual neuroDynamics Stimulation (tIDS), was higher than in the absence of stimulation (baseline). As a check, we compared tIDS with the most efficient non-invasive facilitatory cortico spinal tES known so far, which is 20 Hz transcranial Alternating Current Stimulation (tACS). The control conditions were as follows: i) Sham, ii) transcranial random noise stimulation (tRNS) in the same frequency range as tIDS (1-250 Hz), and iii) a low current tIDS (tIDSlow). Cortico-spinal excitability was measured with motor evoked potentials (MEP) under transcranial magnetic stimulation (TMS). The mean MEP amplitude increase was 31% of the baseline during tIDS ($p<.001$), and it was 15% during tACS ($p=.096$). tRNS, tIDSlow and Sham induced no effects. While tACS did not produce an enhancement in any subject at the individual level, tIDS was successful in producing an enhancement in 8 of the 16 subjects.

The results of the present proof-of-principle study showed that proper exploitation of local neurodynamics can enhance the efficacy of personalized tES.

Here, we introduced a model that hypothesized the mechanisms causing cortico-spinal neurons to enhance excitability during tIDS. We considered a set of nodes and their connections as a neural network (NN). In our case, the node is the hand section of pyramidal neurons of the primary motor area. In our model, each node of a nn develops 'typical' dynamics of neuronal activity during its lifespan. In agreement with this model, we observed that has 'its own' dynamics (Cottone et al., 2017). A nn implements a function via the communication among its nodes, i.e., the nodes of a nn share a common 'language'. Once a signal arrives to a node ('word-in'), it automatically answers with a 'word-out' determined by a fixed 'word-in → word-out' correspondence until plasticity modifies that correspondence. In other words, in a nn each node cannot stay silent; it necessarily produces the 'word-out' once the 'word-in' arrives. Our hypothesis in this model was that using tIDS, we 'speak' to M1 neurons with their 'typical language', i.e., the one they use to produce their physiological output. In our hypothesis, the neuronal pools change their probability to activate, i.e., to send their message to other neuronal pools when a signal similar to the one they typically send impinges their membranes.

Conclusions

The approach of listening to the Body&Brain system to personalize interventions led us to a double need. 1. To find the proper measures to identify alterations to be compensated; 2. To develop devices to implement the proper communication with the Body&Brain system to compensate these alterations.

Here we reported 1. how a measure that takes into account the pattern generated by neuronal pools is sensitive to physiological phenomena and alterations by a single number. 2. How is it possible to exploit this measure and its basic principle to develop interventions able to compensate brain alterations typical of multiple diseases.

Author contribution statement (for the final and published version of the manuscript).

FT conceived the paper and supervised the writing. FT and FZ contributed to the writing of the original draft. MB contributed to figures creation. MB, TL, EG, and LP contributed to the writing and the editing of the manuscript. All authors reviewed and approved the final manuscript.

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3.3 Neuronal electrical ongoing activity studied trough its fractal dimension as a hallmark for identifying cortical regions*

The following authors contributed to the final and published version of this work (here synthetized) *:

Karolina Armonaite [1], Massimo Bertoli [2,3], Luca Paulon [2], **Eugenia Gianni [2,4]**, Marco Balsi [5], Livio Conti [1,6], Franca Tecchio* [2]

[1] Uninettuno University, Rome, Italy

[2] Laboratory of Electrophysiology for Translational NeuroScience, Institute of Cognitive Sciences and Technologies - Consiglio Nazionale delle Ricerche, Rome, Italy

[3] Department of Neuroscience, Imaging and Clinical Sciences, University 'Gabriele D'Annunzio' of Chieti-Pescara, Chieti, Italy

[4] Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy

[5] Department of Information Engineering, Electronics and Telecommunications, Sapienza University, Rome, Italy

[6] INFN – Istituto Nazionale di Fisica Nucleare, Sezione Roma Tor Vergata, Rome, Italy

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Introduction

The human brain is now widely acknowledged to be a dynamic complex system made up of interacting sub-components formed over various space-time scales. (Bullmore et al. 2009; Bassett and Sporns 2017). As a result, brain system may be thought of as a system of connected neural networks, where each node might be a single neuron, a group of the same neurons, a region of nearby neurons, or a broad area of the brain (Tecchio et al. 2020).

The scalp electroencephalography (EEG) is the product of hundreds of neurons' activity continuously fluctuating in response to excitatory and inhibitory inputs (Lopes Da Silva 2011; Buzsáki and Watson 2012), observable at the scalp level as rhythmic activity of the brain that are represented throughout a range of frequencies and are linked to particular processes of the brain (Thut et al. 2012; Buzsáki et al. 2013). Results from earlier studies (Cottone et al. 2017) that analyzed local neuronal activity derived from EEG scalp data supported the theory that each cortical area generates an electrical activity that results in a specific time course, the local neurodynamics, that represents a signature of that area.

As it directly senses the electrical activity of the brain with the same appropriate temporal resolution of the neuronal electric exchanges, EEG is the preferable investigation method for the non-invasive examination of the local neurodynamics. Since Hans Berger's groundbreaking research (Berger 1929), non-invasive EEG has made it possible to gain a comprehensive knowledge of the physiological and pathological aspects of brain activity as well as the behavioral implications of these features. Studies on humans also made use of intracranial stereo-encephalographic (sEEG) recordings.

Study aim

Utilizing the multicenter data collection of sEEG dense coverage recordings made possible by the renowned Montreal Neurological Institute during open-eye awake in normal cortical areas (MNI) (Frauscher et al. 2018), the purpose of our

study was to deepen knowledge of the brain neurodynamics as cortical area signature. Following the non-invasive EEG-derived successful attempt to distinguish the neurodynamics of the primary motor (M1) and somatosensory (S1) hand representations (Cottone et al. 2017), we now aimed to strengthen the results by conducting a similar analysis on MNI sEEG assessments, expanding the investigation to additional primary cortical areas.

We investigated the neurodynamics of primary motor (M1), somatosensory (S1), and auditory (A1) cortices using intra-cranial stereo-electroencephalographic (sEEG) recordings from the public Montreal Neurological Institute (MNI) atlas, measuring Higuchi fractal dimension (HFD) in the same subject.

Results and conclusions

In M1, which predominated above beta band, S1 in the alpha band, and A1 in the delta band, we noticed distinct spectral peculiarities. M1 HFD was more than S1, which was also greater than A1.

The efforts in cortical parcelling based on this manifestation of the local cytoarchitecture and connection are supported by a clear differentiation between the neurodynamic features of different main cortices.

Potential clinical significance principally resides in utilizing such exchange patterns to boost the effectiveness of neuromodulation interventions to treat symptoms brought on by imbalances in neuronal activity.

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3.4 DopByTIDS: a research Protocol

To give breath to the project of continuous research in personalization and innovation in the field of neuromodulation (that is one of the relevant objectives of my research grant), we designed a project that aims to better explore the potential of tIDS (Chapter 1 and 3). Even if it hasn't been possible to realize the project during pandemic, we wish to realize it in the next years.

In previous studies (see Chapter 1, Section 1.1 and Chapter 3, Section 3.1-2) my Lab team realized that a tES with a current that mimics the endogenous activity of a target neuronal pool, can modify the excitability of that specific region.

In our project we aimed at confirming this relevant finding, adding novel explorations. We aimed at testing – basing on previous observations - whether a current with the same dynamics but with lower amplitude can induce inhibition in a target neuronal pool. Moreover, we aimed at verifying whether the increase in excitability corresponds to a relevant increase in the behavioral performance of the subject, and viceversa whether inhibiting elicits a decrease in the performance. Specifically, here our hypothesis is that facilitating the controlling networks, the excitatory tIDS will enhance the task performance and conversely for inhibitory tIDS.

To this aim we planned to perform a randomized controlled cross-over study by enrolling, basing on sample size estimation, 13 healthy volunteers.

First, we planned to determine the tIDS current by applying EEG to our subjects and subsequently extract the individual dynamics with the algorithm FSS. Secondly, we planned to probe the tIDS effects by gathering the MEP induced by TMS during tIDS stimulation in four different conditions: an inhibitoy tACS at 20 Hz, a tIDS at full intesity that is expected to have an excitatory effect a tIDS at low intensity that is expected to have an inhibitory effect and the sham condition. Moreover, we plan to add two behavioral conditions in which the subject will perform a behavioral task during tIDS but in absence of TMS. In this case we

planned to observe how well the subject performs the task both with excitatory and inhibitory tIDS.

The MEP amplitude is going to be studied as primary outcome and the behavioral performance as secondary outcome.

SUMMARY

1. GENERAL INTRODUCTION OF THE RESEARCH PROJECT

1.1 Electroceuticals

The World Economic Forum 2018 named Electroceuticals[26], i.e., the treatment of disorders using electrical signals (Famm et al. 2013; Reardon 2014), among the 10 emerging technologies for global social and economic development. Electroceuticals encompasses neuromodulation techniques, i.e., the modification of the excitability of specific neural targets, capable of modifying the relationship with related regions to achieve desired behavioural effects. At the brain level, this modification can be mediated electrically either by means of invasive techniques or by means of what is known as Non-Invasive Brain Stimulation (NIBS). Among NIBS, transcranial electrical stimulation (tES) represents a technique based on the use of painless electrical current applied to the scalp and includes the most widely used transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS). This non-pharmacological approach has proven to be safe and reliable (Lefaucher 2014; Antal 2017) and has no side effects typical of systemic drug therapies. In this perspective, electrophysiology acquires a key role in understanding the temporal dynamics of neuronal electrical activity, neurodynamics, which reflects the complexity of the brain with its structure and functionality emerging at spatial and temporal levels and inherently modular in nature (Bassett & Gazzaniga 2011).

1.2 Exhitatory and Inhibitory neuromodulation

For the set up of effective neuromodulation treatments -the modification of the excitability of the target region, and thus of its relationship with the other

[26] The increase in our knowledge about the neurobiological correlates of psychophysical well-being has paved the way for new therapeutic possibilities, among which electroceuticals, treatment using electrical signals, has recently been identified [1]. This nonpharmacological approach has been shown to be safe and reliable [3,4] and has no side effects typical of systemic pharmacological therapies. Electroceuticals is implemented through neuromodulation, the change in excitability of the target region. At the brain level, such a change can be mediated electrically either through invasive techniques or through the set that goes by the name of Non Invasive Brain Stimulation (NIBS). Among NIBS, transcranial electrical stimulation (tES) represents a technique based on the use of painless electrical current applied to the scalp, and includes the most widely used transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS).

nodes of the networks with which it communicates-, it is necessary to be able to induce an increase or reduction in the excitability of the neuronal target. In fact, in various pathologies it is possible to find an alteration of local excitability, either in the direction of increased excitability (delirium, hallucinations, epilepsy) or in the one of an excessive reduction (depression). Neuromodulations aim to rebalance these alterations. As far as tDCS is concerned, among the parameters influencing the effects - current intensity, reference montage, treatment duration (Dedoncker et al.2016) - the positioning of cathode and anode determine the inhibition and excitation of the underlying region, respectively. The application of tDCS for specific psychological and neurological disorders is widely studied, with promising results also in our experience (Cancelli et al. 2018; Tecchio et al. 2015), although still not reaching the level of standard clinical application (Lefaucheur 2017).

There is increasing evidence that tACS modulates cognitive performance (Schutter & Wischnewski 2016) in healthy subjects and patients (Wu et al. 2016). The rationale of tACS is to interact with cortical rhythms, synchronising/desynchronising fluctuations in brain neuronal electrical activity. tACS induces a 'synchronisation' of neuronal activity, defined as an effective transfer of delivered energy, i.e., an effective change in the activity state of the neuromodulation target. The effects of different frequencies are manifold and affect somatosensory processing, memory, perception, and decision-making processes (Herrman et al. 2013; Antal et al. 2017). tACS with 20Hz beta frequencies is frequently used when interacting with the primary motor cortex (M1). The application of this tACS frequency on M1 induces an increase in corticospinal excitability, as assessed by TMS motor evoked potentials (MEPs), whereas other frequencies do not affect MEPs (Feurra et al. 2011). It has been observed that a gamma frequency tACS in M1 modulates inhibitory gabaergic networks, with neuromodulation levels associated with sensorimotor learning levels (Nowak et al. 2017). At a fixed frequency, a tACS that induced excitation at

high current intensity inhibited the target region if at low intensity (Moliadze et a. 2012; Cancelli et al. 2015).

1.3 Transcranial Individual neuroDynamics Stimulation, tIDS

Fundamental research by Steriade and colleagues (Crochet et al. 2006) demonstrated that stimulus trains delivered to the neocortex of cats produce a modification of postsynaptic potentials if the stimulation is applied at frequencies corresponding to endogenous brain rhythms of the individual cat. In humans, the efficacy of neuromodulation depends on the frequency of stimulation applied in a stimulated region-dependent manner (Feurra et al. 2011; Kanai et al. 2008; Brinkman et al. 2016) and on the activity state of the network (Moliadze et al. 2012; Feurra et al. 2013; Feurra et al. 2019). Neuromodulations with time-varying transcranial current (tRNS) support cognitive domains more effectively than direct current (Fertonani et al. 2011). In vitro studies have shown that both oscillatory (Fröhlich & McCormick 2010) and scale-free stimulation (Gal & Marom 2013) can induce 'entrainment', defined as the effective modification of target excitability induced by stimulation with modulated currents.

The efficacy of neuromodulation induced by modulated currents therefore depends on the dynamic characteristics of the current modulation.

We have shown (Cottone et al. 2018) that a current tES with a time course that mimics the endogenous activity of its target, its neurodynamics, is able to modify the excitability of the target region with high efficacy. We call this 'transcranial individual neurodynamics-based stimulation' - transcranial Individual neuroDynamics Stimulation, tIDS). Notably, tIDS achieves individual-level efficacy in half of the subjects, whereas tACS, so far, the most effective technique in a similar online protocol, does not reach the significance threshold in any single subject.

This new strategy of tES in modulated current delivers stimulation, which is derived from non-invasive electroencephalographic (EEG) recordings provided by the Functional Source Separation (FSS, (Tecchio et al. 2007)) algorithm that

allows the identification of specific brain regions on the basis of a functional fingerprint. In particular, we observed that in healthy people it is possible to identify the region by considering the population as a whole (Porcaro et al. 2018) or individual subjects (Cottone et al. 2017) and we will test here whether the neuromodulation effects are similar in the two cases.

Considering the greater effectiveness in neuromodulation of individual dynamics compared to optimal sinusoidal stimulation for the target region, we proposed a model in which the nodes of a neuronal network communicate with each other through a 'typical' language (Cottone et al. 2017) made up of patterns, which we can call words, whose dynamic form carries information. If we send a signal to a node in the network that is similar to the one spoken by it, as in the case of tIDS, we effectively change the probability that this node transmits, becomes active (Cottone et al. 2018). While our tIDS approach exploits the local dynamics of the resting region's activity to enhance the effectiveness of neuromodulation, other authors are investigating the relevance of the timing of the stimulus phase with respect to ongoing neuronal activity (Fiene et al. 2020) and cortico-cortical communication times (Momi et al. 2020; Huang et al. 2019).

1.4 Objectives

1. **Document that tIDS is able to induce a reduction in corticospinal excitability in addition to the increase.** We will provide higher and lower current amplitude values while maintaining the same current dynamics derived via FSS from the individual EEG. We have unpublished observations to support this choice.

2. **Strengthen the tIDS ability to enhance excitability of the target region** as demonstrated in Cottone et al. 2018.

3. **Document that inhibition/enhancement changes in excitability correspond to reduction/improvement in task performance** where the target

region is a primary node in the task execution network. It is well known that execution of a movement is accompanied by an increase in M1 excitability (Rossi et al. 2009), but this does not mean that an increase in M1 excitability corresponds to better task performance. In fact, a recent meta-analysis found no significant effect on the quality of movement execution induced by anodal tDCS applied on M1 before or during the execution of an isometric task by the upper or lower limb (Machado et al. 2019). We pose here the hypothesis that tIDS facilitates/inhibits M1's communication with the other nodes of the sensorimotor network, thus increasing/decreasing the performance of motor task execution.

4. **To compare the neuromodulation effects induced by neurodynamics identified via FSS from the individual subject or population.**

People will be comfortably seated on an armchair, in a quiet environment, together with two experimenters, with whom they can converse, maintaining a state of silent relaxation only during the five-minute periods associated with each experimental condition. At the end of the session, they will be able to leave independently, although they may stay a few more moments if they wish. No drugs will be administered.

The protocol requires two sessions: a preliminary one to obtain the tIDS dynamics, in which we will collect the EEG data to which the FSS algorithm will be applied, identifying the neurodynamics of the primary motor cortex that will constitute the dynamics of the current delivered by tIDS. And a secondary one in which we will probe the tIDS effects by gathering the MEP induced by TMS stimulation in four different conditions: an inhibitoy tACS at 20 Hz, a tIDS at full intesity that is expected to have an excitatory effect a tIDS at low intensity that is expected to have an inhibitory effect and the sham condition. Moreover, we will add two behavioral conditions in which the subject will perform a behavioral task during tIDS but in absence of TMS. In this case we will observe how well the subject perform the task both with excitatory and inhibitory tIDS.

2. PROCEDURE

2.1 The study design

To quantify the effects of tIDS we will perform a randomised, controlled, cross-over study in healthy volunteers. We will quantify the ability of tIDS to increase/decrease corticospinal tract excitability using TMS-induced MEP in an online protocol (tES On vs Off) as the primary outcome. As control conditions, in addition to Sham we will use a tES capable of inhibition in an online protocol (tACS 20 Hz inhibitory).

As a secondary endpoint, we will use the performance of a simple isometric grasping task performed with the left hand to assess the performance quality of a motor task.

2.2 Participants

Healthy volunteers will be enrolled according to the following eligibility criteria:

2.2.1 Inclusion criteria

- Age between 25 - 50 years

- Dextremity (Edinburgh Handedness Inventory 0.5-1)

2.2.2 Exclusion criteria

- Psychoactive substances taken within 6 months prior to enrolment

- Presence of metal implants in the skull

- Reported pathologies

- Not having signed the informed consent form.

2.3 Sample size numerosity

Our main objective is to show that tIDS neuromodulation (tIDS on) is able to increase/decrease the excitability of the corticospinal tract compared to the baseline condition (tIDS off: baseline), as quantified by the evoked motor potentials (MEPs) from transcranial magnetic stimulation (TMS). We will therefore

consider relevant a 25% increase in MEP amplitude during stimulation compared to baseline. For example, we consider an increase from an average of 800 μV to 1000 μV during stimulation to be relevant. However, by pooling MEPs from different subjects, the variability between subjects and the interaction Stimulation (Off= Off, On= On) * Subjects must be taken into account. Studies that have used MEPs as an outcome measure are typically characterized by wide variability within and between subjects (Feurra et al. 2011; Moliadze et al. 2012; Feurra et al. 2013; Cancelli et al. 2015; Tecchio et al. 2008). Based on data already available in our laboratory, we calculated that the standard deviation (SD) of within-subjects MEPs was approximately 80% of the mean. With respect to the variability between subjects, we again used the available data and estimated that the Intra-Class correlation was 0.53, indicating that the percentage of variance explained by the subjects was 47%. To deal with this inter-subject variability, we decided to normalise each MEP for each subject in both stimulation conditions (Off, On), thus calling them MEP_Normalised. Thus, the mean of the amplitudes of these MEP_Normalised MEPs corresponded to 1 when tES was off (Off) in each subject and in each of the 5 conditions, i.e., the between-subject variability from the mean in the tES Off condition was zero by definition. In summary, the between-subject variability of the MEP_Normalised in the baseline condition was 0, while the within-subject variability was 0.8. To have a probability of 0.8 (power 1-b=0.8) of recognising a 25% increase in MEP_Normalised as statistically significant (with two-tailed alpha error set at 0.05), assuming a homogeneous standard deviation of 80%, the number of MEPs should be 330 (165 with stimulation on and 165 with stimulation off). Therefore, by recruiting 11 subjects, the number of MEPs for each subject is 30 (15 + 15). Since the procedure is not based on simple random sampling but on cluster sampling (one subject represents a cluster), the sample size should be increased by the design effect to consider intra-class correlation. However, since inter-subject variability in baseline conditions is cancelled out by the normalization of MEPs, the design effect does not increase the number of MEPs required.

Between-subject variability of the effect (tES Off vs tES On) is assessed through the interaction Stimulation * Subjects. With 11 subjects and 15 measures per subject and each stimulation level (Off, On), an average effect (conventionally corresponding Cohen's f=0.25) has a strength greater than 0.95 to be recognized as significant (with alpha =0.05). In summary, with 30 MEPs for each of the 11 subjects, there is sufficient strength to detect a 25% increase in MEP_Normalised (tIDS Off vs tIDS On) and also to test the homogeneity between subjects of the expected effect of tIDS.

The second objective is to show that tIDS produces different effects compared to the other tES and in particular compared to Sham. Thirty MEPs for each of the 11 subjects (15 with tES on, 15 with tES off) in each of the 5 experimental conditions (tACS, tRNS, tIDS, tIDSlow, Sham) produces a matrix of 1.650 MEPs which allows us to test the effect of the tES*Stimulation Off/On interaction (even with a small effect size) with a power >0.99. Furthermore, this sample size allows us to test the similarity of such interactions between subjects with a power of 0.8 (triple interaction Subjects*tES*Stimulation Off/On) with an average effect size of f=0.25. Then, taking into account 10% drop-out, we recruit 13 volunteers according to the enrolment criteria defined above.

2.4 tES Protocol

We will deliver resting tES through two standard transcranial electrical stimulation electrodes (7x5 cm 2) placed over C4 and Oz according to the international EEG 10-20 system. The waveforms will be produced by a function generator (2 MHz USB PC Function Generator PCGU1000, Velleman Instruments) connected to a current stimulator (STMISOLA linear isolated stimulator, Biopac System). We will provide in random order between subjects, the following tES (500 points per second):

● tIDS with dynamics reproducing the individual neurodynamics of M1 (see attachments, tIDShigh)

● tIDSlow identical to tIDShigh but with sufficiently low intensity to inhibit

● tACS at 20 Hz sinusoidal with inhibitory current intensity as reported in Cancelli et al. 2015b

● population tIDS with excitatory intensity tIDShigh (see Appendices, tIDSpophigh)

● Sham, i.e. current delivery for 6 seconds at the beginning and end of a 1.5-min period.

2.5 TES Estimate of the effects

We perform a paradigm that tests the efficacy of neuromodulation during stimulation (Feurra et al. 2011; Cancelli et al. 2015; Cottone et al. 2018; cancelli et al. 2015b; Tecchio et al. 2013). For each experimental condition, we will follow 1.5 minutes of no stimulus/compound, and then 3 minutes of TMS/compound stimulation with the tES off for 1.5 minutes obtaining the baseline values and 1.5 minutes with the tES on obtaining the values in the experimental condition of interest.

2.5.1 Excitability of the CST via TMS: primary outcome

We will deliver single TMS stimuli through a standard focal coil (HP 90 mm Coil 9784-00) connected to a Super Rapid stimulator (Magstim), with the coil superimposed on the electrode used for tES positioned in the rolandic area. We record the TMS-induced MEPs in the left thumb opposing muscle (OP) by means of surface electrodes with a muscle-tendon assembly 2.5 cm apart. The hot spot of the left OP muscle is identified with the TMS coil superimposed on the tES electrode. Next, we determine the resting motor threshold (RMT) and stimulate at an intensity of 120% of the RMT. For each experimental condition, after 1.5 minutes of no stimulus (TMS off, tES off), a stimulus is delivered every 4.5 - 5.5 seconds (random interstimulus interval) for the next 3 minutes, of which 1.5 minutes with tES off collecting approximately 18 MEPs in baseline conditions, and in the next 1.5 minutes, tES is switched on, obtaining another 18 MEPs

approximately in the experimental condition of interest. The four tES conditions are provided in random order among the different subjects.

2.5.2 Quality of the motor execution: secondary endpoint

The motor task performed with the left hand consists of compressing an air bulb to a level equal to 5% of maximum voluntary contraction (MVC). To determine the MVC, the volunteer is asked to compress the bulb as hard as possible for a few moments (300 ms) three times in succession. This is followed by at least 2 minutes of rest in order to avoid fatigue effects. After resting, the subject performs the task for periods of 20 s at the appearance of a green rectangle (go signal), interspersed with rest for 10 s at the appearance of a red rectangle (stop signal). The subject receives continuous visual feedback of the pressure exerted, which is recorded and sent to the monitor via the position of a segment.

2. 6 Safety and adverse events

The safety of the procedure will primarily be ensured by the use of a stimulation protocol with parameters (intensity, duration, surface current density) that are well within internationally recognised safety limits when applied to persons meeting the present inclusion criteria (Antal et al. 2017). The application of EEG is completely non-invasive and harmless. The TMS protocol for testing effects is an established practice (Rossini et al. 2015).

The investigators, who are present during all procedures of the study, undertake to provide adequate assistance to the participant in case adverse events occur and to institute appropriate corrective intervention, regardless of the causal link with the treatment. The trial will be stopped in any case if requested by the subject. In addition, at the end of each stimulation, the investigator will note any sensations of discomfort during the stimulation in order to monitor the subjects' reactions.

Definition of Adverse Event: An adverse event is any undesirable, unpleasant or harmful event that may or may not be related to the procedures being performed.

2.7 Statistical Analysis

2.7.1 Excitability of the CST via TMS

We transform the MEP amplitudes for each subject with logarithms, typically obtaining the normal distribution (as verified with Shapiro-Wilk).

For each experimental condition, we subject the normalised MEPs to an analysis of variance (ANOVA) model with the factors Stimulation (On, Off) as a fixed factor and Subject as a random factor. For the tES that had shown the Stimulation effect we will evaluate differences in the effects with the ANOVA model that will include the tES factor (tIDShigh, tIDSlow, tACS, Sham) and Stimulation (Off, On) as fixed factors, keeping the Subject factor as random. If the tES*Stimulation interaction is significant, the post-hoc analysis will evaluate the direction of the differences.

Furthermore, in order to extend our knowledge with respect to personalisation strategies able to increase the efficacy of neuromodulation, for the tES that had shown the Stimulation effect, we will compare in each individual subject the active condition with its baseline, using a two-tailed independent samples t-test obtaining indications of intra-individual as well as population significance.

2.7.2 Motor Execution Quality

A similar analysis will be conducted on the performance of the sensorimotor task, submitting to ANOVA the distance between the required pressure level and that obtained over periods of 500 ms overlapping by 50% understood as the standard deviation divided by the mean of the pressure levels exerted.

3. ETHICAL, NORMATIVE AND ORGANIZATIONAL ASPECTS

3.1 Reference normative

The trial may only be started after authorisation by the Ethics Committee and will be conducted in accordance with the Declaration of Helsinki, in

compliance with the European Union's Good Clinical Practice Guidelines and in accordance with Italian law.

3.2 Informed Consent

The recruitment of volunteers to take part in the study can only take place with prior written informed consent. Volunteers will be informed of the aims, benefits, and risks of the trial. This information will be set out concisely but fully in writing on the informed consent form. A copy of the informed consent, signed by the volunteer and the investigator collecting the consent, will be given to the subjects. They may withdraw their willingness to participate in the study at any time.

3.3 Record Keeping

All documentation relating to the study will be kept in accordance with current regulations and accessible only to the investigators and the Ethics Committee. The documentation will be kept for a period of 25 years after the end of the trial.

3.4 Protocol Deviations and Amendments

Any deviation from the protocol not approved by the project coordinator and the Ethics Committee will result in suspension of the trial. Amendments to the protocol must be submitted for approval to the study coordinator and the Ethics Committee that approved the study.

3.5 CRF

The researcher will be responsible for the accuracy of the data entered in the data collection sheet. All entries must be written in black or blue ink and additions/corrections must include the initials of the investigator making them and the date. In addition, the data collection sheet must be accessible to the Ethics Committee for review at all times.

3.6 Property of the experimental data

The independently conceived and designed trial data belong to the investigators, who will be responsible for writing one or more scientific papers to be submitted for publication. At the end of the trial, the investigators will send the Ethics Committee a report on the conduct and outcome of the study.

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5. ATTACHMENTS: M1 DYNAMIC EXTRACTION

To obtain the individual neurodynamics of M1 (hand section), each volunteer undergoes EEG (64-channel actiCHamp System; Brain Products) and EMG recording of the opposing muscle of the right thumb (OP). EEG and EMG are sampled at 5 kHz (pre-sampling analogue band-pass filtering 0.1-2000 Hz) and archived for offline analysis. Through FSS, we identify the neurodynamics of M1 pyramidal neurons dedicated to the control of the contralateral hand, particularly those of the thumb, at rest (Figure 1).

The following is an in-depth description of the experimental design and procedure of FSS, a technique that has already proven capable of identifying the activity proper to M1 pyramidal neurons (Porcaro et al. 2008; Betti et al. 2009; Melgari et al. 2013].

Experimental EEG paradigm

EEG traces will be recorded while volunteers perform an isometric manual grasp against a semi-resistant air bulb connected to a digital card capable of recording the pressure exerted and displaying visual feedback on a computer screen positioned in front of the subject (Interactive Pressure Sensor, National Research Council). The task described above is also performed during EEG for the identification of M1 via FSS.

The details of the FSS algorithm have been published previously (for a review, see (Tecchio et al. 2007). In summary, the FSS algorithm follows the classical assumption of independent component analysis (ICA) according to which EEG traces, recorded by n electrodes placed on the scalp, are a linear combination of signals generated by an unknown neuronal source mixed within an unknown matrix (mixing matrix).

The goal of ICA is to find the mixing matrix, and consequently the sources, from the available recorded signals, without making any assumptions beyond the statistical independence of the unknown sources. FSS adds one or more functional constraints to the standard assumptions of ICA by exploiting the known functional characteristics of the areas of interest. Similarly, to ICA, FSS starts from the original EEG data and returns the functional source FS (t) through its distribution on the scalp and its trend over time. Unlike the ICA, the FSS identifies one source at a time, i.e., the one that maximises the functional constraint. Using the maximisation algorithm recursively, FSS then estimates the function that maximises this constraint.

Figure 1. Detection of resting neurodynamics of M1.

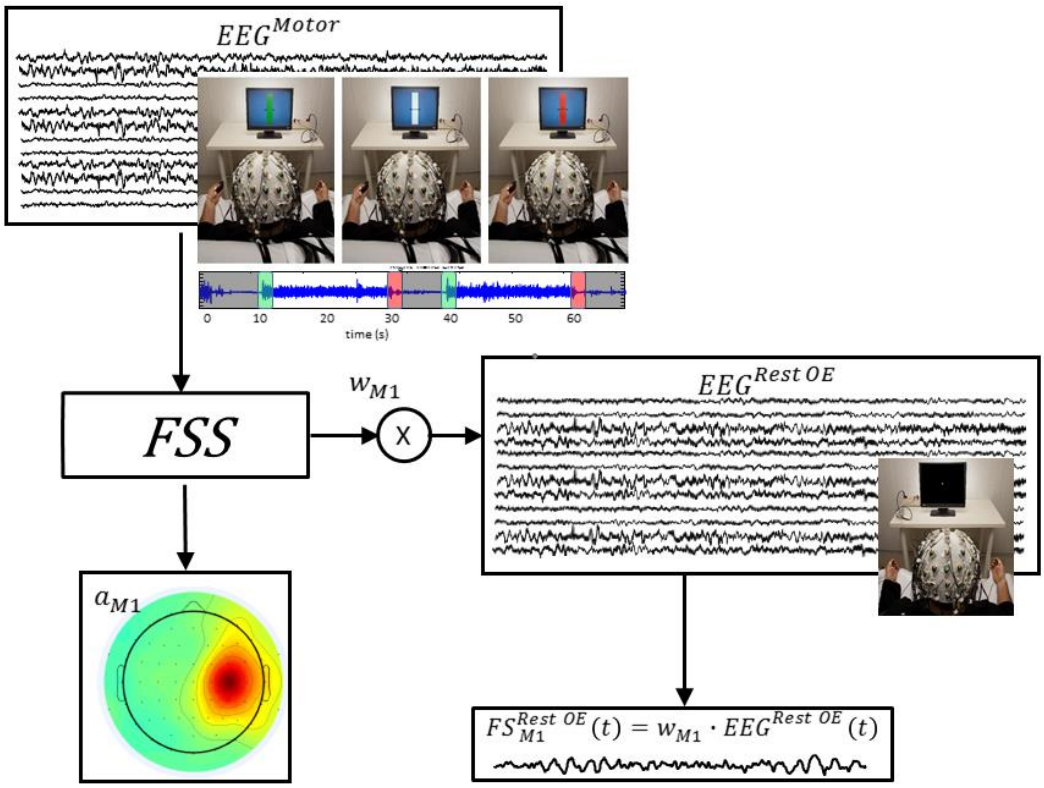

Figure 1. Detection of resting neurodynamics of M1. The image represents how FSS extracts the functional constraint from the activity of the primary motor cortex of the left hand (FSM1) for each subject in resting condition with open eyes (Rest OE). FSS receives as input EEG data recorded during an isometric hand grip performed with the left hand (motor condition, corresponding to the white background intervals in the top inset) and provides aM1 as outcome (i.e., a 64-dimensional vector, similar to wM1). Bottom left, the topographic map of FSM derived from aM1. Bottom right, FSM1 at Rest OE was obtained from wM1 multiplied by the EEG data collected during the open-eye resting condition.

PART II: AN ELECTROCEUTICAL SERVICE

Chapter 4: Towards an electroceutical service

4.1 Introduction: assessing the clinical validity of Faremus and verifying its domiciliary use

Multiple sclerosis is the most common inflammatory disease in young adults affecting the CNS.

Almost 80% of individuals affected by multiple sclerosis (MS) complain about the symptom of fatigue and for half of them fatigue is the most disabling symptom independently of their level of disability, significantly interfering with everyday usual activities (Bakshi 2003; Khan 2014). This is reflected in the MS Council's definition of fatigue as "a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities" (MSCCPG 1998). Despite the spread of this symptom, the etiology of fatigue in MS remains largely debated (the current hypothesis span from axonal damage, low hormones' level, immunological processes, to comorbidities with depression and sleep disorder; Heesen et al. 2006; Kos et al. 2008; Mohr et al. 2003; Tartaglia et al. 2004; Tèllex et al. 2006; Trojan et al. 2007; see also Chapter 2) and it hampers the development of a proper therapeutic approach (Braley 2010).

From the therapeutic point of view, two fundamental approaches have been proposed: physical activity together with behavioral change interventions and pharmacological treatment (Moss-Morris 2021).

While producing, at some degree, an amelioration of fatigue symptoms, physical training programs as well as behavioral change interventions' studies present some important limitations. Exercise training applies to MS patients who are less disabled (Asano et al. 2015; Heine et al. 2015) and both physical training and behavioral change interventions produce small and non-robust effects (Motl 2012; Pilutti et al. 2013).

On the other side, drug therapies proved to be poorly effective in fatigue treatment and there is none specifically indicated for this symptom (Kesselring & Beer 2005). Among the several medications Amantadine seems to be the most indicated for the treatment of fatigue, but clinical findings on this treatment remain strongly debated (Pilling 2021) and, most of all, the medication presents non-negligeable side-effects (Branas et a. 2000). Side effects of Amantadine may range from hallucinations, nausea, gastric irritation, early morning wakening and hyperactivity (Murray 1985); moreover, recent studies indicated that several randomized controlled trials have shown that the improvement in MS fatigue by amantadine is not better than placebo (Nourbakhsh 2021 for a review).

The inefficacy of these two therapeutic approaches led us to further question the literature about the neuronal mechanisms and networks' dynamics (Bertoli et al. 2020, Chapter 2) at the basis of fatigue in MS patients and to lay the grounds for a novel therapeutic approach.

Existing literature might suggest that the symptom of fatigue is associated with a combination of structural alterations as revealed through MRI and DTI studies (see Chapter 2) and functional alterations as revealed through electrophysiological studies (targeting in particular the cortical excitability and functional connectivity within the sensorimotor network, see Chapter 2). From a functional point of view, in fatigued patients, M1 (primary motor cortex) shows an altered hyperexcitable profile counterbalanced by a low excitability of the S1, i.e., the primary sensorimotor area, along with an alteration of the functional connectivity between the temporo-parietal hemispheric homologues and a generally reduced connectivity between S1 and M1. Nonetheless, further studies considering both structural and functional features in the same MS patients have shown either that the functional damage correlates strongly with the symptom of fatigue even in the absence of major structural alterations or that morphological changes correlated weekly with patient's fatigue levels despite an evident, strong correlation between fatigue levels and functional abnormalities observed throughout electrophysiological measurements (see Chapter 2).

This led my Lab team to the hypothesis (Bertoli & Tecchio 2020) that the functional alteration has the prevalent role in the etiology of fatigue and, consequently, that intervening directly by modulating the neurodynamics of the sensorimotor network (as the region which is most involved in fatigue according to previous literature; see Chapter 2) can have a potential effect on modulating the levels fatigue in MS patients. Therefore, the team finally decided to implement a novel therapeutic strategy able to directly influence the neuronal networks' dynamics and their excitability or inhibitory cortical levels, namely the electroceuticals technique.

The Faremus treatment

In two recent studies (Tecchio et al. 2014; 2018), the authors have modulated the neural activity of the bilateral whole body primary somatosensory areas by a personalized anodic transcranial Direct Current Stimulation (tDCS). The treatment, applying tDCS 15 min/day for 5 consecutive days was proved to be effective against the symptoms of fatigue in both studies. To the aim of personalizing the treatment, the anodic electrode (RePE-Regional Personalized Electrode) was modeled on the patient's shape of S1 circonvolution derived from individual 3D-rendered brain magnetic resonance images (Cancelli, 2015; Tecchio, 2013) and the cathode was positioned on the occipital area. They decided to target S1, carefully avoiding M1 based on the above-mentioned literature, reporting specific functional alterations in the sensorimotor network (in particular hyperexcitability of M1 and lowered excitability of S1) and impaired connectivity between S1 and M1 (Tomasevic 2013).

In this case they presumed tDCS to be able to enhance S1 cortical excitability and parietal-frontal functional connectivity (Polania, 2011).

The intervention was able to reduce MS fatigue and was called 'Fatigue Relief in Multiple Sclerosis (FaReMuS]'.

The therapeutic electroceutical service

In 2019, driven mainly by the various contacts that the scientific divulgation related to the treatment had solicited[27]; we decided to devote our efforts to the creation of a therapeutic service for multiple sclerosis patients to combat fatigue. With the aim, in the future, of extending the neuro-modulation service to other diseases (see Section 1.2). Such a service was conceived as including both an in-clinic treatment and the possibility for the patient to obtain home treatment to be used with the subsidy of digital helpers[28].

Section 4.2: The review

A very relevant preliminary step in creating an electroceutical service was to assess the clinical validity of Faremus treatment. In line with this scope, we wrote a quantitative review of tDCS randomized controlled trials in no-structural diseases, whereby no structural diseases we mean diseases mostly involving electrical activity unbalances that we think can mostly benefit from tDCS treatments, thus excluding diseases involving relevant structural alterations such as Alzheimer or stroke. I actively contributed to realizing this review as its First Author (first authorship was shared with my colleagues Massimo Bertoli and Ilaria Simonelli). The general purpose of the review was to evaluate whether it is possible to include tDCS treatment in the framework of medical therapies under the indications of the international competent authorities. Within this framework we had 3 objectives:

1. to estimate the recommendation strength of tDCS treatments in no-structural diseases as dictated by the international competent authorities.
2. to indicate the PICO variables values for effective tDCS treatments.
3. estimation of Sham effect in trials planned to assess the efficacy of tDCS treatments.

[27] The TEDTALK we talked about in the first Chapter and we will talk broadly about in the following Chapter solicited patients affected by diverse pathologies, not only MS, to contact our Professor Franca Tecchio for asking either more information or properly to try out the medical treatment.
[28] Barbieri et al. 2019.

In our review we followed the GRADE classification criteria (Goldet 2013; Section 4.2; https://gdt.gradepro.org/app/handbook/handbook.html) where GRADE stands for Grading of Recommendation, Assessment, Development and Evaluation of a medical treatment. It is a set of criteria established by the competent authorities (and recognized also by our health ministry) for defining a medical treatment as recommendable. According to the GRADE system a treatment is highly recommendable if there exist at least one randomized controlled trial that can be classified as a Class I RCT according to the CONSORT checklist for RCTs of non-pharmacologic treatments. According to these criteria and excluding structural diseases we selected 18 class I studies relating to the pathologies of pain, fatigue in MS and depression and we carried out meta-analysis separately for each pathology.

The GRADE system outlines the Treatment Rating Criteria for assigning the position of a procedure within a continuum of recommendation strength ranging from 'strong against' to 'strong for'. The meta-analysis results, integrated with extensive evidence of negligible side effects and low-cost, easy-to-use procedures, indicated that tDCS treatments for depression, fatigue in MS and pain ranked between moderately and highly recommendable.

For these interventions we reported the PICO[29] variables, for example PICO variable for depression and fatigue in MS. Recommended PICO variables for depression result to be left vs. right dorsolateral prefrontal target for 30 min for 10 days; PICO variables for MS fatigue result to be bilateral somatosensory vs occipital target for 15min for 5 days, like in the Faremus treatment. Therefore, the montage used in the Faremus treatment results to be efficacious, and the recommandability as well results to be high.

Section 4.3: The Press release

[29] Where PICO stands for Population, Intervention, Comparison and Outcome. Leonardo (2018).

To disseminate our relevant findings as well as the beneficial effects of the neuromodulation approach, we divulgated our results through a press release (Section 4.3).

Section 4.4: The FaremusH

A second relevant step towards the building up of an electroceuticals service was to test a home-based version of our treatment Faremus.

Three main considerations contributed for us to the relevance of developing a home-treatment: reaching the hospital or other treatment locations on a daily basis generates itself fatigue, especially within congested areas, in extreme weather conditions and in pandemic conditions as well; the set-up we developed is simple and easily manageable by the patient without special assistance; the effectiveness and simplicity of home treatments promise to achieve sustainable repetitions over time.

In this study I contributed to writing, the aim here was to assess the feasibility, efficacy and acceptance by the patients of the FaReMuS treatment at patients' home. The same MRI-based procedure to shape and position RePE (**re**gional **p**ersonalized **e**lectrode) was used, taking advantage of the scalp-space distances calculated on the 3D MRI reconstruction of the subject's head. To reposition RePE at patients' home, an easy and accurate procedure developed by adjustable helmet frame (AHF) was used to maintain the position of RePE during stimulation, and to allow an easy repositioning of RePE in multisession tDCS treatments.

No matter how severe their clinical condition, MS patients responded favorably to a 5-day bilateral S1 anodal tDCS stimulation delivered via a customized electrode created from an MRI and embedded in an adaptive helmet frame that allows for exact placement.

We concluded FaremusH is a successful treatment that is suitable for use at home, according to research into safety and usability issues we carried out in this

study as well as consistent indications of efficacy comparable to that gained in clinical settings.

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4.2 Randomized Clinical Trials using tDCS in no-structural disorders: a quantitative review *

The following authors contributed to the final and published version of this work*:
For details see "author contribution statement" at the end of the manuscript

Gianni E[* 1,2], Bertoli M[* 1,3], Simonelli I[* 4], Paulon L[1], Tecchio F [1], Pasqualetti P [5].

* These Authors equally contributed to this work.

[1] LET'S–ISTC–CNR, Rome, Italy

[2] University Campus Bio-Medico of Rome, Rome, Italy

[3] Department of Imaging and Neuroscience and Clinical Sciences, University 'G. D'Annunzio' of Chieti-Pescara, Chieti, Italy

[4] Service of Medical Statistics and Information Technology, Fatebenefratelli Foundation for Health Research and Education, Rome, Italy

[5] Department of Public Health and Infectious Diseases, Section of Medical Statistics,

Sapienza Università di Roma

This paragraph contains an extract reformulated by Eugenia Gianni of an article published in Scientific Reports in 2021. Figures and tables are from the article.

Gianni, E., Bertoli, M., Simonelli, I., Paulon, L., Tecchio, F., & Pasqualetti, P. (2021). tDCS randomized controlled trials in no-structural diseases: a quantitative review. *Scientific Reports*, *11*(1), 1-18.

Introduction

In the first Chapter we introduced and largely spoke about the cutting-edge field of Electroceuticals [1-2], the treatment of pathologies by electrical signals, its impact, and clinical implications. We said how it opened new therapeutic options when the Body-Brain System suffer from electrical activity imbalances[3],[4]. Indeed, the body-brain system is becoming increasingly well understood as a multidimensional network[5] in which neuronal electrical transmission maintains connection across various nodes, which in turn interacts with the operation of the system at various levels (immune, behavioural and hormonal)[6-7]. It is acknowledged that it is possible to communicate with the body-brain network by sending the right signals directly to some particular control network nodes, strengthening bridges where communication has deteriorated due to a pathological condition [8-9]; this is the principle at the basis of the Electroceutical approach. In the First Chapter we mentioned how non-invasive brain stimulation (NIBS) is an important subfield in electroceuticals. Its two primary methods are transcranial direct current stimulation (tDCS)[10-12] and repeated transcranial magnetic stimulation (rTMS)[11]. Although there is substantial evidence supporting the clinical efficacy of rTMS in treating a variety of pathologies[12], the purpose of this article is to focus on the use of tDCS because, in our opinion, whereas rTMS is better suited for high intensity stimulations focused on small cortical areas, tDCS exhibits its benefits when focused on wider cerebral areas and typically delivers small currents[13-14-15-16-17-18]. In the very last years, motivated by a large bulk of descriptive investigations with promising results, the international community started tDCS randomized controlled trials (RCTs)[19,20].

As long as no-structural disorders, which are the subject of the current review, contain electrical activity imbalances that may diffuse to larger cortical regions and be addressed superficially by small currents, we think that tDCS therapies may be most effective in treating them.

In this work, focusing on tDCS, we questioned the literature on these RCTs

proceeding in agreement with the PICO framework Population, Intervention, Comparison, Outcome- of the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) of clinical practices[21] (Table 1) which presents a well-accepted methodology for framing health care questions endorsed by the Cochrane collaboration for EBM (evidence-based medicine) assessments.

In this review, we set out to accomplish three goals: 1. To determine the GRADE recommended strength of tDCS against symptoms caused by imbalances in neuronal electrical activity. Instead of providing a list of diseases to include, we provided a list of structural disorders to be omitted in order to be as comprehensive as possible and to identify all areas where tDCS has been applied for clinical purposes. We used a more cautious approach when evaluating the clinical efficacy of the therapies, taking into account only Class 1 RCTs, and we subjected each set of studies related to the same pathology to a meta-analysis quantitative assessment. We presented all of those RCTs regardless of the effectiveness level; this decision was made to assess the validity of each treatment in relation to the stimulation settings or demographic characteristics; 2. to list the values of the PICO variables for successful tDCS treatments, i.e., the clinical conditions most likely to benefit, the stimulation parameters, and the outcome measurements; 3. The assessment of the Sham effect in trials intended to evaluate the effectiveness of tDCS is another contribution made by this quantitative evaluation of tDCS RCTs.

Table 1 Quality of recommendation of nonpharmacologic treatments

Rating criteria of Treatments
Balance between desirable and undesirable outcomes (trade-offs) taking into account: - best estimates of the magnitude of effects on desirable and undesirable outcomes

- importance of outcomes (estimated typical values and preferences)
Confidence in the magnitude of estimates of effect of the interventions on important outcomes (overall quality of evidence for outcomes)
Confidence in values and preferences and their variability
Resource use

The Treatment Rating criteria from GRADE.

Table from Gianni et al. 2021.

Methods

Eligibility criteria for studies' search and selection

Selection of PICO variables

Adults who have no-structural disorders were the participants. Patients with stroke, dementia, Alzheimer's, Parkinson's, or palsy were not included. The intervention was restricted to tDCS, with specified delivery method, current, and session length. The Sham procedure was always used as the comparative condition. The outcome measure served as a criterium for choosing studies that were included in the meta-analyses.

Quality of treatments' recommendation

For conducting the review, we consulted the competent regulatory guidelines that published the GRADE of medical treatments[21] to outline the Treatment Rating Criteria (Table 1) for determining where a procedure falls on a continuum of recommendation strength ranging from "strong against" to "strong for," (GRADE Chapter 6, Going from evidence to recommendations, Figure 1).

Figure 1 Treatment recommendation strength

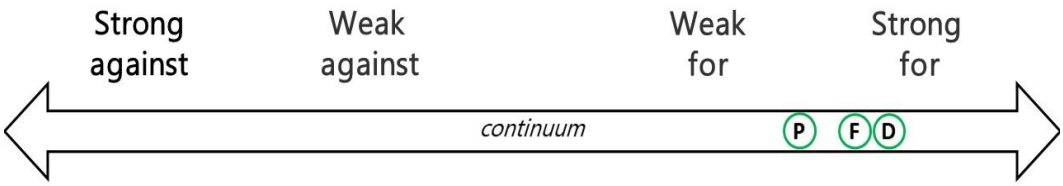


Figure 1. The level of confidence a guideline panel has that desirable outcomes exceed unfavorable consequences is reflected in the intensity of a recommendation for a clinical practice within a continuum of categories.

Figure and caption from Gianni et al. 2021.

According to the findings of the current meta-analysis, the GRADE recommendation for tDCS therapies ranges from moderate to strong for the following diseases: depression (D), fatigue from multiple sclerosis (F), and pain (P) (pain meta-analysis will not be reported in this extract; for more information see the article Gianni et al. 2021).

In reviewing the study, we adhered to a crucial criterion, which is that the evidence supporting the use of the treatment is of high quality: according to the methods' section of the CONSORT (Consolidated Standards of Reporting Trials) checklist for RCTs of non-pharmacologic therapies[23], we only chose RCTs in Class 1, which are those that meet every criterion given in Table 2 RCT classification.

In GRADE the role of strength and quality of evidence is highlighted in Chapter 5. GRADE clearly indicates criteria for the strength of the evidence, referring to specific cases for the classification of the RCTs on which the evidence is based. Therefore, GRADE does not indicate a strict classification of clinical studies, leaving this point open. For the classification of neurological studies, we reasoned based on the table of Brainin et al. 2004[23a] (see Appendix).

Table 2 Classification criteria of RCTs, *from Gianni et al. 2021*.

The RCT classification criteria from CONSORT

Sample size estimate to enrol adequately powered groups
Control condition
Randomization assignment
Masking
Randomization concealment
Primary outcome clearly defined
Exclusion/inclusion criteria clearly defined
Adequate accounting for dropouts and crossovers with numbers sufficiently low to have minimal potential for bias
Relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences

Electronic Repository search strategy

We used the following search terms and keywords in PubMed: Filters: Randomized Controlled Trial, Search: ((((((tDCS transcranial direct current stimulation) NOT stroke) NOT dementia) NOT Alzheimer) NOT Parkinson) NOT palsy) NOT "brain injury" AND later than Sept 2016.

These criteria generated a list of 266 articles. Based on the titles of these papers, we eliminated any non-RCT and structural pathology-related studies that weren't already rejected by our first search. Animal studies were not included. The publications from before 2016 were collected from Leufaucher et al. 2017[24], who used our selection criteria to report tDCS RCTs up until September 2016. After reading the papers, we eliminated those that didn't meet the RCT Class1 requirements. In doing so, we used the search (size OR sample OR power) to apply the "adequately powered" condition (table 2 and Appendix).

Study selection

Three review writers (EG, MB, and IS) separately sought for and evaluated eligible studies. Any discrepancies among the authors were discussed with the author FT and addressed through discussion.

We only considered one data set, usually the most current or largest, when numerous publications from the same experiment occurred.

Meta-analysis design

Data collection

We conducted a meta-analysis to determine the global dimension of the effect when the inclusion criteria resulted in several studies for a given disease.

For each trial that was included in the meta-analysis (Real, Sham), we retrieved the number of participants who were randomly assigned to either Real of Sham treatment and then analyzed in each treatment group. Since the outcomes were continuous, we gathered the scale used to measure the

intervention's effectiveness and the pre- and post-treatment mean and standard deviation (sd) or standard error, as appropriate. If baseline and post-intervention data were not provided, *graphreader.com* extracted it from the graph. The standard deviation and % mean variance were extracted.

For one study[25] data points on respondents and non-responders were merged.

If further longitudinal measurements of the result were present, we took only the first into consideration.

Quantitative treatment description - Summary measures

We measured the effects of each treatment—Sham and Real tDCS—in accordance with PRISMA guidelines[26] by calculating the effect size and the relative Standard Error (SE) using the methods described in Chapter 4 of Borenstein, Hedges, Higgins, and Rothstein's (2009) book[27].

We analyzed the difference between mean change scores observed in Sham and in Real tDCS groups. Since multiple studies utilized different scales of assessment, the summary statistics used was the standardized mean difference (SMD). The SMD was determined in accordance with the instructions in Cochrane Handbook Chapter 6 ([28]). When none of the studies revealed the correlation between pre-intervention and post-intervention data, we estimated it using the data that were available. A sensitivity analysis was then conducted with a 0.5 correlation.

A random-effect meta-analysis was used. All effects were shown with a 95% Confidence Interval (CI). Values for ES (SMD) were classified as follows: 0.2 denotes a minor effect, 0.5 a moderate effect, and 0.8 a high effect[29].

Assessment of heterogeneity

The Cochran's Q test was used to assess the heterogeneity, and the I^2 was used to quantify it. The I^2, which ranges from 0 (no heterogeneity) to 100, represents the rate of difference between studies caused by heterogeneity rather

than chance (maximal heterogeneity).

PICO variables' values of efficacious tDCS treatments

Based on findings from meta-analyses on the effectiveness of treatment for each clinical condition, we provided a qualitative analysis of the procedures used. We developed a schematic depiction of the PICO variables for each pathology included in the quantitative analysis, with the inclusion criteria specifying the population of interest, the outcome measure, the tDCS montage, delivered current amplitude, and stimulation time.

Results

Methods and selection flowchart

By the search and selection process depicted in the flowchart (Figure 2), we started from 330 arriving to 18 analysed RCT papers (4 depression, 4 fatigue in MS, 1 pain, 5 addictions, 4 fibromyalgia). For seek of synthesis we will report in this work only results for depression and fatigue; for the complete analysis see Gianni et al. 2021. Sham analysis will follow.

Figure 2 Flow chart

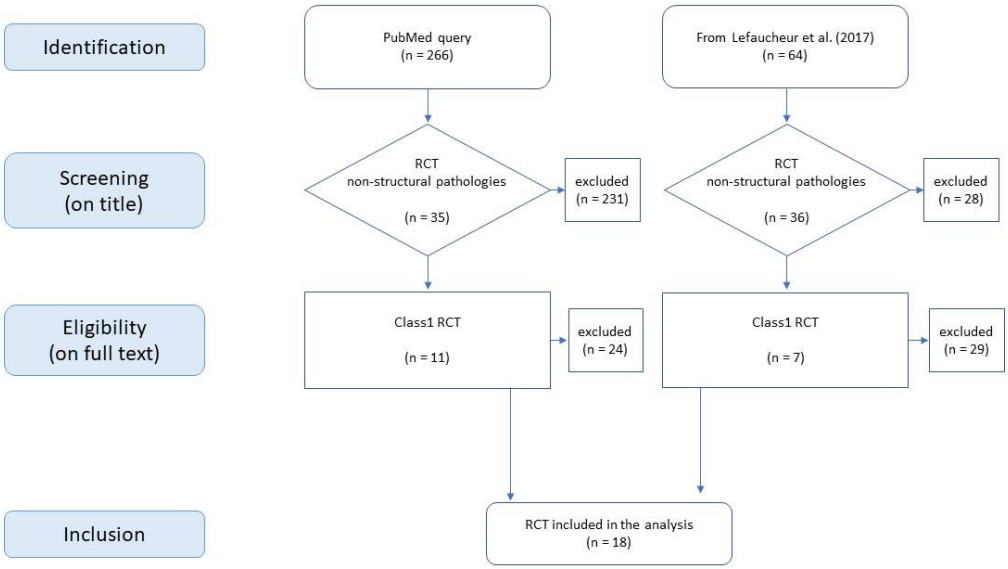


Figure 2. PRISMA flow diagram illustrating search strategy, inclusion and exclusion process. *Figure from Gianni et al. 2021.*

Overview of the Class 1 tDCS RCT in no-structural disorders

We provided a summary of all non-structural pathologies related studies arranged according to pathology.

We calculated the effectiveness of the treatment for each pathology for Sham (Tables 3, 6, 9 and Forest plot in Figures 3, 6), Real (Tables 4, 7, 10, and Figures 4, 7), and their relationship (Tables 5, 8, 11 and Figure 5, 8), and we provided the population inclusion criteria, the outcome measure, and the stimulation parameters in Tables 12, 13, and 14.

DEPRESSION

Meta-analysis

Only one research[30] published the % mean and standard deviation (SD) change; however, none of the studies gave the mean change score and the SD, so that the data were generated as specified in the statistical analysis section. In the Sham group (Table 3, Figure 3), the correlation (Pearson's r) was equal to 0.86, while in the Real group, it was equal to 0.96. (Table 4, Figure 4).

The Hamilton Depression Rating Scale (HDRS-17) scores were used in the Sampaio-Junior et al. research[31] to present data regarding depression, but the Montgomery Asberg Depression Rating Scale (MADRS) was used in the other three studies[30, 32, and 33]. Data points were taken from a graph for one study[33]. Combining the trials, there were a total of 132 patients in the Sham group and 135 patients in the Real group.

Depression - Sham effect

There was a significant Sham effect, with the pooled effect size equal to 0.93 (95% CI 0.52-1.33; p=0.001). The degree of heterogeneity was high (I2=92.4%, p=0.001).

Because the data were taken from the graph in the Loo et al. research (2018), we did a sensitivity analysis without including that study. There was a moderate Sham effect, with the pooled effect size equivalent to 0.72 SDs (95% CI 0.57-0.87; p0.001). (I2=26.1%, p=0.259) The heterogeneity was not significant.

By lowering the correlation to 0.5, a second sensitivity analysis was conducted, and the results about the decrease after Sham were consistent (ES=1.15, 95% CI 0.59-1.71; p=0.001). There was significant heterogeneity (I2=84.5%, p=0.001). If Loo et al. 2018 were not included in the analysis, the pooled effect would have been 0.85 (95% CI 0.61 to 1.095; p0.001) and the heterogeneity across trials would not have been significant.

Figure 3 Depression. Sham

Figure from Gianni et al. 2021

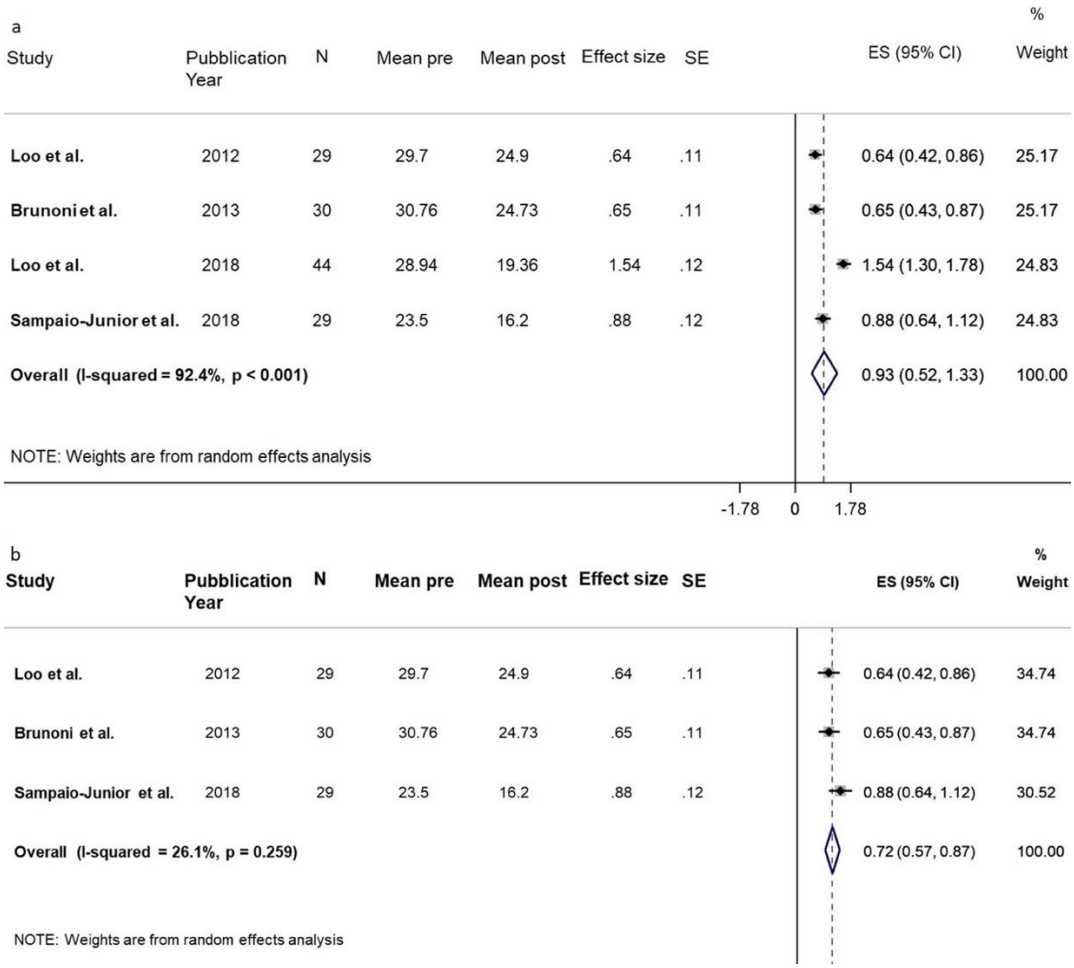


Figure 3. a) Forest plot of meta-analysis results considering all studies. The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study. b) Forest plot of sensitivity meta-analysis. Note: SE= Standard Error; ES=Effect Size. CI= Confidence Interval.

Table 3 Depression. Sham, *table from Gianni et al. 2021*

Study	PY	Study designed	Scale	n	Mean pre	SD pre	Mean post	SD post	Mean Diff	SD Diff[a]	SD within[a]	Effect Size[a] (SMD)	SE of effect size[a]	SD Diff[b]	SD within[b]	Effect Size[b] (SMD)	SE of effect size[b]
Loo et al.	2012	Parallel	MADRS	29	29.7	5.7	24.9	7.6	4.8	3.96	7.48	0.64	0.11	6.86	6.86	0.70	0.21
Brunoni et al.	2013	Factorial Randomized Controlled	MADRS	30	30.8	5.3	24.7	8.7	6.03	4.90	9.26	0.65	0.11	7.56	7.56	0.80	0.21
Loo et al.	2018	Parallel	MADRS	44	28.9	2.6	19.4	5.28	9.58	3.29	6.22	1.54	0.12	4.57	4.57	2.10	0.27
Sampaio-Junior et al.	2018	Parallel	HDRS-17	29	23.5	4.7	16.2	7.7	7.3	4.37	8.27	0.88	0.12	6.72	6.72	1.09	0.23
Pooled analysis				**132**								**0.93**	**0.21**			**1.15**	**0.29**
Pooled analysis without Loo et al. 2018				**88**								**0.72**	**0.08**			**0.85**	**0.13**

Data about pre and post data in Sham group. PY: Pubblication year. SD: Standard Deviation. SMD= Standardized Mean Difference. SE=Standard Error. Note: [a] Correlation pre-post r= 0.86. [b] Correlation pre-post r= 0.

Depression - Real effect

According to the pooled effect size of 0.99 (95% CI 0.56-1.42; p0.001), there was a large effect after tDCS. The degree of heterogeneity was high (I2=98.2%, p0.001).

The Loo et al. paper from 2018 was excluded from the sensitivity analysis since the data were taken directly from the graph.

According to the pooled effect size of 1.05 (95% CI 0.41-1.70; p=0.001), there was a large effect after tDCS. The degree of heterogeneity was high (I2=98.8%, p0.001).

The high heterogeneity levels may have been brought on by the various tDCS administration conditions.

Another sensitivity analysis was conducted with the correlation set to 0.5, and the results of the decrease after tDCS were consistent (ES=1.78, 95% CI 1.13-2.43; p0.001). There was significant heterogeneity (I2=82.5%, p=0.001) (Table 4, Figure 4).

Figure 4 Depression. Real, *figure from Gianni et al. 2021*

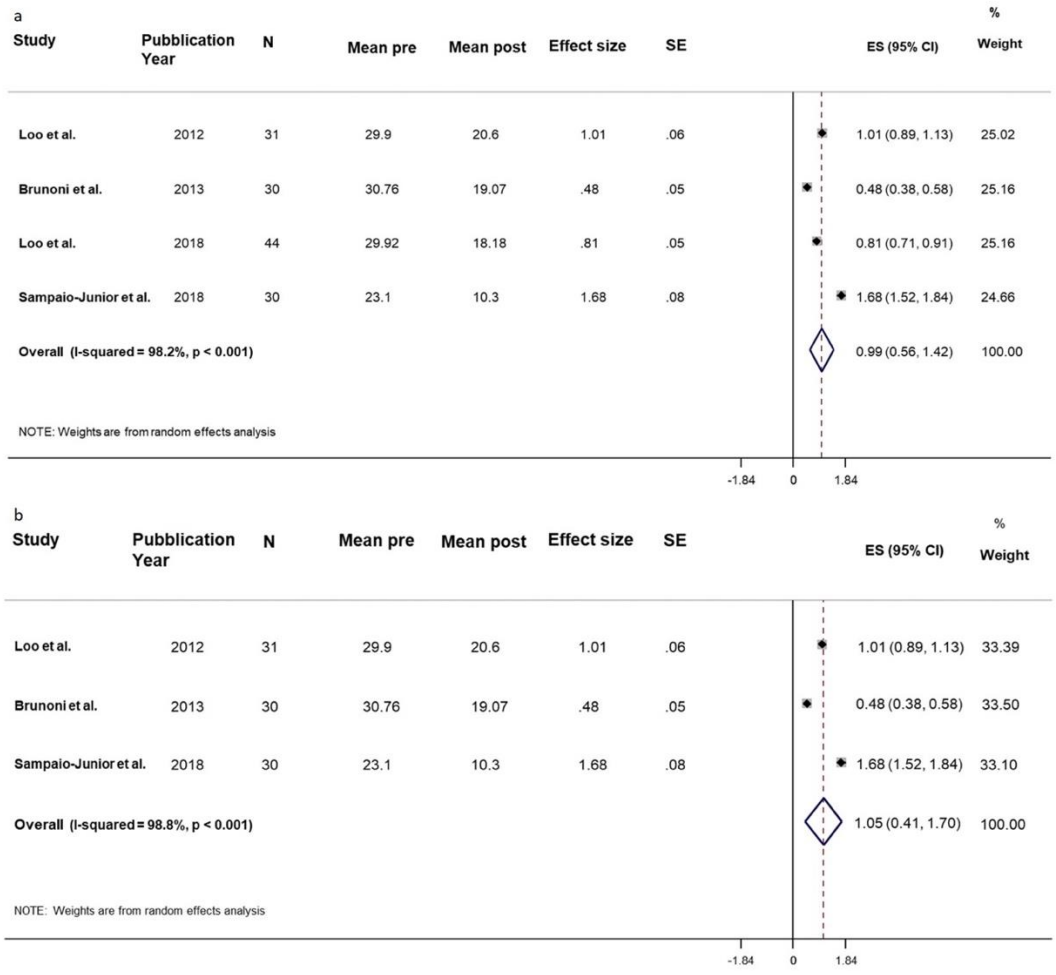


Figure 4. a) Forest plot of meta-analysis results considering all studies. The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study. b) Forest plot of sensitivity meta-analysis.

Note: SE= Standard Error; ES=Effect Size. CI= Confidence Interval.

Table 4 Depression. Real, *from Gianni et al. 2021*

Study	PY	Study designed	Scale	n	Mean pre	SD pre	Mean post	SD post	Mean Diff	SD Diff[a]	SD within[a]	Effect Size[a] (SMD)	SE of effect size[a]	SD Diff[b]	SD within[b]	Effect Size[b] (SMD)	SE of effect size[b]
Loo et al.	2012	Parallel	MADRS	31	29.7	5.7	20.6	7.6	9.3	2.6	9.24	1.01	0.06	6.84	6.84	1.36	0.25
Brunoni et al.	2013	Factorial Randomized Controlled	MADRS	30	30.8	5.78	19.07	12.2	11.7	6.9	24.24	0.48	0.05	10.58	10.58	1.11	0.23
Loo et al.	2018	Parallel	MADRS	44	29.9	1.96	18.18	5.9	11.7	4.1	14.47	0.81	0.05	5.24	5.24	2.24	0.28
Sampaio-Junior et al.	2018	Parallel	HDRS-17	30	23.1	3.9	10.3	5.6	12.8	2.2	7.61	1.68	0.08	4.97	4.97	2.57	0.38
Pooled analysis				**135**								**0.99**	**0.22**			**1.78**	**0.33**
Pooled analysis without Loo et al. 2018				**88**								**1.05**	**0.33**			**1.62**	**0.38**

SD=Standard Deviation. SMD=Standardized Mean Difference. SE=Standard Error. Note: [a] Correlation pre-post r=0.96. [b] Correlation pre-post r=0.5.

Depression - Real vs Sham effect

The findings demonstrated that tDCS had a large effect; the average reduction in depression shown after tDCS was 1.09 SDs, that is greater than that seen after sham (95% CI=0.63-1.54; p0.001) (Fig. 1). Heterogeneity was discrete and significant (I^2=66.8%, p=0.029).

There was no indication of publication bias by the funnel plot.

For the previously stated rationale, we did a sensitivity analysis removing Loo et al. paper 2018. The combined effect size after tDCS was 1.28 SDs (95% CI 0.91-1.65; p=0.001), which is a large effect. (I2=23.5%, p=0.271) The heterogeneity was not significant (I2=23.5%, p=0.271).

The results were consistent, according to the sensitivity analysis, by taking a correlation of 0.5 into account (SMD=0.63, 95% CI=0.39-0.88; p=0.001). The depression decrease shown after tDCS was substantially greater than that seen after Sham.

Figure 5 Depression. Real Vs Sham*, figure from Gianni et al. 2021*

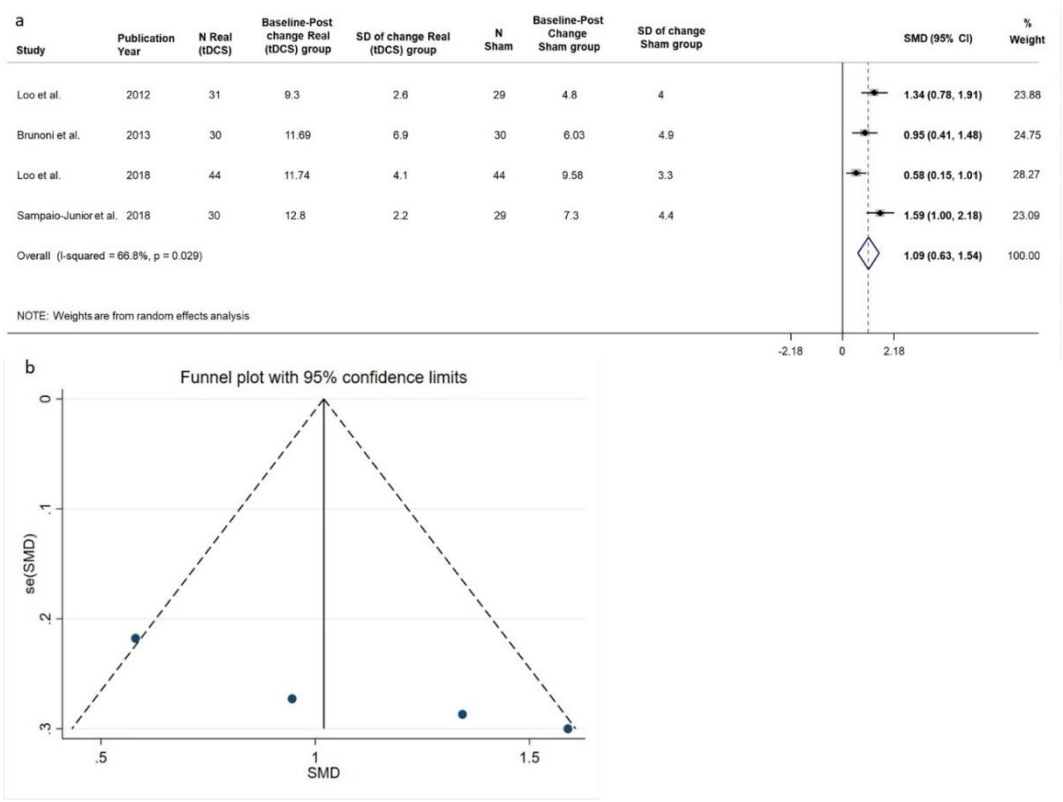


Figure 5. a) Forest plot of meta-analysis results considering all studies. The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study. b) On y-axis is represented the standard errors of the studies from lowest to higher, on x-axis the estimated SMDs; the solid vertical line represents the pooled SMD and diagonal dashed line represents its 95% CI.

Table 5 Depression. Real Vs Sham, *from Gianni et al. 2021*

				Baseline-post mean change						95% CI		
Study	**PY**	**Study designed**	**Scale**	**N Sham**	**N Real**	**Sham group**	**SD Sham group**	**Real group**	**SD Real group**	**SMD**	**LL**	**UL**
Loo et al.	2012	Parallel	MADRS	29	31	4.8	4	9.3	2.6	1.34	0.78	1.91
Brunoni et al.	2013	Factorial, Randomized, Controlled	MADRS	30	30	6.03	4.9	11.69	6.9	0.95	0.41	1.48
Loo et al.	2018	Parallel	MADRS	44	44	9.58	3.3	11.74	4.1	0.58	0.15	1.01
Sampaio-Junior et al.	2018	Parallel	HDRS-17	29	30	7.3	4.4	12.8	2.2	1.59	1.00	2.18
Pooled analysis										**1.09**	**0.63**	**1.54**

SD=Standard Deviation.SMD=Standardized Mean Difference. CI=Confidence Interval. LL=Lower Limit. UL=Upper Limit.

PICO variables' values for tDCS against depression

The depressed population that benefits from tDCS, experience moderate to severe symptoms (Table 12). Since all but one study utilized MADRS as the major outcome and found identical findings when analyzed by HDRS, the data point out to the use of MADRS as an outcome measure. All investigations placed the anode on the left dorsolateral prefrontal cortex (dlPFC), which is targeted using the 10-20 international method of EEG electrode placement by centering the anode on F3. This is how we were able to determine the parameters for the most effective tDCS intervention.

Since the two trials employing F4 instead achieved the best and worst results, it appears that F8 drives the cathode's most steady outcome. However, as the F8 cathode position findings are in-between, we contend that there is no benefit to having the anode and cathode positions asymmetric in the two hemispheres. As a result, we recommend mounting the anode on F3 and the cathode on F4 (Figure 10).

Figure 10 tDCS montage for depression and MS fatigue, *figure from Gianni et al. 2021*

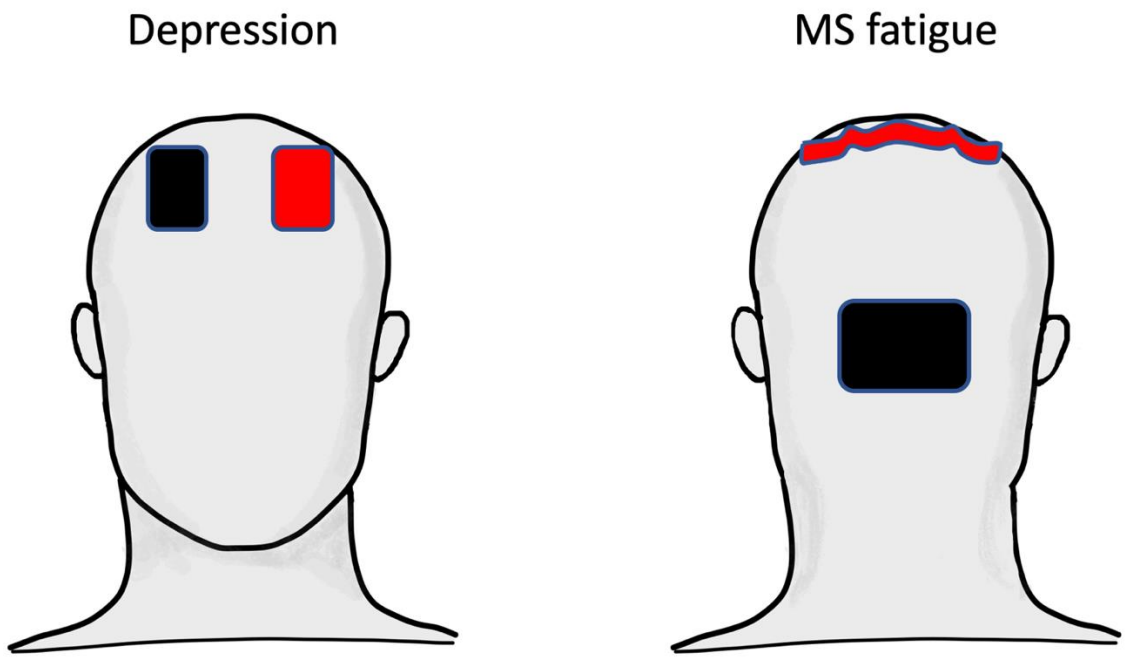


Figure 10. Graphical representation of the tDCS electrodes position and shape. In Depression the rectangular (7x5 cm^2) anode (red) is centred on F3 and the cathode (black) on F4. In MS fatigue, the anode is an electrode with 35 cm^2 area shaped as the individual central sulcus cortical folding and the occipital cathode a double area rectangle (7x10 cm^2).

The current provided varies only slightly between experiments, and the greater current surface density (csd) employed in two of the research yields the greatest and poorest effectiveness, respectively. Therefore, the proposed csd may be calculated as the average of the four studies, or around 0.072 mA/ cm2.

In terms of time, 10 days of therapy appear adequate; in fact, Sampaio et al. found that the best outcome occurs after 10 days, while Loo and colleagues found that a 20-day treatment had a worse outcome than a 15-day one. Even though Loo et al. found that a 20-minute session had results similar to those of 30-

minute sessions, we recommend a 30-minute daily session time. Therefore, a proposed time frame of 30 minutes every day for 10 days.

In conclusion, it would be interesting to investigate if tDCS may be used in the phases just before the manifestation of severe depression, given that rTMS has been approved by the FDA as a clinical therapy[34] after demonstrating clear and substantial effects against this disease.

Table 12 Parameters. Depression, *from Gianni et al. 2021*

Study	Outcome	Population	Intervention						
			Electrode position		Stimulation intensity			Duration	
			Anode	Cathode	Electrode size	Ci	Csd	Daily	Days
Loo 2012	MADRS	MADRS >20	F3	F8	35/35	2	0.057	20	15
Brunoni 2013	HDRS	HDRS > 17	F3	F4	25/25	2	0.080	30	10
Loo 2018	MADRS	MADRS >20	F3	F8	35/35	2.5	0.071	30	20
Sampaio 2018	MADRS	MADRS >20	F3	F4	25/25	2	0.080	30	10

PICO variables for tDCS against depression. The parameters of tDCS intervention include the electrodes' position (El position) expressed by the site of the 10-20 EEG International System where the electrode was centered; the stimulation intensity considering the area of the electrodes (El size, cm2) for anode/cathode, the current intensity (Ci, mA), and the current superficial density (Csd, mA/cm2); the stimulation duration with the daily session duration (min) and the number of days (Days).

MS FATIGUE

Meta-analysis

Five studies[25,35, 36,37] met the requirements for Class 1 studies. The 2015 study by Tecchio et al. used the same sample as the 2014 study by Tecchio et al. for the S1 target, hence only data from the most recent study were taken into account in the meta-analysis (Table 6, 7, 8, Figure 6, 7, 8). Methodological guidelines provided by Elbourne et al.[38] were followed because all of the studies were cross-over. As measure of correlation between the two conditions (Sham and Real), the coefficient indicated by one of the individuated studies (Cancelli et al., r = 0.55) was assumed. The standardized mean difference served as the summary statistic (SMD). According to the instructions in the method-statistics section, we compute the effect size (ES) and the relative Standard error (SE). When all the trials were combined, there were 46 patients.

Ms Fatigue – Sham effect

The pooled ES showed a minor but significant effect of the sham (p=0.00; ES = 0.27; 95% CI: 0.11 to 0.42). It was heterogeneous (I2=69.7%, p=0.037). The locations of the electrodes were one potential cause of heterogeneity. We conducted a meta-analysis again, only taking into account two trials with identical electrode placements (Tecchio et al 2015[36] and Cancelli et al. 2018[37]). The outcomes supported the earlier findings that there was a minor but significant effect of Sham (ES=0.18, 95% CI: 0.07 to 0.3; p=0.002). The heterogeneity was not significant I2=0%, p=0.803 (Table 6, Figure 6).

Figure 6 MS Fatigue. Sham, *from Gianni et al. 2021*

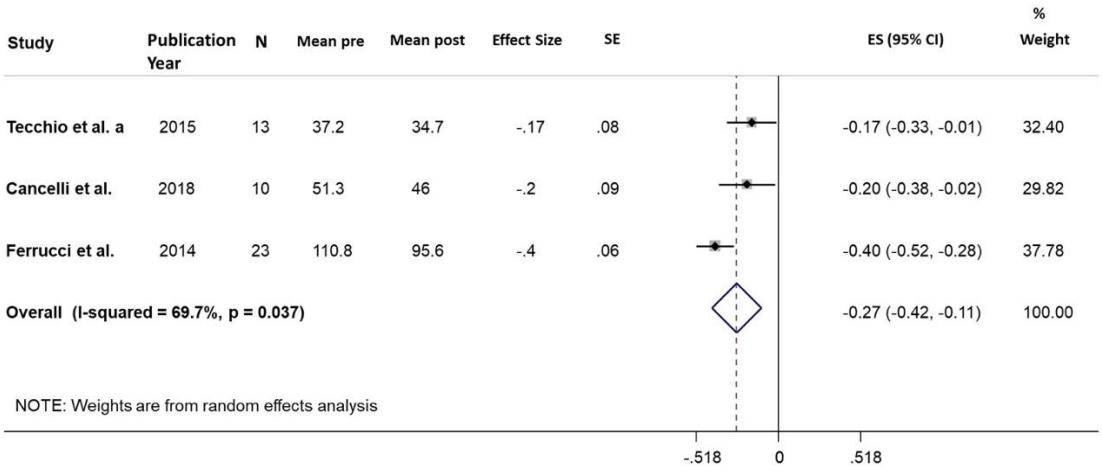


Figure 6. Forest plot of meta-analysis results considering all studies. The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study.

Note: SE= Standard Error; ES=Effect Size. CI= Confidence Interval.

Table 6 Fatigue. Sham, *from Gianni et al. 2021*

Study	PY	Study designed	Scale	n	Mean pre	SD pre	Mean post	SD post	Baseline-post mean change	SD Diff	Effect size [a] (SMD)	SE of effect size [a]
Tecchio et al.	2015	Crossover	MFIS	13	37	7	35	10	-3	4	0.17	0.08
Cancelli et al.	2018	Crossover	MFIS	10	51	12	46	19	-5	8	0.20	0.09
Ferrucci et al.	2014	Crossover	FIS	23	111	42	96	42	-15	12	0.36	0.06
Pooled analysis											**0.27**	**0.08**

PY= Publication Year. SMD=Standardized Mean Difference. SE=Standard Error.

Note: [a] Correlation pre-post r=0.96 was assumed to calculate the effect size and the corresponding standard error.

Ms Fatigue – Real effect

As described in the method-statistics section, we compute the Standardize Mean Difference as the effect size (ES) and the relative Standard error (SE). A statistically big effect of Real (tDCS) was shown by the pooled ES (ES= 0.80, 95% CI 0.42 to 1.17; p=0.001). Significant and substantial heterogeneity existed among the studies (I2=71%, p=0.032). The locations of the electrodes were one potential cause of heterogeneity. We conducted a meta-analysis again, only taking into account two trials with identical electrode placements (Tecchio et al 2015[36] and Cancelli et al. 2018[37]). The findings showed a strong effect of Real that was statistically significant (ES=0.98, 95% CI 0.69 to 1.27; p=0.001). I2=0%, p=0.503, showed that the heterogeneity was not significant (Table 7, Figure 7).

Figure 7 MS Fatigue. Real, *from Gianni et al. 2021*

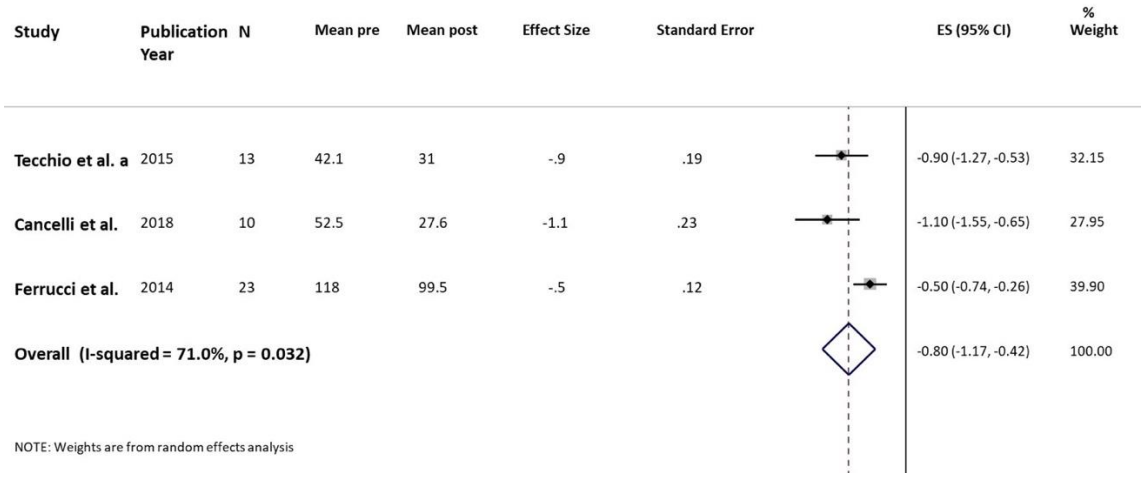


Figure 7. Forest plot of meta-analysis results considering all studies. The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study.

Note: SE= Standard Error; ES=Effect Size. CI= Confidence Interval.

Table 7 Fatigue. Real, *from Gianni et al. 2021*

Study	PY	Study designed	Scale	n	Mean pre	SD pre	Mean post	SD post	Baseline-post mean change	SD Diff	Effect size (SMD)	SE of effect size
Tecchio et al.	2015	Crossover	MFIS	13	42	8	31	12	11	7	0.9	0.19
Cancelli et al.	2018	Crossover	MFIS	10	53	10	28	19	25	13	1.1	0.23
Ferrucci et al.	2014	Crossover	FIS	23	118	41	100	34	19	22	0.5	0.12
Pooled analysis											**0.80**	**0.19**

PY= Publication Year. SMD=Standardized Mean Difference. SE=Standard Error

Ms Fatigue – Real vs Sham effect

All investigations on fatigue in MS patients included crossover designs. We calculated the mean difference, or SMD, between the mean value post-Sham and the mean value post-Real.

The results showed that the Real tDCS had not a significant effect compared to Sham, the pooled standardized mean difference of mFIS observed after tDCS was 0.34 standard deviation (sd) lower than the fatigue observed after Sham (95% CI= 0.24 to 0.92; p=0.247) (Fig. 1). Significant heterogeneity existed and was large (I2=71.7%, p=0.029).

We conducted a meta-analysis again, only taking into account two trials with identical electrode placements (Tecchio et al 2015[36] and Cancelli et al. 2018[37]). Results revealed a modest impact of Real (tDCS) compared to Sham (ES=0.61, 95% CI 0.02 to 1.23; p=0.056), which was marginally significant. (I2=50.6%, p=0.155) The heterogeneity was not significant (Table 8, Figure 8).

Figure 8 MS Fatigue. Real Vs Sham, *from Gianni et al. 2021*

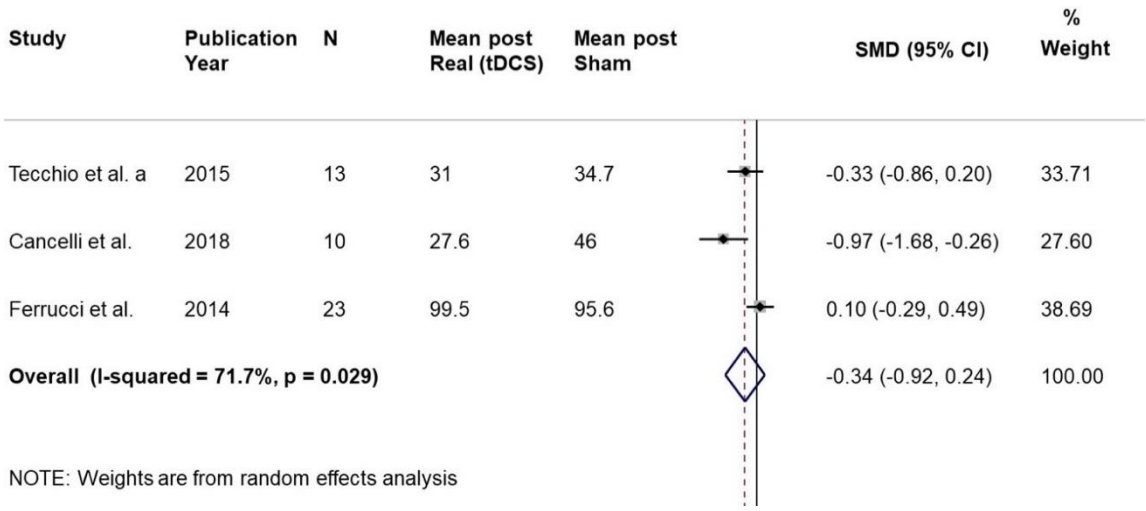


Figure 8. Forest plot of meta-analysis results considering all studies. The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study.

Table 8 Fatigue. Real Vs Sham

Study	PY	Study designed	n	Mean post Real	SD post Real	Mean post Sham	SD post Sham	Effect Size (SMD)	SE
Tecchio et al.	2015	Crossover	13	31	12	35	10	0.33	0.27
Cancelli et al.	2018	Crossover	10	28	19	46	19	0.97	0.36
Ferrucci et al.	2014	Crossover	23	100	34	96	42	0.10	0.20
Pooled analysis								**0.34**	**0.30**

PY= Publication Year. SMD=Standardized Mean Difference. SE=Standard Error

PICO variables for tDCS against MS fatigue

In light of the limited patient populations in the few Class 1 RCTs, we derived the indicated parameters (Table 13), mindful of the necessity for confirmation in larger groups.

The Modified Fatigue Impact Scale (mFIS) > 35 (indicating severe fatigue sympthoms) cut-off appeared to be an appropriate cut-off, since beneficial treatment benefits were shown from this inclusion level.

All of the cross-over studies had small sample sizes, and the findings indicate that at this time, it would be more appropriate to include patients with minimal to moderate clinical severity (Expanded Disability Status Scale, EDSS 3.5) than to provide tDCS treatment to patients with a wider range of disease-related impairment. A treatment that is effective against fatigue becomes crucial for the patient's quality of life even though it will be important to evaluate the

effectiveness in the presence of increasing disability in larger populations. Notably, MS is frequently accompanied by severe fatigue even in the absence of other disabling symptoms.

Current research, which is supported by a recent large-scale multi-center RCT[39], points to the mFIS as the right outcome measure. Its 21 items are adequate to detect induced alterations, therefore there is no need to collect the lengthier Fatigue Impact Scale, which has 40 questions (FIS).

When determining the most effective tDCS intervention parameters, we found a significant difference in efficacy when the anode was focused on the bilateral whole-body primary somatosensory cortex, S1[36,37] , as opposed to the left and right hand sensorimotor regions[25], where Real tDCS had no discernible difference from Sham.

The reduced effectiveness in Ferrucci et al. research[25] may have been caused by the half-current superficial density given over the two electrodes focused on C3 and C4, each measuring 35 cm2. Thus, 0.04 mA/cm2 is the proposed value.

In terms of time, five days of therapy at 15 minutes each day should be adequate.

The findings of a recent systematic review and meta-analysis on non-invasive brain stimulations against fatigue[40,] which showed that tDCS had significant short- and long-term treatment effects but that real TMS and transcranial random noise stimulation were not superior to sham stimulation, are further supported by the results of the current meta-analysis of the Class 1 tDCS RCTs. The bilateral whole body S1[35–37] showed the highest effectiveness of the 11 tDCS RCTs.

According to promising results of tDCS against fatigue when directed at the primary somatosensory cortex, we think beneficial effects may also be elicited in dystonia. Although dystonia is a highly heterogeneous neurological condition that

mostly presents as a movement issue, from a physiological perspective, the major cause of the illness is a dysfunction in sensorimotor integration[41]. Multiple levels of the sensorimotor circuit[42,43] can exhibit abnormalities, such as a lack of inhibition, sensory dysfunction, and changes in synaptic plasticity.

There are important effectiveness findings from studies focusing on certain dystonic diseases[24], even if there are no Class 1 RCTs to suggest a regular usage of tDCS in dystonia. With the help of available and reliable RCTs, we can make educated guesses about potential future directions in the treatment of dystonia by employing electroceutical interventions intended to restore the pathological functional imbalances.

Study	Outcome	Population	Intervention						
			Electrode position		Stimulation intensity			Duration	
			Anode	Cathode	Electrode size	Ci	Csd	Daily	Days
Tecchio 2015	mFIS	EDSS ≤ 3 mFIS > 38 BDI < 19 No clinical relapse	S1	Oz	35/70	1.5	0.04	15	5
Cancelli 2018	mFIS	EDSS ≤ 2 mFIS >35 BDI < 19 No clinical relapse	S1	Oz	35/70	1.5	0.04	15	5

Ferrucci 2014	FIS	EDSS 0-6.5 mFIS > 45	C3+C4	Right deltoid	(35+35)/35	1.5	0.02	15	5

Table 13 Parameters. Fatigue

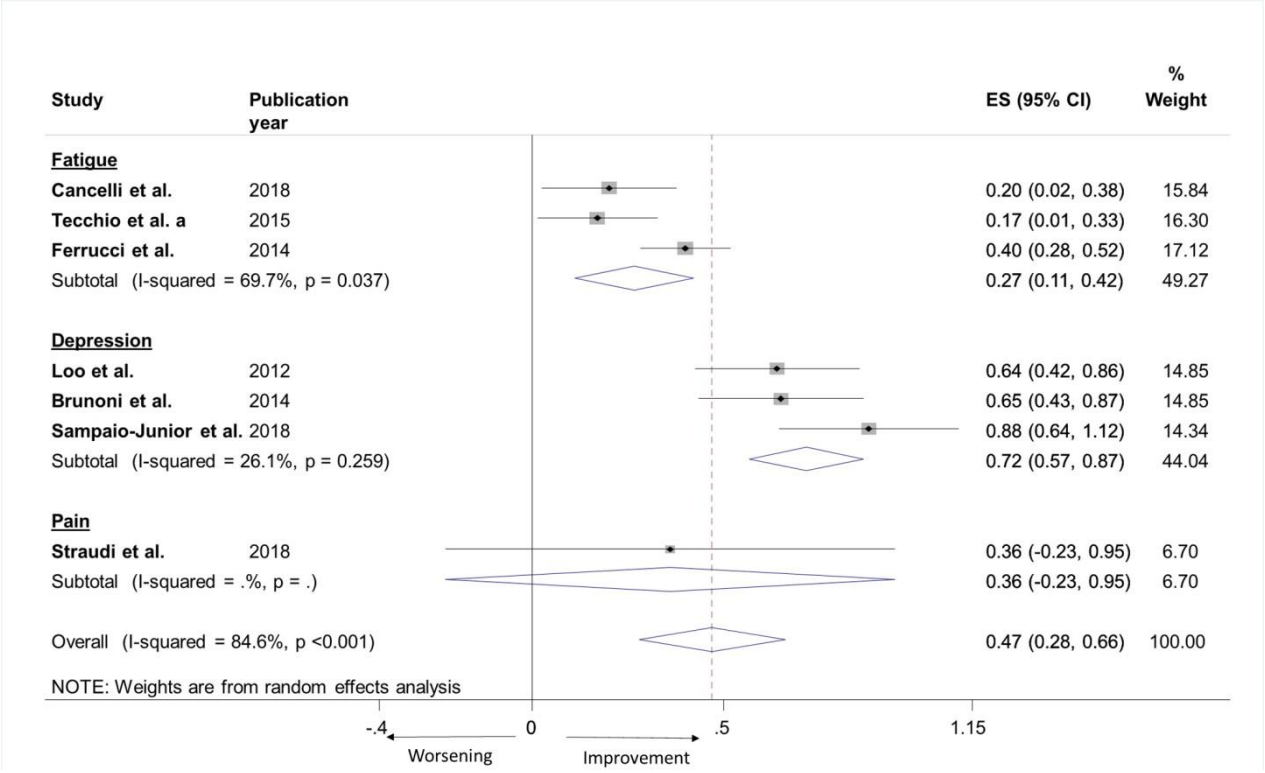
PICO variables for tDCS against MS fatigue, see the legend of Table 12 Depression, from Gianni et al. 2021

ESTIMATION OF SHAM EFFECT

The assessment of the Sham effect in trials intended to define the effectiveness of tDCS is another contribution made by this quantitative review.

Figure 9 shows that the Sham effect was obviously influenced by the disease or symptom. It is clear that the Sham effect was significantly different in fatigue and depression (test for subgroup differences: I2=94%, Chi2(1)=16.75, p=0.001). We calculated the Sham effect size to be 0.27 (a minor effect) for fatigue and 0.72 for depression (a quite large effect).

Figure 9 Estimation of Sham effect, *from Gianni et al. 2021*



a) Forest plot of meta-analysis results considering all studies by pathology (MS fatigue, Depression, Pain). The diamond represents the pooled standardized mean difference (SMD, dashed red vertical line) and its 95% confidence interval; vertical solid dark line is the line of equivalence. The estimates for each study and their 95% confidence intervals are represented by a box with whiskers, the dimension of grey box is proportional to the precision of the study. Note: ES=Effect Size. CI= Confidence Interval.

Researchers may use them in upcoming RCTs that try to show the effects of a Real stimulus. Different possibilities are shown in Table 15. Based on our estimation of the Sham effect on fatigue (standardized Effect Size, sES=0.27), a sample size of almost 800 patients will be required for a two-arm parallel design in order to achieve a 90% probability (power) of recognizing as statistically significant (at two-sided alpha level set at 0.05) an increase of Real stimulation up to a medium effect size (sES=0.50). In the event of a one-sample design, this number drops to around 200 patients.

A cross-over design, for which the correlation between pre-post Sham and pre-post Real changes was assumed to be equal to r=0.7, might result in an even smaller sample size (n=122). In the case of large Real Stimulation effect (sES=0.98) even smaller populations are needed.

In accordance with this reasoning, Table 15 lists the appropriate sample size for various Real stimulation for depression effect sizes.

Aware of the small populations involved in this sample sizes' estimate, we open the door for future investigations in which the evaluation of Real efficacy will eliminate the requirement for using extensive efforts and long hours for patients and experimenters in the Sham assessment.

Symptom/ pathology	Two-sided alpha	Power (1-beta)	Expected sham-effect (from this meta-analysis)	Effect size of Real stimulation (minimal clinically relevant difference)	Target effect size of Real stimulation	Design	Sample size
Fatigue	0.05	0.9	0.27	0.5	Medium	one-sample	201
						parallel two-samples	399+399
						cross-over*	122
				0.8	Large	one-sample	40
						parallel two-samples	76+76
						cross-over*	25
				0.98	Based on this meta-analysis	one-sample	23
						parallel two-samples	43+43
						cross-over*	15
Depression	0.05	0.9	0.72	0.8	Large	one-sample	1644
						parallel two-samples	3285+3285
						cross-over*	987
				1	Very large	one-sample	136
						parallel two-samples	270+270
						cross-over*	83
				1.79	Based on this meta-analysis	one-sample	12
						parallel two-samples	20+20
						cross-over*	8

Table 15 Sham Power Analysis, *from Gianni et al. 2021*

Sample size estimation for different effect size of Real stimulation vs. estimated effect size of Sham stimulation, according to the present meta-analysis. Sham effect size in fatigue resulted =0.27, thus effect size of Real stimulation was set at 0.5 (medium) and 0.8 (large). Sham effect size in depression resulted =0.72, thus effect size of Real stimulation was set at 0.8 (large) and 1.0 (very large). In addition, the effect size of Real stimulation (estimated with this meta-analysis) was considered in order to indicate that effects of this magnitude (and thus also smaller) could be considered realistic. * a correlation equal to 0.7 is assumed.

rTMS as opposed to tDCS

While some researchers (e.g.,[61, 62]) expressed doubt about the efficacy of tDCS (as opposed to the validity of rTMS) we believe that the fundamental technological and physical differences in the generated stimulation make rTMS preferable when focused and high intensity stimulation are required while tDCS can be used when targets are wider cortical areas and even small currents are effective.

Study limits

Knowing that all search engines would need to be included in a systematic study, we conducted a quantitative review instead. However, as the PubMed repository is the largest collection of biomedical and life sciences journal literature, we chose to limit our search to it and assumed that it would contain all of the publications on RCTs. Notably, in addition to the domains represented in PubMed of the covered scientific subjects, additional sources like WebOfScience and Scopus frequently provide abstracts and conference communications.

Three experts with diverse backgrounds meticulously evaluated the quality of all RCTs returned by the query, validating each GRADE criterion that defines Class 1 research (stated in Table 2) (Biostatistician, Psychologist, Philosopher with a Neuroscience PhD).

Conclusions

Our investigation demonstrates that there is an increasing number of RCTs using tDCS, and that at least some of them may be categorized as Class 1, confirming strong reliability of the reported therapeutic effectiveness in diseases associated with imbalances in neuronal activity. For depression and MS fatigue, our quantitative review indicates a treatment recommendation based on the GRADE classification criteria between moderate and high (Figure 1). This suggests that tDCS treatment is a promising tool in these cases, and we also provided the treatment parameters within the PICO model. For future research designs where the Sham effect is known a-priori without carrying out experiments, we also performed a meta-analysis of the Sham effect magnitude and gave the resulting sample size quantifications.

Further research is required to strengthen the tDCS treatment GRADE recommendation by increasing the samples and opening the door to the intervention personalization.

Appendix

Table 14 Evidence classification scheme for a therapeutic intervention (from Brainin et al. 2004)

Class I: An adequately powered prospective, randomized, controlled clinical trial with masked outcome assessment in a representative population or an adequately powered systematic review of prospective randomized controlled clinical trials with masked outcome assessment in representative populations. The following are required: (a) randomization concealment (b) primary outcome(s) is/are clearly defined (c) exclusion/inclusion criteria are clearly defined (d) adequate accounting for dropouts and crossovers with numbers sufficiently low to have minimal potential for bias (e) relevant baseline characteristics are presented and substantially equivalent among treatment groups or there is appropriate statistical adjustment for differences

Class II: Prospective matched-group cohort study in a representative population with masked outcome assessment that meets a–e above or a randomized, controlled trial in a representative population that lacks one criteria a–e
Class III: All other controlled trials (including well-defined natural history controls or patients serving as own controls) in a representative population, where outcome assessment is independent of patient treatment
Class IV: Evidence from uncontrolled studies, case series, case reports, or expert opinion Rating of recommendations

The table reports the classification of RCTs by Brainin et al. 2004[23a].

Authors' contributions statement

FT conceptualized the study; EG and MB performed the data collection and curation; IS and PP performed the quantitative analysis and contributed to data visualization. EG, MB and FT contributed to the writing of the original draft. LP contributed to the reviewing and editing of the manuscript. FT and PP supervised the writing and revised it critically. All authors reviewed the draft and approved the final version of the manuscript.

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4.3 Disseminating our results: the press release

Following the publication of the paper I presented in paragraph 4.2, we wrote a press release for CNR for disseminating our results. This was an important step in my pathway that allowed me to acquire new skills in to make our results known to a wide audience with an easy and understandable language, but at the same time of impact. I report it here in both its italian original version and english translation.

Italian version

Neuromodulazione per contrastare la fatica nella sclerosi multipla*

Questa tecnica di stimolazione cerebrale transcranica, sperimentata da un gruppo di ricercatori dell'Istituto di scienze e tecnologie della cognizione del Cnr sul sintomo dell'affaticamento nelle persone affette da Sclerosi Multipla, ha mostrato un grado di affidabilità tra alto e moderato. *La ricerca è stata pubblicata sulla rivista* Scientific Reports *del gruppo Nature*

Mentre gli esseri umani per comunicare si scambiano parole, i neuroni del loro cervello, la cui unica funzione è comunicare, ricorrono invece a segnali elettrici. In alcune patologie neurologiche (sclerosi multipla), psichiatriche (depressione) o neuropsicologiche (dipendenze) i meccanismi di trasmissioni di questi segnali però si alterano e in questi casi può essere utile ricorrere all'elettroceutica, la cura con segnali elettrici. Questa terapia ha un vasto campo di applicazioni che va dai pacemakers cardiaci alla stimolazione cerebrale sia profonda (Dbs-Deep Brain Stimulation) che non-invasiva, utilizzando due tecniche transcraniche: la stimolazione magnetica transcranica ripetitiva (rTMS) e la stimolazione in corrente continua (tDCS). Proprio la tDCS si sta rivelando utile per alleviare il sintomo

dell'affaticamento in pazienti affetti da sclerosi multipla. La ricerca, condotta da un team dell'Istituto di scienze e tecnologie della cognizione del Consiglio nazionale delle ricerche (Cnr-Istc), è stata pubblicata su *Scientific Reports*.

"Applicando una debole corrente elettrica (1-2 milliampere) tramite elettrodi posti sui capelli, la tDCS sembra risultare efficace nella cura di sintomi legati a scompensi dell'attività elettrica cerebrale", spiega Franca Tecchio del Laboratorio di elettrofisiologia per la neuroscienza transazionale (Let's) del Cnr-Istc. "Attraverso una revisione della letteratura, applicando le linee guida internazionali della medicina basata sull'evidenza (EBM-Evidence based medicine) che forniscono modelli e criteri di adeguatezza delle procedure diagnostiche e mediche, un team di neuroscienziati del Let's Cnr, in collaborazione con statistici biomedici della Fondazione Fatebenefratelli per la Ricerca e dell'Università Sapienza, hanno stabilito che interventi con tecnologia tDCS sono raccomandabili a livello tra moderato e alto per curare la fatica nella sclerosi multipla".

Nel caso del contrasto alla fatica, la tDCS efficace è stata messa a punto da Let's Cnr con un trattamento personalizzato chiamato Faremus. "Il miglioramento indotto è del 30-40% in media rispetto al livello di partenza, con grande variabilità da persona a persona: delle 35 persone con sclerosi multipla trattate, 26 sono migliorate più del 20% rispetto al livello di fatica prima del trattamento (responders), nessuna è peggiorata e in chi ha risposto la durata dei benefici del trattamento Faremus, eseguito un quarto d'ora al giorno per cinque giorni, è stata di 2-3 mesi. I miglioramenti del sintomo fatica sono stati molto simili per il trattamento Faremus effettuato in clinica o a casa dei pazienti, col solo aiuto di un famigliare".

I dati emersi dalla ricerca hanno messo in luce che la tDCS, oltre a essere un valido alleato nel trattamento dei sintomi di alcune patologie quali la fatica nella sclerosi multipla, è utile anche nella depressione e nel dolore cronico quando la cura farmacologica si dimostra inefficace.

La scheda

Chi: Laboratorio di elettrofisiologia per la neuroscienza transazionale dell'Istituto di scienze e tecnologie della cognizione del Cnr

Che cosa: Una review sugli studi controllati randomizzati che usano la tDCS come trattamento per alcune patologie neurologiche e psichiatriche :

Gianni E, Bertoli M, Simonelli I, Paulon L, Tecchio F, Pasqualetti P. tDCS randomized controlled trials in no-structural diseases: a quantitative review. Sci Rep. 2021; 11(1):16311.

DOI: https://doi.org/10.1038/s41598-021-95084-6

Faremus applicato a casa dei pazienti:

Tecchio F, Cancelli A, Pizzichino A, L'Abbate T, Gianni E, Bertoli M, Paulon L, Zannino S, Giordani A, Lupoi D, Pasqualetti P, Mirabella M, Filippi MM. Home treatment against fatigue in multiple sclerosis by a personalized, bilateral whole-body somatosensory cortex stimulation. Mult Scler Relat Disord. 2022;63:103813.

doi: 10.1016/j.msard.2022.103813

*This manuscript is to be published in CNR (Consiglio Nazionale delle Ricerche) website.

English translation

Neuromodulation to counteract fatigue in multiple sclerosis

This transcranial brain stimulation technique, tested by a group of researchers from the Institute of Cognition, Science and Technology at the CNR on the symptom of fatigue in people with Multiple Sclerosis (MS), showed a high to moderate degree of reliability. The research was published in the journal Scientific Reports of the Nature Group

While humans exchange words to communicate, the neurons in their brains, whose sole function is to communicate, resort instead to electrical signals. In some neurological (multiple sclerosis), psychiatric (depression) or neuropsychological (addictions) diseases,

however, the mechanisms of transmission of these signals are altered, and in these cases, it may be useful to resort to electroceuticals, the treatment with electrical signals. This therapy has a wide range of applications from cardiac pacemakers to both deep (DBS-Deep Brain Stimulation) and non-invasive brain stimulation using two transcranial techniques: repetitive transcranial magnetic stimulation (rTMS) and direct current stimulation (tDCS). It is precisely tDCS that is proving useful in relieving the symptom of fatigue in patients with multiple sclerosis. The research, conducted by a team from the Institute of Cognition Science and Technology of the National Research Council (CNR-Istc), was published in Scientific Reports.

"By applying a weak electric current (1-2 milliamps) via electrodes placed on the hair, tDCS appears to be effective in treating symptoms related to imbalances in brain electrical activity," explains Franca Tecchio of the Laboratory of Electrophysiology for Transactional Neuroscience (Let's) at CNR-Istc. "Through a review of the literature, applying international guidelines of evidence-based medicine (EBM-Evidence based medicine) that provide models and criteria for appropriateness of diagnostic and medical procedures, a team of neuroscientists from Let's Cnr, in collaboration with biomedical statisticians from Fatebenefratelli Foundation for Research and Sapienza University, determined that interventions with tDCS technology are recommendable at a moderate to high level to treat fatigue in multiple sclerosis."

In the case of combating fatigue, effective tDCS was developed by Let's CNR with a personalized treatment called Faremus. "The induced improvement is 30-40% on average from baseline, with great variability from person to person: of the 35 people with multiple sclerosis treated, 26 improved more than 20% from their pre-treatment fatigue level (responders), none worsened, and in those who responded, the duration of benefit from the Faremus treatment, performed a quarter hour a day for five days, was 2-3 months. Improvements in the symptom of fatigue were very similar for Faremus treatment performed in the clinic or at the patients' home, with only the help of a family member."

The data from the research highlighted that tDCS, in addition to being a valuable ally in treating symptoms of some conditions such as fatigue in multiple sclerosis, is also useful in depression and chronic pain when pharmacological treatment proves ineffective.

Tab

Who: Laboratory of Electrophysiology for Transactional Neuroscience of the Institute of Cognitive Science and Technology, CNR

What: A review on randomized controlled trials using tDCS as a treatment for some neurological and psychiatric disorders

Gianni E, Bertoli M, Simonelli I, Paulon L, Tecchio F, Pasqualetti P. tDCS randomized controlled trials in no-structural diseases: a quantitative review. Sci Rep. 2021; 11(1):16311.

DOI: https://doi.org/10.1038/s41598-021-95084-6

Faremus applied at patients' home:

Tecchio F, Cancelli A, Pizzichino A, L'Abbate T, Gianni E, Bertoli M, Paulon L, Zannino S, Giordani A, Lupoi D, Pasqualetti P, Mirabella M, Filippi MM. Home treatment against fatigue in multiple sclerosis by a personalized, bilateral whole-body somatosensory cortex stimulation. Mult Scler Relat Disord. 2022;63:103813.

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4.4 A customized, bilateral whole-body somatosensory cortex stimulation used at home to combat fatigue in people with multiple sclerosis*

The following authors contributed to the final and published version of this work*:
For details see author contribution statement at the end of the manuscript:

Tecchio Franca[1*], Cancelli Andrea[1], Pizzichino Arianna[2], L'Abbate Teresa[1], **Gianni Eugenia[1,3],** Bertoli Massimo[1,4], Paulon Luca[1,5], Zannino Silvana[2], Giordani Alessandro[1], Lupoi Domenico[2], Pasqualetti Patrizio[6], Mirabella Massimiliano[7], Filippi Maria Maddalena[2]

[1] Laboratory of Electrophysiology for Translational neuroscience (LET'S) – ISTC – CNR, Rome, Italy

[2] Neuroscience and Imaging, Fatebenefratelli Hospital, Isola Tiberina, Rome, Italy

[3] Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma

[4] Department of Neuroscience, Imaging and Clinical Sciences, University 'G. D'Annunzio' of Chieti-Pescara, Chieti, Italy

[5] WebMonks S.R.L, Rome, Italy

[6] Department of Public Health and Infectious Diseases, Section of Medical Statistics, Sapienza Università di Roma, Rome, Italy

[7] Department of Geriatrics, Neurosciences & Orthopedics, Institute of Neurology, Catholic University of the Sacred Heart, Fondazione Policlinico A. Gemelli, Rome, Italy

This paragraph contains an extract reformulated by Eugenia Gianni of an article published in Multiple Sclerosis Related Disorders in 2022.

Tecchio F, Cancelli A, Pizzichino A, L'Abbate T, Gianni E, Bertoli M, Paulon L, Zannino S, Giordani A, Lupoi D, Pasqualetti P. Home treatment against fatigue in multiple sclerosis by a personalized, bilateral whole-body somatosensory cortex stimulation. Multiple Sclerosis and Related Disorders. 2022 Jul 1;63:103813.

1. *Introduction*

The clinical neuroscientific community was induced by the various benefits of tDCS neuromodulation technology, which is simple to use, adaptable, and without side effects (Antal et al., 2017; Lefaucheur et al., 2017), to remotely controlled and supervised tDCS application at home (Palm et al., 2018). Guidelines for home tDCS therapy have just been published thanks to long-term experience gained in the field (Charvet et al., 2020). Our plan combines the Faremus tailored treatment's therapeutic efficacy with the chance to provide it in a home environment.

In this study, we seek to evaluate the practicality, safety, acceptability among patients, and effectiveness of the Faremus treatment at Home (FaremusH).

2. *Materials and methods*

2.1. Participants

We included MS patients who met the diagnostic criteria established in (Polman et al., 2011). Self-reported Fatigue (modified Fatigue Impact Scale, mFIS ≥ 35) was the inclusion criterion. Patients reporting the following conditions were excluded from the study: (a) clinical relapse or radiological evidence of disease activity for the previous three months; (b) assumption of symptomatic medications for fatigue (suspended for at least three months prior to inclusion in the study) and depression; (c) epilepsy or other comorbidities of the central or peripheral nervous system; and (d) systemic conditions that may cause fatigue—assessed by clinical examination and history collection: anemia, pregnancy, infectious diseases, hypo or hyperthyroidism, cardiovascular disease, pulmonary disease, renal disease, and hepatic disease.

In order to avoid clinical severity confounding the fatigue assessment and contrasting the symptom in those who could benefit the most from the amelioration as fatigue was the most invalidating condition, we applied the EDSS<3 inclusion criterion in investigation studies on MS fatigue mechanisms (Buyukturkoglu et al., 2017; Cogliati Dezza

et al., 2015; Tecchio et al., 2015; Tomasevic et al., 2013; Vecchio et al 2017). Here, we considered the possibility that home therapy would be helpful even for patients with more severe clinical problems, thus we did not include the criterion.

MS patients were registered at the Fatebenefratelli 'San Giovanni Calibita' hospital in Rome, where they were treated and followed up in the MS Unit, a division of the Neuroscience Department.

All patients had brain magnetic resonance imaging screening. The MRI data collection was utilized to shape the S1 customized electrode (Cancelli et al., 2018a).

2.2. Study design

Given that a 5-day intervention did not significantly interfere with potential activities, which should instead be continued, the Faremus treatment was suggested without changing current therapies (unless fatigue-related) or rehabilitation programs. To assess the outcomes of prospective future research involving larger populations, every day of Faremus treatment we asked within the Case Report Form (CRF) about 'Today had you physiotherapy (0,0.5, 1,2 h) ____, sport (0,0.5,1,2 h) ____, walking (0,0.5,1,2 h) ___'.

A randomized double-blind cross-over trial with the same sample size and design as in (Cancelli et al., 2018b) was what was intended to be done. So, in September 2016, the recruiting process began, randomly assigning half of the initial patients to Real stimulation and the other half to Sham stimulation. However, soon after the trial started, a series of significant logistical changes at the leader laboratory prevented it from being completed as intended after the first 6 patients. After that, despite receiving the identical Informed Consent document at recruitment and having the option of either Real or Sham stimulation, all patients got Real stimulation. We kept the goal of evaluating the effectiveness of the Faremus therapy used at home even when a reliable Sham control group was not present, as was the case in the original research goals. In other words, we kept the post-treatment decrease in mFIS as our primary outcome. A quantitative evaluation of the tDCS randomized controlled trials (RCTs) in clinical conditions, such as

fatigue in MS, has just been published, deepening the estimation of the Sham effect (Gianni et al., 2021). In this case, we took use of the meta-analysis suggestion and compared it to the scenario without Sham treatment because we were aware of the limitations, we had in the research execution due to the absence of internal control. The sample size of 14 offers an 80% probability (power) of identifying as statistically significant (at two-sided alpha level set at 0.05) a mFIS relative change after Real stimulation at the level consistent with previous observations (sES = 1.1 (Cancelli et al. 2018 b; Tecchio et al. 2015)) based on the estimation of Sham effect on fatigue (standardized Effect Size, sES=0.27).

Figure 1. Study design

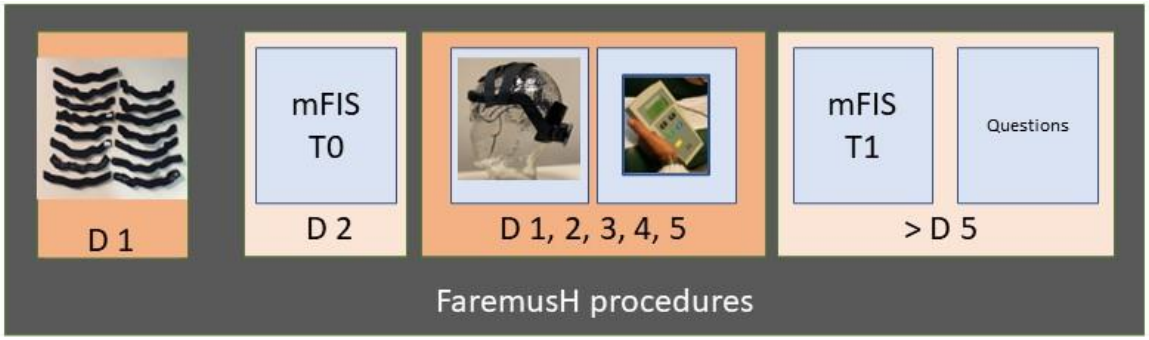


Fig. 1 On Day 1 following recruiting, we prepared each patient's regional customized electrode using an automated process (even from our home of lab, yellow code). The level of weariness was then measured on Day 02, the day the patient (green code) arrived at the clinic to get the stimulator and instructions. After smearing the gel on the electrodes and carefully mounting the adjustable helmet frame in reference to the locations of the nasion and the top edge of the ears, she or he performed the tDCS stimulation at home (orange code) for 5 consecutive days. The feasibility, safety, and acceptability questionnaires were also gathered when she or he returned to the clinics to return the stimulator.

Feasibility of the treatment was evaluated by confirming that less than 10% of research participants dropped out. For each item with a score between 1 and 10, safety was determined by checking for any skin itchiness or redness; side effects were tracked by checking for discomfort during stimulation or in the hours and days that followed; and personal acceptance by filling out a report detailing how each person felt about the 5-day

treatment, including the burden (heaviness) and level of discomfort during stimulations (nuisance).

Additionally, three years later, we gathered patient memories of the actual Faremus -home (FaremusH) therapy. The technician (AP) who trained the patients and provided them with assistance during the FaremusH therapy, made telephone calls to each of them and completed the ad-hoc questionnaire that we created. The patient's recall of having completed Faremus at home was graded on a scale of 1 to 10 by AP, along with whether or not that recollection was favorable. Thereafter, AP asked the following three questions: did you have any difficulty organizing the treatment at home? Would you like to use this fatigue treatment again? Do you think neuromodulation techniques are safe?

2.3. Experimental protocol

Following patient enrollment, we gathered the Beck Depression Inventory (BDI), the Expanded Disability Status Scale (EDSS), and a thorough clinical history (Table 1).

Table 1. MS patient demographic and clinical profile

	Sex	Age	DisDur	ARR	EDSS	BDI
Mean/*Median*	9F/6M	40.5	9.5	0.5	*1.5*	11.4
SD/(Range]		13.1	7.3	0.71	(0, 7]	6.4

M=male, F=female; Mean or *Median* in italics and standard deviations (SD) or range in squared parentheses of: DisDur=disease duration; ARR= annual relapse rate; Scores of: EDSS=Expanded Disability Status Scale, BDI=Beck Depression Inventory.

We used the computational method described in (Cancelli et al., 2018a) to design the bilateral whole body S1 electrode as a 2-cm-wide band along the central sulcus trace using clinical brain MR images (1.5 Tesla, Philips Medical Systems, Best, The Netherlands). Notably, the unique electrode form enabled delineating a significant portion of the central sulcus cortical folding with an electrode area set to 35 cm^2, which is the standard size for

tDCS electrodes. The personalized S1 served as the anode, and the cathode electrode (7 x 10 cm2) was placed on the EEG 10-20 system's Oz location with its longer side pointing left to right.

2.4. RePE electrode position and AHF setting in clinical environment for home treatment

The frontal edge of the customized electrode was placed 1.5 mm posterior to the central sulcus. We developed the appropriate protocol for precise and repeatable electrode positioning at home in accordance with the worldwide recommendations (Charvet et al., 2020). When patients arrived at the hospital, a technician placed the customized electrode based on anatomical landmarks obtained from each patient's 3D model of their scalp. The two electrodes were securely kept in place during each stimulation by an ad-hoc adaptable helmet frame (AHF), which also enabled a simple and repeatable adjustment throughout the course of the following days. With the assistance of a non-expert familiar caregiver, the neurophysiopathology technician demonstrated how to apply the gel to the electrodes and place the AHF utilizing nasion and periauricular references for exact repositioning at the patients' homes (Cancelli et al., 2018a). Before giving the patient and caregiver a manual to use as a reference at home and her phone number as well as the numbers of the other two experimenters (AC and FT), the committed technician also demonstrated how to turn on and off the tDCS stimulator (Fig. 2).

2.5. AHF-equipped tDCS at home in asynchronous modality

We supported the treatment through written instructions and the ability to contact the technician or experimenters during the stimulations in accordance with prior trials, where tDCS applied at home appeared to be acceptable and safe if provided with adequate preparation and monitoring (Sandran et al., 2019). We did this because for treatments of short duration (5 days) in people with MS who have minimal disability or who are supported by their caregiver, real-time videoconferencing was not required.

The device was adjusted for remote usage, only delivering one current supply per day, and setting stimulation duration and strength by fixed preset settings. It also included a suitable recording mechanism to remember when the stimulations were really done.

For five days in a row, people performed the tDCS at the same time of day. Through electrodes connected to an electrical stimulator, tDCS was administered (BrainSTIM, EMS srl, Bologna, Italy; Fig. 1). For five days in a row, a steady 1.5 mA intensity current was used for 15 minutes each day (Cancelli et al., 2018b; Tecchio et al., 2014).

Figure 2 Adaptable Helmet Frame for Faremus treatment at home

Fig. 2 The adaptable helmet consists of a simple customizable perimeter that can be applied with extreme precision to the tops of the ears and the base of the nose. The occipital cathode is placed in the posterior while Velcro bands are employed to hold the anode electrode on the S1 area. ***(Figure and caption reformulated from Tecchio et al. 2022).***

2.6. Statistical analysis

Setting responders to tDCS treatments as those changing more than 20% of the pre-treatment value (Ferrucci et al., 2014; López-Alonso et al., 2015; Saiote et al., 2014), we evaluated the effects of the treatment on fatigue in terms of two-tailed paired-sample t-test change post- vs. pre- Faremus at home.

We evaluated the Real treatment's effectiveness as measured by the effect size (ES), which was calculated using Cohen's d coefficient (Cohen, 1988). A ≤ 0.2 denotes a minor ES, a ≤ 0.5 a medium ES, and a ≤ 0.8 a significant ES.

3. *Results*

The MS patients showed only minimal depression, which was consistent with the inclusion criteria (Table 1). The mFIS did not correlate with any clinical parameter ($p>.200$ consistently for mFIS with EDSS, BDI, Disease Duration, and yearly recurrence rate.

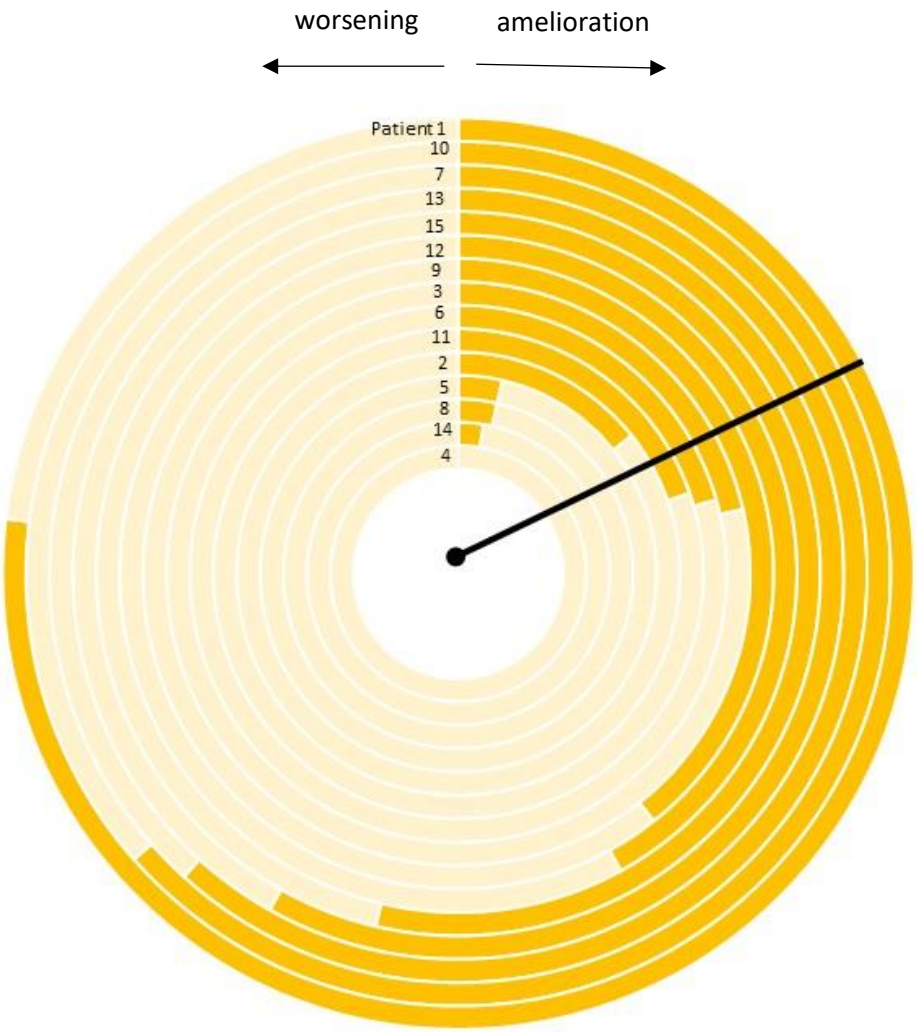
The mFIS score distributions did not deviate from a Gaussian distribution, according to the Shapiro-Wilk test ($p>.500$). The results of the two-tailed paired-sample t-test comparing the baseline and post-Faremus at home mFIS scores were $t(14) = 4.717$, $p=.0003$ (Table 2, Fig. 3 left). The average reduction in fatigue symptoms was 36% from pre-treatment levels, with a wide range between 0% and 86%. We identified 10 out of 15 patients as Responders, defined as a change in fatigue level higher than or equal to 20% of baseline (Fig. 3). Patient P6, a Responder with extremely dense, thick, and wavy hair, was able to utilize the device successfully.

Notably, three of the five non-responders (P4, P5, P8) had wire connection-related technical issues that were compatible with the prototype device we employed.

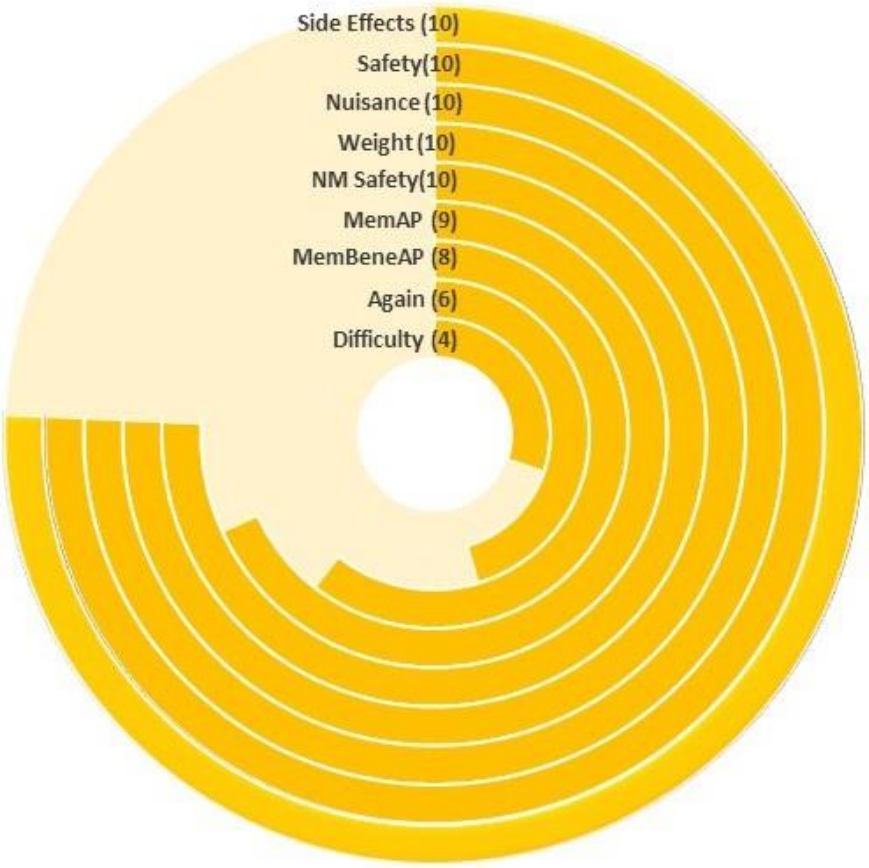
Since Sawilowsky's (Sawilowsky, 2009) threshold for an effect size is 1.2, the Cohen's d coefficient used to evaluate the Effect Size (ES) of Faremus therapy at home yielded a result of 1.21, suggesting an effect size classed as very large.

Since none of the 15 patients dropped out of the study, this indicates good treatment feasibility. All 15 patients completed the home stimulation. Patients carried out the procedures during the intervention based on the manual we gave them, and the explanations given by AP at the clinics; only one patient used AP's phone number owing to issues commencing the stimulation, which were resolved with AP's assistance. At the conclusion of the five-day Faremus home treatment, patients rated the difficulty of carrying it out on average as 1 out of 10 (maximum 8, minimum 0), and as 0 bothersome out of 10 (maximum 6, minimum 0). (Table 3, Fig. 3 bottom).

Figure 3 Faremus-H efficacy and perception



A. EFFICACY



B. PERCEPTION

Fig.3 A Effectiveness of the Faremus-H is measured as a percentage change from the initial mFIS value. The black radius denotes the 20% responsiveness level. Notably, none of the patients had worsening fatigue after receiving Faremus-H.

Fig. 3 B In order to accurately portray Faremus-H perception, we opted to invert all scores to a value of 10 with regard to the score provided in response to our questions. Subjects' scoring for treatment: Side Effects, Safety, Niusance, Weight, Safety with the treatment ongoing and inquired by the technician 3 years later (MN Safety), Memory of the treatment after 3 years (MemAP); Fine memory after 3 years (MemBeneAP); wish to repeat it (Again); difficulty in applying the treatment (difficulty). Figure and captions reformulated from Tecchio et al. 2022.

After around 3 years from the treatment (Table 3, Fig. 3 right), when AP phoned the Patients on the phone to inquire, she thought they remembered it very well because the median Memory score was 9, and they still had a favorable view of it (8). Patients contemplated continuing the therapy (6) even though they thought the procedures weren't particularly simple (Difficulty 6). Years later, they recalled that the effects wore off quickly following the course of therapy (2). Nobody had any negative side effects after taking Faremus at home (the highest score in every case was 1), and nearly everyone retained in memory a belief in the safety of neuromodulation procedures (10) (Fig. 3, right).

Table 2. Fatigue levels and Faremus treatment

	mFIS		
	pre	post	change (%)
P1	37	5	**86**
P2	56	47	16
P3	38	29	**24**
P4	40	40	0
P5	50	48	4
P6	43	33	**23**
P7	65	20	**69**
P8	55	53	4
P9	59	33	**44**
P10	45	13	**71**
P11	77	60	**22**

P12	47	25	**47**
P13	63	22	**65**
P14	66	64	3
P15	35	14	**60**
Mean/*Median*	***51,6***	***33,7***	***35,9***
SD/*(Range]*	***12,5***	***17,9***	***(0, 86]***

Fatigue levels as scored by mFIS before (pre) and after (post) Faremus-H and percentage change with respect to the pretreatment estimated as (mFISpre-mFISpost)/mFISpre. In the last two rows mean or median and standard deviations or range across the 15 patients.

Table 3 Individual evaluation of Faremus at home

	Perception during treatment		
	median	**min**	**max**
Safety	10	10	10
Heaviness	0,5	0	8
Nuisance	0	0	6
Side Effects	1	1	1
	Perception at long-term		
	median	**min**	**max**

Memory - AP	9	6	10
Pleasant memory - AP	8	5	10
Difficulty	6	1	10
Again	6	1	10
NM safety	10	8	10

Median, minimum and maximum of the score ranging from 1 to 10 of the 7-dimension vector about the long-term memory patients had of Faremus performed at their home. The technician who assisted patients in the home treatment called them by telephone about 3 years later. We report the perception that AP had of how much the patient remember to have done Faremus at home (Memory), and if that memory was positive (Pleasant memory). Thereafter, AP asked the following five questions and the patient gave the score: Did you have any difficulty organizing the treatment at home (Difficulty)? Would you like to use this fatigue treatment again (Again)? Do you remember that the effect of the treatment lasted a long time (Duration)? Were there any side effects in the weeks following the stimulation (Side Effects)? Do you think neuromodulation techniques are safe (NM safety)?

4. *Discussion*

In this study, we evaluated the viability, acceptability, safety, and effectiveness of FaremusH, a Faremus treatment that is administered at home. Here we will detail each of these issues in accordance with the crucial procedures connected to the caliber of help for home tDCS as it appears in international guidelines (Charvet et al., 2020).

No matter how severe their clinical condition, MS patients respond well to a 5-day bilateral S1 anodal TDC stimulation delivered via a customized electrode created from an MRI and positioned in an adaptive helmet frame that allows for exact placement. FaremusH is also a successful therapy that is acceptable for use at home, according to research into safety and usability issues as well as consistent indications of efficacy equivalent to that gained in clinical settings.

4.1 Viability/feasibility

All of the patients were successful in ending up the therapy, which was the feasibility goal of the current study.

In contrast to the two RCTs that helped setting up the Faremus therapy, which only included patients with minimum clinical severity (EDSS 3.5), we chose to include patients with a wider variety of clinical symptoms in the current investigation. This deliberate decision was taken to test the therapy on patients who, because of their limitations, may most benefit from a home-delivered modality, arguing that FaremusH treatment is feasible independently of disability-related difficulties. Three of the four patients with an EDSS between 4.5 and 7 were responders, and we discovered that all patients finished the course of therapy.

4.2 Safety

The equipment and the setup and administration of the treatment have both been done in accordance with international standards, enabling for the monitoring of tDCS protocol adherence.

We took care to evaluate the treatment safety using ad-hoc queries inside the dedicated CRF in accordance with worldwide regulatory guidelines (Fertonani et al., 2015). The safety was praised by all of the patients, who all expressed minimal discomfort either during or after the procedure.

In our current study, the technician trained patients to properly set up the device and was available to the patient if needed during the course of treatment, in addition to the written instruction manual that was coherent with the most recent data regarding best practices in the use of devices. The technician was quite familiar to the patients as part of the MS-unit caring for their clinical history (Sandran et al., 2019).

4.3 Acceptability

Along with determining a new therapy framework's efficacy, it's important to gauge how practical and comfortable it is for patients during the entire process.

The nine dimensions we employed represented a range of personal perceptions, both throughout the course of therapy and at a point far enough in the future to allow for evaluation of Faremus's memory.

Patients stated that they had completed the treatments without difficulty or bother at the end of the five-day course of therapy, providing evidence that the process may be successfully repeated at home without putting undue strain on the patients' capacity to handle it. Since the procedure's favorable perceptions continued over time, neuromodulation therapy was viewed as a non-invasive, secure, and adaptable method. According to our observations, the Faremus effect lasted one to two months in 60% of patients who responded, or in 6 out of 7 patients in Tecchio et al(2014) .'s study and 4 out of 9 patients in Cancelli et al(2014) .'s study (Cancelli et al., 2018b). Because of this, it is crucial to ensure that the therapy can be administered to patients in a safe, convenient, and pleasant manner while still being effective. It is very helpful to have a home-based version because it must be repeated frequently.

4.4. FaremusH effectiveness

Faremus therapy still shows consistent effects when compared to other trials that also try to combat the symptom of fatigue with non-invasive electroceutical therapies like tDCS.

Leight Charvet and her team developed a telerehabilitation protocol that targets MS fatigue through tDCS of the dorsolateral prefrontal cortex (left anode, right cathode) with different block durations (10 or 20-day sessions, 20 min per day) and intensity (1 or 2 mA), whether or not associated with cognitive training. This follows extensive experience in successful home treatments supporting cognitive function in MS patients (Charvet et al., 2018). Only when cognitive training was added to the neuromodulation did positive benefits appear. Frontal tDCS did not lessen fatigue in its absence. The FaremusH therapy, on the other hand, which is intended to be administered while the patient is at rest and relaxed, showed effectiveness superior to frontal tDCS coupled with cognitive training with a Cohen's d = 1.21 vs. 0.71 of Charvet et al (Charvet et al., 2018). Notably, compared to the

cognitive combined frontal tDCS, Faremus provides half the superficial current density (0.04 vs. 0.08 mA/cm2) for a fourth the time (5 vs. 20 days). Faremus demonstrated meaningful efficacy even when used in a clinical setting, with d = 1.0 (Tecchio et al., 2015) and 1.3 (Cancelli et al., 2018b), respectively. In terms of responsiveness, we saw 5/13 (36%) responders after the combined frontal tDCS, compared to 7/10 (70%) and 9/10 (90%) and 10/15 (67%) responders. These findings support the Faremus settings as the most effective ones currently available for treating MS fatigue.

However, it is conceivable to think that tactics combining neuromodulation with other behavioral states, such as listening to music or reading a book of the patient's choice, may be used to increase the effectiveness of the Faremus intervention by fostering a more responsive patient state (Carvalho et al., 2020).

4.5. Electronic mFIS

In the clinic, patients completed an electronic questionnaire to score the mFIS. By visiting the ad-hoc created website from home, the patient outside of Rome was able to return the Faremus device without wasting time or energy going to the Hospital and score the mFIS.

The information is gathered in a unique semi-anonymous database. This functionality is extremely helpful when providing the therapy at several locations.

4.6. Therapeutic continuity

With cheap costs, user-friendliness, safety, and tolerability, as well as promising outcomes in treating debilitating symptoms like MS fatigue, tDCS is an appropriate technology to develop a patient-centered treatment paradigm that is simple to use at home. The stress of traveling to clinical institutions for repeated therapy sessions is reduced with home-delivered tDCS therapies like FaremusH. This is especially true for patients who have severe physical or mental disabilities or when the health care system is under stress from a crisis like the Covid-19 outbreak (Bikson et al., 2020), as it provides a useful tool to establish and maintain treatment continuity.

4.7. Limitations of the present research

4.7.1. Prototypical system

One of the drawbacks of the current study is the use of the AHF at a prototype level, despite the use of a high-quality equipment approved for home treatments. Indeed, three of the five patients who were labeled as non-responders experienced technological difficulties.

4.7.2. No telemedicine devices

Patients were simply given the experimental research referents' phone number and a booklet that contained instructions and commonly asked questions in order to complete the home therapy. Only one patient indicated a need to speak with the technician, thus this guidebook appears to be comprehensive (AP). We are aware of the importance of developing complete telemedicine systems to support communication between patients and clinicians as well as between patients themselves. Although the execution of the treatment was initially satisfactorily supported by the training received in the clinic and later by the instruction manual at home.

5. *Conclusions*

A user-friendly, widely approved treatment against MS-fatigue is offered by FaremusH, and it has an effectiveness that is equivalent to that of the application in a clinical setting.

Authorship contribution statement

Franca Tecchio: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing – original draft, Supervision, Project administration, Writing – review & editing. Andrea Cancelli: Investigation, Data curation, Writing – review & editing. Arianna Pizzichino: Investigation, Data curation, Writing – review & editing. Teresa L'Abbate: Writing – original draft, Writing – review & editing. Eugenia Gianni: Writing – original draft, Writing – review & editing. Massimo Bertoli: Writing – original draft, Writing – review &

editing. Luca Paulon: Writing – review & editing. Silvana Zannino: Investigation, Data curation, Writing – review & editing. Alessandro Giordani: Software, Methodology, Writing – review & editing. Domenico Lupoi: Writing – review & editing. Patrizio Pasqualetti: Methodology, Writing – review & editing. Massimiliano Mirabella: Investigation, Writing – review & editing. Maria Maddalena Filippi: Investigation, Writing – review & editing.

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Chapter 5: Building up the service and carrying on the engineering of the device

5.1 The meetings with the enterprise Igea

To pursue the objective of developing the therapeutic service we asked the enterprise Igea which funded half of my grant and is specialized in developing electroceutical domiciliary treatments for a set of meetings.

First Meeting – June 2020

During the first meeting, I exposed the advancement of my Phd and set up the basis for a collaboration in developing and commercializing a Faremus-Igea device.

During my presentation I outlined both the results of our studies and the considerations on the market potentialities of the treatment Faremus that prompted us to move enthusiastically in the direction of creating a service.

First, I reminded that the World Economic Forum 2018 listed electroceuticals as one of the Top10 emerging technologies, as a key technology therefore for economic and social development (along with Personalised Medicine and Digital Helpers). I stressed how electroceuticals represents a highly innovative sector with enormous therapeutic potential, on which neuroscience and enterprise interest has recently converged.

I reminded also how, in 2016, Kevin Tracey, a world-renowned neuroscientist who boasts major publications in Nature on the topic of electroceuticals with over 2000 citations and who has been the contact person in the World Economic Forum for electroceuticals, presented a TED talk on the value and medical applicability of electroceuticals, which was highly successful. Where TED - which stands for **t**echnology, **e**ntertainment and **d**esign - refers to a live lecture that is subsequently distributed free online on youtube and whose topic are ideas that are relevant to disseminate. Almost at the same time, our Laboratory developed a TEDx, a European version of the TED, in which the mechanisms and advantages

of the Faremus electroceutical treatment were explained, and it is interesting to see how this, despite being in Italian, reached over 400,000 views, more than ten times the number of views that K. Tracey's TED got. This tells us that the topic of electroceuticals, with its innovative potential as a medical treatment, is attracting strong interest.

Within this framework I talked about our vision and our mission.

In this field of applied research, our mission, moving towards a fatigue-free world, is to build and provide electroceutical treatments to combat chronic fatigue and in this context to foster cooperation with patients suffering from the same clinical condition, with other patients, relatives and friends, and with clinical staff and health professionals.

Within this framework, I proposed our project: the set up of a national service that we have called FaremusS, which also qualifies as a business opportunity. For the building up of this service, we will start from the Faremus treatment developed by our laboratory, whose validity and market potential are based on certain premises: fatigue is a disabling

symptom in people suffering from multiple sclerosis, which has a negative effect on their quality of life, there are no effective pharmaceutical treatments, and the few that exist have strong side effects. The aim is therefore to offer a service against fatigue in MS with an easy, fast and side-effect-free treatment that also has great potential in other areas, as it can be extended to other pathologies besides MS, as fatigue is a common symptom in many diseases as well as in healthy individuals. Indeed, the treatment can be effectively extended, as the evidence shows, to pathologies such as for example depression, tinnitus, addiction (see Chapter 1.2).

Then I shared the features of the device in use by our Laboratory. I reminded how our laboratory has developed the Faremus treatment over the years. This is a 15-minute direct current transcranial stimulation treatment for 5 days against fatigue in multiple sclerosis. The target area for this stimulation is S1: the somatosensory cortex of the whole body which, based on previous literature we have learned is hypo-excitable in fatigue sufferers. For stimulation, our laboratory has developed a customised electrode whose shape is modelled on the circumvolution of S1 of each individual patient derived from the individual MRI and reconstructed by means of the neuronavigator. 2 randomised, crossover and sham-controlled clinical trials have verified the effectiveness of the treatment on patients with multiple sclerosis, finding a lowering of mFIS values after treatment.

Finally, I started outlining the potential economic benefits of the FaremusS. Making a forward-looking assessment of the target market of the FaremusS service, we estimated that this could potentially affect MS sufferers worldwide who are around 2 million, of whom 750.000 in Europe, 122.000 in Europe and, to make examples 11.650 in Lazio and 19.900 in Lombardy.

People with MS (Barometro AISM 2019)

	Total	Women
World	2.000.000	1.400.000
Europe	750.000	500.000
Italy	122.000	80.000
Lazio	11.650	7.750
Lombardy	19.900	13.250

Thus, we have estimated that, starting from the percentage of people with MS in Italy, i.e., 122,000 people, 50% of these may feel fatigued, i.e., 61,000, and the FaremusS service can reach 15% of these, i.e., 9,000. If we estimate that each of these patients receives one treatment per year worth 500 euros, we can see that the service can yield a total income of 4.5 million euros per year.

People with MS

In Italy	100%	122.000
Fatigued	50%	61.000
Reachable by Faremus	15%	9.000

With these considerations in mind, we asked if the Igea company was interested in developing a Generation II stimulator perhaps in collaboration with us.

In response to our question the enterprise Igea asked us to prepare a documentation about Faremus treatment they could reason upon whether to

commercialize it. We prepared a pitch also graphically cured with text and pictures of impact, in which we tried to expose a relevant set of motivations for which we think it is advantageous for the enterprise to invest in the Faremus treatment.

In our pitch (Chapter 4.1) we tell a common story, the story of Maria who, due to MS fatigue, feels tired and cannot pursue the everdyday normal activities up to refuse going out with friends. Since fatigue is the most invalidating synthom in half of the MS population, we estimate that in Italy about 61.000 people feels like Maria. In this context we present our vision that is to fight chronic fatigue and we delineate our mission that is to offer an efficacious, safe, easy and rapid treatment without side effects that can be applied autonomously by the patient even at home. With the same aims we also wish to open a therapeutic service in sinergy with other MS units in Italy, where patients like Maria can benefit from the Faremus treatment.

Second meeting – June 2021

After an attentive evaluation of our pitch the enterprise asked for a second meeting which happened in June 2021. During the meeting the enterprise director asked us for some clarifications on the operational aspects of the initiative of producing the device. First of all, they expressed their interest in developing a market line related to electroceutical applications in the field of neurophysiology and said that our project could fully fit into this line.

Starting from this assumption, they specified that Igea is interested in following not only the production of the device, but also its launch on the market. Therefore, they proposed the creation of a dedicated spin off with the aim of placing the product on the market. The operational step that remained to be thought about is what will be the relationships between CNR and the spin-off company throughout the production and market launch process, so we left ourselves with the idea of finding an answer to this question and organized a new meeting for mid-September.

Third meeting – November 2022

The commercialisation process of our device came to a halt for the following reason: Igea former President informed us that he was no longer the president of Igea and that his role is now honorary president. He said he is interested in the project of opening a business line dedicated to neurology that would be supported by Igea but as an external body. For this he is actively seeking funding. He reiterated his interest in our work and told us that this could be part of the new entrepreneurial line.

He suggested as a first step that we patent the product; to this end, he proposes a meeting with a patent team to be conducted in 2023.

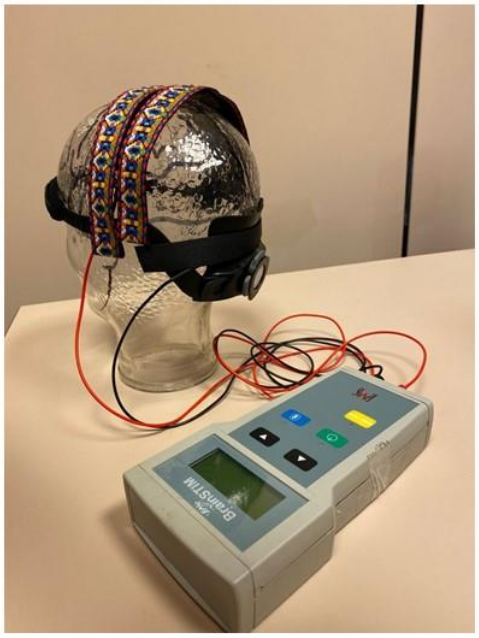
5.2 The Pitch

"The pitch serves as a marketing tool for an innovative company endeavour and must include data that will help advance a business concept. The facts presented during a startup pitch must be backed up by citations, conveyed clearly and completely, and serve as the foundation for determining the viability and potential of the product. The key components of the ideal startup pitch include presenting the problem or need that the company concept seeks to address or satisfy, the solution to that need, the product to that solution ratio, a team and investment research, the aspect connected to rivals, and the target market. Once more, the creation of the pitch requires the development of a business model (how the company concept develops, distributes, and captures value), as well as a roadmap outlining the essential activities and milestones for the development of the business project. To grab the attention of the interlocutor in the normal duration of an elevator journey, a project that may be demonstrated in the period of two to three minutes is known as an "elevator pitch[30]."

In our pitch (an extract of which is attached, English translation follows italian[31]) we tell a common story, the story of Maria who, due to MS fatigue, feels tired and cannot pursue the everdyday normal activities up to refuse going out with friends. Since fatigue is the most invalidating synthom in half of the MS population, we estimate that in Italy about 61.000 people feels like Maria. In this context we present our vision that is to fight chronic fatigue and we delineate our mission that is to offer an efficacious, safe, easy and rapid treatment without side effects that can be applied autonomously by the patient even at home. With the same aims we also wish to open a therapeutic service in sinergy with other MS units in Italy, where patients like Maria can benefit from the Faremus treatment.

[30] https://www.economyup.it/glossario/pitch-definizione/
[31] We decided to report just an extract of our Pitch for confidentiality purposes.

This was an important production for me because I had the opportunity to apprehend how to communicate our projects and results in a manner that is scientific, tough relevant and attractive for the enterprise world.



IGEA vuole, in collaborazione con Let's Cnr, sviluppare un dispositivo elettroceutico domiciliare Faremus contro la fatica nella SM?

A cura di Franca Tecchio, Luca Paulon ed Eugenia Gianni

Sommario

La Storia di Maria

'Pronto, **Maria**, vieni con noi per un giro nel parco?' '**Sono tanto stanca**, magari la prossima volta...', risponde Maria, 26 anni, malata di sclerosi multipla, che ancora prima della diagnosi ha iniziato a sentirsi così stanca, senza motivo... e ora non riesce ad andare al lavoro per colpa della fatica.

Come lei in Italia 60.000 persone con SM sentono la fatica come il sintomo più invalidante. I farmaci che il medico le prescrive, in assenza di indicazione specifica, le creano effetti collaterali come nausea, vomito, giramenti di testa, insonnia, dolori, ansia.

E se esistesse un’altra soluzione?

Il servizio che serve a Maria

Maria può curare la fatica periodicamente, magari a casa, 5 giorni ogni 3-4 mesi grazie al trattamento Faremus[32], che si inscrive in un servizio terapeutico[33] in via di sviluppo.

In ottica partecipativa e costruttiva di Citizen Science, la rete Cnr IGEA garantisce il top in termini di assistenza sanitaria domiciliare, customer care, e ricerca scientifica.

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[32] Specifiche del trattamento Faremus e parametri PICO

Faremus (fatigue relief in multiple sclerosis) **è un trattamento di stimolazione trascranica in corrente continua (tDCS) con intensità di corrente 1.5mA della durata di 15 minuti al giorno per 5 giorni consecutivi.** L'area target di questa stimolazione è standard 35cm^2, raggiunta con un elettrodo di forma personalizzata modellata sul solco centrale derivato dalla risonanza magnetica cerebrale MRI individuale con procedura computerizzata.

Miriamo così a compensare le alterazioni tipiche della fatica SM, inviando la corrente eccitatoria alla corteccia somatosensoriale di tutto il corpo (S1), ipo-eccitabile, minimizzando gli effetti nell'adiacente corteccia motoria (M1), iper-eccitabile.

Il catodo è in posizione occipitale (7x10 cm^2, lato lungo in direzione longitudinale).

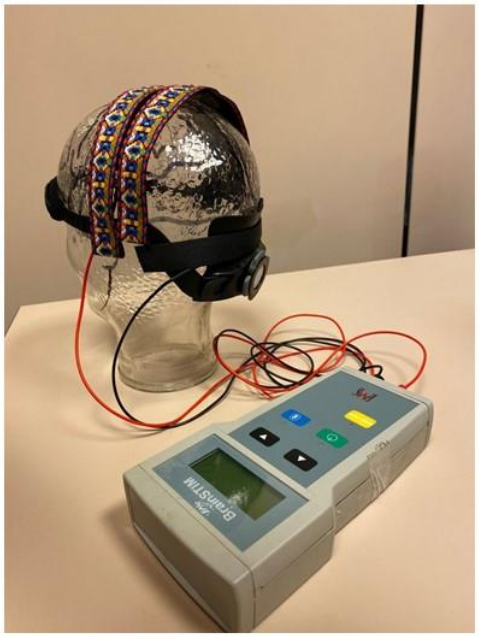
L'attuale stimolatore Faremus comprende: elettrodo personalizzato e referenza occipitale, montati nel caschetto adattabile e connessi con lo stimolatore in corrente a norma CE (E.M.S. Bologna, Italia).

Secondo il modello PICO (popolazione, intervento, confronto, outcome), rivolto a pazienti con SM affaticati, l'intervento Faremus è confrontato con la stimolazione sham e individua come misura di outcome la mFIS (modified fatigue impact scale).

[33] Progetto Let's di un Servizio Elettroceutico personalizzato nazionale contro la fatica nella SM

Il World Economic Forum del 2018 ha indicato l'Elettroceutica, la cura con segnali elettrici, tra le tecnologie Top10 per lo sviluppo economico e sociale del mondo, assieme alla Medicina Personalizzata e i Digital Helpers.

Nel nostro laboratorio Let's (Laboratory of Electrophysiology for Translational neuroScience)-Istc-Cnr implementiamo e sperimentiamo sistemi elettroceutici per la cura personalizzata di sintomi secondari ad alterazioni dell'attività elettrica cerebrale. Con provata efficacia, abbiamo messo a punto Faremus. Forti della sua validità e potenzialità clinica e di mercato, stiamo avanzando sul piano della accessibilità terapeutica effettiva. Miriamo a potenziare qualità ed efficacia della cura costruendo dispositivi ergonomici e strumenti di e-community e Digital Helper, abilitando il paziente ad informarsi, esprimere il proprio consenso, dialogare, condividere i risultati raggiunti e scambiare i dati per la personalizzazione continua così come monitorare le dimensioni espresse dalle linee guida contro la fatica nella SM.



Does IGEA want, in collaboration with Let's CNR, to develop a Faremus home electroceutical device against fatigue in MS?

Cured by Franca Tecchio, Luca Paulon ed Eugenia Gianni

Summary

The history of Maria

‘Hello, Maria, will you come with us for a walk in the park?' 'I'm so tired, maybe next time...', replies Maria, 26, a multiple sclerosis sufferer, who even before her diagnosis started feeling so tired, for no reason... and now cannot go to work because of fatigue.

Like her, 60,000 people with MS in Italy feel fatigue as the most disabling symptom. The drugs that the doctor prescribes to her, in the absence of a specific indication, create side effects such as nausea, vomiting, dizziness, insomnia, pain, anxiety.

What if there was another solution?

The service that Maria needs

Maria can treat fatigue periodically, perhaps at home, 5 days every 3-4 months thanks to the Faremus treatment[34], which is part of a developing therapeutic service[35].

In a participative and constructive Citizen Science perspective, the CNR IGEA network guarantees the top in terms of home healthcare, customer care, and scientific research.

[34] **Faremus treatment specifications and PICO parameters**
Faremus (fatigue relief in multiple sclerosis) is a transcranial direct current stimulation (tDCS) treatment with 1.5mA current intensity lasting 15 minutes per day for 5 consecutive days. The target area of this stimulation is standard 35cm^2, reached with an electrode of customized shape modelled on the central sulcus derived from individual brain MRI with a computerized procedure.
We thus aim to compensate for the alterations typical of MS fatigue by sending the excitatory current to the whole-body somatosensory cortex (S1), which is hypo-excitable, while minimizing the effects in the adjacent motor cortex (M1), which is hyper-excitable.
The cathode is in the occipital position (7x10 cm^2, long side in longitudinal direction).
The current Faremus stimulator comprises customised electrode and occipital reference, mounted in the adaptable helmet and connected to the CE-compliant current stimulator (E.M.S. Bologna, Italy).

[35] **Let's project a national personalised Electroceutical Service against fatigue in MS**
The 2018 World Economic Forum named Electroceuticals, the treatment with electrical signals, among the Top10 technologies for economic and social development in the world, together with Personalized Medicine and Digital Helpers.
In our laboratory Let's (Laboratory of Electrophysiology for Translational neuroScience)-Istc-Cnr we implement and test electroceutical systems for the personalized treatment of symptoms secondary to alterations in electrical brain activity. With proven efficacy, we have developed Faremus. On the strength of its clinical and market validity and potential, we are advancing in terms of effective therapeutic accessibility. We aim to enhance the quality and efficacy of care by building ergonomic devices and e-community and Digital Helper tools, enabling patients to inform themselves, express their consent, dialogue, share their results and exchange data for ongoing personalization as well as monitor the dimensions expressed by the guidelines against fatigue in MS.

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PART III: DEEPENING THE MECHANISMS OF FAREMUS

Chapter 6: Personalization and comprehension of the mechanisms of Faremus and of fatigue generation: current studies and publications

6.1 Introduction: exploring the effects of Faremus on parietal connectivity and motor control

We already largely spoke about the treatment Faremus (Chapter 1, 2, 4, Tecchio et al. 2014; Cancelli et al. 2018). In the two studies we present here our aim was to explore Faremus effects, on other syndromes like depression and on cortico-muscular coherence (a mechanism subtending motor control). In our intentions these studies could, in turn, lead us to a better comprehension of the mechanisms of fatigue generation and of the functioning of the motor and sensorimotor systems inducing a further amelioration of our personalized system Faremus. Moreover, the relevant observation that Faremus normalizes a measure like cortico-muscular coherence (Section 6.3) led us to better explore this measure, to try to unravel its significance as index of the functioning of the motor system and to try overcoming its limitations (Chapter 7).

Section 6.2: Faremus and parietal connectivity

In a study of 2019, Jeager et al. found a pattern of fatigue related parietal dysfunctional connectivity. As a secondary finding, they observed that, as depression symptoms increase, the alteration of the functional connectivity in parietal areas increases. As we said in the Second Chapter a pattern of dysfunctional connectivity in parietal areas is strongly present also in fatigue in MS and we expect Faremus treatment, among other effects, to re-balance this dysfunction.

Performing a preliminary analysis in MS patient who underwent FaReMuS, we observed that the BDI[36] was significantly reduced. In our Letter article, I contributed to writing, we formulate two alternative hypotheses for explaining this effect. Either

[36] BDI stands for Beck Depression Inventory and it is the most common clinically validated scale to measure depression. Beck, A. T., Steer, R. A., & Brown, G. (1996). Beck depression inventory–II. *Psychological assessment*.

depression is a sympthom secondary to fatigue and resolves by fighting fatigue targeting parietal areas, or the parietal alteration plays an important role in depression. If so, it could be relevant to evaluate enriching the protocols that typically aim to balance the excitability of the right vs. left dorsolateral prefrontal regions (Lefaucheur et al. 2017), considering the possible parietal involvement.

Section 6.3: Faremus and cortico-muscular coherence subtending motor control

In a 2021 study (Padalino et al. 2021) I contributed to writing, we tested whether a functional alteration previously observed in multiple sclerosis (MS) fatigue during a simple movement normalizes when fatigue reduces.

In fact, my Lab team previously investigated the handgrip execution in two MS patient groups with low and high fatigue respectively (Tomasevich et al. 2013). The cortico-muscular coherence (CMC), measured through simultaneous electroencephalographic (EEG) and surface electromyographic (EMG) recording was studied. Physiologically, CMC expresses in the beta-band when executing an isometric contraction (Gross et al. 2000). Despite a comparable execution quality of the task, CMC worked at significantly higher frequencies in the group with higher fatigue (27.5 ± 4.8 Hz) than in that with lower fatigue (16.7 ± 3.6 Hz). Moreover, CMC frequency increased along with fatigue.

Here, we tested the working hypothesis that the personalized neuromodulation FaReMuS reverts CMC to lower physiological frequency. Thus, we measured CMC in eleven fatigued patients during a weak handgrip before and after Faremus treatment.

What we found out was that before FaReMuS, the CMC was observed at a high frequency of 31.5 ± 1.6 Hz (gammaband) and positively correlated with the level of fatigue. After FaReMuS the rate of fatigue reduction was 28% ± 33% and the CMC frequency reduced, thus forthcoming the physiological beta band as observed in healthy people. We concluded tha the personalized neuromodulation treatment targeting S1 was able to ameliorate the central-peripheral communication, which subtends simple everyday movements. We believe this finding to strengthen the appropriateness of neuromodulations aiming at increasing the parietal excitability in fighting MS fatigue.

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6.2 Parietal dysfunctional connectivity in depression in multiple sclerosis*

The following authors contributed to the final and published version of this work*:

Franca Tecchio[1] , Massimo Bertoli[1,2] , **Eugenia Gianni[1,3]**, Teresa L'Abbate[1], Elvira Sbragia[4], Silvia Stara[4], Matilde Inglese[4,5]

1. Laboratory of Electrophysiology for Translational neuroScience (LET'S), Istituto di Scienze e Tecnologie della Cognizione (ISTC), Consiglio Nazionale delle Ricerche (CNR), Rome, Italy

2. Department of Imaging and Neuroscience and Clinical Sciences, University 'G. D'Annunzio' of Chieti-Pescara, Chieti, Italy

3. University Campus Bio-Medico of Rome (UCBM), Rome, Italy

4. Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy

5. Ospedale Policlinico San Martino – IRCCS, Genoa, Italy

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For their study, "Multiple sclerosis-related fatigue: Altered resting-state functional connectivity of the ventral striatum and dorsolateral prefrontal cortex," Jeager and colleagues (Jeager et al. 2019) enrolled MS patients with mild clinical severity (Extended Disability Status Scale, EDSS 2.5) and low depression level (assessed by Beck Depression Inventory, BDI). Along with the fatigue-related characteristics, which were in line with the FaReMuS treatment approach, the authors demonstrated that when depressive symptoms worsen, there is an increase in the functional connectivity between the left hemisphere's upper ventral striatum and post-central gyrus.

Performing a preliminary analysis on a group of 14 MS patients with eligibility criteria similar to those of Jeager et al. (MS patients with EDSS <2.5 at low BDI levels), who underwent FaReMuS in parallel 7 Real and 7 Sham, we observed that the BDI was reduced by Real (9 pre vs. 5 post, two-tail t-test for paired samples p = .0005) in the absence of any Sham effect (7 pre vs. 7 post, p = .8902).

According to Jeager's findings and our observation of the FaReMuS effects, this observation leads to two alternative hypotheses: a) the depression profile of these patients—low depressive symptoms and MS diagnosis—is decisive in pointing to parietal involvement; alternatively, the parietal alteration plays a significant role in the most severe depression and is not just secondary to MS. If so, it could be pertinent to think about enhancing the procedures that normally aim to balance the excitability of the right vs. left dorsolateral prefrontal regions (Lefaucheur 2017), taking into consideration the potential parietal involvement.

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6.3 FaReMuS modifies cortico-muscular coherence subtending motor control*

To tthe final and published version of this work contributed the following authors*:
For details see the "author contribution statement" at the end of the manuscript

Matteo Padalino*[1], Carla Scardino*[1-2], Giancarlo Zito[5,6], Andrea Cancelli[1], Carlo Cottone[1], Massimo Bertoli[1-3], Eugenia Gianni[1-4], Teresa L'Abbate[1], Elisabetta Trombetta[1], Camillo Porcaro[1,7,8,9], Fabiano Bini[2], Franco Marinozzi[2], Maria Maddalena Filippi[5], Franca Tecchio[1]

1 LET'S – ISTC – CNR, Rome, Italy;

2 Department of Mechanical and Aerospace Engineering, "Sapienza" University of Rome, Rome, Italy;

[3] Department of Imaging and Neuroscience and Clinical Sciences, University 'G. D'Annunzio' of Chieti-Pescara, Chieti, Italy;

[4] University Campus Biomedico of Rome, Rome, Italy;

[5] 'S. Giovanni Calibita' Fatebenefratelli Hospital, Rome, Italy;

[6] Innovation and Pharmaceutical Strategy Division, Italian Medicines Agency (AIFA), Rome, Italy

[7] Research Center for Motor Control and Neuroplasticity, KU Leuven, Leuven, Belgium

[8] Department of Information Engineering, Università Politecnica delle Marche, Ancona, Italy

[9] S. Anna Institute and Research in Advanced Neurorehabilitation (RAN) Crotone, Italy

* These Authors contributed equally to this work

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Introduction

In the previous Chapter we largely spoken about the functioning of the system Faremus (Chapter 1; Chapter 4, Introduction).

In the present study, we tested whether FaReMuS, which reduces the fatigue levels (Tecchio et al. 2014; Cancelli et al. 2018) and normalizes the dynamics of the S1 and M1 cortical regions (Porcaro et al. 2019), also reverts to normal the cortico-muscular synchronizations underlying the execution of everyday simple movements. Thus, we measured CMC in eleven fatigued patients during a weak handgrip before and after Faremus treatment. In the current study, we only investigated the hypothesis after Real FaReMuS since the two randomized controlled trials (RCTs) (Tecchio et al. 2014; Cancelli et al. 2018) showed that the Real treatment reduced fatigue, but the Sham treatment did not.

Subjects and methods

Subjects

By meeting the following eligibility requirements, we were able to recruit 11 patients with relapsing-remitting MS from the Fatebenefratelli Isola Tiberina Department's MS Center. Inclusion criteria: absence of clinical relapse or radiological evidence of disease activity over the last three months; low clinical disability (Expanded Disability Status Scale, EDSS ≤ 2) and depression (Beck Depression Inventory <15). Exclusion criteria: assumption of symptomatic drugs, which may affect the level of fatigue, depression and anxiety within the past three months; epilepsy or other central/peripheral nervous system comorbidities; any systemic conditions which may cause fatigue (anaemia and pregnancy).

Methodology

The Expanded Disability Status Scale (EDSS), signs of the continuing Disease Modifying Therapy (DMT), and Beck Depression Inventory were all part of the comprehensive clinical history the neurologist gathered (BDI).

Electroencephalography (EEG), surface electromyography (EMG), and the Modified Fatigue Impact Scale (mFIS) were used to measure each participant's degree of fatigue both before and after receiving the FaReMuS therapy (Figure 1). FaReMuS involves five days of anodal tDCS (1.5 mA, 15 minutes each day) applied to the body's somatosensory representation brain regions (S1, 35 cm2). The cathodic electrode was a rectangle of 70 cm2 with Oz as its center. FaReMuS is customized by the choice of the target and the anodal electrode's shape to meet the unique central sulcus cortical folding as determined by the cerebral MRI of every single patient and positioning it 0.5 cm anteriorly and 1.5 cm posteriorly to the central sulcus.

Electrophysiological study

EEG and EMG Data collection Using a 64-channel actiCHamp System (Brain Products GmbH, Munich, Germany) and electrodes positioned in accordance with the 10-10 EEG International System, EEG data were captured. A belly-tendon montage (2.5 cm inter-electrode spacing) was used to record the surface electromyogram (EMG) of the right and left opponens pollicis muscles. We sampled EEG and EMG at 5 kHz (pre-sampling analogical bandpass filtering 0.1–2000 Hz) and collected them for off-line processing.

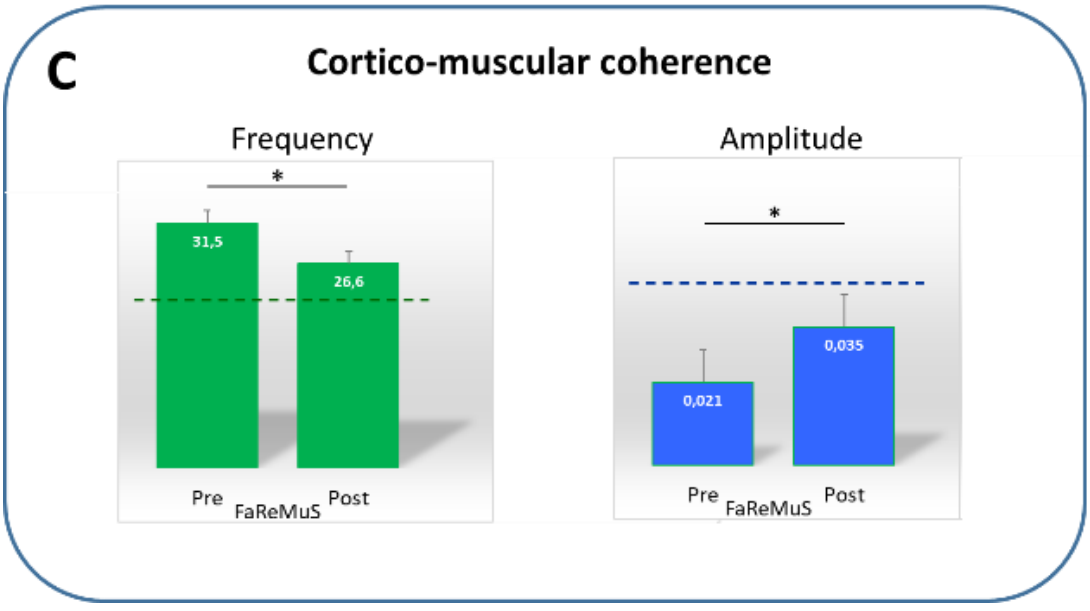
Motor task The participant, who was comfortably sitting on an armchair, was requested to apply intermittent and repetitive self-paced handgrip pressure on a semi-compliant air-bulb against resistance during the EEG-EMG session, while getting continuous visual feedback.

To determine the appropriate degree of contraction to employ for the activity, the exercise was originally done three times for a total of roughly 300 ms at maximum

voluntary contraction (MVC). For the purpose of reducing task-dependent fatigue, the goal threshold was established at 5% MVC. The individual was instructed to begin the 20-second block of the weak isometric handgrip after receiving a visual cue (green screen). Every 10 seconds, a new visual indication (a red screen) signaled the beginning of the relaxation phase. A total of 240 s of contraction was recorded to ensure a minimum of 200 s of artifact-free data. That is, the task lasted about 6 minutes.

CMC measurement The CMC was measured by the amplitude and the frequency of the maximum amplitude peak of the spectral coherence function between the EMGOP and the contralateral bipolar EEG derivation identified as displaying maximal coherence with the muscle activity among the bipolar derivations in fronto-posterior and medio-lateral directions between the channels indicated in Figure 2. CMC as well as Power Spectral Density (PSD, Figure 2) of the EEGSM1 and the EMGOP during right and left hand grip were estimated by Welch procedure with the following parameters: 2048 ms duration, Hanning window, no overlap, number of artifact free trials fixed across patients.

Figure 1. Cortico-muscular coherence frequency and amplitude



Mean and standard deviations (half values in vertical segments) prior to (Pre) and following (Post) FaReMuS therapy against fatigue of the CMC frequency (left, green) and amplitude in multiple sclerosis (right, blue). ***(Figure reformulated from Padalino et al. 2021).***

Statistical analysis

We performed an analysis of variance (ANOVA) on the peak frequency of the CMC using the within-subject variables FaReMuS (Pre, Post FaReMuS), and Body Side (left hemisphere-right hand, right hemisphere-left hand). The FaReMuS effect, which shows that the intervention against fatigue also altered the frequency of cortico-muscular synchronization, was the major focus of our attention. We also looked at how FaReMuS affected ongoing activity in the cortical sensorimotor and muscle systems. Using Pearson's correlation analysis, we examined the correlation between CMC and fatigue levels.

Results

None of the clinical or demographic characteristics and the mFIS before or after FaReMuS were statistically significantly correlated (Table 1, correlation with age, disease duration, yearly relapse rate, EDSS, and BDI: p>.400 consistently).

The CMC estimations were estimated on a number of epochs set to 110 across patients using at least 226 seconds of artefact-free data. The subject-specific confidence limit ranged from 0.004 to 0.01. According to the Shapiro-Wilk test, the distribution of the frequency corresponding to the maximum CMC amplitude (CMC f) in either the pre- or post-FaReMuS hemisphere did not deviate from a Gaussian distribution.

Faremus (Left hemisphere-right hand, right hemisphere-left hand) and Body Side (Left hemisphere-left hand, Right hemisphere-right hand) were included in an ANOVA design as within-subject factors. This resulted in a reduction of the CMC frequency 31.5±1.6 Hz pre-treatment to 26.6±1.5 Hz (Figure 2, Table 2).

The FaReMuS treatment did not have a difference effect on task performance with the left or right hand, according to the lack of a FaReMuS*Body Side interaction effect (p=.211).

As a result, there was no Body Side effect before or after treatment (p=.775), indicating that the CMC frequency did not differ in the two homolog cortico-spinal circuits while moving either the right or left hand. When looking at the CMC amplitude through a similar design, we found a significant FaReMuS effect (F(1,10)=6.068; p=.033], corresponding to an increase of the CMC amplitude from 0.021 ± 0.005 pre- to 0.035±0.007 post-FaReMuS (Figure 1).

No effects were found after analysing through a similar ANOVA the EMG and the EEG PSDs by adding Band as within-subject factor (p>.500 consistently).

Table 1. FaReMuS effects on central-peripheral synchronization

	Cortico-muscular coherence (CMC)			
	Frequency (Hz)		Amplitude (dimensionless)	
	Pre FaReMuS	Post FaReMuS	Pre FaReMuS	Post FaReMuS
mean	**31.5**	**26.6**	**0.021**	**0.035**
SD	1.6	1.5	0.005	0.007

Average (mean) and standard deviation (SD) of the frequency and amplitude of the cortico-muscular coherence before (pre) and after (post) the treatment against MS fatigue (FaReMuS). In bold the values changed after vs. before FaReMuS (see text).

Fatigue and CMC features

The frequency of CMC during the pre-treatment period, particularly during the left handgrip, corresponded well with the degree of fatigue (Pearson's correlation.727, p=.027).

After the therapy, there was no longer any association (p>.200).

Discussion

The main finding of our study is that a neuromodulation intervention properly tailored on individual neuroanatomic characteristics in MS patients experiencing fatigue did not only improve their symptom but also modify the pattern of synchronization between cortical and muscular neuronal activities during the execution of straightforward movements resembling those made in daily life. Furthermore, the direction in which such a shift was detected shows that the motor control goes through a "normalization" process.

When performing isometric contractions under normal settings, the cortico-muscular coupling operates in the beta band (frequency range 13–25 Hz), but it operates in the gamma band (above 26 Hz) when modulating contractions at time-varying force levels (Omlor et al. 2007). The normalized synchronizations between cortical and muscular activities in MS patients after FaReMuS suggests that although simple isometric contractions may become more laborious as fatigue increases, they may revert to physiological and in a way smoother execution scheme when subjected to the neuromodulation treatment, that turns out to be efficacious against fatigue.

FaReMuS treatment significantly modified this impaired feature of motor control. In fact, pre-FaReMuS mean CMC frequency was 31 Hz in fatigued MS patients, 8 Hz more than the mean 23 Hz in healthy subjects performing the same task (Tecchio et al. 2006; Bigland-Rithchie & Woods 1984). The neuromodulation intervention according to FaReMuS paradigm reverted the CMC frequency to 26 Hz, thus inducing a mean amelioration of 5 Hz, which, compared to the above 8 Hz, can be quantified as an improvement of 62% of the pre-treatment alteration.

Of note, it has been demonstrated that the CMC frequency alterations related to MS fatigue may also occur without changes in CMC amplitude (Tomasevich et al. 2013) while in healthy people undergoing fatiguing tasks, the CMC increases in amplitude and not in frequency (Bigland-Ritchie et al. 1984). These observations emphasize the possibility that the fatigue mechanisms involved in MS differ from those observed in physiological state (Sheean 1197). Here, pre-FaReMuS MS patients displayed CMC alteration not only in frequency but also in amplitude. This discordance is coherent with the nature of fatigue

experienced by subjects with MS in our experiment, for whom the symptom was defined as exhausting, long-lasting and involving the whole body (mean mFIS 50.7), therefore different from those observed in Tomasevic et al group (mean mFIS 36.6). FaReMuS partially reverted also CMC amplitude, but the CMC frequency is confirmed as the core feature as it correlated with MS-fatigue levels, while no association was found with CMC amplitude (Pearson's correlation rho=.182, p=.640).

Notably, before FaReMuS treatment, the CMC frequency increased along with the fatigue levels for the non-dominant body side. Previous observations of dominance-dependency in association with fatigue indicated more pronounced signs of locally impoverished organization of the dominant somatosensory representation (Porcaro et al. 2019; Vecchio et al. 2017; Buyukturkoglu et a. 2017). The dissociation between hemi-lateral parietal (S1-dominant) and frontal (M1 with muscle-non dominant) imbalances due to fatigue gives rise to a further directionality of the recovery pathways involved in MS-related fatigue: in addition to the well-known left vs. right hemispheric homologs (Deco et al. 2011), an important additional role is played by the functional matching between frontal (motor) vs. parietal (sensory) regions.

The planned action beneath voluntary movement execution, i.e., the centrally generated intention to act, translates into descending motor commands sent to the pattern generating circuitry in the spinal cord. Concurrently, it is also continuously subjected to a tuning of motor control derived by the feedback signals arriving from the peripheral proprioceptive circuitry (Fink et al. 2014), accomplished through the fine interplay between S1 and M1 (Tecchio et al. 2008; Gandolla et al. 2014). A two-fold explanation strengthens the hypothesis that the functional inflow from S1 to M1 is crucial in the generation of fatigue in MS: 1. The cortical alteration increases with MS fatigue increase, as expressed by the distortions of the dynamics of the neuronal electric activity and is more evident in S1 than M1 (Porcaro et al. 2019). 2. The neuromodulation FaReMuS that selectively targets S1 ameliorates the movement execution (present results). Indeed, these data give greater confidence that the impoverished parietal synchronizations involving the primary

somatosensory cortex prevent adequate sensorimotor feedback processing at the origin of MS fatigue.

Conclusions

A personalized intervention directed to sustain the excitability of primary somatosensory areas devoted to the whole-body representation, showed ability in normalizing the motor control strategy of everyday activities in people with MS, together with ameliorating their fatigue symptoms.

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Chapter 7: Personalization and comprehension of the mechanisms of the functioning of the motor and sensorimotor system: current studies and publications

7.1 Introduction: how to measure cortico-muscular synchronization

Neurones communicate each other via signals displaying fluctuations, which produce 'synchrony' among the activities of the involved brain areas (Bowyer, 2016) both during rest and while performing different tasks (Deco *et al.*, 2011). By sustaining communication among networks (Varela *et al.*, 2001) synchronization of neural activity mediates information processing in the brain (Singer, 1993; Borisyuk *et al.*, 1998; Fries, 2009). In other words, if neurons display correlated behaviour, even if they find place in spatially discrete and/or distant areas, the integration of their signals allows for sensory (Gray, 1994), attentional (Womelsdorf and Fries, 2007) or motor processing as well as for memory (Axmacher *et al.*, 2006) and for other fundamental cognitive processes (Daffertshofer et al. 2020).

Motor functioning in healthy subjects also emerge from the interaction and synchronous activation within and among multiple central nervous system areas such as the motor cortex, frontal cortex, parietal cortex, premotor cortex and subcortical and cerebellar areas, as well as the spinal cord (van Wijk *et al.*, 2012). The key role of such an important mechanism in motor control is further underlined by the evidence showing abnormal patterns of synchronization in diseases involving movement impairments such as Parkinson (Hammond *et al.*, 2007) or Dystonia (Brown, 2007; McClelland *et al.*, 2020). As we said, not only synchronization of oscillatory activity subtending motor control occurs locally within the motor cortex or among different cortex regions (cortico-cortical synchronization) but also between the cortex and the spinal cord (cortico-spinal synchronization) i.e. synchronization occurring between motor areas and spinal moto-neurons (MNs) (van Wijk *et al.*, 2012). Throughout this mechanism, the cortex controls

muscle contraction (Lemon, 2008) therefore the electrical activity recorded on the surface of the muscles results correlated with the cortex activity (Negro and Farina, 2011).

There is ample evidence from MEG and EEG - coupled with EMG - studies of cortico-spinal synchronization, (Conway *et al.*, 1995; Salenius *et al.*, 1997; Halliday *et al.*, 1998; Mima and Hallett, 1999; Gross *et al.*, 2000; Kilner *et al.*, 2000; Negro and Farina, 2011; Liu *et al.*, 2019; Suzuki and Ushiyama, 2020). These studies showed how neurons synchronize their firing patterns at different frequencies according to diverse behavioural states (Mima and Hallett, 1999) as for example initiating movement (Ramayya *et al.*, 2021), exerting either a static force (Kristeva *et al.*, 2007) or a dynamic force (Omlor *et al.*, 2007) or even according to different force levels of contraction (Brown *et al.*, 1998; Mima *et al.*, 1999; Brown, 2000).

Cortico-muscular coherence

Cortico-muscular coherence (CMC) has traditionally been used to assess the degree of synchronization between the brain and the associated muscles (Mima and Hallett, 1999; Liu et al., 2019). This is the spectral coherence between the EEG or MEG signal designating the contralateral brain and the EMG signal recorded by relevant muscles while the subject is performing a simple motor task (Mima and Hallett, 1999).

In 2022 we published a study (L'Abbate et al. 2022) (to which I contributed to writing) that examined the CMC's sensitivity to visual feedback information and handedness. To do this, we recorded participants' EEG and MEG as they used either their right or left hand to complete a weak handgrip task with or without visual feedback. Despite the evident right-handed dominance of our subjects, we highlighted how, despite our observations of CMC's sensitivity to visual feedback, neither prior research nor our findings indicated any substantial variation of CMC due to handedness (Tecchio et al., 2006).

We predicted that the CMC parameter itself is the source of the assessment limit, given the essential role of asymmetries in the operation of our body-brain system and the significance of handedness in our daily lives. Therefore, we hypothesized that

measurements sensitive to the complex character of the exchanged signals could also be sensitive in how the cortical regions controlling the two hands are organized.

Section 7.2: Normalized Compression distance

In a published paper I contributed to writing among the first authors (first Authorship was shared, for details see the following paragraph); the method we suggested (Pascarella et al. 2022) for measuring the cortex-muscle synchronization was designed to get over the drawbacks associated with conventional Fourier analysis or autoregressive models. Indeed, these estimations are insensitive to important components of the internal dynamics of the neural pool activity while assuming the stationary signal and representation with sinusoids functions (Buzsáki et al., 2013; Cottone et al., 2017).

We use here the normalized compression distance (NCD), which is computed from the lengths of compressed signals, singly and in pairwise concatenation.

As well as in the previous study (L'Abbate et al. 2022) we collected EEG and EMG simultaneously when subjects were performing a weak isometric handgrip task, with either the right or left hand, with or without visual feedback of their exerted pressure. Our aim was to test the working hypothesis that NCD was sensitive to visual information level, as for CMC, but also, differently from CMC, it captures even subtle variations of cortex-muscle communication features dependent on manual dominance. What we found was that, equally to CMC, NCD is sensitive to visual feedback but, additionally, also to manual dominance. As a result, we concluded that NCD can serve as an appropriate enrichment tool to evaluate synchronization phenomena between two nodes.

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7.2 Cortico-muscular synchronization measured using Normalized Compression Distance*

The following authors contributed to the final and published edition of this work*:
For details see the "author contribution statement" at the end of the manuscript

Annalisa Pascarella*[1], **Eugenia Gianni***[2-3], Matteo Abbondanza*[4-2-1], Karolina Armonaite[5-2], Francesca Pitolli[4], Massimo Bertoli[6-2], Teresa L'Abbate[5-2], Joy Grifoni[5-2], Domenico Vitulano[4-1], Vittoria Bruni[4-1], Livio Conti[7-8], Luca Paulon[9-2], Franca Tecchio[2]

*These Authors equally contributed to the work

[1] Institute for the Applications of Calculus (IAC) 'M.Picone', National Research Council,

Via dei Taurini 19, 00185, Rome

[2] Laboratory of Electrophysiology for Translational neuroScience LET'S, Institute of Cognitive Sciences and Technologies ISTC, Consiglio Nazionale delle Ricerche CNR, Via Palestro 32, 00185 Rome, Italy

[3] Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, University Campus Bio-Medico of Rome, 00128 Rome, Italy

[4] Department of Basic and Applied Sciences for Engineering (SBAI), University of Roma "La Sapienza", Via Antonio Scarpa 16, 00161 Roma, Italy

[5] Faculty of Psychology, Uninettuno University, Rome 00186, Italy

[6] Department of Neuroscience, Imaging and Clinical Sciences, University 'Gabriele D'Annunzio' of Chieti-Pescara, 66100 Chieti, Italy

[7] Faculty of Engineering, Uninettuno University, Rome 00186, Italy

[8] INFN - Istituto Nazionale di Fisica Nucleare, Sezione Roma Tor Vergata, Rome 00133, Italy

[9] Eng. Luca Paulon, Ph.D., Free Lance, Rome, Italy

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Introduction

The neuroscience community is increasingly focusing its interest and studies around the concept of functional connectivity (FC) (Bullmore and Sporns, 2012; Wang et al., 2014) as a phenomenon of the brain functioning. FC is evaluated as a statistical dependency between the signal time series of different nodes and can be measured using various methodologies such as electro-encephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI) (Hutchison et al., 2013; Wang et al., 2014).

The degree of functional connectivity across distinct nodes has historically been measured using linear measurements (coherence and correlation) (Gross et al., 2001; Broyd et al., 2009; Jensen and Mazaheri, 2010). However, these measurements present limitations. For example, researchers pointed out that only because there isn't a linear statistical relationship between two nodes it means that FC is not present (Fingelkurts et al., 2005). This is one of the factors contributing to the increased interest in non-linear FC measurements like mutual information (Hlinka et al., 2011; Wang et al., 2016).

We already told how, in the case of cortico-muscular synchronization, a classically used electrophysiological measure is the cortico-muscular coherence (CMC) (Mima and Hallett, 1999; Liu et al., 2019). This is the spectral coherence between the EEG or magneto-encephalographic (MEG) signal from the contralateral cortex and electro-myographic (EMG) signal recorded by involved muscles while executing a motor task.

Although the CMC is regarded as a reliable indicator of the flow of information between the brain and muscles in both healthy and pathological settings (Mima & Hallet 1999; Liu et al. 2019), several limitations have been pointed out (Yang et al. 2017).

We have already spoken, in this Chapter's Introduction, about the study of L'Abbate et al. 2022) in which we found sensitivity of CMC to visual feedback, but no sensitivity to manual handedness.

We need to add that, some authors further highlighted the limits of linear electrophysiological studies in light of the sensorimotor system's recognized characteristics (Yang et al., 2016, 2018; Tan et al., 2022). For instance, they found that while ascending somatosensory feedback and descending motor commands are the sources of synchronization in the sensorimotor system (Kilner et al., 2004; Witham et al., 2011), CMC is unable to distinguish between this bidirectional contribution in cortico-muscular interaction. Additionally, they noted that recent research suggests the sensorimotor system is nonlinear and exhibits cross-frequency coupling (Chen et al., 2010; Yang et al., 2018), opening the way to non-linear measures able to complement linear ones (Palva et al., 2005; Yang et al., 2016; Siebenhühner et al., 2020).

Here, we suggest utilizing the Normalized Compression Distance (NCD), a unique non-linear metric, to explore FC. By comparing the compression length of a file created by concatenating two signals, it is a parameter-free metric that determines the information shared by two signals. As it produces outstanding results whether comparing genomes, grouping languages, or classifying music, NCD appears to be a good fit for biological systems (Li and Vitányi, 1990).

Notably, NCD is robust and resilient in that its functionality seems to be relatively unaffected by the type of compressor that was used to code the data. No special characteristics or prior understanding of the data is necessary for NCD. We chose this synchronization measure since it does not need signal stationarity or any representation of the single in harmonics and estimates the information shared by the two signals. In fact, the estimations are insensitive to important components of the internal dynamics of the neural pool activity according to the hypothesis of the stationarity of the signal and representation with sinusoid functions (Buzsáki, 2009; Buzsáki et al., 2013; Cottone et al., 2017; Armonaite et al., 2022).

The goal of our study was to evaluate how sensitive the NCD is to basic physiological characteristics. As a paradigmatic example, we investigated, using the NCD, how the cortex and muscle (CMncd) synchronized while carrying out a straightforward movement typical

of daily activity. To this aim we employed the same protocol of L'abbate et al. (2022) in which subjects performed a weak handgrip during an EEG-EMG session with either the left or the right hand, with or without visual feedback of their exerted pressure. In this case Lower NCD estimates should correspond to higher synchronization levels.

Therefore, we specifically state the working hypothesis that the degree of visual feedback and the hand used for the motor task influence CMncd's behavior. In other words, according to the behavior of cortico-muscular coherence, we anticipate that: i) left non-dominant hand control would express greater CMncd than right dominant hand control; and ii) supplying undirect visual feedback will increase CMncd as suggested by the behavior of cortico-muscular coherence (L'Abbate et al., 2022).

Methods

Study design

It is a cross-over study with two interacting conditions (moved hand, visual feedback).

Subjects

Fifteen healthy volunteers (10 females and 5 males, age range from 20 to 48 years with mean 29 ± 7 years) participated in the study after signing a written informed consent. All subjects were right-handed (as tested by Edinburgh Handedness Questionnaire Oldfield, 1971), and had normal or corrected-to normal vision.

Experimental procedure

Data recordings

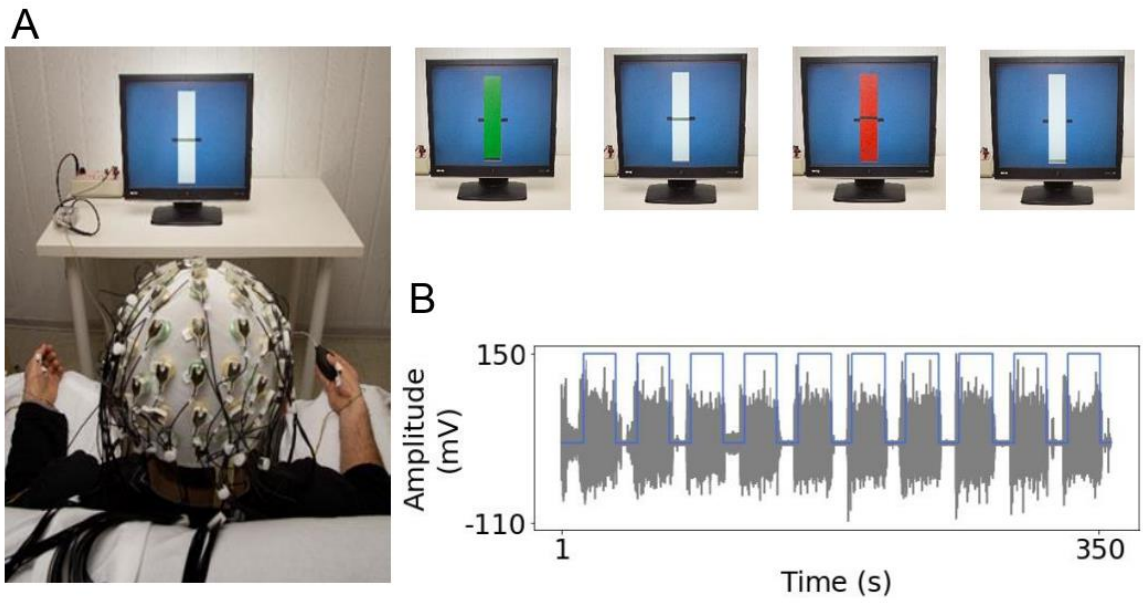
Using a 64-channel acti-CHamp System with montage in accordance with the 10-10 EEG International System and referred to the Fz electrode, the individual's EEG was recorded. A belly tendon montage was used to capture the surface EMG of the right and left opponents pollicis muscles (EMGOPr and EMGOPl) using Ag-AgCl cup electrodes. For

off-line processing, EEG and EMG were sampled at 5 kHz (pre-sampling analog band pass filtering 0.1-2000 Hz).

Visuo-motor task

In front of a monitor, each participant sat in a chair about one meter away (Figure 1A). The patient gripped a semi-compliant air-bulb with either their left or right hand, as shown in Figure 1's legend, while the air-bulb was attached to a digital board that recorded the applied pressure (Interactive Pressure Sensor, InPresS) (Tomasevic et al., 2013). A noteworthy difference between the physiological information our visual system typically provides while performing a movement, such as a weak hand grip, and the visual information about the exerted pressure provided as a horizontal segment vertically oscillating on the monitor is that the latter implies “transposed” feedback. Each of the four handgrips took around five minutes to complete, and all participants performed them in the same order: first, with the dominant hand receiving visual feedback (DxYes), next, with the dominant hand without it (DxNo), and finally, with the non-dominant hand receiving visual feedback (SnYes) and without visual feedback (DxNo) (SnNo).

Figure 1. Experimental setting



A. EEG recordings and task

The typical setup to capture the EEG during the weak hand grip consisted of 20-second sequences that began with a go signal (green rectangle) and ended with a stop signal (red rectangle), separated by 10-second rest periods. A horizontal segment in the visual feedback "yes" ("no" condition reveals the amount of pressure being applied to the bulb via vertical oscillations (blocked).

A rest interval of at least two minutes was given after calculating the handgrip maximal voluntary contraction (MVC). The weak isometric handgrip exercise was followed by a 5-minute rest period. To reduce task-related fatigue, the goal level was established at 5% MVC.

B. Example of EMG acquisition during isometric contraction execution

The EMG trace of one sample subject's opponents pollicis (OP) muscle for the whole task length, with 20 s contraction cycles interspersed by 10 s during rest, is shown in gray. The temporal segments chosen for study are shown by a light blue line. (***Figure and caption from Pascarella et al. 2022).***

Data analysis

EEG data pre-processing

Prior to analysis, EEG data were filtered (1-250 Hz). To find and eliminate biological (cardiac, ocular, and muscular) and non-biological (power line, instrument, and ambient noise) artifacts from the whole recordings, a semi-automatic fast independent component analysis (fastICA)-based approach was used (Barbati et al., 2004). For the analysis, we chose 180 seconds of artifact-free data for each individual.

Normalized Compression Distance (NCD)

The NCD is a quasi-universal metric, in the sense that it has been defined to simultaneously detect all similarities between signals that other effective distances detect separately (Cilibrasi and Vitányi, 2005). In other terms, NCD is based on the concept that two signals are similar if we can significantly "compress" one using the information of the other. NCD captures the dominant similarity over all possible features for every pair of signals compared, up to the stated precision.

We must remember that a lossless compressor acts as an invertible mapping function of a signal into a binary sequence. The length of this binary sequence reveals the amount of compression. Hence, the NCD computed between two signals x and y, i.e. NCD(x,y) is defined as

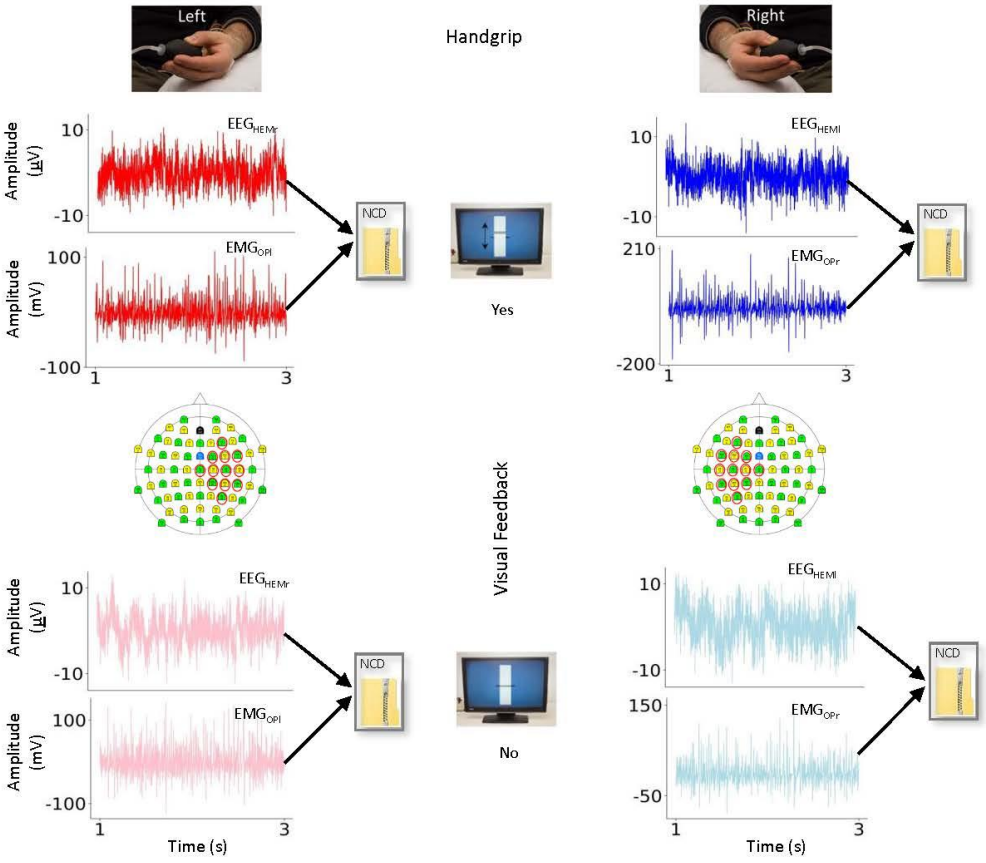
$$\mathrm{NCD}(x,y) = \frac{C(xy) - \min(C(x), C(y))}{\max(C(x), C(y))},$$

where C(xy) denotes the compressed size (length of the binary sequence that has been obtained by applying the compressor C) of the concatenation of x and y, wherein C(x) denotes the compressed size of x, and C(y) denotes the compressed size of y. NCD assumes values between 0 and 1, where 0 indicates maximum similarity and 1 the opposite.

In this work, the compressed size has been measured in terms of number of bits per sample, which is the average number of bits used for coding each sample of the considered signal. We used as compressor C the Huffman coding implemented in Matlab environment.

For each subject and condition (Figure 2), we computed the NCD between the cleaned EEG and EMG signals, with EEG being the selected bipolar channel, for epochs of 180 s length, windowed in segments of 18 s, obtaining 10 estimates for each subject and condition.

Figure 2. Variables of interest



Representation of the functional connectivity measure calculated by NCD from the ongoing EEG and EMG data in the four circumstances of interest. The circumstances with visual feedback are shown in red (left movement) and blue (right movement), respectively, whereas the situations without are shown in light red and light blue (No). We selected the 64-EEG recording channels with the maximum cortico-muscular coherence throughout the task using the contralateral hand as the EEG representative, and we highlighted those channels in the topographical depiction of the 64-EEG recording channels. (***Figure and caption from Pascarella et al. 2022).***

Statistical Analysis

The Shapiro-Wilk test and Levene test were used to determine the normality and homogeneity of variance of each variable's distribution.

Initially, we evaluated the variation coefficient of the roughly ten quantifications in subsequent 18-second epochs in the same condition, in all participants and conditions, in order to test the stability of the CMncd estimate.

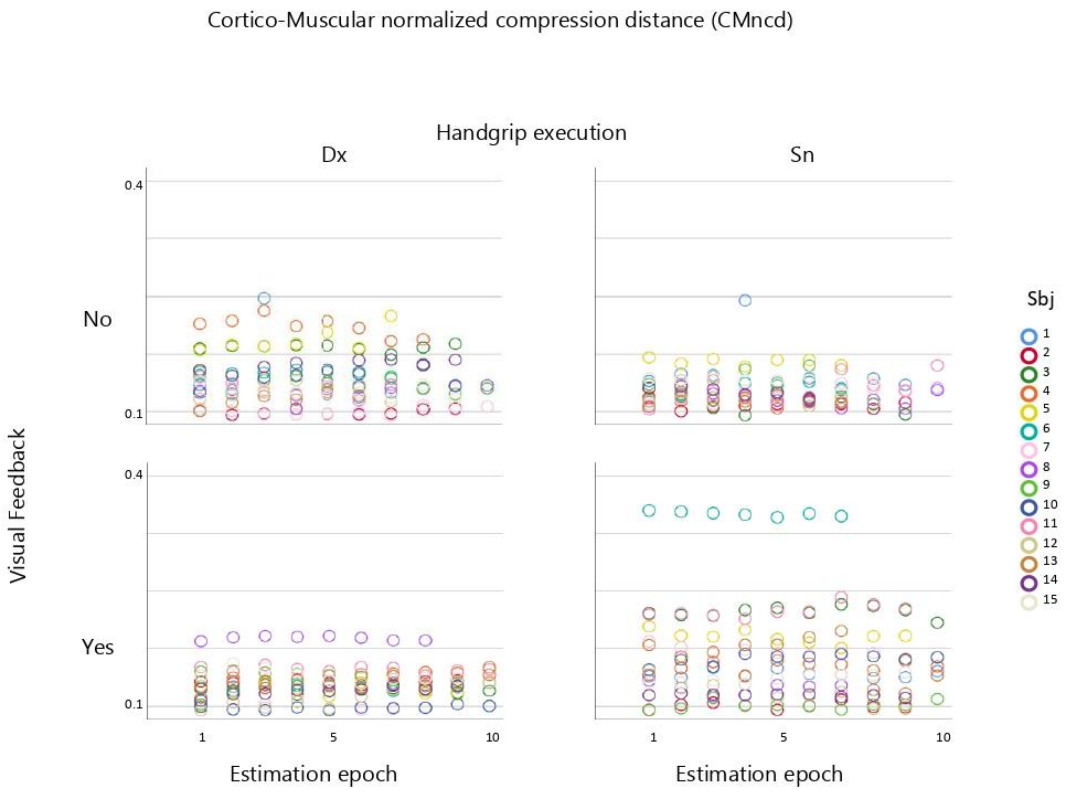
We analyzed the data by taking into account two factors, hemi-body and visual feedback, which resulted in four different conditions: hemi-body (left hemisphere-right hand, right hemisphere-left hand), and visual feedback. This allowed us to assess the sensitivity to the behavioral condition of the cortico-muscular synchronization estimated by CMncd (Yes, No).

Results

CMncd stability intra-individually across conditions

Stability of the CMncd estimate within each subject resulted high, as the mean variation coefficient was 0.20, with first and third quartiles 0.10 and 0.34 across the 10 estimate epochs across the 15 subjects and the four conditions (Figure 3).

Figure 3. CMncd stability intra-individually across conditions



CMncd data points for the 10 repetitions (in some cases some epochs lacked) of epoch estimate for each of the 15 subjects in the four conditions: while subjects were performing the task with the right hand in absence (DxNo) or presence of visual feedback (DxYes) and with the left hand (SnNo and SnYes). The intra-subject stability of the estimate is appreciable.

CMncd behaviour across conditions

The distribution of CMncd measurements among patients in the four situations (DxNo, DxYes, SnNo, and SnYes) did not fit a Gaussian, according to Shapiro-Wilk statistics. Levene tests further revealed that the variations in CMncd were not uniform across the four conditions. Because of this, we used non-parametric statistics.

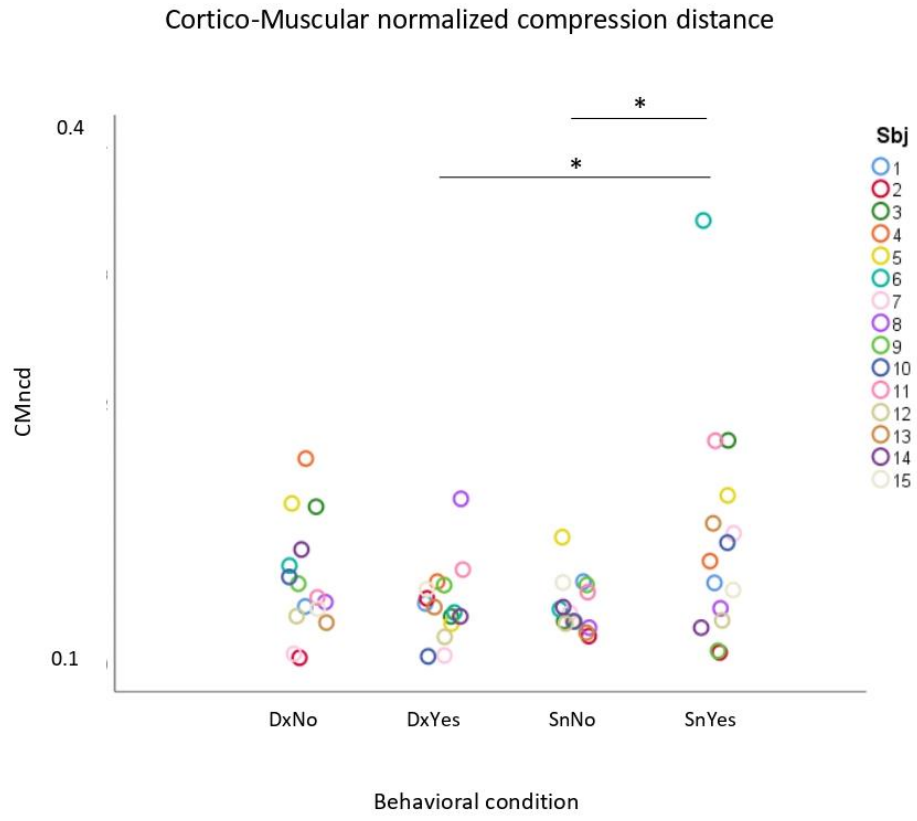
We took into account the 10 CMncd measurements for each individual and determined the median value for each condition (DxNo, DxYes, SnNo, SnYes). Since the medians for every subject remain the same, we chose not to delete outliers.

We compared the medians using the non-parametric Wilcoxon matched-pairs test (Z), looking for differences both between hemibodies under the same feedback condition and between hemibodies in the absence and presence of visual feedback (to assess the dependence of CMncd on the various behavioral conditions (absence or presence).

While executing the task with the left hand, the CMncd was lower in absence of visual feedback than in presence of it (SnNo vs. SnYes, Z=-2.101; p=0.036; see Figure 4 and 5A). Controlling the right-hand movement was accompanied by lower CMncd that controlling the left hand when the execution was in presence of visual feedback (DxYes vs. SnYes, Z=-1.988; p=0.047; see Figure 4 and 5B).

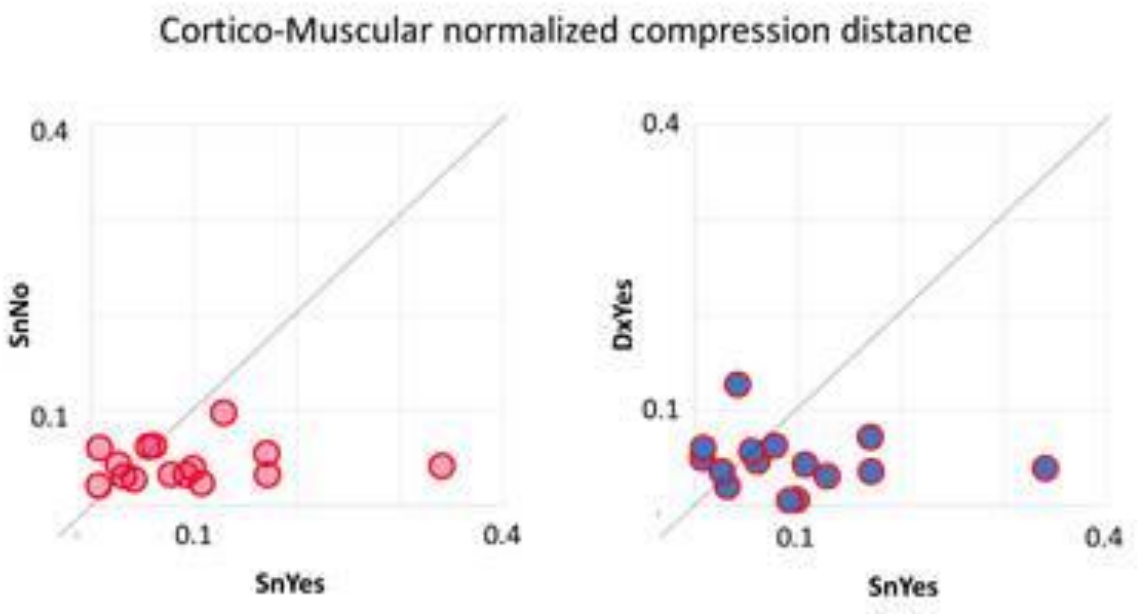
No significant differences were found when comparing the two overall conditions of the left vs. right hand movement (including both presence and absence of visual feedback, Z=0.751; p=0.453) and presence vs. absence of visual feedback (including both the left and right hand executions, Z=-1.059; p=0.289).

Figure 4. CMncd of individual subjects across conditions



Median values of CMncd for each of the 15 subjects in the four conditions. Black lines with asterisks: conditions differing for p <0.05. ***Figure and caption from Pascarella et al. 2022.***

Figure 5. CMncd dependence on behavioral condition



Boxplot of CMncd during the left handgrip with visual feedback (abscissa of both panels) compared with the left handgrip without visual feedback (Left panel) and right handgrip with visual feedback (Right panel). We reported these comparisons, since when moving the right hand the visual feedback had no effect (DxNo vs. DxYes, Z=-1.136; p=0.256) and in absence of visual feedback there were no differences when moving the two hands (DxNo vs. SnNo, Z=-1.647; p=0.100).

Discussion

Our findings demonstrate that when performing the left handgrip, CMncd exhibits higher values in the absence compared to the presence of visual feedback, reflecting minor synchronization between the cortex and muscles when the task necessitates the integration of transposed visual feedback about the applied pressure. Additionally, in the presence of visual feedback, CMncd differs from CMC (L'Abbate et al., 2022) in that it exhibits larger values for the left handgrip than the right. As predicted, the non-dominant side's hand movement exhibits less cortico-muscular synchronization than the dominant side.

The overall findings demonstrate that CMncd is sensitive to motor control dexterity, distinguishing the dominant vs. non-dominant sides for routine motions and demonstrating the difficulties of the non-dominant side to integrate unexpected information during an unfamiliar task.

CMncd sensitivity to visual feedback

Bottom-up somatosensory and visual feedback can influence our actions while we are performing voluntary movements, both during planning and execution (Scott et al., 2015). When motor and sensory area synchronization occurs within the expected range, the movement continues as planned; when the level of synchronization is insufficient, the movement is corrected (Wolpert et al., 2011). The gaze plays a critical function in the planning and execution of motor activities: it both comes before and directs our daily actions (Jovancevic-Misic and Hayhoe, 2009). When we carry out a routine task, we implement eye-motor programs simultaneously with the spatial changes of the body parts we are moving (Flanagan and Johansson, 2003). Visual feedback is considered to play a crucial part in the motor regulation of hand motions (Saunders and Knill, 2004). The findings demonstrates that visual feedback is important for the online regulation of reaching motions and that a lack of visual feedback results in significant endpoint variability

(Saunders and Knill, 2003). Visual feedback modifies the direction and distance from the endpoint in pointing movements (Saunders and Knill, 2005). There is also strong evidence that vision is used to train and control gripping movements and to handle objects (Connolly and Goodale, 1999; Johansson et al., 2001).

The task "without" visual feedback implemented the typical physiological condition of everyday life: although people did not look at their hands, the grasping task while looking at the fixed monitor reflects the typical situation in which we eat at the table while looking at each other and not constantly at our cutlery. In our experiment, simple grasping movements with the hand were used. The cortico-muscular synchronizations in this case were the same whether the dominant or non-dominant hand was moved.

When using any light instrument, we calibrate the strength based on visual and proprioceptive information, but in this work, we gave information about the executed pressure via the location of the horizontal section that fluctuated (Sober and Sabes, 2005). In other words, our "visual feedback" situation necessitates a quick learning that involves adaptive processes. Our research showed that whereas the dominant side had identical characteristics while performing routine or atypical movements, the non-dominant hemi-body displayed less cortico-muscular synchronization when attempting to handle novelty. CMncd may be sensitive to the challenges that less dexterous systems face when attempting to take use of the indirect transposed information with respect the manipulated object.

CMncd sensitivity to manual handedness

Based on knowledge from the resting state, our working hypothesis predicted that handedness effects would manifest independently of the behavioral test. On the other hand, when the two dominant and non-dominant controlling networks were engaged in a task with new processing needs, the impact appeared for motions that did not belong to the ordinary repertory.

The cortico-muscular synchronization is believed to be considerably more behavior-dependent than the intra-cerebral networks' activities because, contrary to central networks, where the resting state originates with a continuous ongoing neuronal pool activity, the muscles are electrically silent at rest.

As with other biological systems, the human brain exhibits asymmetries in its structure and operation (Toga and Thompson, 2003). According to some theories, lateralization developed as a result of variables related to evolution, development, genetic makeup, and personal experience. The most notable and well-known example is lateralization of language (Bishop, 2013), but there is also a lot of evidence for hemispheric asymmetry in motor control (Haaland and Harrington, 1996). According to brain imaging studies and EEG (Serrien et al., 2012; Serrien and Sovijärvi-Spapé, 2016), lateralization of motor control has a cerebral counterpart (Schluter et al., 2001). Our findings support the idea that hand control is carried out by hemi-body homologous networks in a variety of functional configurations. Indeed, the network controlling the non-dominant left hand exhibits signs of less tuned coordination in comparison to the dominant homolog when the subject is required to complete a task with a relevant novelty processing component, such as adjusting the handgrip pressure according to a distant and independent visual information.

NCD for 2-node functional connectivity in the nervous system

We offer the NCD as a measure of synchronization in this study to account for the complex character of the ongoing neuronal electrical activity, the neurodynamics, and the motor-associated synchronization between cortical neuronal activity and that of spinal moto-neurons. NCD sensitivity implies that it can enhance the evaluation of communication events within the nervous system in the studied settings, opening a new window to evaluate network functional connectivity qualities. Due to its definition, NCD may be used to compare activity in various locations, even when data is acquired at various times (Sarasa et al., 2019). NCD is also appropriate for comparing signals of different

durations, such as when engaging in activities where artifacts occur over a range of time periods and cause inconsistent epoch rejections (Li and Vitányi, 2008).

Conclusions

As a result, we think NCD can serve as an effective enrichment tool to evaluate synchronization phenomena between two nodes, improving the estimation of functional connectivity within the brain networks that underlie brain processing.

Author contribution statement

FT and EG drafted the manuscript. AP, DV, and VB grounded the normalized compression distance theory and algorithm. MA and FP coordinated the analysis on the present data set. LC cured the manuscript writing. MB and JG deepened the clinical perspective and LP the conceptual framework. KA and TL'A shared the neurophysiology-psychotherapy connection. All authors contributed to the final writing.

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Conclusions and future directions

In the first part of this work, namely in the First Chapter, we provided the reader with a broad overview of the concept of electroceuticals; the treatment of ailments by electrical signals. From this overview emerged not only the importance and therapeutic potential of this new field, but also the relevance within the medical framework of devising personalized electroceutical treatments. In our laboratory, we moved along two personalization tracks: personalization of the montage (Faremus) and personalization of the current (tIDS). With regard to the first track, the Faremus treatment has proven to be valid and effective against fatigue in multiple sclerosis. In fact, fatigue, as emerges from the Second Chapter, reveals itself as a functional alteration of the sensorimotor cortex that can certainly benefit from electroceuticals treatment. As for the second avenue, work is still in the planning stage, as shown in Chapter Three. Although we have seen that personalized current can increase the efficacy of electroceutical treatment, we still need to accumulate more evidence in this regard, and this will be an important future line of research in our laboratory.

In the second part of this work, we showed how in these years we were able to set up relevant milestones in the direction of creating an electroceutical service. With my contribution, as described in Chapter Four, we were able to establish, throughout a quantitative review of randomized controlled trials using tDCS, that it is possible to classify Faremus treatment between highly and moderately recommendable under the indications of the international authorities. Moreover, we were able to write up and publish an important work testing the use of Faremus at home, which revealed its feasibility, acceptance and efficacy at home as well as in clinical settings. In the direction of engineering and commercialization of our device we took contact with relevant enterprises for setting a pathway along this way, as detailed in Chapter Five. Next step, as we were suggested, could be to explore the option of a patent for our system.

From the third part of this work, we can confirm that fatigue, as demonstrated in Chapter Six, is originated from an imbalance of sensorimotor areas that can be rebalanced

through appropriate neuromodulation. As demonstrated in Chapter Seven, advances in the understanding of the nervous system and its communication with muscular effectors appear to be enriched by measures that also take into account the complex, non-oscillatory dimension of neural activity. Regarding this stream of research, it would be interesting to a) explore physiological markers of balance/imbalance in connectivity between multiple areas, even homologous areas, and their role in brain alterations along with potential therapeutic interventions b) to use novel consolidated measures such as normalized compression distance (see Chapter Seven) to explore fatigue mechanisms.

List of publications

Armonaite K, Bertoli M, Paulon L, **Gianni E**, Balsi M, Conti L, Tecchio F. Neuronal electrical ongoing activity as cortical areas signature: an insight from mni intracerebral recording atlas. Cerebral Cortex. 2022 Jul 1;32(13):2895-906.

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List of conference participation and posters

Conference Participation: AISC Conference 2019 – Roma Tre University, via Ostiense 234, 11-13 December 2019.

Poster Presentation: FaReMuS modifies the control of everyday movements, BrainSTIM 2020, the 6th annual Brain Stimulation and Imaging Meeting, online, May 19-20 2020.

Poster presentation: Effects on motor control of personalized neuromodulation against multiple sclerosis fatigue, NYC Neuromodulation 2020 online conference, December 18-22 2020.

Poster presentation: tDCS randomized controlled trials in no-structural diseases: a quantitative review (TdcsRctRev]. NonInvasive Mathematics, On-line INDAM Workshop, April 13-16 2021.

Poster presentation: Effects on motor control of personalized neuromodulation against multiple sclerosis fatigue, NonInvasive Mathematics, On-line INDAM Workshop, April 13-16 2021.

Conference Participation: Baci conference, 5th Conference on Basic and Clinical Multimodal Imaging, online 14-17 October 2021.

Brief Talk on my research experience: IEEE International leadership summit – WIE Women in Engineering (WIE) Online event 02 December 2021.

Poster Presentation: tDCS randomized controlled trials in no-structural diseases: a quantitative review (TdcsRctRev]. ICCN 2022 International Federation of Clinical Neurophysiology 4-8 September 2022, Geneve.

Poster Presentation: Effects on motor control of personalized neuromodulation against multiple sclerosis fatigue, ICCN 2022 International Federation of Clinical Neurophysiology, 4-8 September 2022, Geneve.