THE ROLE OF PRIMARY MOTOR CORTEX AS A MARKER FOR AND MODULATOR OF PAIN CONTROL AND EMOTIONAL-AFFECTIVE PROCESSING

EDITED BY: Jorge Leite, Sandra Carvalho, Linamara R. Battistella, Wolnei Caumo and Felipe Fregni

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THE ROLE OF PRIMARY MOTOR CORTEX AS A MARKER FOR AND MODULATOR OF PAIN CONTROL AND EMOTIONAL-AFFECTIVE PROCESSING

Topic Editors:
Jorge Leite, Harvard Medical School, United States; University of Minho, Portugal; Universidade Portucalense, Portugal
Sandra Carvalho, Harvard Medical School, United States; University of Minho, Portugal
Linamara R. Battistella, University of São Paulo, Brazil
Wolnei Caumo, Universidade Federal do Rio Grande do Sul, Brazil
Felipe Fregni, Harvard Medical School, United States

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The sensory and motor cortical homunculi proposed by Walter Penfield were a major landmark for the anatomical mapping of the brain. More than 60 years after, the development of new tools to investigate brain function non-invasively has increased our knowledge about the structure and functions of the primary motor Cortex (M1) beyond motor control in both humans and animals.

This book highlights the role of the motor cortex that goes way beyond motor functioning. We were interested in both theoretical and empirical contributions related to electrophysiological, pharmacological, neuroimaging, and neuromodulatory studies exploring the role of M1 on non-motor functions, such as pain, abnormal neuroplasticity that may lead to chronic pain conditions; or the relationship between M1 and mental imagery or emotion.

This book is comprised of 15 articles published in this edited volume as a research topic collection in Frontiers in Human Neuroscience titled "The Role of Primary Motor Cortex as a Marker and Modulator of Pain Control and Emotional-Affective Processing."

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EDITORIAL
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Editorial: The Role of Primary Motor Cortex as a Marker and Modulator of Pain Control and Emotional-Affective Processing

Jorge Leite[1,2,3], *Sandra Carvalho*[1,2], *Linamara R. Battistella*[4], *Wolnei Caumo*[5,6] and *Felipe Fregni*[1]*

[1] Spaulding Neuromodulation Center, Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA, United States, [2] Neuropsychophysiology Laboratory, Psychology Research Center (CIPsi), School of Psychology (EPsi), University of Minho, Braga, Portugal, [3] Portucalense Institute for Human Development (INPP), Universidade Portucalense, Porto, Portugal, [4] Physical and Rehabilitation Medicine Institute, Medical School General Hospital, University of São Paulo, São Paulo, Brazil, [5] Post-Graduate Program in Medical Sciences, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [6] Laboratory of Pain and Neuromodulation, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

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Editorial on the Research Topic

The Role of Primary Motor Cortex as a Marker and Modulator of Pain Control and Emotional-Affective Processing

In the 1940–50's Wilder Penfield and colleagues applied cortical electrical stimulation to patients undergoing epilepsy surgery to define what has become one of the landmarks on neuroscience: a map of the anatomical divisions of the body, divided in two cortical homunculi: sensory and motor (Penfield and Boldrey, 1937).

Ever since, the development of new tools to investigate brain function non-invasively increased knowledge about the structure and functions of the primary motor Cortex (M1) beyond motor control in both humans and animals. For instance, the role of M1 in visuomotor transformations, mental imagery, or mental rotation has been shown in studies dating more than 30 years ago (Georgopoulos and Pellizzer, 1995; Kosslyn et al., 1998). Also, M1 seems to be activated during memory retrieval of sensory information or finger tapping sequences after a short delay (Kaas et al., 2007), suggesting the M1 involvement with memory processes; as well as involved in language processing of action related words (de Lafuente and Romo, 2004; Hauk et al., 2004; Pulvermuller, 2005 for review). Furthermore, the involvement of the M1 region in higher cognitive functions has also been demonstrated in emotional processing. There seems to be a correlation between sensorimotor activation and empathy (Lamm et al., 2007), as well as relationship between sensorimotor activation and emotional processing in silent reading of emotionally laden words (Papeo et al., 2012). Moreover, M1 seems to be asymmetrically modulated by here emotionally laden sounds, with unpleasant sounds resulting in higher facilitation od motor evoked potentials in the left hemisphere, whereas pleasant sounds resulted in higher excitability in the right side (Komeilipoor et al., 2013).

The involvement of the M1 region in higher cognitive functions was also supported by a recent meta-analysis of neuroimaging findings in which an activation likelihood estimation was used to determine topographic convergence (Tomasino and Gremese, 2016). In the meta-analysis, the M1 subregion 4a was commonly activated during motor imagery and working memory, emotion/empathy, and language. But the potential role of M1 in higher cognitive functions is not limited to the activation of specific brain regions during task performance. By understanding how

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Edited by:
Stephane Perrey,
Université de Montpellier, France

Reviewed by:
Stephane Perrey,
Université de Montpellier, France
Yelena Granovsky,
Rambam Health Care Campus, Israel

***Correspondence:**
Felipe Fregni
felipe.fregni@gmail.com

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M1 modulates distant neural structures and its relationship with respective brain behavior, M1 can also be used as a potential marker for clinical applications, as well as to guide neuromodulatory therapeutic options (DaSilva et al., 2012; Carvalho et al., 2015). It is well known, for instance, that M1 has connections with several areas of the brain, and the stimulation of the motor cortex can induce changes in other systems (e.g., pain: Fregni et al., 2006; Castillo-Saavedra et al., 2016). Moreover, stimulation of motor cortex may actually improve cognitive functioning by the activation of cortico–striatal–thalamo–cortical loops (CSTC) (Leite et al., 2011).

Considering the role of M1 in cognitive functioning that surpass the motor processing, we proposed a research topic about the relationship between M1 and behavior, namely those related to pain and emotional-affective processing. We were interested in both theoretical and empirical contributions related to electrophysiological, pharmacological, neuroimaging, and neuromodulatory studies.

This special topic comprises 15 articles from a diverse group of scientists that provide a robust contribution for the development to the field. We also want to acknowledge the invaluable help that all reviewers provided during this process—many of them leaders in their field—whose contribution improved significantly the manuscripts. The reviews in this special issue investigate the role of motor cortex when using stimulation techniques to M1 to investigate pain modulation (Brasil-Neto) and how noninvasive brain stimulation can be used for reverting abnormal neuroplasticity associated with chronic pain (Naro et al.). This focus of M1 neuromodulation on pain modulation is also the focus of original studies in different types of pain, such as chronic musculoskeletal and post stroke pain, pain related to chemotherapy, fibromyalgia, or neuropathic pain (Botelho et al.; Caumo et al.; Hu et al.; Luu et al.; Mendonca et al.; O'Brien et al.). Additionally, a framework addressing the contralateral inhibition of the impaired hemisphere following stroke and its potential relationship with central post stroke pain is proposed (Morishita and Inoue). A second common theme was the use of EEG to understand changes in M1, and correlate this neural signal with pain and emotional processing in stroke patients (Doruk et al.) and chronic pain secondary to rheumatoid arthritis (Meneses et al.). Furthermore, the use of neuroimaging was also the topic of one study assessing connectivity alterations in patients with rheumatoid arthritis and correlating increased pain perception with increased connectivity for the supplementary motor areas, mid-cingulate cortex, and the primary sensorimotor cortex (Flodin et al.). Finally roles of the motor cortex on other cognitive domains were also explored, namely M1 activation with real or mental imagery (Galdo-Alvarez et al.), kinematic changes associated with pain in patients with fibromyalgia (Costa et al.), or changes in motor cortex activity following observation of emotionally laden pictures (Nogueira-Campos et al.).

This special topic highlights the role of the motor cortex that goes way beyond motor functioning. Also that we need to expand our knowledge about this particular region, its cortico–cortico and cortico–subcortico interactions, and how it can modulate or be modulated by different bottom-up (such as median nerve stimulation) or top down (such as TMS or tDCS) interventions. Despite that, this special topic clearly emphasizes methods to probe and neuromodulate motor cortex functioning and its potential impact for comprehensive rehabilitation (such as pain). But those are only a few examples of how motor cortex is involved in pain processing and higher order cognitive processing.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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MINI REVIEW
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Motor Cortex Stimulation for Pain Relief: Do Corollary Discharges Play a Role?

Joaquim P. Brasil-Neto *

Laboratory of Neurosciences and Behavior, Department of Physiological Sciences, Universidade de Brasília, Brasília, Brazil

Both invasive and non-invasive motor cortex stimulation techniques have been successfully employed in the treatment of chronic pain, but the precise mechanism of action of such treatments is not fully understood. It has been hypothesized that a mismatch of normal interaction between motor intention and sensory feedback may result in central pain. Sensory feedback may come from peripheral nerves, vision and also from corollary discharges originating from the motor cortex itself. Therefore, a possible mechanism of action of motor cortex stimulation might be corollary discharge reinforcement, which could counterbalance sensory feedback deficiency. In other instances, primary deficiency in the production of corollary discharges by the motor cortex might be the culprit and stimulation of cortical motor areas might then be beneficial by enhancing production of such discharges. Here we review evidence for a possible role of motor cortex corollary discharges upon both the pathophysiology and the response to motor cortex stimulation of different types of chronic pain. We further suggest that the right dorsolateral prefrontal cortex (DLPC), thought to constantly monitor incongruity between corollary discharges, vision and proprioception, might be an interesting target for non-invasive neuromodulation in cases of chronic neuropathic pain.

Keywords: transcranial direct current stimulation (tDCS), chronic pain, pain neuromatrix, transcranial magnetic stimulation (TMS), motor cortex stimulation

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Edited by:
Felipe Fregni,
Harvard Medical School, USA

Reviewed by:
Toshiki Tazoe,
University of Miami, USA
Filippo Brighina,
University of Palermo, Italy

***Correspondence:**
Joaquim P. Brasil-Neto
neurounb@gmail.com

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INTRODUCTION

Chronic pain usually presents a therapeutic challenge. Its pathophysiology, however, is often obscure. Recently, there have been important advances in the understanding of chronic pain, and central mechanisms have been increasingly implicated in its initiation and perpetuation (Melzack, 1990, 2001; Harris, 1999; McCabe et al., 2005, 2008).

The first reports of chronic pain control by means of motor cortex stimulation were published more than two decades ago (Tsubokawa et al., 1991a,b). Since then, favorable effects of motor cortex stimulation upon chronic pain have been repeatedly reported, both with invasive and non-invasive techniques (García-Larrea et al., 1999; Nguyen et al., 2000; Brown, 2001; Nuti et al., 2005; Cioni and Meglio, 2007; Klein et al., 2015). The neurosurgical implantation of epidural or subdural electrodes yields the best results, but non-invasive techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) pose fewer risks to the patient and have been increasingly studied (Klein et al., 2015).

The mechanisms of action of motor cortex stimulation for pain relief are not well understood. However, it has been demonstrated that repeated cortical stimulation, by various techniques, is capable of inducing cortical excitability changes (Hoogendam et al., 2010). In addition to that, it has been shown that, even in the adult brain, changes in sensory afferences due to disease or experimental manipulation lead to cortical reorganization (Merzenich et al., 1983; Sanes et al., 1988; Donoghue et al., 1990).

Several central nervous system (CNS) structures constitute the pain neuromatrix (Melzack, 1990, 2001). According to this theory, chronic pain should not be conceived as a direct consequence of noxious stimulation acting upon sensory pathways, but rather as the result of complex processing of information in the neuromatrix, influenced by its existing synaptic architecture, which is determined by genetic and sensory factors, as well as by influences from other parts of the brain. Significant cortical reorganization might, therefore, strongly influence pain processing in the neuromatrix.

Here, we review clinical and experimental evidence of an important role for cortical reorganization in the pathophysiology of chronic pain. We propose that, in physiological induction of motor cortex plasticity, as in the case of motor learning, the mechanisms responsible for sensory-motor integration remain intact, whereas in disease conditions that same plasticity may be maladaptive and lead to conflict between motor intention and sensory feedback. It has already been suggested that such conflict might lead to chronic pain (Harris, 1999; McCabe et al., 2005; Ramachandran et al., 2007).

COROLLARY DISCHARGES AND CENTRAL PAIN

Corollary discharges play a role in attenuating perception of voluntarily generated movement and also of self-inflicted pain (Berner et al., 2007; Voss et al., 2007; Therrien et al., 2011; Wang et al., 2011). This is similar to the suppression of vision during voluntary saccadic eye movements to avoid blurred vision and to the attenuation of auditory perception during speech. Both phenomena are produced by corollary ("re-afferent") discharges.

In an interesting experiment, McCabe et al. (2005) studied 41 healthy adult volunteers without a history of motor or proprioceptive disorders who performed a series of bilateral upper and lower limb movements while viewing a mirror or a whiteboard, which created varied degrees of sensory-motor conflict during congruent and incongruent limb movements. Sixty-six percent of their subjects reported anomalous sensations in the limbs during performance of the incongruent condition; when they reported pain, this was described as numbness, pins and needles, moderate aching and/or a definite pain.

Harris (1999) hypothesized that central pain might result from an incongruity between intention to move, visual feedback and proprioception. The same author also proposed the existence of a cortical incongruity monitoring region in the right cerebral hemisphere that would also be responsible for the production of nausea in cases of conflict between vestibular and visual afferences. An interesting hint pointing towards a common mechanism implicated in nausea and painful sensations arising from sensory conflict is the analgesic effect of motion-sickness drugs, such as scopolamine and orphenadrine, in some cases of chronic pain (Goldstein, 2002).

Intention to move, as described by Harris (1999), probably relates to corollary discharges in the motor system. We might then substitute corollary discharges for intention to move and hypothesize that central pain might arise whenever mismatches occur between motor corollary discharges, visual feedback and proprioception. According to the PET studies of relative regional cerebral blood flow performed by Fink et al. (1999), "a ventral right lateral prefrontal region is primarily activated by discrepancies between signals from sensory systems, while a more dorsal area in right lateral prefrontal cortex is activated when actions must be maintained in the face of a conflict between intention and sensory outcome".

Transcranial Magnetic Stimulation and Corollary Discharges

Patients sometimes describe the illusion of movement of paralyzed limbs during TMS, a fact that might be explained by production of corollary discharges.

Ellaway et al. (2004) compared the timing of perception of peripherally produced muscle twitches in response to nerve electrical stimulation to that of similar twitches evoked centrally by TMS. Since perception of TMS-evoked twitches occurred, on average, 20 ms later than was the case for those produced by direct nerve stimulation, the authors concluded that the sensation of movement elicited by TMS was due to proprioceptive feedback rather than to intracortical corollary discharges.

In a more recent study, however, Christensen et al. (2010) studied TMS-induced sensation of movement of completely anesthetized limbs. They used repetitive TMS at a frequency of 20 Hz. Afferent and efferent neural signaling was abolished in the arm with ischemic nerve block, and in the leg with spinal nerve block. Under those conditions, they were able to demonstrate persistent sensation of movement, thus confirming its central origin. Both dorsal premotor and motor cortical stimulation produced such corollary discharges, but dorsal premotor cortex stimulation was more effective than motor cortex stimulation. Their conclusion was that "repetitive TMS over dorsal premotor cortex produces a corollary discharge that is perceived as movement".

EXAMPLES OF CHRONIC CENTRAL PAIN AND POSSIBLE SENSORY-MOTOR MISMATCHES

Phantom Pain

A remarkable example of dissociation of motor intention and sensory feedback is given by the phantom of an amputated limb. Such phantoms are frequently painful (Ramachandran et al., 2007). In such a situation, the patient vividly perceives the phantom limb and may or may not be able to move it.

In both cases, there is a mismatch between intention to move and the non-existent sensory feedback. It has been pointed out that paralyzed phantoms are usually painful (Ramachandran et al., 2007). Remarkably, when mirror therapy was used by Ramachandran et al. (2007) to provide visual feedback of the moving phantom (albeit artificially), many patients experienced striking decrease or complete resolution of their phantom pain.

In such cases, there is maladaptive plasticity of the somatotopic representation of the missing limb in the somatosensory cortex, usually with incorporation of the hand and arm area to the face area in the case of upper limb amputation; after leg amputation, sensations are referred from genitals to the phantom foot, likewise indicating functional union of those somatotopic areas (Kaas et al., 1983; Sanes et al., 1988; Ramachandran et al., 2007; de Villers-Sidani and Merzenich, 2011).

Spinal Cord Injuries

Patients with spinal cord injuries often report movement sensation or pain below the level of the spinal lesion, which also amounts to a phantom phenomenon (Melzack, 1990). Siddall and McClelland (1999), in a study of 103 patients, reported movement illusions in nine of them. They found that "four of the nine reported that the legs felt as though they were swinging. The other reports included a sensation of movement in the hands and fingers, including one in which there was a sensation of picking up something between the fingers". This might be explained by central production of corollary discharges. However, in that same series, patients with phantom limb sensations were not able to voluntarily change phantom limb position.

In spinal cord injuries there are also important plastic changes in the CNS. Topka et al. (1991) demonstrated, by TMS mapping of the motor cortex, that there was enhanced excitability of muscle representation areas of body parts rostral to the spinal cord lesion (e.g., of abdominal muscles).

Early Stages of Repetitive Strain Injury (RSI)

RSI is commonly found in workers who perform repetitive, low-amplitude movements with little or no visual feedback, such as typing on a computer keyboard. The low movement amplitude decreases the amount of proprioceptive feedback. Thus, there is a discrepancy between intention to move (i.e., corollary discharges), visual and proprioceptive feedback (Harris, 1999).

In a monkey model of RSI, Byl et al. (1997) trained the animals to perform a repetitive task: closing a handpiece against an 8% force (3–400 trials per day, training at 80–90% accuracy). There was a degradation and dedifferentiation of the normally sharply segregated areas of the hand representation in area 3b. Individual fingers did not have separated cortical representation areas anymore, and this interfered with motor control.

Byl et al. (1996) have examined patients with RSI, and have shown defects in kinaesthesia, stereoacuity, and graphaesthesia, suggesting that those patients had changes in cortical representation similar to those in the monkey model.

Central changes and discrepancy between motor intention, visual feedback and proprioception could explain the presence of persistent pain in RSI before any detectable pathological changes in the affected hand.

Complex Regional Pain Syndrome (CRP)

The first report of possible cortical sensory somatotopic reorganization in patients with CRP was that of McCabe et al. (2003). Five of 16 subjects recruited by them demonstrated referred sensations (RFs) to different body parts during clinical tests of sensation. Such RFs were experienced in real time, were modality specific (touch and pinprick) and were located on the body part immediately adjacent, on Penfield's cortical homunculus, to the stimulated site. This is similar to RFs described in amputees (Ramachandran et al., 2007) and suggests encroaching of cortical representations of body parts affected by CRP upon adjacent cortical somatotopic areas. One possible explanation for such a phenomenon might be that, due to the greatly hiperexcitable afferences from the limb with CRP, its cortical representation area increases and becomes functionally connected to adjacent areas in the homunculus.

Motor symptoms in CRP include weakness, tremor, dystonia and myoclonia. Maihöfner et al. (2007) demonstrated a significant reorganization of central motor circuits in CRP patients, with an increased activation of primary motor and supplementary motor cortices (SMA) as revealed by functional magnetic resonance imaging, during finger tapping of the affected extremiy. Additionally, the ipsilateral motor cortex showed a markedly increased activation.

POSSIBLE THERAPEUTIC APPROACHES FOR PAIN ARISING FROM MALADAPTIVE BRAIN PLASTICITY

Restoration of Normal Cortical Somatotopy

Aberrant somatotopy secondary to maladaptive brain plasticity probably results in changes in corollary discharges, leading to sensory-motor incongruities. Depending on the underlying condition, sensory feedback might also be abnormal. Rehabilitation strategies aiming at normalization of cortical somatotopy might also decrease pain. Harris (1999) suggested, for example, that typists suffering from RSI might benefit from the performance of daily exercises involving individual fingers, coupled with sensory stimulation, so as to restore a normal cortical map of the involved hand. Special keyboards allowing for longer finger excursions during typing, as well as keyboard visualization during typing, would also be advisable.

Mirror Therapy

Mirror therapy has been successfully tried in amputees with chronic phantom pain (Ramachandran et al., 2007) and might also be used in other cases where visual feedback is difficult or impossible (e.g., chronic back pain). According to Ramachandran et al. (2007), in cases of stroke or other conditions leading to movement impairment, the paralysis might be in part

learned, and visual feedback of a normally moving limb on a mirror might help the motor system overcome such learned component.

Neuromodulation Techniques

TMS and tDCS have been tried, with variable success, in cases of chronic pain (Lefaucheur et al., 2001, 2011; Fregni et al., 2006a,b; André-Obadia et al., 2014). The motor cortex has been the most frequent target of all studies, given the success achieved by neurosurgical stimulation (Tsubokawa et al., 1991a,b).

Mechanisms suggested for the beneficial effects of motor cortex stimulation include: (1) stimulation of parallel fibers involved in top-down control of pain perception rather then direct stimulation of motor neurons (Nguyen et al., 2011); (2) indirect stimulation of distant areas, accounting for modulation of emotional aspects of pain (Strafella et al., 2003; Sacco et al., 2014); (3) restoration of defective intracortical inhibition in the motor cortex of chronic pain patients (Lefaucheur et al., 2006); (4) release of endogenous opioids (Maarrawi et al., 2007); and (5) changes in various neurotransmitters in the motor cortex, striatum and limbic system (DosSantos et al., 2016).

On the other hand, the right dorsolateral prefrontal cortex (DLPC) has been mainly targeted, with inhibitory stimulation (either low-frequency repetitive transcranial magnetic stimulation (rTMS) or cathodal tDCS) to treat depression. One study applied high-frequency rTMS to the left DLPC and found a beneficial effect on capsaicin-induced pain (Sacco et al., 2014). However, given the neuroradiological evidence of a role of the right DLPC in the continuous monitoring of sensory-motor incongruities (Fink et al., 1999), that same strategy might also decrease chronic pain. Another interesting possibility for neuromodulation would be to enhance the neuroplastic effects of exercises aimed at restoring normal brain maps by concomitant tDCS, as has already been done in other forms of motor learning (Hashemirad et al., 2015).

CONCLUSION

In conclusion, motor cortex stimulation for treatment of chronic pain with non-invasive neuromodulatory techniques such as rTMS and tDCS had variable degrees of success. A better understanding of the effects of increasing M1 excitability by these techniques upon the physiology of the complex pain neuromatrix is clearly needed.

Modulation of motor corollary discharges might be one such mechanism, and there is evidence that neuromodulatory techniques may have diferent effects on the populations of neurons that generate motor output in M1 and on those neural structures that are involved in generating corollary discharges (Voss et al., 2007). If there is indeed a link between modulation of corollary discharges and analgesia, concomitant stimulation of the dorsal prefrontal area might increase the beneficial effect, since this area has been shown to produce more motor corollary discharges than M1 after rTMS (Christensen et al., 2010). Moreover, left DLPC stimulation by rTMS has been shown to have an analgesic effect of its own, even in the absence of simultaneous M1 stimulation (Sacco et al., 2014).

However, new neuromodulatory strategies might be attempted. More specifically, since PET studies have implicated the right DLPC as a monitoring center for sensory-motor mismatch, it would be interesting to investigate a possible beneficial effect of inhibiting this area, using either low-frequency rTMS or cathodal tDCS, in cases of chronic pain. In fact, Graff-Guerrero et al. (2005) described an analgesic effect of 1 Hz rTMS of the right DLPC. It might have been the result of direct inhibition of this area or of reciprocal inhibitory connections between right and lef DLPC through the corpus callosum. Further neuromodulation studies targeting the right DLPC might eventually help to clarify this issue.

Finally, when the underlying disease causes potentially reversible changes of cortical somatotopic maps, as in RSI cases, exercise programs to restore normal cortical representation of the involved body parts might benefit from adjuvant neuromodulatory treatments, such as tDCS.

AUTHOR CONTRIBUTIONS

JPB-N wrote this article. The author confirms being the sole contributor of this work and approved it for publication.

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frontiers in Human Neuroscience

REVIEW
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Non-invasive Brain Stimulation, a Tool to Revert Maladaptive Plasticity in Neuropathic Pain

*Antonino Naro[1], Demetrio Milardi[1,2], Margherita Russo[1], Carmen Terranova[3], Vincenzo Rizzo[3], Alberto Cacciola[1,2], Silvia Marino[1], Rocco S. Calabrò[1] and Angelo Quartarone[1,2]**

[1] IRCCS Centro Neurolesi "Bonino-Pulejo", Messina, Italy, [2] Department of Biomedical, Dental Sciences and Morphological and Functional Images, University of Messina, Messina, Italy, [3] Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

Neuromodulatory effects of non-invasive brain stimulation (NIBS) have been extensively studied in chronic pain. A hypothetic mechanism of action would be to prevent or revert the ongoing maladaptive plasticity within the pain matrix. In this review, the authors discuss the mechanisms underlying the development of maladaptive plasticity in patients with chronic pain and the putative mechanisms of NIBS in modulating synaptic plasticity in neuropathic pain conditions.

Keywords: TMS, neuropathic pain, NIBS, plasticity, tDCS

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****Correspondence:***
Angelo Quartarone
aquartar@unime.it

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INTRODUCTION

Despite plasticity of the central nervous system is considered a positive adaptive phenomenon related to structural modifications as well as changes in afferent inputs and target outputs, sometimes it may become detrimental causing significant dysfunctions. In this case, functional impairment is the result of maladaptive plasticity (Pascual-Leone et al., 2005).

The best example of maladaptive plasticity in human pathology is focal dystonia where sensory motor plasticity impairment occurs as a consequence of excessive practicing of a stereotyped movement leading to musician's dystonia or writer's cramp (Quartarone et al., 2006).

Non-invasive brain stimulation (NIBS) has a therapeutic potential in focal dystonia, as revealed by clinical studies that have demonstrated the efficacious and long-lasting neuromodulatory effects of repetitive transcranial magnetic stimulation (rTMS) at 1 Hz over primary somatosensory area (S1; Havrankova et al., 2010) and rTMS at 0.2 Hz or 1 Hz over the premotor cortex (Murase et al., 2005; Borich et al., 2009).

Chronic pain is another classic example of maladaptive plasticity in neurology and provides the ideal model to discuss the use of NIBS in the prevention of this pathological event.

Therefore, in the present review, we would like to discuss the potential role of NIBS in blocking and possibly reverting maladaptive plasticity, which is associated with several models of chronic pain, such as central post-stroke pain, pain after spinal cord injury or post-surgical pain.

MALADAPTIVE PLASTICITY IN CHRONIC PAIN

The detection of noxious stimuli (Sherrington, 1906) is a protective process that helps to prevent injury by generating both a reflex withdrawal from the stimulus and a sensation so unpleasant that culminates in complex behavioral strategies to avoid further contact with such noxious stimuli. If

stimuli are particularly intense, sensitization of the nociceptive system may lower the threshold for nociception, increasing the amplitude of withdrawal responses to subsequent inputs (Woolf and Salter, 2000). In this sense, nociceptor-induced sensitization of the somatosensory system is a very efficient adaptive plastic mechanism that makes the system hyper alert in conditions in which a risk of further damage is high, for example, immediately after exposure to an intense or damaging stimulus.

In many clinical syndromes, pain is no longer protective. The pain in these situations arises spontaneously, can be elicited by normally innocuous stimuli (allodynia), is exaggerated and prolonged in response to noxious stimuli (hyperalgesia), and spreads beyond the site of injury (secondary hyperalgesia). Overstimulation of nociceptive pathways induced by chronic conditions (such as inflammatory pain, neuropathic pain, or deafferentation syndromes) in predisposed patients (depending on the influence of the individual genotype on the predisposition to pain chronicity and, consequently, the response to treatment; Baron, 2006) may lead to a massive maladaptive re-arrangement in pain-related structures, called central sensitization, which culminates in secondary hyperalgesia and allodynia. When neurons in the dorsal horn of spinal cord are subject to central sensitization, they develop: (i) an increase in spontaneous activity; (ii) a reduction in the threshold for activation by peripheral stimuli; (iii) an increase in response to supra-threshold stimulation; and (iv) an enlargement of their receptive fields (Woolf and King, 1990; Woolf and Salter, 2000; Ji et al., 2003). Central sensitization induces conversion of nociceptive-specific neurons to wide-dynamic-range neurons that now respond to both innocuous and noxious stimuli (Woolf, 1983, 2007).

In this way, spinal dorsal horn neurons undergoing central sensitization become hyper-excitable and hyper-responsive to nociceptive inputs from already sensitized or injured first order neurons. They also show hyper-responsiveness to inputs from other non-sensitized neurons outside the lesioned area (secondary hyperalgesia) and become responsive to non-nociceptive inputs to the nociceptive pathway (allodynia; Woolf, 2011).

At molecular level, central sensitization of pain is characterized by two different phases: (i) the phosphorylation-dependent stage, resulting in rapid changes of glutamate receptors and ion channel properties. This stage is induced with a short latency (seconds) by intense, repeated, or sustained nociceptor inputs and typically lasts from tens of minutes to several hours in the absence of further nociceptor input. (ii) the transcription-dependent stage, where synthesis of new proteins take place for longer-lasting effects. Both these stages depend on N-methyl-D-aspartate (NMDA) receptors and glutamate signaling modifications and contribute to the induction and maintenance of acute activity-dependent central sensitization (Woolf and Thompson, 1991). Multiple triggers can contribute to the establishment of this process, such as substance P, Calcitonin Gene Related Peptide (CGRP), bradykinin, Brain-Derived Neurotrophic Factor (BDNF), and nitric oxide (Latremoliere and Woolf, 2009). Indeed, these different triggers are released or induced in response to nociceptor activity, and each trigger can initiate the activation of multiple intracellular signaling pathways that lead to a hyperexcitability in dorsal horn neurons. The elevation in intracellular Ca^{2+} has a key role since it activates multiple Ca^{2+}-dependent kinases acting on receptors and ion channels, which increases synaptic efficacy.

Finally, glutamate receptor phosphorylation during central sensitization increases the activity/density of NMDA receptors, leading to an increase in membrane excitability, a facilitation of synaptic strength, a decrease in inhibitory influences in dorsal horn neurons, and the strengthening of nociceptive transmission at the dorsal horn. The role of glutamate in central sensitization is suggested by animal studies that have revealed that NMDA receptor blockade by microinjection of 2-amino-5-phosphonopentanoate in the rostral ventromedial medulla (RVM) attenuated signs of central sensitization (Coutinho et al., 1998; Urban et al., 1999). Similarly, microinjection of MK-801 (a NMDA receptor antagonist) within the thalamus reduces signs of central sensitization (Kawamura et al., 2010; Kaneko et al., 2011). The NIBS-induced plasticity modulation is achieved though several mechanisms, including changes in threshold, in kinetics and trafficking to the membrane of glutamate receptors, increase in inward currents and reduction in outward currents of ion channels, and reduction in inhibitory neurotransmission. Altogether, such mechanisms may lead to changes in the excitability of nociceptive neurons (Carvalho et al., 2000; Fang et al., 2003).

On the other hand, transcription-dependent changes are required for longer-lasting effects; these do not occur only in response to nociceptor activity but also as a consequence of peripheral inflammation and nerve injury (see below). In this stage, different mechanism of synaptic plasticity with some resemblance to long-term potentiation (LTP) and long-term depression (LTD) phenomena occur in central nervous system, thus activating either active synapses (homosynaptic potentiation) or non-activated synapses (heterosynaptic potentiation). The main mediators of these mechanism are thought to be the metabotropic glutamate receptors and the nitroxide (Fagni et al., 2000).

Even though the role of neural circuit remodeling and structural synaptic plasticity in the "pain matrix" in chronic pain has been thought as a secondary epiphenomenon to altered nociceptive signaling in the spinal cord, brain imaging studies on human patients and animal models have suggested the possibility that structural plastic changes in cortical neural circuits may actively contribute to the development of chronic pain symptoms (Kim and Kim, 2016). Indeed, activity-dependent central sensitization is basically an adaptive mechanism, since it prevents, e.g., the use of an injured body part. Nonetheless, central sensitization is pathological when tissue damage persists or if it becomes autonomous and it is maintained in absence of real signaling (Koltzenburg et al., 1992b).

At central level, the abovementioned plastic changes indeed occur in at least six supra-spinal structures of the pain matrix, including the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), anterior cingulate cortex (ACC), insular cortex, and thalamus, which are involved in the phenomena of central sensitization (Urban and Gebhart, 1999; Zhuo, 2007). In addition, neuroimaging studies of induced

secondary hyperalgesia have shown significant activations in the prefrontal cortex, periaqueductal gray (PAG), nucleus cuneiformis, superior colliculi, cerebellum and somatosensory and parietal associative cortices (Iadarola et al., 1998; Baron et al., 1999; Witting et al., 2001; Maihöfner et al., 2004; Zambreanu et al., 2005; Lee et al., 2008; Seifert et al., 2009). On the other hand, pathological and experimentally induced allodynia appear to be associated with enhanced activity of ACC, thalamus, RVM, PAG, insula, orbitofrontal cortex, dorsolateral prefrontal cortices (DLPFCs), putamen, somatosensory cortex, and dorsomedial midbrain. These supra-spinal structures may exert facilitatory pain mechanisms that have been implicated in the generation and maintenance of central sensitization and, possibly, the establishment of chronic pain (Lorenz et al., 2002, 2003; Becerra et al., 2006; Mainero et al., 2007; Seifert and Maihöfner, 2007; Geha et al., 2008).

By a molecular point of view, the NMDA-mediated mechanisms of central sensitization also contributes to the longer-lasting and sometimes persistent pain hypersensitivity (Latremoliere and Woolf, 2009). With regard to neuropathic pain, damaged and non-damaged Adelta- and C-fiber generate spontaneous action potentials after a peripheral nerve injury (ectopic input; Devor and Seltzer, 1999; Djouhri et al., 2006). Such activity in C- and also Adelta-fiber can initiate and maintain activity-dependent central sensitization in the dorsal horn (Koltzenburg et al., 1992a; Devor, 2009). Injured and also non-injured sensory neurons in the dorsal root ganglion exhibit massive changes in transcription, thus altering their membrane properties, growth, and transmitter functions (Xiao et al., 2002). Affected fibers express new transmitters and neuromodulators, including substance P, BDNF, and a cofactor for nitroxide synthase (namely, synthetic enzymes for tetrahydrobiopterin). On the other hand, the stimulation of non-nociceptive fibers triggers the release of factors that can further drive central sensitization (Xiao et al., 2002). The release of these mediators induces a substantial disinhibition in the dorsal horn with loss of Gamma-Aminobutyric-Acid(GABA)ergic and glycinergic inhibitory currents leading to a NMDA-dependent excitotoxicity neuronal death (Moore et al., 2002; Scholz et al., 2005). In addition, there is also an increase in descending excitatory controls from the RVM in the brainstem after peripheral nerve injury, as well as a reduction of descending inhibitory controls (Vera-Portocarrero et al., 2006).

Of note, there are also structural changes as a consequence of the molecular processes described above, which consist in a trans-ganglionic degeneration of C-fiber terminals in lamina II. This degeneration determines the myelinated Abeta-fibers sprouting from laminae III-IV into laminae I-II and making contact with nociceptive-specific neurons (Woolf et al., 1992, 1995). Finally, astrocytes become hyper-active after nerve injury and may play a role in the maintenance of neuropathic pain hypersensitivity (Zhuang et al., 2005).

It is likely that chronic pain, regardless of the etiology (inflammatory or neuropathic) and pain model, may trigger various forms of maladaptive structural plasticity at cortical and sub-cortical level, which in turn could be directly or indirectly involved in the development of sensory, emotional and cognitive symptoms of chronic pain. Since it is well known that structural plasticity of neuronal connections in the brain occurs after a period of several weeks or months after the functional changes, it is mandatory in the future, to intervene as soon as possible before these permanent changes may take place. In this perspective, the use of NIBS in the transition from acute to chronic pain should be explored in the near future to optimize a time window for new efficient therapeutic strategies (Andrade et al., 2013).

GENERAL OVERVIEW ON NON-INVASIVE BRAIN STIMULATION AND CORTICAL PLASTICITY: TMS AND TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS)

TMS and tDCS are methods to painlessly stimulate the cerebral cortex through the intact skull, and can be used to induce long-term effects in several cortical areas.

TMS was conceived as a method to investigate the integrity of the corticospinal outflow from cerebral motor cortex to the spinal cord (Rothwell, 1997). Indeed, TMS pulses readily penetrate the skull and carry an electric stimulating current into the cortex near the surface, thus activating the axons of interneurons of layers II and III that synapse onto the pyramidal neurons of layers V. In this way, the size of the response produced by a given stimulus is sensitive to the excitability of synaptic connections within the cortex, giving an indirect measure of the excitability of intrinsic cortical circuits within the conscious brain. This provides a reliable indicator of any changes produced by neural plasticity within the motor cortex. In addition, when probing motor cortex excitability with single pulses, TMS can also produce long-term changes in excitability if the TMS pulses are applied repetitively (Siebner and Rothwell, 2003). In general, low-frequency stimulation (1 Hz or below) depresses cortical excitability, whereas high-frequency application (5 Hz) increases cortical excitability (Quartarone et al., 2005a). Although the duration of the effects of brief rTMS is short-lasting, longer-lasting after-effects can be achieved by using protocols that include longer periods of stimulation or multiple sessions of rTMS (Quartarone et al., 2006).

Most researchers believe that the long-lasting therapeutic effects of rTMS and the effects of magnetic stimulation on the processes described above are related to two phenomena: LTP and LTD (Ziemann, 2004). The possibility that rTMS induces changes in brain excitability that outlast the stimulation period has prompted its use for therapeutic purposes. The long lasting effects are probably mediated through NMDA synaptic plasticity. Indeed, it has been demonstrated that the long lasting after effects of continuous and intermittent theta burst stimulation on the M1 of healthy volunteers are abolished by using memantine, an NMDA-receptor antagonist (Huang et al., 2008). According to the classical model of induction of LTP- and LTD-like effects, postsynaptic NMDA receptors induce Ca^{2+} influx into neurons. This ionic shift triggers a series of reactions that prompt long-term changes in synaptic strength (Malenka and Bear, 2004). An

important upstream regulator of NMDA synaptic plasticity is the BDNF- Tropomyosin receptor kinase-B (TrkB) system. Indeed, we showed that a 5-day rTMS stimulation enhanced BDNF binding affinity for TrkB, BDNF-TrkB signaling, and NMDA receptor-TrkB interaction in rat prefrontal cortex (Wang et al., 2011). Interestingly, in the same study, we showed that the same protocol could induce an increased BDNF binding affinity for TrkB and enhanced BDNF-TrkB signaling in rats and humans peripheral lymphocytes (Wang et al., 2011). These results suggest that the long lasting excitatory effects of rTMS are at least in part mediated through an upstream regulation of glutamatergic NMDA interaction.

Another important issue about the mechanism of action of rTMS at a system level is that the effects of rTMS are not only restricted to the stimulated region but tend to spread over distant interconnected cortical, subcortical, and spinal structures (Kobayashi and Pascual-Leone, 2003). This possibility opens a window to reach subcortical structures of the pain matrix that are involved in the mechanism of central sensitization. Indeed, neuroimaging studies have revealed that rTMS applied over the primary motor cortex (M1) can modulate the activity in cortical and subcortical regions such as M1, premotor cortex supplementary motor area, thalamus, ACC, somatosensory cortex, insula, red nucleus, and cerebellum (Fox et al., 1997; Siebner et al., 2000; Baudewig et al., 2001; Lee et al., 2003; Okabe et al., 2003; Speer et al., 2003; Takano et al., 2004; Rounis et al., 2005; Gaynor et al., 2008; Cárdenas-Morales et al., 2011). Similarly, DLPFC, ACC, somatosensory cortex, basal ganglia, thalamus, insula, cerebellum and parahippocampus were the main targeted areas when rTMS was applied over DLPFC (Zheng, 2000; Paus et al., 2001; Loo et al., 2003; Michael et al., 2003; Ferrarelli et al., 2004).

tDCS does not induce any action potential, in contrast to other NIBS techniques. It instead modulates membrane excitability by the application of weak electrical currents through two oppositely charged electrodes. The amount of polarization is small, but it can bias the membrane potential of cells, changing the threshold for synaptic activation. When a positively charged electrode (anode) is applied to the surface of the scalp, a fraction of the current is thought to enter the brain and to polarize neurons in proximity of the electrode, thus increasing neuronal firing. Conversely, a negatively charged electrode (cathode) decreases cortical excitability and induces neuronal hyperpolarization (Nitsche and Paulus, 2000; Quartarone et al., 2006).

Application of a small current (1–2 mA), using two electrodes on the scalp for 5–10 min, changes cortical excitability up to 30–60 min afterward (Nitsche and Paulus, 2000). Animal experiments show that this leads to changes in firing rates of neurons while the stimulus is applied, and it is thought that this causes long-term effects on excitability that outlast the stimulation (Fritsch et al., 2010). Similar to the effects of rTMS, after-effects of tDCS are abolished by NMDA receptor antagonists and, hence, are likely to reflect changes in synaptic effectiveness (Nitsche et al., 2003). In addition to NMDA receptors, it is also possible that dopamine and GABA receptors are involved in tDCS-mediated neuroplasticity. Indeed the administration of sulpiride (a D2 receptor antagonist) abolishes tDCS after-effects in normal humans (Nitsche et al., 2006). In addition, lorazepam enhances and prolongs the plastic effects of anodal tDCS (Nitsche et al., 2004). Finally, the effects of tDCS can also be non-synaptic, possibly involving transient changes in the density of protein channels localized below the stimulating electrode or alterations on cAMP and calcium levels (Nitsche et al., 2008). Indeed, the tDCS-induced constant electric field can locally change ionic concentrations, induce a migration of transmembrane proteins (similarly to gel electrophoresis), thus causing steric and conformational changes, and locally alter the tissue acid–base balance (Ardolino et al., 2005). The latter may mainly affect NMDA signaling (Tang et al., 1990).

PUTATIVE MECHANISMS OF TMS IN PAIN TREATMENT

Best practices for neurostimulation on neuropathic pain have been standardized and are available in the European Federation of Neurological Societies for neurostimulation therapy for neuropathic pain (Cruccu et al., 2007). Nonetheless, it is difficult to determine which specific parameters are best for clinical use, since the TMS treatment parameters vary among the published studies. Effectiveness of rTMS depends on the type of neuropathic pain (Lefaucheur, 2006; Leung et al., 2013), although many types of intractable chronic pain have been treated with NIBS. On note, before rTMS can be applied in a patient, it is necessary to accurately determine timing, amount, and duration for each stimulation session, thereby ensuring the optimal duration of effect. Significant results have been reported when employing rTMS at 20 Hz (Fricová et al., 2009; Leung et al., 2013). Nonetheless, rTMS has also been tested at low-frequency stimulation (1 Hz), thus reducing the activity of excitatory circuits in the human motor cortex. However, the best frequency of stimulation for the most effective pain treatment has not yet been resolved. The most commonly targeted area is represented by the M1 contralateral to the position corresponding to the somatotopic location of the pain source; the DLPFC is also of interest, since it seems to have a substantial influence on neuronal circuits involved in the processing of cognitive and emotional aspects of pain (Rokyta and Fricová, 2012).

Beyond frequency and protocol duration, the orientation of the figure-of-eight–shaped coil used to perform the stimulation can influence the nature of the descending volleys elicited by the TMS itself. It is well known that the best analgesic effect is obtained using an antero-posterior orientation (André-Obadia et al., 2008). Taking into account the effects of magnetic field orientation on cortical fibers, pain relief after stimulation of M1 is thought to be produced by activating fibers running superficially within the precentral gyrus, parallel to the convexity of the cortical surface. This pattern of activation is similar to that produced by cathodal epidural motor cortex stimulation (EMCS) at the crown of the precentral gyrus.

Another important issue when designing a NIBS protocol for pain treatment is the timing of rTMS application. It is generally thought that rTMS should be applied as soon as possible in case of intractable pain (Treister et al., 2013).

There are many uncertainties regarding the mechanism of pain relief induced by TMS and the nature and connections of the TMS-activated neuronal circuits (Nguyen et al., 2011). It is thought that NIBS may target the 'top–down' regulatory system controlling anti-nociception. TMS may induce a variety of changes concerning LTD and LTP mechanisms, activation of feedback loops, and changes in neuronal excitability. In fact, neurostimulation can activate axons more easily than cell bodies (Nowak and Bullier, 1998) and, therefore, the mechanisms of action of neurostimulation must be modeled in terms of neural circuits rather than local brain activity changes. Axons recruited by cortical stimulation can be short fibers of intracortical interneurons of layers II and III and afferent or efferent fibers connected with distant structures. Altogether, these changes may decrease sensory pain threshold and inhibits the transmission of sensory information in the spinothalamic tract, depending on the stimulation duration and frequency of each treatment (Lefaucheur et al., 2004; Lefaucheur, 2008).

Of note, the fact that motor but not sensory cortex stimulation relieves pain is not fully understood. Since TMS only directly affects the superficial cortex, the currents rapidly dissipate, the triggered action potentials propagate to distributed neural networks, and M1 projections directly reach pain-modulating structures (including medial thalamus, anterior cingulate/orbitofrontal cortices, and PAG), it is possible that parallel fibers within motor areas may be more suitable than the sensory ones to be targeted by TMS (Irlbacher et al., 2006; Wasserman et al., 2008; Mylius et al., 2012; Peterchev et al., 2012). Indeed, experimental evidence suggests that either epidural stimulation or NIBS may act through an antidromic modulation of the thalamo-cortical pathways (Tsubokawa et al., 1991), thus confirming the important role of the connections between afferent fibers from thalamic nuclei and pyramidal cells concerning nociception control (Villanueva and Fields, 2004). In keeping with this notion, recent studies confirmed that the integrity of the thalamo-cortical tract is required to mediate the anti-nociceptive effects of high-frequency rTMS of M1 (Ohn et al., 2012). In addition, there is evidence suggesting that rTMS may exert a descending modulation within the brainstem, triggered by the cortico-thalamic output (Lefaucheur et al., 2004).

Finally, it should be considered that rTMS of M1 also can act on structures involved in the affective, cognitive, and emotional aspects of pain, such as the cingulate, prefrontal, and orbitofrontal cortices involving opioidergic mechanisms (Tamura et al., 2004). In line with this view, an elevation of serum beta-endorphin concentration was found in patients with phantom limb pain successfully treated by high-frequency rTMS of M1 (Ahmed et al., 2011). Last, naloxone (an opioid receptor antagonist) significantly reduces the analgesic effect of high-frequency rTMS on either M1 or left DLPF in normal volunteers (de Andrade et al., 2011; Taylor et al., 2012). Regarding the neurotransmitters, the mechanisms of action of motor cortex stimulation could also involve inhibitory GABA transmission. This is suggested by some data reporting that intracortical inhibition, a TMS marker of $GABA_A$ transmission in the motor cortex, is reduced in the hemisphere contralateral to neuropathic pain. High-frequency rTMS of M1 can restore intracortical inhibition in correlation with the amount of induced pain relief in patients with neuropathic pain (Lefaucheur et al., 2006, 2012; Fierro et al., 2010; Mhalla et al., 2011).

PUTATIVE MECHANISMS OF tDCS IN PAIN TREATMENT

As compared to TMS, tDCS after-effects are less well characterized (Ngernyam et al., 2013). There is growing evidence confirming the effectiveness of tDCS in treating different types of neuropathic pain (Knotkova et al., 2013), including refractory orofacial pain, fibromyalgia, phantom pain, and back pain (Rokyta et al., 2012; Bolognini et al., 2013; Zhang et al., 2013). Several papers have used different sites of stimulation, including the DLPFC and M1 (Fregni et al., 2006a,b; O'Connell et al., 2013), intensity of stimulation (1–2 mA), time (from 10 up to 30 min; Boggio et al., 2009) and duration of application (i.e., number of sessions per week; Soler et al., 2010).

The mechanism of action of tDCS differs from that of rTMS or epidural motor cortex stimulation, since tDCS-induced current intensity is not high enough to generate action potentials into the brain by itself alone (Lefaucheur, 2016). As outlined above, tDCS increases or decreases the value of axon membrane potential (depolarization or hyperpolarization), according to the polarity (anodal or cathodal) of the stimulation. However, tDCS may exert local and remote effects that, like rTMS, extend well beyond the time of stimulation, reversibly, painlessly, and safely (Nitsche and Paulus, 2001).

Similarly to EMCS and rTMS, the analgesic effects of tDCS may result from the modulation of distant neural structures involved in sensory-discriminative, cognitive, or emotional aspect of chronic pain (Yoon et al., 2014). Indeed, tDCS has preferential analgesic efficacy when the motor cortex receives anodal stimulation, whereas EMCS-induced analgesia is mediated by the placement of cathode over M1 (Holsheimer et al., 2007b; Foerster et al., 2015). This is supported by a recent study showing decreased levels of glutamate in the ACC and thalamus and increased levels of N-acetyl-aspartate and GABA in the posterior and anterior insula after anodal tDCS delivered over the left M1 in patients with non-neuropathic pain (DosSantos et al., 2012). In addition, similarly to rTMS, tDCS may also target the opioid system. In particular, the posterior thalamus was activated by anodal tDCS of M1 in a patient with trigeminal neuropathic pain (Holsheimer et al., 2007a).

EXPERT COMMENTARY AND FUTURE PERSPECTIVES

In line with the current lines of research, we hypothesize that NIBS over M1 could exert its modulation of descending facilitatory pathways and the subsequent disruption of ongoing plastic changes in cortical and sub-cortical structures of the pain matrix, before they consolidate in maladaptive structural phenomena.

Considering that central sensitization is a mechanism mediated by NMDA related synaptic plasticity, it is tempting to consider the possibility of using rTMS at early stages to shift the threshold of plasticity and to trigger homeostatic mechanisms that could reset abnormal plasticity and may prevent the development of maladaptive plasticity phenomena.

In line with this hypothesis, one opportunity of manipulating the abnormal plasticity in acute pain would be to prime the effects of rTMS. Indeed, preconditioning M1 using tDCS prior to 1 Hz rTMS of M1 effectively modulated experimental thermal pain thresholds. In addition, the direction of pain threshold modulation after 1 Hz rTMS depended on the polarity of tDCS priming. For the cathodal (inhibitory) tDCS before 1 Hz rTMS, heat and cold pain thresholds significantly increased. Consistently with the concept that pre-conditioning with tDCS controls the direction of the effect of subsequent rTMS, pain threshold decrease was observed after the anodal (excitatory) tDCS before 1 Hz rTMS (Moloney and Witney, 2013).

Further studies are needed to provide direct evidence of the efficacy of NIBS to prevent the development of maladaptive plasticity at an early stage, using the prime technique. In particular, it would be important to evaluate the homeostatic control of plasticity in patients with neuropathic pain, especially in the acute phase, in order to better define the priming protocol of stimulation.

It is interesting to note that patients suffering from migraine have an alteration of the homeostatic regulation plasticity within the motor cortex between the attacks (Antal et al., 2008), similarly to patients with focal dystonia, another condition characterized by maladaptive plasticity (Quartarone et al., 2005b; Kang et al., 2011).

Finally, since there are no reliable serum biological markers that can assess neuroplasticity, it will be useful to validate surrogate outcomes for neuroplasticity using TMS, high-density electroencephalography, and neuroimaging methods (including tractography), in the attempt to better correlate functional and structural maladaptive plastic changes with clinical outcomes.

AUTHOR CONTRIBUTIONS

AN: work conception and design, work revision, final approval, global agreement. DM: data acquisition, data analysis, data interpretation, work revision, final approval, global agreement. MR: work conception and design, work revision, final approval, global agreement. CT: data acquisition, data analysis, data interpretation, drafting the work, final approval, global agreement. VR: work conception and design, drafting the work, final approval, global agreement. AC: data acquisition, data analysis, data interpretation, drafting the work, final approval, global agreement. SM: work conception and design, work revision, final approval, global agreement. RC: data acquisition, data analysis, data interpretation, work revision, final approval, global agreement. AQ: work conception and design, drafting the work, final approval, global agreement.

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frontiers in Human Neuroscience

GENERAL COMMENTARY
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Commentary: Non-invasive Brain Stimulation, a Tool to Revert Maladaptive Plasticity in Neuropathic Pain

Arman Taheri[1], Mahbod Lajevardi[1], Sara Emami[1], Sanaz Shabani[1] and Hassan Sharifi[2, 3]*

[1] Department of Anesthesiology and Pain Medicine, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran, [2] Department of Medical Surgical Nursing, School of Nursing and Midwifery, Iranshahr University of Medical Sciences, Iranshahr, Iran, [3] School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran

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A commentary on

Non-invasive Brain Stimulation, a Tool to Revert Maladaptive Plasticity in Neuropathic Pain
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Pain as a plastic neurophysiological process is a multifaceted sensory and emotional experience. Recent investigations have demonstrated that the neuroplastic changes occur in the structure and function of the brain, particularly in affective and somatosensory regions, in response to chronic pain. These neuroplastic changes could contribute to the maladaptive plasticity (Apkarian et al., 2011); however, recent evidence suggests that these changes may be modifiable and in some cases reversible with specific targeted interventions (Bushnell et al., 2013). In this regard, several promising interventions such as behavioral interventions, human brain stimulation (HBS), feedback and pharmacological interventions were identified for promoting adaptive neuroplastic changes (Cramer et al., 2011).

HBS has been proven effective for treatment of several chronic painful conditions by improving brain function and/or disrupting its activity. Depolarizing neurons, triggering action potentials and changing the brain cortex excitability are among the most important underlying mechanisms of HBS (Davis and van Koningsbruggen, 2013). In their comprehensive and detailed paper regarding non-invasive brain stimulation (NIBS), Naro et al. (2016) argued that NIBS is an effective treatment for preventing and possibly reverting maladaptive plasticity. They suggested that NIBS could stimulate the several cortical areas of the brain and disrupt the ongoing of plastic changes in cortical and sub-cortical structures of the pain matrix.

We would like to congratulate the authors for their exhaustive review. We want to highlight the promising effects of intermittent fasting for improving adaptive neuroplastic changes. In addition, we want to propound the idea of combining NIBS and intermittent fasting as a promising multifaceted therapeutic approach for preventing or possibly reverting the maladaptive plasticity induced by chronic pain.

The efficacy of fasting in reducing weight, delaying aging, and enhancing health status as well as diminishing pain in rheumatoid arthritis is well-documented (Longo and Mattson, 2014). From the perspective of molecular mechanisms, fasting or food deprivation challenges brain function to manage energy efficiently. It has been argued that intermittent fasting may enhance brain function by improving synaptic plasticity, promoting neurogenesis (Longo and Mattson, 2014), enhancing neuronal stress resistance, increasing synaptogenesis, reducing inflammation, promoting motor

Edited by:
Felipe Fregni, Harvard Medical School, USA

Reviewed by:
Aurore Thibaut, University of Liège, Belgium
Mirret M. El-Hagrassy, Spaulding Rehabilitation Hospital, USA

****Correspondence:***
Sara Emami
saraemami19@gmail.com

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and cognitive function, as well as exerting complex integrated adaptive responses in the brain and enhancing resistance of the brain to injury (Van Praag et al., 2014). According to Van Praag et al. (2014), fasting affects brain areas by changing the expression of genes that encode proteins involved in *"synaptic plasticity, neurotrophic factor signaling, and cellular bioenergetics, disposal of damaged proteins and organelles, and cellular stress resistance."*

From the perspective of pathophysiological mechanisms, the most recent evidence suggests that intermittent fasting could improve chronic pain induced neuroplastic changes (Sibille et al., 2016). According to Sibille et al. (2016), the intermittent fasting and administration of glucose could improve cognitive function, neuroplasticity and activate pain-associated paths in brain and ultimately could increase pain treatment effectiveness. In addition, some animals and humans studies have acknowledged that intermittent fasting could prevent brain dysfunction, improve cognitive function and enhance neuroplasticity (Messier, 2004; Fusco and Pani, 2013).

In summary, available evidence supports the benefits of both NIBS and intermittent fasting for preventing and possibly reverting the maladaptive plasticity in chronic neuropathic painful conditions. Therefore, one question has been raised that merits further attention: Could the addition of intermittent fasting improve the effectiveness of NIBS in preventing, reducing and/or reverting the maladaptive plasticity in patients with chronic pain in comparison to each approach separately?

Considering all of aforementioned evidence, it seems that combining brain stimulation and intermittent fasting appear to be promising strategy for improving adaptive neuroplastic changes. This combination has potential to open new windows for better treatment of chronic pain. Therefore, future well-designed studies will be required to confirm the benefit and clinical efficacy of combining brain stimulation and intermittent fasting in preventing or possibly reverting the development of maladaptive brain plasticity in patients with chronic neuropathic pain.

AUTHOR CONTRIBUTIONS

All of the authors declare that they have all participated in the writing of the paper, and that they have approved the final version.

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Brain Stimulation Therapy for Central Post-Stroke Pain from a Perspective of Interhemispheric Neural Network Remodeling

Takashi Morishita and Tooru Inoue*

Department of Neurosurgery, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

Central post-stroke pain (CPSP) is a debilitating, severe disorder affecting patient quality of life. Since CPSP is refractory to medication, various treatment modalities have been tried with marginal results. Following the first report of epidural motor cortex (M1) stimulation (MCS) for CPSP, many researchers have investigated the mechanisms of electrical stimulation of the M1. CPSP is currently considered to be a maladapted network reorganization problem following stroke, and recent studies have revealed that the activities of the impaired hemisphere after stroke may be inhibited by the contralesional hemisphere. Even though this interhemispheric inhibition (IHI) theory was originally proposed to explain the motor recovery process in stroke patients, we considered that IHI may also contribute to the CPSP mechanism. Based on the IHI theory and the fact that electrical stimulation of the M1 suppresses CPSP, we hypothesized that the inhibitory signals from the contralesional hemisphere may suppress the activities of the M1 in the ipsilesional hemisphere, and therefore pain suppression mechanisms may be malfunctioning in CPSP patients. In this context, transcranial direct current stimulation (tDCS) was considered to be a reasonable procedure to address the interhemispheric imbalance, as the bilateral M1 can be simultaneously stimulated using an anode (excitatory) and cathode (inhibitory). In this article, we review the potential mechanisms and propose a new model of CPSP. We also report two cases where CPSP was addressed with tDCS, discuss the potential roles of tDCS in the treatment of CPSP, and make recommendations for future studies.

Keywords: transcranial direct current stimulation, post-stroke central pain, interhemispheric inhibition, motor cortex, pain suppression

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****Correspondence:***
Takashi Morishita
tmorishita@fukuoka-u.ac.jp

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INTRODUCTION

Stroke is a vascular disorder of the brain causing various symptoms including motor weakness, sensory disturbances, balance problems, and spasticity. Pain after stroke can be caused by various conditions secondary to spasticity, and a recent study reported that as many as 39.0% of stroke patients experienced new-onset chronic pain after stroke (Klit et al., 2011). Among various pain etiologies, central post-stroke pain (CPSP) is an especially debilitating, severe disorder characterized by intractable pain with abnormal sensations such as burning and allodynia, which severely affect the quality of life (QOL).

CPSP was first described by Dejerine and Roussy as a consequence of stroke-related lesions in the thalamus (Dejerine and Roussy, 1906); however, lesions in other brain structures in the somatosensory pathway may result in CPSP (MacGowan et al., 1997; Klit et al., 2009). In the somatosensory pathway, lesions in the ventrocaudalis portae nucleus of the thalamus and lateral medulla particularly predispose patients to CPSP (Sprenger et al., 2012). The prevalence of CPSP has been reported to be 1–12% (Andersen et al., 1995; MacGowan et al., 1997; Lampl et al., 2002; Weimar et al., 2002; Widar et al., 2002; Appelros, 2006; Kuptniratsaikul et al., 2009; Lundström et al., 2009).

Even though the mechanisms of CPSP remain unclear, CPSP has been considered to be a maladapted network reorganization problem after stroke (Hosomi et al., 2015), as CPSP usually occurs in a delayed fashion from weeks to months after the initial insult (Nasreddine and Saver, 1997). To explain the abnormal network conditions of CPSP, various circuit models have been proposed (Klit et al., 2009; Hosomi et al., 2015). In this article, we review the potential mechanisms and propose a new model of CPSP. We also report two cases where CPSP was ameliorated with transcranial direct current stimulation (tDCS) and discuss the potential roles of tDCS in the treatment of CPSP and future studies.

MALFUNCTIONING NEURONAL CIRCUITS

CPSP is characterized by either spontaneous or evoked unpleasant feelings described as allodynia, hyperalgesia, and dysesthesia. Insults to the central nervous system (CNS) induce various responses including neurochemical reactions, cytotoxicity, and inflammation at the cellular levels, and these changes have been considered to induce maladapted neuroplasticity resulting in the abnormal sensations of CPSP (Yezierski, 2005; Costigan et al., 2009).

Hyperactivities in pain-related structures have been described in various studies and are supported by the fact that medications suppressing neuronal activities were reported to be effective for CPSP (Leijon and Boivie, 1989; Attal et al., 2000; Vestergaard et al., 2001; Canavero and Bonicalzi, 2004; Vranken et al., 2008). In particular, spontaneous pain has been considered to be due to hyperexcitability in the pain circuits of the brain (Vestergaard et al., 1995), and neurophysiological studies revealed hyperactive thalamic bursting activities in CPSP cases (Lenz et al., 1994, 2004). These findings were also supported by neuroimaging studies that showed increased regional cerebral blood flow (rCBF) in the thalamus of patients with Wallengerg syndrome and CPSP (Peyron et al., 1998).

The mechanisms of abnormal hyperactivities in the pain network could be also explained by "disinhibition theory" (Craig and Bushnell, 1994). The CNS is controlled by a delicate balance between excitation and inhibition (Vanegas and Schaible, 2004; Hull and Scanziani, 2007; Bee and Dickenson, 2008; Costigan et al., 2009; Heinricher et al., 2009), and the pain sensations in CPSP are considered to be caused by an imbalance. Burning pain could be explained by the damage to the transmission system for cold sensations, for instance.

Conversely, it has been reported that additional stroke lesions may either aggravate (Kim, 1999) or alleviate the preexisting pain (Soria and Fine, 1991; Helmchen et al., 2002). These cases illustrated that CPSP is a network reorganization disorder. It should be noted that there are affective and sensory components in pain sensation (Sewards and Sewards, 2002a,b). Limbic structures including the amygdala and insular cortex are a part of the affective pain circuit (Price, 2000), and there is a possibility that CPSP involves the malfunctioning of the circuit. Additionally, a recent resting-state functional magnetic resonance imaging (fMRI) study has shown changes in the default mode network activities in chronic pain states (Baliki et al., 2014).

INVASIVE BRAIN STIMULATION PROCEDURES

In a classic clinical experience, the applications of thalamotomy (Menon, 2014) and postcentral gyrectomy were described (Erickson et al., 1952). These procedures were performed based on a theory that the thalamus and somatosensory cortex are the "center of the pain perception," and removing these structures might decrease pain sensations. These procedures are no longer performed in modern neurosurgery practice. Currently, there are two neurosurgical approaches to CPSP: deep brain stimulation (DBS) and invasive motor cortex (M1) stimulation (MCS). These brain stimulation therapies have been widely used, as they are considered to be safer than destruction surgery, due to the possibility of reversibility.

Various brain structures have been stimulated with DBS methods to treat intractable pain. The most frequently reported DBS targets have been the periaqueductal gray matter (PAG), periventricular gray matter (PVG), and ventroposterior (VP) nucleus of the thalamus (Hosobuchi, 1983; Tsubokawa et al., 1984; Owen et al., 2006). The mechanism of action of PAG/PVG stimulation was originally reported to involve activation of the μ-opioid system (Hosobuchi et al., 1977) even though increases in endogenous opioid levels were not consistently found in these cases (Dionne et al., 1984; Young and Chambi, 1987). Electrical stimulation of the VP nucleus has also been considered to suppress the abnormal firing in the thalamus. However, no randomized controlled studies have definitively demonstrated favorable outcomes with these methods (Bittar et al., 2005). In both procedures, PAG/PVG and Vc DBS leads were unilaterally implanted in the ipsilesional hemisphere. Another classic DBS target was the septal nuclei, which were considered to be associated with pleasurable feelings (Heath, 1963). However, Gol reported that electrical stimulation of the septal nuclei was only effective in one of six cases (Gol, 1967).

Recently, neuropsychiatric DBS approaches have been applied to address the affective components of pain in pain disorders. The DBS targets included limbic structures, the anterior cingulate cortex (ACC; Boccard et al., 2014a,b), and the ventral capsule/ventral striatum (VC/VS; Machado et al., 2013; Morishita et al., 2015a). The ACC stimulation was applied based on the experience of anterior cingulotomy for intractable pain and obsessive-compulsive disorder (OCD; Brotis et al., 2009).

Boccard et al. reported favorable outcomes in the pain levels and QOL of 11 patients who had follow-up evaluations after bilateral ACC DBS (Boccard et al., 2014a). However, Morishita et al. (2015a) reported an unsuccessful case of unilateral VC/VS stimulation. Currently, a bilateral VC/VS DBS study is underway (clinicalTrial.gov Identifier: NCT01072656).

In the early 1990's, MCS was first introduced by Tsubokawa et al. (1991a,b). Since then, many researchers have replicated the effects of electrical stimulation of the M1 using either invasive or non-invasive methods (Lima and Fregni, 2008). Nguyen et al. (2011) reported that MCS showed greater than 40% pain reduction on the visual analog scale (VAS) in 60% of CPSP patients in their literature review. The efficacy of MCS has been proven by several controlled trials as well (Nguyen et al., 2008; Velasco et al., 2008).

Tsubokawa proposed the descending pain inhibitory mechanism in his report and suggested that electrical stimulation of the upper level structures in the sensory pathway may inhibit deafferentation pain from lower level lesions (Tsubokawa et al., 1993). Peyron et al. (2000, 2007) revealed that MCS activated remote areas, including the cingulate gyrus. A recent animal study showed that MCS suppressed activity in the primary somatosensory cortex and prefrontal cortex (Jiang et al., 2014). Interestingly, pain relief usually is delayed several days to weeks following the start of MCS therapy (Nguyen et al., 2011). These findings may indicate that pain relief by MCS can be achieved by global pain network modulation involving corticocortical and thalamocortical loops rather than merely activating the primary M1. Katayama et al. (1998) reported that MCS more effectively addressed CPSP in patients with better motor functions. This finding may indicate that the degree of damage in the corticospinal tract (CST) is associated with the integrity of the pain inhibitory network involving the M1.

INTERHEMISPHERIC INTERACTIONS

Various animal and neuroimaging studies have shown post-stroke neuroplastic changes in the neural network involving the contralesional hemisphere (Xerri et al., 2014). For example, a recent animal study demonstrated enhanced activity in the somatosensory cortex of the contralesional hemisphere only 30–50 min after a small ischemic lesion was induced in the somatosensory cortex (Mohajerani et al., 2011). Additionally, compensatory remodeling with functional recovery reportedly occurred in the contralesional hemisphere 1 month after the functional loss of the ipsilesional hemisphere in the recovery process after complete infarction of the somatosensory cortex (Takatsuru et al., 2009).

fMRI studies have shown contralesional M1 activation during tasks using the impaired upper extremity in stroke cases (Rehme et al., 2011; Grefkes and Fink, 2014). Recent studies using transcranial magnetic stimulation (TMS) and MRI revealed that abnormal activity of the contralesional M1 might inhibit motor recovery after stroke (Grefkes and Fink, 2014; Volz et al., 2015), and a resting-state fMRI study revealed increased interhemispheric M1-M1 functional connectivity in stroke patients compared with that in healthy volunteers (Liu et al., 2015). All of these findings underpin the importance of the role of the contralesional hemisphere in the network reorganization after stroke. In this context, there is a possibility that the maladapted neuroplasticity in the contralesional hemisphere may partly contribute to the abnormal pain sensations in CPSP. In fact, it has been reported that additional stroke lesions in the contralateral hemisphere to the first stroke lesion influenced the preexisting CPSP (Kim, 1999; Helmchen et al., 2002). We hypothesized that the inhibitory signals from the contralesional hemisphere may suppress the activities of the M1 in the lesioned hemisphere, and therefore pain suppression mechanisms may be malfunctioning in the CPSP patients (**Figure 1**).

tDCS FOR CPSP

As mentioned above, past studies have shown that recovery of the impaired limb may be inhibited by abnormal contralesional M1 activities. This interhemispheric inhibition (IHI) theory has been applied for neurorehabilitation therapy using tDCS to improve motor functions (Lüdemann-Podubecká et al., 2014). In tDCS therapy, the bilateral motor cortices can be stimulated simultaneously using an anode (excitatory) and cathode (inhibitory). tDCS, therefore, has been considered to be a reasonable treatment modality to address interhemispheric imbalance due to stroke. Based on the IHI theory and the fact that anodal M1 stimulation suppresses the CPSP, we considered that tDCS may address both interhemispheric imbalance in neural activities and pain at the same time.

Only a few reports have concerned the use of tDCS for CPSP, even though tDCS have widely used for the treatment of other types of neuropathic pain (Fregni et al., 2006; DosSantos et al., 2012; Mehta et al., 2015). Most studies placed the anode over the contralateral M1 to the painful site and the cathode over the supraorbital area on the other side, and continuous stimulation was administered for 20 min at 2000 μA. Bae et al. used the same tDCS method for CPSP cases and reported the clinical effects of active tDCS therapy group compared to a sham stimulation group (Bae et al., 2014). In the same report, the authors concluded that pain reduction was achieved only in the active stimulation group. Another report, from our group, showed that tDCS improved CPSP as well as motor functions, and an imaging study demonstrated improved interhemispheric balance (Morishita et al., 2015b).

Here we present two representative CPSP cases where pain reduction was successfully achieved with tDCS therapy using a commercially available stimulator (DC-Stimulator plus, neuroConn, Germany). For the tDCS procedure, we positioned the electrode aiming at the M1, and the anode and cathode were placed on the lesional and the contralesional sides, respectively, on C3 and C4 of the international 10–20 electroencephalography system. We administered 2500 μA of continuous stimulation for 20 or 25 min. These parameters were selected based on the previous tDCS report concerning safety (Poreisz et al., 2007).

The first case was a 72-year-old woman with dysesthesia in her right hemibody, who had had a left thalamic hemorrhage 1 year prior. The pain started 3 months after the left thalamic hemorrhage, and she rated the pain as 60/100 on the VAS.

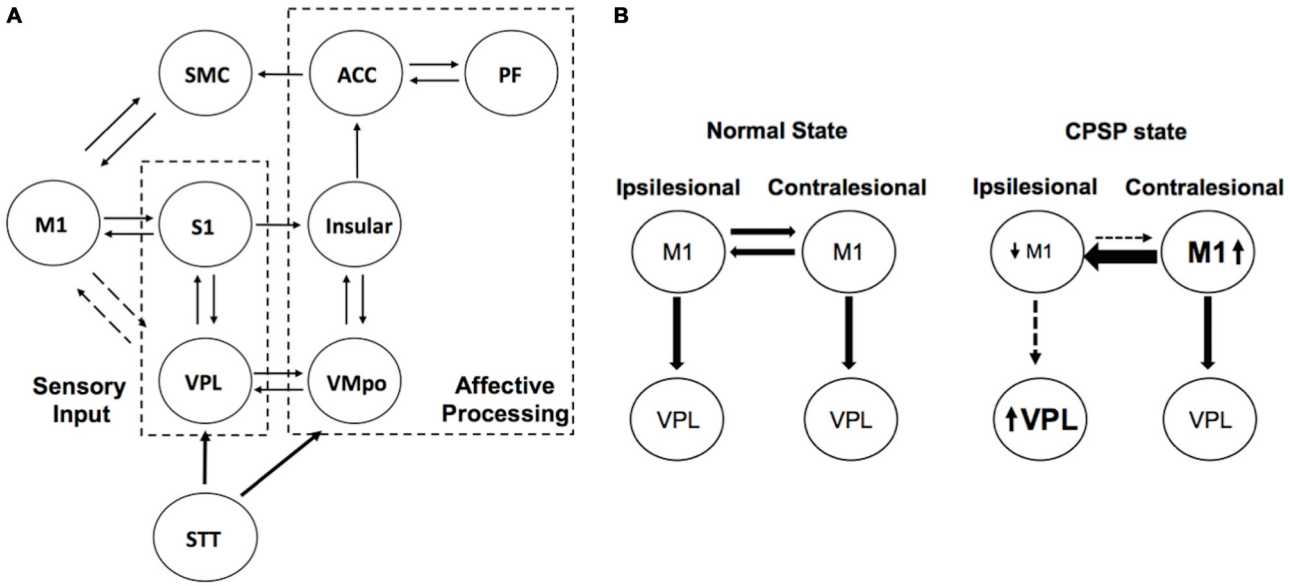

FIGURE 1 | Schema explaining interhemispheric inhibition (IHI) in central post-stroke pain (CPSP). (A) Simplified pain circuit model composed of lateral and medial thalamic pain pathways. The motor cortex (M1)-VPL connection is described as dotted lines as there is an indirect connection. It should be also noted that there is an indirect somatosensory projection from the S1 to insular cortex through posterior parietal cortex (Price, 2000). **(B)** Impaired descending inhibition pathways from primary M1. Ipsilesional M1 activity is decreased due to not only stroke lesion but also inhibitory signals from the contralateral M1. ACC, anterior cingulate cortex; PF, prefrontal; SMC, supplementary motor cortex; STT, spinothalamic tract; VMPo, posterior ventromedial nucleus of the thalamus; VPL, ventral posterolateral nucleus of the thalamus.

In this case, we administered 10 sham stimulations and 10 active stimulations during 2 weeks at a hospitalized setting. Her pain level was evaluated in a double-blinded fashion, such that the rater and the patient did not know whether sham or active stimulation had been administered at each session. The pain level was significantly lower with active stimulation than sham stimulation (active vs. sham: 26.9 ± 5.49 vs. 39.5 ± 13.4, $p = 0.006$). Motor function was evaluated using an action research arm test (ARAT), which demonstrated improvement from 30 (baseline) to 37 (after all sessions). We also performed functional near infrared spectroscopy (fNIRS) to evaluate the interhemispheric balance at baseline and after all tDCS sessions. The fNIRS study showed improvement in the imbalance of the motor activity between the left and right hemispheres, and the activated motor area was more focused on the left hemisphere (**Figure 2**). This fNIRS finding was consistent with the results of previous fMRI studies (Grefkes and Fink, 2014). This case was previously reported elsewhere (Morishita et al., 2015b).

The second case was a 66-year-old man who started having burning pain and allodynia in his left hemibody 3 months after a right thalamic hemorrhage (**Figure 3**). He visited us 16 months after the onset of CPSP. We administered tDCS therapy twice a week on an outpatient basis. The tDCS settings were the same as in case 1. Before the tDCS therapy, he rated his pain in his upper extremity as a 96 on the VAS; however, he rated his pain as 48 on the VAS following 15 sessions of tDCS therapy. In this case, we evaluated the motor function of the impaired upper extremity using the Fugl–Meyer Assessment scale, and the upper extremity score improved from 57 (baseline) to 62 (after all sessions).

As presented in our illustrative cases, tDCS may be a promising treatment option for CPSP cases. Interestingly, our cases showed improvements in motor function as well as pain. It may be debated whether the motor recovery was secondary to the pain reduction or not, however, we consider that electrical stimulation of the M1 itself results in motor recovery, as shown by various studies (Lüdemann-Podubecká et al., 2014). To test our theory and prove the effectiveness of tDCS for CPSP, further clinical studies are warranted. Additionally, even though case reports are not enough convincible to conclude that addition of contralateral cathodal tDCS had any additional effect over ipsilateral anodal stimulation alone, we believe this bilateral tDCS approach may address the abnormalities in the interhemispheric neural network.

CONCLUSIONS

In this article, we briefly reviewed the basic theories concerning the mechanisms of CPSP and proposed a CPSP neurocircuit model involving the contralesional M1. Malfunctioning neuronal circuits in CPSP may involve the contralesional hemisphere, and IHI may play an important role in pain mechanisms. Most brain stimulation therapies in the past have targeted the ipsilesional hemisphere, but we hypothesize that intervening in both hemispheres may be more effective to address CPSP. Further investigation of network abnormalities in the contralesional hemisphere may shed light on the potential mechanisms of CPSP.

Rather than trying to address the "abnormal region" in the brain, a neural network modulation approach to the global

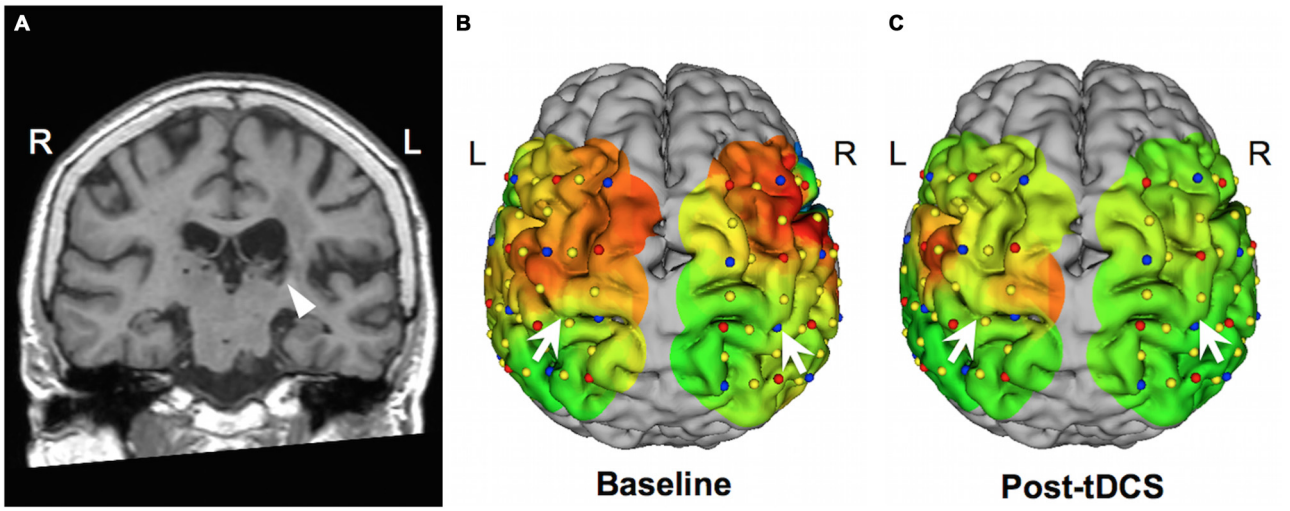

FIGURE 2 | Neuroimaging studies in case 1. (A) Coronal view of a T1-weighted image. The arrow indicates a post-hemorrhagic lesion in the left thalamus. **(B,C)** Functional near infrared spectroscopy (fNIRS) results showing oxyhemoglobin level mapping during a right fist closure and opening task over a 3-D reconstructed image of the patient's brain. Red and green indicate higher and lower functional activity levels, respectively. Arrows indicate the central sulci. Following all transcranial direct current stimulation (tDCS) sessions, activity in the right hemisphere was reduced. (This figure was adapted from Morishita et al. (2015b) with permission).

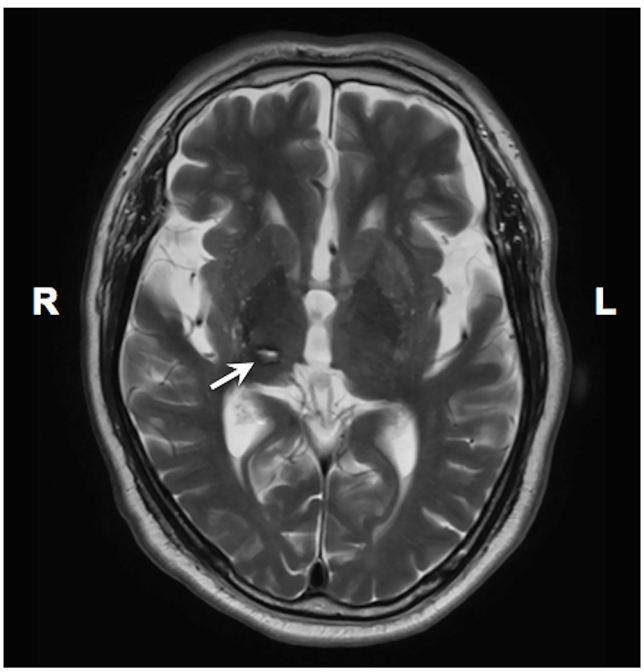

FIGURE 3 | A T2 weighted MRI image showing thalamic lesion in case 2. An arrow indicates the stroke lesion in the right thalamus.

pain system would be desirable in future studies (Thompson et al., 2012). In this context, non-invasive brain stimulation techniques such as TMS and tDCS are excellent treatment options as well as research tools. Since a number of studies have already shown the efficacy of electrical stimulation of the M1 in the ipsilesional hemisphere, neuroplastic changes following magnetic or electrical stimulation of the contralesional may also be observed. Based on these findings, more effective brain stimulation parameters may be found.

Due to the heterogeneous nature of stroke, CPSP etiology varies among patients, and the number of patients who receive brain stimulation therapy is limited. Therefore, cross-over study designs having active and sham stimulation periods for each case might be desirable to test the efficacy of new stimulation approaches. For future clinical trials using brain stimulation techniques, we also propose formation of a registry database recording clinically important variables including: (1) anatomical location of the stroke lesion; (2) time between the stroke onset and CPSP onset; (3) detailed pain assessment using universal measures; (4) details of stimulation methods and parameters; and (5) clinical outcomes, inclusive of post-procedure pain scores and adverse events. This will allow us to analyze the data from a standardized cohort and lead to better understanding of CPSP etiology.

AUTHOR CONTRIBUTIONS

TM contributed to conception of the article and data collections, and wrote the manuscript. TI supervised the manuscript writing and reviewed the manuscript.

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A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal, and Neuroplasticity Regulation Systems in Chronic Myofascial Pain

Leonardo M. Botelho[1,2,3], *Leon Morales-Quezada*[4], *Joanna R. Rozisky*[1,3], *Aline P. Brietzke*[1,3], *Iraci L. S. Torres*[1,5], *Alicia Deitos*[1,3], *Felipe Fregni*[4,6] and *Wolnei Caumo*[1,2,3,7]*

[1] Post-Graduate Program in Medical Sciences, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [2] Pain and Palliative Care Service at Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3] Laboratory of Pain and Neuromodulation, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil, [4] Laboratory of Neuromodulation of Spaulding Rehabilitation of Harvard Medical School, Boston, MA, USA, [5] Pharmacology Department, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [6] Department of Physical Medicine and Rehabilitation, Harvard Medical School, Boston, MA, USA, [7] Surgery Department, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

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***Correspondence:**
Wolnei Caumo
wcaumo@hcpa.edu.br

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Myofascial pain syndrome (MPS) is a leading cause of chronic musculoskeletal pain. However, its neurobiological mechanisms are not entirely elucidated. Given the complex interaction between the networks involved in pain process, our approach, to providing insights into the neural mechanisms of pain, was to investigate the relationship between neurophysiological, neurochemical and clinical outcomes such as corticospinal excitability. Recent evidence has demonstrated that three neural systems are affected in chronic pain: (i) motor corticospinal system; (ii) internal descending pain modulation system; and (iii) the system regulating neuroplasticity. In this cross-sectional study, we aimed to examine the relationship between these three central systems in patients with chronic MPS of whom do/do not respond to the Conditioned Pain Modulation Task (CPM-task). The CPM-task was to immerse her non-dominant hand in cold water (0−1°C) to produce a heterotopic nociceptive stimulus. Corticospinal excitability was the primary outcome; specifically, the motor evoked potential (MEP) and intracortical facilitation (ICF) as assessed by transcranial magnetic stimulation (TMS). Secondary outcomes were the cortical excitability parameters [current silent period (CSP) and short intracortical inhibition (SICI)], serum brain-derived neurotrophic factor (BDNF), heat pain threshold (HPT), and the disability related to pain (DRP). We included 33 women, (18–65 years old). The MANCOVA model using Bonferroni's Multiple Comparison Test revealed that non-responders ($n = 10$) compared to responders ($n = 23$) presented increased intracortical facilitation (ICF; mean ± *SD*) 1.43 (0.3) vs. 1.11 (0.12), greater motor-evoked potential amplitude (μV) 1.93 (0.54) vs. 1.40 (0.27), as well a higher serum BDNF (pg/Ml) 32.56 (9.95) vs. 25.59 (10.24), ($P < 0.05$ for all). Also, non-responders presented a

higher level of DRP and decreased HPT ($P < 0.05$ for all). These findings suggest that the loss of net descending pain inhibition was associated with an increase in ICF, serum BDNF levels, and DRP. We propose a framework to explain the relationship and potential directionality of these factors. In this framework we hypothesize that increased central sensitization leads to a loss of descending pain inhibition that triggers compensatory mechanisms as shown by increased motor cortical excitability.

Keywords: BNDF, cortical excitability, CPM, MEP, TMS, QST, chronic pain

INTRODUCTION

Myofascial pain syndrome (MPS) is a leading cause of chronic musculoskeletal pain (Simons et al., 1999). MPS has been associated with disability, and also with dysfunction of corticospinal conduction as assessed by motor evoked potential (MEP; Vidor et al., 2014). As with other chronic pain syndromes, the mechanisms of MPS are not entirely elucidated. A major barrier to the understanding of these mechanisms is that pain is an experience orchestrated by a network of cortical regions, elements of the limbic system and the spine-bulbospinal loop. The ascending portion of this circuit involves the spine reticular tract (Willer et al., 1999), which comprises modulatory systems such as the opioidergic (Le Bars et al., 1981; Willer et al., 1990), noradrenergic (Sanada et al., 2009; Makino et al., 2010), and serotonergic systems (Chitour et al., 1982). Given this complex interaction, our approach to provide insights into the neural mechanisms of pain was to investigate the relationship between neurophysiological, neurochemical, and clinical outcomes such as corticospinal excitability as indexed by transcranial magnetic stimulation (TMS) measurements, conditioned pain modulation (CPM) to measure the descendent endogenous inhibitory pain system and serum brain-derived neurotrophic factor (BDNF) as a critical marker of neuroplasticity. Corticospinal excitability as indexed by TMS has become a reliable marker in chronic pain syndromes, including MPS (Vidor et al., 2014). It has been shown that pain and disability are associated with an imbalance between excitatory and inhibitory systems as assessed by increased intracortical facilitation (ICF) and by a reduced current silent period (CSP; Vidor et al., 2014; a proxy of glutamatergic activity), a higher pain catastrophizing score (Volz et al., 2013a) and a higher trait anxiety score (Vidor et al., 2014).

The CPM (Yarnitsky, 2010) involves the diffuse noxious inhibitory control (DNIC) system. The DNIC system assesses the reduction in the pain sensation on the stimulus by a simultaneous pain input from distant sites of the body (Le Bars, 2002). While the CPM assesses how much, a conditioning stimulus can reduce the pain response evoked by the other strong, painful stimuli at a distant large body surface area (the test stimulus; Volz et al., 2013a). When the CPM-task increases pain, this indicates a disruption of endogenous pain-inhibitory processes and a summation effect (King, 2014), which amplifies the pain response and it is a process of the central sensitization (Boyer et al., 2014). It appears that these pain-related neural changes maintain the dysfunction of endogenous descending inhibitory mechanisms as observed in many chronic pain syndromes including knee osteoarthritis (Arendt-Nielsen et al., 2010), chronic pancreatitis (Olesen et al., 2010), rheumatoid arthritis (Leffler et al., 2002a), long-term trapezius myalgia (Leffler et al., 2002b), irritable bowel syndrome (King et al., 2009), temporomandibular disorder (King et al., 2009), fibromyalgia (Staud et al., 2003), and MPS (Pielsticker et al., 2005).

BDNF, a critical molecule for the development and maintenance of cortical neurons and cortical synapses, interacts with the descendant modulatory system. Clinical studies have found higher levels of BDNF in the blood (Deitos et al., 2015) and cerebrospinal fluid in patients with chronic pain (Bø et al., 2009), and in fibromyalgia has been associated with a lower pain threshold (Zanette et al., 2014). This set of evidence demonstrates that there are three main neural systems involved in chronic pain: (i) the corticospinal motor system; (ii) the internal descending pain modulation system; and (iii) the system regulating neuroplasticity. Our hypothesis is that disruption of the infra cortical medulator system, as assessed by pain scores during the CPM-task, is correlated with dysfunction of corticospinal conduction and disinhibition at the cortical level, due to increases in the MEP amplitude, ICF, and serum BDNF level. We aimed to analyze the relationship between these three central systems in chronic MPS patients in responders and non-responders to Quantitative Sensory Testing (QST) during the immersion of her non-dominant hand in cold water (0−1°C) to produce a heterotopic nociceptive stimulus (CPM-task). To determine the CPM we used the difference between the pain score on NPS (0–10) QST during cold water immersion (QST+CPM) at the temperature of the point at which subjects felt 6/10 pain on the NPS scale [during the initial time period (T0)]. Our primary outcomes were the MEP and ICF as assessed by TMS. The secondary outcomes were the cortical excitability parameters [current silent period (CSP) and short intracortical inhibition (SICI)], serum BDNF level, heat pain threshold (HPT), and the disability related to pain.

MATERIALS AND METHODS

This exploratory study was performed at the Hospital de Clinicas de Porto Alegre in Porto Alegre, Brazil. The study protocol was approved by the Institutional Review Board (IRB 0000921) at the Hospital de Clinicas de Porto Alegre and conducted according to the Declaration of Helsinki. All subjects provided written informed consent for their participation. We administered

clinical assessment scales validated in the Brazilian population. Additionally, we collected behavioral measurements (i.e., several pain assessments) and neurophysiological measurements (i.e., motor córtex excitability as indexed by TMS) to establish baseline data.

Design Overview, Settings, and Participant

We recruited the participants from the general population through public postings in different health care units and physicians' referrals from the Chronic Pain Service at the Hospital de Clínicas de Porto Alegre. The inclusion criteria included the following: (1) right-handed females (2) aged 19–65 years old, (3) confirmed the diagnosis of MPS in the upper body segment for at least 3 months before enrollment, and (4) limitation in routine activities due to MPS. Furthermore, patients needed to present with a pain score of the visual analog scale (VAS) at least of 4 cm (i.e., moderate or severe pain; Palos et al., 2006), associated with functional disability in most days of the 3 months before enrollment. Disability associated with MPS was evaluated using a questionnaire that included six specific questions (yes/no). These questions aimed at assessing interference with work, personal relationships, pleasure obtained during activities, personal goals, clear thinking (i.e., problem solving, concentrating, or remembering), and responsibilities at home during the past 3 months. For enrollment, an affirmative answer to one or more of these questions was necessary to ensure that chronic pain was decreasing the patient's quality of life. Moreover, the diagnosis of MPS was confirmed by a second experienced independent examiner with significant clinical experience related to chronic pain. MPS criteria were the presence of regional pain, normal neurological examination, stiffness in the target muscles; decreased the range of motion, the presence of palpable nodules, tender points, trigger points, taut bands, and pain characterized as hollow, dull, or deep that was exacerbated by stress. To standardize the severity of MPS and to distinguish neuropathic pain from ongoing nociception, were included only patients with the Neuropathic Pain Diagnostic Questionnaire (DN4) with a score equal to or higher than four (Bouhassira et al., 2005). The presence of previous surgery on the affected areas or other pain disorders such as rheumatoid arthritis, radiculopathy, and fibromyalgia; and frequent use of steroidal and non-steroidal anti-inflammatory medications were exclusion criteria.

Anticipating an effect size (f) of 0.4 for a multiple regression analysis allowing for two predictors and a type I and II errors of 0.05 and 0.20, respectively, and the minimum sample size was 30 patients. Finally, considering the likely attrition rate and other unexpected factors, the required sample size was determined to be 33 patients (**Figure 1**).

Instruments and Assessments

The tools used to assess psychological state were validated in the Brazilian population (Staud et al., 2003; Kaipper et al.,

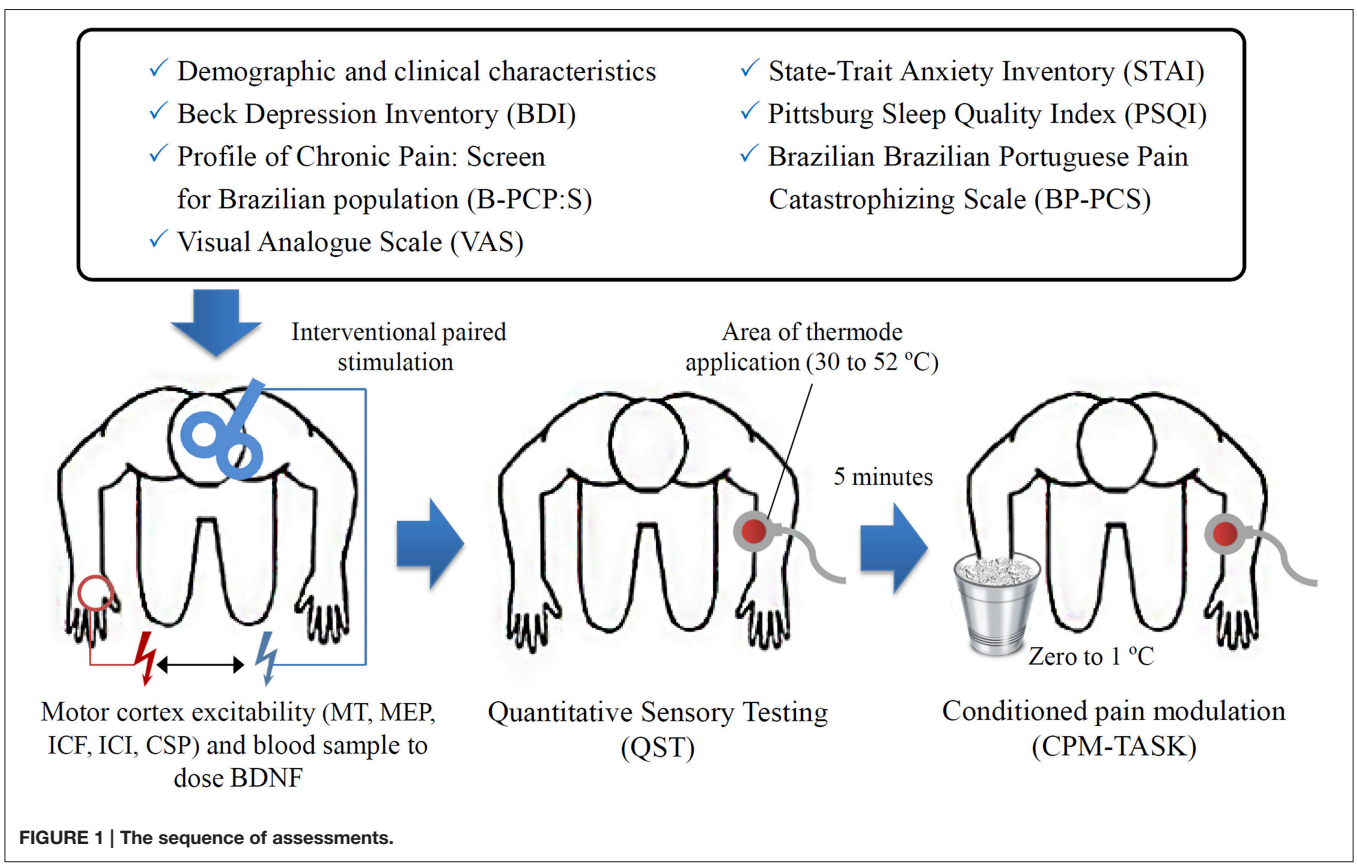

FIGURE 1 | The sequence of assessments.

2010; Sehn et al., 2012; Caumo et al., 2013). Two independent medical examiners that were blinded to the aim of the study were trained to conduct the psychological tests and to administer the pain scales. The patients' baseline depressive symptoms were assessed using the Beck Depression Inventory (BDI II; Warmenhoven et al., 2012), and the Pittsburgh Sleep Quality Index to assess the sleep quality (Buysse et al., 1989). To measure the anxiety, we used the refined version of the State-Trait Anxiety Inventory (STAI; Kaipper et al., 2010) obtained using the Rasch model, which derivates shorter state-trait STAI-Form X scales free of threshold disorders and for differential item functioning (DIF) problems. The scores in the state- and trait score ranges from 13 to 52, and 12 to 36, respectively. The catastrophic thinking related to pain was assessed using the Brazilian Portuguese Catastrophizing Scale (BP-PCS; Sehn et al., 2012). To measure the pain intensity during the most part of time in the last week was used the VAS, ranging from 0 cm (no pain) to 10 cm (worst possible pain). We used a standardized questionnaire to assess demographic data and medical comorbidities.

As subjects with chronic pain usually use rescue analgesics changes from week to week according to pain level, the analgesic use was defined as the self-reported average used per week during the last 3 months. For data analysis, we included the analgesic use as a dichotomous variable: the analgesic was coded one when they used more than 4 days per week while the analgesic uses less than 4 days per week it was coded as zero (reference value).

Outcomes

The primary outcomes were the MEP and ICF as assessed by TMS. The secondary outcomes were the cortical excitability parameters CSP and SICI, serum BDNF level, HPT, and the disability related to pain assessed by Brazilian Profile of Chronic Pain: Screen score (B-PCP:S). The primary factor of interest, the score on NPS (0–10) during the conditioned pain modulated (CPM-task), are described in detail below.

(a) The Brazilian Profile of Chronic Pain: Screen (B-PCP:S; Caumo et al., 2013) was used for quick identification of an individual's multidimensional pain experience. The B-PCP:S includes a severity scale (four items; possible score range of 0–32), an interference scale (six items; possible score range of 0–36), and an emotional burden scale (five items; possible score range of 0–25). The disability related to pain (DRP) regarding severity, interference with daily activities, and the emotional burden was evaluated using the B-PCP:S (Caumo et al., 2013). It accepted as a criterion to define disability a presence of chronic or recurrent pain or discomfort causing restriction (Caumo et al., 2013); thus, we assumed that higher scores on the B-PCP:S indicated more severe disability or greater functional deficits at work, at home, and during social situations and a higher emotional burden (Vidor et al., 2014).

(b) To measure the cortical excitability parameters we used a surface electromyography. The recordings were gathered at the contralateral right first dorsal interosseous muscles using Ag/AgCl electrodes. First, the resting motor threshold (RMT) was determined by obtaining five motor evoked potentials (MEPs) with a peak-to-peak amplitude of 50 μV from 10 consecutive trials. To define the MEP we recorded 10 MEPs with an intensity of 130% of the individual RMT. Moreover, the cortical silent periods (CSPs) were assessed during muscle activity by a dynamometer to maintain them at ∼20% maximal force. Accordingly, the CSPs were 10 records using an intensity of 130% of the RMT. Short intracortical inhibition (SICI) using an inter-stimulus interval of 2 ms was also assessed. The conditioning stimulus was set at 80% of the RMT while the test stimulus was set at 100% of the individual MEP intensity. The intracortical facilitation (ICF) was assessed with an inter-stimulus interval of 12 ms. We conducted the paired-pulse in a randomized order for a total of 30 trials (ten each for ICF, SICI and control stimuli). To calculate the RMT we used the lowest stimulus intensity that was able to evoke an MEP of at least 50 μV in 5 out of 10 consecutive trials. Off-line analyzes included the collection of the duration of the CSPs as well as the amplitudes of all of the MEPs, SICIs, and ICFs. The corresponding units for these parameters included MEP in μV, SICI, and ICF in their ratios to MEP and CSP in ms (Pascual-Leone et al., 1994).

(c) The laboratory outcome measured was the serum level of BDNF. We collected the blood samples before starting the assessment. We centrifugate the blood samples for 10 min at 4500 × g at 4°C, and we stored the serum at −80° C for the hormone assay. We determined the serum BDNF using an Enzyme-Linked Immunosorbent Assay (ELISA) using a ChemiKine BDNF Sandwich ELISA Kit, CYT306 (Chemicon/Millipore, Billerica, MA, USA). The lower detection limit of the kit for BDNF is 7.8 pg/mL.

(d) We used the Quantitative Sensory Testing (QST) to assess HPT. This measure use the method of limits with a computer Peltier-based device thermode (30 × 30 mm; Schestatsky et al., 2011) attached to the skin on the ventral aspect of the mid-forearm. The set at 32°C and was increased at a rate of 1°C/s to a maximum of 52°C. The heat pain threshold (HPT) of each patient was defined as the mean of three assessments performed with an inter-stimuli interval of 40 s (Schestatsky et al., 2011). The thermode position was slightly altered between trials, to avoid, either sensitization or response suppression of the cutaneous heat nociceptors.

(e) To measure the CPM-task we evaluated the pain intensity in two tonics HPT test stimuli separated by a CPM-task. We used the HPT as conditioning pain stimulus to elicit a prolonged pain sensation to trigger CPM. The CPM-task consisted of immersion of non-dominant hand in cold water at a temperature of 0−1°C for 1 min. To maintain the water temperature zero to 1°C was used a thermostat to control the temperature variation. The QST procedure was introduced after 30 s of cold-water immersion. To determine the CPM we used the difference between the pain score on NPS (0–10) QST during cold water immersion (QST+CPM) at the temperature of the point at which subjects felt 6/10 pain on the NPS scale [during the initial time period (T0)]. An accepted criterion to define responders to the CPM-task

is the reduction of NPS pain scores under a heterotopic stimulus compared with NPS pain scores under a nociceptive stimulus without a heterotopic stimulus. If the patients did not report a reduction or report an increase in their pain score during the CPM-task, the descendent modulatory systems were considered to have failed to modulate the nociceptive response. For the data analysis, non-responders showed a difference in the score on NPS, HPT1–HPT0, of zero or higher, and for responders, these values were lower than zero.

Statistical Analysis

Descriptive statistics were used to summarize the main socio-demographic features of the sample. *T*-Tests for independent samples and Chi-squared and Fisher's exact tests were used to compare continuous and categorical variables between groups respectively. To test for normality was used the Shapiro-Wilk test. To ensure that the data were normally distributed, we performed a log transformation for BDNF level.

After verifying the corresponding assumptions, the Pearson correlation coefficient (r) was used to assess the relationship between covariates (age, sleep quality, catastrophic thinking about pain; state-trait anxiety, and depressive symptoms) with the outcomes related to cortical excitability parameters, BDNF, and pain measures (see **Table 3**). To maintain the assumption of independence between covariates and to control for collinearity when the Pearson correlation coefficients (r) for two variables were higher than 0.5 (moderate), in the multivariate analysis model was included only one of the variables (see **Table 4**). Based on this criterion the catastrophizing thinking related to pain and trait-anxiety were included in the multivariate analysis model, taking into account that they have been shown to be correlated with cortical excitability in previous studies on MPS (Volz et al., 2013b; Vidor et al., 2014) (**Table 4**). The covariates not included in the multivariate analysis model were age, depressive symptoms, sleep quality, and state-anxiety. A multivariate covariance analysis (MANCOVA) model was used to explore the relationship between the responders and non-responders to multiple outcomes [cortical excitability (MEP, ICI, ICF, CSP), BDNF, HPT, and disability related to pain on B-PCP:S. Bonferroni's Multiple Comparison Test was used to identify the source of significant differences. The data were analyzed using SPSS software version 22.0 (SPSS, Chicago, IL).

RESULTS

Patient Characteristics

We screened 54 potential participants with a diagnosis of MPS, and we included 33 in the study. The reasons for exclusion were not fulfilling the diagnostic criteria for MPS, not present a neuropathic component according to the DN4 (Neuropathic Pain Diagnostic Questionnaire), lacking disability as defined in the protocol, and the presence of another diagnosis (fibromyalgia). All enrolled subjects participated in all aspects of the study and were included in all of analyses (**Table 1**).

Univariate Analysis

Relationships between the Function of the Corticospinal Modulatory System, Motor Córtex Excitability, Pain Measures, and BDNF Level

Relationships between the function of the corticospinal modulatory system, motor córtex excitability, pain measures, and BDNF level according to a spectrum of responders and no responder to CPM-task. The non-adjusted means and standard deviation (SD) of the cortical excitability parameters, BDNF, pain threshold and disability related to pain were presented in **Table 2**.

Assessment of Relationship between Independent Variables to Identify Potential Confounders

The Pearson correlation was used to identify potential confounding factors in the relationships between outcomes (cortical excitability, BDNF, HPT, and disability). The correlated parameters were the scores of the Brazilian Portuguese Catastrophizing Scale (B-PCS); Beck Depression Inventory (BDI); Pittsburgh Sleep Quality Index (PSQI); and Short State-Trait Anxiety Inventory (STAI-E-T), and age (**Table 3**). The covariates included in the multivariate analysis model (**Table 4**) were the trait-anxiety and catastrophizing scores.

Multivariate Analysis of the Relationship between the Corticospinal Modulatory System, Cortical Excitability, BDNF, HPT, and Disability According to Spectrum of Responders and Non-Responders to CPM-task

The results of the MANCOVA model analysis with multiple outcomes as dependent variables, including cortical excitability parameters (MEP, ICF, SICI, CSP), BDNF, HPT, and disability related to pain according to spectrum of responders and non-responders to CPM-task, and the STAI-E-T score and catastrophizing score, as independent variables, are presented in **Table 4**. The MANCOVA model using Bonferroni's Multiple Comparison Test revealed a significant relationship between the responders and non-responders groups and the outcomes related to cortical excitability measurements (ICF and MEP), BDNF, disability related to pain and HPT [Hotelling's Trace $= 1.84$, $F(34) = 6.05$, $P < 0.001$]. This analysis presented a power of 0.99. The adjusted determination coefficient of this model was $R2 = 0.57$; thus, the variables included in the model explain 57% of the variance in the outcome variables. The results of this adjusted multivariate model are presented in **Table 4**. Non-responders showed higher cortical excitability (ICF, MEP), greater disability related to pain, higher BDNF level, and lower HPT. However, no effect was observed in other cortical excitability parameters (CSP and ICI; see **Table 4**).

In **Figures 2A–C** are presented the relationships according to a spectrum of responders and non-responders to CPM-task and intracortical facilitation and MEP (primary outcomes) and BDNF (secondary outcome). The means were compared using

TABLE 1 | Demographic and clinical characteristics of the study sample.

Variables	Non responders ($n = 23$)	Responders ($n = 10$)	*P*
Age (years)	43.36 (14.78)	48.30 (9.13)	0.33
Marital status (married/unmarried)	13/10	4/6	0.31
Education (years)	13.91 (4.25)	12.57 (3.88)	0.37
Smoking (yes/no)	1/22	0/10	0.69
Alcohol consumption (yes/no)	22/1	10/0	0.69
Duration of pain (years)	6.04 (1.64)	6.4 (0.97)	0.42
Pain on visual analog scale (cm)	8 (1.33)	6.5 (1.85)	0.01
Trait-anxiety (STAI-T)	28.82 (6.14)	25.17 (6.41)	0.13
State-anxiety (STA-T)	30.0 (8.42)	29.91 (5.75)	0.98
Beck depression inventory	13.45 (7.04)	15.83 (7.12)	0.37
Brazilian Portuguese Catastrophizing Scale (BP-PCS)	33.82 (6.98)	31.52 (8.14)	0.42
Number of days analgesics were used per week in the last 3 months (<4 times/= 4 times)[a]	8/15	2/8	0.33
Presence of other chronic diseases before appearance of pain (yes/no)[b]	4/19	2/8	0.6
Diagnosis of psychiatric disorders (yes/no)	8/15	5/5	0.32
Active use of central nervous system medication (yes/no)[c]	20/3	7/3	0.25

Values are given as the mean (SD) or frequency (n = 33).
[a] The same patient may have used more than one medication.
[b] Chronic diseases other than pain: hypertension (n = 12); ischemic heart disease (n = 1); heart attack (n = 1); diabetes mellitus (n = 5); thyroid diseases (n = 2); other chronic diseases listed (n = 0).
[c] Central nervous medication: tricyclic antidepressant (n = 2); topiromate (n = 1); tylex (n = 1).

TABLE 2 | Measurements of motor córtex parameters by TMS, HPT, B-PCP:S, and BDNF ($n = 33$).

Cortical Excitability Measures	Non-responders ($n = 23$)		Responders ($n = 10$)		*P*[&]
	Mean ± *SD*	Median (Q25, Q75)	Mean ± *SD*	Median (Q25, Q75)	
Motor threshold (MT)	44.46 (8.04)	44 (32: 65)	41.1 (5.53)	40.5 (32; 50)	0.15
Motor evoked potential (mV)	1. 93 (0.54)	2.06 (0.98; 3.14)	1.40 (0.27)	1.42 (1.03); 1.81)	0.01
Intracortical facilitation (ratio: ICF/ test stimulus)	1.43 (0.3)	1.35 (0.71; 1.99)	1.11 (0.12)	1.09 (0.94; 1.24)	0.00
Short interval intracortical inhibition (ratio: SICI/ test stimulus)	0.25 (0.02)	0.25 (0.23;0.27)	0.27 (0.10)	0.25 (0.08; 0.42)	0.38
Cortical silent period (CSP)	69.36 (21.74)	79.00 (38.0;120.0)	61.91 (15.49)	62.50 (33.25; 91.75)	0.17
Profile of chronic pain: screen for Brazilian population (B-PCP:S)	71.00 (10.02)	73.00 (55.0;91. 0)	59.22 (11.23)	63.00 (51.0; 75.0)	0.00
Quantitative sensory testing (°C)	42.78 (4.27)	44 (35;50)	38.0 (3.03)	38.00 (37: 41)	0.00
Brain-derived neurotrophic factor (BDNF) pg/ml (*log*)	32.56 (9.95)	33.0 (20.0;36. 0)	25.59 (10.24)	22.5 (5.5; 39.5)	0.02

Motor evoked potential: (MEP); Interquartile interval (Q). Intra-cortical inhibition (ICI) expresses the relationship between the amplitude of wave and motor evoked potentials (relative amplitude, express in %), at inter-stimuli intervals (ISIs) of 2 ms with paired-pulse. The first is a sub-threshold stimulus [80% of the rest motor threshold (rMT)] followed by the second one which is a suprathreshold stimulus (130% rMT).
(A) Cortical silent period (CSP) expressed in milliseconds (ms);
(B) Motor-evoked potentials (MEP) expressed in mV, evoked by a stimulus of 130% the intensity of the rMT, and should have peak-to-peak MEP amplitude of at least 1 mV.
&, Comparisons of mean using t-test for independent samples.

MANCOVA with Bonferroni's Multiple Comparison test (the model was shown in **Figures 2A–C**; **Table 4**).

DISCUSSION

This study confirmed our hypothesis that the descending pain modulation system as assessed according to a spectrum of responders and non-responders to CPM-task is simultaneously correlated with a disinhibition at the cortical level, as measured by ICF and with global neuroplasticity levels as determined by serum BDNF. Also, the disengagement of descending pain modulatory system was correlated with a dysfunction of the corticospinal pathway as indexed by MEP, a lower HPT, and a greater disability.

The current study expanded on the data available in the literature showing that the magnitude of disinhibition in regulating sensory information was associated with changes in the cortical and subcortical levels. This disinhibition state occurs through multiple neurobiological systems, which can amplify sensory pain signals to the neural pain matrix. Additionally, the level disengagement of descending pain modulatory system was correlated with changes in serum BDNF level, which is involved in the modulation of the excitatory/inhibitory central nervous system balance. Thus, the variation in the spectrum of dysfunction of internal modulator system in chronic pain

TABLE 3 | Pearson correlation coefficient (*r*) between potential confounding factors and outcomes (*n* = 33).

	Age	STAI-T	STAI-E	BPC-S	B-PCP:S	BDI	PSQI	MEP	ICF	SICI	CSP	BDNF
Age	*r = 0.05*											
STAI-T	r = 001	*r = 0.15*										
STAI-E	r = −0.04	r = 0.65**	*r = –0.07*									
BP-PCS	r = 0.06	r = 0.32	r = 0.29	*r = –0.08*								
B-PCP:S	r = −0.13	r = 0.40*	r = 0.19	r = 0.62**	*r = –0.11*							
BDI	r = 0.18	r = 0.58**	r = 0.43**	r = 0.66**	r = 0.54**	*r = –0.25*						
PSQI	r = −0.07	r = 0.34*	r = 0.24	r = 0.54**	r = 0.36*	r = 0.46**	*r = –0.11*					
MEP	r = −0.26	r = 0.05	r = −0.08	0.11	r = 0.26	r = −0.10	r = −0.05	*r = 0.33**				
ICF	r = −0.01	r = 0.15	r = −0.06	0.14	r = 0.45**	r = 0.09	r = −0.07	r = 042*	r = 0.25			
SICI	r = −0.13	r = −0.12	r = −0.27	–0.01	r = −0.03	r = −0.03	r = −0.06	r = −0.15	r = 0.04	*r = –0.27*		
CSP	r = −0.05	r = −0.14	r = 0.05	–0.18	r = −0.13	r = −0.13	r = −0.21	r = 0.01	r = 0.27	r = 0.18	–0.38*	
HPT	r = 0.32	r = 0.03	r = −0.08	0.28	r = 0.07	r = 0.20	r = 0.11	r = −0.35*	r = −0.05	r = 0.34*	r = −0.11	*r = 0.20*

***Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). Brazilian Portuguese Catastrophizing Scale (BP-PCS); Beck Depression Inventory (BDI); Pittsburgh Sleep Quality Index (PSQI); Short State-Trait Anxiety Inventory (STAI-E-T); Brazilian Profile of Chronic Pain: Screen (B-PCP:S); Intra-cortical inhibition (ICI); Cortical silent period (CSP): Motor-evoked potentials (MEP) expressed in mV, Brain-derived neurotrophic factor (BDNF) pg/ml (log).*

conditions could be understood as a signal from a balance in the neuroplasticity mediators involved in the modulation of the excitatory/inhibitory central nervous system (CNS; Deitos et al., 2015). While that the variation of BDNF could be interpreted as a signal from a "diseased balance," once such balance differs between the spectrum of responders and responders to CPM-task. However, persists the concerns how good is this signal to identify the chronic pain imbalance in the CNS and how is its predictive properties for the evaluation of the MPS.

These results demonstrated that this integrative pattern to assess changes in the pain pathway highlights that a cross talk between the neural network of cortical regions and the spine-bulbospinal loop occurs along with changes in the BDNF secretion, which is the central marker of neuroplasticity process mechanisms. Thus, this set of changes reinforcing the hypothesis, that, if we improve the understanding of underlying neurophysiological mechanisms of chronic MPS, this could give support for the clinical decision based on practical approaches for its recognition (Nijs et al., 2010). Additionally, these findings provide some theoretical support for the mechanism involved in the effect of interventions that improved pain and enhanced the function of the descendent modulatory system in studies that used melatonin, amitriptyline (de Zanette et al., 2014), rTMS (Dall'Agnol et al., 2014), and the combination treatment of CPM and duloxetine (Yarnitsky et al., 2012). Although human studies permit us to determine only the effect in the network, our findings allow a new way to construct the rational to combine therapeutic approaches to improve functional of descending pain modulatory systems. Such techniques include pharmacological (i.e., antidepressant, anticonvulsant, etc.) and non-pharmacological approaches (i.e., Transcranial direct current stimulation (tDCS), TMS, electroacupuncture and other physical therapy).

We observed greater MEPs amplitude in non-responders to CPM-task (**Table 4**). Although a significant portion of the corticospinal input to the motoneuron pool is relay via lumbar group II interneurons (Marchand-Pauvert et al., 1999), the MEP amplitude is a reflection of the latency of depolarization of the spinal motor neuron pool. Its amplitude reflects the integrity and function of conduction along the efferent pathway, which form part of the lumbar propriospinal system and it express the excitability of the cortical and spinal motor neuron pool (Marchand-Pauvert et al., 1999; Pierrot-Deseilligny and Burke, 2005; Iglesias et al., 2008). Thereby, this result suggests that an enhanced activity of descending tracts, whose motor portion is assessed by the MEP, suggests that the inhibitory capacity of the corticospinal modulator system is reduced (Vidor et al., 2014), resulting in increased amplitude of MEP. One critical issue here is whether corticospinal excitability is a compensatory or a causal mechanism of pain. Given our data does not allow us to clarify the temporal relationship between these two variables; we can only hypothesize the correlation between these two variables (MEP and CPM response). We have proposed before that increased motor cortex excitability is a compensatory mechanism aiming to reduce thalamic overactivity and thus pain (Castillo Saavedra et al., 2014); though this mechanism is not enough to control pain (an anology here would be increased insulin in a subject with hyperglycemia; increased insulin levels would be the compensatory mechanism). Therefore, increased pain increases corticospinal excitability and when pain is controlled this marker becomes normalized. The data from ICF supports this hypothesis. We have proposed before that increased motor cortex excitability is a compensatory mechanism aiming to reduce thalamic overactivity and thus pain (Castillo Saavedra et al., 2014).

Either increased ICF or decreased ICI suggest an involvement of cortical mechanisms in the dysfunction of the descendent modulatory system, which facilitate the activity of the corticospinal system. Although the ICF is a complex phenomenon, it reflects increase in the activity within glutamatergic circuits, it also may arise through a loss of GABA-A-mediated modulation (Di Lazzaro et al., 2000; Fedi

TABLE 4 | Relationship between outcomes (cortical excitability parameters, pain measures and BDNF), and responders and no responders according change in NPS (0–10) during the CPM-task ($n = 33$).

Dependent variable	Type III Sum of Squares	*df*	Mean Square	*F*	*P*	*Partial eta Squared*
Motor evoked potential (mV)	1.15	3	0.38	2.79	0.03	0.22
Intracortical facilitation (ratio: ICF/ test stimulus)	2.52	3	0.84	5.81	0.00	0.38
Short intracortical inhibition (ratio: SICI/test stimulus)	0.94	3	0.31	9.19	0.00	0.49
Cortical silent period	0.004	3	0.001	0.17	0.91	0.01
Brazilian profile of chronic pain: screen (B-PCP:S)	929.06	3	309.69	1.10	0.36	0.10
Quantitative sensory testing (°C)	184.73	3	61.58	4.86	0.00	0.33
Brain-derived neurotrophic factor (BDNF) pg/ml (*log*)	2881.96	3	960.65	15.40	0.00	0.61
Parameter	**SEM**		**βa**	***t***		***P***
MOTOR EVOKED POTENTIAL (mV)						
Conditioned pain modulation (CPM) during CPM/task						
No responder[a]	0.61		0.15	4.09		0.00*
Brazilian Portuguese catastrophizing scale (BP-PCS)	0.007		0.009	0.74		0.46
State-anxiety (STAI-T)	−0.01		0.01	−1.50		0.14
INTRACORTICAL FACILITATION (RATIO: ICF/ TEST STIMULUS)						
Conditioned pain modulation (CPM) during CPM/task	0.33		0.07	4.50		0.00*
No responder[a]						
Brazilian Portuguese catastrophizing scale (BP-PCS)	0.004		0.004	0.88		0.38
State-anxiety (STAI-T)	0.004		0.006	0.70		0.48
SHORT INTRACORTICAL INHIBITION (RATIO: SICI/TEST STIMULUS)						
Conditioned pain modulation (CPM) during CPM/task	−0.02		0.03	−0.62		0.54
No responder [a]						
Brazilian Portuguese catastrophizing scale (BP-PCS)	−0.01		0.02	−0.34		0.80
State-anxiety (STAI-T)	1.85		0.003	0.007		0.99
CORTICAL SILENT PERIOD						
Conditioned pain modulation (CPM) during CPM/task	10.82		6.66	1.62		0.11
No responder[a]						
Brazilian Portuguese catastrophizing scale (BP-PCS)	−0.19		0.40	−0.47		0.64
State-anxiety (STAI-T)	−0.47		0.50	−0.94		0.36
BRAZILIAN PROFILE OF CHRONIC PAIN: SCREEN (B-PCP:S)						
Conditioned pain modulation (CPM) during CPM/task	7.81		3.13	2.48		0.01*
No responder[a]						
Brazilian Portuguese catastrophizing scale (BP-PCS)	0.80		0.18	4.24		0.00*
State-anxiety (STAI-T)	0.51		0.24	2.17		0.03*
QUANTITATIVE SENSORY TESTING (°C)						
Conditioned pain modulation (CPM) during CPM/task	−3.82		1.41	−2.70		0.01*
No responder[a]						
Brazilian Portuguese catastrophizing scale (BP-PCS)	0.08		0.08	0.99		0.33
State-anxiety (STAI-T)	−0.19		0.10	−1.82		0.07
BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) PG/ML (*log*)						
Conditioned pain modulation (CPM) during CPM/task	0.39		0.14	2.66		0.01*
No responder[a]						
Brazilian Portuguese catastrophizing scale (BP-PCS)	−0.05		0.09	−0.61		0.54
State-anxiety (STAI-T)	−0.01		0.01	−1.47		0.15

[a]*Reference category is no responder, hence a positive value mean that the mean was higher in no responder.*
*CPM-task [no responder (NPS (0–10) HPT1–HPT0 ≥ 0) or responder (NPS (0–10) HPT1–HPT0 <0] *P < 0.05.*

et al., 2008). Additionally, the disinhibition involves the loss of inhibitory pyramidal cells. MEP amplitude is also an indicator of primary motor córtex excitability: larger amplitudes indicate higher excitability of the motor córtex, which may modulate intracortical excitability and the transmission efficiency of corticospinal neurons, resulting in less facilitation. Overall,

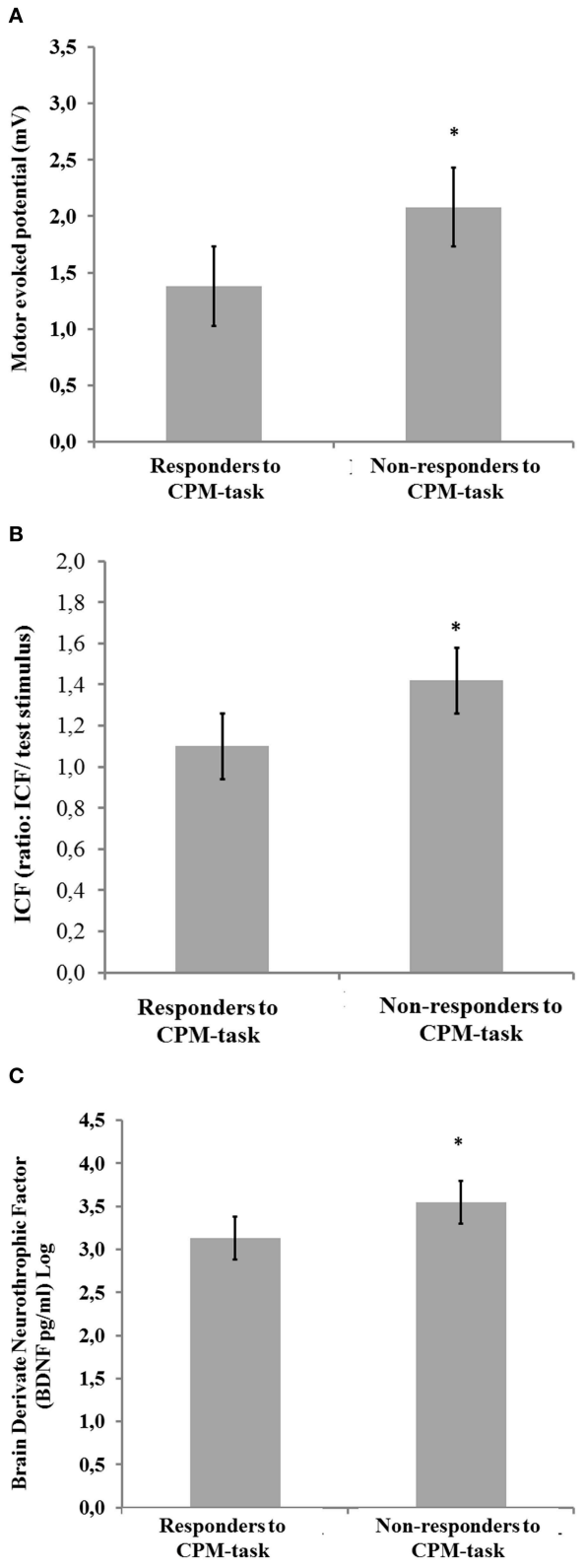

FIGURE 2 | Comparisons between [non-responders (NPS (0–10) HPT1–HPT0 ≥ 0; $n = 10$) and responders (NPS (0–10) HPT1–HPT0 < 0; $n = 23$)]. (A) Motor evoked potential (mV); **(B)** Intra-cortical facilitation (amplitude/MEP amplitude ratio = ICF); and **(C)** Brain derived neurotrophic factor (BDNF) ng/ml (Log). Error bars indicate standard error of the mean (S.E.M.). Asterisks positioned above the bars indicate differences between groups (responders and non-responders to CPM-task) assessed by MANCOVA with *post-hoc* Bonferroni's Multiple Comparison test.

as proposed above these changes in cortical plasticity could be explained as a compensatory mechanism to downregulate increased excitability in the pain neural networks such as thalamic structures.

The higher serum BDNF in non-responders suggests that this neurotrophin may be a marker of severity of CS. The CS involves a proliferation of synaptic activity due the trophic factors, to support maladaptive plasticity that perpetuates the sensation of pain. Our findings give neurophysiological support (MEP) to understand the link between serum BDNF and the severity of dysfunction of the descendent modulatory system. Even though this relationship is complex, they support the idea that the activity of the descending inhibitory system is related to central sensitization (Schwenkreis et al., 2003; Deitos et al., 2015) and a greater activation in the brainstem (Graven-Nielsen et al., 2012). This assumption, supported by an experimental study with rats exposed to chronic pain, demonstrates that the BDNF effect on pain pathways may change according to the region of central nervous system (i.e., spine, brainstem, hippocampus, and cortex, etc.; Spezia Adachi et al., 2015). The mentioned study demonstrated that the tDCS decreased the BDNF levels in the spinal cord and brainstem, whereas BDNF levels did not change in the hippocampus (Spezia Adachi et al., 2015). These differents effects according to site suggest that BDNF activates distinct pathways (i.e., descending systems) and that its effect is pleiotropic. Although previous findings show that the increase in excitatory activity and the decrease in inhibitory synaptic activity in the córtex related to BNDF level (Ren and Dubner, 2007; Tao et al., 2014), the present results do not allow for a conclusion regarding a cause-effect relationship between BDNF level and descendent modulatory system dysfunction.

Overall, the findings of this study corroborate the idea that the BDNF modulates the synaptic plasticity in an activity-dependent manner to strengthen a nociceptive transmission, recruits non-nociceptive input to the pain pathways and it binds to high-affinity trkB receptors. This BDNF effect enhances the response that NMDA-mediated C-fibers evoke, which in turn causes activation of several signaling pathways in spinothalamic tract neurons. Thereby, this strength excitatory synapsis promotes the disinhibition of descending pathways (Zanette et al., 2014). This statement is also supported indirectly by clinical findings, where the serum BDNF was correlated inversely with the pressure pain threshold in fibromyalgia (Zanette et al., 2014). Equally, we showed that the BDNF increase would be favoring pain transmission because greater scores in the CPM indicates a lower function in the descending pain modulatory system and a higher propensity for pain. This finding is biologically plausible because the enhance in the BDNF activates signaling pathways in the

spinothalamic tract, which reduces the GABAergic inhibitory effect (Spezia Adachi et al., 2015). These findings support the hypothesis that the chronic pain induces reorganization in circuits involved in pain processing at cortical and in descending pain modulatory system. Although the relationship between BDNF with the physiopathology of pain is complex, it has important functions in the processes of neurogenesis and neuroplasticity. Thereby, efforts are being made to understand its role in the pain modulatory system.

In the current study, a lower HPT and higher DRP in non-responders were observed (**Table 4**). These results are congruent with evidence from previous studies that a lower pain threshold in patients with long-term chronic pain may be a signal of lack of function of the inhibitory system (Kwon et al., 2013; Defrin et al., 2015). Another explanation for this finding is a potential protective effect if one considers that the hippocampus amplifies signals to the neural representation (Ma et al., 2012).

A greater disability according to scores on the B-PCP:S was associated with the disinhibition of the descendent pain modulatory system. The B-PCP:S dominions indicate pain severity, restriction for daily activities (at work, at home, during social situations) and the emotional burden. According to a spectrum of responder and non-responders to CPM-task, the disability was correlated positively with the catastrophizing and trait-anxiety. In previous study we demonstrated the relationship between greater disability related to pain and a higher trait anxiety in MPS (Vidor et al., 2014). While in another study with healthy subjects was observed that the perceived intensity of the conditioning stimulus was associated with the pain catastrophizing and trait anxiety (King, 2014). In fact, the current findings suggest that the relationship between the descending modulatory system and the disability related to pain is regulated by brain regions that are involved not only with pain but also with cognitive and emotional functioning in general (Pessoa, 2008). Similar dysfunction was observed when there were lesions in brain regions implicated in descending pain modulation (i.e., traumatic brain injury and multiple sclerosis), including the medial prefrontal córtex (PFC) and rostral anterior cingulate córtex (ACC; Bushnell et al., 2013). Additionally, it has been demonstrated that the alterations in the biological integrity and functioning of brain regions were involved in both pain control and cognitive and emotional functioning. Thereby, the changes in this network could explain the relationship between the severity disability and the dysfunction of the corticospinal pain modulatory system (**Table 4**).

This study had some limitations: Firstly, TMS is an indirect neurophysiological evaluation of neurotransmitter system activity. Secondly, only females were evaluated, as gender differences in pain perception and modulation are controversial. Thirdly, psychiatric disorders are a potential confounding factor in chronic pain syndromes, and they cannot have been adequately controlled. The psychiatric symptoms (anxiety, depression, catastrophizing, and psychiatric diagnosis) were equally distributed between the groups (responder vs. non-responder). In fact, 39.39% (14/33) of patients suffered from mental illnesses. However, this finding is expected, because the emotional disturbance is part of chronic pain syndromes, and they can worsen the sensitization and chronification. Moreover, the results of this study need to be carefully interpreted because it was an exploratory study. Further, research on chronic pain of different psychopathologies is required to confirm our initial findings and the impact of our findings on patients' responses to different therapeutic approaches.

These results suggest that a non-response to the CPM-task is likely due to increased plasticity to in central structures associated with pain that control endogenous inhibitory control and that in this case compensatory mechanisms are activated as reflected by increased cortical excitability. This failure to respond to CPM-task is associated with higher serum BDNF, lower HPT, and a greater level of disability related to pain. Overall, these findings suggest that the CPM-task is a test that allows for inference regarding the loss of net descending pain inhibition. Thus, this short and simple test might be useful for predicting a patient's response to therapy, and it helps in the clinical decision-making process for individual patients. The results of this study may also assist in the development of individualized treatment.

AUTHOR CONTRIBUTIONS

AB participated in the sequence alignment and drafted the manuscript. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived the study, participated in its design and coordination and helped drafting the manuscript.

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Motor Cortex Neurostimulation Technologies for Chronic Post-stroke Pain: Implications of Tissue Damage on Stimulation Currents

Anthony T. O'Brien[1†], Rivadavio Amorim[1†], R. Jarrett Rushmore[2,3], Uri Eden[4], Linda Afifi[2,3], Laura Dipietro[5], Timothy Wagner[5,6‡] and Antoni Valero-Cabré[2,3,7,8*‡]*

[1] Neuromodulation Lab and Center for Clinical Research and Learning – Department of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA, USA, [2] Laboratory of Cerebral Dynamics, Plasticity and Rehabilitation, Boston University School of Medicine, Boston, MA, USA, [3] Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, USA, [4] Department of Mathematics and Statistics, Boston University, Boston, MA, USA, [5] Highland Instruments, Cambridge, MA, USA, [6] Division of Health Sciences and Technology, Harvard Medical School/Massachusetts Institute of Technology, Boston, MA, USA, [7] Université Pierre et Marie Curie, CNRS UMR 7225-INSERM U1127, Institut du Cerveau et la Moelle Epinière, Paris, France, [8] Cognitive Neuroscience and Information Technology Research Program, Open University of Catalonia, Barcelona, Spain

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Wolnei Caumo, Universidade Federal do Rio Grande do Sul, Brazil

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****Correspondence:***
Timothy Wagner
twagner@alum.mit.edu
Antoni Valero-Cabre
antoni.valerocabre@icm-institute.org

[†] These authors have contributed equally to this work as first authors.

[‡] These authors shared senior author responsibilities for this manuscript.

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Background: Central post stroke pain (CPSP) is a highly refractory syndrome that can occur after stroke. Primary motor cortex (M1) brain stimulation using epidural brain stimulation (EBS), transcranial magnetic stimulation (TMS), and transcranial direct current stimulation (tDCS) have been explored as potential therapies for CPSP. These techniques have demonstrated variable clinical efficacy. It is hypothesized that changes in the stimulating currents that are caused by stroke-induced changes in brain tissue conductivity limit the efficacy of these techniques.

Methods: We generated MRI-guided finite element models of the current density distributions in the human head and brain with and without chronic focal cortical infarctions during EBS, TMS, and tDCS. We studied the change in the stimulating current density distributions' magnitude, orientation, and maxima locations between the different models.

Results: Changes in electrical properties at stroke boundaries altered the distribution of stimulation currents in magnitude, location, and orientation. Current density magnitude alterations were larger for the non-invasive techniques (i.e., tDCS and TMS) than for EBS. Nonetheless, the lesion also altered currents during EBS. The spatial shift of peak current density, relative to the size of the stimulation source, was largest for EBS.

Conclusion: In order to maximize therapeutic efficiency, neurostimulation trials need to account for the impact of anatomically disrupted neural tissues on the location, orientation, and magnitude of exogenously applied currents. The relative current-neuronal structure should be considered when planning stimulation treatment, especially across techniques (e.g., using TMS to predict EBS response). We postulate that the effects of altered tissue properties in stroke regions may impact stimulation induced analgesic effects and/or lead to highly variable outcomes during brain stimulation treatments in CPSP.

Keywords: epidural brain stimulation, transcranial magnetic stimulation, transcranial direct current stimulation, motor cortex, neurological model, stroke, pain, analgesia

INTRODUCTION

Central post stroke pain (CPSP) results from stroke lesions to any region of the somatosensory pathway (Klit et al., 2009; Kumar et al., 2009; Creutzfeldt et al., 2012; Mozaffarian et al., 2015). Between 8 and 25% of the ∼18 M/year new cases of stroke develop CPSP (Strong et al., 2007; Klit et al., 2015). CPSP leads to poor quality of life (Kumar and Soni, 2009; Oh and Seo, 2015). Patients are often refractory to pharmacotherapy and can become drug dependent (Kumar and Soni, 2009). Such limitations have motivated researchers to explore brain stimulation therapies to treat CPSP.

Epidural Brain Stimulation (EBS), Transcranial Magnetic Stimulation (TMS), and Transcranial Direct Current Stimulation (tDCS) have all been investigated. Stimulation of primary motor cortex (M1) appears to be the most effective cortical target (Nguyen et al., 1999; Kumar and Soni, 2009; Hirabayashi et al., 2011; DosSantos et al., 2012; Fregni et al., 2014; Brietzke et al., 2015; Cioato et al., 2015; Morishita et al., 2015; Oh and Seo, 2015). Analgesia is believed to be achieved through the stimulation of M1-thalmic relays to reduce hyperactivity in thalamic linked pain networks (Tsubokawa et al., 1993; Mertens et al., 1999; Khedr et al., 2005; Garcia-Larrea and Peyron, 2007; Peyron et al., 2007; Lima and Fregni, 2008; Nguyen et al., 2008; Fontaine et al., 2009; Lefaucheur et al., 2009; Ohn et al., 2012; Bae et al., 2014; Hasan et al., 2014; Lefaucheur, 2016).

While EBS, TMS, and tDCS have shown some clinical success in treating CPSP, high variability across studies has impeded their widespread acceptance (Mertens et al., 1999; Lefaucheur et al., 2004, 2009; Lima and Fregni, 2008; Nguyen et al., 2008; Fontaine et al., 2009; DosSantos et al., 2012; Bae et al., 2014; Lefaucheur, 2016). Upward of 30% of EBS patients do not respond to stimulation (Tsubokawa et al., 1993; Katayama et al., 1998; Mertens et al., 1999; Nguyen et al., 1999). However, it should be noted that this is highly dependent on patient characteristics, and even lower response rates have been reported in certain patient classes (Katayama et al., 1998). Meta-analyses by O'Connell et al. (2014) and Vaseghi et al. (2014) demonstrated limited evidence supporting the use of TMS or tDCS in chronic pain and CPSP. Vaseghi et al. (2014), who focused on tDCS, commented that stimulation could induce significant analgesic effects, but due to the heterogeneity across studies it is difficult to support its use in chronic pain (O'Connell et al., 2014; Vaseghi et al., 2014).

Such variable levels of efficacy have been associated with several factors such as lesion location and extent, the impact of altered neuronal excitability, and the shrinkage of gray and white matter (Hossman, 2009). Infarction based changes in brain tissue conductivity could also impact stimulation based CPSP treatments. Necrotic brain tissue in the infarction region is phagocytized by inflammatory cells and replaced by a cerebral spinal fluid (CSF) (De Girolami et al., 1999). CSF produces a sixfold increase in the tissues' electrical conductivity and a drastic disruption of the tissue geometry (Yunokuchi et al., 1998; Jacobs et al., 2001; Brown et al., 2003; Soltanian-Zadeh et al., 2003; Wagner et al., 2004, 2006, 2007a; Harris-Love and Cohen, 2006). Such altered electrical tissue properties have been shown to perturb the stimulating currents during TMS and tDCS (Wagner et al., 2006, 2007b, 2009).

Nevertheless, as emphasized by Plow and others, the role of such variables in influencing the distribution of current fields and ultimately impacting therapeutic efficacy in focally injured brain models needs further consideration, and remains to be compared across different brain stimulation techniques (Plow et al., 2009). Comparisons across stimulation techniques, which differ by electrode/source size, focality, invasiveness, proximity to lesion borders and specific features of the delivered electrical currents, are fundamental to evaluating and optimizing their clinical use (Plow et al., 2009). Furthermore, this comparative information is important for assessing the use of non-invasive stimulation techniques to identify responders to CPSP stimulation treatments prior to implanting invasive stimulation devices (Khedr et al., 2005; Lefaucheur, 2013, 2016).

The aim of this study is to determine how infarctions and/or complex neuroanatomy could alter the neurostimulation currents of the three primary neurostimulation techniques used in CPSP and potentially impact their clinical significance.

MATERIALS AND METHODS

Simplified magnetic resonance imaging (MRI) guided Finite Element Models (FEMs) of the stimulating current density distributions elicited through EBS, TMS, and tDCS were generated. The models were generated following methods previously outlined (Wagner et al., 2004, 2007b), and following foundational physics reviewed in the appendix of Wagner et al. (2014).

Briefly, we developed a FEM head/brain model with a healthy brain (developed from the MRI of a 38-year-old male) and a second model that included a circumscribed frontal cortical lesion within the head, specifically modeling a middle cerebral artery (MCA) based occlusion (Wagner et al., 2004). For simplification purposes, we focused on the comparison across stimulation techniques most commonly used to treat CPSP, and thus the head models did not include sulci and gyri, but only the presence of the lesion. Furthermore, we assumed static fields during stimulation for tDCS and EBS and sinusoidal steady state solutions during TMS.

The models were developed with Ansoft's Maxwell software (Ansoft Inc, Pittsburg, PA, USA). We specifically solved a modified magnetic diffusion equation for the TMS models:

$$\nabla \times \left(\frac{1}{\sigma(\omega) + j\omega\varepsilon(\omega)} \nabla \times \hat{\mathrm{H}} \right) = -j\omega\mu \hat{\mathrm{H}}$$

where H is the magnetic field in phasor form, sigma the tissue conductivity, epsilon the tissue permittivity, and omega the angular frequency of the source. The Ansoft package numerically solves the problem via a modified T-Ω method (Wagner et al., 2004). For the tDCS and EBS models, the Ansoft FEM solver was set to solve for the current densities in terms of the electric potential (ϕ), by solving the equation: $\nabla \cdot (\sigma_i \nabla \phi) = 0$, where σ_i is the conductivity of the tissue (Ansoft) (Wagner et al.,

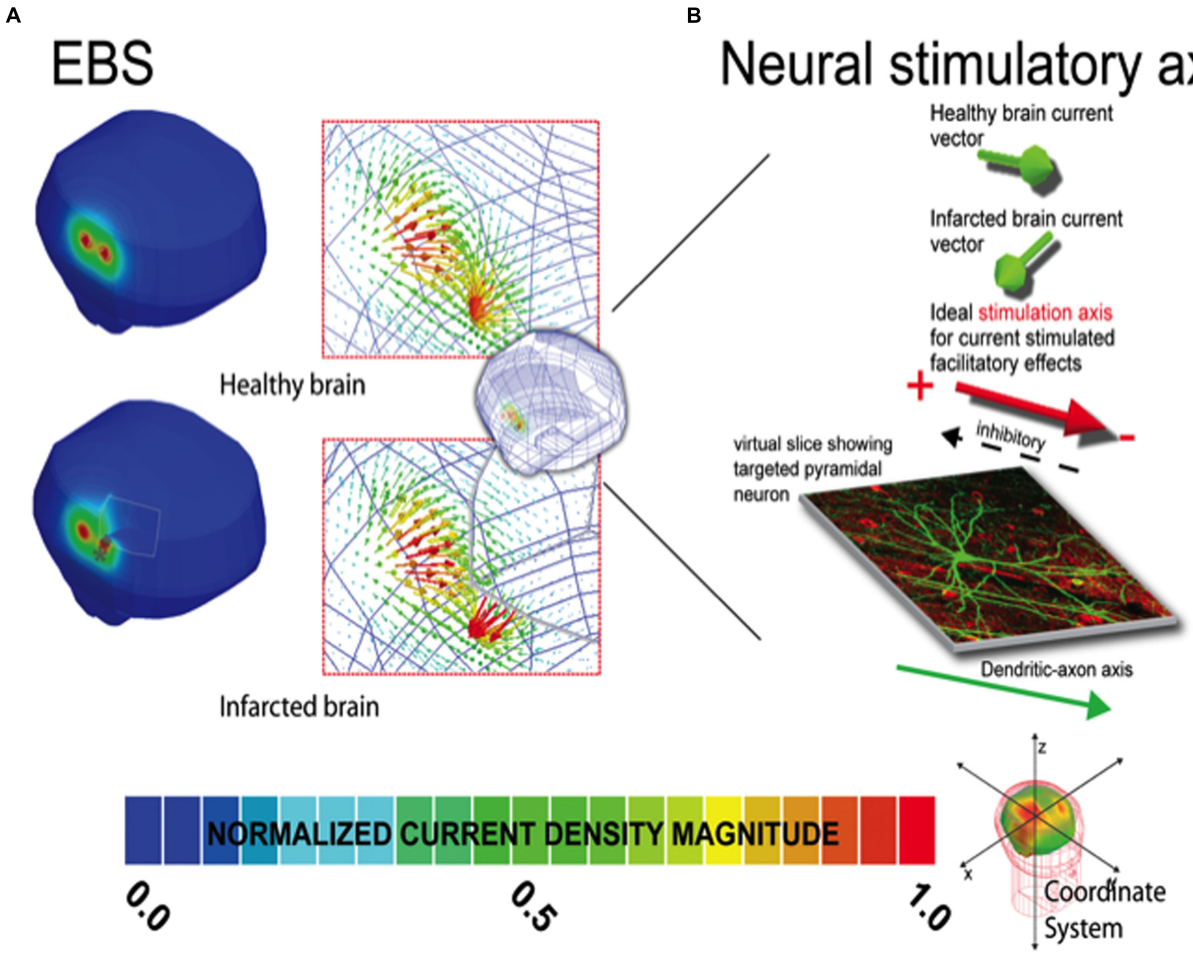

FIGURE 1 | Current density distribution maps induced by EBS stimulation. In **(A)**, the left column depicts the current density magnitude for the corresponding healthy intact (top) and infarcted (bottom) brains stimulated with EBS. The borders and limits of the infarcted region are demarcated with a thin white line. Note that the scales in **(A)** are normalized to the maxima of the solution in each case (i.e., the maximum in the healthy brain is 1.19 A/m^2 and 1.35 A/m^2 in the infarcted brain). See location of the maxima in the infarcted (gray ◆) and healthy brains (gray *) indicating the location shift due to the infarction. Exact quantitative estimations on maxima shifts can be found in **Table 2**. In the right column of each panel, the vector distribution demonstrating the orientation of the currents is provided for both the intact and damaged brains. Note the direction of the currents can change substantially in the region of the perturbation. **(B)** Demonstrates how the distribution of EBS induced currents can be altered such that facilitatory stimulation might become inhibitory in select neural populations in the lesion region, when applying **subthreshold** polarizing currents where the stimulatory effect is dependent on the relative current density orientation to the axo-dendritic axis (Terzuolo and Bullock, 1956; Landau et al., 1964). In our results for select regions of tissue near the lesion border, the current orientation is altered relative to the neural axis such that the neural effect would be opposite of that predicted for the healthy brain. Note herein, the inhibitory/facilitatory axis is simplified for graphical representation, and will ultimately depend on the complexity and relative position of the neural structure, related to the axo-dendritic axis of the neuron. The total net effect across the total tissue stimulated could be comprised of a mix of areas receiving inhibitory and facilitatory stimulation (based on the relative neural cell and current density orientations in each individual patient relative to the stimulator source). Furthermore, such effects could potentially be seen in areas of in areas of complex sulcal anatomy even in healthy subjects. Unique solutions based on each individual patient's stimulation criteria are thus recommended for individual patient dosing considerations.

2007b). For each model, the Ansoft FEM solver was set to follow an adaptive iterative process with convergence limits determined by the energy error in the system, further detailed in Ansoft (2002, 2005). The criterion for model convergence was defined as an energy error below 1.0% (Wagner et al., 2004, 2007a).

The current source device parameters correspond to those typically used in clinical studies and trials (Brown et al., 2006; Fregni et al., 2007; Lima and Fregni, 2008). The TMS source current was set as in prior modeling studies at 5 kHz with a 1.8×10^3 A peak current on a figure-of-eight coil with two 3.5 cm radius copper windings (Wagner et al., 2004). The tDCS source current was set at 1 mA across a 5 × 7 cm anode (on a scalp area overlying the motor strip) and cathode (above the contralateral *orbital*) (Wagner et al., 2007a). The EBS source was set at 1 mA, with the anode and cathode placed above the M1 (18 mm inter-contact distance, 1 mm radius) (Brown et al., 2006). Note that those EBS parameters are based on *Adtech* 1 mm radius electrodes mounted on a 3 × 3 grid over an 18 × 18 mm area (where the inner row is inactive) which generates three separate bipolar

TABLE 1 | Maximum current density magnitude (in A/m^2) in the healthy and the infarcted brain.

Neurostimulation modality and polarity	Healthy brain max current density (A/m^2)	Infarcted brain max current density (A/m^2)	Infarcted vs. healthy brain. Relative change in max current density (%)
EBS			
Cathode	1.15	1.35*	+17.4%*
Anode	1.19	1.22	+2.50%*
tDCS			
Anode	0.098	0.129*	+31.6%*
Cathode	0.082	0.084	+2.40%*
TMS			
	2.40	4.16*	+73.30%*

Corresponds to location of stimulation source proximal to the infarction border.

arrangements (distanced 18 mm)- (Adtech Medical Instrument Corp) (Brown et al., 2003).

While, we used a 1 mA source magnitude for EBS, it should be noted that the EBS solutions are linear in the region of interest and simple multiplicative scaling can be used to account for varied source magnitudes (Woodson and Melcher, 1968; Zahn, 2003; Wagner et al., 2014). Furthermore, as the EBS electrostatic solutions are addressable by superposition, we focused on one bipolar section at a time (Woodson and Melcher, 1968; Zahn, 2003; Wagner et al., 2014). As EBS and tDCS were modeled based on the same static approximations, the modeling and solution procedures were equivalent, except for the source properties (e.g., location and geometry). Finally, tissue material properties (i.e., conductivity and permittivity), including those of the infarction region, were assigned impedances as detailed in Wagner et al. (2006, 2007a).

The analyses then focused on determining the current density distributions for the head models (i.e., healthy vs. infarction) and specifically determining the current density magnitude, maximum current density location in the cortex, and current density vector orientation for the EBS, TMS, and tDCS sources. Full details of the analysis are given in Wagner et al. (2004, 2006, 2007a,b, 2014).

Briefly, the stimulation source location and stimulation device orientation were normalized for the three techniques, such that the stimulation sources were located with their device source centers above the same physical target location (M1) and equally distanced along the brain surface from the modeled lesion borders, which in our case was the caudal border.

To determine the current density maximum, we ran an algorithm that scanned the current density magnitudes in the brains, and determined the magnitude and location of the maxima for the healthy head and stroke models for each stimulation source. Where the results are reported as current density magnitudes, they indicate the magnitude of the sinusoidal steady state current density for TMS and the magnitude of the steady state current densities for EBS and tDCS, all of which are provided in units of A/m^2 unless otherwise stated.

The relative change between the healthy and infarcted brains is reported as the value of the difference between the current density maxima in the infarction and healthy head models divided by the current density maxima in the infarction model. Further, the individual models all shared the same Cartesian coordinate system, with an origin at the heads' center, and thus the relative change in maxima locations between the various healthy brain and infarction models was determined by the Euclidean distance equation. The current density vector field directional patterns were also analyzed in the models, and focused on comparing the change in the current density fields' vector orientation proximal to the current source and the lesions the healthy and infarction models [see **Figure 1**, and (Wagner et al., 2006) for further details]. The angular perturbation of the current densities between the healthy and infarction models was used to determine the relative current density orientation shift that would occur along a fixed axonal axis between the models (see **Figure 1B**). Finally, as the models were deterministic, we did not conduct statistical testing between the different solution sets.

RESULTS

Current density distributions (magnitude, location, and orientation) were altered in the presence of our idealized model of focal right frontal infarction for TMS, tDCS, and EBS, as compared to solutions in the intact brain models (**Tables 1–2** and

TABLE 2 | Coordinates of the locations (relative to the x,y,z head coordinate system) of the current density maxima in the healthy and the infarcted brain.

Neurostimulation modality and polarity	Stimulating source radius or equivalent length (mm)	Healthy brain *maxima* location x,y,z (mm)	Infarcted brain *maxima* location x,y,z (mm)	Absolute distance shift (mm)
EBS				
Cathode	∼1 mm	53.9, 22.9, 193.8	53.1, 24.7, 197	4.0 mm*
Anode	∼1 mm	53.7, 6.8, 194.1	53.6, 7.2, 194.8	<1.0 mm
tDCS				
Anode	∼25 mm	56.0, 18.2, 17.5	47.1, 27.5, 26.9	15.9 mm*
Cathode	∼25 mm	−14.5, 50.8, 27.3	−15.4, 50.5, 27.5	<1.0 mm
TMS				
	∼35 mm	−4.8, −7.2, −23.1	−15.1, −20.5, −17.0	17.9 mm*

Corresponds to alterations in predicted current density maxima location if the effects of the infarction on stimulation currents were ignored.

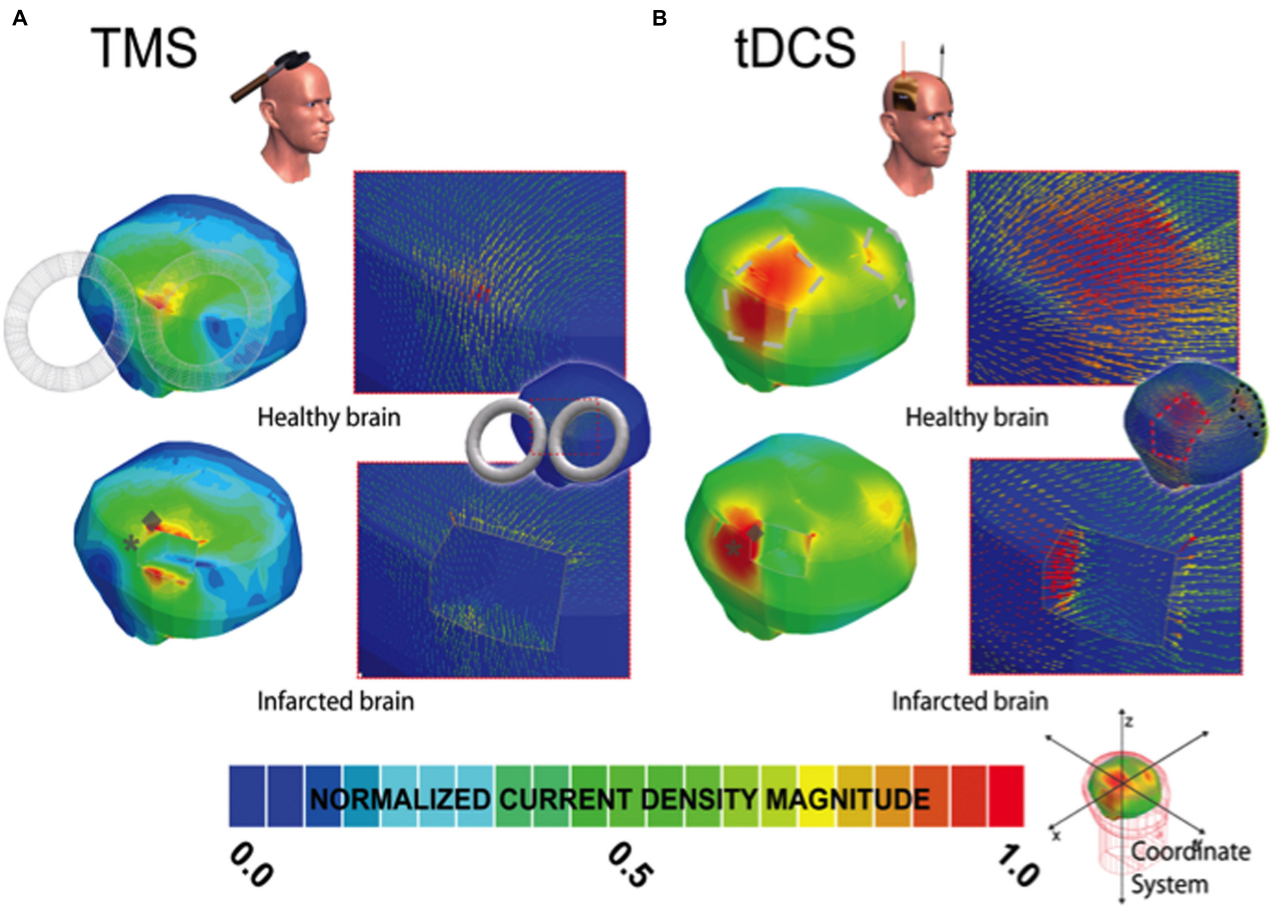

FIGURE 2 | Current density distribution maps induced by TMS and tDCS stimulation. In **(A,B)**, the left column depicts the current density magnitude for the corresponding healthy or intact (top) and infarcted (bottom) brains stimulated with TMS and tDCS, respectively. The borders and limits of the infarcted region are demarcated with a thin white line. The modeled lesions presented for EBS (see **Figure 1A**), TMS (2A), and tDCS (2B) all have the same size and volume and occupy the exact same location in the right hemisphere in the infracted brain. As in **Figure 1A**, note that the scale of **(A,B)** is normalized to the maxima in the corresponding solution pictured (i.e., the maximum current density in the TMS healthy brain solution is 2.4 A/m^2 and 4.16 in the infarcted brain, and 0.098 and 0.129 in the tDCS healthy and infarcted cases, respectively). The location of the maxima in the infarcted (gray ◆) and healthy brains (gray *) are both marked symbolically on the injured brain to indicate the estimated site shift (please zoom on the image for a better appreciation if needed). Note, as in EBS, the direction of the currents changes substantially in the region of the perturbation for both techniques.

Figures 1–**2**). For all three techniques, currents were increased in magnitude and directed toward the infarction border. Increases of peak current density in a damage brain compared to the healthy one were less drastic for EBS (+18%) than for tDCS (+32%) or TMS (+73%) (see **Table 1**). Furthermore, the vector current orientation was altered at the infarction borders, such that the net sign of the neuromodulation effects (i.e., lasting inhibition or facilitation) could be reversed (e.g., **Figure 1B** and further discussion below).

The overall absolute distance between the expected target and the actual site of the current maxima (comparing the healthy brain and infarction brain models) were less remarkable in overall magnitude for EBS (a 4 mm shift from the expected vs. the real maximum site) than for TMS (17.9 mm shift) or tDCS (15.9 mm shift) – see **Figures 1**–**2** and **Table 2**. However, relative to the size of the stimulation source, the shift of the current maxima was more drastic for EBS (∼1 mm radius contacts) than for TMS (∼35 mm radius contact source) or tDCS (∼25 mm shortest center-edge segment for a 50 × 70 mm electrode) (see **Table 2**, and in **Figures 1A** and **2A,B,** distances between the gray ◇ and * icons displayed on the brain models).

DISCUSSION

This study suggests that EBS, tDCS, and TMS neurostimulation current density distributions are altered in the presence of strokes in a manner that may explain discrepancies in CPSP treatment outcomes across the different stimulation techniques (André-Obadia et al., 2008, 2011, 2014; Hosomi et al., 2008, 2013; Lefaucheur et al., 2008, 2011a,b; Velasco et al., 2008; Tanei et al., 2011; Sachs et al., 2014). Currents flow down the path of least resistance, in the highly conductive CSF at an infarction location, and impact the current density distributions in magnitude,

location, and orientation for EBS (**Figure 1**), TMS (**Figure 2A**), and tDCS (**Figure 2B**) (Wagner et al., 2006, 2007a,b, 2009).

Although the overall absolute perturbation effects in the current densities were greatest in TMS and tDCS, EBS currents were still significantly affected when the stimulatory contacts were close to irregular tissue borders of the modeled chronic stroke lesion. Moreover, the change in the location of maximal stimulation between the infarcted and healthy brains was greatest with EBS relative to the size of the stimulator (see **Figures 1** and **2,** and **Table 2**). The lower focality of TMS and tDCS, as compared to EBS, could make them less sensitive to relative mislocalizations around the targeted location. This difference could reconcile the relevance of our current findings with the fact that TMS and tDCS studies in perilesional stroke regions have generally reported beneficial therapeutic effects with potentially less variability than EBS studies (Lima and Fregni, 2008; O'Connell et al., 2014; Hosomi et al., 2015; DosSantos et al., 2016).

The altered orientation of the stimulation currents relative to the targeted neurons could impact the degree and/or the direction of inhibitory/excitatory response of the involved networks, particularly for sub-threshold stimulation conditions- see **Figure 1B** (Terzuolo and Bullock, 1956; Landau et al., 1964; Wagner et al., 2007b; Radman et al., 2009a,b; Wongsarnpigoon and Grill, 2012). The net sign of the neuromodulation effects (i.e., lasting inhibition or facilitation) could potentially be reversed in cases where the lesion boundary alters the currents' orientation relative to the targeted cell's axo-dendritic axis [particularly for sub-threshold stimulations (Terzuolo and Bullock, 1956; Landau et al., 1964)].

Ultimately, the varied stimulation current perturbations between the techniques could in part explain inter-technique discrepancies between tDCS, TMS, and EBS in treating CPSP. Low-intensity EBS M1 cathodic stimulation currents are postulated to affect axons parallel and superficial over the crown of the precentral gyrus (Lefaucheur, 2013). In pain treatment, maximal pain relief is postulated to be associated with late indirect waves (recorded at the spinal cord level) produced from cathodic M1 EBS and also anteroposterior M1 TMS. On the other hand, anodal M1 EBS and lateromedial M1 TMS stimulation lead to early direct waves, suggesting that the polarity and orientation of the current in these techniques activates different axonal tracts and pathways (Lefaucheur, 2016). Unlike EBS, tDCS shows more analgesic effect during anodal stimulation, potentially due to different neuronal structures being activated, or due the relative current vector orientations having similar orientations in the targeted neurons, see **Figures 1–2** (Lefaucheur et al., 2010; Lefaucheur, 2013, 2016). This suggests that the relative current-neuronal structure orientations between tDCS, TMS, and EBS should be considered when planning stimulation treatments for CPSP, especially across techniques (e.g., using TMS to predict EBS response). Proper planning of the stimulation protocol with a MRI-integrated field solver-tracking device could be helpful to address the current-tissue interactions, but only with systems that track and predict current vector orientations (i.e., systems which predict field strengths alone could not be used to overcome discrepancies between the techniques).

Although the conclusions of the current study could apply to a large number of cases, any extension of the current results to other lesion features, such as subcortical locations and single or multiple lacunar strokes, which have been explored in neurostimulation therapeutic CPSP studies, would need to be specifically evaluated for individual dosing considerations. It is clear from the present study that electromagnetic tissue properties differently affect brain stimulation dosing for different stimulation methods, and introduce a technique-dependent variability in potential therapeutic benefit. Ignoring the effects of altered neural tissue properties on the M1 stimulating currents in stroke may contribute to contradictory outcomes in CPSP neurostimulation trials (O'Connell et al., 2014; Hosomi et al., 2015). Finally, our results highlight the need for new forms of brain stimulation that can overcome these limitations and provide effective treatment for chronic pain syndromes and other disorders where brain stimulation is used.

AUTHOR CONTRIBUTIONS

Respective roles of each author are as follows: RR and AV-C wrote the initial version of the manuscript. AO and RA had substantial contribution in the adaptation of the final manuscript to the challenges of neurostimulation technologies and approaches in CPSP. Finally, RR, UE, LA, LD, TW, and AV-C provided substantial contribution to the design of the work, and the revised versions of the manuscript. All authors provided their final approval of the submitted version and agreed to be accountable for all aspects of the work.

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Motor Cortex Excitability and BDNF Levels in Chronic Musculoskeletal Pain According to Structural Pathology

Wolnei Caumo[1,2,3,4], Alícia Deitos[1,2], Sandra Carvalho[5], Jorge Leite[5], Fabiana Carvalho[1,2], Jairo Alberto Dussán-Sarria[1,2], Maria da Graça Lopes Tarragó[1,2], Andressa Souza[6], Iraci Lucena da Silva Torres[1,7] and Felipe Fregni[8]*

[1] *Post-graduate Program in Medical Sciences, School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil,* [2] *Laboratory of Pain and Neuromodulation at UFRGS, Porto Alegre, Brazil,* [3] *Anesthesiologist, Pain and Palliative Care Service at Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil,* [4] *Pain and Anesthesia in Surgery Department, School of Medicine, UFRGS, Porto Alegre, Brazil,* [5] *Neuropsychophysiology Laboratory, CIPsi, School of Psychology (EPsi), University of Minho, Campus de Gualtar, Braga, Portugal,* [6] *Post-graduate Program in Health and Human Development, La Salle University Center, Canoas, Brazil,* [7] *Department of Pharmacology, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, Brazil,* [8] *Berenson-Allen Center for Noninvasive Brain Stimulation, Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*

The central sensitization syndrome (CSS) encompasses disorders with overlapping symptoms in a structural pathology spectrum ranging from persistent nociception [e.g., osteoarthritis (OA)] to an absence of tissue injuries such as the one presented in fibromyalgia (FM) and myofascial pain syndrome (MPS). First, we hypothesized that these syndromes present differences in their cortical excitability parameters assessed by transcranial magnetic stimulation (TMS), namely motor evoked potential (MEP), cortical silent period (CSP), short intracortical inhibition (SICI) and short intracortical facilitation (SICF). Second, considering that the presence of tissue injury could be detected by serum neurotrophins, we hypothesized that the spectrum of structural pathology (i.e., from persistent nociception like in OA, to the absence of tissue injury like in FM and MPS), could be detected by differential efficiency of their descending pain inhibitory system, as assessed by the conditioned pain modulation (CPM) paradigm. Third, we explored whether brain-derived neurotrophic factor (BDNF) had an influence on the relationship between motor cortex excitability and structural pathology. This cross-sectional study pooled baseline data from three randomized clinical trials. We included females (n = 114), aged 19–65 years old with disability by chronic pain syndromes (CPS): FM (n = 19), MPS (n = 54), OA (n = 27) and healthy subjects (n = 14). We assessed the serum BDNF, the motor cortex excitability by parameters the TMS measures and the change on numerical pain scale [NPS (0–10)] during CPM-task. The adjusted mean (SD) on the SICI observed in the absence of tissue injury was 56.36% lower than with persistent nociceptive input [0.31(0.18) vs. 0.55 (0.32)], respectively. The BDNF was inversely correlated with the SICI and with the change on NPS (0–10) during CPM-task. These findings suggest greater disinhibition in the motor cortex and the descending pain inhibitory system in FM and MPS than in OA and healthy subjects.

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***Correspondence:**
Wolnei Caumo
wcaumo@hcpa.edu.br

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Likewise, the inter-hemispheric disinhibition as well as the dysfunction in the descending pain modulatory system is higher in chronic pain without tissue injury compared to a structural lesion. In addition, they suggest that a greater level of serum BDNF may be involved in the processes that mediate the disinhibition of motor cortex excitability, as well as the function of descending inhibitory pain modulation system, independently of the physiopathology mechanism of musculoskeletal pain syndromes.

Keywords: short intracortical inhibition, brain-derived neurotrophic factor, central sensitization, conditioned pain modulation, osteoarthritis, fibromyalgia, myofascial pain syndrome

INTRODUCTION

A central sensitivity syndrome (CSS) is a cluster of symptoms including psychological distress, sleep disturbances, fatigue, pain, allodynia, hyperalgesia, and expansion of the receptive field (Yunus, 2007, 2008), which overlap with many chronic pain disorders. Despite the substantial overlapping, there is no consensus on the presence of these symptoms and structural pathology. For instance, in fibromyalgia (FM), chronic tensional headache and myofascial pain syndrome (MPS) the evidence of structural pathology is scarce. In contrast, in other conditions like osteoarthritis (OA), there is strong evidence of anatomic structural pathology that accounts for persistent nociceptive input. Nonetheless, irrespective of the amount of visible injury, these chronic pain conditions share a cluster of symptoms that support the hypothesis of the presence of central sensitization (CS) phenomenon. This neuronal event comprises an abnormal state of responsiveness for nociceptor stimuli. Thus, the pain arises as a consequence of changes within the central nervous system (CNS) that amplifies the response to nociceptive inputs across many organ systems and fails to suppress noise signals (Woolf and Salter, 2000; Ji et al., 2003).

At the cellular level, the CS comprises an impaired function of neurons and circuits in nociceptive pathways, in which exist increased membrane excitability, synaptic efficacy, or reduced inhibition (Latremoliere and Woolf, 2009). Sensitized neurons of the spinal dorsal horn exhibit increased spontaneous activity and response to subthreshold stimulation, reduction in the threshold for activation, and an enlargement of their receptive fields (Latremoliere and Woolf, 2009).

Thus, CSS pain arises from different abnormal mechanisms, including increased presynaptic release of excitatory neurotransmitters that will, in turn, elicit a greater postsynaptic response, by increasing the excitability of the postsynaptic membrane (Woolf et al., 1994; Craig, 2003). Then, changes in the microglia, astrocytes, gap junctions and gene transcription, contribute to the maintenance of this general state of excitation. Moreover, as part of the spinal microglial activation, the brain-derived neurotrophic factor (BDNF) is released, further contributing to the induction and maintenance of the CS (Trang et al., 2011).

Besides clinical complaints, the CSS also shares pathophysiological mechanisms. In OA, lower pain threshold and punctual hyperalgesia has been shown in areas of referred pain rather than on the original area of tissue injury (O'Driscoll and Jayson, 1975; Bajaj et al., 2001), which has been thought to reflect brainstem activation, as shown by functional magnetic resonance imaging (fMRI; Gwilym et al., 2009). In FM, the CS has been associated with the widespread reduction in thermal and mechanical pain thresholds (Gibson et al., 1994), inducing temporal summation, muscular hyperalgesia and pain, which are attenuated experimentally with the use of ketamine (Graven-Nielsen et al., 2000). Similarly, the persistent muscular tension experienced in the MPS has been hypothesized to induce CS (Fernández-de-las-Peñas et al., 2009), which could progressively produce the lead changes in the SNC. Although the BDNF is a common key player in the CS process, our research team recently showed that its serum levels might differ among chronic pain syndromes (CPS) (Deitos et al., 2015). In fact, the BDNF is a neurotrophic factor capable of strengthening glutamatergic synapses, while it weakens GABAergic synapses. The increase of this neurotrophic factor inverts the polarity of GABA currents in dorsal horn neurons (Coull et al., 2005). Thereby the GABAergic system loses the capacity to downregulate of the Cl-cotransporter K^+-Cl^- exporter (KCC2) expression in the dorsal horn (Rivera et al., 2002; Zhang et al., 2008). Thus, the accumulation intracellular of Cl^- limits the GABAergic inhibitory effect on these nociceptors, thereby promoting the disinhibition (Latremoliere and Woolf, 2009), which results in a persistent and amplified response to nociceptive inputs and fails to suppress noise signals (Woolf and Salter, 2000; Ji et al., 2003). Although the cross-talk among BDNF and chronic pain is a complex phenomenon, and the underlying mechanisms responsible for such observations remain poorly understood, the differential BDNF levels might be utility in distinguishing CS syndromes with and without structural pathology (Deitos et al., 2015).

Considering that the CSS is the utmost clinical picture of dysfunctional neuronal circuits where the defective inhibitory function stands out, it is reasonable to consider the use of neuronal inhibition indexes to increase our mechanistically knowledge about the underlying neural substrates of CSS. Fortunately, it is nowadays possible to clinically assess the motor intracortical inhibition (ICI) and probing neural plasticity (Schwenkreis et al., 2011) using the motor cortex excitability by transcranial magnetic stimulation (TMS) paradigms. Accordingly, in the neuropathic pain compared

to healthy controls (HCs) the repetitive transcranial magnetic stimulation (rTMS) improved the cortical disinhibition indexed by the ICI and by a shorter cortical silent period (CSP; Lefaucheur et al., 2006). Similar results were observed in other chronic pain conditions, such as in FM (Salerno et al., 2000) and complex regional pain (Schwenkreis et al., 2003). While in healthy subjects' experimental pain using capsaicin, the rTMS applied over the dorsolateral prefrontal cortex (DLPFC) decreased the short intracortical inhibition (SICI; Fierro et al., 2010) and induced a significant anti-nociceptive effect in the capsaicin pain model (Brighina et al., 2011). Thereby, this set of findings suggest that the TMS, permit us modulate and also assess the state of the balance of excitatory and inhibitory system involved in the physiopathology of the CSS, which is a fundamental process to develop and maintain the CPS.

Therefore, the present study aims to explore tools to assess clinically some of the mechanisms likely associated with the CS. We evaluated the cortical excitability, the function in the descending pain modulatory system, and their relationships with the BDNF in three CS syndromes of chronic pain with different pieces of evidence of tissue injury: OA, FM, and MPS. Thus, we explored three hypotheses. First, we hypothesized that these syndromes present differences in their cortical excitability parameters assessed by TMS, namely motor evoked potential (MEP), CSP, SICI and short intracortical facilitation (SICF). Second, considering that the CS in the absence of tissue injury could be detected by serum neurotrophins, we hypothesized that the spectrum of structural pathology (i.e., from persistent nociception like in OA, to the absence of tissue injury like in FM and MPS), could be detected by differential efficiency of their descending pain inhibitory system, as assessed by the conditioned pain modulation (CPM) paradigm. Third, we explored whether BDNF had an influence on the relationship between motor cortex excitability and structural pathology.

MATERIALS AND METHODS

Design Overview, Settings, and Participants

This protocol was approved by the Institutional Ethics Committee at the Hospital de Clínicas de Porto Alegre (HCPA; application no. 1005-55, Post-Graduate Research Group). All of the trials ran their respective protocols with the approval of the HCPA Ethics Committee and obtained written informed consent from all subjects. We conducted a cross-sectional study pooling baseline data from three clinical trials. The sample involved women with chronic pain conditions associated with CS syndromes but without evidence of structural pathology (FM NCT01904097 and MPS NCT01964729), and chronic pain conditions with CS symptoms due to known organic pathology (i.e., OA NCT01747070). Details of the inclusion of each study can be seen in **Figure 1**. All subjects were recruited by directly contacting them from the institutional chronic pain clinic, by referrals from other clinic units, and through media advertising. Besides their particular criteria, all trials excluded subjects who failed to understand Brazilian Portuguese.

The baseline data from the samples of three experiments conducted in our institution were retrieved. All the studies used the 100 mm visual analog scale (VAS) for pain, ranging from no pain (0 mm) to the worst pain imaginable (100 mm). Only subjects that reported pain equal or higher than 40 mm in the VAS [i.e., moderate or severe pain (Palos et al., 2006)] for more than 3 months, and that were associated with functional disability, were included in this study. Functional disability was assessed by a structured questionnaire containing dichotomous questions (yes/no) about how the chronic syndrome had interfered with their activities in the past 3 months, namely with: (1) work; (2) responsibilities at home; (3) enjoyable activities; (4) relationships; (5) personal goals; and (6) thinking clearly, problem-solving, concentrating, or recall. To be included, patients had to have at least one affirmative answer (i.e., Yes) to the questionnaire. Additionally, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used in the OA trial, as it is a reliable, and sensitive instrument commonly used to assess pain and disability in other studies of knee OA (Nunes et al., 2013). Only OA subjects with a disability score on the WOMAC were included.

Each clinical trial had rigorous inclusion criteria, and the diagnoses were confirmed by a physician with over 15 years of experience in chronic pain conditions. Specifically, the diagnosis was determined by the presence of clinical complaints, current and past medication, medical and psychiatric history, and current medical and psychiatric diagnosis. Trials criteria are summarized as follows:

1. Diagnosis criteria for chronic MPS included regional dull, achy, or deep pain with normal neurological examination; the presence of trigger or tender points, taut bands, palpable nodules; and exacerbation by stress, which could involve decreased range of motion and ropiness in the muscle (Tough et al., 2007). To distinguish neuropathic pain from ongoing nociception, the Neuropathic Pain Diagnostic Questionnaire (DN4) was applied to all subjects. Only those with a neuropathic component (score Z4) were included to standardize the severity of MPS.
2. FM diagnosis adhered to 2010 American College of Rheumatology criteria (Wolfe et al., 2011).
3. OA required the clinical and radiographic criteria of the American College of Rheumatology.
4. Pain-free control volunteers were invited using media advertisement and were prospectively recruited. We used a standard screening questionnaire to assess if they fulfilled the inclusion criteria. To be included, subjects had to be free of any acute or chronic pain; without recent use of analgesics, corticosteroids or medications with known effects on the CNS. Also, volunteers were not included if had abused of alcohol or psychotropic substances in the 6 months previous to the screening; and if had any rheumatologic, psychiatric, or neurological disorder. After obtaining written informed

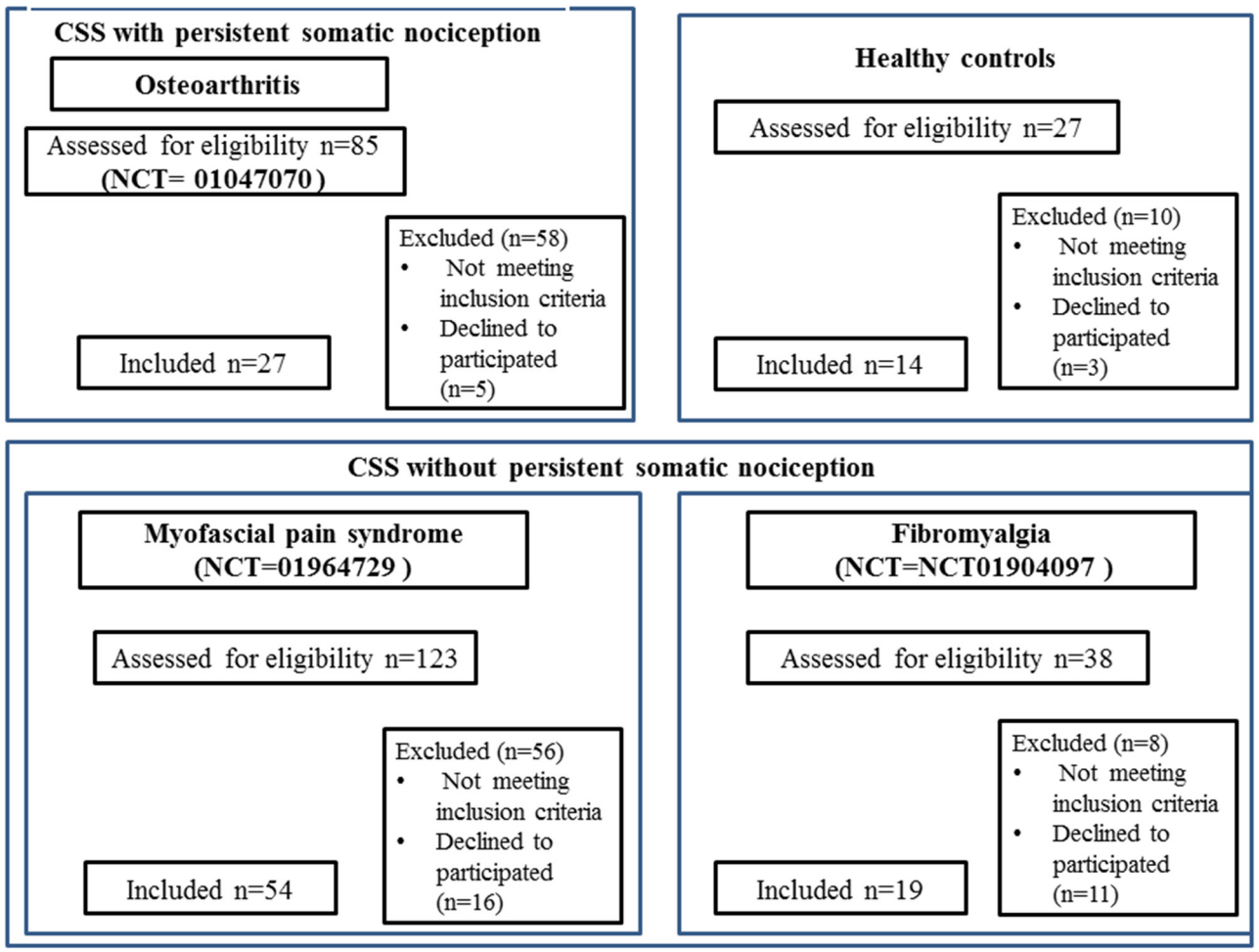

FIGURE 1 | CONSORT 2010 flow diagrams. The present cross-sectional study pooled baseline data from three clinical trials run at Hospital de Clínicas de Porto Alegre (HCPA), and that recruited women with fibromyalgia (FM; NCT01904097), myofascial pain syndrome (MPS; NCT01964729), osteoarthritis (OA; NCT01747070), and CSS indicates central sensitivity syndrome; VAS, visual analog scale.

consent, a structured interview, and a blood sample was obtained. All biological samples were collected at the HCPA in agreement with institutional policies. None of the healthy volunteers underwent a thorough physical examination. Although apparently a healthy population might have an underlying disease, or asymptomatic tender points, the lack of pain symptoms, or the lack of analgesics or other drugs use in the last 6 months makes highly unlikely the presence of current disease, particularly during the cross-sectional evaluation of our study. None of the patients neither the pain-free volunteers received monetary or any other compensation for participating in the studies.

Dependent and Independent Variables of Main Interest

The primary dependent variable was the measurement of the SICI and the change on the numerical pain scale (NPS 0–10) during a heterotopic stimulus. Secondary outcomes were other cortical excitability measures (MEP, SICF, and CSP). The independent variables of primary interest were the spectrum of structural pathology from persistent somatic nociception (i.e., OA) to absence of tissue injuries such as FM and MPS.

Instruments and Assessments

(a) The motor cortex excitability was assessed using TMS with a MagPro X100 (MagVenture Company, Lucerne marked, Denmark) and a figure-8 coil. The coil was centered over the motor cortex (M1), held tangentially to the scalp to reach the midline at 45°. To ensure a relaxation of arms and correct positioning of the hand, subjects were sat in a comfortable reclining chair. Cortical excitability parameters were registered through surface electromyography recordings gathered at the contralateral right first dorsal interosseous muscles using Ag/AgCl electrodes. First, the resting motor threshold (RMT) was determined by obtaining five out of 10 consecutive trials MEPs with a peak-to-peak amplitude of 50 μV. Next, 10 MEPs were recorded with an intensity of 130% of the individual

RMT. Moreover, the CSPs were assessed during muscle activity measured on a dynamometer set to approximately 20% of the maximal force. Accordingly, 10 CSPs were recorded using an intensity of 130% of the RMT. The SICI was assessed using an inter-stimulus interval of 2 ms. The conditioning stimulus (first) was set at 80% of the RMT while the test stimulus (second) was set at 100% of the individual MEP intensity. To assess the SICF was used an inter-stimulus interval of 12 ms. In total, 30 trials of paired-pulse were conducted in a randomized order (10 for each SICI, SICF, and control stimuli). We included the collection of all amplitudes of the MEPs, SICI and SICF and the duration of the CSPs in an off-line analyze. The units for these parameters included MEP in mV, SICI and SICF in their ratio to the MEP, and the CSP in milliseconds (Pascual-Leone et al., 1994).

(b) Quantitative sensory testing (QST) was used to assess heat pain thresholds using the method of limits with a computer Peltier-based device thermode (30 × 30 mm; Schestatsky et al., 2011). The thermode was attached to the skin on the ventral aspect of the mid-forearm, with an increase of temperature at a rate of 1°C/s, from 30°C to a maximum of 52°C. We asked the participants to press a button as soon as their felt mild pain (6/10) on the NPS ranging from 0 (no pain) to 10 (the worst pain). A single training session was offered before so participants could get familiar with the device. The thermode remained on the right ventral forearm, even though, it was slightly altered on trials to avoid either response suppression or sensitization of the cutaneous heat nociceptors. To evaluate the degree to which pain perception is modulated following the presentation of an initial heterotopic noxious stimulus (CPM), we used the QST during cold water immersion. This sensation was assessed raising the temperature to the point at which subjects felt mild pain (6/10) on the NPS. Thus, they immersed their non-dominant hands into cold water (zero to four degree Celsius) for 1 min. The QST was administered after 30 s of the cold-water immersion. During this time, subjects were asked to rate the pain of the stimulated arm (pain sensation by heat) using the same NPS. During the experiment for each participant, the temperature was held constant. The CPM was defined as the difference (presented in percentage) between the average pain rating before and after cold water immersion. To control for individual variation concerning baseline values, we used the proportion of difference from baseline.

Potential Confounding Factors

The psychological tests used in the current study had been validated for the Brazilian population (Gomes-Oliveira et al., 2012; Sehn et al., 2012). Two independent medical examiners were trained to administer the pain scales and to conduct the psychological tests. The patients' depressive symptoms were assessed using the Beck Depression Inventory II (Gomes-Oliveira et al., 2012). The catastrophizing thinking related to pain was evaluated using the Brazilian Portuguese of the Catastrophizing Scale (B-PCS; Sehn et al., 2012). We used a standardized questionnaire to assess demographic data and medical comorbidities.

Serum Neuroplasticity Mediators' Concentration

All of the trials used standard procedures for biological samples, by collecting blood at minimum 8 h after fasting early in the morning. All biological materials were collected before applying any intervention. Plastic tubes were centrifuged for 10 min at 5000 g at 4°C. Serum was frozen at −80°C until assays were performed. Serum neuroplasticity mediators concentrations were determined using specialized BDNF kits (catalog no. CYT306, the lower detection limit of the kit = 7.8 pg/mL, Chemicon/Millipore).

Efforts to Address Potential Sources of Bias

To reduce assessment bias only two researchers (MGT; WC) with a practicing of the outpatient pain clinic at HCPA with vast clinical expertise were responsible for making the diagnostics according to pre-specified criteria. Three evaluators with specific training in performing TMS were responsible for all TMS measures of cortical excitability. The same evaluators applied clinical scales and performed the QST.

Sample Size

The power of the study was estimated based on type II and type I error of 0.15 and 0.05 respectively and in anticipation of an effect size (f_2 = determination coefficient) of 0.15 for the multiple hierarchical regression analysis allowing for four predictors (the *Post hoc* statistical power calculator for hierarchical multiple regression: 46[1]. A sample of 100 patients would detect an effect size for correlations of 0.15, with a power of 93% at a 0.05 alpha level.

Statistical Analysis

To summarize the main characteristics of the sample we used traditional descriptive statistics, and performed Shapiro-Wilk tests to evaluate a normal distribution, we used Shapiro-Wilk tests. We used ANOVA to compare continuous variables with parametric distribution, and Chi-Square or Fisher's exact test for categorical variables. Variables not normally distributed were log transformed for further inclusion into regression models.

To compare each cortical excitability parameter (MEP, CSP, SICI, SICF) among CPS (MPS, FM, OA) and healthy subjects we used ANOVA. While an MANCOVA was used to compare the relationship between the cortical excitability parameters as dependent variables (MEP, CSP, SICI, SICF) and the spectrum of structural pathology as a binomial independent variable (where, OA represents ongoing tissue injury and FM and MPS grouped together represent the absence of structural pathology). Another MANCOVA model was used to assess the relationship between the SICI and the CPM (dependent variables) with the BDNF, according to the spectrum of structural pathology. Taking into account that the pain severity, the age, the degree of depressive symptoms, and the use of psychotropic medications differed

[1]http://www.danielsoper.com/statcalc3/calc.aspx?id

between the pain syndromes and that these factors can affect the biological process of BDNF secretion, we constructed an adjusted index. A multivariate regression model controlled by multicollinearity was used to obtain an adjusted index used as the surrogate of the BDNF. We adjusted for multiple comparisons using Bonferroni correction. Cohen's f2 effect size was calculated using an effect size calculator for multiple regressions given the values of R2 [A-priori Sample Size Calculator for Hierarchical Multiple Regression[2]]. The data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL, USA).

RESULTS

One hundred records were retrieved from three different trials ran in the HCPA. The sample of CS syndrome without structural pathology was composed of subjects with MPS ($n = 54$) and FM ($n = 19$). The sample of CS syndrome with persistent nociceptive input included subjects with OA ($n = 27$). The flow chart of this study is presented in **Figure 1**.

We screened 123 potential participants with a diagnosis of MPS, and 54 of them were included in the study. Subjects were excluded if they did not fulfill the criteria for MPS or due to the presence of another diagnosis (e.g., FM). We screened 38 potential participants with a diagnosis of FM, and 19 of them were included in the study. Subjects excluded did not fulfill the diagnostic criteria for FM or had other diagnoses (e.g., rheumatoid arthritis, chronic use of corticosteroids). We screened 85 potential participants with a diagnosis of OA, and 27 of them were included in the study. We excluded them for not fulfilling diagnostic criteria for severe OA, and due to the presence of other diagnoses (i.e., surgery in the segment, chronic use of corticosteroids, among others). We screened 27 HCs, and 14 were included in the study. They were excluded in the presence of depressive symptoms in moderate to severe intensity, history of epilepsy, chronic headache or use of psychotropics. Final sample characteristics are presented in **Table 1**.

Motor Cortex Excitability Parameters According to Chronic Pain Syndromes

The cortical excitability parameters are presented in **Table 2**. The mean (SD) between CPS and healthy subjects were compared using ANOVA followed by Bonferroni adjustment for multiple comparisons. Compared to healthy volunteers, subjects with MPS presented greater corticospinal tract excitability as shown by elevated MEPs. They also exhibited higher SICF but reduced SICI and CSP. As a matter of fact, MPS showed the largest MEPs amplitude among the subjects with CPS. Furthermore, the SICF was higher, while the SICI and CSP were lower in MPS compared to healthy volunteers. Except the MEP, the cortical excitability parameters of the MPS subjects were similar to those with FM, but different to those of OA subjects. Except for MEPs, FM subject's cortical excitability was different to those of the HCs in the same direction as MPS were. Likewise, the cortical excitability parameters of the subjects with OA differed to the one of the HCs in the same direction as MPS and FM, which means having higher MEPs and SICF and lower SICI and CSP.

[2]http://www.danielsoper.com/statcalc

Cortical Excitability According to the Spectrum of Structural Pathology to Absence of Tissue Injury

The multivariate regression model with the cortical excitability parameters as dependent variables (MEP, SICF, SICI, CSP) using the spectrum of structural pathology to an absence of tissue injury as independent binomial variable, where structural pathology (i.e., OA) is one level, and the absence of tissue injury (MPS and FM pooled together) is the other level (please see **Table 3**). This analysis showed a significant relationship between the spectrum of structural pathology to an absence of tissue injury and SICI (Wilks' $\lambda = 0.93$, $F = 3.16$ (2) = 85, $P < 0.04$). The power of this analysis was 0.80. Subjects with an absence of structural pathology presented greater disinhibition than those with persistent nociceptive input. The adjusted mean (SD) on the SICI observed in the absence of tissue injury was 56.36% lower than in those with persistent nociceptive input [0.31(0.18) vs. 0.55 (0.32)], respectively.

Relationship Between Intracortical Inhibition and Descendent Pain Modulatory System with The BDNF According to Structural Pathology

The lack of significant differences in cortical excitability parameters between MPS and FM supports the hypothesis that both pathologies do not differ significantly in their cortical facilitatory and inhibitory profile. Therefore, FM and MPS were grouped under the label of CSS with an absence of tissue injury for the subsequent analysis. As presented in **Table 1**, serum BDNF differs between healthy volunteers and subjects. When pooling subjects according to the presence of tissue injury, we observed that serum BDNF in those with the spectrum of structural pathology had significantly lower levels in comparison to those with absence of tissue injury, with 17.91 (7.27) and 35.29 (21.22), respectively ($t = 3.830$; $P < 0.0001$ of the comparison after log transformation of both serum BDNF). To account for the influence of pain severity, age, depressive symptoms, and use of psychotropic medications on the secretion of BDNF, an index was constructed using multivariate regression. The factors included explained 30% of the variance of the model.

The multivariate linear regression model included the SICI and the CPM as dependent variables, and the used as independent variables the structural pathology (absence vs. presence, binomial) and the BDNF adjusted index. The model is presented in **Table 4**. This analysis showed a significant relationship between the presence of structural pathology, the SICI and the CPM (Wilks' $\lambda = 0.90$, $F = 4.83$, $P < 0.01$). The power of this analysis was 0.81. Subjects with an absence

TABLE 1 | Characteristics of the sample.

Characteristic	Healthy subjects (*n* = 14)	Myofascial pain syndrome (*n* = 54)	Fibromyalgia (*n* = 19)	Osteoarthritis (*n* = 27)	*P*
Age (years)	32.43 (10.81)	46.13 (12.10)	50.42 (8.84)	64.42 (7.81)	0.00
Body mass index (kg/m^2)	23.12 (2.93)	25.22 (4.37)	31.81 (7.08)	28.52 (5.52)	0.00
Years of education	17.14 (2.53)	10.16 (3.61)	13.29 (4.04)	10.37(5.61)	0.00
Employed (yes/no)	14/0	45/9	16/3	19/8	0.19
Smoking (yes/no)	0/14	2/52	5/14	0/27	0.14
History of psychiatric disorder (yes/no)*	NA	19/35	7/12	5/22	0.24
Drug active on the nervous system (yes/no)**	NA	17/37	11/8	12/15	0.11
Tricyclic antidepressant (yes/no)	NA	7/47	7/12	1/26	–
Selective serotonin reuptake inhibitor (yes/no)	NA	8/46	9/10	6/21	–
Anticonvulsants (yes/no)	NA	4/50	1/18	0/27	–
Benzodiazepine	NA	1/53	2/17	5/22	–
Chronic disease (yes/no)	NA	11/8	16/38	20/7	0.00
Hypertension (yes/no)	NA	14/40	10/9	14/13	–
Diabetes mellitus (yes/no)	NA	5/49	1/18	4/23	–
Asthma (yes/no)	NA	1/53	3/16	3/24	–
Number of analgesic doses used per week (≥4 doses-week/<4 doses)	NA	27/26	14/5	20/6	0.04
Pain on the VAS (last 7 days)	NA	7.23 (2.19)	7.94 (1.89)	6.26 (2.15)	0.03
Pain on the VAS (24 h)	NA	6.11 (2.59)	7.10 (1.88)	5.37 (2.47)	0.06
Beck depression inventory II	NA	13.92 (8.85)	24.47 (11.67)	10.04 (7.18)	0.00
Brazilian portuguese catastrophizing scale (B-PCS)	NA	28.26 (12.51)	34.68 (11.69)	22.89 (11.59)	0.00
Serum BDNF (ng/mL)	19.00 (8.79)	29.28 (20.01)	50.78 (16.06)	17.91 (7.27)	0.00

*Data are presented as mean (standard deviation) or number of subjects (n = 114). *Patients could have none or more than one psychiatric disorder. **Some patients were using more than one type of drug. Abbreviations: visual analog scale for pain, from 0 to 10 (VAS); brain-derived neurotrophic factor (BDNF).*

of structural pathology presented greater disinhibition than those with persistent nociceptive input. The increase in BDNF was associated with the lower efficiency of the descendent pain modulatory system. However, it was not observed any difference of the BDNF effect in the SICI when we compared it according to the presence of tissue injury. Thus, to address this important issue we conducted an additional regression analysis to assess the relationship between the SICI and BDNF despite the condition sustaining pain. This model revealed a β coefficient = −0.22; t = −2.14; P = 0.03, suggesting that the relationship between BDNF and ICI is independent of the chronic pain mechanism.

Figures 2A,B presents the relationships between the SICI and the CPM (primary outcomes) according to the presence of structural pathology. The means were compared using MANCOVA, and *post hoc* were adjusted for multiple comparisons using Bonferroni correction (the model is presented in **Table 4**).

TABLE 2 | Cortical excitability parameters presented by chronic pain syndrome (CPS).

	Chronic pain syndrome			
	Fibromyalgia (*n* = 19)	Myofascial pain syndrome (*n* = 54)	Osteoarthritis (*n* = 27)	Healthy subjects (*n* = 14)
Motor evoked potential (mV)	1.13 (0.11)[a]	1.64 (0.49)[c]	1.46 (0.62)[b]	1.25 (0.38)[a]
Short intracortical facilitation (ratio: SICF/test stimulus)	0.96 (0.44)[c]	1.13 (0.23)[c]	0.97 (0.41)[c]	0.71 (0.36)[a]
Short interval intracortical inhibition (ratio: SICI/test stimulus)	0.32 (0.22)[c]	0.31 (0.17)[c]	0.59 (0.30)[b]	0.92 (0.07)[a]
Cortical silent period (CSP)	68.07 (18.43)[c]	68.44 (20.55)[c]	62.20 (16.68)[b,c]	76.67 (21.35)[a]

Data are presented as mean (SD; n = 114). Different superscripts (a, b, c) indicate significant difference among treatment groups after post hoc analysis adjusted by Bonferroni (P < 0.05). Analysis of variance (ANOVA) to compare mean (SD).

TABLE 3 | Relationship between motor cortex excitability according to the spectrum of structural pathology and absence of tissue injury (*n* = 100).

Dependent variable	Type III sum of squares	*df*	Mean square error	*F*	*P*	*Partial eta squared*
Motor-evoked-potential amplitude (mV)	2.10	2	1.05	4.11	0.02	0.09
Short intracortical facilitation (ratio: SICF/test stimulus)	0.19	2	0.09	0.86	0.42	0.02
Short interval intracortical inhibition (ratio: SICI/test stimulus) *log*	5.42	2	2.71	7.42	0.001	0.15
Cortical silent period (CSP)	2668.54	2	1334.27	3.55	0.03	0.08
	B		**SEM**	***t***	***P***	***Partial eta squared***
Motor evoked potential (mV)						
Intercepted	2.06		0.37	5.64	0.000	0.27
Absence of structural pathology (*n* = 73)	−0.008		0.17	−0.05	0.96	0.00
Presence of structural pathology (*n* = 27)	0[a]		–	–	–	–
Age (years)	−0.01		0.005	−2.27	0.02	0.06
Short intracortical facilitation (ratio: SICF/test stimulus)						
Intercepted	1.05		0.24	4.30	0.000	0.18
Absence of structural pathology (*n* = 73)	0.09		0.11	0.81	0.41	0.008
Presence of structural pathology (*n* = 27)	0[a]		–	–	–	–
Age (years)	−0.001		0.003	−0.30	0.76	0.001
Short intracortical inhibition (ratio: SICI/test stimulus) log						
Intercepted	−0.79		0.43	−1.82	0.07	0.00.
Absence of structural pathology (*n* = 73)	−0.58		0.20	−2.84	0.006	0.09
Presence of structural pathology (*n* = 27)	0[a]		–	–	–	–
Age (years)	0.002		0.006	0.27	0.79	0.001
Cortical silent period						
Intercepted	68.74		14.03	4.89	0.001	0.22
Absence of structural pathology (*n* = 73)	8.60		6.54	1.31	0.19	0.02
Presence of structural pathology (*n* = 27)	0[a]		–	–	–	–
Age (years)	−0.19		0.19	−0.99	0.32	0.01

DISCUSSION

This study assessed the motor cortex excitability by using different TMS measures, such as MEP, CSP, SICI, SICF, and BDNF levels in chronic musculoskeletal pain according to structural pathology. These data suggest that there is a relationship between the motor cortex disinhibition and conditions of chronic musculoskeletal pain compared to healthy subjects. This disinhibition is greater in subjects with chronic pain without tissue injury compared to the ones with structural lesion (**Table 4**). Additionally, after adjusting for relevant confounders, higher levels of BDNF were significantly correlated with decreased inhibitory system as assessed by CPM.

We observed greater disinhibition at the cortical level when the CS syndrome occurred without evidence of structural pathology (i.e., MPS and FM) compared to those with persistent nociceptive input (i.e., OA). This finding suggests that a different activation of the nociceptive system leads to distinct plastic changes in the pain pathways. It is possible that this disinhibition process is a common feature of CPS, which could be further increased in the absence of structural pathology due to lack of opposition. Furthermore, the cortical inhibition could be used as an additional tool to infer, to a certain extent, the level of severity of the CS phenomena. This needs, however, further confirmation with large clinical trials.

This hypothesis is biologically plausible because the disinhibition results from the imbalance between excitability and inhibition induced by GABA activity reduction, an increase in glutamate activity, and activation of NMDA-dependent activity (Nitsche et al., 2010). These dysfunctions in excitatory/inhibitory systems at pain pathways are nothing but the biological grounds of the clinical picture known as CSS.

Our study suggests that the supraspinal reorganization in different chronic musculoskeletal pain conditions, which is in agreement with previous studies (Gracely et al., 2002; Flor, 2003). Despite being conceivable that due to the structural lesion, pain occurs by specific activation of pain pathways, a sustained activation of the nociceptive system leads to an involvement of different brain circuitries. While in the CS syndromes in the absence of an obvious source of nociception, a self-driven stimulus activates the pain circuits autonomously. Thereby, the present findings suggest the evidence for a disinhibition spectrum that was presented according to the pathophysiology of chronic musculoskeletal pain.

TABLE 4 | Relationship between intracortical inhibition and descendent pain modulatory system with the brain-derived neurotrophic factor (BDNF) according to the spectrum of structural pathology and absence of tissue injuries (*n* = 100).

Dependent variable	Type III sum of squares	*df*	Mean *square error*	*F*	*P*	*Partial eta* squared
Change of NPS (0–10) during CPM-task	73.097	3	24.36	4.60	0.005	0.30
Short intracortical inhibition (*log*)	3.88	3	1.29	3.75	0.014	0.11
	B		**SEM**	***t***	***P***	**Partial eta *squared***
Change of NPS (0–10) during CPM-task						
Intercepted	0.10		0.69	0.16	0.880	0.00
Absence of structural pathology (*n = 73*)	4.46		0.76	5.90	0.001	0.28
Presence of structural pathology (*n = 27*)	0[a]		–	–	–	–
BDNF (*adjusted index*)	−0.09		0.03	−3.02	0.003	0.09
Interaction						
Absence of structural pathology *vs*. BDNF (*index adjusted; n = 65*)	−0.07		0.03	−2.31	0.020	0.06
Presence of structural pathology *vs*. BDNF (*index adjusted; n = 27*)	−0.29		0.07	−4.33	0.001	0.18
Short intracortical inhibition (*log*)						
Intercepted	−0.80		0.18	−4.55	0.001	0.19
Absence of structural pathology (*n = 73*)	−0.45		0.19	−2.34	0.020	0.06
Presence of structural pathology (*n = 27*)	0[a]		–	–	–	–
BDNF (*index adjusted*)	0.00		0.008	0.05	0.95	0.00
Interaction						
Absence of structural pathology *vs*. BDNF (*index adjusted; n = 65*)	−0.001		0.003	−0.29	0.78	0.001
Presence of structural pathology *vs*. BDNF (*index adjusted; n = 27*)[§]	0.01		0.006	1.77	0.08	0.04

[§] *Take in account that the severity of pain, age, depression symptoms, and use of psychotropic medication as dummy variable.*

Our data also shows that the neuroplastic changes as assessed by the TMS measurements were related to both, the musculoskeletal CPS and its severity (interpreted as the gradient in evident structural pathology). An indirect evidence supporting these claims is that there are morphometric differences in prefrontal and thalamic gray matter between subjects with neuropathic and non-neuropathic chronic pain, which can suggest differential CNS neuroplasticity according to the etiology involved in the development of chronic pain (Apkarian et al., 2004). Also, corresponding neuroplastic changes were experimentally observed in a transient deafferentation-induced by an anesthetic nerve block (Theuvenet et al., 2011). Taken together, these findings suggest that the pathophysiological mechanisms and the severity of the disease moderate the disinhibition process. This hypothesis is also supported by further evidence in neuropathic pain, in which a more pronounced motor cortex disinhibition was observed in the moderate/severe pain (NRS $\geq$ 4) compared to the mild pain. The cortical reorganization has also been suggested following the use of rTMS applied to the motor cortex restored the equilibrium between the excitatory and inhibitory system in parallel to the reduction in pain intensity (Lefaucheur et al., 2006; Mhalla et al., 2011). Moreover, increased ICF and a decreased SICI in the contralateral hemisphere following limb amputation was shown (Chen et al., 1998; Schwenkreis et al., 2000), thus supporting a cortical reorganization. Likewise, subjects with complex regional pain syndrome (CRPS) presented a reduced SICI, not only in the ipsilateral but also in the contralateral motor cortex (Lenz et al., 2011). Similarly, SICI reduction was shown in several other different chronic pain conditions, such as MPS (Dall'Agnol et al., 2014), FM (Mhalla et al., 2010), OA (da Graca-Tarragó et al., 2015) and neuropathic pain syndromes (Schwenkreis et al., 2010).

The cortical excitability pattern of FM and MPS is not significantly different. The only difference is in the MEPs, which is thought to represent the corticospinal tract excitability (Petersen et al., 2003). In fact, these two entities might be the same syndrome, which represents a continuum at different moments at long of time. According to other authors have previously proposed such interpretation, although they offered scarce evidence supporting this point (Ge et al., 2009).

In the present study, the heterotopic nociceptive stimulus during the CPM-task induced a greater response in the descending modulatory system in subjects with persistent

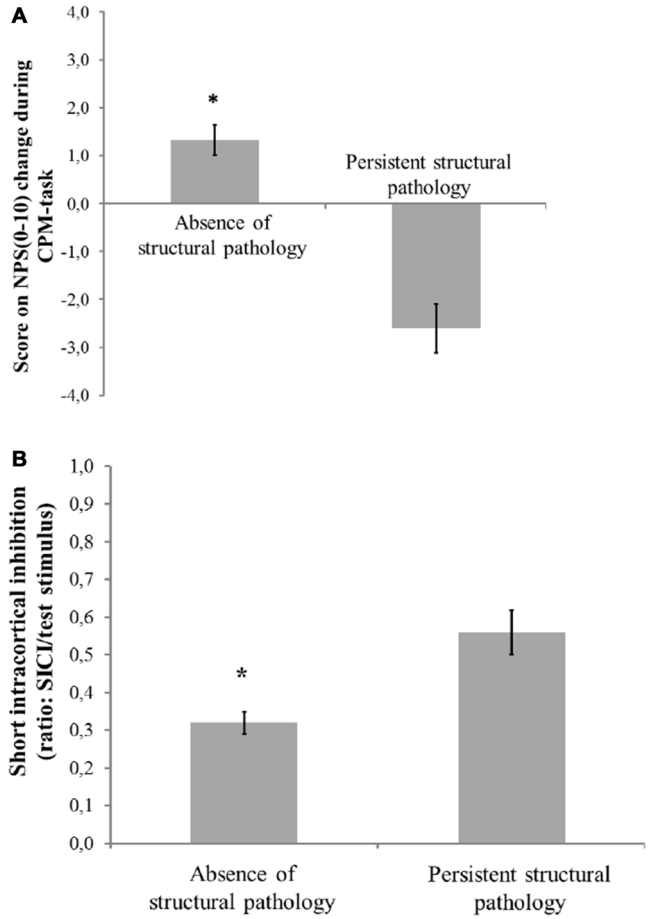

FIGURE 2 | Mean and standard error of mean (SEM). (A) Change on numerical pain scale [NPS (0–10)] during conditioned pain modulation (CPM)-task; **(B)** Short intra-cortical inhibition (SICI) expresses the relationship between the amplitude of wave and motor evoked potentials (MEPs) (relative amplitude, express in %), at inter-stimuli intervals (ISIs) of 2 ms with paired-pulse. The first is a sub-threshold stimulus [80% of the rest motor threshold (rMT)] followed by the second one which is a suprathreshold stimulus (130% rMT). Spectrum of structural pathology to absence of tissue injury to persistent nociceptive input. The error bars indicate the SEM. An asterisk indicates a significant difference between groups according to spectrum of structural pathology to absence of tissue injury to persistent nociceptive ($P < 0.05$). A MANCOVA was used to perform the comparisons, followed by the Bonferroni correction for *post hoc* multiple comparisons.

nociception compared to an absence of tissue injury. Although the mechanisms underlying this phenomenon are unknown, it is known that during inflammation the periaqueductal gray (PAG) suffers structural changes (Guan et al., 2002; Miki et al., 2002; Imbe et al., 2005). Furthermore, in the spinal cord, an increased turnover of noradrenaline (Weil-Fugazza et al., 1986) and the number of alpha(2)-adrenergic receptor has been associated with the inflammation (Brandt and Livingston, 1990). All these changes likely contribute to a rise in descending pain inhibition. It is possible that the inflammation contributes to triggering and maintenance of increased inhibitory controls. However, there are mixed results. Chronic arthritis induced experimentally strengthens the excitatory drive caused by conditioning stimulus (Danziger et al., 1999). Whereas in clinical studies subjects with FM presented a reduction of CPM, which potentially contributes to hyperalgesia (Kosek and Hansson, 1997) and in neuropathic pain the effect of CPM varied from a particular influence on on-going vs. evoked pain (Witting et al., 2003).

Although the mechanisms of facilitation induced by chronic pain prompt to disengagement in the descending modulation, according to the present findings this process can have a distinct level of severity according to the spectrum of structural pathology to an absence of tissue injury. The descendent modulation involves some mechanisms to inhibit the neurotransmission at the PAG, and at the spinal cord. These mechanisms include the activation of inhibitory of interneurons (Millar and Williams, 1989); reduction the quantity of amino acids, neuropeptides, and monoamines (Jensen and Yaksh, 1984); and postsynaptic inhibition of pain-relay neurons (Giesler et al., 1981). Additionally, the role of monoamines in order to increase the inhibition has already been demonstrated in preclinical studies, in which the duloxetine use reduces the amine uptake (Wong et al., 1993), and also in clinical research (for instance in knee OA), where the duloxetine decreased pain more than placebo (Chappell et al., 2009).

In this report, the reduced inhibition was inversely correlated with the BDNF despite the pain condition. This result highlights the remarkable effect of this neurotrophic factor in the cortical neuroplasticity process. Equally, the BDNF had an inverse correlation with the CPM, thus suggesting that decreased function in the descending pain modulatory system (greater scores in the CPM) prone to a higher propensity for pain. In this way, we showed that the variability of serum BDNF and the dynamic state of inter-hemispheric cortical excitability was independent of the mechanism underlying the chronic musculoskeletal pain. Thus, the correlation between the disinhibition in the motor cortex and the dysfunction of descending pain modulation system observed in this study might have clinical relevance because the CPM is a marker with a large size effect to identify impairment of descendent pain modulatory system in populations with long-term pain conditions (Lewis et al., 2012). Hence, it is biologically plausible that the BDNF enhancement activates signaling pathways in the spinothalamic tract due to a reduction of the GABAergic inhibitory effect (Spezia Adachi et al., 2015). Even so the design of this study prevents establishing if the disinhibition in the motor cortex could result in the higher serum levels of this neurotrophic factor, or vice-versa, it does permit us to a better comprehension of the dysfunctional disinhibition at cortical and intra-cortical regions in severe musculoskeletal chronic pain. Thereby, the pieces of evidence these findings possess may hold clinical implications such as to understand that the effects of BDNF in GABAergic/glycinergic system engage multiple molecular and cellular mechanisms that are largely complementary (i.e., increased excitation and reduced inhibition) in spinal, midbrain and peripheral structures associated with nociceptive processing. The secretion of BDNF by microglia downregulates

of the Cl-cotransporter K^+-Cl^- exporter (KCC2) expression in the dorsal horn resulting accumulation of intracellular chloride shifts the chloride equilibrium potential (ECl) to a less negative value. Hence, the activation of GABAA receptors produces a less hyperpolarization and a less inhibition (Prescott and De Koninck, 2005; Prescott et al., 2006). If ECl is sufficiently displaced, GABA may exert an excitatory effect and augments nociceptive transmission at the level of descending control mechanisms by a tyrosine receptor kinase B (TrkB)-dependent mechanism (Guo et al., 2006). In fact, there is compelling evidence that BDNF is a ubiquitous pain mediator at many levels of the nervous system. Given this, it is hard to conclude that the generation of BDNF is indeed a compensatory mechanism specifically associated with both, chronic inflammatory and neuropathic pain. So, these results support the hypothesis that the chronic pain induces reorganization in circuits involved in pain processing at cortical and in descending pain modulatory system. Also, they suggest that the pain thresholds are in opposite direction of BDNF level in severe chronic musculoskeletal pain independently of the pain mechanism.

However, several potential limitations in this study need to be addressed. From a biological perspective, the integration of cortical excitability, descendent modulatory system, and neuroplasticity makers has been showed in conditions other than pain, including inflammation, cancer, learning, memory, epilepsy, neurodegenerative, and neuropsychiatric disorders. Therefore, differences among different populations deserve additional consideration. Furthermore, a set of factors such as pain severity, age, analgesic and antidepressant use and depressive symptoms differed between our samples. As we demonstrated in a previous study, BDNF is a marker to distinguish to some extent the level of CS among several types of non-neuropathic chronic pain (Deitos et al., 2015). In previous studies, an association between BDNF levels and cortical excitability measures was shown (Kleim et al., 2006; Cheeran et al., 2008). In fact, in the clinical setting is not possible to assess directly and isolate the effect of each one of these potential confounding factors in the BDNF secretion, neither in the neurophysiological measures. Thus, to control for the potential concealed influence of these set of factors in the BDNF secretion and in the measures of cortical excitability we constructed an adjusted index. This approach allows us to evaluate the contribution of BDNF as the independent variable on the cortical excitability (SICI) and in the descending pain modulatory system function on a standard scale. To the best of our knowledge, the relationship between BDNF, cortical excitability, descending pain modulatory system and the mechanism of musculoskeletal pain syndrome have not been explored before. But the cross-sectional nature does not allow establishing a cause-effect relationship. Nonetheless, this is certainly a good starting point to generate hypotheses for future studies. Also, the present results should be carefully considered in male samples because BDNF levels seem to be sex-dependent (Stefani et al., 2012). Moreover, the chronic pain samples studied in this study do not (neither pretend) represent all cases of CS syndromes, such as neuropathic pain and disorders with little pain involvement (e.g., conditions with less structural involvement, such as hand OA).

The aim of this study, was to comprehend the changes associated with the CSS in the neurophysiological and neurobiological measures. Thus, from a musculoskeletal pain perspective, the MPS in initial stages may be triggered by peripheral nociceptor stimuli, which will induce changes in brain networks, which in turn will begin to generate self-inputs to sustain the pain sensation (Mense, 2010). Therefore, to reduce the heterogeneity in the sample with the absence of peripheral nociception, only subjects with MPS that had a neuropathic component and functional disability were initially included. Significant cortical alterations have already been demonstrated in this sample by our group, in which increased intracortical facilitation and a dysfunction in the descendent pain system was observed (Dall'Agnol et al., 2014). In fact, it is possible that the chronic pain syndrome with an absence of nociception tends to induce more disinhibition by the lack of contra-regulatory effects induced by the sustained nociception. Thus, from a clinical perspective, the classification of CSS according to the spectrum of tissue injuries provides a substrate for rehabilitation, because it was shown that CSS subjects with an absence of nociception current worst catastrophizing thinking related to pain (Soriano-Maldonado et al., 2015). Hence, therapeutic approaches could then also change maladaptive illness beliefs, and thus altering maladaptive pain cognitions. This can help in the clinical decision process, as well as helping in the construction of practical approaches for "unexplained" chronic musculoskeletal pain for both, clinical recognition (Nijs et al., 2010) and treatment (Nijs and Van Houdenhove, 2009; Nijs et al., 2010). Specifically, CSS therapies could target the neuroplasticity process using pharmacological (i.e., antidepressant, anticonvulsant, etc.) and non-pharmacological techniques such as transcranial direct current stimulation (tDCS), TMS, electro-acupuncture and other physical and cognitive therapies.

Also, in the present study, the serum level of BDNF can overestimate the central sources because we cannot isolate its source of other structures besides the brain. In this way, a recent study showed BDNF gene in primary cultures of megakaryocytes of rats and human, which suggests that the platelets could represent the largest source of BDNF (Chacón-Fernández et al., 2016). In spite of this; it has also been demonstrated, that the circulating BDNF represents 70–80% of the one produced in the CNS (Rasmussen et al., 2009). Also, an experimental study in rats showed a correlation of about 0.8 between the serum levels of BDNF and its concentration in the cerebral cortex (Karege et al., 2002). Although the transport of BDNF produced in the CNS occurs through the blood-brain barrier (BBB) via saturable systems, this data suggests that the fluctuations of this neurotrophin in the blood reflect changes in the nervous system (Poduslo and Curran, 1996; Asmundson et al., 1999). Furthermore, there are a significant number of studies demonstrating that occur variations in the

serum levels of BDNF after interventions with effect in the CNS (Okamoto et al., 2008; Solati et al., 2015; Jeong et al., 2016; Kawazu et al., 2016; Niimi et al., 2016; Wens et al., 2016). These approaches therapeutics include antidepressant drugs (Brunoni et al., 2008), electroconvulsive therapy (ECT; Brunoni et al., 2014), TMS (Dall'Agnol et al., 2014) and tDCS (Brietzke et al., 2015). Haile et al. (2014) demonstrated similar results post-infusion of ketamine, where they observed a higher increase in serum levels in responders compared to non-responders. Thus, this set of evidence shows that changes in peripheral BDNF levels are associated with clinical outcome involving a neuroplasticity process, and they suggest that at least part of BDNF is produced in CNS. Nevertheless, we should have parsimony in the interpretation these results because we can infer only indirectly changes of BDNF from the brain.

In sum, the present findings showed greater disinhibition in the motor cortex and the descending inhibitory pain modulation system in FM and MPS than in OA. Likewise, the inter-hemispheric disinhibition as well as the dysfunction in the descending pain modulatory system is higher in chronic pain with the absence of tissue injury compared to chronic pain with a structural lesion. Finally, increased level of serum BDNF mediated the disinhibition of motor cortex excitability, as well as the function of descending inhibitory pain modulation system, independently of the physiopathology mechanism involved in these musculoskeletal pain syndromes.

AUTHOR CONTRIBUTIONS

AD: participated in the sequence alignment, participated in the design of the study, drafted the manuscript and approved the final version to be published. FC: participated in the sequence alignment, participated in the design of the study and approved the final version to be published. JAD-S: participated in the sequence alignment, participated in the design of the study, drafted the manuscript and approved the final version to be published. MdG and LT: participated in the sequence alignment, participated in the design of the study and approved the final version to be published. AS: participated in the sequence alignment and approved the final version to be published. ILdST: drafted the manuscript and approved the final version to be published. SC: drafted the manuscript and approved the final version to be published. JL: drafted the manuscript and approved the final version to be published. FF: drafted the manuscript and approved the final version to be published. WC: conceived the study, performed the statistical analysis, participated in the design of the study, drafted the manuscript and approved the final version to be published.

FURTHER INFORMATION

Registration in ClinicalTrials.gov: FM NCT01904097; MPS NCT01964729; OA NCT01747070.

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Feasibility of Non-invasive Brain Modulation for Management of Pain Related to Chemoradiotherapy in Patients with Advanced Head and Neck Cancer

Xiao-Su Hu[1,2‡], Clayton A. Fisher[1,3‡], Stephanie M. Munz[4], Rebecca L. Toback[1], Thiago D. Nascimento[1], Emily L. Bellile[1,5], Laura Rozek[5], Avraham Eisbruch[6], Francis P. Worden[7], Theodora E. Danciu[3] and Alexandre F. DaSilva[1,2†]*

[1] Headache and Orofacial Pain Effort Lab, School of Dentistry, Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, MI, USA, [2] Center for Human Growth and Development, University of Michigan, Ann Arbor, MI, USA, [3] Division of Oral Pathology, Department of Periodontics and Oral Medicine, University of Michigan, Ann Arbor, MI, USA, [4] Department of Oral and Maxillofacial Surgery/Hospital Dentistry, University of Michigan, Ann Arbor, MI, USA, [5] Biostatistics Department, University of Michigan, Ann Arbor, MI, USA, [6] Department of Radiation Oncology, University of Michigan, Ann Arbor, MI, USA, [7] Department of Internal Medicine Oncology, University of Michigan, Ann Arbor, MI, USA

Patients with head and neck cancer often experience a significant decrease in their quality of life during chemoradiotherapy (CRT) due to treatment-related pain, which is frequently classified as severe. Transcranial direct current stimulation (tDCS) is a method of non-invasive brain stimulation that has been frequently used in experimental and clinical pain studies. In this pilot study, we investigated the clinical impact and central mechanisms of twenty primary motor cortex (M1) stimulation sessions with tDCS during 7 weeks of CRT for head and neck cancer. From 48 patients screened, seven met the inclusion criteria and were enrolled. Electroencephalography (EEG) data were recorded before and after tDCS stimulation as well as across the trial to monitor short and long-term impact on brain function. The compliance rate during the long trial was extremely high (98.4%), and patients mostly reported mild side effects in line with the literature (e.g., tingling). Compared to a large standard of care study from our institution, our initial results indicate that M1-tDCS stimulation has a pain relief effect during the CRT that resulted in a significant attenuation of weight reduction and dysphagia normally observed in these patients. These results translated to our patient cohort not needing feeding tubes or IV fluids. Power spectra analysis of EEG data indicated significant changes in α, β, and γ bands immediately after tDCS stimulation and, in addition, α, δ, and θ bands over the long term in the seventh stimulation week ($p < 0.05$). The independent component EEG clustering analysis showed estimated functional brain regions including precuneus and superior frontal gyrus (SFG) in the seventh week of tDCS stimulation. These areas colocalize with our previous positron emission tomography (PET) study where there was activation in the endogenous μ-opioid system during M1-tDCS. This study provides

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***Correspondence:**
Alexandre F. Dasilva
adasilva@umich.edu

†Present Address:
Alexandre F. DaSilva, H.O.P.E. Lab, Biologic and Materials Sciences Department, School of Dentistry, and Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI, USA

‡*Co-first authorship.*

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preliminary evidence demonstrating the feasibility and safety of M1-tDCS as a potential adjuvant neuromechanism-driven analgesic therapy for head and neck cancer patients receiving CRT, inducing immediate and long-term changes in the cortical activity and clinical measures, with minimal side-effects.

Keywords: tDCS, EEG, head and neck cancer, pain management, chemotherapy, adjuvant

INTRODUCTION

More than 50,000 Americans are diagnosed with head and neck cancer every year (Siegel et al., 2012). These patients struggle with feeding, changes in physical appearance, speech, and psychological well-being (Epstein et al., 1999; Rose-Ped et al., 2002; Sonis, 2004a). Despite advancements in treatment options, a majority of patients experience emotional and physical distress (Ichikura et al., 2016), especially during treatment as chemoradiotherapy (CRT) itself induces mucositis and excruciating local pain that impairs food intake, leading to escalating opioid overuse and, consequently, drug associated side-effects (Schaller et al., 2015). Especially, radiotherapy (RT) induces inflammation of the mouth and mucous membranes of the throat, oral-mucositis, leading to odynophagia or painful swallowing. In some cases, further dose increases do not provide analgesia (Schaller et al., 2015). These treatment-induced side-effects often result in hospitalization and breaks in treatment, which translate to lower locoregional control and survival rates (Sonis, 2004a).

Recent studies have shown the efficacy of non-invasive brain stimulation in acute and chronic pain alleviation (Hosobuchi, 1986; Nitsche and Paulus, 2000; Fregni et al., 2006; Zaghi et al., 2011; Dasilva et al., 2012; Luedtke et al., 2012; O'Connell et al., 2014; Vaseghi et al., 2014). Transcranial direct current stimulation (tDCS) is a brain stimulation technique that applies a weak direct current to the scalp that flows from anode to cathode electrodes, which tend to increase and decrease cortical excitability, respectively. Studies revealed that half of tDCS current diffuses across the scalp while sufficient current penetrates the scalp and skull to influence transmembrane neuronal potentials and modulate neuronal excitability in the cortex without eliciting action potentials (Wagner et al., 2007). The immediate effects of tDCS are due to modulation of neuronal membrane potentials at subthreshold levels, which increases or decreases the rate of action potential firing. Usually, anodal stimulation will depolarize membranes to subthreshold levels and increase cortical excitability while cathodal stimulation will hyperpolarize membranes and decrease cortical excitability (Nitsche and Paulus, 2001). Therefore, the efficacy of tDCS is influenced by parameters such as electrode position and current strength (Nitsche et al., 2005; Fregni et al., 2006). Previous studies suggest that primary cortex stimulation using tDCS was an effective tool for alleviating chronic pain. While the precise mechanism of this analgesia is unclear, growing evidence suggests that motor cortex stimulation triggers rapid phasic activation in the lateral thalamus, which results in modulation of activity in other pain related regions such as the medial thalamus, ventrolateral thalamus, insula, anterior cingulate gyrus, and upper brainstem (e.g., periaqueductal gray matter) (García-Larrea et al., 1999; Garcia-Larrea and Peyron, 2007). More specifically, lateral thalamic modulation leads to inhibition of thalamic sensory neurons, cingulate modulation leads to decreased emotional appraisal of pain, and periaqueductal gray modulation leads to descending inhibition toward the spinal cord (Garcia-Larrea and Peyron, 2007). Evidence suggests motor cortex stimulation may also cause endogenous opioid release and directly inhibit the somatosensory cortex (Garcia-Larrea and Peyron, 2007). Besides the anode placement at motor cortex, studies also suggested the prefrontal cortex (PFC) appears to mediate affective networks associated with pain (Boggio et al., 2008). Recently, our group investigated, using the μ-opioid specific radiotracer [^{11}C] carfentanil, the immediate effect of conventional primary motor cortex - supraorbital (M1-SO) tDCS application in healthy subjects with positron emission tomography (PET) imaging (Mendonca et al., 2011). We demonstrated that tDCS application induced μ-opioid system activation in several pain-related regions, including the periaqueductal gray matter (PAG), dorsolateral prefrontal cortex (DLPFC) and pre-cuneus (DosSantos et al., 2012, 2014). Such findings suggest that we could potentially activate the endogenous μ-opioid system, one of the most important analgesic-related mechanisms in the brain, in head and neck cancer patients undergoing treatment, with the intent to decrease their pain suffering and improve quality of life during CRT.

Electroencephalogram (EEG) is an inexpensive and non-invasive measure of brain activity, with the advantage of high temporal resolution (milliseconds) and direct measure of neuronal activity in the human brain. EEG is frequently used to address the dynamics of brain processing of pain perception. Particular pain presents characteristically in EEG demonstration in terms of frequency and region. Moreover, using independent component analysis (ICA) and the independent component clustering (ICC) method, it is possible to estimate the stimulus evoked functional brain regions. These features greatly increase the value of using EEG in clinical pain studies. Researchers have been able to show the use of EEG in pain mechanism studies for both acute and chronic pain (Chen et al., 1983; Bromm and Lorenz, 1998; Seidel et al., 2015), including tonic cold pain (Chang et al., 2002) and chronic neuropathic pain (Bromm and Lorenz, 1998; Sarnthein et al., 2006). In addition, analgesic drugs can also trigger particular EEG alterations in the brain (Hartley et al., 2014; Graversen et al., 2015), and the pain-relieving effect varies according to individual baseline brain activity (Jensen et al., 2014).

In this feasibility study, our aim was to investigate and modulate the CRT induced mucocitis pain (inflammatory) regulatory cortical mechanisms in advanced head and neck

cancer patients. While understanding central mechanisms related to pain using neuroimaging is important, it is equally important to develop novel clinical protocols aimed at relieving CRT-induced pain in head and neck cancer patients.

MATERIALS AND METHODS

Subjects

Patients with a head and neck malignancy were recruited through the University of Michigan Health System (UMHS) Department of Medical Oncology and weekly tumor board meetings. Patients were screened by the Medical Oncology clinical studies team and then approached by H.O.P.E. lab study team members for discussion of the protocol and informed consent. Inclusion criteria were (1) AJCC Stage III-IV head and neck malignancy scheduled for definitive chemoradiotherapy or radiation therapy only; (2) patients capable of understanding and adhering to the protocol requirements; (3) patients between the ages of 18–75 years. Emphasis was placed on patients with no current chronic pain conditions or use of narcotic medications; (4) all patients entered into the current protocal had biopsy confirmed head and neck squamous cell carcinoma (HNSCC). Exclusion criteria: (1) substantial dementia; (2) actively being treated for another cancer at the time of enrollment; (3) any condition that would prevent the use of tDCS, including skull abnormality, implanted metal, implanted electronic device, seizure disorder, or other neurologic conditions;(4) the use of an investigational drug or device within 30 days of study screening.

Patients were evaluated for dental clearance at the University of Michigan Hospital Dentistry Clinic as part of the pre-established standard of care. This protocol (HUM00078942) was approved by the University of Michigan Institutional Review Board. Written informed consent was obtained from all participants.

From 48 patients screened, seven met the inclusion criteria and were enrolled. Six patients were placed into the stimulation arm of the study, with the fifth patient withdrew during the third week of CRT due to CRT-related side effects (**Table 1**). Five patients completed the study according to the outlined protocol. The seventh patient was placed into the control arm of the study and completed the study according to the protocol, however, this patient was excluded from further EEG data analysis because of lack of sufficient data, since the signal quality in half of the data files of the specific patient failed to pass the visual data examination. A possible reason for this might be loose contact between EEG electrode and scalp. A brief report of chemotherapy agents used as well as the total radiation dose delivered can be found in **Table 1**.

For comparison we used data from two previous studies at our Institution consisting of head and neck cancer patients who did not receive tDCS or any other investigational drug or device (HUM 000221 and 000584), which was provided to us by the Department of Radiation Oncology. These two studies examined MRI techniques for patients with head and neck cancer, and only the UMHS standard of care was provided to this group, which provided additional data from a patient cohort similar to our control patient cohort. Baseline and 1-month follow-up quality of life questionnaires (UWQoL), toxicity evaluations, and weight tracking were performed for these patients, similar to baseline and 1-month follow-up information collected from patients enrolled in our study. Of the 97 patients enrolled in that study, 93 met the requirements of our study and the data from these patients were analyzed. These patients received the standard of care while undergoing CRT and served as controls for our study.

Neuroimaging and Transcranial Direct Current Stimulation (tDCS)

Once placed into their assigned study arms, patients presented 1 week prior to the start of CRT for the pre-study visit, consisting of a 20 min EEG recording, as well as questionnaires and data collection. During the first week of CRT, only questionnaires and data collection were completed. During weeks 2 and 3 of CRT, patients received daily tDCS stimulations (five per week), and completed weekly and daily questionnaires. During weeks 4 and 5 of CRT, patients received three tDCS stimulations per week; and During weeks 6 and 7, patients received two tDCS stimulations per week. In total, 20 tDCS sessions were applied across 6 weeks (5/5/3/3/2/2)(**Figure 1**). EEG was recorded for 10 min prior to, during, and for 10 min after tDCS stimulation at the first appointment of the second, third and seventh stimulation weeks. Additionally, EEG recordings were taken at the 1-week and 1-month follow-up appointments. If patients were unable to complete the stimulation or missed an appointment (due to weather, emergency, holiday, etc.), the stimulation appointment was rescheduled for the day before or the day after. If two stimulations were required on the same day to make up a missed appointment, one stimulation was performed in the morning and one in the afternoon, with a minimum of 3 h in between stimulation appointments.

The 25 cm^2 sponge-pad tDCS montage consisted of the anodal electrode placed on the left primary motor cortex (M1) at the location of C5 (according the 10–20 intermediate Modified Combinatorial Nomenclature EEG system) and the cathode electrode placed on the right DLPFC at the location of F4 (**Figure 2**).

Stimulation consisted of 2 mA of tDCS for 20 min, with a 30 s ramp up and cool down. During simultaneous stimulation/EEG, the stimulation electrodes were placed at C5 (anode) and F4 (cathode), and Ag/AgCl ring electrodes for EEG were placed at P3, Cz, Fz, F3, FP1, and FP2 (**Figure 2**). A prefabricated cap (Neuro Electrics, Spain), with previously perforated holes and a chin-strap was used to mount the electrode. The proper size cap, small, medium, or large, was determined at the pre-study visit. Approximately 6 mL of 0.9% Saline solution was used per sponge electrode for conductivity. Approximately 12 mL of Lectron II Conductivity Gel was then injected into the EEG electrode sites when applicable. Neuroelectrics StarStim (Neuro Electronics, Spain) software was used to control stimulation and EEG settings, monitor impedance and time intervals, and record EEG data.

Pain Level Assessment

We selected the visual analog scale (VAS), percentage of weight loss, and common terminology criteria for adverse

TABLE 1 | Basic characteristics of stimulation patients.

ID	ENROLL DATE	AGE	SEX	GROUP	Disease site	Tobacco history	Quit?	Quit year	Planned cumulative radiation dose (Gy/day)	Carboplatin (AUC 1)	Paclitaxel (mg/m^2)	Cisplatin (mg/m^2)
1	11/21/14	56	Male	Stimulation	Oropharynx	Yes	Yes	2014	70.00	Yes	30	N/A
2	12/15/14	67	Male	Stimulation	Oropharynx	Yes	Yes	1994	70.00	Yes	30	N/A
3	01/21/15	63	Female	Stimulation	Oral Cavity	No			66.00	No	N/A	40
4	02/20/15	69	Male	Stimulation	Oropharynx	No			70.00	Yes	30	N/A
6	03/04/15	58	Male	Stimulation	Oropharynx	Yes	No		70.00	Yes	30	N/A

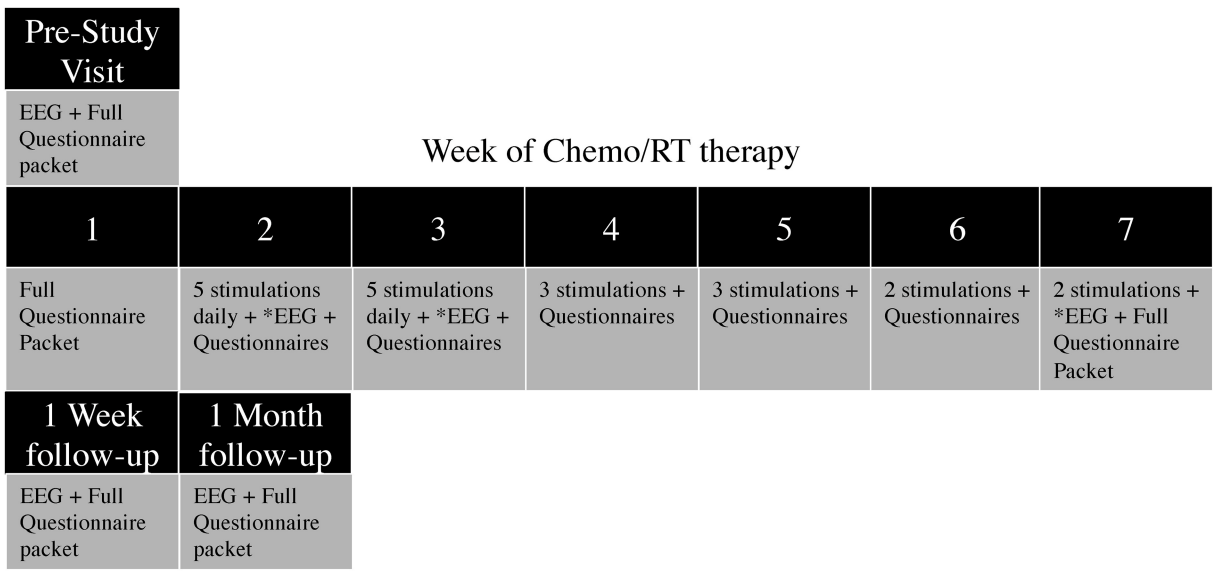

FIGURE 1 | Study Protocol (* denotes simultaneous stimulation and EEG recording).

events (CTCAE) as indices to reflect the level of pain patients experienced during CRT. To further evaluate the tDCS treatment effect, we also used the McGill and positive and negative affect schedule (PANAS) questionnaires before and after each tDCS session. For patients in the stimulation arm, weight was measured regularly as an objective measure of nutritional status, and has been used in numerous other head and neck radiotherapy trials, including oral mucositis mitigation trials (Gellrich et al., 2015). While for patients in control arm, weight was measured at baseline and 1 month following the regular treatment. Dysphagia was recorded at weekly oncology visits for all patients in the stimulation arm. The CTCAE v3.0 has been used since 2006 at University of Michigan Health System (UMHS), and varied little from v2.0 regarding the grading of dysphagia. During the CRT process, the patients were taking oral morphine equivalent as analgesic drug.

EEG Data Analysis

The EEG data analysis was completed in EEGLAB (a matlab based software, Mathworks) (Delorme and Makeig, 2004). For preprocessing, the raw data were firstly high-pass filtered at >1 Hz using basic FIR filter function. Then automatic channel rejection function was applied to reject the channel using kurtosis measure and Z-score threshold at 5. Then the filtered data were visually inspected to remove the artifacts and noisy parts. Next, the Run ICA function was used to conduct an ICA process on the data. Finally the functional EEG dipoles were estimated for each patient using DIPFIT 2.x tool in EEGLAB based on the ICs calculated from the previous step.

The post-processing steps consisted of two parts. The first part was power spectrum comparisons for all channels respectively between pre/post tDCS stimulation and between pre-study visit week/seven of tDCS stimulation. We used the Precompute channel measures and Plot channel measures functions to compare the EEG power spectra before/after as well as pre-study visit week/week 7 of tDCS. The comparison frequency bands ranged from 0 to 50 Hz. The EEG lab statistics were used and the threshold was set to be $p < 0.05$. The second part was to cluster independent components across patients and estimate the locations of group-level functional EEG dipoles. The Build pre-clustering array function was used following the Precompute component measures. The centered MNI coordinates (with a 5 mm radius voxel) of each identified IC cluster was examined for their related brain region within the automatic anatomical labeling (AAL) database (Tzourio-Mazoyer et al., 2002).

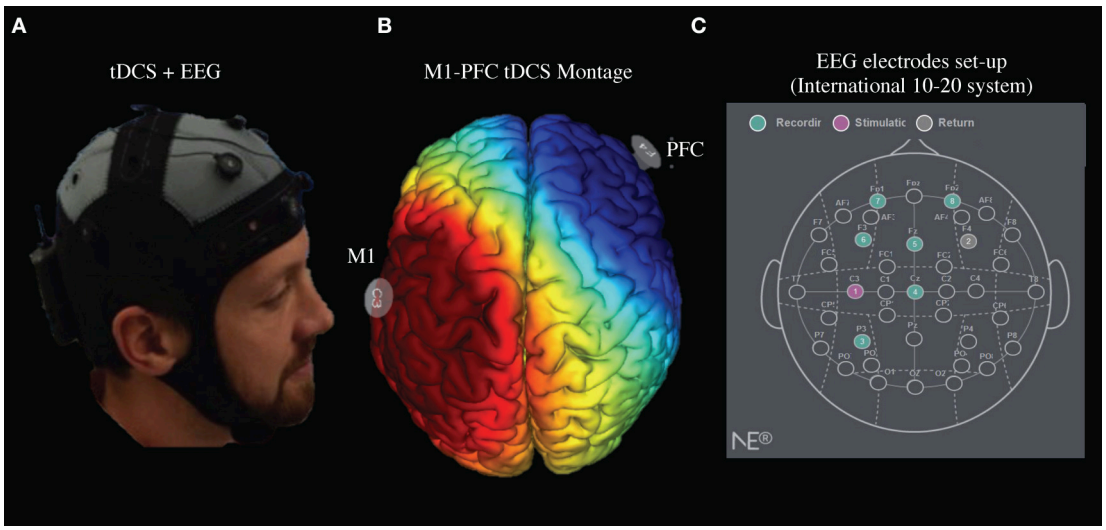

FIGURE 2 | tDCS Stimulation and EEG recording setup. (A) M1-PFC tDCS set up with concurrent EEG. **(B)** Electric current pathway from M1 anode in red to PFC cathode in blue. **(C)** tDCS anode/cathode and EEG channel locations set up.

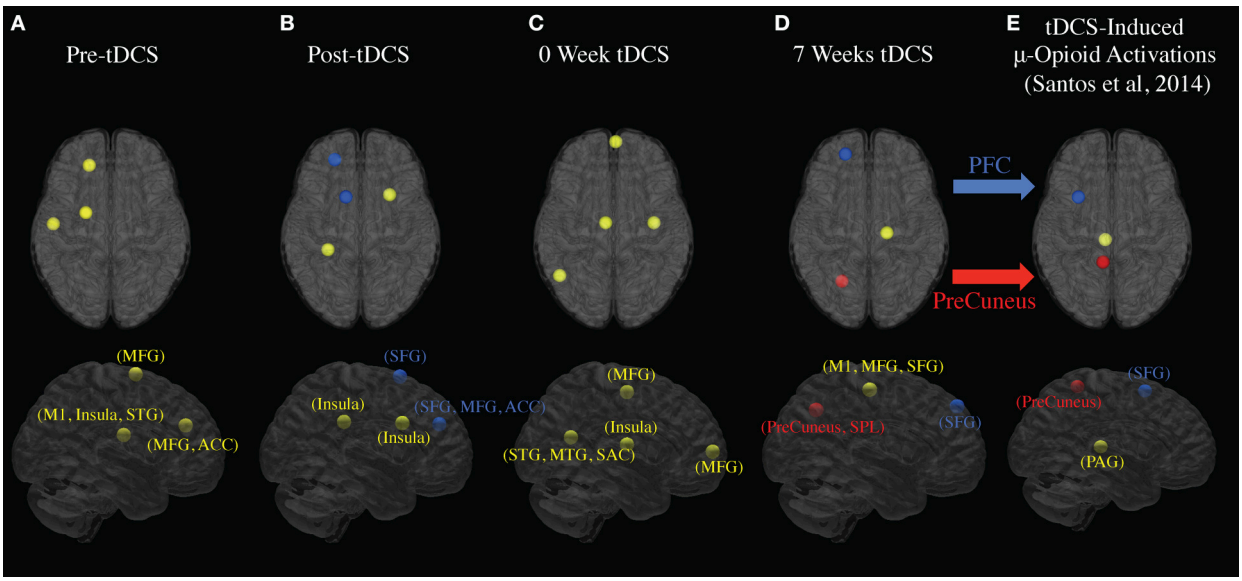

FIGURE 3 | EEG signal sources changes to PFC and PreCuneus following 7 weeks of tDCS stimulation. The estimated locations of the EEG sources are marked out for each stage. The blue, red, and yellow dots indicate, respectively, the sources with possible locations at SFG, PreCuneus, and other areas. **(A)** Estimated signal sources before tDCS stimulation (average of week 2, 3, and 7). **(B)** Estimated signal sources immediately after tDCS stimulation (average of week 2, 3, and 7). **(C)** Estimated signal sources in pre-study visit week. **(D)** Estimated signal sources in week seven. **(E)** Estimated tDCS-induced mu-opioid activation locations (DosSantos et al., 2014).

RESULTS

Locations of Estimated Functional EEG Sources

Figure 3 shows the clustered ICs from 5 subjects by common properties of their EEG data spectrums and scalp maps. The pre-tDCS stage had three clusters separately that included estimated functional brain regions (Week 2, 3, and 7 EEG data included): left Anterior Cingulate Cortex (ACC) and left Medial Frontal Gyrus (MFG) centered at (MNI: −15, 40, 16); left MFG and Sub-Gyral centered at (−18, −2, 59); Insula, left Precentral Gyrus (primary motor cortex), and left Superior Temporal Gyrus (STG) centered at (−47, −12, 8). The post-tDCS stage had four clusters separately and included estimated functional brain regions (Week 2, 3, and 7 EEG data included): left Superior Frontal Gyrus (SFG) centered at (−9, 12, 57); left SFG, MFG,

and ACC centered at (−19, 45, 17); Extra-Nuclear and Insula centered at (−47, −12, 8); Sub-Gyral, Extra-Nuclear and Insula centered at (García-Larrea et al., 1999; Zaghi et al., 2011; Hartley et al., 2014). The pre-study visit week tDCS stage had four clusters separately and included estimated functional brain regions in: Insula, Extra-Nuclear, and Sub-Gyral centered at (40, −11, 20); left STG, Supramarginal Gyrus (somatosensory association cortex), and middle Temporal Gyrus (MTG) centered at (−44, −58, 26); left MFG centered at (6, 61, 14); left MFG centered at (−3, −11, 64). The week 7 tDCS stage had three clusters separately AND included estimated functional brain regions: Sub-Gyral, right Precentral Gyrus, Middle Frontal Gyrus, and SFG centered at (20, −20, 66); left SFG centered at (−17, 50, 50); and left Precuneus and Superior Parietal Lobule centered at (−20, −63, 49). All coordinates reported were MNI coordinates.

EEG Channel Spectrum Analysis

Figure 4 indicates EEG data spectrum change right before and after tDCS stimulus (Week 2, 3, and 7 EEG data included, power between 0 and 50 Hz frequency band were compared). Power spectra at γ band significantly decreased immediately after tDCS application at location F3, Fz, Cz, and P3 ($p < 0.05$). Power spectra at β band significantly decreased immediately after tDCS application at location Fz and P3 ($p < 0.05$). In α band, the power spectra significantly decreased after tDCS stimulus at location Cz and P3 ($p < 0.05$). **Figure 5** compares EEG data spectrum in pre-study visit week and week 7 in a long term. Power spectra at δ band significantly increased after 7 weeks tDCS stimulation at location Fp1, F3, Fz, Cz, and P3 ($p < 0.05$). Power spectra at θ band significantly increased in week 7 at locations Fp1, Fz and Cz compared with pre-study visit week ($p < 0.05$). In α band, the power spectra significantly increased in week 7 at locations Fp1, Fz and Cz ($p < 0.05$). In γ band, the power spectra significantly increased in week 7 at location P3 ($p < 0.05$).

Pain Level Assessment Results

The pain level assessments were completed in primarily three primary measures: VAS scores, weight loss and graded dysphagia between the tDCS stimulus cohort and control cohort. **Tables 2–4**, respectively, show VAS scores, weight loss and graded dysphagia.

The five patients who completed the tDCS protocol reported VAS at the beginning of each week. In average, 2.94 and 1.59 out of 10 were reported, respectively at baseline and 1-week follow-up.

The five patients lost 10.12, 9.60, 9.33, 5.11, and 4.51% body weight from baseline to 1-week follow-up (**Table 2**). One out of five (20%) patients examined in this study lost >10% of their body weight from baseline through the end of treatment. For patients in the stimulation arm, the average body weight loss was

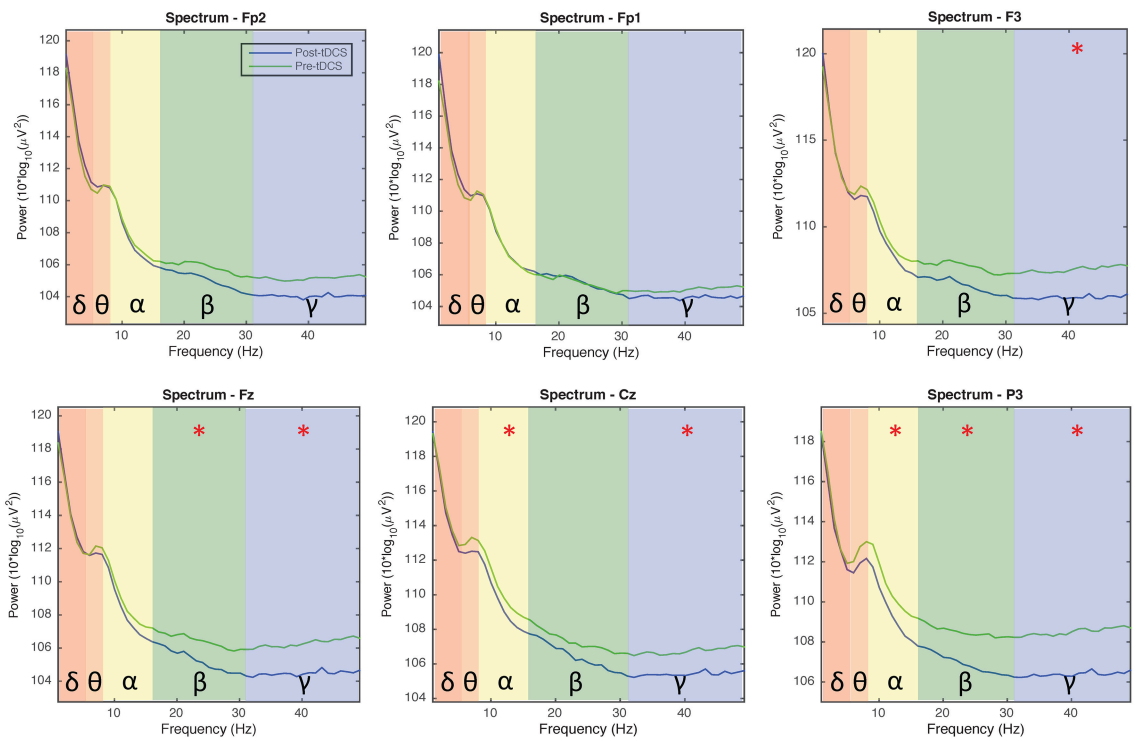

FIGURE 4 | EEG power spectra analysis results comparison for all channels before and immediately after tDCS stimulation (1 - 50 Hz; Fp1, Fp2, Fz, F3, Cz, and P3; average of week 2, 3, and 7). The background colors indicate EEG frequency bands: red, δ wave; orange, θ wave; yellow, α wave; green, β wave; blue, γ wave. The green and blue lines, respectively, indicate power spectra before and after tDCS stimulation. Generally the power decreased immediately after tDCS stimulation for α, β, and γ waves.

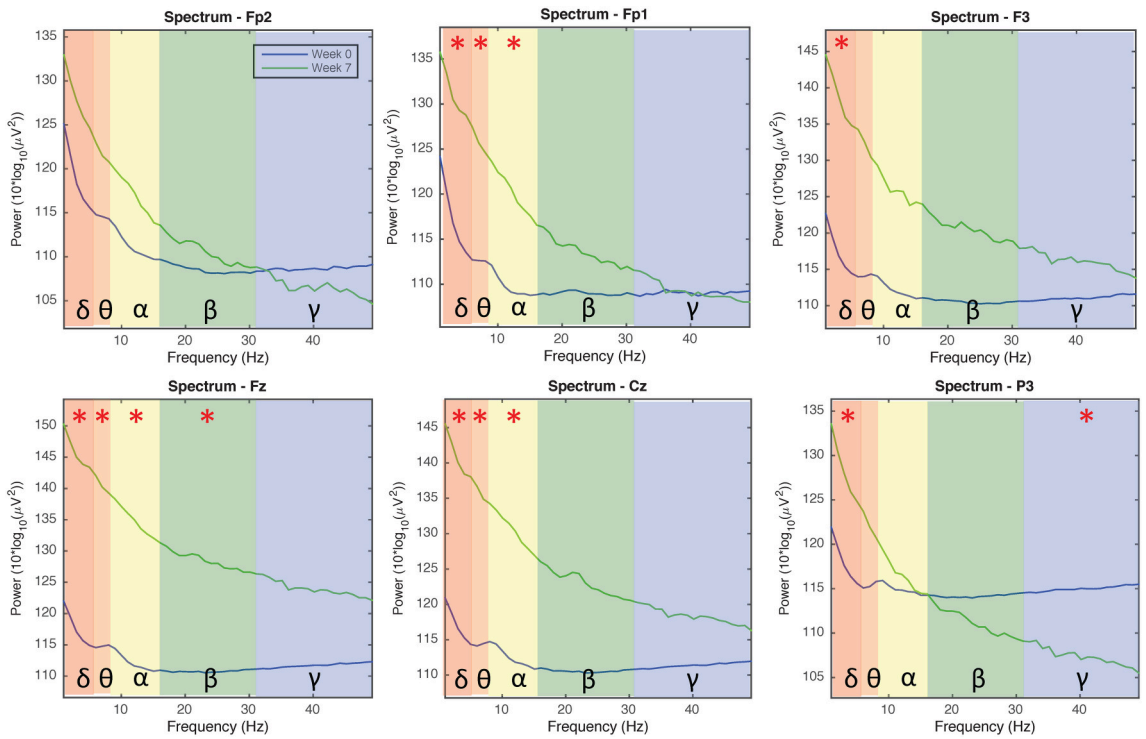

FIGURE 5 | EEG power spectra analysis results comparison for all channels in the pre-study visit week and seventh week (1–50 Hz; Fp1, Fp2, Fz, F3, Cz, and P3). The background colors indicate the EEG frequency bands: red, δ wave; orange, θ wave; yellow, α wave; green, β wave; blue, γ wave. The green lines and blue lines, respectively, indicate power spectra in the seventh week and pre-visit week of tDCS stimulation. Generally the power of δ, θ, α, and β waves increased, while the power of γ wave decreased in channels Fp1/Fp2/P3 and increased in channels F3/Fz/Cz, after 7 weeks of tDCS stimulation.

TABLE 2 | Patient-reported pain was measured using VAS at baseline (pre-visit week), week 1–week 7 of CRT process, both one-week, and 1-month follow-up.

ID	PRE	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	WkFu	MoFu
1	5.30	0.00	0.80	2.55	4.62	2.05	3.80	3.65	1.25	5.00
2	2.40	0.00	0.00	0.45	1.00	0.60	1.10	2.60	2.40	0.65
3	7.00	6.00	5.70	5.00	9.80	9.00	6.00	6.00	N/A	4.00
4	0.00	0.00	0.25	0.80	0.90	1.80	0.65	0.30	0.60	2.35
6	0.00	0.00	0.00	0.60	1.15	1.30	2.00	1.95	2.10	1.05
Ave	2.94	1.20	1.35	1.88	3.49	2.95	2.71	2.90	1.59	2.61

7.52%. Of the 93 control patients examined, 72 (77.4%) lost >10% of their body weight, with a mean weight loss of 12.9%.

Four out of five stimulation patients reported scores of 0 at baseline, thus only control subjects with reported scores of 0 at baseline were used for optimal comparable analysis of the CTCAE grading system, and 64 of the control patients met these criteria. While none of the four stimulation patients had grade 3 dysphagia (0%), nine out of the 64 control patients that met the criteria reached grade 3 dysphagia (14.1%). Of those nine patients, some developed grade 3 dysphagia at week 2, while a majority developed grade 3 dysphagia between weeks 4 and 5, barely past their halfway mark of treatment. The difference between grade 2 (symptomatic eating/swallowing that alters eating habits) and grade 3 dysphagia (severely altered eating/swallowing habits), which lead to inadequate intake and possible indication for feeding tube placement, is clinically significant. **Figure 6** shows intraoral pain area and intensity during chemoradiation/tDCS trial for all four patients receiving tDCS stimulation.

Assessment of oral mucositis grades, as assigned by the WHO Oral Mucositis Grading Criteria, revealed similar scores for both control and stimulation patients. Of the 93 control patients examined, 90 patients had mucositis grades recorded during CRT, with an average grade of 2.4 based on the worst grade given during therapy. The patients in our study had an average WHO mucositis grade of 2.5 based on the worst grade given.

TABLE 3 | Patient weights along time course (Pre-visit, Week 1–Week 7, Following Week, Following month).

ID	PRE	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk Fu	Mo Fu
STIMULATION GROUP										
1.00	252.90	247.00	247.00	239.70	238.00	240.90	239.80	243.30	227.30	216.10
2.00	200.10	193.00	190.00	191.40	188.00	186.00	183.70	179.70	180.90	176.60
3.00	165.00	168.90	168.90	159.30	158.00	157.80	154.00	149.60	149.60	147.00
4.00	213.40	212.30	214.70	213.90	213.40	212.90	210.90	207.60	202.50	197.70
6.00	286.00	283.90	286.00	281.00	277.50	274.60	270.80	271.00	273.10	266.10
AVE ($N = 5$)	223.48	221.02	221.32	217.06	214.98	214.44	211.84	210.24	206.68	200.70
PWC%	N/A	−1.10	−0.97	−2.87	−3.80	−4.05	−5.21	−5.92	−7.52	−10.19
CONTROL GROUP										
AVE ($N = 91/72$)	199.95	N/A	N/A	N/A	N/A	N/A	N/A	N/A	175.58	N/A
PWC%	N/A								-12.9	

The weight variation table shows the patients' weight at each time. The weight variation in percentage table shows the variation of weight at each time based on the pre-visit week weight in percentage.

TABLE 4 | Weekly dysphagia grading of stimulation patients and control patients during CRT.

Group	Dysphagia	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Control	0	60	34	9	4	3	0	0
	1	1	18	28	17	17	9	5
	2	0	7	21	32	33	43	39
	3	0	2	2	3	7	9	7
	4	0	0	0	0	0	0	0
	total	61	61	60	56	60	61	51
	missing	3	3	4	8	5	3	14
tDCS Stimulation	0	4	4	0	0	0	0	0
	1	0	0	3	0	0	0	0
	1/2	0	0	1	2	1	2	0
	2	0	0	0	2	2	2	3
	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	total	4	4	4	4	3	4	3
	missing	0	0	0	0	1	0	1

The Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 has been used regarding to the grading of dysphagia. The selection criteria for this comparison is that the patient reported 0 score at baseline. Four stimulation patients in the intervention arm are summarized and compared to the sixty-four patients in the control arm.

This suggests that tDCS has no impact on the development of mucositis in head and neck cancer patients as expected.

To further evaluate the immediate treatment effect of tDCS treatment on pain, **Tables 5, 6** show the VAS, present pain intensity (PPI) and PANAS scores before and after the tDCS sessions from week 2 to week 7, respectively. Generally, the VAS reported by patients reduced in every week after the tDCS sessions (average decrease range: 0.19–0.57). While the PPI indices generally decreased one grade. Both positive and negative scores in PANAS questionnaire decreased in average after receiving tDCS stimulation (positive decrease range: −0.25–6.5, negative decrease range: 0.5–3.5).

DISCUSSION

The aim of our study was to test the feasibility and safety of M1-tDCS as an adjuvant neuromechanism-driven analgesic therapy for head and neck cancer patients receiving CRT. We observed immediate superior frontal gyrus (SFG) activation in response to acute tDCS stimulation and activation of the SFG and precuneus, documented up to the seventh and final week of tDCS stimulation. In addition, power spectra analysis of EEG data showed significant changes in different frequency bands indicating possible evidence of central modulatory effect on pain. Of immediate clinical significance, the tDCS patient group

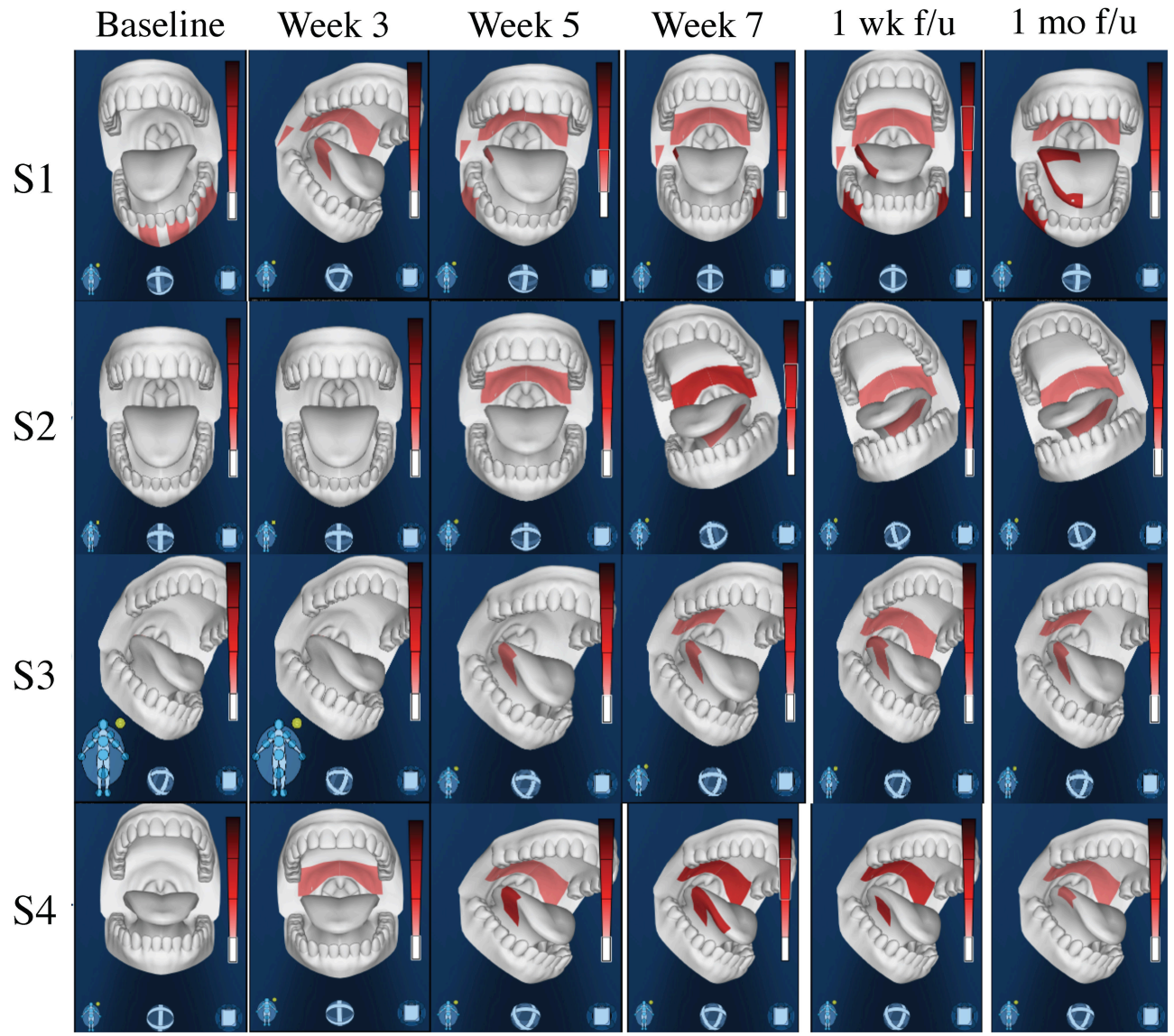

FIGURE 6 | Intraoral Pain Area and Intensity During Chemoradiation/tDCS trial. All four patients reported only mild-to-moderate pain throughout their 7-week course of CRT. Using the GeoPain technology (MoxyTech LLC, MI), patients are able to quickly and efficiently illustrate their pain locations and pain intensity, allowing healthcare providers to both acknowledge current pain, as well as easily access and evaluate the patients pain history.

showed less weight loss and dysphagia during the CRT process compared with the non-tDCS patient group, indicating less functional effects from pain for the patients in the tDCS group.

Our first finding demonstrates that long-term EEG changes induced by 7 weeks of tDCS colocalize with acute changes during tDCS stimulation observed in the endogenous μ-opioid system. In a previous study using PET, our lab used a radiotracer with specific affinity for μ-opioid receptors, [11C]carfentanil, to test the immediate pain threshold variation after applying M1-tDCS (DosSantos et al., 2012). We found that a significant increase in tDCS-induced mu-opioid receptor mediated neurotransmission in the precuneus, PAG, and left PFC. In the current study, we found the left precuneus and left PFC were activated in week 7, however, we did not observe any functional activation in PAG or other deeper regions. A possible explanation for this is that EEG as a non-invasive imaging technique produces a weaker signal from regions in the midbrain (Klein and Thorne, 2006).

We also documented a change in EEG power spectra in different frequency bands immediately and after long-term use of tDCS stimulation. Although few studies investigated clinical pain with EEG, there were several reports of EEG in different kinds of experimental pain (Chen and Rappelsberger, 1994; Ghione et al., 2005; Nir et al., 2010; Peng et al., 2015; Wang et al., 2015), however, the reported results were not consistent. α activities seem to be most commonly reported among these studies. Generally the lower amplitude of α activity indicates greater cortical excitability (Peng et al., 2015). Moreover, Wang et al. (2015) reported θ/β activity decreased in a cognitive behavior

TABLE 5 | VAS and PPI reported by patients before and after tDCS treatment in week 2–week 7.

ID		1	2	3	4	6	Average
W2 BtDCS	VAS	1.68	0.00	5.88	0.10	0.06	1.54
	PPI	MI/DIC	NO	DIT/HOR	NO	NO	N/A
W2 AtDCS	VAS	0.90	0.00	5.80	0.04	0.02	1.35
	PPI	NO/MI	NO	DIC/DIT	NO	NO	N/A
W3 BtDCS	VAS	1.80	0.50	6.40	1.20	1.04	2.19
	PPI	MI/DIC	MI	DIT/HOR	NO/MI	MI/DIC	N/A
W3 AtDCS	VAS	2.14	0.44	6.22	0.58	0.76	2.03
	PPI	MI/DIC	MI	DIT/HOR	NO/MI	MI/DIC	N/A
W4 BtDCS	VAS	2.90	0.90	8.20	0.47	1.17	2.73
	PPI	MI/DIC	MI	DIT/EX	NO/MI	DIC	N/A
W4 AtDCS	VAS	1.80	0.87	8.63	0.20	1.10	2.52
	PPI	MI/DIC	MI	DIT/EX	NO	DIC	N/A
W5 BtDCS	VAS	2.27	0.67	8.90	1.07	1.00	2.78
	PPI	MI/DIC	MI	HOR/EX	MI	DIC	N/A
W5 AtDCS	VAS	0.00	0.67	9.10	0.47	0.83	2.21
	PPI	NO/MI	MI	HOR/EX	MI	DIC	N/A
W6 BtDCS	VAS	2.00	1.60	7.20	0.45	1.85	2.62
	PPI	DIC	DIC	DIT/HOR	NO/MI	DIC	N/A
W6 AtDCS	VAS	1.00	1.55	7.40	0.60	1.60	2.43
	PPI	NO/MI	DIC	DIT/HOR	NO/MI	DIC	N/A
W7 BtDCS	VAS	3.80	2.65	8.60	0.30	1.50	3.37
	PPI	MI/DIC	DIC	DIT/EX	NO	DIC	N/A
W7 AtDCS	VAS	2.20	2.45	9.05	0.35	1.15	3.04
	PPI	NO/MI	DIC	DIT/EX	NO	DIC	N/A

therapy group. In our study, immediately after tDCS stimulation, the α activity at Cz and P3 positions decreased significantly, indicating cortical excitability increases in the proximity of the tDCS electrodes. We also observed β activity decrease at positions Fz and P3 and γ activity decrease at positions F3, Fz, Cz, and P3. Arguably γ waves are implicated in creating the unity of conscious perception and meditation (Singer and Gray, 1995) and its sequence of heightened sense of consciousness, bliss, and intellectual acuity. Notably, meditation is known to have a number of health benefits including pain relief (Zeidan et al., 2015). In the long term, after 7 weeks of tDCS stimulation, our EEG results revealed a different pattern. α activity increased at locations Fp1, Fz and Cz, showing that cortical excitability under the path from anodal to cathodal decreased after long-term tDCS stimulation. δ/θ activities generally increased, and in channel P3 close to tDCS anodal γ activity decreased. Increased slow-wave activity, especially θ activity, and reducing fast-wave activity was detected in mental therapies for pain including neurofeedback treatment, hypnosis and meditation (Sime, 2002; Fell et al., 2010). Considering that all these therapies involved cognitive changes in the brain, it would be reasonable, based on these findings, to suggest that the increases in slower wave activity (e.g., θ and α) and decreases of faster wave activity (e.g., β) in our tDCS study, provides physical neuromodulation to reduce clinical pain. Further studies are warranted to investigate specific mechanisms of tDCS stimulation in pain relief.

Stimulation using tDCS in the current clinical study reduced patients' pain during CRT and improved quality of life. We monitored patients' weight loss and reported dysphagia as indices of pain level during their CRT. Studies have shown that during CRT for head and neck cancer, the pain level correlated with patients' weight loss and dysphagia (Gellrich et al., 2015). The average weight loss of our tDCS cohort was 7.52% compared to 12.9% of the standard of care cohort. The CTCAE graded dysphagia at grade >2 were 0% for the tDCS stimulation cohort and 14.1% for the control cohort. The severity of mucositis in our patients ranged from grade 0 to grade 4, and the amount of patient-reported pain varied greatly from patient to patient. Of the four stimulation patients examined, mucositis grades of 2 and 3 were seen, but none were scored higher than a grade 2 on the CTCAE dysphagia scale. Meanwhile, 14.1% of the 64 control patients reached scores of grade 3 on the CTCAE scale. Additionally, all four reported only mild-to-moderate pain throughout their 7-week course of CRT.

Oral Mucositis is characterized by ulceration of the mucosa, leading to pain and dysphagia, and has been reported to occur in 75–90% of patients undergoing chemo and radiation therapy for head and neck cancer (Trotti et al., 2003; Sonis, 2004a,b;

TABLE 6 | PANAS results reported by patients before and after tDCS treatment in week 2–week 7.

		ID	1.00	2.00	3.00	4.00	6.00	Average
WEEK 2	Stim1BtDCS	Positive	N/A	N/A	N/A	30.00	42.00	36.00
		Negative	N/A	N/A	N/A	10.00	11.00	10.50
	Stim1AtDCS	Positive	N/A	N/A	N/A	18.00	41.00	29.50
		Negative	N/A	N/A	N/A	10.00	10.00	10.00
WEEK 3	Stim1BtDCS	Positive	N/A	N/A	20.00	13.00	41.00	24.67
		Negative	N/A	N/A	18.00	10.00	14.00	14.00
	Stim1AtDCS	Positive	N/A	N/A	13.00	13.00	40.00	22.00
		Negative	N/A	N/A	12.00	10.00	10.00	10.67
WEEK 4	Stim1BtDCS	Positive	N/A	N/A	12.00	11.00	39.00	20.67
		Negative	N/A	N/A	15.00	10.00	11.00	12.00
	Stim1AtDCS	Positive	N/A	N/A	11.00	10.00	40.00	20.33
		Negative	N/A	N/A	13.00	10.00	11.00	11.33
WEEK 5	Stim1BtDCS	Positive	N/A	N/A	10.00	10.00	39.00	19.67
		Negative	N/A	N/A	14.00	11.00	11.00	12.00
	Stim1AtDCS	Positive	N/A	N/A	10.00	10.00	39.00	19.67
		Negative	N/A	N/A	11.00	10.00	11.00	10.67
WEEK 6	Stim1BtDCS	Positive	N/A	34.00	10.00	10.00	38.00	23.00
		Negative	N/A	14.00	12.00	10.00	11.00	11.75
	Stim1AtDCS	Positive	N/A	34.00	10.00	10.00	39.00	23.25
		Negative	N/A	12.00	13.00	10.00	11.00	11.50
WEEK 7	Stim1BtDCS	Positive	N/A	31.00	21.00	14.00	39.00	26.25
		Negative	N/A	12.00	32.00	10.00	11.00	16.25
	Stim1AtDCS	Positive	N/A	28.00	13.00	13.00	40.00	23.50
		Negative	N/A	12.00	18.00	10.00	11.00	12.75

Scully et al., 2006). Mucositis most commonly affects the movable mucosa, including the tongue and buccal mucosa. The pain associated with mucositis results in a significant decrease in the patients' ability to eat, swallow, and talk (Sonis, 2004a). The severity of the pain can lead to treatment breaks or dose reduction of chemotherapy (Scully et al., 2006; Elting et al., 2008). By the second week of therapy, ulcerations develop throughout the oral cavity and oropharynx, requiring opioid treatment (Trotti et al., 2003; Sonis, 2004b; Murdoch-Kinch and Zwetchkenbaum, 2011). Multiple studies and drugs are in development to relieve patients of OM, but little success has been found (Sonis, 2004b; Scully et al., 2006). Because of this, opioids are the primary method of current analgesic relief. Patients that do develop oral mucositis are four times more likely to be hospitalized due to pain and malnutrition compared to patients that do not develop OM. The symptoms can last 1–2 weeks after the end of treatment, but may last longer depending on the severity implying that head and neck cancer patients may suffer from pain and discomfort for 5–10 weeks. Although we did not see changes in oral mucositis prevalence or severity as a result of tDCS application, we noticed that tDCS application reduce pain in patients with CRT-induced oral mucositis.

Four out of the five stimulation patients analyzed had decreases in body weight <10%, with a mean loss of 7.52% with standard deviation 2.7%, an excellent sign of long-term prognosis. The average weight loss of patients with head and neck cancer undergoing CRT was found to be 12.9% with standard deviation 5.6% in the control cohort for this study at our institution. Loss of total body weight >10% often produces higher co-morbidities and a worse prognosis in CRT patients (van Bokhorst-de van der et al., 1999; Argiris et al., 2004; Liu et al., 2006; Capuano et al., 2008). Platek et al. retrospectively reviewed 140 patients receiving chemoradio- or radiotherapy and found a median weight loss of 8.56%, classified as clinically severe (Platek et al., 2013). Ottosson et al. retrospectively examined 203 patients and found that at 5-months post-RT 77.8% of their patients suffered from weight loss >10% (Ottosson et al., 2014). Both studies emphasized multiple contributing factors to the weight loss, including dysphagia, xerostomia, radiation-induced mucositis, and other related toxicities. The mean weight loss percentages from these studies are higher than our stimulation patient average, but lower than our control average. As patients undergoing tDCS treatment may have altered pain perception, these patients may be able to better tolerate food intake, and thus report reduced weight loss at the end of therapy.

To further evaluate the tDCS treatment effect, we also used the McGill (VAS and PPI scores) and positive and negative

affect schedule (PANAS) questionnaires before and after the each tDCS session. Patients generally reported reduced VAS scores and lower grade PPI indices after receiving the tDCS stimulation, indicating the tDCS has immediate effect of pain relief effect. However, the reduction scale is relatively small and due to limited number of patients, it is hard to statistically compare the scores before and after the stimulations. Both the positive and negative scores reported by patients reduced after tDCS stimulation in every week. These results indicate that cathode PFC tDCS applied in the current study was associated with analgesia for both unpleasantness and intensity ratings. Which aligned with a previous repetitive transcranial magnetic stimulation (rTMS) study that applied stimulation on DLPFC (Borckardt et al., 2011). These findings may potentially justify the DLPFC placement of tDCS stimulation.

This study has several limitations: First, the patient number recruited in the current protocol was small, leading to difficulties in statistics across patients; and second, MRI scanning information did not accompany EEG data for clustering analysis. We used a unique MNI 152 brain template for the EEG channel alignment. This may generate certain bias in group EEG functional sources location estimation. Since this was a preliminary feasibility test study, these entire limitations can and will be addressed in subsequent studies.

CONCLUSION

This study gives preliminary evidence that demonstrates the feasibility and safety of M1-tDCS as an adjuvant neuromechanism-driven analgesic therapy for head and neck cancer patients receiving CRT, inducing immediate and long-term changes in the cortical activity and clinical measures, with minimal side-effects.

AUTHOR CONTRIBUTIONS

All authors contributed to study design and manuscript writing. XH, CF, RT, TN, TD, AD contributed to data collection and analysis. AE provided information about the control cohort in the current study.

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Slow-Frequency Pulsed Transcranial Electrical Stimulation for Modulation of Cortical Plasticity Based on Reciprocity Targeting with Precision Electrical Head Modeling

Phan Luu[1,2], Easwara Moorthy Essaki Arumugam[1], Erik Anderson[1], Amanda Gunn[1], Dennis Rech[1], Sergei Turovets[1,3] and Don M. Tucker[1,2]*

[1] Electrical Geodesics, Inc., Eugene, OR, USA, [2] Department of Psychology, University of Oregon, Eugene, OR, USA, [3] NeuroInformatics Center, University of Oregon, Eugene, OR, USA

In pain management as well as other clinical applications of neuromodulation, it is important to consider the timing parameters influencing activity-dependent plasticity, including pulsed versus sustained currents, as well as the spatial action of electrical currents as they polarize the complex convolutions of the cortical mantle. These factors are of course related; studying temporal factors is not possible when the spatial resolution of current delivery to the cortex is so uncertain to make it unclear whether excitability is increased or decreased with anodal vs. cathodal current flow. In the present study we attempted to improve the targeting of specific cortical locations by applying current through flexible source-sink configurations of 256 electrodes in a geodesic array. We constructed a precision electric head model for 12 healthy individuals. Extraction of the individual's cortical surface allowed computation of the component of the induced current that is normal to the target cortical surface. In an effort to replicate the long-term depression (LTD) induced with pulsed protocols in invasive animal research and transcranial magnetic stimulation studies, we applied 100 ms pulses at 1.9 s intervals either in cortical-surface-anodal or cortical-surface-cathodal directions, with a placebo (sham) control. The results showed significant LTD of the motor evoked potential as a result of the cortical-surface-cathodal pulses in contrast to the placebo control, with a smaller but similar LTD effect for anodal pulses. The cathodal LTD after-effect was sustained over 90 min following current injection. These results support the feasibility of pulsed protocols with low total charge in non-invasive neuromodulation when the precision of targeting is improved with a dense electrode array and accurate head modeling.

Keywords: cortical plasticity, head tissue conductivity, transcranial electrical stimulation, transcranial direct current stimulation, transcranial alternating current stimulation, transcranial pulsed current stimulation

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****Correspondence:***
Phan Luu
pluu@egi.com

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INTRODUCTION

Over the last two decades there has been a resurgence of interest in non-invasive transcranial electrical stimulation (TES) for the modulation of neural function in humans (Nitsche and Paulus, 2000). In addition to bringing the promise of electrical manipulation of the brain back to modern neuroscience, researchers have made important advances in understanding the underlying

mechanisms at the macroscopic level (Stagg and Nitsche, 2011). Progress is also being made in understanding the effects of electrical currents at both mesoscopic and microscopic levels (Bikson et al., 2004; Kabakov et al., 2012; Ranieri et al., 2012; Rahman et al., 2013).

The current for TES can be direct current (DC or polarizing) or alternating (AC). Direct current can be applied in intervals as an "oscillatory" or variable manner, with similar effects as transcranial direct current stimulation (tDCS) in some studies (Groppa et al., 2010), and driving endogenous EEG rhythms, such as slow waves in sleep, in others (Marshall et al., 2011). More recently, it has been shown that transcranial pulsed current stimulation (tPCS) can also be used to alter cortical excitability (Jaberzadeh et al., 2014). The pulsed protocols are particularly important because they suggest the ability to draw from the literature on long-term depression (LTD) and long-term potentiation (LTP) with supra-threshold, pulsed protocols in animal studies (Froc et al., 2000; Bear, 2003) to improve lasting effects that may be more relevant for neurorehabilitation than the transient polarization of the cortex observed in many tDCS studies.

Exogenous current sources appear to affect neuronal excitability (and ultimately neural plasticity) in the same way as endogenous electrical fields generated by populations of active neurons (Frölich and McCormick, 2010), with both direct and alternating currents affecting neural activity by regulating up (firing) and down (quiescence) states. In addition to evidence that non-invasive neuromodulation alters immediate cognitive function (Wassermann and Grafman, 2005; Jacobson et al., 2012) some findings have suggested that LTD and LTP may be extended over several weeks (Reis et al., 2009). With the ability to induce long term changes in neural function, researchers have explored clinical applications, such as treatment of epilepsy (Fregni et al., 2006), stroke rehabilitation (Boggio et al., 2007), treatment of depression (Loo et al., 2012), and the specific topic of this special issue, pain management (Castillo-Saavedra et al., 2016).

Despite these advances, TES as a technology can still be regarded as being in its early stages, with many issues to still be resolved (Horvath et al., 2014). Because current flow cannot be focused, but rather follows the path of least resistance through the head tissues, an accurate model of electrode positions and head conductivity is required (Wagner et al., 2007). Furthermore, because current is likely to have different effects when aligned with the neuronal columns (normal to the cortical surface) than when crossing them (tangential flow; Bikson et al., 2004; Rahman et al., 2013), it is important to model the individual's cortical geometry with cortical surface extraction from anatomical MRI (Li et al., 2016) in order to compute the components of induced current flow that are normal vs. those that are tangential. Moreover, there is now increasing interest in moving beyond the use of two large sponge electrodes, such as with the "high-definition" pattern of one source electrode surrounded by four sinks (Kuo et al., 2012), to improve precision of TES. Improving the specification of current density at the target, thereby computing the effective dosage, may be important to account for the considerable variability that is observed across individuals (Lopez-Alonso et al., 2014; Wiethoff et al., 2014). As described by Wiethoff et al. (2014) only about 36% of the participants showed the canonical pattern of anodal-facilitatory/cathodal-inhibitory after-effects that are typically assumed in the literature. Furthermore, the evidence of a non-linear relation between current dosage and measured after-effects for both motor (Batsikadze et al., 2013; Monte-Silva et al., 2013; Simis et al., 2013) and cognitive functions (Benwell et al., 2015) implies that consistency of treatment may be highly sensitive to dosage precision, even though underlying mechanisms that produce the non-linear effects may differ between motor and cognitive functions.

The goal of the present research was to evaluate the feasibility of more effective neuromodulation through improving targeting precision with a number of technical advances and use of a slow-frequency pulsed-stimulation protocol. We employ the standard protocol for assessing the effects of tDCS by targeting the hand area of the primary motor cortex and use of transcranial magnetic stimulation (TMS) as the cortical excitability probe. To minimize after-effect variability that may be attributable to previous technological and methodological limitations, in the present study for each participant we (1) identify the TMS motor hotspot through use of a neuronavigation system, (2) construct a high-resolution electric head model to determine direction of current distribution at the cortical surface, (3) select the optimal scalp electrode montage for current injection based on the reciprocity theorem, and (4) use dense-sensor arrays and multiple current sources to optimize current flow to the targeted cortical region. These technological and methodological procedures enable us to account for variations in individual anatomy and ensure that the target region always has the intended radial current direction.

The slow-frequency pulsed electrical stimulation protocol was modeled after *in vivo* animal work indicating that supra-threshold, low-frequency (0.5–3.0 Hz) stimulation induces LTD (Froc et al., 2000; Bear, 2003). Such findings motivated the development of low-frequency TMS protocols that were then shown to produce depression of motor cortex excitability (Chen et al., 1997). Following on those findings, slow (0.5 Hz) pulsed repetitive TMS was then shown to reduce cortical excitability and decrease the frequency of seizures for up to 6 months in epileptic patients (Sun et al., 2012).

Jaberzadeh et al. (2014, 2015) showed that *sub-threshold*, pulsed stimulation with a duty cycle that approaches tDCS determines the level of corticospinal excitability. Although we are not aware of direct evidence of pulsed, sub-threshold stimulation modulating plasticity in the same way that has been demonstrated with supra-threshold, low-frequency pulsed stimulation studies, the evidence that tDCS induced plasticity are Ca^{2+} dependent (Stagg and Nitsche, 2011), like supra-threshold findings, and results from human TMS work lead us to hypothesize that, even at *sub-threshold* stimulation, low-frequency stimulation is the important factor. Specifically, *we hypothesize that sub-threshold low-frequency (0.5 Hz) pulses will produce consistent inhibitory responses, regardless of the direction of current.* Moreover, based on the first hypothesis, we also examine a second hypothesis: *total charge required to affect cortical excitability will be minimal, compared to levels required in previous tDCS (including pulse and oscillatory) studies.*

MATERIALS AND METHODS

Participants

Twelve participants took part in the study and completed all five sessions. Participants were recruited from Electrical Geodesics Inc. (EGI) and the University of Oregon. All participants were screened for MRI and TMS contraindications prior to acceptance into the study. Ten participants were male and the average age was 37 ($SD = 10$) and all were right handed. No participants were excluded from the study for any reason, including non-canonical after-effect responses.

Study Design

Institutional Review Boards (IRB) at EGI and the University of Oregon approved the human subject use protocol for the present study. Prior to each session, participants provided informed consent. The study required five sessions (1 day per session) to complete. The first involved MRI acquisition and took approximately 20 min per participant. The second session involved TMS mapping to determine the location in primary motor cortex that elicited the strongest (i.e., "hotspot") index finger EMG response. The second session also involved application of the HydroCel GSN (HC GSN) and Geodesic Photogrammetry to determine the 3-dimensional position of each sensor (see below). After the second session, the electric head model and stimulation plan were constructed. The three remaining sessions involved either a placebo (sham), anodal, or cathodal protocol; the order was counter-balanced across participants using a 3 × 3 latin square design. Participants were informed that one of three stimulation sessions would be a placebo. Both participants and TMS operator were blind to the electrical stimulation condition for any given session. A minimum of 48 h separated the three electrical stimulation sessions (Mean = 10 days, $SD = 11$).

Structural MRI

Structural MRI data were obtained in all participants for use with Neuronavigated TMS and construction of high-resolution electrical head models. T1-weighted scans were obtained using Siemens' MPRAGE sequence [repetition time (TR) = 2.5 s; echo time (TE) = 3.4 ms; flip angle (FA) = 8°] with a 1 mm × 1 mm × 1 mm resolution covering 256 voxels in each spatial direction. Data were acquired in Siemen's 3T Skyra (Siemens Medical Systems, Erlangen, Germany) scanner using a 20-channel, head-neck coil. Sequence time was approximately 10 min. Foam padding was used to minimize head movements, and all participants were highly cooperative.

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation was accomplished with the Brainsight neuronavigation system (Rogue Research, Montreal, QC, Canada) and the STM9000 TMS system (EBNeuro, Florence, Italy). Each participant's T1 MRI data was used in the Brainsight system to reconstruct the scalp surface. The scalp surface was registered with the participant's head for each TMS session. A figure-of-eight coil (diameter of one winding = 70 mm, peak magnetic field = 3.2 T) was used and all stimulation employed a monophasic pulse. Motor evoked potentials (MEP) were recorded from the right first dorsal interosseous (FDI) muscle with Ag-Ag Cl electrodes arranged in the belly tendon montage and connected to Brainsight's integrated EMG module. The MEP signal was bandpass filtered between 16 and 470 Hz, amplified by 4444, and digitized with a 12 bit ADC at a 3 kHZ sampling rate.

To identify the location that elicited the strongest index finger response, the cortical surface was characterized with Brainsight's curvilinear reconstruction method, and the hand region was identified using anatomical landmarks (Yousry et al., 1997). Once the hand region was identified, a virtual 5 × 5 grid (5 mm spacing between each position) was placed over the region to systematically guide TMS coil placement. For each location, the TMS coil was positioned with the handle pointing 45° posterolaterally relative to midline. At each site, two monophasic pulses (separated by at least 6 s) were delivered, with participants instructed to keep the hand and fingers in a relaxed state, and the MEP was qualified as a peak-to-peak measurement. After sampling of all of the grid positions, if the hotspot was at the edge of the grid, the grid was moved such that the hotspot was at the center of the grid and mapping was performed once again. Once this was completed, 3–5 additional pulses were applied over the hotspot for verification. A sample of the MEP amplitude map and identified hotspot is provided in **Figure 1A**.

Resting motor threshold (rMT) was defined as a percentage of TMS power output required to elicit MEP amplitudes of 50 μV in 5 out of 10 stimulation pulses, and this was determined with the hand and fingers in the relaxed state. Baseline MEP was specified as the TMS power output of 130% of rMT and the MEP amplitude (peak-to-peak) was specified as the average MEP amplitude of 10 stimulation pulses (each separated by at least 6 s). On average, this translated to 75% of maximum machine output. In certain participants, 130% of rMT still produced MEPs that were below 1 mV. In order to allow MEP decrement due to TES (i.e., minimize potential for floor effects), in these participants TMS power was increased to achieve an MEP average of 1 mV over 10 stimulation pulses. In some participants, 1 mV MEP could not be obtained even as we increased the power output up to 87%, and we accepted the MEP amplitude at 87% (arbitrarily set limit) as the baseline. On average, this was equal to 122% of rMT in these participants. Across the 12 participants, the average baseline MEP amplitude across all three sessions (see Procedure) was 0.97 mV ($SD = 0.35$).

High-Resolution Electrical Head Models

Each voxel of the structural MRI data was segmented and classified into seven tissue types using the Modal Image Pipeline (EGI, Eugene, OR, USA): eyeball, flesh, skull, cerebral-spinal fluid (CSF), gray matter (GM), white matter (WM), and air. Because the skull is the most electrically resistive tissue, it is important to model, and yet bone can not be accurately obtained from MRI data. To estimate the skull, an atlas skull model derived from CT (1 mm × 1 mm × 1 mm) was non-linearly warped to the participant's MRI tissues (using the other tissues as a

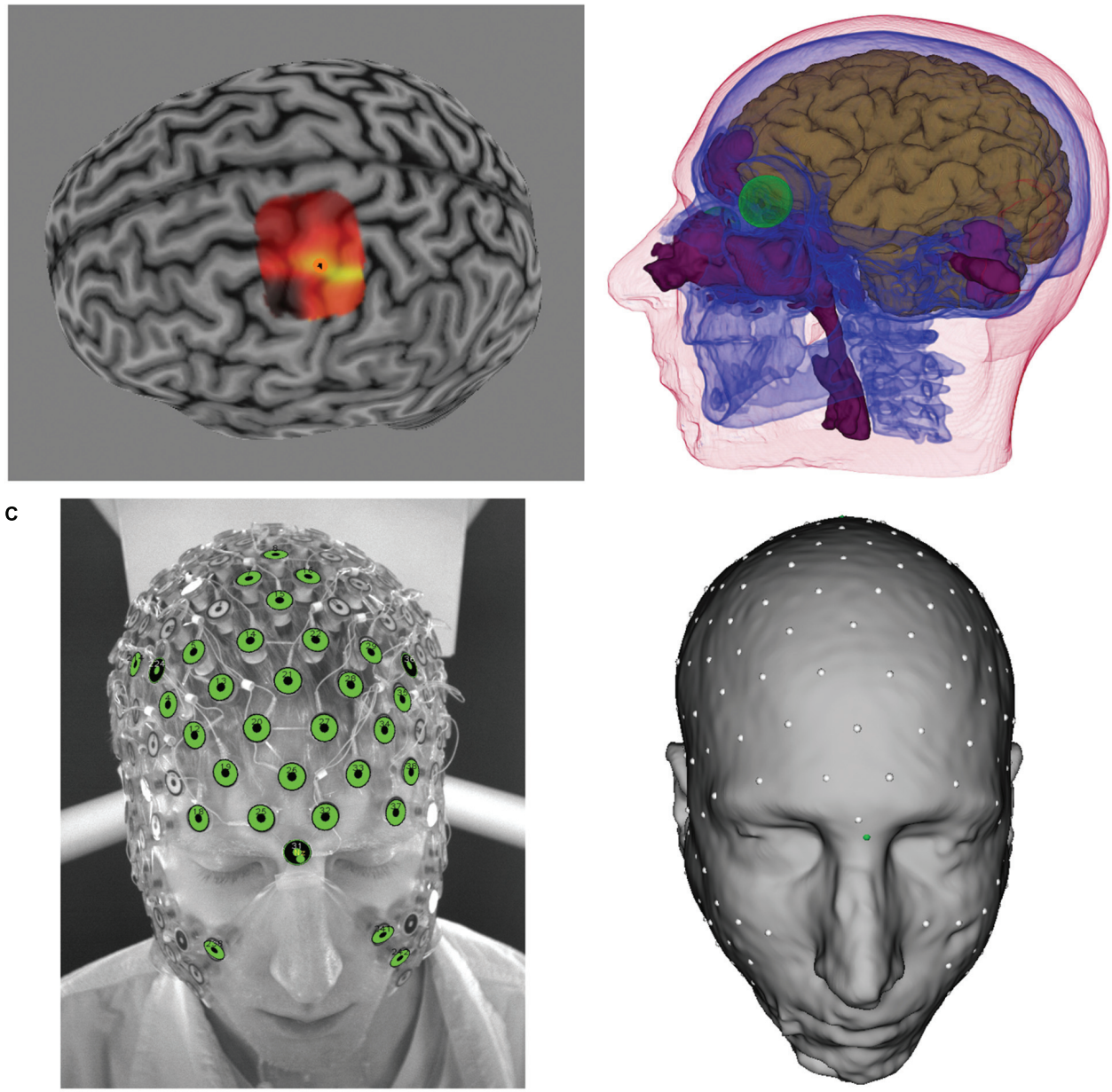

FIGURE 1 | (A) Transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEP) heat map over M1. Brighter colors represent larger MEP amplitudes. Orange circle denotes "hotspot." **(B)** Tissues identified from MRI and atlas CT, including brain (brown), skull (blue), eyes (green), air (purple), and scalp. Not shown are white matter and CSF. **(C)** photographic image from GPS (left) and sensors registered to the scalp surface (right).

guide). Detailed information about tissue segmentation and CT warping procedures is described in Li et al. (2016), and a complete characterization of the various tissues from these procedures are illustrated for one participant in **Figure 1B**.

To describe current flow from the cortex to the scalp, the cortical surface was first characterized through the use of triangular meshes, which were then parceled into patches of approximately equal size. All models used in the present study contained 1200 dipole patches per hemisphere, with each patch $\sim$1 cm^2 in size. For each patch, perpendicular directions of vertices within the patch were averaged to derive the average, perpendicular orientation for that cortical patch. This average, perpendicular orientation is used to describe the direction of current flow. Electrode sensor positions of the 256-channel HC GSN 100 (EGI, Eugene, OR, USA) were digitized using the Geodesic Photogrammetry System (GPS, Russell et al., 2005, EGI, Eugene, OR, USA). The digitized sensor positions were then registered, using the Modal Image Pipeline, to the scalp surface

of the head model, and the registration was verified against the photographic images (see **Figure 1C**).

From the complete head model, a lead-field matrix (LFM), which describes the propagation of current from each cortical patch to each sensor position, was computed using the finite difference method (FDM, Salman et al., 2015). The following conductivity values (in Siemens/meter) were assigned to each tissue type and are based on previously reported literature values: Eyeball = 1.5, Scalp = 0.44, Skull = 0.018, CSF = 1.79, GM = 0.25, WM = 0.35, and Air = 0.0 (Ferree et al., 2000). The total time required for construction of the high-resolution head models from MRI to completion of the LFM took approximately 60 min per participant.

Selection of Optimal Current Injection Electrodes

Present approaches to targeting the primary motor cortex with TES employ the standard M1-contralateral supraorbital placement with two large electrode patches. This standard placement is a limitation because current paths and cortical distribution are estimated based on scalp placement, but the spatial relation between electrode on the scalp and underlying cortex does not accurately characterize current flow through the head. Therefore, this approach can not ensure that current will be optimally delivered to the intended target. Accurate head models are required for selection of optimal current injection electrodes for each individual (Wagner et al., 2007). In the present study, selection of current injection electrodes were performed using high-resolution head models and the neuronavigated TMS results.

In order to select the optimal current injection electrode montage and determine the appropriate amount of current to deliver through each of the active electrodes for a given target, we rely on the Lorentz reciprocity relating current densities at differing points and their electromagnetic fields in a complex resistive volume in the Rayleigh-Carson formulation, which assumes that all current sources have compact support (for detailed information see Tai, 1992). This theorem can be extended to analysis of linear passive electrical networks (King, 1963), and further applied to EEG by relating the electric field at the cortical dipole location created by injecting a current on the scalp with the electric potentials at the scalp injecting points caused by the same dipole (Rush and Driscoll, 1969; Malmivuo and Plonsey, 1995; Nunez and Srinivasan, 2006). However, only recently has it been realized (Tucker, 2003; Salman et al., 2015; Fernandez-Corazza et al., 2016) that the reciprocity principle can be used for efficient computational solution for EEG source analysis and TES optimization. Specifically for TES, the reciprocity principle dictates that injection of the given current amplitude based on the scalp voltage field produced by a dipole at the target location maximizes the directional current density on the target location. To implement the reciprocity principle in our Geodesic Transcranial Electrical Neuromodulation (GTEN) Planning Module (EGI, Eugene, OR, USA) together with safety constraints, we identify the scalp topography and then shape the injecting current patterns in accordance with the scalp voltage amplitudes around the positive and negative ends of the voltage field. To do so, we first assign the number of source (anode) electrodes, N, and sink (cathode) electrodes, M, to use for current delivery. We then sort the electrodes according to the voltage derived from the lead-field projection from dipoles representing a given cortical target to the scalp, assigning the electrodes with the N largest voltages to be sources and those with M largest in absolute value negative voltages to be sinks. These electrode values are then normalized such that the largest source voltage is assigned a weight 1.0 and the largest sink voltage is assigned a weight −1.0. We then calculate the current at each electrode by multiplying each electrode's weight with the maximum allowable current per channel. These values are summed to ensure that the total anodal and cathodal currents sum to 0.0. If this is not the case, the current values are re-normalized using the smaller of the two values to ensure all safety criteria are strictly adhered to. A final normalization is then used to ensure that the total current delivered does not exceed the total current requested by the plan, or by safety constraints, whichever value is smaller.

In the present feasibility study, we used a 16 channel prototype of the GTEN 100 system, such that the total number of electrodes used for each participant was eight anodes and eight cathodes. Maximum current at any given electrode (1 cm^2) was limited to 200 μA. Given the weighting scheme described, this resulted in variable total current for each participant (mean = 1.16 mA, SD = 0.19) given the set number (eight) of electrodes. The average current density across all electrodes and participants is 0.15 mA/cm^2 (SD = 0.02). Two examples from this procedure are illustrated in **Figure 2**.

Transcranial Electrical Stimulation

Pulsed, direct current was applied using the prototype GTEN 100 system (EGI, Eugene, OR, USA) with the 256-channel HC GSN 100, which is an evenly spaced network of Ag-Ag Cl electrodes. The GTEN 100 has a double-fault Sentinel Circuit® that monitors the sum total current such that the total current cannot exceed a 2 mA limit.

With targeting formulated mathematically through use of the reciprocity principle (as described above), the targeting is achieved in GTEN 100 via hardware that drives multiple constant current circuits to be balanced in the presence of multiple electrode impedances that are changing in time (through current-induced electroosmosis, iontophoresis, and electroporation of the electrode-skin interface). GTEN uses a proprietary balancing circuit, the AccuCharge Circuit®, capable of maintaining the designed balanced source-sink configurations over time.

Elefix conductive paste (Nihon Kohden, Tokyo, Japan) was mixed with over-the-counter lidocaine cream (5%) and used as the conductive material between the electrode and scalp. All electrode starting impedance were below 100 KΩ. Due to the iontophoretic mechanisms (Prausnitz, 1996), lidocaine was delivered to minimize physical perception of current stimulation at the scalp (Saliba et al., 2011), and electrode-scalp impedances were reduced over time as well (Oh and Guy, 1995). Note that with the constant current multichannel AccuCharge Circuit, the desired current level is maintained even as impedances drop with

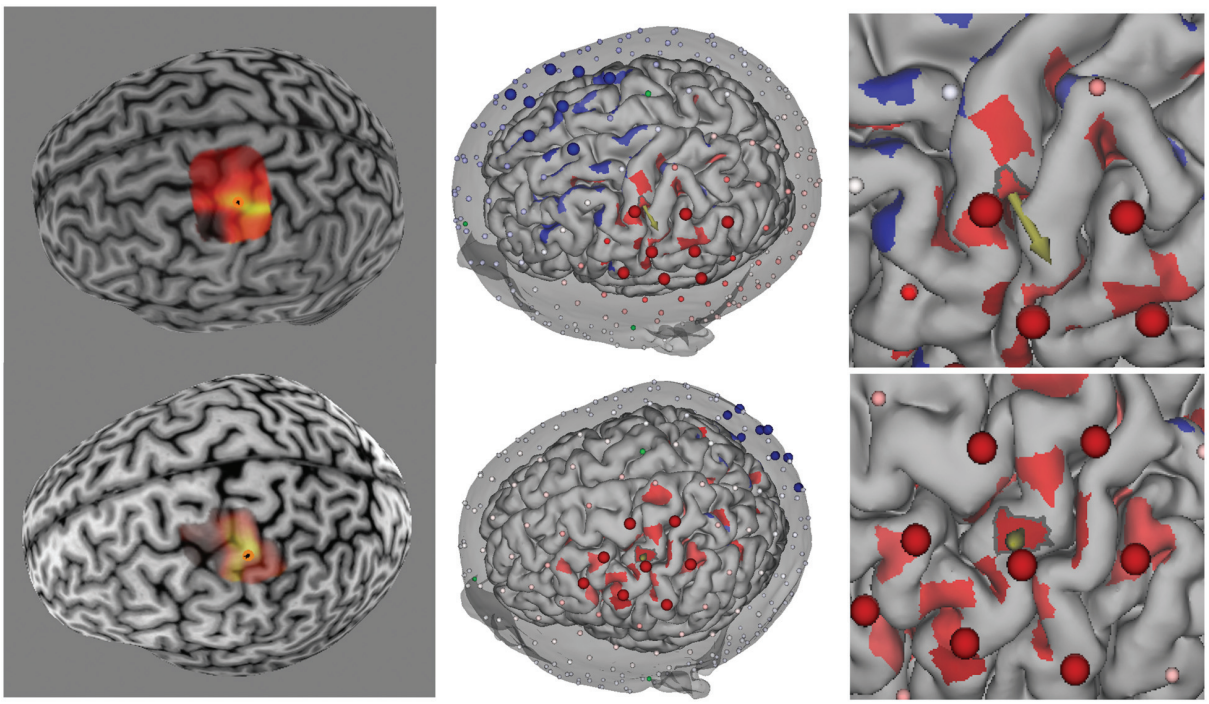

FIGURE 2 | (Left) TMS-induced MEP heat map over M1 for two participants (top and bottom rows). Brighter colors represent larger MEP amplitudes. Orange circle denotes "hotspot." **(Middle)** Reciprocity-based optimized selection of current injection electrodes for hotspot targets. Shown are eight anode (large red electrodes) and eight cathode (large blue electrodes). Note that the electrode montage is substantially different between the two participants because of the cortical geometry of the target (cortical patch with outline and arrow pointing in the average direction for the given cortical patch). Color intensity on the cortex represents current density normal to the cortical surface (red = anodal current direction, blue = cathodal current direction). Note that the current density was thresholded to remove the lowest 50% values in order to highlight the locations of high current density. **(Right)** Zoomed in view of the hotspot location to show the normal orientation (arrow) of the target (hotspot).

continued skin hydration. The stimulation protocol consisted of individual pulses (100 ms duration) at 0.5 Hz for 17 min.

Cathodal and anodal stimulation used the same electrode montage for each participant with current direction reversed. In addition to cathodal and anodal stimulation, a placebo condition was also employed in the present research. To minimize current flow to the targeted region while still maintaining potential for sensory perception associated with current flow in the placebo condition, stimulation used the same anodal-cathodal electrode clusters employed in the non-placebo conditions with the following modification. First, within each anodal and cathodal cluster, the electrode with the lowest current level used in the actual stimulation conditions were selected and the closest electrode neighbor was used to pass current. Therefore, in each cluster there was one pair (one anode and one cathode), and current (100 μA per pair and 200 μA total) passed through these pairs are prevented from penetrating deeply to affect the targeted region. Second, current was only delivered for five pulses (over 10 s) to further reduce the likelihood of any charge accumulation in the targeted brain region.

Procedures

In sessions 2–5, participants were seated comfortably in a TMS chair (Rogue Research, Montreal, QC, Canada). Across sessions 2–5, participants were always scheduled for the study at the same time of day. Sessions 3–5 (the experimental electrical current stimulation sessions) started with the determination of MEP baseline followed by application of the Sensor Net and electrode-scalp impedance verification of the 100 KΩ threshold (Net Station 5.0, EGI, Eugene, OR, USA). Application of the Sensor Net in sessions 3–5 was guided by sensor positions in the 11 GPS images acquired in the second session (hotspot mapping). The GPS images provide 11 different views of the Sensor Net on the participant's head. Following this procedure ensures that the sensors for a given session maintains the original sensor positions used to create the head model and stimulation plan.

Each electrical stimulation session (including placebo) started with a 30-s direct current conditioning period in one direction followed by another 30 s period in the opposite direction to facilitate the uptake of lidocaine through iontophoresis. The current level for each electrode was 50 μA (400 μA total). This brief duration of stimulation (with 1 mA) has been shown not to significantly affect neuronal excitability (Nitsche and Paulus, 2000). Next, current was delivered in 0.5 Hz pulses for 17 min, unless it was a placebo session. During this time, participants were instructed to sit comfortably in the TMS chair with their eyes open and hands and fingers in a relaxed state.

Participants were asked if they felt sensations (tingling, poking, burning, heating, and itching) during the direct current conditioning period, and during the pulse stimulation, participants were also asked if they felt any sensation at the following intervals: at start of stimulation and 4, 9, 13, and 17 min after start of the pulse stimulation. Upon cessation of stimulation, the HC GSN 100 was quickly removed (about 15–30 s) and TMS MEPs were immediately sampled followed by measurements at 5-min intervals for 30 min and at 60 and 90 min. Between the immediate to 30 post-stimulation measurement interval, participants remained seated in the TMS chair. After 30 min, participants were allowed to leave the room and return for the two remaining intervals. At each measurement interval, 12 stimulation pulses (separated by at least 6 s) were applied and the smallest and largest MEP amplitudes were excluded prior to averaging the remaining 10 MEPs.

RESULTS

Report of Sensation during Current Stimulation

None of the participants reported adverse effects from participation in the study. Across the 72 (12 participants × 3 sessions × 2 polarities), 30-s current condition blocks (i.e., prior to pulse stimulation), participants reported sensations in 67 conditioning blocks. During pulse stimulation, participants reported feeling sensations in 21 blocks at the start of stimulation, 12 blocks after 4 min of stimulation, eight blocks after 9 min of stimulation, eight blocks after 13 min of stimulation, and three blocks after 17 min of stimulation. These data show that the conditioning period used for lidocaine delivery was effective in reducing sensations produced by the current by approximately 33%, and after 4 min of pulse stimulation, sensation was eliminated in approximately 66% of the sessions. By the end of the study, only one participant continued to report any sensation; this participant continued to experience slight sensations in all three sessions (including placebo). However, in the placebo session, pulses were only delivered for the first 10 s. No other participant reported sensations in the placebo session beyond the 1st minute after stimulation.

Only three participants reported experiencing phosphenes during pulse stimulation, and then only during anodal and cathodal sessions (and not for placebo). In these participants, the current injection electrode configuration included more frontal electrodes (e.g., top row in **Figure 2**), suggesting that they experienced retinal phosphenes. All of these three participants also correctly identified the placebo condition. An additional five participants were also able to identify the placebo condition; only four participants were not able to identify it.

Modulation of Cortical Excitability

The first hypothesis was that TES applied at 0.5 Hz would produce a reduction in MEP amplitude, relative to baseline, regardless of the direction of the current. Of particular importance is that the polarity of the current is not defined by the direction of the current at the scalp (i.e., over primary motor cortex) but rather by the direction of current at the cortical surface of the target region as determined by each participant's head model. Therefore, there is no ambiguity concerning cortical current direction, as would be the case without a model and the ability to optimize the stimulating electrode configuration.

Figure 3 shows the average MEP (as a percentage of the baseline MEP) for each condition. Consistent with our hypothesis, over the post-stimulation course MEP amplitude for both cathodal and anodal stimulation protocols were reduced compared to placebo, with cathodal stimulation producing a larger reduction. Based on our hypothesis, we performed two one-tailed, paired *t*-test comparisons across the entire post-stimulation period: Anodal vs. Placebo and Cathodal vs.

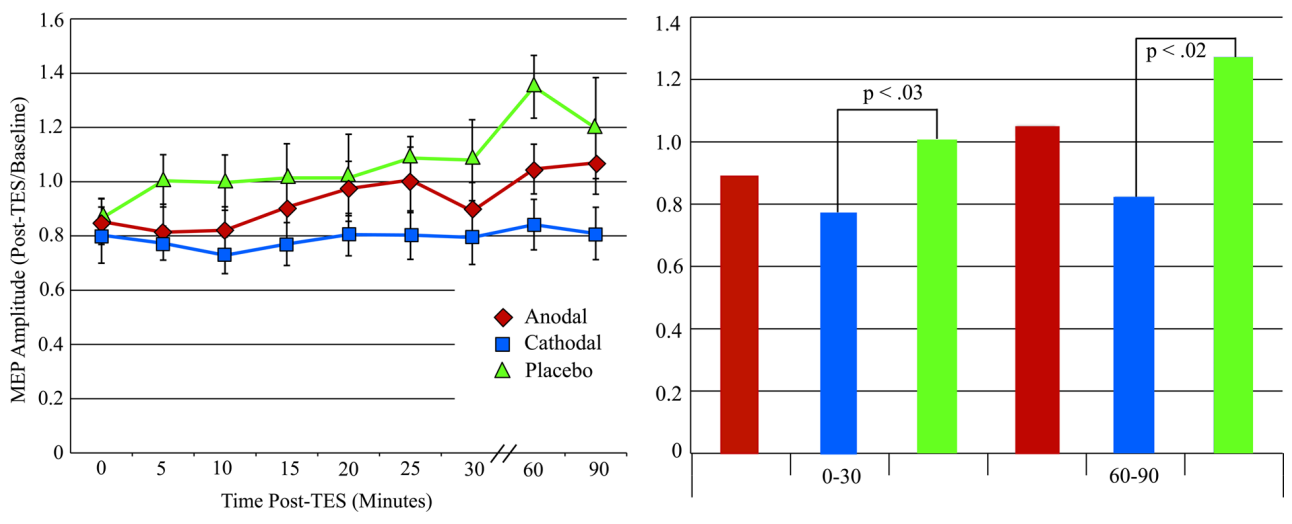

FIGURE 3 | (Left) Motor evoked potentials amplitude changes after tPCS with error bars (standard error of the mean). **(Right)** MEP amplitude changes after tPCS grouped by time after stimulation.

Placebo. The results showed that the difference between anodal stimulation and placebo was not significant, although the mean MEP amplitude after anodal stimulation also decreased (mean MEP amplitude relative to baseline = 0.93, $SD = 0.08$) relative to placebo (mean MEP amplitude relative to baseline = 1.1, $SD = 0.36$). In contrast, cathodal stimulation produce a significant reduction (mean MEP amplitude relative to baseline = 0.79, $SD = 0.21$) compared to placebo, $t(11) = -2.41$, $p < 0.02$. To explore the time course of the cathodal stimulation effect, we compared the post-stimulation MEP amplitude against MEP placebo amplitude for 0–30 and 60–90 min intervals. Paired t-test revealed that the difference was significant for the 0–30 min interval, $t(11) = -2.26$, $p < 0.03$, and 60–90 min interval, $t(11) = -2.43$, $p < 0.02$. As can be seen in **Figure 3**, the placebo condition showed a large increase at 60 and 90 min. Examination of the data showed that this increase was mainly due to one participant (11, see **Figure 4**). Therefore, we performed an analysis of the 60–90 min interval with this participant excluded to confirm the result. The paired t-test result showed that with this participant excluded, the effect is still significant, $t(10) = -2.17$, $p < 0.03$.

Figure 4 shows the average MEP (across all post-stimulation measurement intervals) change relative to baseline for each participant. Of the 12 participants, eight demonstrated MEP amplitude decreases after cathodal stimulation in comparison to the placebo condition. The MEP response to anodal stimulation is more variable, with only five participants showing an amplitude reduction relative to placebo.

To test whether the cathodal stimulation produced more consistent inhibitory responses than conventional low spatial resolution methods, we compare our results to those reported by Wiethoff et al. (2014). Wiethoff et al. (2014) found that 22 out of 53 (∼41%) participants showed MEP amplitude reduction after cathodal stimulation (2 mA for 10 min). Because Wiethoff et al. (2014) did not include a placebo condition in their study, MEP changes were defined relative to only the pre-stimulation MEP baseline. As can be seen in **Figure 4**, relative to the baseline, in our sample there are a total of 10 participants who have inhibitory responses. Given the sample size, we computed an exact goodness-of-fit test. The test revealed that the observed distribution (i.e., more participants responded with a reduction in MEP amplitude) is indeed significantly different, $p < 0.01$ (one tailed).

We also performed an exact goodness-of-fit test to determine whether the number of participants showing an inhibitory response to anodal stimulation differed from the 26% reported by Wiethoff et al. (2014). In our sample, eight participants showed reduced MEP amplitudes after anodal stimulation, and this is significantly different than expected from the findings of Wiethoff et al. (2014) $p < 0.004$ (one tailed).

Total charge and Excitability Modulation

A second hypothesis was that total charge is not the critical factor for determining the effect of TES. Given the present study's stimulation protocol, the total stimulation time (100 ms at 0.5 Hz over 17 min) is 51 s and the total charge is 59.16 milli-coulombs

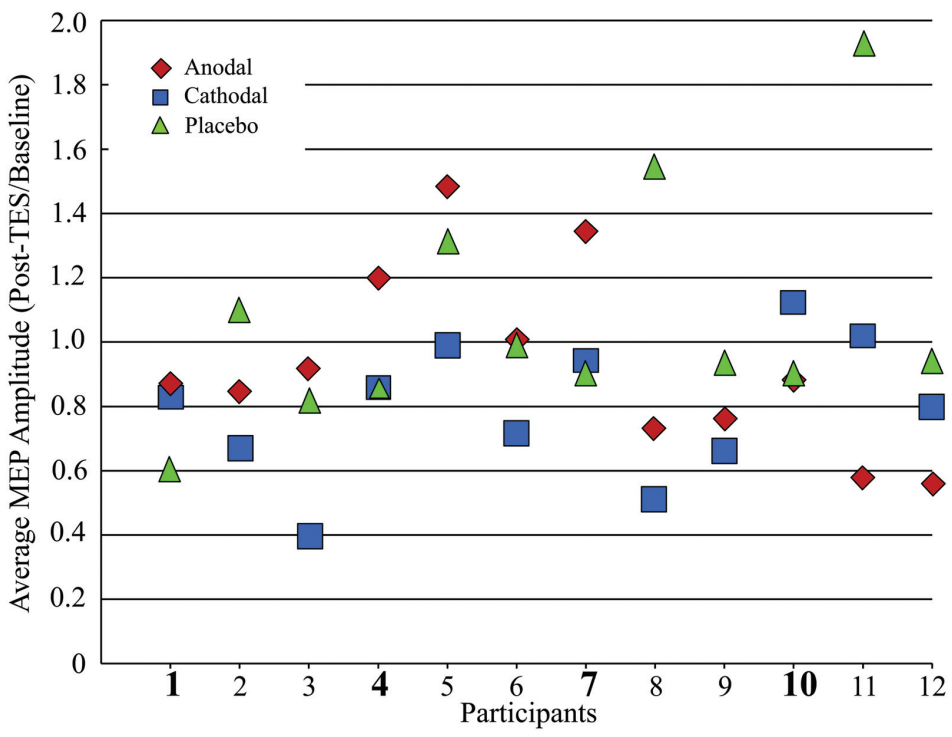

FIGURE 4 | Average MEP amplitude changes after tPCS plot for all 12 participants. Participant numbers in bold highlight those participants with cathodal MEP amplitudes equal to or greater than placebo MEP amplitudes.

(see **Table 1**). A previous study by Nitsche and Paulus (2000) concluded that anodal tDCS must be applied for at least 3 min (at 1 mA) for significant MEP changes after current cessation. For cathodal stimulation, Nitsche et al. (2003) showed that 5 min of stimulation at 1 mA produced very short lasting (1 min) after-effects; by 5 min the MEP returned to baseline. Examination of the total charge reveal that the total charge is similar between anodal stimulation in the present study and that used by Nitsche and Paulus (2000). However, for cathodal stimulation, the total charge in the present study is approximately five times less than the level used by Nitsche et al. (2003), and yet it was still effective in reducing the MEP amplitude for at least 90 min after stimulation.

Table 2 also compares total charge used in the present study with two studies that use similar pulse-like stimulation protocols. Groppa et al. (2010) applied slow oscillatory tDCS (so-tDCS) using both anodal and cathodal currents at two different current levels and assessed its affect on cortical excitability. They found that post-stimulation MEP amplitudes were only affected by tDCS and the higher current level so-tDCS protocol. Jaberzadeh et al. (2014) only used anodal stimulation but employed both tDCS and tPCS. For tPCS, these authors manipulated the inter-pulse interval while keeping the pulse duration and total charge constant. They found that short inter-pulse intervals produced significantly larger MEP responses compared to both the placebo condition and standard tDCS. However, long inter-pulse interval tPCS produced only a small and non-signifiant increase in MEP amplitudes.

In summary, with improved targeting and the brief slow pulse protocol, the present methods achieved significant LTD with much lower total charge than required in previous studies with conventional low spatial resolution tDCS, so-tDCS, and tPCS methods.

DISCUSSION

Participants in the present feasibility study did not report any adverse side effect from the tPCS protocol using the dense-array electrode configuration. Given that the current density for the electrode with the maximum current (200 $\mu A/cm^2$) is greater than those used in previous studies, it is important that participants did not report uncomfortable sensations during the conditioning period (before lidocaine became effective at reducing sensations). The time-course of reported sensations showed that lidocaine became increasingly effective at reducing reports of sensations. It is expected that with constant current (rather than tPCS), the time course would be accelerated because of a more sustained iontophoretic application of the lidocaine.

Our use of lidocaine was intended to minimize discomfort over the full session; even if participants report the stimulation is not painful in the beginning, it may become uncomfortable when continued for many minutes. Waiting longer ($\sim$ 20 min) after lidocaine application would minimize sensation. In the present experiment, 8 out of 12 participants correctly guessed the placebo condition, but this was due in part to the greater experience of phosphenes during tPCS compared to the placebo condition.

TABLE 1 | Comparisons of transcranial pulsed current stimulation (tPCS) and transcranial direct current stimulation (tDCS) after-effects as a function of total charge.

	Modulation type	Polarity	Total current (mA)	Area (cm^2)	Total duration (seconds)	Pulse duration (seconds)	Hz	Total current injection time (seconds)	Total charge (mCoulomb)	Findings
Present study	tPCS	Anodal/Cathodal	1.16	8	1020	0.1	0.5	51	59.16	Significant MEP decrease for cathodal stimulation
Nitsche and Paulus, 2000	tDCS	Anodal	1.0	35	60	NA	NA	60	60	No significant change
Nitsche et al., 2003	tDCS	Cathodal	1.0	35	300	NA	NA	300	300	Significant MEP changes lasting for only 1 min after stimulation (returned to baseline at 5 min)

Stimulation parameters are also listed for comparison.

TABLE 2 | Comparisons of tPCS and so-tDCS after-effects as a function of total charge.

	Modulation type	Polarity	Total current (mA)	Area (cm^2)	Total duration (seconds)	Pulse duration (seconds)	Hz	Total current injection time (seconds)	Total charge (mCoulomb)	Findings
Present study	tPCS	Anodal/ Cathodal	1.16	8	1020	0.1	0.5	51	59.16	Significant decrease for cathodal
Groppa et al., 2010	tDCS	Anodal/ Cathodal	0.75	12	600	NA	NA	600	450	Significant increase/decrease
	so-tDCS		0.75	12	600	tACS	0.8	tACS	225	No significant change
	so-tDCS		1.5	12	600		0.8		450	Significant increase/decrease
Jaberzadeh et al., 2014	tDCS	Anodal	0.7	24	600	NA	NA	600	420	Significant increase
	tPCS (short)		1.5	24	300	0.5	1.8	272.7	409.1	Significant increase
	tPCS (long)		1.5	24	600	0.5	0.9	260.9	391.3	No significant change

Stimulation parameters are also listed for comparison.

Modulation of Cortical Excitability

An important theoretical question for the present approach is whether, given improved spatial targeting of the oriented cortex, it is possible to manipulate cortical plasticity through more complex temporal parameters of activity-dependent neural plasticity. The Bienenstock–Cooper–Munro theory describes how LTP and LTD occur as a function of a modification threshold (for a historical overview see Bear, 2003). According to this theory, LTP and LTD occur when presynaptic activity is associated with post-synaptic activity that is above or below a certain threshold, respectively. The physiological mechanism of the modification threshold has been shown to be the level of Ca^{2+} flux, which is controlled via voltage gated channels, into the postsynaptic cell (Mulkey and Malenka, 1992). In slice preparations, the level of Ca^{2+} influx can be electrically manipulated through variations in the rate of stimulation (Mulkey and Malenka, 1992; Kirkwood et al., 1993), with low-frequency stimulation producing LTD. These findings motivated human studies that show low-frequency stimulation using TMS produce a reduction in motor cortex excitability (Chen et al., 1997). Based on this evidence, we predicted that low-frequency tPCS also would result in reduced cortical excitability, regardless of the current direction. Moreover, we also predicted that the after affects of low-frequency tPCS should be more consistent across subjects.

Consistent with these predictions, we found that both anodal and cathodal pulsed stimulation at 0.5 Hz produced inhibitory after-effects that were below baseline and, more importantly, below the level observed for the placebo condition. Moreover, when we examined the proportion of participants who exhibited inhibitory responses to both anodal and cathodal stimulation against previously reported proportions (Wiethoff et al., 2014), the difference was significantly greater in the present study.

In the present study, we found that the reduction in MEP amplitude was significantly different from placebo only for cathodal stimulation, raising the theoretical question of why the directional polarization (surface-cathodal) of the cortex is relevant for the induction of LTD by slow pulses. In this regard, the anodal and cathodal pulses were not equivalent, suggesting polarization with respect to the cortex is important. The observation of relatively weak anodal after-effects has been reported previously in several studies with tDCS. In a study by Dieckhöfer et al. (2006) examining the effects of tDCS on the N20 SEP component from primary somatosensory cortex, only cathodal stimulation produced a significant N20 amplitude reduction. Anodal after-effects were not observed. A recent tDCS study with rabbits showed a similar effect: cathodal stimulation of somatosensory cortex reduced N1 amplitude, whereas anodal stimulation did not (Márquez-Ruiz et al., 2012). Rogalewski et al. (2004) found that only cathodal stimulation over sensorimotor cortex reduced tactile discrimination performance (up to 7 min post stimulation) when compared to placebo, whereas anodal stimulation had no effect. Antal et al. (2004) noted that cathodal tDCS after-effects lowered beta and gamma power after stimulation in response to a visual stimulus whereas anodal tDCS had no effect.

Sommer et al. (2013), using TMS, induced orthodromic (analogous to anodal TES) or antidromic (analogous to cathodal TES) current flow with 1 and 5 Hz TMS pulses. These researchers reported that for 1-Hz, monophasic pulse protocol, antidromic current flow produced significant inhibitory after-effects and orthodromic current flow did not (note that 5-Hz monophasic stimulation did not produce any significant after-effects). These findings are consistent with our findings of a significant after effect only for cathodal stimulation (see below). It is worth noting that Sommer et al. (2013) also found significant facilitatory after-effects for 1-Hz stimulation when a biphasic pulse was used. How this latter finding may be related to the present results (or the model discussed below) is unknown.

Transcranial current delivery produces diffuse current flow and thus affects synapses and cells distributed across all cortical layers (Fritsch et al., 2010) and over a broad area of cortex (including beyond primary motor cortex, Stagg and Nitsche, 2011). Afferent inputs not perpendicular to the cortical surface are also affected (i.e., they can also be polarized or depolarized) because of the substantial tangential current component of the current flow (Rahman et al., 2013). Therefore, the observed after-effects represent the summed influence on neuronal compartments over a relatively large area. When current flow is estimated for a specific cortical target, as with the high resolution electric head model, there is an overall polarization of the cortex, where the large vertical pyramidal neurons experience somatic depolarization and hyperpolarization in the presence of cortical-surface-anodal and cortical-surface-cathodal current fields, respectively. Therefore, the asymmetry of anodal and cathodal stimulation in the present study suggests a differential effect of the direction of polarization on the neuronal populations of the motor cortex.

Recognizing that LTD and LTP effects are mediated by Ca^{2+} influx, it is important to consider a regulatory mechanism [Ca^{2+}-activated small conductance (SK) channels] at the dendritic spine that prevents over activation produced by Ca^{2+} influx. Tigaret et al. (2016) showed that LTP at the dendritic spine depends on overriding this regulatory mechanism by slow-acting group 1 metabotropic glutamatergic receptors (mGluR1). Because the apical dendrites are hyperpolarized and depolarized in anodal and cathodal electrical fields, respectively, and because SK channels (Maciaszek et al., 2012) and mGluR1 receptors (Luján et al., 1996) are densely distributed at the dendritic spines (and sparsely at the dendritic trunk and soma), the mechanisms of plasticity induction may be biased toward the dendritic spine compartment of the neural network. As noted by Gee and Oertner (2016), Ca^{2+} flux in dendritic compartments and the interaction with Ca^{2+} levels in the soma must be considered for a complete picture of how long term plasticity is induced. Although this interaction is not fully understood at present, it may be that the more powerful effect of cortical-surface-cathodal current on the dendritic region may explain why this direction of polarization is more effective in producing both tDCS and tPCS after-effects.

Thus, in a static DC surface-anodal electric field, there is very little change in activity (i.e., minimal Ca^{2+} flux) in the apical compartments. The summed effect reflects plasticity changes centered on the somatic compartment (where activity is summed in the initial segment). For surface-cathodal current, changes occur primarily in the apical compartment, and the soma's integration of activity is affected by the polarized state induced by a cathodal electric field. Because the effects are primarily in the apical dendritic compartment, surface-cathodal current may be more effective due to the dense distribution of plasticity inducing mechanisms (such as mGluR1 and SK channels) in this compartment. Consistent with this reasoning is the observation that only cathodal tDCS significantly modulates TMS input-output curves (Nitsche et al., 2005; Stagg and Nitsche, 2011). The input-output curve metric is believed to reflect the activation of corticospinal tract neurons as well as intracortical neurons over a wide area, which would engage feedforward and feedback connections between neurons at superficial layers and across the apical neuronal compartment. Also consistent with this reasoning are findings by Sommer et al. (2013), who showed the directionality of current flow induced by TMS is important to the induction of neuroplasticity. As noted above, these researchers demonstrated that when current flows from layer I (apical) to layer VI (basal), analogous to anodal electrical stimulation, plasticity changes were not observed. On the other hand, when current flow was directed from layer VI toward layer I (analogous to cathodal stimulation) plasticity changes were significantly demonstrated. Sommer et al. (2013) attributed the plasticity changes induced by antidromically flowing current to changes in excitability in the apical dendritic compartment.

Another possible explanation for the observed after-effect asymmetry for anodal and cathodal stimulation relies on the concept of homeostatic plasticity. Homeostatic plasticity describes the fact that neurons have mechanisms that restore baseline levels of neuronal function (Davis, 2013; Tigaret et al., 2016). Using this concept, it is plausible that low-frequency anodal pulsed stimulation indeed inherently produce facilitation but "over excites" the affected neurons such that homeostatic compensatory mechanisms are engaged and thus, result in low-level inhibitory responses. However, this appears unlikely. Given that (1) cathodal stimulation provided the same amount of current, (2) SK and mGluR1receptors that form part of the homeostatic compensatory mechanism are densest in the apical compartment, and (3) cathodal stimulation depolarizes the apical compartment, one would expect homeostatic mechanisms to be most sensitive to cathodal stimulation, and yet no compensation appeared to have occurred.

The variability of responses observed in previous research may be due to the poor precision of cortical targeting. Given the variability in participants' anatomy and the use of large M1 electrodes vs. contralateral supraorbital stimulation electrodes, it is possible that current at the target area of the cortical surface (such as the finger area) is not of the desired polarity. Additionally, given the demonstration of non-linear effects of total charge on after-effects direction (Batsikadze et al., 2013; Simis et al., 2013; Benwell et al., 2015), the inability to account for total charge variations may be a significant factor contributing to the variable responses.

In the present study, we observed greater consistency of LTD across participants. By employing the reciprocity principle between EEG and TES with high resolution subject-specific head models, the optimal stimulating electrode montage was always individually adjusted such that current delivered to the motor cortex hotspot was maximized radially. Because of the very nature of the low-frequency pulsed protocol, total charge is relatively low. Moreover, low-frequency stimulation is a well-established protocol for inducing LTD because it directly affects the rate of Ca^{2+} influx, which in turn affects the cascade of neurophysiological events that induce LTD. In the present feasibility study, we did not manipulate these factors separately, so we cannot determine the extent to which these three variables contributed to the reduced variability.

The Influence of Pulsed Stimulation vs. Total Charge on Cortical Excitability

We also predicted that total charge is not the critical factor in determination of the effectiveness of tPCS, and that brief pulses would be adequate. As shown in **Tables 1** and **2**, results from prior tDCS and so-tDCS studies suggest that total charge is important to the after-effects. In tDCS, low total charge produces little to no significant after-effects. In the so-tDCS study, Groppa et al. (2010) showed that anodal stimulation at 0.75 μA produced no significant after effects. However, keeping the duration the same but increasing the current to 1.5 mA produced significant after-effects for both cathodal and anodal stimulation.

Jaberzadeh et al. (2014) performed a study most similar to the present study. In their study, using anodal pulses that are 500 ms wide separated by short (50 ms, 1.8 Hz) or long (650 ms, 0.9 Hz) inter-pulse interval (changing total stimulation duration to keep total charge approximately equal), the authors found that cortical excitability was changed only for the short inter-pulse interval (longer duty cycle) protocol. The authors concluded that it is the inter-pulse interval that is important to the observed effects and not the total charge or pulse width, because these two variables were controlled to be approximately equal across the short and long inter-pulse interval protocols. Their conclusion that total charge is not the relevant factor for determination of significant after-effects is consistent with our proposal.

The explanation proposed by Jaberzadeh et al. (2014) for the lack of effect with the long inter-pulse interval protocol (43.5% duty cycle) was that this protocol prevented accumulation of charge. However, the inter-pulse interval employed in the present study is more than twice as long (1900 ms, 5% duty cycle), and yet participants showed significant after effects. Moreover, given that the frequency used by Jaberzadeh et al. (2014) is within the slow frequency range that should produce inhibition (i.e., LTD, Bear, 2003), it is surprising that these researchers observed facilitation for anodal stimulation, which is contrary to our findings. This discrepancy may be attributable to the pulse width difference between their study (500 ms) and our (100 ms) study. However, in a more recent study, Jaberzadeh et al. (2015) used a similar pulse width (125 ms), but with very short inter-pulse interval (50 ms, 5.7 Hz), and the results showed no significant after-effects. Given these results, it is likely that short pulse duration stimulation has to be coupled with low-frequency stimulation to induce significant after-effects, consistent with evidence from animal LTD studies (Froc et al., 2000) and TMS findings with low-frequency stimulation (Chen et al., 1997).

Study Limitations and Future Directions

In the present research, we demonstrated feasibility of a pulsed protocol with low current when high resolution modeling of cortical targeting is employed, yet we did not include experimental manipulation of each of the potentially contributing factors. Future studies will be required to clarify the contribution of each variable. Although we showed that very low total charge can still induce significant after-effects, it is still possible that there exists a relation between total charge and magnitude as well as duration of after-effects using a tPCS protocol. Future studies should address this possibility. We, as well as Jaberzadeh et al. (2014), showed that the inter-pulse interval parameter appears to be important, and yet opposite effects are observed for anodal stimulation in the two studies; pulse width and frequency is the obvious variable that may contribute to the observed difference and this should be tested in future studies.

By considering the greater concentration of plasticity mechanisms at dendritic spines rather than at soma levels, we have suggested a model to understand the effects of the direction of polarization on cortical excitability that generates testable predictions. For example, one prediction is that faster frequency stimulation, known to induce LTP, will also show greater facilitation for cathodal stimulation pulses.

CONCLUSION

In this feasibility study, we were able to implement a number of improvements in the spatial targeting of cortical-surface-normal electrical current to the specific finger motor area of each participant, and to observe that the slow pulse electrical stimulation was effective for the induction of LTD. As hypothesized, LTD was achieved with much lower total charge levels than are required for tDCS protocols. Although not significantly different from placebo in this sample of 12 participants, 0.5 Hz anodal tPCS also reduced cortical excitability. The consistency of reduced cortical excitability was greater across participants than has been reported in previous research. Finally, we proposed a model for how to understand the apparent asymmetry of anodal and cathodal stimulation effects of tDCS and tPCS. For non-invasive TES to contribute maximally to clinical applications particularly, it is clearly important to understand how to achieve reliable induction of cortical plasticity in each person.

AUTHOR CONTRIBUTIONS

PL, EMEA, and DT contributed to the design of the study. DT, EA, and ST contributed to the design of the Reciprocity

algorithm. DR designed of hardware system for current injection. EMEA, AG, and PL were responsible for data acquisition and analysis. All authors contributed to the interpretation of the results and preparation of the manuscript. All authors acknowledge that they are accountable for all aspects of the work.

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Conflict of Interest Statement: Authors of this paper are employees of a commercial EEG company, Electrical Geodesics, Inc., and EGI holds several patents for technologies used in the present research including: US Pat. No. 7,840,250, No. 6,594,521, No. 7,190,826, and 8,478,011.

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Transcranial Direct Current Stimulation Combined with Aerobic Exercise to Optimize Analgesic Responses in Fibromyalgia: A Randomized Placebo-Controlled Clinical Trial

Mariana E. Mendonca[1], Marcel Simis[2], Luanda C. Grecco[3,4], Linamara R. Battistella[2], Abrahão F. Baptista[5†] and Felipe Fregni[1,4]*

[1] Department of Neuroscience and Behavior, Institute of Psychology, University of São Paulo, São Paulo, Brazil, [2] Physical and Rehabilitation Medicine Institute of the University of São Paulo, Medical School General Hospital, São Paulo, Brazil, [3] Pediatric Neurosurgical Center - Rehabilitation (CENEPE), São Paulo, Brazil, [4] Laboratory of Neuromodulation, Department of Physical Medicine and Rehabilitation, Harvard Medical School, Spaulding Rehabilitation Hospital and Massachusetts General Hospital, Boston, MA, USA, [5] Laboratory of Functional Electrostimulation, Department of Biomorphology, Federal University of Bahia, Bahia, Brazil

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***Correspondence:**
Felipe Fregni
felipe.fregni@ppcr.hms.harvard.edu

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Fibromyalgia is a chronic pain syndrome that is associated with maladaptive plasticity in neural central circuits. One of the neural circuits that are involved in pain in fibromyalgia is the primary motor cortex. We tested a combination intervention that aimed to modulate the motor system: transcranial direct current stimulation (tDCS) of the primary motor cortex (M1) and aerobic exercise (AE). In this phase II, sham-controlled randomized clinical trial, 45 subjects were assigned to 1 of 3 groups: tDCS + AE, AE only, and tDCS only. The following outcomes were assessed: intensity of pain, level of anxiety, quality of life, mood, pressure pain threshold, and cortical plasticity, as indexed by transcranial magnetic stimulation. There was a significant effect for the group-time interaction for intensity of pain, demonstrating that tDCS/AE was superior to AE [$F_{(13,\ 364)} = 2.25$, $p = 0.007$] and tDCS [$F_{(13,\ 364)} = 2.33$, $p = 0.0056$] alone. *Post-hoc* adjusted analysis showed a difference between tDCS/AE and tDCS group after the first week of stimulation and after 1 month intervention period ($p = 0.02$ and $p = 0.03$, respectively). Further, after treatment there was a significant difference between groups in anxiety and mood levels. The combination treatment effected the greatest response. The three groups had no differences regarding responses in motor cortex plasticity, as assessed by TMS. The combination of tDCS with aerobic exercise is superior compared with each individual intervention (cohen's *d* effect sizes > 0.55). The combination intervention had a significant effect on pain, anxiety and mood. Based on the similar effects on cortical plasticity outcomes, the combination intervention might have affected other neural circuits, such as those that control the affective-emotional aspects of pain.
Trial registration: (www.ClinicalTrials.gov), identifier NTC02358902.

Keywords: transcranial direct current stimulation (tDCS), fibromyalgia, aerobic exercise, combined therapy, motor cortex

INTRODUCTION

Fibromyalgia is a chronic pain syndrome that is characterized by the presence of diffuse pain throughout the body and secondary symptoms, such as sleep disturbances and cognitive dysfunction (Bernardy et al., 2013). The etiology of fibromyalgia is unknown, but its onset is attributed to the continuity of painful stimuli, triggering mechanisms of central sensitization (Cagnie et al., 2014). These processes lead to maladaptive plastic changes in cortical activity in various regions, including the classical areas of the pain neuromatrix circuit and other neural circuits, such as the primary motor cortex.

In this context, the Motor Cortex (M1) is an important area to understand the pathophysiology and treatment of Fibromyalgia Sindrome (FMS). A recent review noted that many studies in other pain syndromes reported increased activation in this region to rest and increased response to nociceptive sensory stimuli, demonstrating its interaction with other areas of pain modulation (Castillo Saavedra et al., 2014). Current studies are using neuromodulation techniques to modify the excitability of the M1 and provide relief from the symptoms of chronic pain (Fregni et al., 2006; Valle et al., 2009; Mori et al., 2010; Mendonca et al., 2011; DaSilva et al., 2012; Yoon et al., 2013).

One such technique is Transcranial Direct Current Stimulation (tDCS). tDCS promotes the modulation of brain activity by subtly altering the excitability of the neuronal membrane, and its prolonged and continuous application can effect plastic modification, with activation of NMDA receptors and the Long Term Potentiation (LTP) phenomenon (Nitsche et al., 2003; Fritsch et al., 2010; Monte-Silva et al., 2013).

Preliminary trials that have tested tDCS of the M1 in reducing fibromyalgia pain have reported positive results, although the effects varied and, in some cases, were small (Fregni et al., 2006; Valle et al., 2009; Mendonca et al., 2011). Based on the mechanisms of tDCS, one approach to optimizing its effects is to combine it with a behavioral intervention that promotes activation in the same neural circuit. Thus, we hypothesize that tDCS of the M1, combined with aerobic exercise, would enhance the effects of tDCS on FMS pain.

Aerobic exercise acts systemically, influencing various aspects of body function. For example, it can affect a large neural circuit via afferent input (bottom-up) from somatosensory stimulation and a neuroendocrine response (Schwarz and Kindermann, 1992; Goldfarb and Jamurtas, 1997; Kramer and Erickson, 2007). This technique has long-lasting effects and can be sustained by the patient to maintain the improvement (Colcombe et al., 2004).

We tested the clinical and neurophysiological effects of the combination of tDCS and aerobic exercise on a treadmill over 1 month to generate results of a new intervention and to understand how modulation of the M1 circuit leads to pain control. Our main aim was to assess whether the combined intervention of tDCS and aerobic exercise would induce significantly greater pain reduction as compared to tDCS alone and aerobic exercise alone.

METHODS

Participants

Participants were recruited through social networks, local health care facilities, and referrals for a waiting list for treatment at the Institute of Physical Medicine and Rehabilitation, Faculty of Medicine, University of São Paulo, Brazil. The study population comprised individuals who were diagnosed with fibromyalgia. The diagnosis was performed by medical specialists taking into consideration the modified criteria from ACR (Wolfe et al., 2011). For the evaluations were taken into account the terms described by the modified evaluation criteria and who fulfilled the following eligibility criteria: (a) completed high school and (b) age between 18 and 65 years. Subjects were excluded if they: (a) were on medication for pain control for less than 2 months; (b) had been treated for depression for less than two months; (c) had epilepsy, psychiatric disorders, or any recent episode of neurological disorders, such as idiopathic syncope; (d) were pregnant and infant-aged; (e) had metallic implants in the brain; (f) were using illicit drugs; or (g) had been undergoing some type of physical treatment for less than 2 months.

All patients signed informed consent forms prior to initiation of the study procedures. This research was approved by the research ethics committee at CONEP under registration number CAAE 08603612.0.0000.5511. The trial was also registered at clinicaltrials.gov (identifier NTC02358902).

Experimental Design

The design was a clinical, randomized, double-blind study with 2 months of follow-up. Data were collected from January 2013 to November 2014. Recruitment was performed during the entire period since interventions were carried out in group each month.

A total of 45 participants were included (**Figure 1**). Randomization was performed by a blinded therapist using sealed envelopes for each individual. The subjects were divided into 3 intervention groups: **tDCS/AE**, which received active intervention of aerobic exercise training and active tDCS intervention; **AE**, which received active intervention of aerobic exercise and placebo tDCS; and **tDCS**, which received placebo AE and active intervention for tDCS.

Participants were blinded to the intervention groups, as were the therapists who performed the evaluation.

Outcomes

All variables were measured 1 week before the beginning of the intervention (baseline), after intervention period (T2) and during the periods of follow-up conducted 1 month (T3) and 2 months (T4) after the end of the intervention period. For variables such as pain intensity and intensity of anxiety, these evaluations were performed every day before the intervention. The assessment of cortical excitability was conducted at baseline, T2, T3, and T4 and after the fifth day of intervention (first week) (T1), which corresponding to the end of the stimulation period. This strategy was chosen to minimize long periods of evaluation during the procedure, reducing burden to subjects.

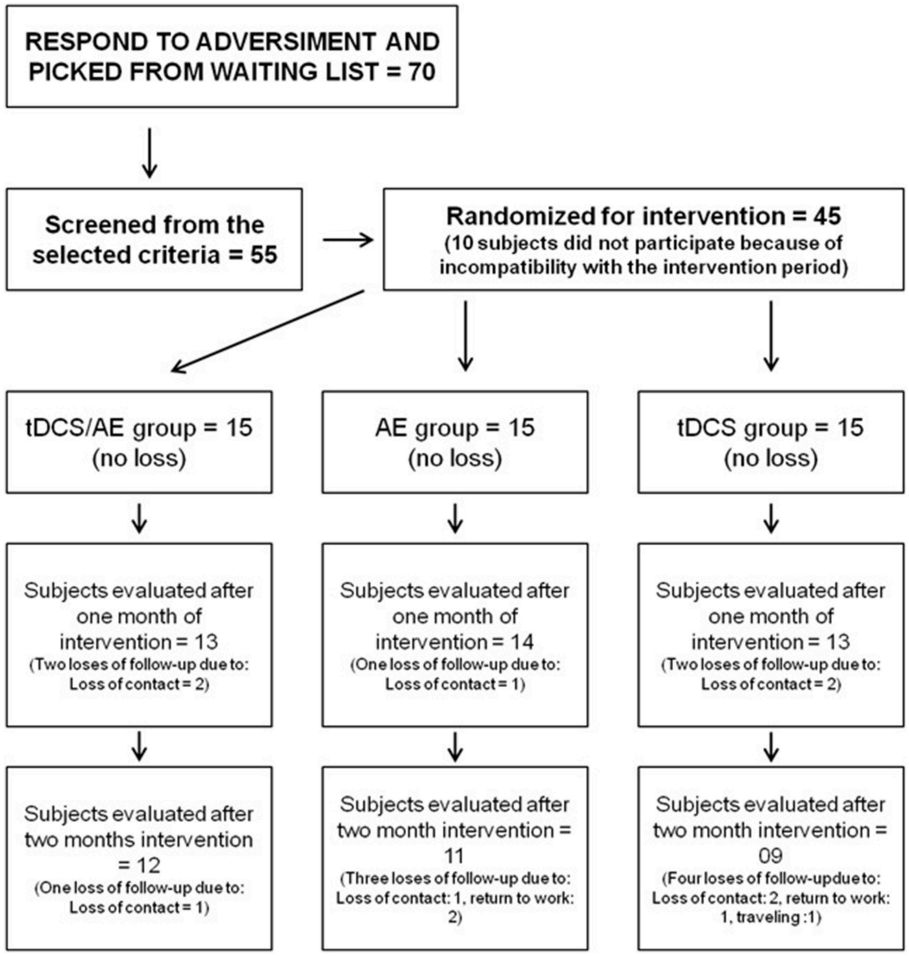

FIGURE 1 | Research flowchart.

Primary Outcome

The Visual Numeric Scale (VNS) was used to assess the intensity of pain, as reported by the patient. This straight 10-cm scale is numbered from 0 to 10, in which 0 represents no pain and 10 is the most pain imaginable. Subjects were asked to mark the number that best reflected the symptoms of pain at that moment.

Secondary Outcomes

Anxiety Levels

Anxiety levels were measured using the VNS for anxiety (from 0 to 10). Also for this outcome, assessments was carried out at baseline, every day before the intervention, post intervention (T2) and follow-up periods (T3, T4).

Pressure Pain Threshold

Pressure pain threshold (PPT) was evaluated with a pressure algometer (Wagner Instruments, USA) to establish the minimum pressure that triggered the pain at the thenar region of the hand and the uppermost portion of the anterior tibialis. These areas were chosen to determine the systemic effects of the interventions. For the statistical analysis, the average of these values was calculated.

Quality of Live

Quality of life was assessed using the SF-36 quality of life questionnaire for all subscales: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health.

Mood

The Beck Depression Inventory was also used to measured symptoms of depression.

Cortical Excitability

Cortical excitability was examined by Transcranial Magnetic Stimulation (TMS) using a figure-of-eight magnetic stimulator coil (BiStim^2 Magstim, UK). Responses to stimuli that were applied to the motor cortex were recorded in the adductor muscle of the thumb of the contralateral hand. The responses of the motor evoked potential (MEP) were amplified and

filtered by surface electromyography (Micromed SpA, Italy). The signals were then transferred to a personal computer for offline analysis using software for data collection (SystemPlus Evolution, Micromed SpA, Italy).

Motor threshold, motor evoked potential, intracortical inhibition, and intracortical facilitation were measured (Kobayashi and Pascual-Leone, 2003). All measures were performed at the left M1 in which the tDCS was held. Motor threshold and motor evoked potentials were evaluated by single-pulse TMS. Motor threshold was found using the lowest intensity for the TMS pulse over the M1 capable to generate a peripheral response of at least 50 microvolts of amplitude at the electromyography. The same technique was used to determine the MEP at 120% of the intensity found for the motor threshold. Ten MEPs were measured at each stage.

Intracortical inhibition (ICI) and intracortical facilitation (ICF) were evaluated by paired-pulse technique. For ICF, a conditional pulse with an intensity of 80% of the motor threshold and a test pulse with the MEP intensity were used. The interstimulus interval was 10 ms for ICF. In measuring intracortical inhibition, the same parameters for the conditional and test stimuli and an interstimulus interval of 2 ms were used. In each individual, 15 measures of ICF and ICI each were made, randomized between inhibition, facilitation, and MEP, totaling 45 pulses for this step.

Adverse Effects

A questionnaire on the adverse effects of tDCS was given to evaluate the adverse effects of transcranial direct current stimulation.

To evaluate the adverse effects of AE, we recorded any musculoskeletal symptoms—such as pain, fatigue, tingling—or cardiovascular symptoms—such as shortness of breath, chest pain, exorbitant increased blood pressure—every day of intervention.

Interventions

The treatment was administered for 4 weeks. During the first week, the subject underwent tDCS every day (Monday to Friday) and aerobic exercise 3 days per week (neuromodulatory phase). On the days on which exercise was performed, the 2 techniques were executed in combination simultaneously. In the following weeks, the subject attended to perform the procedure on 3 days per week for aerobic exercise only (**Figures 2**, **3**).

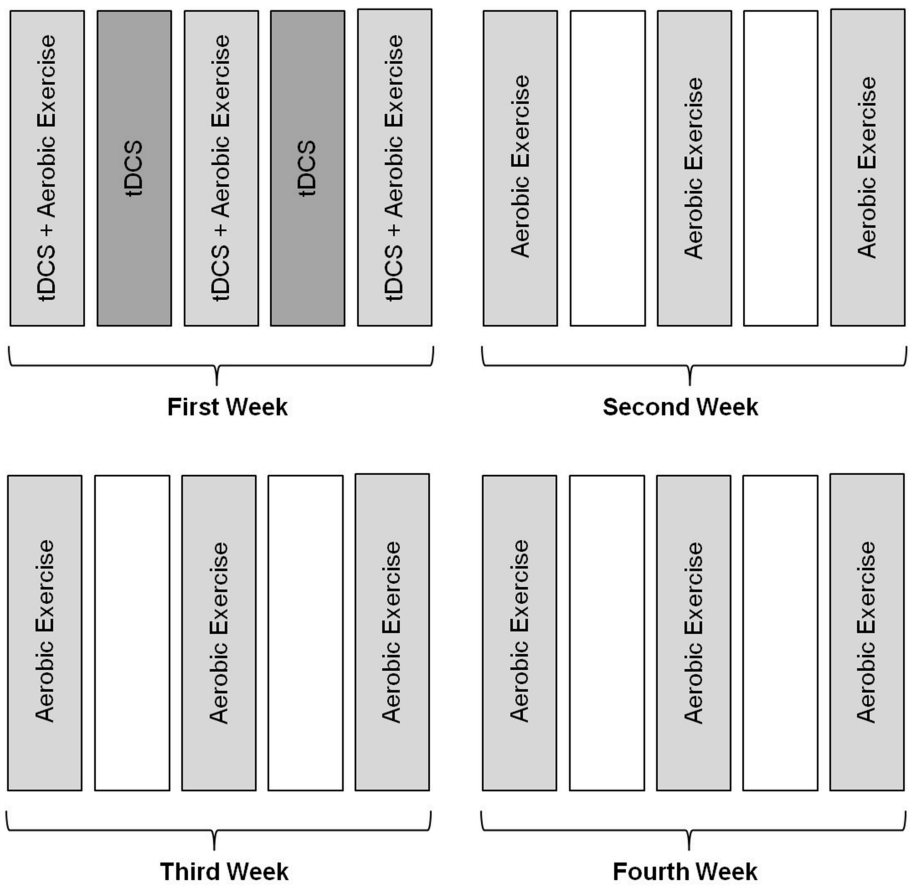

FIGURE 2 | Methodology of intervention. Subjects received intervention with tDCS during the first week for five consecutive days, associated with aerobic exercise training performed three times a week for a month.

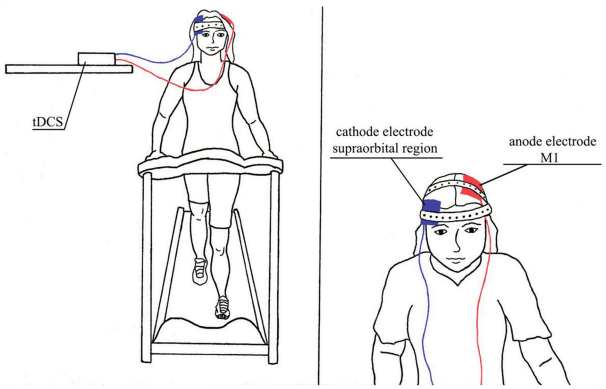

FIGURE 3 | Illustration of interventions in combination, tDCS/AE group which was performed aerobic exercise in combination with tDCS.

Standard safety assessments were performed by the nursing team before and after every visit day including heart and respiratory rate and blood pressure.

Intervention 1: Transcranial Direct Current Stimulation

tDCS was performed using a monophasic current device (DC stimulator, NeuroCom, Germany). Pairs of silicon sponge surface electrodes (35 cm^2) were soaked in saline and positioned as follows: the anode was placed over the region of the primary motor cortex (M1) per the International 10/20 system at point C3 (M1 left), and the cathode was placed over the supraorbital region, contralateral to the anode (right).

The treatment method entailed 5 days of stimulation with monophasic continuous current with an intensity of 2 mA for 20 min. For stimulation a gradual current ramp-up and ramp-down with 30 s duration was used.

The sham procedure for tDCS was performed with same placement of electrodes as in the active group, but the stimulation was administered for only the initial 30 s, with the power turned off for the remaining period.

Intervention 2: Aerobic Exercise

Aerobic exercise was performed on a treadmill (Kikos E100, Brazil) for a period of 30 min per session. The exercise was scheduled to start at an intensity of 60% of the maximum Heart Rate (HR) for each patient. Maximum HR was defined as HRmax = 208 − (0.7 ∗ age; Tanaka et al., 2001). HR was monitored throughout the entire procedure (heart monitor, Oregon Scientific, Brazil). After the second week, the intensity could be increased to 70% of the maximum HR, based on the individual's response. At the beginning and end of the exercise, the lower limbs were stretched in each session.

The sham procedure for AE consisted of subjects undergoing the training on the treadmill, but HR was maintained within 5% of the resting HR at the minimum speed on the treadmill.

Statistical Analysis

All subjects completed the intervention period and carried out the post intervention assessment (T2). There was a loss of 12% of the sample in the first follow-up, and a loss of 28% of the sample in the second period of follow-up. Dropouts during follow-up were similar across groups. Specific missing data per group is described in **Figure 1**. Missing data were treated by intention-to-treat analysis, taking into account the method of the last observation carried forward. Sensitivity analysis was carried out with complete cases analysis. The Kolmogorov-Smirnov test demonstrated normal distribution of the data. Thus, parametric tests were performed, and the data were expressed as mean and standard deviation for the analysis and as mean and standard error in the graphs.

To compare the effects of tDCS and aerobic exercises on the main outcome variable—the VNS—we used mixed ANOVA, including the main effects of time [baseline, each day before the intervention (Days 1–13) and the follow-up period (1 and 2 months)], group (tDCS/AE, AE, and tDCS), and the interaction group X time. *Post-hoc* analyses were conducted using reduced ANCOVA models (for each time point: T1, T2, T3, and T4) adjusted for variables indexing baseline psychiatric and pain characteristics since these variables have an influence on final pain symptoms.

For other outcome variables, as dependent variables in the ANOVA models, we used the SF36 (all subscales), Beck Depression Inventory, pressure pain threshold, and neurophysiological parameters.

The independent fixed variables were time (baseline, post-treatment, follow-up 1, and follow-up 2), group (tDCS/AE, AE, and tDCS), and the group-treatment interaction. The effect size (cohen's *d* effect size) was calculated from the difference in values between baseline and post-treatment comparing the combination group with the other groups.

A similar analysis was conducted for the secondary outcomes. The predictors of outcome were analyzed by linear regression using univariate models, with the difference in pain intensity before and after the intervention as the dependent variable and age, time of pain, VNS values at baseline, SF36 (all subscales), Beck Depression Inventory, and changes in neurophysiological parameters at the post-treatment evaluation as independent variables. A $p < 0.05$ indicated a statistically significant result. The data were organized and tabulated using Stata 12.

RESULTS

Of the study participants, 44 were female. Considering the total sample all were right-handed, with a mean age of 47.4 (±12.1), and mean duration of pain of 138.5 (±94.2) months. Other demographic data are available in **Table 1**. Forty five individuals completed the intervention period. For the follow-up period there were three losses in group tDCS/AE, four losses in group AE, and six losses in group tDCS (**Figure 1**).

Primary Outcome: Visual Numeric Scale

Pain intensity had a significantly effect on interaction time vs. group [$F_{(26, 546)} = 2.08$, $p = 0.0015$]. Similarly, there were significant main effects of group [$F_{(13, 546)} = 6.78$, $p < 0.001$] and time [$F_{(2, 546)} = 32.16$, $p < 0.001$]. By *post-hoc* analysis, there was a difference between the tDCS/AE and AE groups [$F_{(13, 364)} = 2.25$, $p = 0.007$] and the tDCS/AE and tDCS

TABLE 1 | Sample data at baseline.

	tDCS + AE	AE	tDCS	*p*-value*
Age (years) (±SD)	44.5 (±14)	48 (±11.8)	49.9 (±10.6)	0.4
Gender (F/M)	14/1	15/0	15/0	
Regular exercises (Y/ N)	2/13	4/11	3/12	
Pain duration (months) (±SD)	140.6 (±72.2)	149.3 (±111.1)	125.6 (±100.2)	0.7
Hours of sleep (±SD)	5.3 (±1.5)	5.8 (±1.5)	5.7 (±1.8)	0.7
VNS (±SD)	7.3 (±1.7)	6.8 (±2.0)	7.2 (±1.2)	0.72

**Data are presented as mean and standard deviation. Analyzes performed by one way ANOVA.*

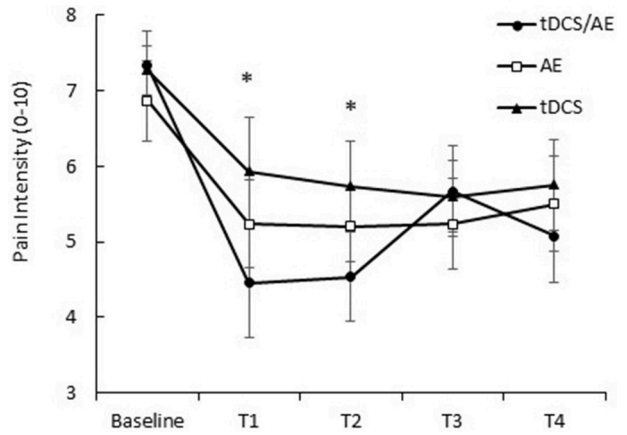

FIGURE 4 | Response of pain intensity (VNS pain). T1, assessment after the fifth day of intervention; T2, assessment after 1 month of intervention; T3, assessment after 1 month of the end of the intervention (follow-up 1); T4, assessment after 2 months of the end of the intervention (follow-up 2). Data presented as mean and standard error. *Statistical analysis demonstrated significant result for T1 ($p = 0.02$) and at T2 ($p = 0.03$) between tDCS/AE group and tDCS group.

TABLE 2 | Mean and standard deviation values of primary outcome (VNS-pain).

	tDCS/AE group	AE group	tDCS group
BASELINE			
Mean (±SD)	7.3 (±1.75)	6.8 (±2.0)	7.2 (±1.27)
T1			
Mean (±SD)	4.4 (±2.85)	5.2 (±2.25)	5.9 (±1.27)
% of improvement from baseline	39.7%	23.5%	18%
T2			
Mean (±SD)	4.5 (±2.29)	5.2 (±1.83)	5.7 (±2.31)
% of improvement from baseline	38.3%	23.5%	20.8%
T3			
Mean (±SD)	5.6 (±2.31)	5.3 (±2.32)	5.5 (±1.91)
% of improvement from baseline	23.2%	22.0%	23.6%
T4			
Mean (±SD)	5.0 (±2.4)	5.5 (±2.45)	5.7 (±2.38)
% of improvement from baseline	31.5%	19.1%	20.8%

groups [$F_{(13,\ 364)} = 2.33$, $p = 0.0056$]. Analysis using covariate adjustment—with baseline psychiatric (anxiety level and mental health—SF-36) and pain characteristics—showed that there are significant changes at day 5 (end of stimulation—T1) and at the end of the protocol (T2) ($p = 0.029$ and $p = 0.030$, respectively), but not at the two follow-ups ($p > 0.5$ for both analyses) (T3 and T4) (**Figure 4**). Values of mean, standard deviation and percentage of improvement are described in **Table 2**.

Subsequent analysis using covariate adjustments demonstrated a difference between the groups tDCS/AE and tDCS at the end of the first week of intervention (effect size = 0.6, $p = 0.02$) and at the end of the 1 month intervention (effect size = 0.56, $p = 0.03$). For the comparison between the groups tDCS/AE and AE, although effect sizes were also large, there was no significant differences at day 5 (effect size = 0.68, $p = 0.14$) and, at the end of the 1 month intervention, although p-value was less than 0.1, it did not reach significance (effect size = 0.59, $p = 0.08$). The comparisons between the groups AE and tDCS revealed no significant differences ($p > 0.5$ for the comparisons between day 5 and end of 1 month). In fact the effect sizes comparing these two groups were very small (effect size day 5 = 0.12 and effect size at end of month = 0.07).

Sensitivity analysis demonstrated no difference in statistical results.

Secondary Outcomes

Anxiety Level

Anxiety level showed a significant result for the time-group interaction [$F_{(8,\ 168)} = 3.86\ p < 0.001$] and time [$F_{(4,\ 168)} = 11.70$, $p < 0.001$] but not for group [$F_{(42,\ 168)} = 7.17$, $p = 0.09$; **Figure 5**).

Pressure Pain Threshold

With regard to pressure pain threshold, the time-group interaction was not significant [ANOVA, $F_{(9,\ 126)} = 2.78$, $p = 0.08$]. but because the p-value of this interaction was less than 0.1, we also calculated the main effects and found that group [$F_{(3,\ 126)} = 4.44$, $p = 0.005$] and time [$F_{(2,\ 126)} = 77.87$, $p < 0.001$] were significant. The results are shown in **Figure 6**.

Quality of Life: SF-36

For the vitality, physical functioning, bodily pain, physical role functioning, emotional role functioning, social role functioning, and mental health subscales, no significant differences for the

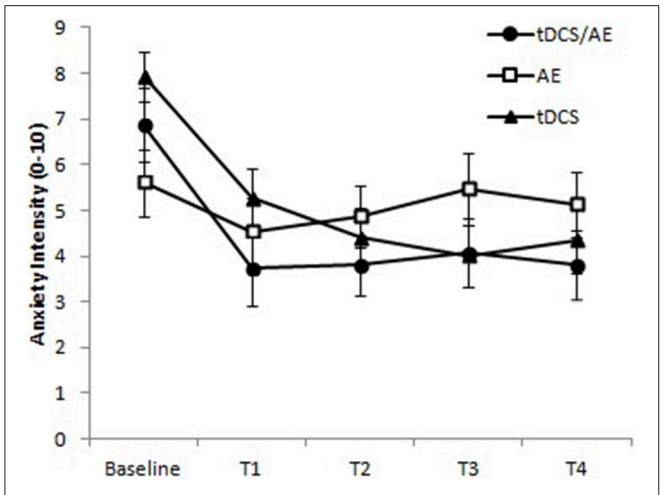

FIGURE 5 | Response for level of anxiety (VNS anxiety). T1, assessment after the fifth day of intervention; T2, assessment after 1 month of intervention; T3, assessment after 1 month of the end of the intervention (follow-up 1); T4, assessment after 2 months of the end of the intervention (follow-up 2). Paired evaluation between groups $p < 0.001$. Data presented as mean and standard error.

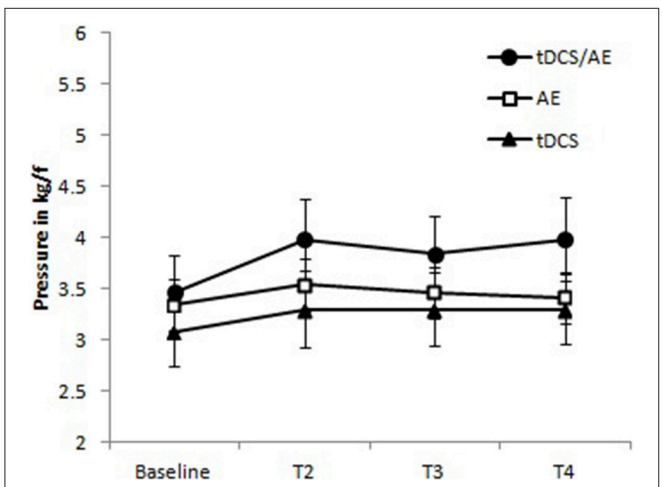

FIGURE 6 | Data for pressure pain threshold. Group tDCS/AE demonstrating a relative of the pressure pain threshold increased, maintained during periods of follow-up. Group AE slight increase after the intervention, apparently not maintained at follow-up period. tDCS group with mild increase in the pressure pain threshold, being held in the follow-up period. No statistical significant data were observed. Data shown as mean and standard error.

main interaction between time and group were observed ($p > 0.05$ for all). The data on the mean and standard deviations are listed in **Table 3**.

For the general heath perceptions subscale, there was a significant result for the time-group interaction [$F_{(6, 84)} = 3.9$, $p = 0.001$] and for group [$F_{(3, 4)} = 7.4$, $p = 0.004$], but no significant differences were noted in the effect of time [$F_{(2, 2)} = 0.6$, $p = 0.549$]. By *post hoc* analysis, there was a difference before the intervention for the combination group vs. the AE and tDCS groups ($p = 0.003$ and $p = 0.012$, respectively) at the end of

TABLE 3 | Mean and standard deviation for SF-36 questionnaire and Beck depression inventory results.

	tDCS/AE group				AE group				tDCS group			
	Baseline	T2	T3	T4	Baseline	T2	T3	T4	Baseline	T2	T3	T4
Physical functioning	41.0(4.8)	48.3(4.0)	49.0(3.9)	48.0(4.5)	45.0(4.1)	49.3(4.8)	53.0(5.2)	50.6(4.9)	37.6(5.0)	41.0(5.8)	42.69(5.6)	45.3(6.0)
Physical role functioning	18.3(7.5)	36.2(9.0)	41.2(8.3)	52.8(10.3)	20.0(7.7)	36.6(10.3)	35.0(10.0)	40.0(10.0)	16.6(7.1)	26.6(9.9)	23.3(9.2)	30.0(10.4)
Bodily pain	25.6(4.6)	44.3(4.5)	43.2(4.3)	49.2(5.4)	27.1(3.0)	40.4(4.1)	39.6(3.8)	40.4(4.1)	21.6(2.6)	30.6(3.7)	29.2(3.7)	30.6(3.3)
General health perceptions	36.3(4.4)	42.8(3.3)	41.4(4.2)	42.1(3.4)	38.2(5.0)	47.5(5.1)	44.9(4.1)	43.9(4.3)	36.0(4.2)	37.1(4.5)	39.8(3.8)	38.1(3.8)
Vitality	30.0(4.7)	39.6(4.2)	43.3(4.6)	40.3(5.0)	39.0(5.4)	48.6(4.0)	47.0(4.2)	49.6(4.2)	24.6(4.1)	31.6(5.4)	32.6(5.3)	31.0(4.8)
Social role functioning	58.8(7.7)	62.7(7.0)	67.2(7.5)	73.5(7.4)	50.8(6.3)	58.3(6.5)	57.5(7.0)	60.7(7.0)	29.9(5.4)	34.6(6.7)	35.5(6.0)	37.1(7.0)
Emotional role functioning	47.4(12.3)	66.6(10.7)	66.6(11.4)	69.6(10.5)	36.0(11.2)	81.3(8.9)	76.0(8.4)	66.5(9.0)	22.2(10.6)	25.1(10.4)	29.5(10.1)	33.9(10.1)
Mental health	42.1(5.1)	57.1(6.4)	54.6(5.5)	53.6(5.8)	46.6(5.9)	56.0(6.3)	53.8(6.0)	53.8(6.1)	32.5(5.8)	41.3(6.2)	43.2(6.0)	48.6(6.6)
Beck depression inventory	20.8(2.1)	13.8(2.1)	14.1(2.1)	13.6(2.4)	21.0(3.1)	18.5(3.2)	16.2(3.2)	16.8(3.4)	25.0(2.7)	20.2(3.0)	19.0(3.0)	19.2(3.6)

Values are represented as mean (± standard deviation).

intervention (T2) (AE vs. tDCS $p = 0.009$) and at follow-up 2 (T4) (tDCS/AE vs. tDCS $p = 0.006$).

Mood

The evaluation of Beck Depression Inventory scores demonstrated no statistical significance for the interaction of time and group [$F_{(6, 123)} = 0.84$, $p = 0.54$], but group [$F_{(2, 123)} = 8.55$, $p < 0.001$] and time [$F_{(3, 123)} = 18{,}26$, $p < 0.001$] had significant effects. In an exploratory analysis of this variable, removing the follow-up periods to better understand the immediate effects of the interventions, significant results were found for the time-group interaction [$F_{(2, 41)} = 3.22$, $p = 0.05$], group [$F_{(2, 41)} = 6.22$, $p = 0.004$], and time [$F_{(1, 41)} = 37.33$, $p < 0.00$]—the combined intervention group experienced the largest decrease in depression intensity ($p = 0.001$) vs. the AE group at the end of the intervention period (T2). Data of mean and standard deviation described in **Table 2**.

Neurophysiological Data

With regard to TMS parameters, there were no significant results for the main analysis of time vs. group for MEP [$F_{(8, 128)} = 0.57$, $p = 0.8$], or time [$F_{(2, 168)} = 2.34$, $p = 0.057$], and group had a significant effect [$F_{(2, 168)} = 5.37$, $p = 0.005$]. These results were similar to those of the other TMS variables. There were no effect of the interaction of time and group for ICF [$F_{(8, 128)} = 0.56$, $p = 0.8$] and for the interaction effect of ICI [$F_{(12, 128)} = 1.6$, $p = 0.9$]. The data for this variable are shown in **Figure 7**.

Regression Analysis

To better understand the influence of demographic, clinical outcomes, and also the baseline pain status on the pain response to the interventions, we initially ran univariate regression models, considering the difference between pain scores before and after the treatment as the dependent variable. The independent variables were the baseline values of the following: duration of pain, intensity of pain, anxiety, pain threshold, mood, subscales of quality of life, and cortical excitability values (MEP, ICI, and ICF), and also intervention group (type of intervention). We defined significance as $p < 0.01$ for this initial analysis. A relationship was observed between the response in pain intensity (difference between pain scores before and after treatment) and baseline pain intensity values ($p = 0.01$) and baseline anxiety levels ($p = 0.01$). The regression data are shown in **Table 4**. Correlation analysis were also carried out for those variables. Results are shown in Table 1, at Supplementary Material.

We then performed multivariate regression analysis, with the difference in pain intensity as the dependent variable and baseline pain level, anxiety and mood scores, and the respective interactions as the independent variables.

The multivariate model with mood and baseline pain levels showed significant results (model $p = 0.0003$). Baseline pain correlated positively with changes in pain after treatment ($b = 0.53$ and $p = 0.002$) and mood scores ($b = 0.07$ and $p = 0.006$), indicating that higher pain and depression scores at baseline were associated with a greater pain response. Baseline pain scores appeared to modify the effect of depression on the response to the

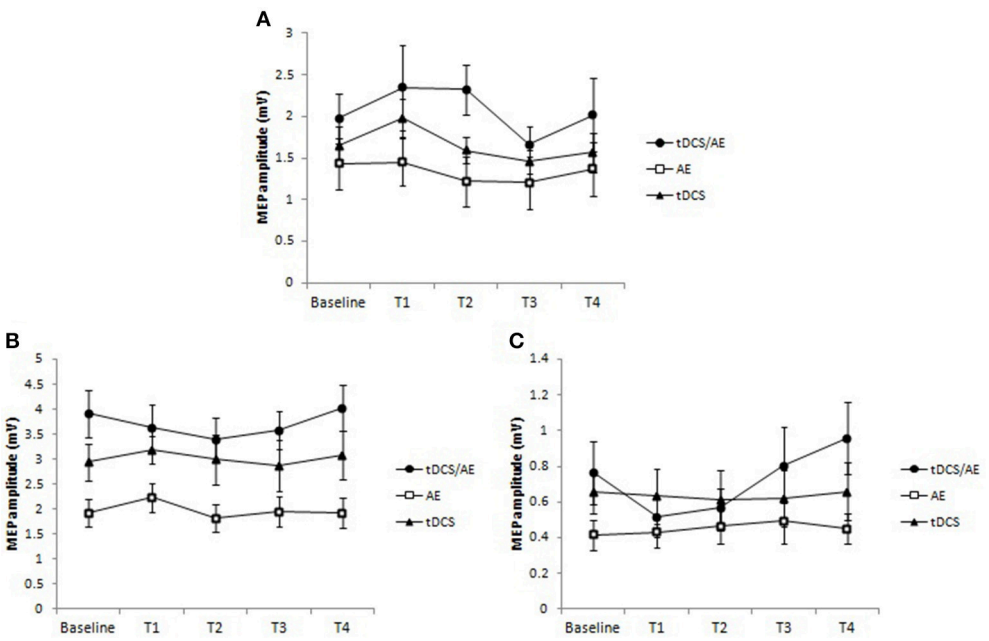

FIGURE 7 | Data for cortical excitability. (A) Data for motor evoked potential (MEP). There was an increased excitability in the tDCS/AE group until the end of the intervention, not being maintained after the period of follow-up. For tDCS group there was an increase in cortical excitability after just 1 week period of intervention that occurred with active stimulation. The EA group showed a slight decrease in MEP after the intervention. **(B)** Intracortical facilitation. There was a slight decrease in intracortical facilitation, represented by decreased amplitude of MEP's only in group tDCS/AE. **(C)** Intracortical inhibition. There was an increase in intracortical inhibition during the protocol period for tDCS/AE group, and in the follow-up periods an increase above the baseline. No statistically significant findings were observed. Data shown as mean and standard error.

interventions, indicating that the association between depression and the response to the interventions weakened with a decrease in baseline pain values (**Table 5**).

Adverse Effects

All adverse effects were mild and did not differ between treatment groups (**Table 6**).

DISCUSSION

This study has demonstrated that neuromodulation with tDCS, in association with aerobic exercise training, in fibromyalgia patients effects greater decreases in pain intensity than the individual techniques. Anxiety levels also improved in the combination therapy group. There was a marginal but significant increase in pain threshold in the combination group compared with tDCS alone. The results for depression were better in the tDCS/AE group vs. the other 2 groups, but did not show significant results in statistic. No significant differences in cortical excitability were observed. Baseline pain and mood scores appeared to be related to the response to these treatments.

The main hypothesis of this study is that the combination of techniques has greater effects in pain intensity in patients with fibromyalgia. Previous studies in neuromodulation for fibromyalgia and aerobic exercise have shown that these techniques yield significant results compared with control interventions and baseline symptoms (Marlow et al., 2013; García-Hermoso et al., 2014; O'Connell et al., 2014; Vural et al., 2014). It is important to underscore that a previous review study concludes that there are no positive results for tDCS when considering the aggregate results for different types of chronic pain, particularly in the long term effects (O'Connell et al., 2014). However in this study we chose to evaluate if there is an additive effect when using two modulating techniques.

In this study, the effect of the combination of techniques was compared between two active techniques and the combination of each individual active intervention and the associated placebo method. Although some of the results for the secondary outcomes were marginally significant, this trial was not powered for the secondary outcomes; also, we compared the combined treatment against each group using one active treatment alone.

Aerobic exercise acts systemically in the body, influencing many domains. For instance, it can alter brain activity through motor cortex activation and neurotransmitter release (Meeusen and De Meirleir, 1995). This concept is known as exercise-induced hypoalgesia, which is regulated by the release of endogenous opioids (Koltyn, 2000). In addition, exercise modifies the activity in certain regions in the cortex through facilitatory and learning mechanisms, leading to long-term potentiation (LTP) mechanisms (Erickson and Kramer, 2009;

TABLE 4 | Results for univariate linear regression models.

	P-value	B Coefficient
Age	0.9	0.001
Intervention Group	0.09	−0.63
Pain duration	0.6	0.001
PPT	0.7	0.07
MEP	0.6	0.14
ICF	0.5	0.12
ICI PRE	0.1	0.79
SF36 Physical functioning	0.9	0.002
SF36 Physical role functioning	0.4	−0.008
SF36 Bodily pain	0.8	−0.006
SF36 General health Perceptions	0.6	−0.009
SF36 Vitality	0.2	−0.02
SF36 Social role functioning	0.3	0.01
SF36 Emotional role functioning	0.5	−0.004
BDI	0.4	0.022
VNS Pain baseline	0.01	0.44
VNS Anxiety baseline	0.01	0.24

TABLE 6 | Side effects occurrence

	tDCS/AE	AE	tDCS	Total	*P*-value*
AEROBIC EXERCISE					
Mild Muscle Pain	**4**	**3**	**0**	**7**	**0.1**
tDCS					
Headache	3	4	3	10	1.0
Neck Pain	1	2	1	4	1.0
Skull Pain	0	0	0	0	-
Skin Injury	0	0	0	0	-
Tingling	3	4	5	12	0.91
Skin Redness	13	7	11	31	0.1
Somnolence	4	3	5	12	0.91
Concentration Issues	1	0	1	2	1.0
Mood changes	0	0	0	0	-

**Statistics was performed by fisher exact test.*

TABLE 5 | Pain intensity data stratified by level of depression.

	tDCS/AE group			AE group			tDCS group		
	BDI	**VNS**	**%***	**BDI**	**VNS**	**%***	**BDI**	**VNS**	**%***
No depression or mild depression	11.8	6.0	26	9.1	6.3	31	11.5	6.3	22
Moderate to severe depression	25.7	7.9	37	29.3	7.2	24	29.9	7.4	20

**Percentage of improvement from the mean values. BDI, Beck depression inventory. VNS, Visual numeric scale.*

Lojovich, 2010). Various studies have reported beneficial results of exercise in many chronic pain syndromes (Nijs et al., 2012).

However, the results are somewhat mixed. Patients with widespread pain experience immediate worsening of symptoms due to dysfunction in endogenous analgesia mechanisms, which might be related to myofibril injury, which causes inflammation and increased nociceptive signaling. Therefore, its use is somewhat limited. To obtain beneficial results it is necessary to overcome this phase, which is not achieved by a majority of patients.

Another method of influencing the motor system is neuromodulation with tDCS (Mendonca et al., 2011). The basic mechanism of action of tDCS is modulation of spontaneous neuronal firing through induced polarization of neural tissue. In this context, anodal tDCS leads to depolarization and thus an increase in spontaneous neuronal firing; cathodal tDCS has the opposite effects. tDCS effects motor cortex activation (M1), resulting in secondary modulation of regions that are associated with pain modulation (Castillo Saavedra et al., 2014).

Continued use of tDCS induces plastic changes and can lead to pain relief for 1 month after the end of the intervention (Fregni et al., 2006). Several studies have shown that five consecutive applications of tDCS over the M1 relieve pain and improve quality of life and sleep in various chronic pain syndromes (Fregni et al., 2006; Roizenblatt et al., 2007; Valle et al., 2009).

Based on the effects of aerobic exercise and the mechanisms of tDCS, we hypothesized that the combined therapy would be more effective than each method alone, because tDCS would prime the system, which would be subsequently modified by aerobic exercise. Another possibility is that these techniques have disparate neural targets and thus do not synergize. Our data on the neurophysiological assessment with TMS, demonstrating no changes in cortical plasticity of the motor cortex between the interventions, supporting that the additional effects of the combination therapy are related to the activity in other neural circuits, independent of the motor system.

Other protocols that have testing the combination of tDCS with other techniques have been reported. Riberto et al. (2011) developed a regimen, comprising tDCS and a multidisciplinary rehabilitation program, observing improvement in only one variable in the SF-36 questionnaire for quality of life (pain domain only). The authors implemented an exercise program, which used stretching and ergonomic and posture instructions 3 times per week, and performed tDCS once per week for 10 weeks. The difference in the use of tDCS might explain the differing results comparing to our results—achieving long-term effects requires a protocol with more days of stimulation in a shorter time (Nitsche et al., 2008).

Another study (Boggio et al., 2009) showed that a single application of tDCS, associated with the use of peripheral TENS, for the treatment of chronic pain had a superior effect compared with tDCS or TENS alone. For other areas, such as cognitive and motor rehabilitation, several trials have combined tDCS with various training methods, including robotics, virtual reality, and computer-based training (Soler et al., 2010; Giacobbe et al., 2013; Martin et al., 2014), the results of which support combination treatment to enhance and guide the effects of tDCS.

We evaluated cortical excitability using single-pulse and paired-pulse TMS to assess motor cortex plasticity changes that were associated with these three groups of treatments. A previous study demonstrated that FM subjects experience alterations in these parameters, such as increased MEPs at rest and reduced ICI and ICF (Salerno et al., 2000; Mhalla et al., 2010). Although, our results were marginally significant, we noted an overall increase in MEP, a decline in ICI, and small changes in ICF. But, we did not observe any significant differences between groups. In contrast, Antal et al. (2010) observed a reduction in ICI after anodal stimulation, but they used different stimulation parameters (smaller electrode size and intensity of 1 mA). In a study using repetitive TMS (rTMS) using 10 Hz of intensity, which also activates the motor cortex, the authors demonstrated an increase in ICI in accordance to our results (Lefaucheur et al., 2006; Dall'Agnol et al., 2014). The lack of difference between groups but the disparate behavioral results suggest that the differential results are attributed to nonmotor neural circuits.

Notably, the initial level of pain and mood appears to be a predictor of outcome. We observed that individuals with higher pain levels and higher levels of depression responded better to the treatment, indicating greater central sensitization that might be responsive to the combined intervention; however the results of the prediction model need to be interpreted carefully given the relatively small sample size for this analysis.

A limitation of this research is related to blinding. There is a debate on the effectiveness of blinding in tDCS studies. Some studies such as the one from Villamar et al. (2013) shows that blinding in single session cross-over studies is not adequate. However, further studies such as the one from Brunoni et al. (2014) showed that blinding in clinical trials in which the treatment effect plays a major role, such as the blinding method of tDCS is comparable to drugs such as sertraline (Brunoni et al., 2014). Regardless we did not conduct blinding assessment given the questionable results of this assessment as patients may correlate stimulation condition with improvement. Finally, we observed that there were no significant differences in adverse effects of tDCS, not even when with regard of skin redness (86% of individuals in the tDCS/AE group, 47% of individuals in the EA group, 73% of individuals in the tDCS group, without significant results $p = 0.1$) shown in **Table 4**. There were also no differences in adverse effects of aerobic exercise. In addition, all groups received two forms of intervention associated, with one active intervention at least which may have also helped to maintain blinding of the other intervention. Another limitation is related to loss of follow-up. Although this is a potential source of bias, missing data were completely at random and distributed equally across groups of treatment. Also it is important to highlight that all patients completed the entire month of intervention and the subsequent final evaluation. In addition, sensitivity analysis showed no significant differences in the results.

Based on these findings, the three groups showed positive effects in many variables, such as pain relief, quality of life,

depression, and anxiety, but there was a larger effect that was associated with the combination treatment. The simultaneous effect of the combination treatment on pain and depression levels in fibromyalgia should prompt larger trials on the effects of this modality with longer follow-up periods.

AUTHOR CONTRIBUTIONS

MM contributed with project creation, data collection, statistical analysis and article writing. MS contributed with data collection, statistical analysis and article writing. LG contribute with data collection, statistical analysis and article writing. LB contributed with article writing. AB contributed with project creation and article writing. FF contributed with project creation, data collection, statistical analysis and article writing.

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SUPPLEMENTARY MATERIAL

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frontiers in Human Neuroscience

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Patients with Rheumatoid Arthritis and Chronic Pain Display Enhanced Alpha Power Density at Rest

*Francisco M. Meneses[1,2], Fernanda C. Queirós[2], Pedro Montoya[3], José G. V. Miranda[4], Selena M. Dubois-Mendes[1,2,5], Katia N. Sá[2,5], Cleber Luz-Santos[2] and Abrahão F. Baptista[1,2,5]**

[1] Graduate Program in Medicine and Health, School of Medicine, Federal University of Bahia, Salvador, Brazil, [2] Functional Electrostimulation Laboratory, Biomorphology Department, Health Sciences Institute, Federal University of Bahia, Salvador, Brazil, [3] Department of Psychology, Research Institute of Health Sciences, University of Balearic Islands, Palma de Mallorca, Spain, [4] Nucleus of Innovation and Technology in Rehabilitation, Institute of Physics, Federal University of Bahia, Salvador, Brazil, [5] Physiotherapy Program, Bahia School of Medicine and Public Health, Salvador, Brazil

Patients with chronic pain due to neuropathy or musculoskeletal injury frequently exhibit reduced alpha and increased theta power densities. However, little is known about electrical brain activity and chronic pain in patients with rheumatoid arthritis (RA). For this purpose, we evaluated power densities of spontaneous electroencephalogram (EEG) band frequencies (delta, theta, alpha, and beta) in females with persistent pain due to RA. This was a cross-sectional study of 21 participants with RA and 21 healthy controls (mean age = 47.20; SD = 10.40). EEG was recorded at rest over 5 min with participant's eyes closed. Twenty electrodes were placed over five brain regions (frontal, central, parietal, temporal, and occipital). Significant differences were observed in depression and anxiety with higher scores in RA participants than healthy controls ($p = 0.002$). Participants with RA exhibited increased average absolute alpha power density in all brain regions when compared to controls [$F_{(1.39)} = 6.39$, $p = 0.016$], as well as increased average relative alpha power density [$F_{(1.39)} = 5.82$, $p = 0.021$] in all regions, except the frontal region, controlling for depression/anxiety. Absolute theta power density also increased in the frontal, central, and parietal regions for participants with RA when compared to controls [$F_{(1,\ 39)} = 4.51$, $p = 0.040$], controlling for depression/anxiety. Differences were not exhibited on beta and delta absolute and relative power densities. The diffuse increased alpha may suggest a possible neurogenic mechanism for chronic pain in individuals with RA.

Keywords: rheumatoid arthritis, chronic pain, EEG, delta rhythm, theta rhythm, alpha rhythm, beta rhythm

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Vasil Kolev,
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University of California, Davis, USA

****Correspondence:***
Abrahão F. Baptista
abrahao.baptista@gmail.com

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, autoimmune disease of unknown etiology (Firestein, 2003). A recent systematic literature review estimated the global prevalence to be 0.24% (95% CI: 0.23–0.25%; Cross et al., 2014). Gender plays an important role, as women are twice as likely to present the condition (mean 0.35%; 95% CI: 0.34–0.37) than males (mean 0.13%; 95% CI: 0.12–0.13; Mikkelsen et al., 1967).

Abbreviations: RA, rheumatoid arthritis; HC, healthy controls; HADS, Hospital Anxiety and Depression Scale; DN4, Neuropathic Pain Diagnostic Questionnaire (*Douleur Neuropathique 4*); DAS28, Disease Activity Score in 28 Joints; ROI, Regions of Interest.

RA is characterized by peripheral and symmetric polyarthritis, affecting the synovial membranes of joints, leading to pain, and joint deformities (McInnes and Schett, 2011). RA was recently associated with neuropathic pain (Mendes et al., 2014; Walsh and McWilliams, 2014; Koop et al., 2015) which may be present, among other factors, because of entrapment neuropathies, the use of certain drugs and central sensitization. Pain is perhaps the most common symptom and the most related to disability in RA patients (Skevington, 1986; Firestein, 2003; Walsh and McWilliams, 2014). However, the quantification and characterization of pain is a challenge for clinicians, since the experience of pain is individual and subjective (Pimenta and Teixeira, 1996; de Vries et al., 2013). Scales and questionnaires have been used in clinical practice to describe pain intensity, as well as its temporal and qualitative aspects.

The electroencephalogram (EEG) is a promising tool for pain evaluation in clinical settings (Jones et al., 2012), since it can provide useful information about the central mechanisms involved in the maintenance of chronic pain in rheumatic diseases (Lee et al., 2011). In general, the assessment of EEG characteristics during wakefulness demonstrated that chronic neuropathic pain usually is associated with EEG slowing, increased power density and peak frequency in the low frequency ranges (theta, alpha; Boord et al., 2008; Olesen et al., 2011; de Vries et al., 2013; Jensen et al., 2013; van den Broeke et al., 2013). Several authors have further argued that EEG abnormalities in chronic pain could be due to a dysfunction of top-down or bottom-up thalamic modulation (thalamocortical dysrhythmia; Llinás et al., 1999, 2005; Sarnthein et al., 2006). Moreover, the fact that patients with chronic low back pain did not show a similar pattern of EEG slowing seems to raise the question of whether this could be a relevant marker for distinguishing between the neuropathic and the nociceptive nature of pain (Schmidt et al., 2012).

RA is fundamentally an inflammatory disease, associated with severe and disabling pain. Although inflammation of joints and other musculoskeletal tissues are the main sources of nociceptive pain in RA (Schaible et al., 2002; Schaible, 2014), recent studies have identified neuropathic pain components within the symptoms of this disease (Ahmed et al., 2014; Mendes et al., 2014; Koop et al., 2015). One of the main candidates to explain the presence of neuropathic pain symptoms is central sensitization (Ahmed et al., 2014). This condition is a consequence of pathological enhancement in nociceptive neuronal function due to maintained nociceptive transmission or decreased endogenous inhibition (Latremoliere and Woolf, 2009). Central sensitization *per se* is associated to the development of neuropathic pain complaints (Mease et al., 2011), which has been identified in patients with RA (Meeus et al., 2012). This maladaptive condition of the central nervous system may be related to spreading of symptoms, decreased pain thresholds, and the poor relation between disease activity and symptoms in RA (Atzeni et al., 2011; Meeus et al., 2012; Hochman et al., 2013). Furthermore, these modifications in the processing of pain at the central level have already been characterized by somatosensory EEG event-related potentials (Wendler et al., 2001), but not by EEG activity at rest.

Given the combination of nociceptive and neuropathic pain in RA, the investigation of quantitative EEG at rest may shed light into its pathophysiology. It may also reveal whether signs of thalamocortical dysrhythmia are present. Therefore, the objectives of the current study were two-fold: (a) to compare EEG activity at rest in patients with RA to healthy controls, and (b) to evaluate the relationship between pain characteristics and EEG activity in patients with RA.

MATERIALS AND METHODS

Participants

Twenty-one women with RA (mean = 47.92, SD = 12.36) and 21 healthy controls (HC; mean = 46.41, SD = 8.30) participated in this cross-sectional study and assessed between August 2013 and October 2014. The participants with RA were recruited from a third-party reference center in Bahia (Brazil) and had received a diagnosis from a rheumatologist, conforming with the criteria from the American College of Rheumatology (Aletaha et al., 2010). Patients were included if they were suffering from chronic pain (pain lasting more than 6 months), during more than 3 days per week, and predominantly located in the joints associated or not with deformities and/or joint range of motion. Participants were excluded if they were diagnosed with any other rheumatologic disease in addition to RA, or reported the use of centrally acting substances. The control group did not report chronic pain and was pain-free on the day of the experiment. Three milliliters of venous blood were collected from the participants with RA in order to analyze the Erythrocyte Sedimentation Rate and C-reactive protein.

Table 1 presents sociodemographic and clinical characteristics for the entire sample and the two groups, as well as the results of tests comparing averages and proportions for the two groups. Significant differences between RA patients HC only appeared in anxiety/depression scores.

The average duration of the disease in the group of participants with RA was 107.4 ± 45.9 months, with an average medical follow-up time of 93.4 ± 43.4 months. The medications most frequently used by these participants were Metotrexate (52.4%), Infliximab (19%), and Prednisone (38.1%). Most of the RA patients reported high ($n = 8$) or moderate ($n = 10$) disease activity. Only two patients were in remission and one presented low disease activity. The main neuropathic pain descriptors (DN4 questionnaire) were numbness (71.4%), tingling (61.9%), and electric shock (57.1%). A total of 57.1% of the RA participants reported neuropathic pain, according to the DN4. The clinical pain characteristics of RA participants are described in detail in **Table 2**.

Participants were verbally informed about the details of the study and all questions answered at the time of recruitment. After agreeing to participate, a written consent was obtained and a printed copy was provided to subjects. The study was conducted in compliance with the principles of the Declaration of Helsinki, and was approved by the Research Ethics Committee at the *Escola Bahiana de Medicina e Saúde Pública* (Bahia School of Medicine and Public Health; reference #1395/2011).

TABLE 1 | Comparison of demographic, behavioral, and clinical characteristics of women with Rheumatoid Arthritis and Healthy Controls.

	Total sample ($n = 42$) *N* (%) or average (SD)	Healthy controls ($n = 21$) *N* (%) or average (SD)	RA Patients ($n = 21$) *N* (%) or average (SD)	*p*-value
DEMOGRAPHIC CHARACTERISTICS				
Age, in years	47.16 (10.42)	46.41(8.30)	47.92 (12.36)	0.64
Variation (min - max)	–	33–59	23–69	
Level of education				0.08
HS incomplete	11 (26.19)	4 (19.05)	7 (33.33)	
Completed HS – some College	20 (47.62)	8 (38.10)	12 (57.14)	
College or higher	11 (26.19)	9 (42.86)	2 (9.52)	
Race/color				0.54
White	3 (7.14)	2 (9.52)	1 (4.76)	
Black/Afro-Brazilian	21 (50)	9 (42.86)	12 (57,14)	
Mixed race	13 (30.95)	6 (28.57)	7 (33.33)	
Others[a]	5 (11.90)	4 (19.05)	1 (4.76)	
Marital Status				0.31
Single	11 (26.19)	3 (14.29)	8 (38.1)	
Married/Live with partner	20 (47.62)	12 (57.14)	8 (38.1)	
Separated/Divorced/ Widow(er)	11 (26.19)	6 (28.57)	5 (23.81)	
HEALTH BEHAVIOR				
Smoking				0.76
No	31 (73.81)	16 (76.19)	15 (71.43)	
Yes	8 (19.05)	3 (14.29)	5 (23.81)	
Former smoker	3 (7.14)	2 (9.52)	1 (4.76)	
Alcohol consumption				0.06
Never	23 (54.76)	8 (38.10)	15 (71.43)	
Occasionally[b]	19 (45.24)	13 (61.90)	6 (28.57)	
Physical activity				0.31
Sedentary	20 (47.62)	8 (38.10)	12(57.14)	
Occasionally	9 (21.43)	4(19.05)	5(23.81)	
Moderate/intense	13 (30.95)	9(42.86)	4(19.05)	
CLINICAL CHARACTERISTICS				
Diabetes Mellitus	3 (7.14)	2 (9.52)	1 (4.76)	1.00
Thyroid problem	10 (23.81)	4 (19.05)	6 (28.57)	0.72
Laterality in upper limb				1.00
Right	39 (92.86)	20 (95.24)	19 (90.48)	
Left	3 (7.14)	1 (4.76)	2 (9.52)	
Depression/Anxiety (HADS)				0.002*
With anxiety and/or depression	19 (45.24)	4 (19.05)	15 (71.43)	

Differences were tested for continuous variables between the groups using the Student's t-test and for categories using Fisher's exact test.
**Significant at level 0.05.*
SD, Standard Deviation; RA, Rheumatoid Arthritis; HS, High School; HADS, Hospital Anxiety and Depression Scale.
[a]Others, the sum of individuals auto-declared "Yellow/Oriental" and "Red/Indian."
[b]Occasionally, weekends without any incidents of drunkenness. The original categories in this variable also included the following options: occasionally with incidents of drunkenness; frequently without any incidents of drunkenness and frequently with incidents of drunkenness. None of the participants selected these options.

Psychological Questionnaires

All participants underwent a semi-standardized interview, including socio-demographic data (age, marital status, level of education, alcohol consumption, smoking, and practice of physical activities) and assessment of mood through the Hospital Anxiety and Depression Scale (HADS). The HADS is a 14-items questionnaire for the assessment of anxiety and depression symptoms (Bjelland et al., 2002). The maximum score on the depression subscale is 21, with a cut-off point at nine. The maximum score on the anxiety subscale is also 21, with a cut-off point at seven. We used an adapted and validated Brazilian version of this scale (Castro et al., 2006).

The following questionnaires were completed by participants with RA only:

McGill Pain Questionnaire. This questionnaire evaluates the subjective and multidimensional experience of pain, providing quantitative measures of clinical pain. It comprises the following

TABLE 2 | Characteristics of the pain in patients with Rheumatoid Arthritis.

CHARACTERISTICS OF THE PAIN	
Disease activity[a]	***N* (%)**
Remission	2 (9.52)
Low	1 (4.76)
Moderate	10 (47.62)
High	8 (38.0)
Neuropathic pain[a]	***N* (%)**
With neuropathic pain	12 (57.14)
Without neuropathic pain	9 (42.86)
	Average (*SD*)
Number of pain descriptors (McGill)	13.57 (5.90)
McGill pain index	28.14 (13.37)

SD, Standard Deviation.

[a]Evaluated using the Douleur Neuropathique 4 (DN4) questionnaire, with a variation of between 0 and 10 and average of 4.10 (DP = 2.51). Patients with neuropathic pain were those with a score equal to or higher than 4.0.

Number of pain descriptors and McGill pain index: measured using the McGill Scale (1996).

categories of pain descriptors: sensitive-discriminative; affective-motivational; cognitive-evaluative; and miscellaneous (Melzack, 1975). In the present study, we used an adapted and validated Brazilian version of this questionnaire (Pimenta and Teixeira, 1996). The maximum "number of pain descriptors" is 20. The "total pain level" was defined as the sum of values for pain intensity, with a maximum score of 78.

The Neuropathic Pain Diagnostic Questionnaire (Douleur Neuropathique 4 - DN4). This 10-item questionnaire was designed to assess neuropathic pain and includes pain descriptors (7 items) and a bedside examination (3 items; Bouhassira et al., 2005). The final score falls within a scale from 0 to 10. Scores higher than three indicate the presence of neuropathic pain. We used a validated Brazilian version of this questionnaire (Santos et al., 2010).

Disease Activity Score in 28 Joints (DAS28). The goal of the questionnaire is to evaluate the level of disease activity in RA patients (Prevoo et al., 1995). It assesses 28 joints (shoulders, elbows, wrists, proximal interphalangeal and metacarpophalangeal, and knees, bilaterally), counting the number of painful joints without considering pain intensity. A joint is considered "painful" if some level of discomfort is present, even if the pain is not intense. The total score varies from 0 to 10. Activity level was classified according to the following cut-off points: remission $\leq$ 2.6; low $\leq$ 3.2; moderate $\leq$ 5.1; and high activity $>$ 5.1 (Pinheiro, 2007).

EEG Recording

EEG data were recorded using a standard amplifier (BRAIN NET 36, EMSA Brazil) from 20 electrodes and two references located on the auricular region (A1 and A2). Active EEG electrodes were placed according to the international 10–20 system at following locations: F7, T3, T5, Fp1, F3, C3, P3, O1, F8, T4, T6, Fp2, F4, C4, P4, O1, Fz, Cz, Pz, and Oz. The sampling rate was 200 Hz and a ground electrode was placed in the frontal region (Fpz). Electrode impedance was kept below 5 kΩ. Participants were instructed to stay relaxed with eyes closed but were monitored so that they were awake throughout the 5 min recording.

The EEG data were analyzed by using the EEGLAB software (version 13). The signals were filtered with a band-pass filter between 0.5 and 50 Hz. Continuous EEG data were segmented in epochs of 1.28 s, which allowed a consistent evaluation of power densities in the frequency range of 1.5–30 Hz. A semi-automated rejection protocol was used to remove artifacts, with an upper limit of 1000 μV and a lower limit of −1000 μV. After the artifact rejection protocol, a minimum of 170 epochs were kept for each participant, an equivalent to roughly 3.5 min. Since we had decided to analyze 2 min for each participant, we selected the central epochs in order to standardize the selection process and avoid selection bias. Thus, only data between epochs 50 and 142 (93 epochs, nearly 2 min) of the EEG recording were analyzed.

Power spectra were calculated by applying a fast Fourier transform for each epoch. Power densities of each epoch and electrode were averaged separately for each participant. The average power densities were grouped into delta [1.5–3.5 Hz], theta [4–7 Hz], alpha [8–12 Hz], and beta [13–30 Hz] frequency bands. In addition, regions of interest (ROI) were computed by averaging power densities at the four frequency bands for the following groups of electrodes: frontal (Fp1, Fp2, F3, Fz, F4), central (C3, Cz, C4), parietal (P3, Pz, P4), occipital (O1, Oz, O2), and temporal (T3, T5, T4, T6). After processing data for absolute power densities, the same was done for relative power densities. These were computed dividing electrode's values in each one of the analyzed frequencies by their values in the total power spectrum. The results for relative power density were also analyzed and displayed by the same ROIs.

Statistical Analysis

Data from the questionnaires were analyzed by using Student *t*-tests to examine differences between the two groups. The differences on the categorical variables were analyzed by using Fisher's Exact Test, as cells with a frequency equal to or less than five were observed in the bivariate analyses. After confirming normality of the data by using Shapiro–Wilk test and Q-Q plots, differences in absolute and relative EEG power densities between the groups was analyzed by using repeated-measures ANOVA with the factors "group" and "region" (ROI) after controlling for anxiety/depression symptoms. Violations of sphericity assumption were corrected by using Greenhouse-Geisser epsilons.

Finally, Pearson zero-order correlations were computed between mean power densities and pain variables (disease activity, neuropathic/nociceptive pain, and McGill outcome variables). All pain variables were normally distributed (using Kolmogorov–Smirnov test). A *p*-value of 5% was used to accept statistically significant differences between the two groups. The *p*-value was corrected for multiple comparisons using the Bonferroni method when

necessary. The SPSS 20.0 software package was used for all analyses.

RESULTS

Difference between Groups on EEG Absolute Power Density

An ANOVA looking at the full power spectrum (1.5–30 Hz) yielded a significant effect of "group" [$F_{(1, 39)} = 5.12$, $p = 0.029$], indicating that patients displayed overall higher power density than HC. We also found a significant "region" effect [$F_{(4, 156)} = 18.27, p < 0.0.0000001$, epsilon GG = 0.595]. Although there were non-significant differences due to the interaction between group and region [$F_{(4, 156)} = 0.51$, $p = 0.605$, epsilon GG = 0.505], mean comparisons in the *post-hoc* analysis (using Bonferroni correction to adjust for multiple comparisons) revealed that RA patients displayed higher power density than HC at frontal (mean difference = 1.589, $p = 0.026$), central (mean difference = 2.006, $p = 0.015$), temporal (mean difference = 1.772, $p = 0.040$), and parietal (mean difference = 2.178, $p = 0.038$) electrodes. After observing this difference in the full power spectrum, we proceeded to look at frequencies of interest. Our discussion will focus on the four frequency bands reported below. **Table 3** and **Figure 1** display the average absolute power density values for the analyzed EEG frequency bands (delta, theta, alpha, and beta) across the five ROIs.

Delta (1.5–3.5 Hz)

The ANOVA yielded only a significant effect due to "region" [$F_{(4, 156)} = 15.39, p < 0.0000001$, epsilon GG = 0.640]. No significant effects of "group" [$F_{(1, 39)} = 2.36, p = 0.132$] or the interaction between "group" and "region" [$F_{(4, 156)} = 0.535$, $p = 0.631$, epsilon GG = 0.470] were observed on absolute delta power densities.

Theta (4–7 Hz)

The ANOVA yielded a significant effect of "group" [$F_{(1, 39)} = 4.51, p = 0.040$], indicating that patients displayed higher absolute theta power density than HC. We also found a significant "region" effect [$F_{(4, 156)} = 18.22$, $p < 0.0000001$, epsilon GG = 0.634], showing that highest power densities were found at parietal, and central electrodes, whereas the lowest ones appeared at frontal electrodes. Although there were non-significant differences due to the interaction between group and region [$F_{(4, 156)} = 0.71$, $p = 0.526$, epsilon GG = 0.634], mean comparisons in the *post-hoc* analysis (using Bonferroni correction to adjust for multiple comparisons) revealed that RA patients displayed higher absolute theta power density than HC at frontal (mean difference = 1.530, $p = 0.039$), central (mean difference = 2.023, $p = 0.024$), and parietal (mean difference = 2.067, $p = 0.043$) electrodes.

Alpha (8–12 Hz)

The ANOVA yielded a significant effect of "group" [$F_{(1, 39)} = 6.39, p = 0.016$], indicating that patients displayed higher absolute alpha power density than controls.

TABLE 3 | Average absolute power density values by regions of interest, controlling for symptoms of anxiety/depression.

Frequency bands (Regions of Interest)	Controls ($n = 21$)	RA Patients ($n = 21$)	$F_{(1.39)}$	*P*-value
Delta (1.5–3.5 Hz)			2.363	0.132
Frontal	28.86 (1.29)	28.88 (2.09)		
Central	27.79 (1.50)	28.07 (1.88)		
Temporal	26.82 (1.75)	26.74 (1.70)		
Parietal	27.96 (1.82)	28.10 (1.76)		
Occipital	27.16 (1.86)	27.14 (1.78)		
Theta (4–7 Hz)			4.505	0.040*
Frontal	25.82 (1.54)	27.03 (2.31)		
Central	25.96 (2.16)	27.57 (2.55)		
Temporal	24.51 (2.13)	25.73 (2.41)		
Parietal	26.28 (2.42)	27.93 (2.98)		
Occipital	25.80 (2.54)	27.23 (2.89)		
Alpha (8–12 Hz)			6.385	0.016*
Frontal	23.31 (3.17)	26.18 (3.30)		
Central	24.68 (4.01)	28.24 (3.76)		
Temporal	23.67 (3.68)	26.47 (4.07)		
Parietal	25.96 (4.72)	29.78 (4.80)		
Occipital	26.20 (4.72)	29.14 (4.93)		
Beta (13–30 Hz)			3.352	0.075
Frontal	16.73 (1.80)	17.87 (1.79)		
Central	17.14 (1.94)	18.36 (1.76)		
Temporal	16.53 (1.97)	17.37 (2.12)		
Parietal	17.74 (2.45)	18.77 (2.35)		
Occipital	17.69 (2.36)	18.38 (2.77)		

**Significant at level 0.05. ANOVA of repeated measures.*

We also found a significant "region" effect [$F_{(4, 156)} = 37.222$, $p < 0.0000001$, epsilon GG = 0.480] showing that highest power densities were found at parietal, occipital, and central electrodes, whereas the lowest ones appeared at frontal and temporal electrodes. Although there were non-significant differences due to the interaction between group and region [$F_{(4, 156)} = 1.69$, $p = 0.192$, epsilon GG = 0.480], mean comparisons in the *post-hoc* analysis (using Bonferroni correction to adjust for multiple comparisons) revealed that RA patients displayed higher absolute alpha power density than HC at all five ROIs: frontal (mean difference = 3.019, $p = 0.015$), central (mean difference = 3963, $p = 0.008$), temporal (mean difference = 3.317, $p = 0.025$), parietal (mean difference = 4.437, $p = 0.015$), and occipital (mean difference = 3783, $p = 0.035$).

Beta (13–30 Hz)

The ANOVA yielded only a significant effect due to "region" [$F_{(4, 156)} = 8.97, p = 0.000207$, epsilon GG = 0.538]. No significant effects of "group" [$F_{(1, 39)} = 3.35, p = 0.075$] or

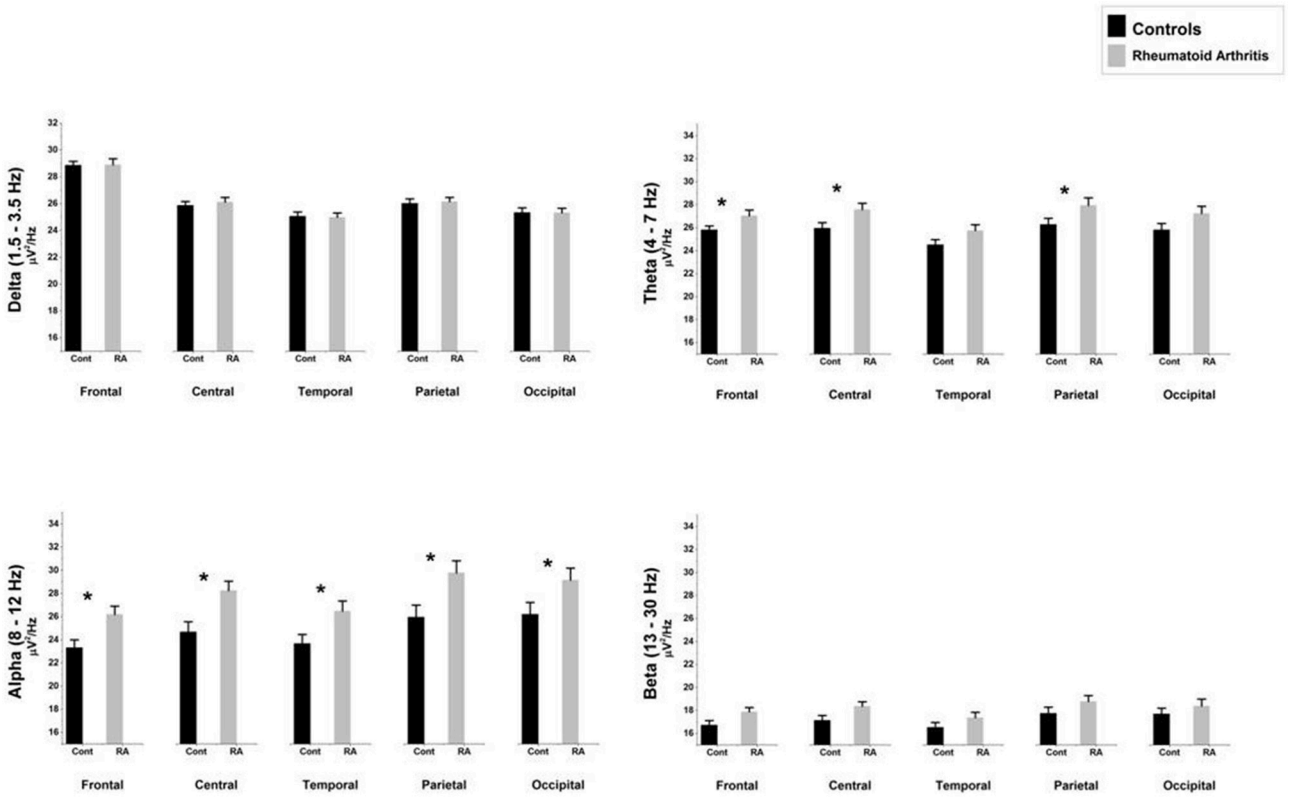

FIGURE 1 | Comparison of absolute mean power densities (μ V^2/Hz) for delta, theta, alpha, and beta bands between patients with rheumatoid arthritis and healthy controls in five brain regions. An asterisk indicates significant statistical difference ($p < 0.05$).

the interaction between "group" and "region" [$F_{(4, 156)} = 0.12$, $p = 0.997$, epsilon GG = 0.538] were observed on absolute beta power densities.

Difference between Groups on EEG Relative Power Density

Table 4 and **Figure 2** show the average relative power density values for the analyzed EEG frequency bands (delta, theta, alpha, and beta) across the five ROIs.

Delta (1.5–3.5 Hz)

The ANOVA yielded only a significant effect due to "region" [$F_{(4, 156)} = 22.89$, $p < 0.0000001$, epsilon GG = 0.582]. No significant effects of "group" [$F_{(1, 39)} = 3.16$, $p = 0.083$] or the interaction between "group" and "region" [$F_{(4, 156)} = 0.021$, $p = 987$, epsilon GG = 0.582] were observed on relative delta power densities.

Theta (4–7 Hz)

The ANOVA yielded only a significant effect due to "region" [$F_{(4, 156)} = 12.63$, $p = 0.000041$, epsilon GG = 0.554]. No significant effects of "group" [$F_{(1, 39)} = 0.56$, $p = 0.457$] or the interaction between "group" and "region" [$F_{(4, 156)} = 0.053$, $p = 960$, epsilon GG = 0.554] were observed on relative theta power densities.

Alpha (8–12 Hz)

The ANOVA yielded a significant effect of "group" [$F_{(1, 39)} = 5.82$, $p = 0.021$], indicating that patients displayed higher relative alpha power density than controls. We also found a significant "region" effect [$F_{(4, 156)} = 23.09$, $p < 0.0000001$, epsilon GG = 0.613] showing that highest relative power densities were found at parietal and occipital electrodes, whereas the lowest ones appeared at central and temporal electrodes. Although there were non-significant differences due to the interaction between group and region [$F_{(4, 156)} = 0.83$, $p = 0.46$, epsilon GG = 0.613], mean comparisons in the *post-hoc* analysis (using Bonferroni correction to adjust for multiple comparisons) revealed that RA patients displayed higher relative alpha power density than HC at four ROIs: central (mean difference = 0.077, $p = 0.020$), temporal (mean difference = 0.064, $p = 0.039$), parietal (mean difference = 0.087, $p = 0.008$), and occipital (mean difference = 0.075, $p = 0.034$).

Beta (13–30 Hz)

The ANOVA yielded no significant effect due to "region" [$F_{(4, 156)} = 1.22$, $p = 0.304$, epsilon GG = 0.580], "group" [$F_{(1, 39)} = 0.44$, $p = 0.511$] or the interaction between "group" and "region" [$F_{(4, 156)} = 0.26$, $p = 0.803$, epsilon GG = 0.580] were observed on relative beta power densities.

TABLE 4 | Average relative power density values by regions of interest, controlling for symptoms of anxiety/depression.

Frequency bands (Regions of Interest)	Controls ($n = 21$)	RA Patients ($n = 21$)	$F_{(1.39)}$	*P*-value
Delta (1.5–3.5 Hz)			3.159	0.083
Frontal	1.44 (0.12)	1.35 (0.11)		
Central	1.36 (0.09)	1.27 (0.11)		
Temporal	1.37 (0.11)	1.29 (0.15)		
Parietal	1.33 (0.12)	1.24 (0.12)		
Occipital	1.30 (0.11)	1.23 (0.14)		
Theta (4–7 Hz)			0.565	0.457
Frontal	1.28 (0.07)	1.26 (0.07)		
Central	1.27 (0.07)	1.24 (0.07)		
Temporal	1.24 (0.07)	1.23 (0.08)		
Parietal	1.24 (0.08)	1.23 (0.08)		
Occipital	1.23 (0.07)	1.23 (0.09)		
Alpha (8–12 Hz)			5.823	0.021*
Frontal	1.15 (0.10)	1.21 (0.08)		
Central	1.19 (0.10)	1.27 (0.08)		
Temporal	1.19 (0.08)	1.26 (0.08)		
Parietal	1.21 (0.09)	1.30 (0.08)		
Occipital	1.23 (0.09)	1.30 (0.10)		
Beta (13–30 Hz)			0.440	0.511
Frontal	0.83 (0.04)	0.83 (0.03)		
Central	0.83 (0.04)	0.83 (0.03)		
Temporal	0.83 (0.03)	0.83 (0.04)		
Parietal	0.83 (0.03)	0.82 (0.03)		
Occipital	0.84 (0.03)	0.82 (0.05)		

Significant at level 0.05. ANOVA of repeated measures.

Relationship between Pain Characteristics and Absolute and Relative EEG Activity

Correcting for multiple comparisons using Bonferroni method, none of the Pearson correlations between pain characteristics and power density in the delta, theta, alpha, and beta EEG frequency bands were significant.

DISCUSSION

This study showed that participants with RA and chronic pain presented higher theta and alpha absolute power densities at rest in comparison to healthy individuals, whereas no group differences were found for absolute power density of the beta and delta EEG band. When looking at relative power densities, we only found group differences in the alpha band.

Our most consistent finding was in the alpha frequency, which was increased among participants with RA for both absolute and relative power densities. We progressed from absolute to relative power analysis because there was an increase in the total spectrum power density for the RA group. If we solely assessed absolute power density, as have the majority of large studies in this area (Pinheiro et al., 2016), we would not be able to state that there were specific differences between groups, since these differences could be related to the general increase in the total spectrum power density.

The increased alpha band power density in RA participants seems to be associated with specific pathological characteristics of the disease. Earlier studies have shown similar results in conditions of mental fatigue (Tran et al., 2014) and emotional stress (Vanneste et al., 2014), which are characteristic symptoms of patients with RA. In this sense, increased alpha power density has already been shown in individuals with tinnitus (Vanneste et al., 2014). Moreover, Sarnthein and Jeanmonod found increased spectral power density in the lower alpha range (7–9 Hz) in all cerebral regions in patients with neurogenic pain (Sarnthein and Jeanmonod, 2008). Similar results have also been evident in individuals with neuropathic pain due to spinal cord injury (Jensen et al., 2013), chronic pancreatitis (Drewes et al., 2008), and breast cancer (van den Broeke et al., 2013).

It is possible that the constant awareness in the expectation of pain may play a role in the increase of alpha power at rest (Babiloni et al., 2008, 2010). Previous studies have already shown that pain expectation activates the pain network, including "emotional" areas (Sawamoto et al., 2000; Koyama et al., 2005), and modulates alpha activity (Franciotti et al., 2009). However, the majority of studies that investigated the association between alpha related synchronization/desynchronization and pain used experimental paradigms (Peng et al., 2015). A recent review (Pinheiro et al., 2016) showed that alpha power may be increased in the resting state EEG, but the mechanisms for such increase still need to be investigated in depth. We did find group differences on depression/anxiety, leading us to control for these variables in the ANOVAs with the EEG data. Thus, we feel that the increase on alpha frequency in RA participants as compared to healthy controls was not influenced by participant's high levels of anxiety/depression.

Our results also revealed an increase in absolute theta power density in the participants with RA. This finding cannot be considered specific because of the increase in total spectral power seen in this group. However, the findings are in agreement with previous studies, showing increased theta power density in patients with migraine, fibromyalgia, neuropathic pain, and chronic pain secondary to low back pain (Sarnthein et al., 2006; Stern et al., 2006; Bjørk et al., 2009; Jensen et al., 2013; Vuckovic et al., 2014). Thalamic dysregulations such as thalamocortical dysrhythmia (TCD) have been described in individuals with neuropathic pain and could possibly explain our findings of enhanced absolute theta power density in RA patients (Sarnthein et al., 2006; Walton and Llinas, 2010; de Vries et al., 2013). Previous studies have shown that increased theta power density in patients with chronic neuropathic pain may be related to thalamic disinhibition due to decreased top-down or bottom-up modulation (Llinás et al., 1999, 2005; Sarnthein et al., 2006). In this sense, Stern et al. described increased theta power density in multiple areas of the pain matrix, including parietal cortices, somatosensory cortices, and mid- and dorsolateral

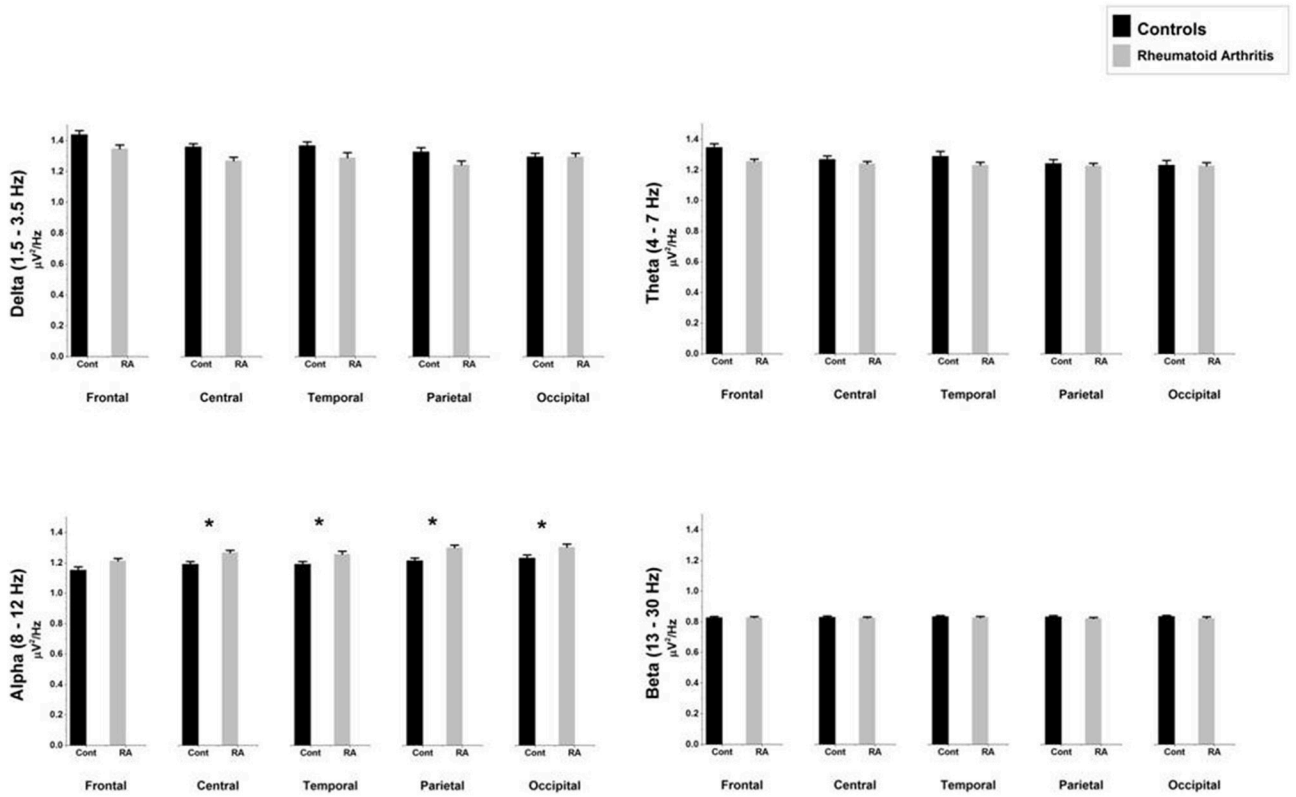

FIGURE 2 | Comparison of relative mean power densities (μ V^2/Hz) for delta, theta, alpha, and beta bands between patients with rheumatoid arthritis and healthy controls in five brain regions. An asterisk indicates significant statistical difference ($p < 0.05$).

prefrontal cortices of patients with neuropathic pain (Stern et al., 2006). These same authors argued that thalamic deactivation could be considered as the neurophysiological basis of chronic neurogenic pain. Furthermore, Sarnthein et al. hypothesized that neurogenic pain could be originated by deafferentation of excitatory inputs in the thalamus, leading to cell membrane hyperpolarization (Sarnthein et al., 2006). In this hyperpolarized state, thalamic interneurons appears to fire at a frequency range similar to the theta activity. Recent studies have shown that many RA patients reported neuropathic pain from different origins (Mendes et al., 2014; Walsh and McWilliams, 2014; Koop et al., 2015), including pain as a consequence of the use of TNF-alpha inhibitors (Birnbaum and Bingham, 2014), neurogenic inflammation (Seidel et al., 2010), and central sensitization (Meeus et al., 2012).

We also observed that RA participants and HC did not differ in delta and beta power densities at any of the ROI. A lack of statistical significance for these group differences may be attributed to a type II error in our study. At one hand, some of the previous studies observed differences on both delta and beta bands between patients with chronic pain and controls. For instance, Sarnthein et al. showed an increase in the total EEG spectrum in patients with neurogenic pain, including the delta and beta ranges (Sarnthein et al., 2006). They attributed these changes to TCD, as described above in the discussion of theta band changes. On the other hand, previous studies in patients with neuropathic chronic pain have failed to find differences between patients and HC in both delta (Bjørk et al., 2009) and beta (Vuckovic et al., 2014) EEG bands.

In this study we did not find any correlations between absolute and relative power densities and McGill scores, after controlling for depression. Correlations between pain characteristics (intensity and/or duration) and EEG power density are controversial. Schmidt et al. found a positive correlation between alpha power density and pain intensity, not at the moment of EEG evaluation but only on the one referenced in the previous 12 months (Schmidt et al., 2012). de Vries et al. only found a positive correlation between alpha peak frequency (but not power density) and pain duration, but not pain intensity (de Vries et al., 2013). On the other hand, other studies failed to find significant correlations between EEG power density and pain intensity (Jensen et al., 2007; van den Broeke et al., 2013).

Although it remains a matter of controversy, the presence of neuropathic symptoms in RA seems to be related to the presence of central sensitization, rather than a lesion of the somatosensory system itself. Since central sensitization involves the spinal cord and brain, neuropathic symptoms may be referred, even if pain is from nociceptive origin. Neuropathic pain symptoms have been identified in RA with another instrument, the PAINDetect (Koop et al., 2015; Christensen et al., 2016), which has different psychometric properties than the DN4. The DN4 has good properties to identify pain due to lesions of the somatosensory

system, but most likely would not identify central sensitization adequately, as seen in Ehlers-Danlos syndrome patients (Di Stefano et al., 2016). Thus, central sensitization may have been underdiagnosed in our sample, which prevented us to identify associations between EEG variables and neuropathic pain. Future studies should use other measures of central sensitization to better classify patients and reveal if this condition has a typical EEG pattern.

As this was an initial exploratory study, the sample size did not allow us to identify whether the main findings were related to the nature of the pain or even to the use of medication to treat RA symptoms, since only two participants in the RA group were not taking medications. A third group of individuals with RA and a low level of disease activity would be required in future studies to establish a clearer relation between the observed findings and the disease itself, independent of the presence of pain.

CONCLUSION

Our data suggest that subjects with RA present electroencephalographic characteristics similar to patients with chronic pain due to other etiologies. Increased absolute and relative alpha power densities at rest could be used as a general marker for the presence of chronic pain in patients with RA. This increase in alpha power density may also help to understand brain dysfunction associated with chronic pain in this population, as well as using it to develop new interventions to treat this condition.

AUTHOR CONTRIBUTIONS

FM substantially contributed with designing the study, data acquisition, analysis and interpretation. He drafted the manuscript and helped revising it critically for important intellectual content. FQ substantially contributed with data analysis and interpretation. She drafted the manuscript and helped revising it critically for important intellectual content. PM substantially contributed with data analysis and interpretation. He helped drafting the manuscript and revising it critically for important intellectual content. JM substantially contributed with the design of the study and data analysis. He helped revising the manuscript critically for important intellectual content. SD, KS, and CL substantially contributed with the conception of the study. They helped revising the manuscript critically for important intellectual content. AB substantially contributed with conceiving and designing the study, and also with data interpretation. He drafted the manuscript and helped revising it critically for important intellectual content. All authors approved the final version to be published and agree to be fully accountable for all aspects of the work.

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Neurophysiologic Correlates of Post-stroke Mood and Emotional Control

Deniz Doruk[1†], Marcel Simis[1,2†], Marta Imamura[2], André R. Brunoni[3], Leon Morales-Quezada[1], Renato Anghinah[2], Felipe Fregni[1,2*] and Linamara R. Battistella[2]*

[1] Spaulding Neuromodulation Center, Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA, USA, [2] Physical and Rehabilitation Medicine Institute of the University of São Paulo, Medical School General Hospital, São Paulo, Brazil, [3] Service of Interdisciplinary Neuromodulation, Laboratory of Neurosciences (LIM-27), Department and Institute of Psychiatry, University of São Paulo, São Paulo, Brazil

Objective: Emotional disturbance is a common complication of stroke significantly affecting functional recovery and quality of life. Identifying relevant neurophysiologic markers associated with post-stroke emotional disturbance may lead to a better understanding of this disabling condition, guiding the diagnosis, development of new interventions and the assessments of treatment response.

Methods: Thirty-five subjects with chronic stroke were enrolled in this study. The emotion sub-domain of Stroke Impact Scale (SIS-Emotion) was used to assess post-stroke mood and emotional control. The relation between SIS-Emotion and neurophysiologic measures was assessed by using covariance mapping and univariate linear regression. Multivariate analyses were conducted to identify and adjust for potential confounders. Neurophysiologic measures included power asymmetry and coherence assessed by electroencephalography (EEG); and motor threshold, intracortical inhibition (ICI) and intracortical facilitation (ICF) measured by transcranial magnetic stimulation (TMS).

Results: Lower scores on SIS-Emotion was associated with (1) frontal EEG power asymmetry in alpha and beta bands, (2) central EEG power asymmetry in alpha and theta bands, and (3) lower inter-hemispheric coherence over frontal and central areas in alpha band. SIS-Emotion also correlated with higher ICF and MT in the unlesioned hemisphere as measured by TMS.

Conclusions: To our knowledge, this is the first study using EEG and TMS to index neurophysiologic changes associated with post-stroke mood and emotional control. Our results suggest that inter-hemispheric imbalance measured by EEG power and coherence, as well as an increased ICF in the unlesioned hemisphere measured by TMS might be relevant markers associated with post-stroke mood and emotional control which can guide future studies investigating new diagnostic and treatment modalities in stroke rehabilitation.

Keywords: chronic stroke, qEEG, emotional disturbance, power asymmetry, inter-hemispheric connectivity

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***Correspondence:**
Marcel Simis
marcelsimis@gmail.com
Felipe Fregni
fregni.felipe@mgh.harvard.edu

† These authors have contributed equally to this work.

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INTRODUCTION

Emotional disturbance is a common complication of stroke (Annoni et al., 2006). About 30% of stroke survivors develop anxiety and depressive symptoms critically affecting functional recovery (Parikh et al., 1990; Hackett and Anderson, 2005) and quality of life (Robinson, 1997; Jonsson et al., 2005). Moreover, a significant number of patients remain undetected and therefore untreated due to difficulties in diagnosis (Dafer et al., 2008; El Husseini et al., 2012; Ayerbe et al., 2013). Investigation of neurophysiological markers associated with post-stroke mood and emotional control could have important implications in the development of new interventions as well as the assessment of current diagnostic and therapeutic modalities in stroke rehabilitation. For example, neurophysiologically guided interventions, such as EEG biofeedback entrainment, has already been shown to be effective in stroke patients with physical and cognitive impairments (Nelson, 2007). Similarly in depression, qEEG has been used to detect inter-hemispheric imbalance in cortical activity that has lead to the application of new therapeutic approaches such as TMS (transcranial magnetic stimulation) and tDCS (transcranial direct current stimulation; Rosenfeld et al., 1996; Linden, 2014).

The exact causes of post-stroke emotional disturbance (PS-ED) are still unknown. Different mechanisms including direct effects of ischemia to mood regulating neural networks (Starkstein et al., 1988; Beblo et al., 1999) and a psychosocial (Gainotti et al., 1999) model have been proposed to explain PS-ED (Whyte and Mulsant, 2002). Additionally, several factors involving the severity of injury, cognitive impairment, pre-morbid depression, disability and localization of the stroke have been identified as predictors of PS-ED (Robinson, 1986; Hackett and Anderson, 2005; Ayerbe et al., 2013). However, some of these factors were inconsistent across studies. For example, earlier studies showed that left sided lesions that are close to the frontal lobe have been associated with depression (Robinson, 1986) whereas more recent studies showed no relation between the localization of stroke and depression after stroke (Carson et al., 2000).

Quantitative electroencephalography (qEEG) is a safe, cost-effective technique used to assess cortical activity and has been valuable in assessing emotion related networks. Among the qEEG parameters, frontal alpha power asymmetry has been especially of interest given its relation to emotional processes and pathological conditions such as major depressive disorder (MDD) and anxiety (Coan and Allen, 2004; Thibodeau et al., 2006; Harmon-Jones et al., 2010). Yet, it is unknown whether emotional disturbance secondary to other neurological conditions, such as stroke, is associated with similar EEG changes. In fact, qEEG has already been used in stroke as a predictive measurement for prognosis and clinical management in motor recovery (Finnigan and van Putten, 2013). However, use of qEEG in non-motor outcomes of stroke is limited (Schleiger et al., 2014) and to our knowledge there is no study assessing the qEEG correlates of post-stroke depression and anxiety.

Transcranial magnetic stimulation (TMS) is another technique that is useful in assessing cortical activity in both MDD and stroke. TMS studies assessing changes in cortical activity in patients with MDD have shown decreased excitability in the left hemisphere (Maeda et al., 2000; Fitzgerald et al., 2004), and decreased motor threshold in the right hemisphere (Bajbouj et al., 2006). In stroke, TMS studies demonstrated that inter-hemispheric asymmetry in cortical activity (Murase et al., 2004) is associated with functional recovery after stroke (Hendricks et al., 2002). Therefore, together with EEG, TMS could potentially help elucidate changes in cortical activity related to PS-ED.

In this cross-sectional preliminary analysis of 35 stroke subjects we investigated the associations between the emotion sub-domain of Stroke Impact Scale (SIS-Emotion) and several neurophysiologic measures obtained by EEG and TMS when adjusted for potential confounders such as age and time since stroke. Given that hemispheric asymmetry plays an important role in both stroke and mood disorders, we hypothesized that post-stroke changes in mood and emotional control is associated with inter-hemispheric imbalance that can be indexed by EEG and TMS.

METHODS

Participants

This study analyzes the secondary data from 35 participants with chronic stroke who were initially enrolled in a larger clinical trial that compares different rehabilitation techniques. All subjects were over the age of 18 years, clinically stable and had clinical and neuro-imaging based diagnosis of stroke within 6–36 months prior to enrollment. The exclusion criteria were: (1) Mini-Mental Examination score lower than 24 or, for aphasic patients, inability to understand the rehabilitation tasks, (2) more than 1 stroke event, (3) psychoaffective disorders that prevented adherence to treatment and (4) joint damage and pain or deformities that makes the implementation of the therapy infeasible. Since the subjects were enrolled to participate in a trial assessing the effectiveness of rehabilitation techniques, some of these criteria were related to application of rehabilitation techniques. The study was approved by the Local Ethics Committee and a written consent was obtained from each subject.

Stroke Impact Scale (SIS)

The Brazilian version of SIS 3.0 was used to measure quality of life and impact of stroke. For the purpose of this study we only analyzed the "emotion" sub-domain of SIS as a measure of mood and emotional control. SIS-Emotion consists of nine-items and has shown good criterion validity when compared to SF-36 Mental Health and Geriatric Depression Scale for SIS 2.0 (Duncan et al., 1999). Emotion sub-domain of SIS 3.0 has also shown good correlation with the Hospital Anxiety and Depression Scale (HADS; Carod-Artal et al., 2008; Vellone et al., 2014).

Assessment of Motor Functions

We used the Fugl-Meyer (FM) assessment to test the association between motor impairment and SIS-Emotion. We also used FM

to show that EEG and TMS findings of this study are specific to mood and emotional control.

Transcranial Magnetic Stimulation (TMS)

TMS assessments included both single pulse and paired-pulse TMS protocols. We measured motor threshold (MT), intracortical inhibition (ICI), and intracortical facilitation (ICF) for both lesioned and unlesioned hemispheres. Motor threshold was defined as the minimum stimulus intensity necessary to elicit a motor-evoked potential (MEP) with the amplitude of at least a 50 μV in %50 of the trials. MEPs were recorded from first dorsal interosseous muscle (FDI). In the absence of MEPs, a maximum value of 100% was accepted as the MT. Paired-pulse protocol was used to measure ICI (ICI at 2 ms inter-stimulus interval) and facilitation (ICF at 10 ms inter-stimulus interval). In this protocol the conditioning pulse was set to 80% of MT while the test pulse was determined as the intensity eliciting an MEP of at least 1 mV. ICI and ICF were calculated as the ratio between the amplitude of MEP elicited during single pulse and during inhibition or facilitation.

Electroencephalography (EEG)

EEG was recorded by using a 128-channel EEG cap with active electrodes (Acti-Champs, PyCorder, Brainvision LLC®) and linked-ear reference for 20 min. During recordings, subjects were asked to close their eyes in a resting position and instructed not to fall asleep. EEG sessions were monitored online for the effects of drowsiness and potential movements. The EEG data was analyzed offline with EEGLab and MATLAB (MATLAB R2012a, The MathWorks Inc. Natick, MA, 2000). To ensure EEG data was not contaminated by the effects of drowsiness only the first 6 min of EEG recordings were included in the analyses. The data was filtered automatically (high-pass at 1 Hz and low-pass at 35 Hz) and then cleaned from artifacts manually by an evaluator blinded to assessments.

Power Asymmetry

Power was calculated using fast Fourier transform (FFT; average of 5-s epochs with a 50% overlap) and averaged for the following EEG bands: theta (4–8 Hz), alpha (8–13 Hz) [including the sub-bands low-alpha (8–10 Hz) and high-alpha (1–13 Hz)], and beta [low-beta (13–20 Hz) and high-beta (21–30 Hz)]. Power asymmetry was determined by calculating the difference in power between the left hemisphere (LH) and right hemisphere (RH; e.g., $L_{alpha} - R_{alpha}$) for 40 pairs of electrodes representing different cortical localizations: frontal (20 pairs), central (11 pairs), and parietal (9 pairs) (**Figure 1**). A positive asymmetry index represents greater left than right hemisphere EEG power, whereas a negative asymmetry index would suggest higher power in the right hemisphere.

Coherence

Coherence was calculated by using a MATLAB function -mscohere- that uses Welch's averaged modified periodogram to calculate magnitude squared coherence estimate. A coherence value between 0 and 1 was calculated for each frequency point and for each electrode pair (40 pairs for inter-hemispheric coherence and 24 pairs for intra-hemispheric coherence; **Figure 1**). We then averaged these values over certain frequency bandwidths including theta (4–8 Hz), alpha (8–13 Hz) [including the sub-bands low-alpha (8–10 Hz), high-alpha (10–13 Hz)], and beta [low-beta (13–20 Hz) and high-beta (20–30 Hz)].

Statistical Analysis

Statistical analyses were completed using STATA 12.1® (StataCorp LP, Texas) and MATLAB.

Covariance Mapping—EEG Data

In order to explore the correlation between SIS-Emotion and EEG data, we used a method introduced by Koening et al. which combines covariance analysis and resampling methods ("TANCOVA") to overcome the issue of multiple testing across EEG electrodes (Koenig et al., 2008). To implement this technique into our analyses, we treated the data from each pair of electrode (left vs. right hemisphere power difference and coherence between each pair) as a single EEG "channel". Initially all selected electrode pairs (40 pairs for inter-hemispheric power asymmetry and inter-hemispheric coherence, as well as 24 pairs for intra-hemispheric coherence) were included in the analysis. Then, we run the same analysis separately within each cortical region: frontal (20 pairs), central (11 pairs), parietal (9 pairs), left hemisphere (12 pairs), and right hemisphere (12 pairs). The main aim of this analysis was to preliminarily explore the association between EEG data and SIS-Emotion by including as many electrodes as possible while eliminating the problems of multiple testing. Following this analysis, we averaged the data over each cortical region (frontal, central, and parietal) in order to simplify the analyses in next steps.

Univariate Analyses

Multiple univariate linear regression analyses were conducted to assess associations between the emotion sub-domain of SIS, SIS-Emotion (as the dependent variable), and each neurophysiologic, demographic, and clinical variable (as an independent variable). Neurophysiologic variables included EEG (i.e., frontal alpha power asymmetry, central alpha coherence) and TMS data (i.e., MT in the lesioned hemisphere). Demographic and clinical variables were age, gender, time since stroke, medication use, lesioned side, and Fugl-Meyer.

Confounders and Multivariate Models

In order to assess and adjust for possible confounders for EEG and TMS models (including the ones that were not significant in the univariate analysis), we added each demographic and clinical variable as an independent variable in multivariate regression models where SIS-Emotion was the dependent variable and each EEG and TMS variable is the main predictor. Each possible confounding variable was tested one at a time and when led to a more than 10% change in the β-coefficient of the main predictor, the variable was kept in the model. Finally, we performed multivariate regression analyses by forcing all significant confounders into final EEG and TMS models. $P < 0.05$ was accepted as significant.

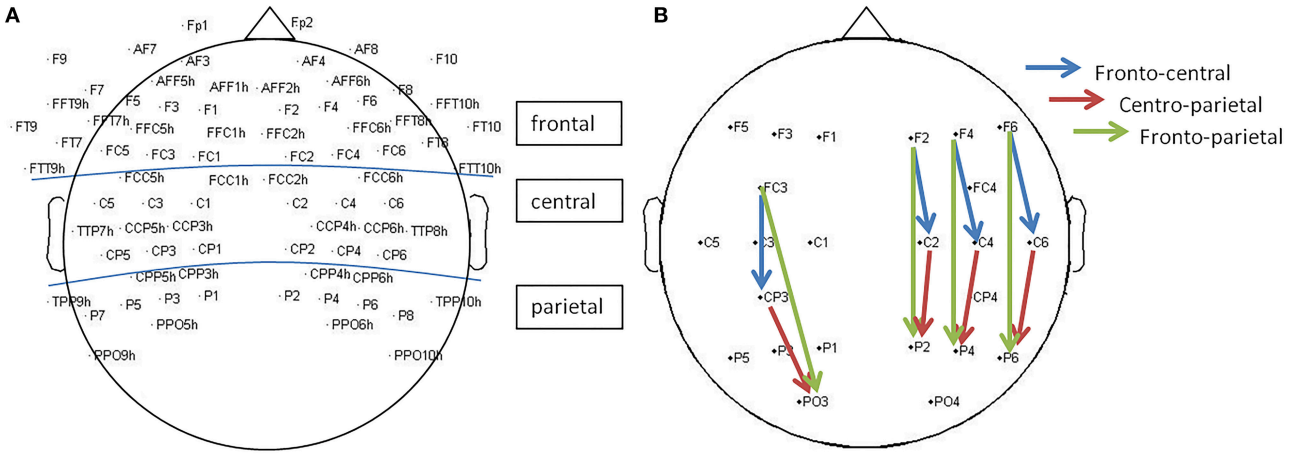

FIGURE 1 | Selected electrodes for EEG analysis. (A) Analysis of inter-hemispheric power asymmetry and coherence: 40 pairs of electrode representing frontal (20 pairs), central (11 pairs), and parietal (9 pairs) areas were included. **(B)** Analysis of intra-hemispheric coherence. For each hemispheric side, 12 electrodes were chosen representing fronto-central (4 pairs), centro-parietal (4 pairs), and fronto-parietal (4 pairs) connections.

Analysis Testing whether Significant Neurophysiological Findings are Associated with Motor Outcomes

In order to assess the specificity of our findings we also tested whether the significant EEG and TMS variables (independent variables) are also predictors of motor (Fugl-Meyer) function (dependent variable). The aim was o show that these variables are not associated with motor disability.

Sensitivity Analysis

In order to control and check whether individual values were driving our final results, we excluded outliers and repeated the analysis for the univariate and multivariate models. For each variable values that were above or below three inter-quartile range (Q1− 3IQR or Q3+ 3IQR) were defined as outliers and excluded.

Effect of Lesion Side on Neurophysiologic and Clinical Parameters

In addition to the above analysis we compared patients with left and right hemisphere damage with regards to neurophysiological and clinical parameters using linear regression in order to further explore the effect of lesion side on these parameters.

TABLE 1 | Demographics and baseline characteristics.

Gender (%)	
Female	42.86
Male	57.14
Age (mean ± SD)	62 ± 13
Lesion (%)	
Cortical	65.71
Subcortical	28.57
Brain stem	5.71
Hemispheric Side (%)	
Right	54.29
Left	45.71
Medications (%)	
Antidepressant	25.71
Neuroleptic	2.86
Anticonvulsant	14.29
Benzodiazepine	5.71
Time since stroke (Months, mean ± SD)	15.3 ± 8.6

RESULTS

Demographics and baseline characteristics of subjects are described in **Table 1**. Motor threshold for the lesioned hemisphere could not be obtained from three participants.

We divided our results into three sections in order to facilitate easy reading: A. Initial analyses of covariance mapping, univariate models and confounders as prerequirements of multivariate analyses; B. Multivariate models; C. Sensitivity Analysis (Sensitivity analysis presents the results in A and B but without outliers); D. Summary of the final multivariate models as also discussed in discussion; E. Other exploratory analyses.

A. Initial Analyses of Covariance Mapping, Univariate Models, and Confounders As Prerequirements of Multivariate Analyses

With regards to results of covariance mapping (**Table 2**), SIS-Emotion significantly correlated with overall power asymmetry in low-beta band regardless of the region. Additional analysis of specific cortical regions revealed that power asymmetry (all

TABLE 2 | Results for covariance mapping.

	Theta *p*-values	Alpha *p*-values	Low-alpha *p*-values	High-alpha *p*-values	Low-beta *p*-values	High-beta *p*-values
POWER ASYMMETRY						
All regions**	0.056	0.186	0.249	0.150	**0.030**	**0.024**
Frontal*	**0.026**	**0.026**	**0.027**	**0.030**	**0.028**	**0.030**
Central*	0.879	0.568	0.380	0.874	0.906	0.827
Parietal*	0.093	0.749	0.781	0.509	0.306	0.556
INTER-HEMISPHERIC COHERENCE						
All regions**	0.359	0.121	0.109	0.137	0.119	0.174
Frontal*	0.174	**0.044**	**0.031**	0.073	0.068	0.133
Central*	0.530	0.199	0.164	0.263	0.340	0.319
Parietal*	0.244	0.110	0.281	0.055	0.054	0.103
INTRA-HEMISPHERIC COHERENCE						
All regions**	0.489	0.732	0.753	0.533	0.753	0.197
Left*	0.139	0.511	0.6104	0.369	**0.039**	**0.011**
Right*	0.814	0.698	0.600	0.861	0.926	0.941

*Showing the p-values for the correlation between SIS-Emotion and EEG data. **Analysis for all regions included 40 pairs of electrodes for power asymmetry and inter-hemispheric coherence, and 24 pairs of electrodes for intra-hemispheric coherence. *Electrodes were further grouped into different cortical regions in order to explore the association between SIS-Emotion and cortical activity in these specific brain regions: frontal (20 pairs), central (11 pairs), parietal (9 pairs), left (12 pairs), right (12 pairs). P < 0.05 are shown in bold.*

frequency bands) and inter-hemispheric coherence (alpha band) over frontal areas as well as intra-hemispheric coherence (beta band) within the left hemisphere are significantly associated with post-stroke mood and emotional control.

Similar results were also found for the univariate linear regression models which showed significant associations between the dependent variable SIS-Emotion, and (1) alpha power asymmetry, (2) alpha coherence, and (3) MT in the unlesioned hemisphere.

We also found that age is a significant predictor for SIS-Emotion (**Table 3**) and is a common confounder (change in β-coefficent more than 10%) for the association between the dependent variable SIS-Emotion, and multiple EEG and TMS variables. Therefore, it was forced into all final multivariate models. Other important confounders were included only for the models that they were confounder for. Models became significant or remained significant discussed below (B. Multivariate models).

TABLE 3 | Main effects of possible confounders.

	p-value	β-coeff
Medication use	0.871	−1.13
Time since stroke	0.398	−0.32
Motor function-FuglMeyer	0.463	0.30
Lesioned hemisphere	0.574	3.64
Gender	0.701	−2.5
Age	**0.016**	−0.56

Results of univariate analyses for the dependent variable SIS-Emotion, showing p-values and β-coefficients for possible confounders. P < 0.05 are shown in bold.

B. Multivariate Models

Multivariate Models-EEG Variables with Confounders

Power asymmetry

We found that SIS-Emotion was significantly associated with beta power asymmetry (low-beta: $p = 0.005$, $\beta = 180.52$, Adj-$R^2 = 0.30$) in frontal regions and alpha power asymmetry (low-alpha: $p = 0.040$, $\beta = 10.67$, Adj-$R^2 = 0.25$) in parietal regions when adjusted for age and time since stroke (only for parietal low-alpha).

EEG Coherence

In addition to power asymmetry, SIS-Emotion was significantly associated with lower functional connectivity measured by inter-hemispheric EEG coherence in *alpha band* over frontal (low-alpha: $p = 0.042$, $\beta = 54.63$, Adj-$R^2 = 0.22$), central (alpha: $p = 0.040$, $\beta = 88.14$, Adj-$R^2 = 0.22$, low-alpha: $p = 0.031$, $\beta = 66.43$, Adj-$R^2 = 0.22$) and parietal areas (alpha: $p = 0.037$, $\beta = 54.35$, Adj-$R^2 = 0.21$) as well as in *beta band* over parietal areas (low-beta: $p = 0.029$, $\beta = 57.51$, Adj-$R^2 = 0.24$). These results were adjusted for age, side of lesion (only for parietal alpha and central low-alpha coherence), time since stroke (only for parietal low-beta), and gender (only for parietal low-beta). No other significant association was found for power and coherence in other frequency bands and locations ($p > 0.05$).

Multivariate models-TMS variables with confounders

A significant association was found between MT in the unlesioned hemisphere and SIS-Emotion ($p = 0.003$, $\beta = -0.73$, Adj-$R^2 = 0.32$) when adjusted for age and gender. ICF in the unlesioned hemisphere (adjusted for age) was also significantly associated with SIS-Emotion ($p = 0.043$, $\beta = -5.15$, Adj-$R^2 = 0.22$). There was no association between SIS-Emotion and other TMS variables.

C. Sensitivity Analysis

P-values for the univariate models after exclusion of outliers are shown in **Table 4**.

TABLE 4A | Univariate analysis-EEG.

	Theta		Alpha		Low-alpha		High-alpha		Low-beta		High-beta	
	p-value	β-coeff	*p*-value	β-coeff	*p*-value	β-coeff	*p*-value	β-coeff	*p*-value	β-coeff	*p*-value	β-coeff
POWER ASYMMETRY												
Frontal*	0.433	43.9	**0.002**	170.6	0.176	50.09	0.327	88.8	0.068	242.1	0.067	290.2
Central*	**0.028**	101.5	0.470	12.5	0.226	−29	**0.002**	−144.3	0.285	−144.5	0.845	70.9
Parietal*	0.929	3.4	0.915	3.9	0.175	29.19	0.710	−15.91	0.327	−160.5	0.670	64.9
Occipital*	0.220	−47.3	0.473	−42.3	0.712	13.8	0.291	−163.8	0.580	−114.0	0.878	55.4
INTER−HEMISPHERIC COHERENCE												
Frontal	0.369	30.2	**0.024**	79.4	**0.020**	65.3	0.073	71.4	0.200	59.3	0.240	43.8
Central	0.324	37.9	**0.006**	114.0	**0.021**	71.5	**0.026**	92.9	0.237	56.2	0.332	29.3
Parietal	0.686	15.8	0.089	79.7	0.292	40.8	0.118	55.1	0.306	41.6	0.215	32.0
Occipital	0.634	7.6	0.598	9.6	0.776	5.2	0.473	12.1	0.638	7.8	0.990	−0.2
INTRA-HEMISPHERIC COHERENCE												
Left Hemisphere												
Fronto-Central	0.658	8.7	0.676	8.9	0.439	15.4	0.991	0.2	0.986	−0.4	0.876	3.1
Centro-Parietal	0.328	23.9	0.391	23.0	0.327	22.6	0.572	14.7	0.624	14.3	0.404	19.8
Fonto-Parietal	0.169	46.0	0.268	38.9	0.160	36.4	0.717	14.0	0.756	−14.0	0.966	−1.2
Right Hemisphere												
Fronto-Central	0.191	32.4	0.052	54.8	0.098	38.2	**0.038**	65.5	0.337	32.5	0.208	36.03
Centro-Parietal	0.782	7.0	0.610	11.6	0.734	7.2	0.545	12.7	0.725	10.2	0.462	17.6
Fonto-Parietal	0.526	25.2	0.227	41.8	0.394	21.5	0.187	48.6	0.852	−9.8	0.319	41.4

Results of univariate linear regression analyses for the dependent variable SIS-Emotion; showing p-values and β-coefficients for each EEG variable after outliers were excluded. P < 0.05 are shown in bold.

TABLE 4B | Univariate analysis-TMS.

	TMS	
	p-value	β-coeff
ICF		
Lesioned	0.760	−1.45
Unlesioned	0.104	−4.48
ICI		
Lesioned	0.510	−5.54
Unlesioned	0.882	1.22
MT		
Lesioned	0.964	−0.01
Unlesioned	**0.022**	−0.54

Results of univariate linear regression analyses for the dependent variable SIS-Emotion; showing p-values and β-coefficients for each TMS variable after outliers were excluded. P < 0.05 are shown in bold.

In the multivariate models without outliers, *alpha power asymmetry* in frontal ($p = 0.008$, $\beta = 145.02$, Adj-$R^2 = 0.29$) and central (only high-alpha, $p = 0.006$, $\beta = -133.2$, Adj-$R^2 = 0.27$) regions, as well as *theta asymmetry* in central areas ($p = 0.025$, $\beta = 95.86$, Adj-$R^2 = 0.25$) became significant for the dependent variable SIS-Emotion when adjusted for age. In beta band, the main effect of power asymmetry in the sub-band low-beta remained significant ($p = 0.045$, $\beta = 247.33$, Adj-$R^2 = 0.21$; **Figure 2**). On the other hand alpha asymmetry over parietal areas became not significant.

With regards to association between SIS-Emotion and EEG coherence, inter-hemispheric coherence over parietal areas (alpha and beta) became not significant when outliers were excluded. The models for frontal and central regions remained same as there were no outliers for these regions (**Figure 3**). Multivariate models with TMS variables also remained unchanged (**Figure 4**).

The results of the sensitivity analysis suggest that the association between SIS-Emotion and EEG variables is more robust for frontal and central areas as compared to parietal areas which seemed to be driven by outliers. Therefore, in our discussion we focused on the results without outliers.

D. Summary of the Final Multivariate Models

In summary, subjects with right greater than left hemisphere EEG power in beta and alpha band over frontal areas as well as in theta band over central areas were found to have more difficulty in post-stroke mood and emotional control (lower SIS-Emotion scores). Interestingly, this relation seems to be inverted for alpha power asymmetry over central areas. The relation between SIS-Emotion and EEG coherence was in line with the results for power asymmetry, and suggests that reduced functional connectivity in alpha band over frontal and central areas is also associated with difficulty in post-stroke mood and emotional control. In addition to EEG variables, the multivariate models with TMS variables showed that MT and ICF in the unlesioned hemisphere could be relevant markers for mood and emotional control after stroke.

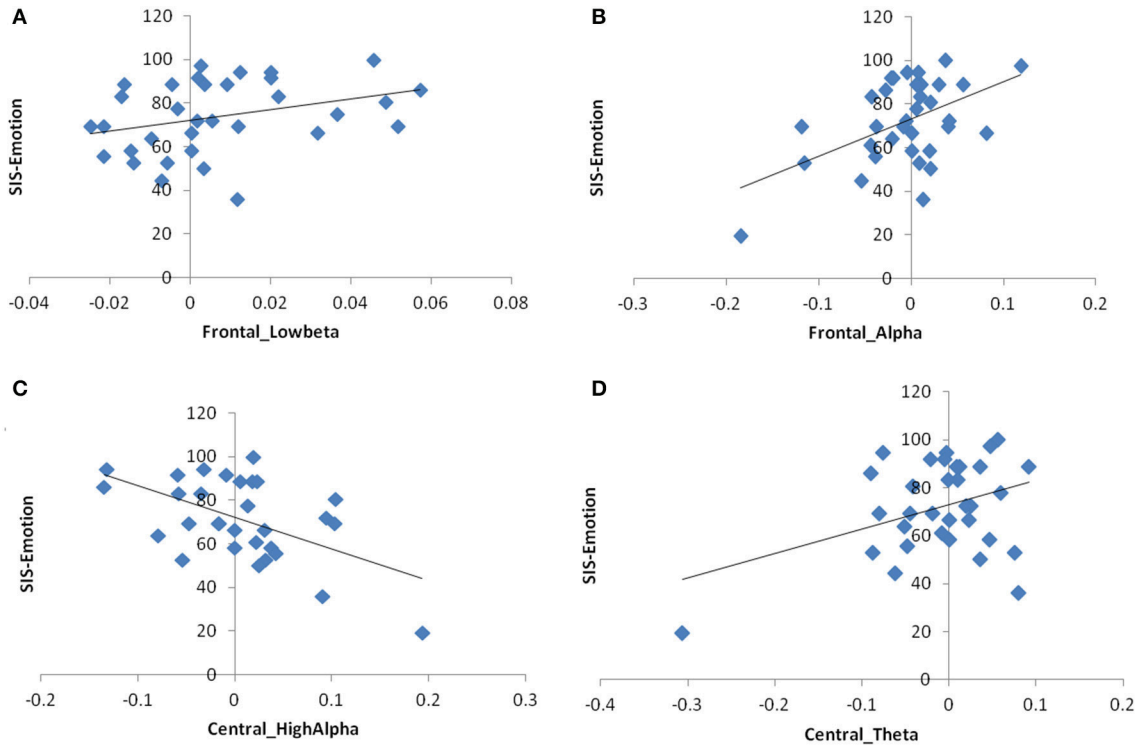

FIGURE 2 | Scatter plot graphs for the relationship between SIS-Emotion and EEG power asymmetry as measured by the difference between left and right hemisphere. (A) Frontal low-beta power asymmetry, **(B)** Frontal alpha power asymmetry, **(C)** Central high-alpha power asymmetry, **(D)** Central theta power asymmetry.

E. Other Exploratory Analyses

Analysis Testing Whether Significant Neurophysiological Findings are Associated with Motor Outcomes

We tested whether the significant EEG and TMS variables from this study are also correlated with motor function outcomes (Fugl-Meyer). In these models Fugl-Meyer was the dependent variable and each EEG and TMS variable was an independent variable. None of the EEG and TMS variables that were significant for SIS-Emotion were associated with Fugl-Meyer (also see the data from Simis et al., 2015).

Effect of Lesion Side on Neurophysiologic and Clinical Parameters

There was a main effect of the lesion side for the following dependent EEG variables: (1) frontal beta power asymmetry (high-beta: $p = 0.037$, $\beta = 0.03$, Adj-$R^2 = 0.09$), (2) parietal inter-hemispheric alpha coherence (low-alpha: $p = 0.037$, $\beta = -0.09$, Adj-$R^2 = 0.09$), and (3) left centro-parietal (low-alpha: $p = 0.019$, $\beta = -0.11$, Adj-$R^2 = 0.13$) and fronto-parietal (low-alpha: $p = 0.049$ $\beta = -0.08$, Adj-$R^2 = 0.09$) intra-hemispheric alpha coherence.

DISCUSSION

In this study we found significant associations between EEG/TMS measures and post-stroke mood and emotional control in patients with chronic stroke. These results indicate that patients who report having more depressive symptoms and anxiety also have (1) higher inter-hemispheric imbalance in cortical activity as measured by EEG power and coherence and (2) higher intracortical excitability and motor threshold in the unlesioned hemisphere as measured by TMS when adjusted for confounders. Furthermore, to our knowledge this is the first study that emphasizes the potential use of EEG and TMS to index neurophysiologic changes associated with post-stroke mood and emotional control.

With regards to the EEG findings in beta band, we found that post-stroke mood and emotional control is associated with greater frontal beta power asymmetry. The direction of the asymmetry index indicates that subjects with relatively greater right than left hemisphere beta power have more difficulties in mood and emotional control. This finding is consistent with previous studies showing higher EEG beta power in the right frontal regions in depressive disorders (Pizzagalli et al., 2002; Volf and Passynkova, 2002; Flor-Henry et al., 2004). Beta oscillations, are inhibition based rhythms that are thought to be produced by GABAergic potentials in inhibitory interneurons and pyramidal cells (Faulkner et al., 1999; Whittington et al., 2000) and are associated with motor control, arousal and attention (Merica et al., 1998; Uhlhaas et al., 2008). They are thought to reflect hyperactive neural circuits (Traub et al., 1999) and increased metabolic activity (Cook et al., 1998). Therefore, increased beta power in the right hemisphere may

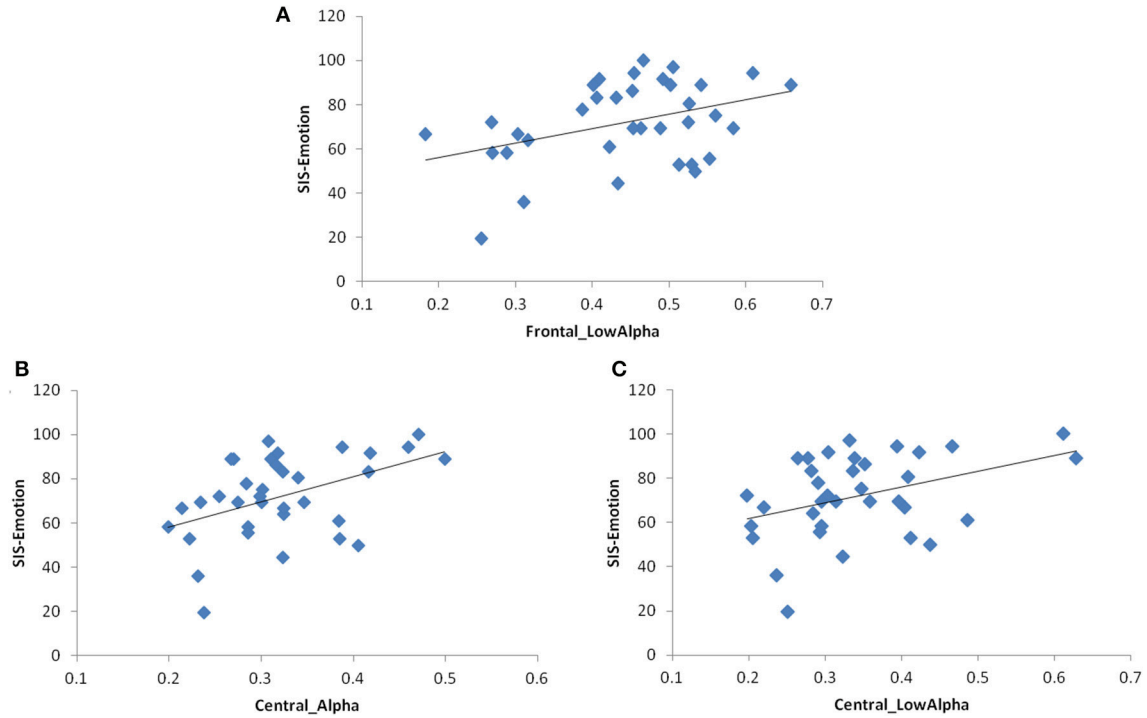

FIGURE 3 | Scatter plot graphs for the relationship between SIS-Emotion and inter-hemispheric EEG coherence. (A) Frontal low-alpha coherence, **(B)** Central alpha coherence, **(C)** Central low-alpha coherence.

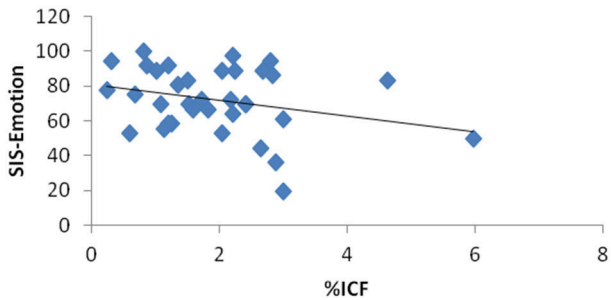

FIGURE 4 | Scatter plot graph for the relationship between SIS-Emotion and intracortical facilitation in the unlesioned hemisphere measured by TMS.

suggest hyperactivity in this region (Flor-Henry et al., 2004). In fact, right frontal hyperactivity has been associated with negative affect and withdrawal (Sutton and Davidson, 1997) as well as the presence of melancholia and anxiety in depressed individuals (Pizzagalli et al., 2002). Moreover, beta oscillations have been suggested as potential marker for plasticity (Rossiter et al., 2014). Therefore, beta oscillation may also reflect post-stroke plastic changes in the networks related to arousal and attention.

In addition to beta band, we also found that post-stroke mood and emotional control is associated with power asymmetry and reduced connectivity in alpha band over frontal and central areas.

Given the inverse relation between alpha power and cortical activity, the direction of the power asymmetry index over frontal regions suggest hypoactivity in the right frontal cortex or hyperactivity in the left frontal cortex, or both. At first this seems to contradict with not only one of the common EEG findings in depression and anxiety (right prefrontal hyperactivity), but also our findings regarding frontal beta asymmetry (which also suggest hyperactivity in the right frontal cortex). However, it is possible that greater activity in the left frontal cortex as measured by reduced alpha power represents different neural interactions and has other implications. For example several authors suggested that greater left prefrontal hyperactivity is involved in anxious apprehension while right sided hyperactivity is related to anxious arousal (Heller et al., 1997; Engels et al., 2007). Indeed it is not uncommon that patients with stroke present with both anxious arousal and apprehension (Mukherjee et al., 2006) which could explain the concurrent hyperactivity in different regions of frontal areas.

The relation between SIS-Emotion and central power asymmetry in high-alpha band was in the opposite direction of what was observed between SIS-Emotion and frontal alpha asymmetry, with patients who have greater left than right alpha power (hypoactivity in the left or hyperactivity in the right) experiencing more difficulty in mood and emotional control. In depression, posterior hyperactivation in right parieto-temporal cortex has been associated with the presence of co-morbid anxiety (Bruder et al., 1997). Even though we did not find any significant correlation over parietal regions in our final models,

hyperactivation in the right central areas may also indicate presence of anxiety in stroke patients. Different topological distribution of power asymmetry (central vs. parietal) may be due to methodological differences (for example we did not group the electrodes for parieto-temporal cortex separately) or due to the shift in brain activity as a result of cortical re-organization after stroke. Another possibility is that, together with theta asymmetry (which was in the opposite direction of alpha asymmetry), alpha asymmetry over central regions represents secondary cognitive impairment in stroke patients. Alpha and theta power are inversely related when they are used to index cognition and memory, and the presence of large power in high-alpha band in addition to small power in theta band is thought to indicate better cognitive performance (Klimesch, 1999). Even though it is not possible to conclude any association without the assessment of cognitive functions, knowing that cognitive impairment is common in stroke and can directly result from depression and anxiety (Mukherjee et al., 2006), one can argue that there might be overlapping EEG findings related to cognition and mood.

Consistent with our findings in power asymmetry we also found that post-stroke mood and emotional control was associated with reduced functional connectivity in alpha band over frontal and central areas. Studies investigating functional connectivity in MDD revealed mixed results (Veer et al., 2010; Zhou et al., 2010; Olbrich and Arns, 2013). Yet, our results are close to Knott et al. (2001) who found that depressive patients have reduced inter-hemispheric coherence in delta, theta, alpha, and beta bands of EEG in all anterior and posterior homologous pairs of channels (Knott et al., 2001). Pathological conditions affecting the integrity of the neural tissue will have structural and functional consequences in the area where the insult occurred. It can be assumed that reduction in inter-hemispheric connectivity reflect anatomical, adaptive, and maladaptive changes to neural connections between the lesioned and unlesioned hemisphere following stroke (Kukke et al., 2015).

One possible explanation for our EEG findings is the "depression network model" that identifies the depressed state as a dysfunction of a "network" rather than single brain region including adaptive and maladaptive compensatory processes. It is possible that in stroke secondary maladaptive changes in remote areas result in changes in the "depression network" which involves connections among neocortex, cingulate, limbic system, striatum, and thalamus (Mayberg, 1997, 2003). In fact, recent neuroimaging studies in stroke confirmed changes to the resting-state networks including the default-mode network (DMN; Wang et al., 2014; Thiel and Vahdat, 2015). Concurring with the "depression network" model, alterations in functional connectivity within DMN are also known to be related to depression (Mayberg, 1997; Greicius et al., 2007; Dutta et al., 2014). Since alpha oscillations (8–13 Hz) are the main regulator for DMN (Knyazev et al., 2011), it is likely that any disruption in the DMN or depression network, such as in stroke, could present as changes in EEG alpha oscillations and synchronization/desynchronization of the connectivity in alpha band.

The observed qEEG findings and its relationship with measurements of mood and emotions offers the possibility to be used as markers for treatment by EEG biofeedback entrainment, as it has been shown to be useful in stroke and memory impairment (Nelson, 2007), information processing (Lee et al., 2015), and motor function rehabilitation (Yilmaz et al., 2014).

We also found that higher ICF and higher motor threshold (MT) in the unlesioned hemisphere are associated with lower (worse) SIS-Emotion scores. TMS is advantageous over EEG considering that it can evaluate the cortical activity within each hemisphere, whereas EEG indices such as power asymmetry and coherence are difficult to interpret regarding which hemisphere mostly contributes to the asymmetry or reduced connectivity. Given that both ICF and EEG beta oscillations reflect interactions between glutamergic excitatory and GABAergic inhibitory neurons (Whittington et al., 2000; Paulus et al., 2008), increased ICF in the unlesioned hemisphere together with decreased inter-hemispheric coherence in beta band might represent the shift in the cortical activity toward unlesioned hemisphere. In stroke, persistent disinhibition in the unlesioned hemisphere has been associated with maladaptive plasticity and motor recovery (Manganotti et al., 2008) and it is possible that same maladaptive changes are related to post-stroke mood and emotional control. However, hyperactivity in the unlesioned hemisphere alone is not sufficient to explain the findings in EEG power asymmetry. One explanation is that both the plastic changes in motor areas and secondary disruption to "depression network" contribute to post-stroke mood and emotional control. In addition to the ICF, we found that MT in the unlesioned hemisphere is also associated with lower scores on SIS-Emotion. Even though this seems to contradict with the relationship between ICF and SIS-Emotion at first; when compared to ICF, MT does not provide information on intracortical connections. Also, while MT in the lesioned hemisphere strongly correlates with motor recovery, it seems to be inadequate to assess the functional changes in the unlesioned hemisphere (Stinear et al., 2015; Simis et al., 2016). Therefore, it is unlikely that the relationship between MT in the unlesioned hemisphere and SIS-Emotion is truly accounted for by maladaptive changes in the unlesioned hemisphere.

Even though there is conflicting evidence, one important factor implicated in post-stroke depression is the lesion side. Therefore, we further compared the patients with left and right sided lesions with regards to neurophysiologic and clinical parameters. We found that three variables were significantly associated with the lesion side: (1) frontal power asymmetry in high-beta band, (2) parietal power asymmetry in low-alpha band, and (3) intra-hemispheric coherence in alpha band within the left hemisphere. These results suggest that patients have relatively greater frontal high-beta power in the lesioned side. Also patients with left sided injuries have reduced connectivity within the left hemisphere as well as in between the two hemispheres over parietal areas as compared to patients with right sided injuries. Even though there was no direct relation between SIS-Emotion and these variables, it seems that the lesion side might have certain effects on brain activity regardless of the change in mood and emotional control.

Altogether, our results support our initial hypothesis suggesting the association between inter-hemispheric imbalance and post-stroke mood and emotional control. Even though

inter-hemispheric imbalance is a common finding in stroke and is associated with motor recovery (Murase et al., 2004; Simis et al., 2015), the lack of correlation between FM and variables that are associated with mood and emotion suggests that observed neurophysiologic changes are not related to motor impairment. Supporting this, we also did not find any association between FM and post-stroke mood and emotional control, and FM was not a confounder for any of the EEG and TMS variables. Indeed, in our recent study (Simis et al., 2015), we showed that EEG variables do not directly correlate with motor function but rather specify the association between motor threshold in the lesioned hemisphere and FM.

It is important to note the limitations of this study. First of all, even though multiple univariate linear regression models were tested, no correction was made for multiple comparisons. On the other hand the results of our initial analysis using covariance mapping and resampling methods were in line with the results of our multivariate models suggesting that it is unlikely that the effects were due to chance. Secondly, it is important to note that measuring coherence based on scalp EEG channels can be confounded with several unwanted effects of volume conduction. Therefore, the results regarding EEG coherence should be interpreted cautiously. Another limitation of this study is the lack of control group. Future studies are needed to compare the findings in healthy subjects and in patients with affective disorders or anxiety only.

CONCLUSION

To our knowledge this is the first study assessing neurophysiologic markers in post-stroke mood and emotional control as indexed by EEG and TMS. Our results suggest that difficulties in mood and emotional control after stroke is associated with greater inter-hemispheric imbalance in EEG power and coherence as well as increased excitability in the unlesioned hemisphere measured by TMS. These results are important for guiding future studies investigating the neurophysiologic mechanisms of post-stroke emotional disturbance, developing diagnostic algorithms, and assessing treatment response.

AUTHOR CONTRIBUTIONS

MS, FF, MI, LB designed the study. MS and RA collected the data. DD, MS, and FF analyzed the data and wrote the first draft. All authors (DD, MS, MI, AB, LQ, RA, FF, and LB) interpreted the results and contributed to the final manuscript.

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Intrinsic Brain Connectivity in Chronic Pain: A Resting-State fMRI Study in Patients with Rheumatoid Arthritis

Pär Flodin[1]*, *Sofia Martinsen*[1], *Reem Altawil*[2], *Eva Waldheim*[2], *Jon Lampa*[2], *Eva Kosek*[1] and *Peter Fransson*[1]

[1] Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, [2] Department of Medicine, Rheumatology Unit, CMM, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Background: Rheumatoid arthritis (RA) is commonly accompanied by pain that is discordant with the degree of peripheral pathology. Very little is known about the cerebral processes involved in pain processing in RA. Here we investigated resting-state brain connectivity associated with prolonged pain in RA.

Methods: 24 RA subjects and 19 matched controls were compared with regard to both behavioral measures of pain perception and resting-resting state fMRI data acquired subsequently to fMRI sessions involving pain stimuli. The resting-state fMRI brain connectivity was investigated using 159 seed regions located in cardinal pain processing brain regions. Additional principal component based multivariate pattern analysis of the whole brain connectivity pattern was carried out in a data driven analysis to localize group differences in functional connectivity.

Results: When RA patients were compared to controls, we observed significantly lower pain resilience for pressure on the affected finger joints (i.e., P50-joint) and an overall heightened level of perceived global pain in RA patients. Relative to controls, RA patients displayed increased brain connectivity predominately for the supplementary motor areas, mid-cingulate cortex, and the primary sensorimotor cortex. Additionally, we observed an increase in brain connectivity between the insula and prefrontal cortex as well as between anterior cingulate cortex and occipital areas for RA patients. None of the group differences in brain connectivity were significantly correlated with behavioral parameters.

Conclusion: Our study provides experimental evidence of increased connectivity between frontal midline regions that are implicated in affective pain processing and bilateral sensorimotor regions in RA patients.

Keywords: rheumatoid arthritis, pain, inflammation, joint, fMRI, resting-state, brain connectivity

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***Correspondence:**
Pär Flodin
parflodin@gmail.com

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease that primarily affects the joints. The prevalence of RA is estimated to be 0.5–1% of the population in the industrialized world, with an overrepresentation of women (McInnes and O'Dell, 2010). The inflammation may lead to dysfunction and destruction of joints, accompanied with joint pain.

Pain severely impacts the patients' perceived subjective health. However, there are often large discrepancies between objective RA inflammatory markers and the degree of subjective pain (Thompson and Carr, 1997). Similarly, although a multitude of efficient immunosuppressive and biologic therapies have proven efficient for a majority of the RA patients, many patients continue to experience significant pain despite improvements in peripheral joint inflammation (Taylor et al., 2010). It is thus reasonable to stipulate that the long-term pain in RA may be accompanied by altered cerebral pain processing, which is also indirectly supported by previous studies showing a generalized increase in pain sensitivity in RA patients compared to controls (Leffler et al., 2002; Fridén et al., 2013). Increased knowledge of the cerebral response to prolonged rheumatic pain could thus be valuable for the development of pharmacological and behavioral therapies aimed at reducing pain in RA. In line with our previous studies of altered resting-state connectivity (Flodin et al., 2014) and abnormal cerebral pain processing in fibromyalgia patients (Jensen et al., 2009), a limited number of studies have investigated pain processing in RA populations. For instance, Wartolowska et al. (2012) used structural MR imaging and reported that RA patient (vs. HC) displayed increased gray matter density in the basal ganglia which is involved in motor control and pain processing. Other studies have targeted brain activation patterns evoked by pain. Jones and Derbyshire (1997) reported reduced brain response to heat induced pain in prefrontal regions and the anterior cingulate cortex. Schweinhardt et al. (2008) on the other hand, found correlations between pain evoked brain activity in the medial prefrontal cortex (MPFC) and depressive symptoms in RA patients. Thus, there are corroborating results from different imaging modalities that RA is associated with an altered state of central pain processing, which likely could be ascribed to the prolonged pain experience. However, to our knowledge, the current study is among the first to investigate spontaneous fluctuation of brain activity in canonical pain brain regions among RA patients using resting state fMRI.

Our main hypothesis was that long-term pain that accompanies RA would influence intrinsic brain connectivity of pain relevant regions. Furthermore, we hypothesized that the intensity of RA related pain and pain sensitivity (e.g., ratings of global pain intensity and pressure sensitivity of affected joints) would correlate with the presumptive group differences in functional connectivity.

METHODS

Subjects

Rheumatoid arthritis patients were recruited through the rheumatology clinic at the Karolinska Hospital in Stockholm, Sweden. Patients fulfilling the inclusion criteria were asked to participate in a randomized, placebo-controlled trial investigating the effects of a tumor necrosis factor (TNF-alpha) blocker on pain and inflammation in RA with baseline comparison with healthy subjects (the PARADE study; www.clinicaltrials.gov; identifier NCT01197144, EudraCT 2009-017163-42).

In this report, only results from the baseline data are described. Inclusion criteria for the RA patients were working age (≥18 years), meeting the ACR 1987 classification criteria for RA (Arnett et al., 1988), clinical indication for use of TNF-blockers and MR examination compatibility. Exclusion criteria were left handedness, fibromyalgia, severe cardiovascular disease, vasculitis, neurological disease, ongoing treatments for anxiety or depression using antidepressants and other reasons based on the judgment of the responsible physician.

For the age- and sex matched healthy controls, exclusion criteria were identical to the RA patients with the additional exclusion criteria of recurrent pain problems, including RA and fibromyalgia.

In total, 27 RA patients were recruited for participation in the study. Two patients were discarded due to excessive head movement during resting state fMRI scanning. Movement outlier participants were identified using mean frame wise displacement (FD) >0.31 mm, corresponding to two standard deviations from the mean of all subjects. Data from one subject had to be rejected due to partial head coverage, rendering 24 RA subjects to be eligible for inclusion in the analysis. Mean age was 53.8 years (range 23–74 years), and 20 were females. Among the 24 RA patients, 17 used Methotrexate, 3 Sulphasalazine and 1 Leflunomide. No patient used higher cortisone dose than 10 mg. See **Supplemental Table S1** for individual medication usage and **Table 1** for further population characteristics.

Twenty-one healthy age- and sex matched control subjects (HC) were recruited through advertisements on noticeboard primarily at the hospital campus. fMRI data from two subjects were discarded due to excessive head movement, leaving 19 HC for further analysis (mean age 50.42 years, range 25–68 years, 16 females).

Screening of RA subjects was performed at the first visit to the hospital. During the first visit, all subject's sensitivity to evoked pressure (P50) was calibrated. Subjects returned the following day for the fMRI scanning.

The study conforms with Swedish legislation regarding clinical pharmacological trials and necessary permit from the Swedish medical products agency has been obtained. The regional ethics committee in Stockholm approved the study and informed consent was obtained from all participants.

TABLE 1 | Data cohort characteristics.

	RA ($n = 24$)	HC ($n = 19$)
Age (mean ± SD)	53.8 ± 14.8	50.4 ± 16.6
Gender (F/M)	20/4	16/3
FD (mean ± SD)	0.15 ± 0.068	0.11 ± 0.036
P50 thumb (mean ± SD)	584.2 ± 186.5	608.7 ± 181.5
P50 joint (mean ± SD)	505.7 ± (262.8)	758.4 ± 126.0
Global Vas (mean ± SD)	33.7 ± (29.3)	0.95 ± 3.44
DAS28 (mean ± SD)	5.20 ± (1.14)	–
RA duration (m) (mean ± SD)	66.0 ± (34.0)	–
Swollen joints (mean ± SD)	7.25 ± (5.06)	–
Tender joints (mean ± SD)	9.79 ± (6.35)	–

Clinical and Behavioral Measures

Assessment of Pressure Pain Sensitivity

To assess pain sensitivity, we applied an automatic, pneumatic computer-controlled stimulator with a plastic piston corresponding to an area of 1 cm^2 (Jensen et al., 2009) on the thumbnail and on the most affected finger joint (or the corresponding joint in healthy controls). Subjects rated the pain intensity of the pressure stimuli on a visual analog scale (VAS). For both locations we first used ascending stimuli to determine the pressure pain threshold and the first pressure rated as >60 mm on VAS. Each subject was then stimulated with five pressure intensities evenly distributed within this interval, 3 times for each intensity, in a randomized order. The stimuli were presented for 2.5 s with a 30 s inter-stimulus interval. A linear polynomial function was fitted to the 15 data points, and from this we derived a measure of 50 mm VAS, that we referred to as P50 (for further details, see Jensen et al., 2009).

Assessment of Global Pain

Prior to the fMRI scan subjects were asked to rate their overall pain intensity using a 100 mm VAS, spanning from "no pain" to "worst imaginable pain" (here referred to as VAS global pain).

RA Disease Activity

For estimating RA activity, we calculated the Disease Activity Score, DAS28, (Prevoo et al., 1995). DAS28 is a composite measure of the number of tender joints in 28 locations, the number of swollen joints, patients' perceived global health and an inflammatory marker of erythrocyte sedimentation rate (ESR). DAS28 was determined on the day before the fMRI scanning.

MRI Data Acquisition

MR imaging was performed on a 3T General Electric 750 MR scanner installed at the MR Research Center, Karolinska Institute, Stockholm. Anatomical MR imaging was acquired with a high-resolution BRAVO 3D T1-weighted image sequence (1 × 1 × 1 mm3 voxel size). For each subject we performed one resting state scan consisting of 200 volumes, using an echo-planar imaging with TR/TE = 2500/30 ms, flip angle = 90°, 49 slices, 96 × 96 matrix size, FOV = 288 × 288 mm, slice thickness = 3 mm and an interleaved mode of slice acquisition. Anatomical (T2-weighted) scans were investigated by radiologist for clinical abnormalities. In the resting state condition, subjects were instructed to lie still and rest, and not to think of anything in particular while keeping their eyes on a fixation cross. Prior to the resting state fMRI data acquisition, subjects underwent two fMRI sessions of a pain exposure paradigms (∼7 min each). Results from the task-evoked fMRI runs will be reported elsewhere.

Resting State fMRI Data Analysis

Group differences in resting state activity, as well as strength of functional connectivity correlating with clinical and behavioral pain measures within the RA group were investigated using a seed-based correlation analysis (SCA). Seed selection was based on 159 uniformly placed spherical ROIs (4 mm radius, 10 mm apart center-to-center) within brain regions that are known to be involved in pain processing. Brain regions related to pain was demarked in a meta-analysis of 314 pain studies indexed in neurosynth (neurosynth.org, retrieved in December 2013), identical to the set of ROIs described in Flodin et al. (2014). This seed selection procedure aimed to enhance sensitivity by restricting SCA to only pain relevant seeds rather than a set of seeds that cover the whole brain. Thereby, we decrease the magnitude of the multiple comparison problem, while at the same time allowing for an extensive set of seed to be used that lessen the influence of seed selection bias. The seed region coordinates and associated anatomical labels are listed in **Supplemental Table S2** and shown in **Supplemental Figure S1**.

Prior to SCA, imaging data were preprocessed using SPM8 (Welcome Trust Center of Neuroimaging, University College London, UK). Image preprocessing included slice-time correction, realignment to the mean image, co-registration of functional and structural images, tissue segmentation of structural images, and direct normalization of functional and structural scans to the MNI template provided by SPM8. Finally, functional volumes were spatially smoothed using an 8 mm FWHM Gaussian kernel. Subject level SCA analyses were carried out using the Conn toolbox (http://www.nitrc.org/projects/conn; Whitfield-Gabrieli and Nieto-Castanon, 2012). Functional volumes were band pass filtered at 0.008–0.09 Hz (default values) simultaneously with nuisance regression (as advocated by Hallquist et al., 2013, in order not to reintroduce nuisance -related variations into a band-pass filtered time-series). Subject specific nuisance regressors included 6 movement regressors and their time derivatives, and 5 regressors pertaining to white matter and CSF signals sources respectively, using a principal component (PCA) based noise correction (CompCorr) approach (Behzadi et al., 2007). Additionally, images that were regarded as movement outliers were regressed out. Image volume outliers were detected using the ART toolbox (nitrc.org/projects/artifact_detect/) and defined as image volumes with a frame wise displacement (FD) value larger than 0.5 mm or signal intensity changes greater than 3 standard deviations (default thresholds). Outlier volumes were modeled at the first level general linear model using dummy variables and regressed out together with the other subject specific nuisance regressors. The mean number of regressed volumes for RA subjects was 13.3 ± 12.5 *SD*, and 5.6 ± 5.4 *SD* among HC. Across the cohort, the number of regressed volumes ranged between 0 and 46 volumes. Thus, all subjects had an equivalent of at least 6 min 25 s of resting state scans (i.e., 77% of the original data points were not regressed out). There was a significant group difference with regard to number of scrubbed volumes [$t_{(41)} = 2.51$, $p = 0.016$].

For each subject and each seed region, z-transformed Pearson correlation maps were used in the second level group analyses. All second level group analyses were controlled for mean FD, age and sex. Independent *t*-tests were used for testing group differences in functional connectivity for each seed region. Furthermore, using measures of pain sensitivity we investigated how pain sensitivity affected functional connectivity across subjects in both groups. All reported SCA results are thresholded at a false discovery rate (FDR) corrected cluster level of $p < 0.05/159 =$

0.00031, accounting for 159 *t*-tests using Bonferroni correction. Cluster defining voxel threshold was $p < 0.001$, uncorrected (to minimize number of false positive and negatives, see Woo et al., 2014).

Additionally, we performed a principal component based multivariate pattern analysis (MVPA) to detect group differences with regard to whole brain connectivity for each voxel. The MVPA analysis complements the SCA in that it is not limited to investigating functional connectivity in pre-selected pain regions, but provides a regionally unbiased mapping of brain areas with abnormal whole brain connectivity patterns. Whereas a SCA conducted on seeds defined in each gray matter voxel requires a very conservative multiple comparison correction that likely would prevent any significant group differences, the MVPA approach enabled us to detect putative abnormal connectivity patterns at a whole brain level using one simple F-test. In detail, the MVPA measure was obtained by reducing each voxels whole brain connectivity matrix into three principal components. The whole brain connectivity matrix for each voxel was reshaped into a row vector and subsequently concatenated over all participants into a matrix NxV, where N was the number of subjects and V is the number of voxels within the brain mask. The dimensionality of the NxV group correlation matrix was reduced by principal component analysis (PCA). This yielded an NxC matrix, where C is the number of maintained principal components. We maintained the first three principal components that explained the most of the variance of the connectivity matrix ($C = 3$), resulting in three component score volumes that best represented the whole brain connectivity pattern for each subject. These volumes were included in an *F*-test on the group level. Thus, we tested for clusters that differed between RA patients and HC with regard to whole brain connectivity as represented by the PCA component volumes. Subsequently, we performed a *post-hoc* seed correlation analysis, using spherical seeds placed at the peak voxels at the three clusters from the MVPA (MNI coordinates x, y, z: −28, 42, 58; 22, −14, 56; 10, 46, 44). The purpose of this analysis was to further probe the nature of putative group related differences in connectivity patterns of these regions (**Figure 2**). All group analyses were controlled for mean frame wise displacement, sex, and age.

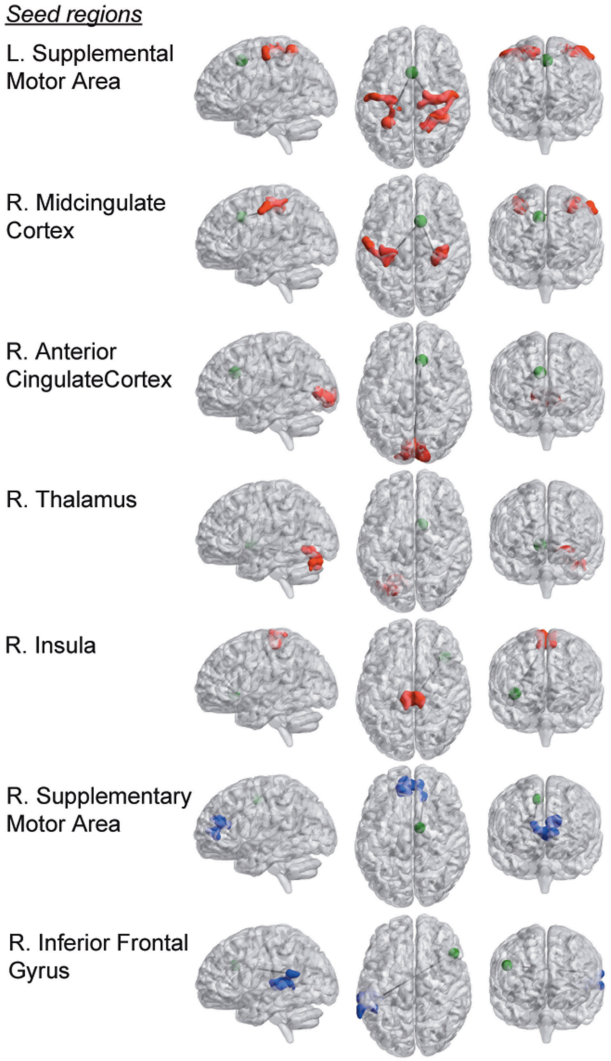

FIGURE 1 | Group differences in functional connectivity of the 159 a-priori defined seed regions within pain processing brain areas. Seed regions are depicted as green spheres (the radius of the seed points has been increased by a factor of 2 for display purposes). Brain areas that display stronger connectivity with respective seed region in the RA compared to the HC group are colored in red, and blue areas represent clusters that are more strongly connected in the HC compared to the RA cohort ($p < 0.0031$, FDR-corrected at the cluster level).

RESULTS

Behavior

RA patients rated higher levels of overall pain (VAS global pain); $t_{(41)} = 4.84$, $p < 0.00001$ than HC. We observed significantly increased pressure pain sensitivity at the affected finger joints (i.e., P50 joint) in RA patients compared to controls $t_{(41)} = -3.85$, $p = 0.0002$. However, the groups did not differ in pain sensitivity at the thumbnail (P50 thumb), $t_{(41)} = 0.43$, $p = 0.69$.

Functional Brain Connectivity

Furthermore, we observed seven group differences with regard to functional connectivity of the original 159 pain regions that were investigated. Overall, the observed pattern of connectivity differences in RA compared to HC was an increase in connectivity between tested pain seeds and other parts of the brain (see **Figure 1**, **Table 2**). Most prominently, RA patients displayed an elevated level of connectivity for seed regions located in both the supplementary motor area and in the mid-cingulate cortex with bilateral primary sensory motor cortices. In addition, we observed an increased level of connectivity for RA between the insula and premotor regions. We also observed an unexpected increased occipital connectivity (to thalamus and ACC) in the RA cohort.

For two a priori seeds regions, we detected weaker connectivity in the RA. The functional connectivity between supplementary motor area (SMA) and dorsal anterior cingulate cortex (dACC), and between inferior frontal gyrus and superior

TABLE 2 | Group differences in functional connectivity.

Contrast	Seed (center of sphere)	Target(s) (peak coordinates)	Clustersize (# voxels)	Cluster p-FWE
RA>HC	Supplementary Motor Area (0, 14,48)	S1/M1 (39, −42, 54)	1603	<0.000001
		S1M1 (−30, −48, 60)	769	0.000078
	Med. Front. Sup. Gyr. [a] (10, 46, 44)	Somatosensory (6, −70, 42)	1579	<0.000001
		Premotor (38, 16, 42)	708	0.00010
	ACC (10, 24, 28)	SVC (0, −82, 0)	1462	<0.000001
	Insula (40, 24, −2)	Premotor (2, −28, 60)	931	0.000025
	MCC (10, 14, 38)	S1/M1 (−30, −32, 50)	797	0.000062
		S1/M1 (30, −32, 50)	665	0.00025
	Thalamus (10, 4, −2)	AVC (−24, −76, −18)	705	0.00013
HC>RA	Postcentral Gyrus [a] (−28, −42, 58)	AVC (34, −56, −10)	822	0.000033
	Supplementary Motor Area (10, −6, 48)	dACC (16, 34, 18)	816	0.000037
	Inf. Frontal Gyrus (50, 24, 28)	Supplementary Temporal Gyrus (−64, −44, 18)	810	0.000066

Target regions are labeled based on the locations of the largest number of voxels within significant cluster, as identified and labeled within the CONN-toolbox. SMA, supplementary motor area; S1/M1, primary sensorimotor regions; MCC, middle cingulate cortex; SVC, secondary visual cortex; (d) ACC, (dorsal) anterior cingulate cortex; AVC, associative visual cortex.

[a]Seed regions defined post-hoc based on results from the MVPA analysis. All results are significant on a corrected cluster level ($p < 0.00031$, FDR), Bonferroni corrected for 159 seed correlation analyses (SCA).

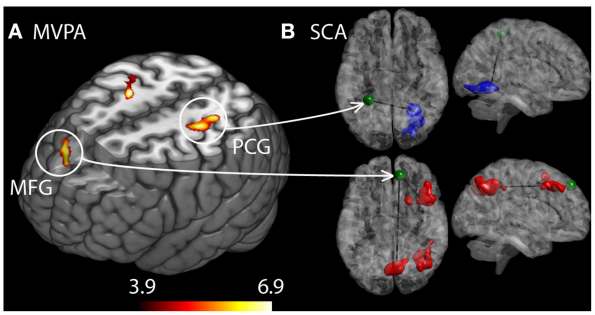

FIGURE 2 | Differences in brain connectivity profile indexed at a voxel level using multivariate pattern analysis (MVPA). (A) Brain regions that are significantly different between the RA and the HC cohorts with regard to connectivity profiles. Colorbar indicate F-statistic of between group differences with regard to the spatial maps of the three first principal components. Three clusters were identified: in left middle frontal gyrus (MFG) and in left and right post central gyrus (PCG). The F-maps are threshold at a $p < 0.05$ FDR corrected cluster level, using an explorative voxel level threshold of $p < 0.01$. **(B)** *Post-hoc* SCA using seed regions defined as spheres placed at the peak coordinates from MVPA, identified RA increased functional connectivity between MFG and premotor areas as well as a cluster spanning the precuneus and somatosensory areas (red clusters). HC displayed stronger connectivity between left PCG and contralateral associative visual areas.

temporal gyrus were both lower in RA compared to HC. (Parametric T-maps for all group differences listed in **Table 2** are available in NeuroVault at: http://neurovault.org/collections/1151).

The MVPA analysis showed group differences with regard to whole brain functional connectivity patterns in three regions, located in medial frontal gyrus (MFG) and bilateral somatosensory cortex in post central gyrus (PCG) (see **Figure 2A**). In the *post-hoc* seed correlation analyses, where we conducted SCA using seed regions based on the significant group differences in the MVPA, we found stronger connectivity between medial frontal gyrus (MFG) and premotor and somatosensory regions for HC, as well as decreased connectivity between post central gyrus (PCG) and associative visual cortex (ASV) in RA patients relative HC.

None of the observed group differences in connectivity correlated with any of the measures (listed in **Table 1**, i.e., P50 thumb, P50 joint, global VAS, DAS28 or RA duration), after Bonferroni correction for multiple comparisons (9 group differences in connectivity rendered a Bonferroni corrected p-value of $0.05/9 = 0.0056$). Associations between clinical symptoms and functional connectivity (controlled for sex, age, and FD) was quantified using Pearson correlation statistic within the RA group. However, in the RA cohort there was a trend [here defined as p-values above the bonferroni corrected p-value, (i.e., $0.0056 < 0.05$)] for a negative correlation between DAS28 scores and the connectivity between SMA and S1/M1, as well as a trend of a positive correlation between DAS28 scores and the connectivity between the inferior frontal gyrus (IFG) and premotor- and sensorimotor areas ($r = 0.44$, $p = 0.033$). Furthermore, there was a trend of a positive correlation between pain sensitivity on thumb (e.g., P50 thumb) and the strength of connectivity between right supplementary motor area and dACC ($r = 0.46$, $p = 0.021$).

Since we observed stronger movement in the RA group compared to the HC, we performed a *post-hoc* control analysis to investigate whether the connectivity differences were related to head movement (i.e., mean framewise displacement). Despite the rigorous set of strategies employed to minimize the effect of head motion in the group comparisons (see the Method section), we did find a significant relationship between head-movement (framewise displacement) and connectivity within the RA group. There was a negative correlation between mean framewise displacement and the SMA-S1/M1 connectivity ($r = -0.58$, $p = 0.0030$), and between the thalamus-AVC connectivity and movement ($r = -0.65$, $p = 0.00058$). We also found a trend for a negative relationship between movement and ACC connectivity ($r = -0.43$, $p = 0.036$), and a positive relationship between movement and the connectivity between inferior frontal gyrus (IFG) and superior temporal gyrus (STG; $r = 0.40$, $p = 0.050$).

DISCUSSION

In the present study we could confirm that RA patients suffer from increased sensitivity to supra-threshold pressure pain (i.e., hyperalgesia) in affected joints, but we found no signs of generalized hyperalgesia. The fact that hyperalgesia was confined to the affected joints in current study is in accordance with peripheral sensitization due to ongoing inflammation, but does not support a more generalized increase in pain sensitivity in our RA patients. The discrepancy between the present study and previous studies could depend on different methodologies (i.e., method of levels vs. method of limits in the previous studies) or on differences in patient cohorts. In addition, as expected, RA patients rated a higher global pain intensity compared to controls.

With regard to functional connectivity, we observed several important abnormalities. The main results are increased connectivity between frontal midline regions [the seeds in SMA, middle cingulate cortex (MCC) and middle frontal gyrus (MFG)] implicated in affective pain processing, to bilateral sensorimotor regions in RA. However, none of the tested group differences in connectivity correlated with measures of symptom gravity within the RA group after Bonferroni correcting for multiple tests. This prevents any firm conclusions on the functional significance of the group differences in functional connectivity to be made. Although the functional interpretation of increased frontal-S1/M1 connectivity remains to be established directly, one could speculate that it reflects increased attribution of emotional valiance to pain stimuli for the cohort of RA patients. Support for this interpretation is obtained from studies on back pain that reports a gradual shift of cerebral pain representations from canonical nociceptive regions toward affective circuits (although non-overlapping with the circuits reported here; Baliki et al., 2012; Hashmi et al., 2013). An alternative but not mutually exclusive interpretation is that the observed hyper-connectivity reflects an increased demand and taxation of prefrontal top-down regulation of sensory areas. For instance (Jones and Derbyshire, 1997) showed a decreased cerebral response in ACC and other prefrontal regions in ACC for induced pain among RA ($n = 6$) patients. The diminished prefrontal pain response among RA patients was interpreted as reflecting an adaptive cognitive and psychological response. A third option is that the altered sensorimotor connectivity is a consequence of life style changes in motor behavior due to a prolonged exposure to pain in the RA cohort. Unfortunately, we did not collect detailed data on motor habits, thus preventing us to test such relationships. It should be noted that the discussion above rely on reverse inference, that is, an inference of the functional significance based on what is known from earlier studies about the functional role of these areas. Since any brain region typically is involved in multiple cognitive processes, such inference is severely limited (for an in depth discussion, see e.g., Poldrack, 2011). Further investigation using complementary measures of for example the degree of daily activity (such as pedometers) would be interesting for determining whether group differences are related to altered movement patterns or other factors besides the exposure to chronic rheumatic pain.

Altered functional connectivity in RA patients shows that prolonged nociception, either alone or in combination with other lifestyle changes associated with RA (e.g., changes in physical activity or mood), modulates the brain connectivity pattern in resting state fMRI. Modulation of resting state brain connectivity in response to behavior and external factors is an intriguing phenomenon that have been confirmed in a wide range of contexts, including cognitive training, physical exercise and motor practice (for a review, see Guerra-Carrillo et al., 2014). Brain imaging of chronic pain patients have identified abnormal pain processing, such as deficiency in inhibitory pain circuits (Jensen et al., 2012), and abnormal resting state brain activity in fibromyalgia patients (Napadow et al., 2012; Flodin et al., 2014). The nature of abnormal cerebral pain processing in chronic pain conditions is far from established, and the heterogeneity of results between different chronic pain studies are likely due to differences in the cohorts investigated, the kind of tasks or no tasks used, and the analytical approaches employed to analyze the MR data. However, a general finding of the neuroimaging literature on rheumatic pain is the central role of the (medial) pain system for sensitization and pain inhibition, according to a review by Jones et al. (2012). They further proposed that studying the brains baseline activity (i.e., resting state fMRI) likely would prove fruitful for increasing our understanding of these mechanisms. Recently we investigated the resting state brain activity in FM by employing similar approaches for fMRI acquisition and data analysis as for the current study (Flodin et al., 2014). The main finding consisted in a reduced connectivity between pain and sensorimotor regions in fibromyalgia (FM). Similarly, Pujol et al. (2014) investigated resting state functional connectivity in FM and concluded that FM displays a general weakening of sensory integration, which could underlie the clinical pain in FM. However, the arguably most recurrent finding with regard to resting state activity in centralized pain is increased connectivity between insula and DMN (Napadow and Harris, 2014). Although we failed to replicate increased insula-DMN connectivity in FM, we observed a significant correlation between pain sensitivity (i.e., inverted P50 thumb) and the functional connectivity between insula and posterior cingulate cortex in the DMN. In contrast, the association of the insula-DMN connectivity and the degree of pain sensitivity was not replicated in the current study. The replication failure could be due to differences in patient cohorts, or other factors contributing to the inherent noise of the both the fMRI measurements and behavioral estimates of pain sensitivity (Barch and Yarkoni, 2013).

In a commendable attempt, Sundermann et al. (2014) used support vector machines to classify resting state brain activity in RA relative FM based on the connectivity within and between nodes of the DMN and the salience network. However, neither the conventional univariate analytical approach, nor the multivariate approaches of support vector machines rendered significant group differences. Interestingly however, the same research group previously identified a pattern of mostly opposing pain evoked brain activation for RA compared to FM. These were located in prefrontal regions and thalamus (Burgmer et al., 2009). Thus, it is worth comparing the current characterization

of resting state connectivity in RA that mainly consisted of increased connectivity between pain-and sensorimotor regions, with the opposing picture that pain in FM is associated with sensory disintegration (Flodin et al., 2014; Pujol et al., 2014). Speculatively, these connectivity differences could relate to the proposal that RA, perhaps in contrast to FM, partly involves an adaptation that serves to decrease the experienced pain (Jones and Derbyshire, 1997).

However, the RA group also displayed decreased connectivity. Importantly, the decreased connectivity between SMA and dACC observed in RA pertains to a part of the supplementary motor area that frequently has been associated with noxious perception (Duerden and Albanese, 2013). The target region in ACC (see **Figure 1** and **Table 2**) is located in the vicinity to the brain area that we previously showed to be under-recruited by chronic pain patients (fibromyalgia) in response to evoked pain. This hypo-connectivity was interpreted as a deficient top-down control of descending pain pathways (Jensen et al., 2009). Furthermore, here we observed a tendency of positive correlation between P50 joint (e.g., pain resilience) with SMA—dACC connectivity ($r = 0.46$, $p = 0.021$) in the RA cohort. Thus, a failure to recruit prefrontal control networks could be present in individual suffering from either of the two rheumatic pain conditions.

A limitation and possible confound in the reported results is the fact that the RA subjects were more prone to move during resting state scanning compared to HC. Typically, micro-head movement is associated with decreased long range anterior-posterior connectivity, and increased bilateral short range connectivity between the hemispheres (Power et al., 2012). Although we have undertaken a set of proven strategies modeling (scrubbing, inclusion of nuisance regressors at the first level and mean FD values at the second level of analysis) to counteract the effect of group differences in movement, there still remained correlations between movement and connectivity. However, matching the groups with regard to movement would have biased the RA group toward a less representative RA cohort. Since the direction of the correlations between movement and connectivity was negative (that is, more movement was associated with less connectivity), movement or the rigorous approach to movement correction likely decreased rather than induce the observed group differences. Movement was, or tended to be related to several of the group differences in connectivity involving occipital regions. For instance, the thalamic- AVC connectivity is normally very weak or absent (as verified in the sample of 1000 subjects available at neurosynth.org), and the enhanced FC between these regions are difficult to interpret in disease relevant terms. Similarly, the observed increased ACC-SCA connectivity in RA had a tendency of correlation with movement, and could partly be confounded by movement.

A second limitation and possible confound in the current study is the fact that resting state scans were acquired subsequent to task-based pain fMRI sessions (that will be reported elsewhere), possibly introducing spill-over effects into the resting-state data (Stevens et al., 2010; Wang et al., 2012). For instance, one could imagine that chronic pain patients experience stronger after-effects from a pain paradigm than healthy controls do, and that such asymmetry would confound group comparisons of the intrinsic brain activity.

Future studies would benefit from larger cohort sizes. In addition to the conventional beneficial effects of greater sample sizes on statistical sensitivity, a larger RA cohort would allow for subdivision of the cohort based on affected joint. Using functionally localizers, one could examine the connectivity of the cortical regions that are involved in processing pain of the primarily affected joints. Additional improvement in the seed selection by using functional localizers could narrow down the number of seeds tested, thus lessen the conservative impact of Bonferroni correction.

CONCLUSION

In the current study we have examined how RA patients differ from HC with regard to resting state functional connectivity. The general pattern that emerged was a stronger connectivity of regions in the medial pain system and regions in sensory- and motor cortex in RA. Additionally, RA related hypo- connectivity was found between frontal control areas and premotor regions that are associated with processing of noxious stimuli. However, the functional role of the group differences in connectivity remains to be established since the associations to subjective pain data and clinical severity scores were absent.

AUTHOR CONTRIBUTIONS

PF analyzed the data and drafted the manuscript. SM, RA and EW collected the data and revised the manuscript. JL and EK designed the study, and partook in interpretation of data and revision of the manuscript. PFr interpreted the data and revised the manuscript. All authors discussed the results and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnhum.2016.00107

Supplemental Table S1 | Individual usage of medication. DMARD, Disease-modifying antirheumatic drugs.

Supplemental Table S2 | The table shows MNI coordinates and anatomical labels of the 159 seed regions used in the seed correlation analyses. Anatomical labeling is performed using the automatic anatomical labeling (AAL) template in MRIcron. Stars (∗) indicate absent AAL labels, in which case anatomical labeling was performed by using the Harvard-Oxford Cortical/Subcortical structural atlas or the Juelich Histological Atlas as provided by FSLview.

Supplemental Figure S1 | Spatial distribution of seed regions placed in pain processing brain regions.

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Functional Equivalence of Imagined vs. Real Performance of an Inhibitory Task: An EEG/ERP Study

Santiago Galdo-Alvarez[1], Fidel M. Bonilla[2], Alberto J. González-Villar[1] and María T. Carrillo-de-la-Peña[1]*

[1] Department of Clinical Psychology and Psychobiology, Universidade de Santiago de Compostela, Santiago de Compostela, Spain, [2] Laboratory of Experimental Psychology, Faculty of Psychology, University El Bosque, Bogotá, Colombia

Early neuroimaging and electrophysiological studies suggested that motor imagery recruited a different network than motor execution. However, several studies have provided evidence for the involvement of the same circuits in motor imagery tasks, in the absence of overt responses. The present study aimed to test whether imagined performance of a stop-signal task produces a similar pattern of motor-related EEG activity than that observed during real performance. To this end, mu and beta event-related desynchronization (ERD) and the Lateralized Readiness Potential (LRP) were analyzed. The study also aimed to clarify the functional significance of the Stop-N2 and Stop-P3 event-related potential (ERPs) components, which were also obtained during both real and imagined performance. The results showed a common pattern of brain electrical activity, and with a similar time course, during covert performance and overt execution of the stop-signal task: presence of LRP and Stop-P3 in the imagined condition and identical LRP onset, and similar mu and beta ERD temporal windows for both conditions. These findings suggest that a similar inhibitory network may be activated during both overt and covert execution of the task. Therefore, motor imagery may be useful to improve inhibitory skills and to develop new communicating systems for Brain-Computer Interface (BCI) devices based on inhibitory signals.

Keywords: functional equivalence, inhibition, stop-signal task, motor imagery, ERPs, time-frequency EEG analyses

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****Correspondence:***
Santiago Galdo-Alvarez
santiago.galdo@usc.es

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INTRODUCTION

During the last decades, Brain-Computer Interface (BCI) communicating systems are being developed successfully for a variety of clinical (Mak and Wolpaw, 2009) and non-clinical (Blankertz et al., 2012) applications. These systems are based mostly on the assumption that the mental rehearsal of an action recruits the same neural mechanisms as its real performance. In particular, the simulation theory, also known as the functional equivalence hypothesis (Jeannerod, 2001), suggests that a similar cortical network, including primary areas, is involved during both mental practice of a movement and its overt execution.

The assumption of a functional equivalence challenges the classical hierarchical view of the motor system. Since Penfield and colleagues reported that stimulation of specific neurons in the primary motor cortex (M1) resulted in movements following a somatotopic representation (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950), it has been generally assumed that M1 plays the role of a pure executor receiving orders from superior motor centers.

In support of this view, former neuroimaging studies on motor imagery confirmed that primary and secondary motor areas were recruited during motor execution, but only secondary areas showed activation during mental practice of the same movements (Roland et al., 1980; Decety et al., 1988). Thus, they concluded that M1 is not activated when motor output is absent.

However, since then, many studies have questioned the hierarchical assumption and provided support for the functional equivalence hypothesis. Thus, various fMRI studies reported that the same network, including M1, was activated in motor imagery (Ersland et al., 1996; Porro et al., 1996; Roth et al., 1996; Lotze et al., 1999; Gerardin et al., 2000; Stippich et al., 2002). In several of these studies, it became clear that this activation could not be explained by subtle motor activity, as trials showing any EMG activity were discarded (Lotze et al., 1999; Gerardin et al., 2000; Lafleur et al., 2002).

Additional support for this hypothesis stems from event-related potential (ERPs) studies using the motor imagery paradigm (Galdo-Alvarez and Carrillo-de-la-Peña, 2004; Carrillo-de-la-Peña et al., 2006, 2008; Kranczioch et al., 2009; Hohlefeld et al., 2011). Although the EEG/ERP technique is characterized by a low spatial resolution, it provides a direct online measure of cortical activation and allows testing whether similar processes are taking place in the same temporal interval (Cohen, 2014; Luck, 2014). Several studies have claimed that one particular ERP component, the lateralized readiness potential (LRP), is generated in M1. The LRP is obtained from central electrodes and reflects the lateralized portion of motor ERPs. The main evidence for M1 as the source of this component is the inversion of polarity found for lower limb movements, as compared to hand movements. Brunia (1980) explained the inversion by the somatotopical distribution of the neurons on the M1: hands are represented in the lateral surface of precentral gyrus, whereas legs are represented in the medial surface. In addition, source reconstruction of LRP activity using EEG (Böcker et al., 1994a,b) and MEG (Praamstra et al., 1999) dipole modeling is consistent with the activation of M1.

Galdo-Alvarez and Carrillo-de-la-Peña (2004) reported that the LRP was present, although with a smaller amplitude, during covert performance, a result that the authors interpreted as evidence for the activation of M1 during motor imagery. Further research (Carrillo-de-la-Peña et al., 2006, 2008) confirmed this finding and provided evidence of functional equivalence of overt and covert actions; e.g., similar timing for simple and sequential or complex movements, inversion of polarity for lower limbs, and similar activation for hand selection. In fact, Hohlefeld et al. (2011) reported that overt and covert movements differed in stimulus processing at early stages of response selection, rather than in motor processing.

From a different perspective, several studies have explored how motor imagery affects EEG oscillations related to movement, i.e., mu and beta bands recorded over the somatosensory and motor areas. Consistent with this, a similar motor-related EEG pattern generally referred to as mu and beta event-related desynchronization (ERD) has been found during motor imagery and actual movement (Pfurtscheller et al., 2006; Stavrinou et al., 2007; Nam et al., 2011). The findings of numerous studies using Transcranial Magnetic Stimulation (TMS) also indicate that motor imagery significantly increases corticospinal excitability (Mizuguchi et al., 2009; Roosink and Zijdewind, 2010; Williams et al., 2012).

Overall, the data on ERPs, EEG dynamics and TMS during motor imagery provide support for the functional equivalence hypothesis. However, the above-mentioned studies analyzed selection, preparation or execution of simple motor responses. In natural situations, motor skills and actions require fine executive processing that involves coding strength, direction and other muscle parameters and also the ability to reset and inhibit ongoing performance. It would therefore be interesting to explore the brain electrical activity during the covert performance of inhibitory tasks.

The go/no-go and the stop-signal tasks are the paradigms most commonly used to study response inhibition, understood as the ability to suppress, withhold, delay or interrupt ongoing or planned actions. The stop-signal task explores inhibition of an already initiated response, i.e., action cancellation, and thus implies greater inhibitory pressure on response-related processes than the go/no-go paradigm (Swick et al., 2011). Two fronto-central ERP components have been associated with performance of the stop-signal task: Stop-N2, a possible index of the conflict between an initiated go response and the stop signal, and Stop-P3, a component whose interpretation is still open to debate. The P3 amplitude is larger in successful than unsuccessful stop (US) trials and in subjects with fast stop performances (requiring greater inhibitory activation; Dimoska et al., 2006), supporting its interpretation as an index of inhibitory efficiency. It has been suggested that the source of Stop-P3 may be in the premotor cortex, a region believed to be responsible for mediating stop-signal inhibition (Kok et al., 2004; Ramautar et al., 2006). Nevertheless, its latency appears to be too late to reflect the initial process of voluntary response inhibition, and it has thus been interpreted as an index of evaluation of the inhibitory process (Huster et al., 2013). It has been also suggested that in no-go and stop trials this positivity may be modulated by the lack of negative activity associated with motor preparation (Kok, 1986; Verleger et al., 2006).

Although the recording of brain activity during the covert performance of an inhibitory task could provide additional support for the functional equivalence hypothesis, as far as we know, there is only one study comparing actual and imagined performance of a stop-signal task (González-Villar et al., 2016). Using auditory stimuli as stop signals, they found similar Stop-N2, Stop-P3 and mu and beta ERD in mental essays and real performance of the task, but did not study the LRP as a possible index of M1 activation.

Thus, the main aim of the present study was to test whether covert performance of a stop-signal task produces the same pattern of motor-related EEG activity observed during real performance. To this end, mu and beta ERD and the LRP were obtained during both imagined and real performance of go and stop trials. A similar pattern on these indices during both

conditions may support the general applicability of the functional equivalence hypothesis to tasks that exert increased executive control over motor performance, as the stop-signal task does.

An additional objective was to replicate the previous study, testing whether the ERP indices that characterize response cancellation (i.e., Stop-N2 and Stop-P3) are also present during the covert performance of the Stop-signal task, using visual stimuli both as targets and as stop signals. Specifically, the presence of Stop-P3 in the covert condition could provide indirect evidence on the activation of an inhibitory network during imagery.

The present study also attempted to clarify the functional meaning of Stop-N2 and Stop-P3. Comparison of ERP components (LRP, Stop-N2 and Stop-P3) produced in US, successful stop (SS) and Imagined Stop (IS) trials may shed some light on the role of motor execution or outcome correction processes in classical ERP inhibition indices.

MATERIALS AND METHODS

Sample

A total of 18 students (5M, 13F) ranging from 19 to 32 years (mean = 20.89; SD = 1.72) participated voluntarily in the study. All were right-handed, according to the Edinburgh handedness inventory, and reported normal or corrected vision. None of them presented a history of neurological or psychiatric disorders, or drug abuse. Informed consent was received from all the participants, in accordance with the Declaration of Helsinki.

Stimuli and Apparatus

The primary task consisted of a choice reaction task in response to white arrows pointing to the left or the right side (stimulus duration: 500 ms; mean interval between stimulus onsets: 2100 ms), which indicated the hand that participants had to respond with. The start of each trial was indicated by the appearance of a fixation cross in the center of the screen. Then, the white arrows substituted the fixation cross. The arrow consisted of an arrowhead and a tail and had a size of 2.1° · 1.4° of visual angle. In 30% of trials, a red arrow (stop signal) indicated that subjects had to cancel the already prepared response.

The task was designed and presented using the STIM program (Neuroscan Labs). The stimuli were presented on a 15″ screen located at a distance of 100 cm from the subjects. Participants responded using a response box held in their hands.

Design and Procedure

Participants were seated comfortably in an armchair in a dimly lit, sound attenuated room. They were instructed to look at the fixation cross in the center of the screen and to press a button with their right or left thumb according to the direction indicated by the white arrow. They were informed that in some trials a red arrow might appear after the white arrow, indicating that the response should be canceled. Subjects were instructed to respond as quickly as possible to the white arrow and not to wait for the appearance of the stop signal. They completed some practice trials before the first block of experimental trials.

In the real condition, the time interval between the onset of go signals and stop signals was 300 ms in the first trial and was then changed according to the subject's performance (ranging from 160 to 400 ms in 40 ms steps). The interval was altered using the staircase-tracking algorithm that adjusts the go-stop interval in a certain trial depending on the results of the previous stop trial (Band and van Boxtel, 1999). This algorithm produces a distribution around $\frac{1}{2}$ of successful and $\frac{1}{2}$ of unsuccessful response-inhibited trials. If the response in the previous stop trial was correctly inhibited, the interval between go and stop signals in the next stop trial was 40 ms longer, also increasing the difficulty of successful inhibition; if the subject responded in the previous stop trial, the interval between signals in the next stop trial was 40 ms shorter, in order to facilitate inhibition (Logan and Cowan, 1984).

In the imagined condition, subjects were instructed to imagine as vividly as possible responding with the hand of the side pointed by the white arrow, and to withhold the response (like braking suddenly) when the stop signal appeared. They had to keep their hands on the response box, as in real performance. In this condition, due to the lack of response feedback, the Go-Stop signal interval was fixed at 300 ms.

The task for each condition consisted of 280 trials, 70% of them were Go (196 trials, 98 for each direction) and 30% Stop (84 trials, 42 for each direction). The order of the tasks was always the same: first, overt execution and then covert performance. This procedure was used to ensure more effective mental rehearsal after real practice, as revealed by previous studies (Cunnington et al., 1996; Carrillo-de-la-Peña et al., 2006). Participants were allowed a 5 min rest between both tasks.

Psychophysiological Recording and Data Analyses

The EEG was recorded from 28 electrode sites (10–20 international system) referenced to the left and right mastoids, using pure tin electrodes attached to a fabric cap (Electro-Cap International, Inc., Eaton, OH, USA). The electrooculogram (EOG) was recorded from sites above and below the left eye and from electrodes lateral to each eye. The AFz electrode served as ground electrode. Electrode impedances were kept below 10 kΩ. The EEG signals were digitized online with Neuroscan equipment (Neuroscan Laboratories, version 4.1), amplified 10,000 times (SynAmp Model 5083 amplifier), filtered using a band-pass between 0.1 and 100 Hz and a notch filter of 50 Hz, and sampled at a rate of 500 Hz.

The EEG data were analyzed using the EEGlab 12.02 toolbox (Delorme and Makeig, 2004). The data were resampled to 250 Hz and re-referenced to an average-reference. Poorly recorded channels were replaced by spherical-spline interpolation and EEG segments containing large ocular or other artifacts were rejected after visual inspection. The data were digitally filtered using a low-pass 30 Hz FIR filter. An Independent Component Analysis algorithm was used to remove components associated with ocular artifacts. The EEG data used for the ERP analyses were baseline corrected from −200 to 0 ms. Epochs were extracted from 200 ms pre-stimulus to 900 ms post-stimulus, and

were extracted time-locked to go stimuli (white arrows) and to the stop stimuli (red arrows; only for N2 and P3 analyses). The ERPs used to measure the N2 wave were filtered with a 2–12 Hz band-pass filter to avoid overlap with other ERP waves.

The stop-signal task is complicated by the fact that the activity to the stop stimuli overlaps with the activity evoked by the previous go signal. To resolve this, we subtracted the activity evoked by go trials from the ERPs obtained in stop trials. First, we calculated the percentage of SS and US trials for each subject, and this percentage was used to select go trials in the following way: if the participant had a 45% of US in all stop trials, the 45% of the fastest go epochs were used as the pool of trials to make the subtraction of the US minus Fast Go trials. The remaining 55% of the slowest go trials were used as the pool to make the subtraction SS minus Slow Go trials. A random go epoch (selected from its respective pool of Go epochs) was then assigned to each stop epoch. Finally, stop and go epochs were aligned by the go signal, and the subtraction was computed. This method was applied in previous studies (Kok et al., 2004; Ramautar et al., 2006).

The LRP was obtained by the average method proposed by Coles (1989), i.e., it was computed by subtracting ERP activity at C3 minus C4 for the right responses and C4 minus C3 for the left responses, and then averaging the resulting difference waveforms. This removes non-motor contribution from this index of lateralized activity associated with response preparation. LRPs were obtained for each trial (go, stop) and task (overt, covert). Also, the topographical distributions of LRPs were calculated using the method described by Praamstra and Seiss (2005), applying the average method to obtain LRP from each pair of contralateral electrodes (e.g., F3/F4, FC3/FC4. . .; only for go trials in both tasks).

Mean amplitudes were obtained for N2 (200–260 ms interval) and P3 (260–450 ms interval) at the FCz electrode site. As different numbers of trials were presented for the different conditions, mean amplitudes were measured instead of peak amplitudes to prevent confusion due to different signal-to-noise-ratios.

Time-Frequency Analysis was performed by convolving the EEG data with a family of complex Morlet wavelets ranging in frequency from 3 to 30 Hz in 27 linearly increasing steps, and with logarithmically increasing cycles, from three cycles at the lowest frequency to eight at the highest frequency. Power data obtained after convolution was baseline corrected by transforming the power change of each time-frequency pixel to dB, relative to the mean power in the baseline interval (−400 to −100 ms) of each frequency.

As the frequencies of interest here are more prominent around Rolandic areas, we first averaged spectrograms of C3 and C4 electrodes. For analysis of mu and beta oscillations, time-frequency windows were selected after averaging the spectrograms for Trial (go, stop) and Task (overt, covert) together, to avoid making assumptions about condition differences. We observed that mu band had two peaks at different latencies (at around 450 and 700 ms, respectively), and we therefore extracted two different windows (from 300 to 550 ms and from 600 to 900 ms) in the 9–13 Hz range. For the beta band, we extracted the mean power from 200 to 550 ms between 18 and 24 Hz.

Statistical Analysis

Behavioral and ERP parameters were analyzed by considering the available measures in the different conditions. Thus, given the lack of motor response in motor imagery conditions, we carried out *t* tests to examine differences in behavioral performance reaction times (RTs) between the overt go response and overt US trials.

In order to assess the possible existence of LRPs during covert motor performance, we carried out one-sample Wilcoxon tests for the mean of five consecutive windows of 50 ms each, with a step size of 10 ms between windows (i.e., each window had an overlap of 40 ms with the prior window), starting 40 ms before the peak latency (approximately 370 ms). If significant differences were found for all the windows, we could conclude that the waveforms deviated significantly from baseline and thus that LRPs were also present during mental rehearsal of movements in the different conditions of the task.

LRP mean amplitudes were measured in the 300–400 interval. The LRP onset latencies were determined using the jackknife procedure. Therefore, 18 different grand averages for each of the experimental conditions were computed by omitting one of the participants from each grand average. The onset was subsequently measured using the method proposed by Schwarzenau et al. (1998), which assumes that the onset of correct preparation corresponds to the intersection point of two straight lines, one fitted to the baseline and another to the rising slope of the LRP.

For the LRP, N2 and P3 mean amplitudes and the beta and mu ERD power, repeated-measures analysis of variances (ANOVAs) were carried out with two within-subject factors (Trial: go, stop; Task: overt, covert). In these analyses, overt response stop trials included only those trials in which successful inhibition was observed. Possible differences between tasks in go LRP topography were analyzed using a repeated measures ANOVA on LRP mean amplitudes (200–400 ms), with task (overt, covert), and electrode pair (F3/F4, FC3/FC4, C3/C4, CP3/CP4, P3/P4) as within-subject factors. The LRP onsets were subsequently analyzed by means of repeated-measures ANOVA with two within-subject factors (Trial: go, stop; Task: overt, covert). The *F* values in the latter case were corrected using the formula $F = \mathrm{F}/(n-1)^2$, as recommended when performing the jackknife procedure for statistical analyses (Ulrich and Miller, 2001).

To clarify the effect of successful vs. unsuccessful performance of the stop-signal task, additional repeated-measures ANOVAs were carried out with the within-subject factor Performance (SS, US, IS) for the same parameters.

RESULTS

Behavioral Performance

Table 1 shows behavioral indices for go and stop trials (as means of left and right hand responses). For go trials, the data included

TABLE 1 | Behavioral parameters for the overt performance of the stop-signal task.

GO	
% Hits	93.4 (7.5)
% Errors	2.5 (3.2)
% Missing	3.7 (6.6)
RTs for hits	453 (94)
RTs for errors	342 (109)
STOP	
% US	50 (17)
US RTs	396 (60)
SSD	250 (47)
SSRT	203 (60)

RTs, reaction times; US, unsuccessful stop trials; SSD, stop signal delay; SSRT, stop signal reaction time.

percentages of hits, errors and missing responses, as well as RTs for hits and errors. For stop trials, the percentage of US trials and their RTs, as well as mean stop signal delay (SSD) values and stop signal reaction times (SSRTs) are provided[1].

The percentage of US was about 50%, as expected given the use of the staircase tracking algorithm. RTs were faster in US trials than in go trials ($t = 5.8$, $p < 0.001$).

LRP

Figure 1 presents the LRP obtained in different pairs of electrode sites and the scalp distribution of the component. **Figure 2** presents the average waveforms of EMG, LRP and stimulus-locked components (N2, P3) obtained from go and stop trials in both overt and covert performance, as well as the scalp distribution for each component.

One-sample Wilcoxon tests were performed to confirm the existence of LRPs in covert response trials. All comparisons revealed significant differences from 0, and therefore we can conclude that the LRP is present in motor imagery for both go and stop trials (**Table 2**). The mean values and standard deviations for all the ERP parameters measured, including LRP, are shown in **Table 3**.

The repeated-measures ANOVA (Trial × Task) for LRP amplitude showed significant main effects of Trial ($F_{(1,17)} = 22.4$; $p < 0.001$) and Task ($F_{(1,17)} = 9.3$; $p = 0.007$), but no interaction effect ($F_{(1,17)} = 3.0$; $p = 0.1$). The LRP amplitude was larger in go than in stop trials, and it was larger when the participants had to perform an overt response task than when they had to imagine the response.

In the analysis of go LRP topography, the ANOVA revealed significant effects for Electrode ($F_{(4,68)} = 12.2$; $p < 0.001$), Task ($F_{(1,17)} = 9.9$; $p < 0.01$), and for the interaction of both factors ($F_{(4,68)} = 4.8$; $p < 0.01$). *Post hoc* comparisons showed that LRP mean amplitude was significantly larger for overt than covert go trials only in fronto-central electrodes ($p < 0.01$ for F3/F4; $p < 0.001$ for FC3/FC4; and $p < 0.01$ for C3/C4) but not in the posterior locations ($p = 0.081$ for CP3/CP4 and $p = 0.28$ for P3/P4). In addition, topographical distribution was similar in both tasks (overt response task: central electrodes > rest of electrode sites except fronto-central electrodes, fronto-central electrodes > frontal and parietal electrodes, and central-parietal electrodes > parietal electrodes; covert response task: fronto-central and central electrodes > central-parietal > frontal and parietal electrodes).

The repeated-measures ANOVA to clarify the effect of successful vs. unsuccessful performance was applied to data from 12 participants, as six of the participants did not produce enough artifact-free US epochs for each hand to yield the LRP. The ANOVA revealed a significant effect of the factor ($F_{(2,22)} = 6.5$; $p = 0.005$), as LRP amplitudes were larger for US than for SS trials ($p = 0.031$) and covert stop trials ($p = 0.033$); however, no differences between the latter two conditions were found ($p = 1$).

The repeated-measures ANOVA (Trial × Task) for LRP onset did not reveal any significant differences for Trial ($Fc_{(1,17)} = 0.1$; $p = 0.7$), Task ($Fc_{(1,17)} = 0.05$; $p = 0.8$) or the interaction between these factors ($Fc_{(1,17)} < 0.01$; $p = 0.9$). The repeated-measures ANOVA with Performance as within-subjects factor did not show a significant effect for LRP onset ($N = 12$) either ($Fc_{(2,22)} = 0.03$; $p = 0.9$).

N2 Mean Amplitude

The repeated-measures ANOVA (Trial × Task) did not reveal any significant effect of Trial ($F_{(1,17)} = 1.1$; $p = 0.3$), Task ($F_{(1,17)} = 0.8$; $p = 0.4$) or the interaction between these factors ($F_{(1,17)} = 0.1$; $p = 0.7$).

The repeated-measures ANOVA showed a significant effect of Performance ($F_{(2,34)} = 10.6$; $p \leq 0.001$). The N2 amplitude was larger for US than for SS trials ($p = 0.019$) and covert stop trials ($p = 0.002$); no differences were found between these two conditions ($p = 1$).

P3 Mean Amplitude

The repeated-measures ANOVA (Trial × Task) revealed a significant effect of Trials ($F_{(1,17)} = 11.3$; $p = 0.004$). The P3 amplitude was larger in stop than in go trials. The ANOVA did not reveal significant effects of Task ($F_{(1,17)} = 3.0$; $p = 0.1$) nor the interaction between Trial and Task ($F_{(1,17)} = 3.0$; $p = 0.1$).

The repeated-measures ANOVA did not reveal a significant effect of the factor Performance ($F_{(2,34)} = 1.1$; $p = 0.3$).

Beta ERD (200–550 ms)

Figure 3 shows the representation of the time-frequency analyses of both beta and mu ERD.

The repeated-measures ANOVA (Trial × Task) revealed a significant effect of Task ($F_{(1,17)} = 20.6$; $p < 0.001$). Beta desynchronization was larger for overt than for covert response trials. The ANOVA did not reveal a significant effect of Trial ($F_{(1,17)} = 1.3$; $p = 0.3$) or the interaction between these factors ($F_{(1,17)} = 1.9$; $p = 0.2$).

[1]The SSRTs represent the point at which the stop process finishes and can be estimated taking into account the go RT distribution and the observed probability of successful/unsuccessful inhibitions to the stop signal for a given SSD (go-stop interval). Using the staircase-tracking algorithm facilitates the estimation of the SSRT since that probability is around 0.50. Thus, it is possible to calculate SSRT by subtracting the observed mean SSD from the observed mean go RT (Logan and Cowan, 1984; Logan et al., 1997).

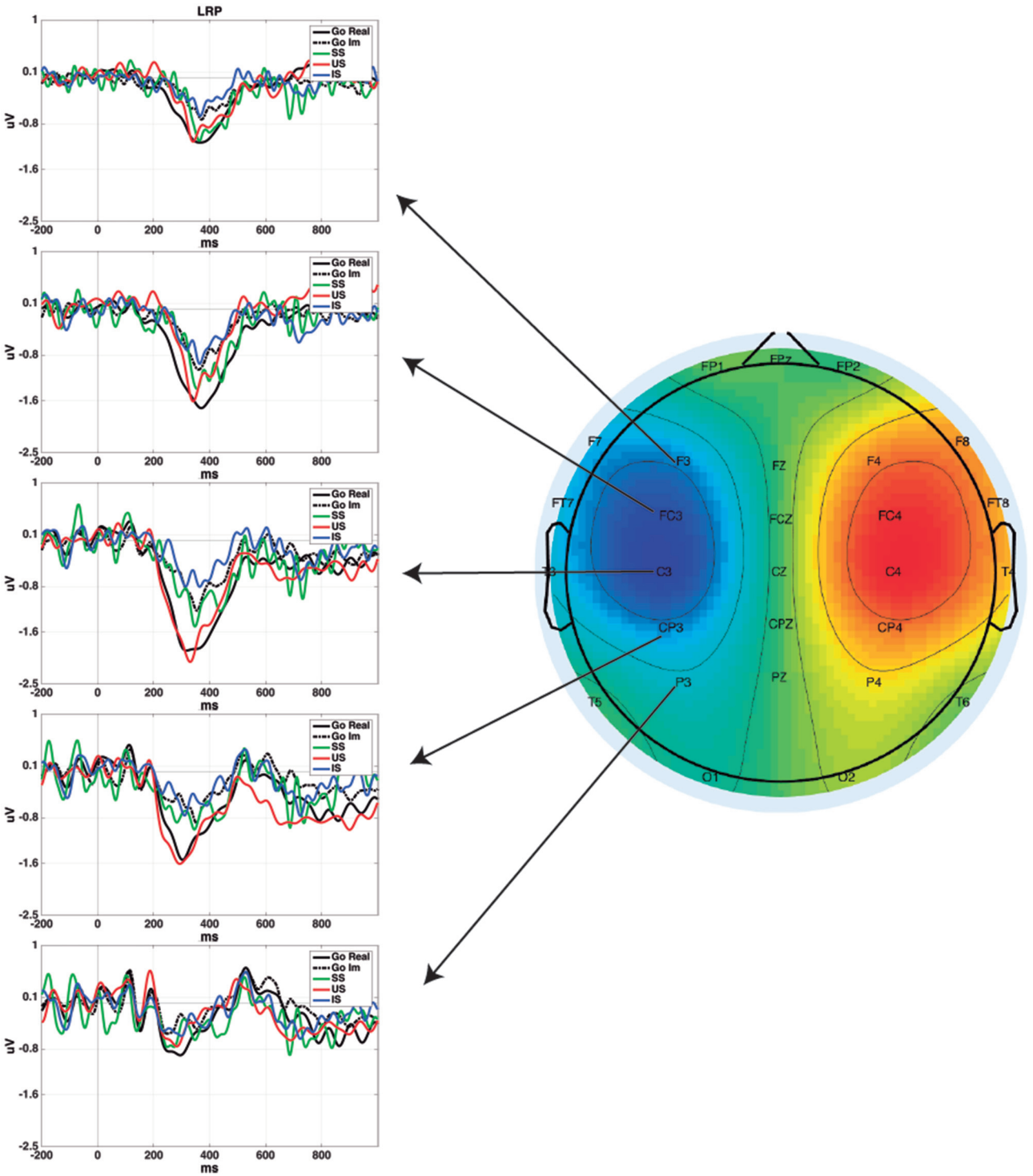

FIGURE 1 | Lateralized Readiness Potential (LRP) time-locked to the go signal for each condition in different scalp locations. Plotted grand averages of Successful Stop (SS) and Unsuccessful Stop (US) were computed using 12 participants, while Go Real, Go Im and Imagined Stop (IS) were computed using 18 participants. Topography represents the mean LRP amplitude of all conditions from 200 to 400 ms.

The repeated-measures ANOVA revealed a significant effect of Performance ($F_{(2,34)} = 9.9$; $p < 0.001$). A larger decrease in power was found in SS ($p = 0.001$) and US ($p = 0.021$) than in IS trials, but no differences were found between successful and US trials ($p = 1$).

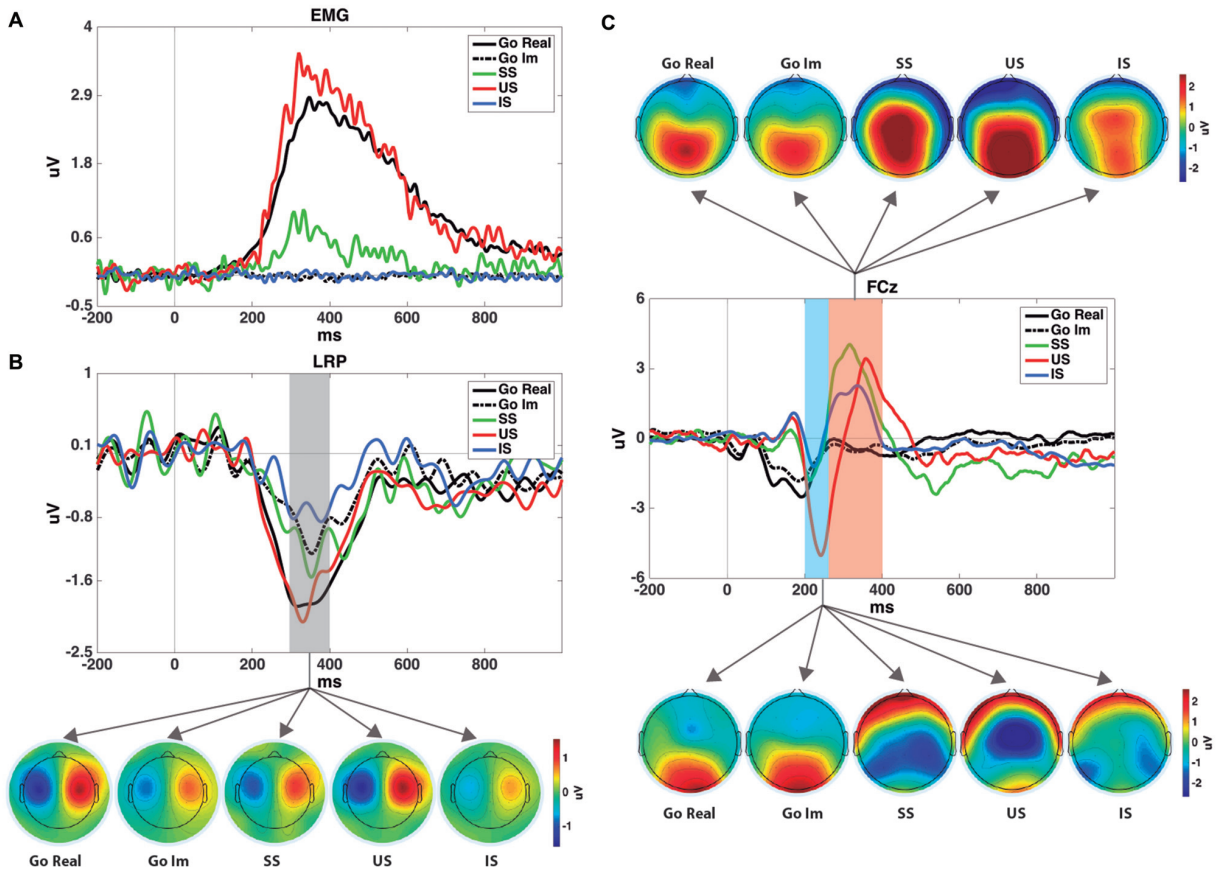

FIGURE 2 | (A) Rectified electromyogram (EMG) for each condition. It shows that no EMG activity was registered after stimulus presentation during the imagined task. **(B)** LRP time-locked to the go signal and the topographies of the shaded area. SS and US grand averages of the LRPs were computed using 12 participants, while Go Real, Go Im and IS were computed using 18 participants. Topographies were calculated using the method described by Praamstra and Seiss (2005). **(C)** Event-related potential (ERPs) for each task and condition at the FCz electrode site and their topographies in the windows selected to measure N2 and P3 components. Note that go trials were averaged time-locked to the go signal, while SS, US and IS were averaged time-locked to the stop signal and with go-stimulus ERPs subtracted.

Mu ERD (300–550 ms)

The repeated-measures ANOVA revealed a significant effect of Task ($F_{(1,17)} = 7.2$; $p = 0.016$). Mu desynchronization was larger in overt than in covert response trials. The ANOVA revealed no significant effect of Trial ($F_{(1,17)} = 0.1$; $p = 0.8$) or the interaction between these factors ($F_{(1,17)} < 0.001$; $p = 0.9$).

The repeated-measures ANOVA revealed a significant effect of Performance on Mu ERD ($F_{(2,34)} = 4.2$; $\varepsilon = 0.69$; $p = 0.041$), although multiple pairwise comparisons (Bonferroni adjusted) did not reveal any significant differences.

TABLE 2 | One-sample Wilcoxon tests for covert trials Lateralized Readiness Potential (LRP) amplitude.

Condition	Interval	Voltage average (microvolts)	Wilcoxon value
Go	340–390	−0.95	−3.7***
	350–400	−1.01	−3.6***
	360–410	−0.87	−3.4***
	370–420	−0.88	−3.3***
	380–430	−0.84	−3.2**
Stop	340–390	−0.34	−2.0*
	350–400	−0.34	−2.3*
	360–410	−0.46	−2.1*
	370–420	−0.40	−2.1*
	380–430	−0.26	−2.1*

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.

Mu ERD (600–900 ms)

The repeated-measures ANOVA (Trial × Task) revealed a significant effect of Task ($F_{(1,17)} = 15.4$; $p = 0.001$). Mu desynchronization was larger for overt than for covert response trials. The ANOVA did not reveal a significant effect of Trial ($F_{(1,17)} = 2.2$; $p = 0.15$) or the interaction between these factors ($F_{(1,17)} = 1.5$; $p = 0.2$).

The repeated-measures ANOVA (Performance) revealed a significant effect of the factor ($F_{(2,34)} = 11.9$; $p < 0.001$). A larger

TABLE 3 | Mean and standard deviations (in parentheses) for the measured event-related potential (ERP) parameters and mu and beta eventrelated desynchronization (ERD).

		LRP Onset (ms)	LRP Amp. (μV)	N2 Amp. (μV)	P3 Amp. (μV)	beta ERD 200–550 (dB)	mu ERD 300–550 (dB)	mu ERD 600–900 (dB)
Overt performance	Go	176 (3)	−1.7 (1.2)	−1.3 (1.8)	−0.5 (1.4)	−1.6 (1.1)	−2.6 (1.5)	−2.2 (1.5)
	Successful stop	204 (21)	−0.9 (0.8)	−0.8 (2.2)	2.0 (3.0)	−1.9 (1.2)	−2.6 (1.7)	−2.9 (1.5)
	Unsuccessful stop	182 (6)	−1.6 (1.3)	−3.7 (4.4)	1.1 (2.9)	−1.7 (1.2)	−2.4 (1.7)	−3.4 (2.2)
Covert performance	Go	196 (8)	−0.9 (0.9)	−1.1 (1.6)	−0.4 (0.9)	−0.7 (0.8)	−1.6 (1.2)	−1.0 (1.0)
	Stop	222 (35)	−0.6 (0.9)	−0.4 (2.0)	1.0 (1.6)	−0.8 (0.7)	−1.6 (1.4)	−1.2 (2.0)

Note: LRP for Unsuccessful Stop (US) data were obtained from 12 participants; for the other parameters, EEG recordings from the 18 participants were used.

decrease in power was observed in SS ($p = 0.004$) and US ($p = 0.004$) than in IS trials, but no differences were found between SS and US trials ($p = 0.5$).

DISCUSSION

The main goal of the present study was to determine whether a similar pattern of motor-related brain electrical activity is shared in the overt and covert performance of the stop-signal task, a paradigm that exerts strong executive (inhibitory) control. To better capture the power and phase dynamics of the EEG, we included time/frequency analyses (mu and beta ERD) in addition to phase-locked averaged responses (i.e., ERPs).

The results of the present study indicate that covert performance of the stop-signal task appears to recruit neural mechanisms in the brain similar to those used during overt execution and with a similar time course.

The presence of lateralized preparatory activity at central electrodes in the motor imagery condition suggested that M1 is actively involved in the simulated performance of the task. Despite the low spatial resolution of EEG, it is generally considered that the neural source of the LRP component is located at the M1, as revealed by dipole estimation from EEG (Böcker et al., 1994a,b) and MEG studies (Praamstra et al., 1999), and given its inversion of polarity depending on the limb that performs the movement (Brunia, 1980; Carrillo-de-la-Peña et al., 2006). The study findings also confirmed that the temporal pattern of activation is the same in covert and overt performance, as no difference was found in LRP onset between conditions.

It could be questioned whether our LRP results certainly support M1 activation during motor imagery. In fact, it has been argued that, depending on the physical setting of visual stimuli, LRP could reflect lateralized posterior activity rather than motor processing (Praamstra, 2007). In addition, with settings of asymmetric stimuli (as it is the case of arrows), other components related to attentional shifts, as the early directing-attention negativity (EDAN), the anterior directing-attention negativity (ADAN) and the late directing-attention positivity

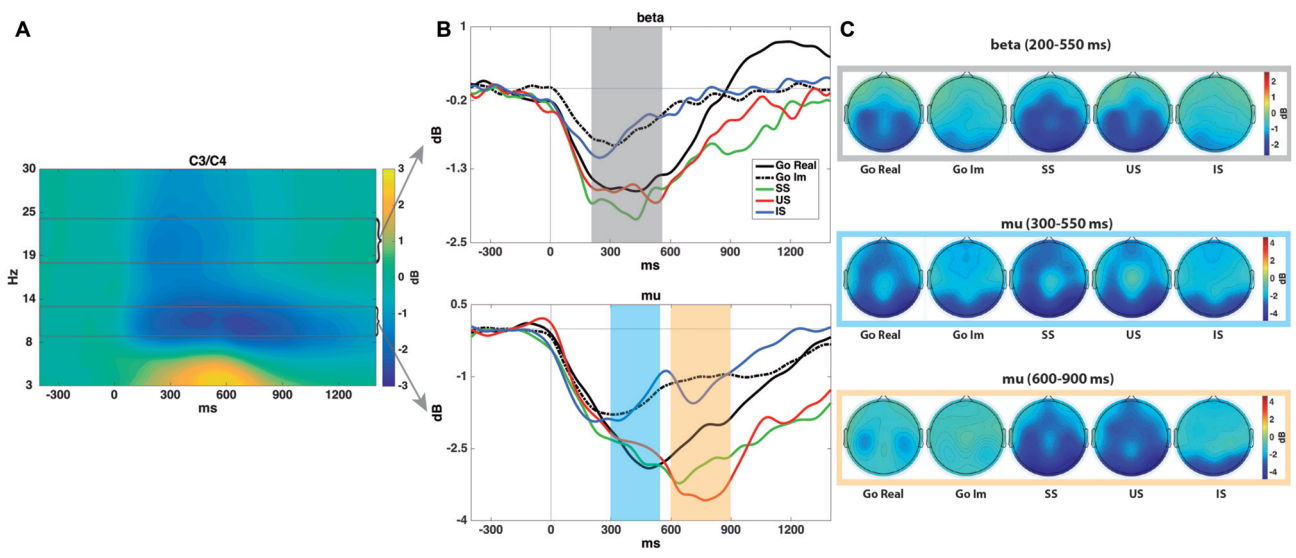

FIGURE 3 | Time-frequency analyses. (A) Spectrogram showing the time-frequency power averaged across all conditions in the C3 and C4 electrodes. This plot was used to select time-frequency windows for statistical comparisons. **(B)** Mean mu (9–13 Hz) and beta (18–24 Hz) power for each task and condition–all time-locked to the go signal. As explained in the "Materials and Methods" Section, mu event-related desynchronization (ERD) presents two peaks (especially in stop trials), in both real and imagined performance. Shaded areas encircle the time intervals submitted to statistical analyses. Mu and beta ERD show a similar time course in covert and overt performance, although with a reduced power decrease in the former. **(C)** Topographies of power modulations in each shaded area and condition.

(LDAP; Verleger et al., 2000; Praamstra et al., 2005; Gherri and Eimer, 2010; Praamstra and Kourtis, 2010), or inhibitory mechanisms, as the N2cc component (Oostenveld et al., 2001; Praamstra and Oostenveld, 2003; Praamstra, 2006; Cespón et al., 2012) might also overlap with LRP.

Given that we did not use eccentric settings of stimuli (all were presented in the center of the screen), the contribution of lateralized brain activity associated to stimulus processing might be ruled out. The LRP scalp distribution, with maximal amplitudes between frontocentral and central electrode sites, and reduced amplitude towards more anterior and posterior sites is also inconsistent with reports of the topographical distribution of attention-shifts ERP waves, as EDAN, ADAN and LDAP. In addition, in a previous study using the same array of stimuli (arrows with the same tail and head sizes), we reported an inversion of polarity when the participants performed the task using feet movements (in both overt and covert trials; see Carrillo-de-la-Peña et al., 2006), an effect that supports the contribution of M1 in the generation of LRP (Brunia, 1980; Böcker et al., 1994a,b). In any case, our results support that a similar brain network is involved in real and imagined inhibition, regardless of whether it is referred to M1 activation, activation of frontoparietal networks, or engagement of premotor inhibitory mechanisms.

The amplitude of the LRP was smaller in motor imagery than in the overt motor execution and inhibition, as consistently observed in previous studies (Galdo-Alvarez and Carrillo-de-la-Peña, 2004; Carrillo-de-la-Peña et al., 2006, 2008). Although this might be interpreted as a sign of weaker motor activation in simulated performance, it is open to alternative explanations. As LRP was also smaller in stop trials than in go trials in the overt condition, it could be argued that the smaller LRP amplitudes in motor imagery are due to the presence of larger or sustained motor inhibition during the task. Alternatively, previous studies have also indicated that differences between overt and covert conditions may be due to stimulus processing (Hohlefeld et al., 2011) or the lack of feedback or control from somatosensory areas (Carrillo-de-la-Peña et al., 2008) rather than to motor activation processes.

Results of time-frequency analyses paralleled those found for LRP and provide a complementary view of the temporal dynamics of motor-related EEG in stop-signal tasks. As in previous studies (Pfurtscheller and Neuper, 1997; McFarland et al., 2000), we observed mu and beta ERD over the lateral central electrode sites during motor imagery; again, the decrease in power of those central rhythms was larger in overt performance. Although some studies have related the power of these bands to motor cortex activation, it has also been demonstrated that bilateral mu and beta ERD may be associated specifically with activation of the somatosensory cortex (Jurkiewicz et al., 2006).

In relation to the ERP components characteristic of the stop-signal task, we found that only P3 was significantly larger for stop than go trials, also in the simulated condition. The presence of Stop-P3 in the latter condition suggests that subjects actually canceled an already prepared response even during motor imagery. This result replicates a previous study with auditory stop signals that found similar P3 amplitude and midfrontal theta in imagined than in successfully stopped trials (González-Villar et al., 2016). As explained below, this finding has practical implications and contributes to understand the functional meaning of Stop-P3.

The inhibition of inappropriate responses is an important part of goal-oriented behavior. From a practical point of view, the observed involvement of similar neural circuits in the covert performance of the stop-signal suggests the possibility of training inhibitory skills through mental rehearsal. Non-invasive methods of recording brain signals, such as the EEG, are widely used in BCI. To date, only brain electrical activity indices of motor activation or stimulus detection have been used as BCI communicating systems. Our findings suggest that the indices of inhibition obtained in motor imagery could also be used as communicating systems and could be useful for developing hybrid BCIs that incorporate various sensing modalities in the brain (i.e., detection of directional movement and inhibition of that movement).

Previous studies have found larger N2 and P3 amplitudes for stop than for go trials. These modulations are usually interpreted as reflecting inhibitory control (De Jong et al., 1990; Dimoska et al., 2003, 2006), although it has also been considered that N2 may reflect conflict detection (Carter et al., 1998; Nieuwenhuis et al., 2002, 2003; Donkers and van Boxtel, 2004; Yeung et al., 2004; Enriquez-Geppert et al., 2010), and P3 the evaluation of the inhibitory process, because of its latency (Huster et al., 2013). Nonetheless, other differences between go and stop trials may contribute to the N2 and P3 modulations reported: first, a motor response, including muscular activation, is only present in go trials (and US trials); second, a stop signal is present only in stop trials, and therefore these trials involve double processing (go stimulus and stop stimulus) that may overlap. Thus, the functional significance of Stop-N2 and Stop-P3 is far from clear.

In the present study, two different experimental manipulations were carried out to clarify these alternative explanations: the inclusion of motor imagery to confirm/dismiss the role of motor execution processes (as no overt response is present during the mental essay of the stop-signal task), and the application of a procedure to remove go stimulus-linked activity from stop trials (see "Materials and Methods" Section).

It has been suggested that P3 in no-go trials may be due to the absence of movement-related negativity (Salisbury et al., 2004), and this could be extrapolated to Stop-P3. In the present study, no movement was present in either covert go or stop trials, but a prominent Stop-P3 appeared only in the latter. After comparing a press no-go and a count no-go condition, Smith et al. (2013) also concluded that P3 is due to motor inhibition related positivity in no-go trials. Thus, the presence of Stop-P3 during the imagery condition in the current study ruled out an interpretation based on differences in motor processes. The analysis of stop trials free from the influence of the go signal also allowed us to conclude that the larger amplitude of P3 in stop trials is not due to the summation of activity evoked by two consecutive stimuli.

In the present study, we failed to replicate the larger N2 to stop than to go trials reported in previous studies. However, in

a comparison of Stop N2 in successful and US trials, Ramautar et al. (2006) found a larger N2 in unsuccessful trials and indicated that Stop N2 resembled an Error-Related Negativity. Our findings are consistent with this interpretation, as we observed larger N2 amplitude in US trials than in SS trials.

Despite the above contributions, there are some limitations in the experimental design; first, the role of M1 in inhibitory control remains unclear. Further research is required to establish whether M1 acts as a passive receptor of inhibitory signals from other components of the executive control network or assumes an active function in the suppression of motor processing. Since previous studies have considered beta rebound as a correlate of inhibition or return to an idling state after termination of a motor program (Neuper and Pfurtscheller, 1996), even after motor imagery (Pfurtscheller et al., 2005; Solis-Escalante et al., 2012), it would be interesting to analyze beta rebound in stop trials, what requires longer ISIs than the ones used in the present study. Our design was also unable to clarify whether Stop-P3 reflects actual inhibitory control or, alternatively, evaluation of the inhibitory process. As Huster et al. (2013) have argued, this process is initiated and controlled before the culmination of P3, suggesting that the component may reflect evaluation of the inhibitory outcome. Similarly, Wessel and Aron (2015) proposed use of the onset of the frontocentral P3 as a better indicator of response inhibition. Finally, we could not rule out the attentional effect produced by the red arrow (stop) in the N2 and P3 amplitudes. Future studies should include a condition in go trials with a second stimulus as a confirmatory signal (e.g., a green arrow to continue with the motor program).

Overall, the present findings add to previous cumulative evidence for the existence of a shared neural substrate between imagined and executed movements (Stavrinou et al., 2007), supporting the functional equivalence hypothesis (Jeannerod, 2001). The results provide a consistent picture: similar lateralized activity (LRP, mu and beta ERD) was observed both in overt and covert responses, with a similar time course (identical LRP onset, and mu and beta ERD temporal windows) and pattern of task-modulation (differences between go and stop trials). Thus, the results suggest that the mental imagery of a motor plan leads to activation of the same network, with similar temporal dynamics and constraints. The use for the first time of a motor imagery paradigm during performance of a stop-signal task allowed us to further conclude that a similar inhibitory network may be also active during covert execution of the task.

As stated above, this finding could contribute to the development of more sophisticated BCI and provides the scientific basis for understanding the efficacy of motor imagery techniques for improving performance in professional athletes (Jones and Stuth, 1997; Ridderinkhof and Brass, 2015) or motor rehabilitation in patients with neurological lesions (Dickstein and Deutsch, 2007; Zimmermann-Schlatter et al., 2008).

AUTHOR CONTRIBUTIONS

SG-A was responsible for the first manuscript draft, manuscript editing and the statistical analyses, and contributed to literature review, and manuscript review. FMB contributed to task design, EEG recording and literature review. AJG-V was responsible for EEG processing and figures, and contributed to literature review and manuscript review. MTC-P was responsible for task design and contributed to statistical analyses, literature and manuscript review. All the authors contributed to interpretation of results.

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Altered Functional Performance in Patients with Fibromyalgia

Isis da Silva Costa[1], *Antoni Gamundí*[1], *José G. Vivas Miranda*[2,3], *Lucas G. Souza França*[2,4], *Charles Novaes De Santana*[5] *and Pedro Montoya*[1]*

[1] *Research Institute of Health Sciences (IUNICS), University of the Balearic Islands, Palma de Mallorca, Spain,* [2] *Department of Physics of the Earth and the Environment, Federal University of Bahia, Salvador, Brazil,* [3] *Motion Analysis Laboratory, Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, MA, USA,* [4] *Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, University College London, London, UK,* [5] *Department of Evolutionary Biology and Environmental Studies, University of Zürich, Zürich, Switzerland*

Fibromyalgia is a common chronic pain condition that exerts a considerable impact on patients' daily activities and quality of life.

Objectives: The main objective of the present study was to evaluate kinematic parameters of gait, functional performance, and balance in women with fibromyalgia syndrome.

Methods: The study included 26 female patients with fibromyalgia (49.2 ± 8.0 years) according to the criteria of the American College of Rheumatology, as well as 16 pain-free women (43.5 ± 8.5 years). Gait and balance parameters were extracted from video recordings of participants performing several motor tasks. Non-linear dynamic of body sway time series was also analyzed by computing the Hurst exponent. In addition, functional performance and clinical pain were obtained by using standardized motor tests (Berg's balance scale, 6-min walking test, timed up and go task, Romberg's balance test) and self-report questionnaires (Fibromyalgia Impact Questionnaire).

Results: Walking speed was significantly diminished ($p < 0.001$) in FM patients as compared to pain-free controls, probably due to significant reductions in stride length ($p < 0.001$) and cycle frequency ($p < 0.001$). Analyses of balance also revealed significant differences between fibromyalgia and pain-free controls on body sway in the medial-lateral and anterior-posterior axes (all $ps < 0.01$). Several parameters of gait and balance were significantly associated with high levels of pain, depression, stiffness, anxiety, and fatigue in fibromyalgia.

Conclusion: Our data revealed that both gait and balance were severely impaired in FM, and that subjective complaints associated with FM could contribute to functional disability in these patients. These findings suggest that optimal rehabilitation and fall prevention in fibromyalgia require a comprehensive assessment of both psychological responses to pain and physical impairments during postural control and gait.

Keywords: fibromyalgia, chronic pain, gait, balance, Hurst exponent, computer vision software

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Universidade do Porto, Portugal
Aurore Thibaut,
University of Liège, Belgium

***Correspondence:**
Pedro Montoya
pedro.montoya@uib.es

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INTRODUCTION

Fibromyalgia (FM) is a chronic syndrome characterized by widespread pain sensitivity and fatigue, as well as by cognitive and affective symptoms (Wolfe et al., 2010). Fibromyalgia also exerts a considerable impact on daily activities and quality of life. In particular, it has been frequently shown that fatigue in fibromyalgia may be severe enough to reduce physical activities and lead to a sedentary lifestyle by reducing physical abilities and increasing risk for disabilities (Bennett et al., 2007; Jones et al., 2008). FM patients often reported functional limitations that were quite similar to those reported by persons with osteoarthritis or rheumatoid arthritis (Hawley and Wolfe, 1991). Furthermore, it has been shown that loss of function could be strongly associated with work disability in these patients (White et al., 1999; Wolfe and Michaud, 2004).

Previous research has also revealed that FM patients may also display deficits in balance or postural stability (Bennett et al., 2007; Jones et al., 2009; Russek and Fulk, 2009), a complex task that involves rapid and dynamic integration of multiple sensory, motor, and cognitive inputs to execute appropriate neuromuscular activity (Horak, 2006; Sousa et al., 2012). Impaired balance has been reported as one of the top ten debilitating symptoms in fibromyalgia with prevalence rates around 45% (Bennett et al., 2007). Moreover, frequency of falls seems to be higher in FM patients (34.4%) (Russek and Fulk, 2009) than in persons aged 65 years and older (25–35%) (Sattin, 1992) and patients with rheumatoid arthritis (Hawley and Wolfe, 1991). Nevertheless, balance and activity level in fibromyalgia have been mostly assessed by using retrospective self-reports (Mannerkorpi et al., 1994; Russek and Fulk, 2009), which are strongly influenced by patients' beliefs about their own physical functioning and pain (Verbunt et al., 2003). In the last decades, different types of recording devices have been developed to monitor and to assess balance and physical activity over long periods of times, providing valid information about subjects' daily activities. Thus, it has been demonstrated that accelerometry-based ambulatory monitoring systems provide more objective measurements of variability in physical activities and pain over several days than self-reports (Verbunt et al., 2009). Biomechanical analysis of gait also constitutes a useful tool for the assessment of motor function, functional capacity and muscle fatigue (Bendtsen et al., 1997; Pierrynowski et al., 2005; Sousa et al., 2012). Previous studies have observed that fibromyalgia women display a reduced walking speed, which could be a consequence of decreases in stride length and cycle frequency, as well as bradykinesia (Auvinet et al., 2006; Heredia Jiménez et al., 2009). Furthermore, it has been suggested that gait at normal speed in these patients may be preferentially achieved by using their hip flexors instead of their ankle plantar flexors, thus increasing metabolic demands and fatigue in comparison to pain-free controls (Pierrynowski et al., 2005). Despite the evidence of altered gait and balance parameters in FM, little is known about how these abnormalities could be linked to clinical variables such as pain, fatigue, stiffness, or depression.

The aim of the present study was to analyze gait and balance parameters in fibromyalgia and to examine the possible relationship between subjective and objective measures of motor function with subjective complaints. In particular, we hypothesized that FM patients would display significant gait and balance deficits as compared with pain-free controls, and that these motor disturbances would be correlated with increased patients' ratings of pain, fatigue, morning tiredness, stiffness, and physical impairment (as measured by the FIQ questionnaire). Furthermore, considering that activity and balance fluctuations have well defined fractal properties in a wide range of time scales, we also aimed to apply nonlinear analyses to evaluate the dynamic of these balance fluctuations in pain-free controls and FM patients. This nonlinear approach allows an evaluation of the autocorrelation in successive displacements, giving us information about possible disturbances in motor control mechanism to correct balance.

MATERIALS AND METHODS

Participants

Twenty-six women diagnosed with fibromyalgia and 16 pain-free women with comparable age and sociodemographic characteristics were recruited from different health centers and patients' associations in Majorca (Spain). The average duration of FM diagnosis was 10.8 ± 7.7 years Patients were included in the study if they fulfilled the 1990 classification criteria of the American College of Rheumatology for fibromyalgia. Participants were excluded from the study if they reported any other musculoskeletal rather than fibromyalgia, or any neurological disorder. Regarding medication intake, most FM patients were taking analgesics, relaxants, or NSAIDs ($n = 18$), followed by antidepressants ($n = 16$), and anxiolytics ($n = 9$). For medical and ethical reasons, medication was not discontinued during the study. At the time of recruitment, all participants were verbally informed about the details of the study and provided written consent. The study was approved by the Ethics Committee of the Balearic Islands (Spain) (reference IB-1284/09).

Self-Report Questionnaire

FM patients completed the *Fibromyalgia Impact Questionnaire (FIQ)* (Burckhardt et al., 1991). The FIQ is a standardized instrument designed to quantify the overall impact of fibromyalgia. Subscales from the 1991 version include 11 physical function items (4-point Likert scale ranging from "always" to "never"), feel good (number of days of the past week), missed work (number of work days in the past week), and 7 symptom-based items (ability to do job, pain, fatigue, rested, stiffness, anxiety, and depression) (100-mm anchored visual analog scale). Test-retest correlations using Pearson's r ranged from 0.56 (pain) to 0.95 for physical function scale. This questionnaire has shown excellent responsiveness to change in clinical studies and a good correlation with other similar questionnaires such as the SF-36 (Bennett, 2005).

Motor Function Tasks

Gait and balance parameters were obtained in FM patients and pain-free controls by using the following functional tasks:

- *Berg Balance Scale* (Berg et al., 1991): This scale is a performance-based assessment tool developed to measure balance during functional activities such as reaching, bending, transferring, and standing. The test is often used for patients who exhibit a decline in function, self-report a loss of balance, or have unexplained falls (Berg et al., 1991). The Berg Balance Scale consists of 14 functional tasks (e.g., sitting unsupported, change of sitting to standing position, and vice-versa, standing with both feet together, standing on one leg, turning 360 degrees) with scores ranging from 0 (unable to perform) to 4 (normal performance). Total scores range from 0 (severely impaired balance) to 56 (excellent balance). Scores below 46 are good predictors for the occurrence of multiple falls (Dibble et al., 2008).
- *Six-minute walking test (6MWT)*: The 6MWT is a functional walking test in which subjects are instructed to walk for 6 min as quickly as possible. This test has been used to assess individuals with mobility deficits (Kosak and Smith, 2005) and FM patients (King et al., 1999; Pankoff et al., 2000a; Latorre-Román et al., 2014). The 6MWT is considered a good indicator of exercise tolerance and aerobic capacity, since it causes a physiological stress without demanding maximum aerobic capacity (Pankoff et al., 2000a). Ratings of perceived exertion were obtained after the 6MWT by using the Borg Effort Scale (Borg, 1982), a 15-point scale ranging from 4 (complete lack of effort) to 20 (maximum effort or exhaustion).
- *Timed up and go task (TUG)*: This task is a standardized test for assessment of functional mobility. The task is performed by using an ordinary armchair (45 cm in height) and a stopwatch. Subjects are seated with their back against the chair and instructed to stand up, walk three meters, turn around, walk back to the chair, and sit down at an ordinary comfortable speed (Shumway-Cook et al., 2000). The stopwatch is started on the word "*Go*" and stopped as the subject sit down. The TUG time is measured in seconds and normal TUG time ranges from 5.4 to 40.8 s (mean = 15 s, $SD = 6.5$) (Khasnis and Gokula, 2003). TUG time appears to be correlated with gait speed, balance, functional level, and the ability to go out (Newton, 1997). After the TUG, overall subjective perception of physical effort was measured by using the Borg Effort Scale (Borg, 1982).
- *Modified version of the Romberg's balance test*: The Romberg's test is an objective measure of patient's standing balance (Khasnis and Gokula, 2003). The original test requires that participants remain in orthostatic position with feet together and eyes closed. In the present study, we modified the procedure by asking the participants to keep the erect position with eyes closed during 1 min. In addition, they were allowed to keep the orthostatic position with feet in parallel and separated and arms extended along the body to avoid that participants fell when they closed their eyes during data collection. The test is based on the fact that maintaining balance while standing with closed eyes should rely on intact sensorimotor integration and motor pathways. The test was repeated twice and motion on the frontal and sagittal planes was captured by using a digital video camera at 30 frames per second (Casio Exilim EX-FS10). For motion detection analysis, a plumb line hanging on the ceiling at a distance of 3 meters was used as reference. Participants were also asked to wear a cap with sticks positioned in the vertical and horizontal planes. For the analysis of body sway in the medial-lateral direction, sticks were aligned with the anatomical position of the glabella of the frontal bone. For the analysis of body sway in the anterior-posterior direction, sticks were aligned with the anatomical position of the pinna (tragus). Unfortunately, we were not able to analyze the Romberg's test videos of eleven FM patients and two pain-free subjects due to poor recording quality.
- *Gait task*: Subjects were instructed to walk on a 3 meters carpet at their normal walking step, without shoes and with flexed arms positioned on the abdomen. Optical markers were attached at the following body positions: anterior superior iliac spine, posterior superior iliac spine, area between the lateral condyle of the femur and the fibular head, bottom of the patella, lateral, and inner malleolus, heel (between the first and second metatarsal), and on the tip of the hallux. Subject's motion was digitally recorded with a video camera at 210 frames per second (Casio Exilim EX-FS10). The camera was positioned at a distance of 3 meters from the carpet to visualize changes in position, velocity, and acceleration of anatomical points along the x-axis. Gait velocity (cm/second), walking duration (seconds), cadence (number of steps/minute), percentage of time in the two phases of the gait cycle (stance and swing phase), and percentage of time with single and double support were computed.

Data Reduction and Pre-processing

Three groups of variables were analyzed in the present study:

- Raw scores obtained from self-report questionnaire (FIQ).
- Performance scores on standardized motor function tasks (TUG, 6MWT, Berg Balance Scale, Borg Effort Scale).
- Kinematic parameters extracted from video recordings: gait velocity (cm/sec), gait duration (sec), cadence (steps/min), stride, and step lengths (cm), percentage of time in the stance/swing phase, and body sway variability in the anterior-posterior and medial-lateral planes (cm). Open-source software for computer vision analysis of human movement (CvMob; Peña et al., 2013; Gea et al., 2014; Quixadá et al., 2016) was used to extract those variables. This software has a high degree of accuracy for calculating body position and movement in the X and Y coordinates recorded by conventional cameras (Peña et al., 2013).

The non-linear dynamic of time series obtained during the balance test was also assessed by computing long term correlations and the *Hurst exponent* (Feder, 1988). This exponent usually ranges between 0 and 1, and describes the tendency of a time series either to cluster in one direction or to regress strongly

to the mean. Thus, it has been assumed that Hurst exponents between 0.5 and 1 would be characteristic of time series with long-term positive autocorrelation (high values will be followed by high values a long time in the future), whereas exponents between 0 and 0.5 would suggest long-term switches between high and low values in adjacent pairs of data. By contrast, Hurst exponents would be around 0.5 if time series describe a pure random oscillation (e.g., Brownian noise or accumulated white noise). Moreover, it has been assumed that exponents lesser than 0.5 would reflect a non-persistent pattern, whereas exponents greater than 0.5 would rather reflect a persistent pattern within the time series (Feder, 1988).

The Hurst exponent was obtained in two steps. First, the deviation of the time series relative to their mean values was computed in a sliding window of size n by using the Root Mean Square (RMS) method (Russ, 1994). The RMS uses the scaling function ($\bar{W}(n)$) defined as follows:

$$\bar{W}(n) = \frac{1}{N-n}\sum_{u=1}^{N-n}\left\{\frac{1}{n}\sum_{i=1}^{n}\left[Z(x_{u+i}) - \bar{Z}_n\right]^2\right\}^{1/2} \quad (1)$$

with the factor N representing the total number of measurements and $\bar{Z}_n$ the average value within each scale. Second, the values for $\bar{W}(n)$ were evaluated for different scales n. The Hurst exponent was obtained by fitting a power-law curve (fractal Brownian motion model) to the scaling function (Feder, 1988; Russ, 1994) as follows:

$$\bar{W}(n) \sim n^H \quad (2)$$

Statistical Analyses

The null hypothesis that data were sampled from a normally distributed population was examined by using Shapiro-Wilk tests, and differences between patients and pain-free controls were analyzed by using parametric Student t-tests for independent samples, or non-parametric two-sample Kolmogorov-Smirnov tests. Pearson correlations were also used to analyze the relationship between kinematic parameters and clinical symptoms in fibromyalgia. A p-value of 0.05 was used for statistical significance. The effect sizes d were interpreted using the classification of Cohen (1988): $0.2 \leq d < 0.5$ small effect, $0.5 \leq d < 0.8$ moderate effect, $d \geq 0.8$ large effect. Means and standard deviations are displayed in the tables. If appropriate, data are reported as mean difference and 95% confidence interval (95% CI).

RESULTS

Fibromyalgia patients and pain-free controls were comparable on age (49.2 years ± 8.0 vs. 43.5 years ± 8.4, respectively), weight (68.6 kg ± 10.9 vs. 64.2 kg ± 10.9), height (161.1 cm ± 6.4 vs. 163.3 cm ± 7.0), and body-mass index (26.5 kg/m^2 ± 4.2 vs. 25.5 kg/m^2 ± 4.1) (all $ps > 0.05$). Mean and standard deviation of FIQ scores in fibromyalgia patients are displayed in **Table 1**.

Table 2 displays mean and standard deviation of gait parameters in fibromyalgia and pain-free controls during performance on several motor tasks. FM patients walked less distance in 6 min (6MWT) [$t_{(29)} = -8.3$, $p < 0.001$], and took more time to stand-up and to walk a distance of 3 meters (TUG) as compared with pain-free controls [$t_{(40)} = 6.7$, $p < 0.001$]. Moreover, ratings on self-perceived effort (Borg Effort scale) after performance on 6MWT (K-S = 1.5, $p < 0.05$) and TUG tests (K-S = 3.02, $p < 0.001$) were significantly higher in fibromyalgia than in pain-free controls. Finally, FM patients reported increased risk of falls (measured by the Berg Balance Scale) in comparison with pain-free controls (K-S = 2.9, $p < 0.001$). The effect sizes were medium-to-large for all group comparisons.

TABLE 1 | Mean and standard deviations of FIQ scores in FM patients.

	Fibromyalgia patients $N = 26$
FIBROMYALGIA IMPACT QUESTIONNAIRE (FIQ)	
Physical impairment (0–3)	1.6 ± 0.7
Feel good (0–7)	5.1 ± 1.7
Missed work (0–7)	2.1 ± 2.2
Do job (10 cm VAS)	8.3 ± 2.4
Pain (10 cm VAS)	8.2 ± 1.9
Fatigue (10 cm VAS)	8.9 ± 2.0
Rested (10 cm VAS)	8.1 ± 3.1
Stiffness (10 cm VAS)	7.6 ± 2.9
Anxiety (10 cm VAS)	7.6 ± 2.9
Depression (10 cm VAS)	6.8 ± 3.4
Total FIQ score (0–100)	71.1 ± 16.1

Analyses of kinematic parameters further indicated that FM patients had significant deficits in gait and balance. Again, the effect sizes were medium-to-large for all group comparisons. FM patients displayed significant reductions in gait velocity [$t_{(31)} = -8.3$, $p < 0.001$], cadence (steps/minute) [$t_{(31)} = -6.2$, $p < 0.001$], stride length [$t_{(31)} = -5.1$, $p < 0.001$), step length [$t_{(31)} = -4.9$, $p < 0.001$], and percentage of single support [$t_{(31)} = -4.3$, $p < 0.001$] and swing phase [$t_{(31)} = 4.2$, $p < 0.001$], as well significant increased gait duration [$t_{(31)} = 5.7$, $p < 0.001$] in comparison with pain-free participants. Same effects were also yielded when values were referenced to each subject's legs (distance between the greater Trochanter and the lateral Malleolus) (**Table 2**). Moreover, FM patients displayed greater body sway in the anterior-posterior [$t_{(27)} = 4.6$, $p < 0.001$] and medial-lateral directions [$t_{(27)} = 5.8$, $p < 0.001$] than pain-free controls.

The non-linear analysis of balance time series also revealed significant group differences on Hurst exponents of anterior-posterior [$t_{(27)} = 2.3$, $p < 0.05$] and medial-lateral axes [$t_{(27)} = 5.1$, $p < 0.001$]. In both cases, the H exponents were close to 0.5 in fibromyalgia patients and around 0.3 in pain-free controls (**Figure 1** and **Table 3**). The effect sizes were medium-to-large for all group comparisons.

In order to further assess if altered motor function was related to clinical symptoms in fibromyalgia, Pearson correlations were computed between motor performance scores and ratings on the different FIQ scales. Results indicated that high ratings on pain intensity were significantly associated with enhanced risk of falls (Berg Balance Scale, $r = -0.52$ and $p < 0.01$), increased time

TABLE 2 | Mean and standard deviations of gait parameters during motor performance in fibromyalgia patients and pain-free controls.

	Fibromyalgia patients $N = 26$	Pain-free controls $N = 16$	Cohen's d	Effect-size r
STANDARDIZED MOTOR FUNCTION TESTS				
Berg scale for risk of falls (0–56)	44.7 ± 5.6	55.4 ± 0.6	−2.68	0.80
TUG (sec)	17.0 ± 5.2	8.2 ± 1.0	2.35	0.76
Perceived effort after TUG (4–20)	12.3 ± 2.3	4.3 ± 0.5	4.80	0.92
6MWT (m)	170.9 ± 46.9	330.1 ± 58.3	−3.00	0.83
Perceived effort after 6MWT (4–20)	14.1 ± 3.6	9.2 ± 3.1	1.45	0.59
GAIT PARAMETERS				
Gait velocity (cm/sec)	67.3 ± 17.3	112.0 ± 12.3	−2.97	0.82
Gait duration (sec)	4.8 ± 1.4	2.7 ± 0.3	2.07	0.72
Cadence (steps/min)	96.6 ± 19.4	115.5 ± 11.6	−1.18	0.51
Stride Length (cm)	69.3 ± 14.3	99.2 ± 12.9	−2.19	0.74
Step Length (cm)	58.6 ± 10.5	79.7 ± 8.6	−2.20	0.74
Single support (%)	57.3 ± 7.0	66.1 ± 4.2	−1.52	0.60
Swing phase (%)	29.3 ± 3.1	33.7 ± 3.0	−1.44	0.58

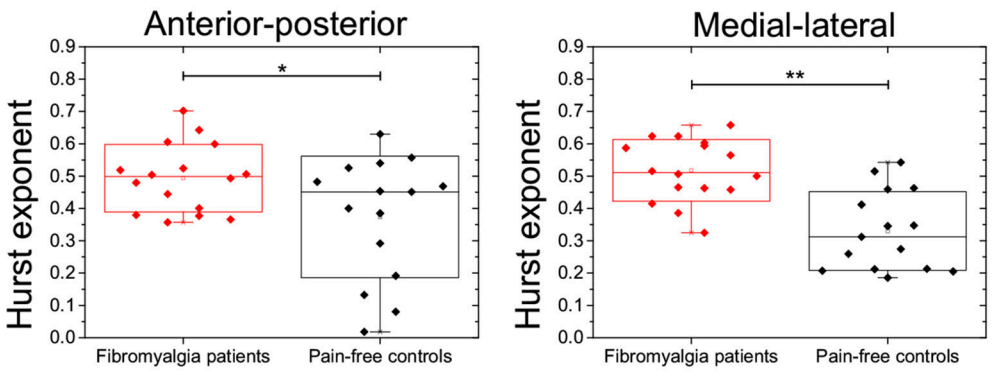

FIGURE 1 | Boxplots showing the distribution of Hurst exponents during body sways in the antero-posterior (left) and medial-lateral axes (right) in FM patients and pain-free controls. Asterisks indicate statistical differences at $^{*}p < 0.05$ or $^{**}p < 0.01$.

TABLE 3 | Mean and standard deviations of balance parameters in the anterior-posterior and the medial-lateral axes during motor performance in fibromyalgia patients and pain-free controls.

	Fibromyalgia patients $N = 15$	Pain-free controls $N = 15$	Cohen's d	Effect-size r
ANTERIOR-POSTERIOR AXIS				
Body sway (cm)	2.31 ± 0.99	1.07 ± 0.11	1.76	0.66
Hurst exponent	0.50 ± 0.10	0.37 ± 0.19	0.85	0.39
MEDIAL-LATERAL AXIS				
Body sway (cm)	2.55 ± 0.92	1.08 ± 0.09	2.25	0.75
Hurst exponent	0.52 ± 0.09	0.33 ± 0.12	1.79	0.67

to perform the TUG test ($r = 0.44$, $p < 0.05$), reduced distance to walk in 6 min in 6MWT test ($r = -0.53$, $p < 0.05$), gait velocity ($r = -0.56$, $p < 0.05$), cadence ($r = -0.50$, $p < 0.05$) and stride lengths ($r = -0.49$, $p < 0.05$), as well as increased gait duration ($r = 0.54$, $p < 0.05$) and body sways in the medial-lateral axis ($r = 0.68$, $p < 0.05$). High ratings on fatigue and stiffness were also associated with reduced percentage of single support ($r = -0.52$, $p < 0.05$ and $r = -0.48$, $p < 0.05$, respectively). In addition, high ratings on stiffness were related to enhanced perceived effort after completion of the 6MWT test ($r = 0.60$, $p < 0.05$), and reduced stride ($r = -0.56$, $p < 0.01$) and step lengths during the gait cycle ($r = -0.54$, $p < 0.05$). High ratings on the physical function scale were significantly associated with high risk of falls (Berg Balance Scale, $r = -0.39$ and $p < 0.01$) and increased perceived effort after completion of the TUG ($r = 0.56$, $p < 0.01$), as well as with reduced distance walked in 6 min during performance of 6MWT test ($r = -0.50$, $p < 0.05$). The number of missed days of work were significantly associated with high risk of falls (Berg Balance Scale, $r = -0.40$ and $p < 0.05$), enhanced perceived effort after completion of the 6MWT test ($r = 0.60$, $p < 0.05$) and reduced distance to walk in 6 min in 6MWT test ($r = -0.50$, $p < 0.05$). Low ratings on ability to do job were significantly associated with high risk of falls (Berg Balance Scale, $r = -0.47$ and $p < 0.05$) and increased body sways in the medial-lateral axis ($r = 0.53$, $p < 0.05$). High ratings on the rested scale

were significantly associated with reduced distance walked in 6 min during performance of 6MWT test ($r = -0.57$, $p < 0.05$). Finally, ratings on depression were correlated with risk of falls (Berg Balance Scale, $r = -0.46$ and $p < 0.01$), increased time to perform the TUG test ($r = 0.49$, $p < 0.05$), enhanced perceived effort after completion of TUG ($r = 0.49$, $p < 0.05$), and reduced stride ($r = -0.53$, $p < 0.05$) and step lengths ($r = -0.49$, $p < 0.05$). High ratings on anxiety were significantly associated with high risk of falls (Berg Balance Scale, $r = -0.55$ and $p < 0.01$) and increased body sways in the medial-lateral axis ($r = 0.54$, $p < 0.05$).

DISCUSSION

We analyzed kinematic parameters of gait and balance, as well as subjective complaints (ratings of perceived exertion, pain, fatigue, stiffness, depression, anxiety) during performance on several motor and balance tasks in fibromyalgia patients and age-matched pain-free controls. Our results indicated that both gait and balance were severely impaired in FM, and that several parameters of motor performance were linked to clinical symptoms associated with FM.

Gait parameters such as speed, cadence, stride and step lengths, percentage of stance, and swing phases, and support base were significantly impaired in FM patients. These findings are in accordance with previous studies showing that FM patients displayed slower cadence during gait compared to pain-free controls (Pankoff et al., 2000b; Auvinet et al., 2006; Heredia Jiménez et al., 2009). Furthermore, it has been reported that FM women spent more time in double than in single support, as well as reduced muscle endurance and both isometric and isokinetic strength in knee joint flexion and extension (Valkeinen et al., 2008; Heredia Jiménez et al., 2009; Cherry et al., 2012). In addition, it has been suggested that generalized pain and overweight could inhibit the single support of body and increase the time of double support in FM (Heredia Jiménez et al., 2009; Cherry et al., 2012). This is of special relevance because the preferential use of hip flexors in comparison to plantar flexors of the ankle in FM patients would also indicate an altered mechanism for maintaining balance during gait (Winter, 1995; Pierrynowski et al., 2005; Valkeinen et al., 2008). Previous studies have also suggested that factors such as level of physical activity, bradykinesia and overweight, together with fatigue and pain could be also responsible for relevant alterations in muscle recruitment patterns during gait in FM (Pierrynowski et al., 2005; Auvinet et al., 2006; Heredia Jiménez et al., 2009). In this sense, our findings were consistent with previous studies showing that patients with chronic pain displayed a reduced level of activity during the morning and the evening compared to pain-free controls (Weering et al., 2009). It was also noteworthy that observed alterations of gait parameters in FM (for instance, a reduction of more than 30% in gait velocity and stride length compared to age-matched healthy individuals) were similar or even greater than those previously reported during aging (for instance, a reduction of 20% in older as compared to young individuals) (Elble et al., 1991; Li et al., 2011). Thus, it seems plausible that an altered pattern of gait could also contribute to the characteristic reductions of daily functioning in FM.

The analysis of body sway during performance on the modified version of the Romberg's balance test further supports the notion that FM may affect some subsystems responsible for postural control and balance. Body sways on the anterior-posterior and medial-lateral axes were significantly greater in FM patients than in pain-free controls. Furthermore, non-linear analyses of body sway time series showed that Hurst exponent values were significantly lower in pain-free controls (values ranging between 0.3 and 0.4) than in FM patients (values around 0.5). These findings were in agreement with previous data observed in healthy individuals (Duarte and Zatsiorsky, 2000) and patients with reduced mobility (Burgunder, 1998; Stylianou et al., 2011). Basically, Hurst exponents below 0.5 would indicate that shifts of the time-series in one direction are followed by shifts in the opposite direction, revealing an anti-persistent trend of body sway to maintain a stable body position along the time. By contrast, Hurst exponents close to 0.5 in FM patients would indicate that time-series were characterized by an uncorrelated pattern of body sway leading to a more unstable balance. This uncorrelated or random behavior may suggest the existence of relevant disturbances in the motor control system which could lead to an increased risk of falls in these patients. Our findings from the Timed Up and Go (TUG) task are also in agreement with this interpretation. We observed that FM patients took significantly more time to complete the task (around 17 s) than pain-free controls (8 s). These values were similar to those obtained in a previous study (Shumway-Cook et al., 2000) showing that older people performing the TUG in more than 13.5 s were more likely to have suffered a fall in the previous 6 months. The analyses of balance during functional activities (reaching, bending, transferring, and standing) further indicated that FM patients displayed higher risk of falls than pain-free controls. In this sense, it has been already reported that balance deficits could be considered as one of the top 10 most debilitating symptoms in FM (Bennett et al., 2007). Moreover, the observed values for risk of falls in the present study were similar to those previously reported in the elderly (Berg et al., 1991; Panton et al., 2006) and in Parkinson patients (Dibble et al., 2008; Fernandes et al., 2015). Taking into account that around 30% of people over 65 may fall at least once a year (Mannerkorpi et al., 1994; Sylliaas et al., 2009), one may speculate that risk of falls in FM patients could represent an important limitation in their elderly life.

Although the influence of psychological factors on motor disturbances observable in chronic pain is still unclear, a common assumption is that pain catastrophizing, hypervigilance, fear of pain, and subsequent avoidance of activities that are known to exacerbate pain (fear-avoidance model) might contribute to reduce physical activity and to alter gait and balance parameters (such as muscle weakness, slower walking, shorter step length, shorter stride time, or higher trunk muscle activity) in chronic back pain (Vlaeyen and Linton, 2000; Leeuw et al., 2007). In line with these previous findings, our data show that FM patients exhibited objective alterations in gait and balance, which were associated with frequent complaints such as pain, stiffness,

fatigue, depression, and anxiety. Nevertheless, our findings seem to suggest that gait and balance deficits could be related to different subjective FM complaints. Thus, for instance, reduced stride length and increased time taken to perform the TUG task were linked to high pain intensity, depression and stiffness, whereas increased body sway in the medial-lateral axis was positively associated with pain intensity and anxiety. In addition, other gait parameters such as gait velocity, gait duration or cadence were only associated with pain intensity, and body sways in the anterior-posterior axis or Hurst exponents of body sways in both axes were even not correlated with pain-related complaints in FM patients. These differences may reflect a differential effect of depression and anxiety on gait and balance and warrant further investigation in FM patients. Moreover, analyses of gait and balance may provide additional information for the identification of subgroups among fibromyalgia patients based on psychosocial and cognitive characteristics (Auvinet et al., 2006). Therefore, multidisciplinary interventions for fibromyalgia should include a focus on correcting functional deficits and instilling greater self-confidence in patients to engage in physical exercise to improve functional outcomes.

The present study has some limitations that should be taken into account for the interpretation of the results. Two-thirds of our FM patients were currently taking analgesic and antidepressant medication during data collection and, therefore, the possible side effects of these drugs on balance and gait cannot be completely discarded. In this sense, a recent study has shown that antidepressant use was one of the possible mediators for the association between obesity and risk of falls in community living older persons (Mitchell et al., 2015). It remains, however, unclear if similar effects could be observable in middle-age FM patients. Moreover, although our sample of FM patients displayed greater body-mass index than age-matched pain-free controls, they could not be considered as obese. Although prevalence of FM in men is significantly lower than in women, future studies should include representative samples of men, as well as medication-free and older participants to examine the mediator role of all these variables on gait and balance. Finally, it should be borne in mind that fatigue was assessed as a subjective symptom from the FIQ questionnaire. Further research is necessary to analyze if more objective and reliable measures of fatigue are also correlated with gait deficits in FM.

In conclusion, our results point toward significant impairments in balance in FM patients as compared with pain-free controls, as assessed by self-reports, standardized motor function tests and kinematic parameters extracted from participants' video recordings. We found that pain intensity, stiffness, fatigue, depression and anxiety were the most relevant factors in explaining some gait and balance deficits in FM. We have also found that FM patients displayed an abnormal pattern of body sways during a balance task, which could be associated with changes in the motor control system and explain a higher risk of falls. All these findings highlight the relevant role of postural control and balance for daily activity functioning in FM. Thus, specific activities directed toward the modification of these altered gait and balance patterns may be included in regular physical intervention programs for FM. This represents a relevant contribution considering that most of previous research on functional disability in FM was based on retrospective reports or on self-report measures rather than on objective measures of gait and balance.

AUTHOR CONTRIBUTIONS

IdSC designed the study, conducted the experiments and wrote the first version of the manuscript. All the authors contributed substantially to data analysis, interpretation of the results and critical revisions of the manuscript. The final version was approved by all authors. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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frontiers in Human Neuroscience

ORIGINAL RESEARCH
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Observing Grasping Actions Directed to Emotion-Laden Objects: Effects upon Corticospinal Excitability

*Anaelli A. Nogueira-Campos[1], Ghislain Saunier[2], Valeria Della-Maggiore[3], Laura A. S. De Oliveira[4], Erika C. Rodrigues[4] and Claudia D. Vargas[5,6]**

[1] Department of Physiology, Federal University of Juiz de Fora, Minas Gerais, Brazil, [2] Laboratory of Motor Cognition, Department of Anatomy, Federal University of Pará, Belém, Brazil, [3] IFIBIO Houssay, Department of Physiology and Biophysics, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina, [4] Post-Graduate Program in Rehabilitation Sciences, Unisuam, Rio de Janeiro, Brazil, [5] Laboratory of Neurobiology II, Neurobiology Program, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [6] Instituto de Neurologia Deolindo Couto, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

The motor system is recruited whenever one executes an action as well as when one observes the same action being executed by others. Although it is well established that emotion modulates the motor system, the effect of observing other individuals acting in an emotional context is particularly elusive. The main aim of this study was to investigate the effect induced by the observation of grasping directed to emotion-laden objects upon corticospinal excitability (CSE). Participants classified video-clips depicting the right-hand of an actor grasping emotion-laden objects. Twenty video-clips differing in terms of valence but balanced in arousal level were selected. Motor evoked potentials (MEPs) were then recorded from the first *dorsal interosseous* using transcranial magnetic stimulation (TMS) while the participants observed the selected emotional video-clips. During the video-clip presentation, TMS pulses were randomly applied at one of two different time points of grasping: (1) maximum grip aperture, and (2) object contact time. CSE was higher during the observation of grasping directed to unpleasant objects compared to pleasant ones. These results indicate that when someone observes an action of grasping directed to emotion-laden objects, the effect of the object valence promotes a specific modulation over the motor system.

Keywords: motor evoked potentials, motor resonance, valence, goal-directed actions, mirror neurons

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Jonas Kaplan,
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University of Minho, Portugal

****Correspondence:***
Claudia D. Vargas
cdvargas@biof.ufrj.br

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INTRODUCTION

One individual's perception of another individual's action and the response this causes in the brain are tightly linked phenomena. The neurophysiological basis of this phenomenon is thought to be based on mirror neurons discovered in the fronto-parietal network, including the premotor cortex, and the intraparietal sulcus (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996; Hari et al., 1998; Buccino et al., 2001; Rizzolatti and Craighero, 2004; Fogassi et al., 2005; Rizzolatti and Sinigaglia, 2010, for review). Mirror neurons are recruited when someone observes an action performed by others and when he/she executes the same action (for review Blakemore and Decety, 2001; Rizzolatti and Craighero, 2004; Rizzolatti, 2005; Fabbri-Destro and Rizzolatti, 2008; Keysers and Fadiga, 2008; Rizzolatti and Sinigaglia, 2010; Sinigaglia and Rizzolatti, 2011). Neurons with mirror-like properties have recently been described in a broader action-perception network involving the primary motor and

somatosensory cortices as well as regions related to memory and emotional processing (Mukamel et al., 2010; Molenberghs et al., 2012; Fogassi and Simone, 2013).

Such a vast action-perception network attests to its crucial role in coding others' actions in the brain (Fadiga et al., 1995; Calvo-Merino et al., 2005), in recognizing their meaning (Avenanti et al., 2005; Rossi et al., 2008; Akitsuki and Decety, 2009; Borgomaneri et al., 2012), predicting their consequences (Kilner et al., 2004; Aglioti et al., 2008; Fontana et al., 2012) as well as their intentions (Becchio et al., 2012; Sartori et al., 2012). Furthermore, there is robust evidence that the observer's motor system codes the expected temporal adjustments when the grasping unfolds over time (Gangitano et al., 2004), suggesting a perfect matching between action observation, and its execution (Gueugneau et al., 2015; Mc Cabe et al., 2015). Thus, motor representations activated by observed actions might allow the anticipation and the processing of the meaning implied in such actions (Umiltà et al., 2001; Urgesi et al., 2010).

Moreover, it has been widely suggested that emotion influences the response of the motor system. Most evidence in support of this statement comes from studies that investigated the effects induced by the observation of emotional pictures upon the motor system (Bradley et al., 1993; Oliveri et al., 2003; Azevedo et al., 2005; Pereira et al., 2006; Hajcak et al., 2007; Coombes et al., 2009; Coelho et al., 2010; Borgomaneri et al., 2012, 2014; Enticott et al., 2012; Hill et al., 2013). However, such studies never measured the activity of the motor system as a real-time action directed to an emotion-laden object unfolds. Enticott et al. (2012), for instance, examined CSE while participants observed videos of a static hand or hand movements after being shown a series of emotion-laden pictures. A higher CSE was found during the observation of hand movements presented after unpleasant pictures. In this study, the hand movement was directed to a mug, i.e., an object totally unrelated to the pictures' emotional content. However, the goal of the action represents a key aspect that modulates the activity of the motor system (Koch et al., 2010; Donne et al., 2011; Rizzolatti et al., 2014; Aihara et al., 2015 for review). In a previous study, we therefore devised a set of experiments in which the activity of the motor system was assessed through a realistic experimental paradigm in which participants had to grasp an emotion-laden stimulus (de Oliveira et al., 2012; Nogueira-Campos et al., 2014). The results showed that preparing to interact with unpleasant stimuli increases the motor system activity compared to pleasant ones. Based on these findings, we suggest that an unpleasant stimulus triggers aversive-like circuits in the brain whose activity has to be overcome so that action can be implemented, whereas a pleasant stimulus facilitates action implementation (de Oliveira et al., 2012; Nogueira-Campos et al., 2014).

Since many of our interactions in the environment rely on our ability to code the actions and/or emotions of others, in this study we designed an experiment to assess the impact of observing actions directed to emotion-laden objects on the motor system. The present study focused on the CSE of the observer's motor system while they watched video-clips depicting grasping directed to emotion-laden objects. We hypothesized that observing grasping directed to emotion-laden objects should induce a specific modulation upon CSE depending on the objects' valence content—unpleasant or pleasant. Accordingly, we expected that the valence of the to-be grasped objects should be taken into account during the observation of grasping directed toward them. More specifically, CSE should be higher when observing grasping directed to unpleasant objects. Thus, reflecting the higher preparatory activity related to the observation of grasping directed to that category of the objects.

MATERIALS AND METHODS

Participants

All volunteers provided informed consent for their participation in the experiments of this study. The experimental protocols were conducted according to the Declaration of Helsinki and were approved by the local ethics committee of the Clementino Fraga Filho University Hospital at the Federal University of Rio de Janeiro (004/09). Volunteers did not present or have a personal or family history of any neurological or psychiatric disorder. Also, they were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971).

Selection of Emotional Video-Clips

Sixty-five video-clips depicting the right-hand of an actor grasping different objects were used. All videos had a duration of 5 s. Movement time lasted approximately 2 s. The objects were grabbed with the index finger and the right thumb (pinch grip). Ninety healthy participants (62 women and 22 men, mean age ± SD: 21.1 ± 2.54 years) were instructed to watch each video-clip presented randomly on a screen positioned in front of them. After each video presentation, they were asked to evaluate each of them by means of the *Self-Assessment Manikin Scale* (Lang et al., 2008), as employed previously for emotional-laden stimuli (de Oliveira et al., 2012). In this affective rating scale, each video-clip was classified in their valence and arousal dimensions. Ratings of valence are indicated by the graphical representation of facial expressions ranging from a severe frown (most negative) to a broad smile (most positive). For arousal, this scale varies from a state of low to high alert. Participants may select any of the five figures, or the four blank spaces in between, on a nine-point rating scale for each dimension. In the valence dimension, nine represents the extreme of pleasantness, and one represents the extreme of unpleasantness. Likewise, for arousal, nine represents a high rating, and one represents a low rating. Upon each video-clip presentation, participants had 10 s to rate it based on these two measures. When a video-clip was rated between 4.5 and 5.5 for valence dimension with a low level of arousal (1–3) it was classified as neutral. Video-clips with lower and higher valence value with respect to the neutral set were then categorized as unpleasant and pleasant video-clips, respectively (**Table 1**).

A one-way Anova revealed a main effect of *valence* (neutral, pleasant, and unpleasant) [$F_{(2,\ 62)} = 168.17$, $p < 0.001$; $n_p^2 = 0.84$; $\beta = 0.81$]. *Post hoc* comparisons revealed that the observation of the unpleasant video-clips (mean ± SE: 3.49 ± 0.11) scored significantly lower than the neutral (5.20 ± 0.05) and the pleasant ones (6.64 ± 0.15), whereas the observation of the neutral video-clips scored significantly lower than the pleasant

TABLE 1 | Valence and arousal ratings for each video-clip.

Pleasant			Neutral			Unpleasant		
Objects	**Valence**	**Arousal**	**Objects**	**Valence**	**Arousal**	**Objects**	**Valence**	**Arousal**
Rolled money	8.35	6.97	Television remote control	5.59	2.72	Spider	2.74	6.44
Chocolate candy	7.76	6.06	Calculator	5.39	2.78	A guava with worms	2.84	6.21
A piece of Brazilian cake	7.50	5.67	Sunglasses case	5.36	2.73	An embalmed rat	2.84	6.20
Car key	7.46	5.60	Video tape	5.34	2.70	An embalmed mouse	2.96	5.93
A can of chocolate milk	7.37	5.47	Floss box	5.34	2.70	An embalmed frog	3.09	5.63
A piece of chocolate	7.24	5.27	Ink cartridge	5.30	2.62	Artificial excrement	3.18	5.44
Packet of condom	7.14	5.12	Spool of thread#2	5.19	2.45	A piece of cake with hair	3.39	4.95
Ipod	7.14	5.11	Charger	5.10	2.31	A denture	3.47	4.79
A piece of sweet bread	7.04	4.96	Soap dish	5.10	2.30	Toast with a fly	3.51	4.70
Cell phone	6.88	4.71	A Rubber stamp	5.09	2.29	Mousetrap	3.53	4.65
Credit card	6.84	4.65	Adhesive tape	5.08	2.27	An embalmed fetal skull	3.54	4.62
Jewelry box	6.73	4.47	Spool of thread	5.05	2.22	An embalmed fetal head	3.59	4.51
Toast with cheese	6.60	4.28	Band-aid box	5.02	2.17	A pack of cigarettes	3.67	4.32
Credit card#2	6.57	4.23	Foot emery	5.00	2.14	An embalmed human eye	3.76	4.14
Computer mouse	6.35	4.34	Gate remote control	4.96	2.07	A piece of bread	3.86	3.91
Car key#2	6.22	3.70	Staples box	4.90	1.97	Kidney	3.96	3.68
Flower	6.09	3.50	Box of clips	4.83	1.86	An embalmed gizzard	4.02	3.55
Deodorant	6.09	3.49	Pencil case	4.83	1.85	An embalmed fish head	4.22	3.09
A little teddy bear	6.04	3.41	White box	4.82	1.85	Cockroach	4.37	2.77
A pack of candy	5.91	3.21				Medicine box	4.41	2.66
Soap	5.87	3.56				Kidney#2	4.42	2.65
Wristwatch	5.85	3.13						
Hairbrush	5.70	3.28						
Ball	5.62	2.76						
A guava	5.60	2.74						

ones. In addition, there was a main effect for *arousal* [$F_{(2,\ 62)} = 32.01$, $p < 0.001$; $n^2_p = 0.51$; $\beta = 0.88$]. *Post-hoc* comparisons revealed that the observation of the unpleasant (4.52 ± 0.26) and pleasant video-clips (4.39 ± 0.22) scored similarly in terms of arousal ($p = 0.89$), and both scored significantly higher than the neutral ones (2.32 ± 0.07, $p < 0.01$; **Table 1**).

Ten of the video-clips classified as pleasant and 10 as unpleasant were selected to study the effect of valence on CSE during action observation (**Figure 1**). In terms of valence, the observation of pleasant video-clips (7.31 ± 0.15) was scored as significantly higher than that of the unpleasant (3.21 ± 0.10; $p < 0.001$; $\beta = 0.99$). In the arousal dimension, the observation of pleasant video-clips (5.38 ± 0.23) was comparable to the unpleasant ones (5.36 ± 0.23; $p = 0.95$). This precaution was taken as distinct neurobehavioral responses can be triggered depending on the arousal level for a same emotional category (Calvo and Avero, 2009; Leite et al., 2012; Wiens and Syrjänen, 2013).

In addition, the hand aperture used by the actor to grasp each object was measured for each video-clip. For this purpose, the specific frame in which the actor touched, and grabbed the object was identified by means of Movie Maker software. After that, the frame was assessed using the Irfanview program and a line between the index finger and thumb was traced to measure the distance between them. There was no significant difference in grip aperture when manipulating pleasant (5.47 ± 0.20 cm) and unpleasant (5.14 ± 0.26 cm) categories ($p = 0.25$). These objects were also balanced in weight so that pleasant (45.86 ± 3.72 g) and unpleasant (37.64 ± 3.32 g) objects did not differ ($p = 0.20$). This allowed for control of the crucial elements involved in grasping actions, since both the degree of muscle strength and the type of grasping required to manipulate the objects influence the level of recruitment of the motor system (Hendrix et al., 2009; Alaerts et al., 2010a,b).

Procedure

A further 14 volunteers (eight women and six men; mean age $\pm$ SD: 23.77 ± 4.75 years) were invited to passively observe the emotion-laden video-clips (pleasant and unpleasant) in order to examine the effect upon CSE. In a dimly lit room, the participants sat on a comfortable chair at a table where a 19-inch screen was positioned 60 cm away from them (**Figure 2**). At the beginning of the experiment, the right hand of the participant was positioned with the palm facing down over a pillow placed under the table, while the left arm was positioned over their leg. This position was kept throughout the experimental session. The experimenter read the following instructions before the experiment started: "Your task is to watch the video-clips that will be presented on the screen. These video-clips depict the hand of an actor grasping different objects. Please pay attention to them in order

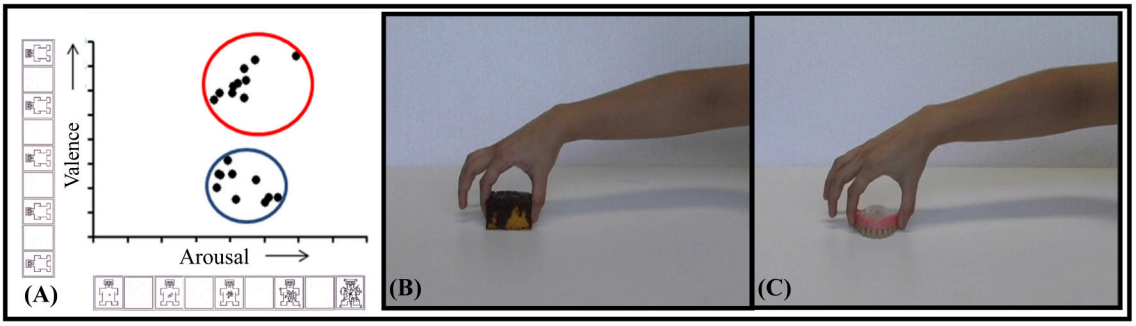

FIGURE 1 | Selected video-clips. (A) Distribution of selected video-clips in valence and arousal dimensions. The blue circle indicates the *unpleasant*, and the red one, the *pleasant selected video-clips*. Snapshot examples of pleasant **(B)** and unpleasant **(C)** video-clips.

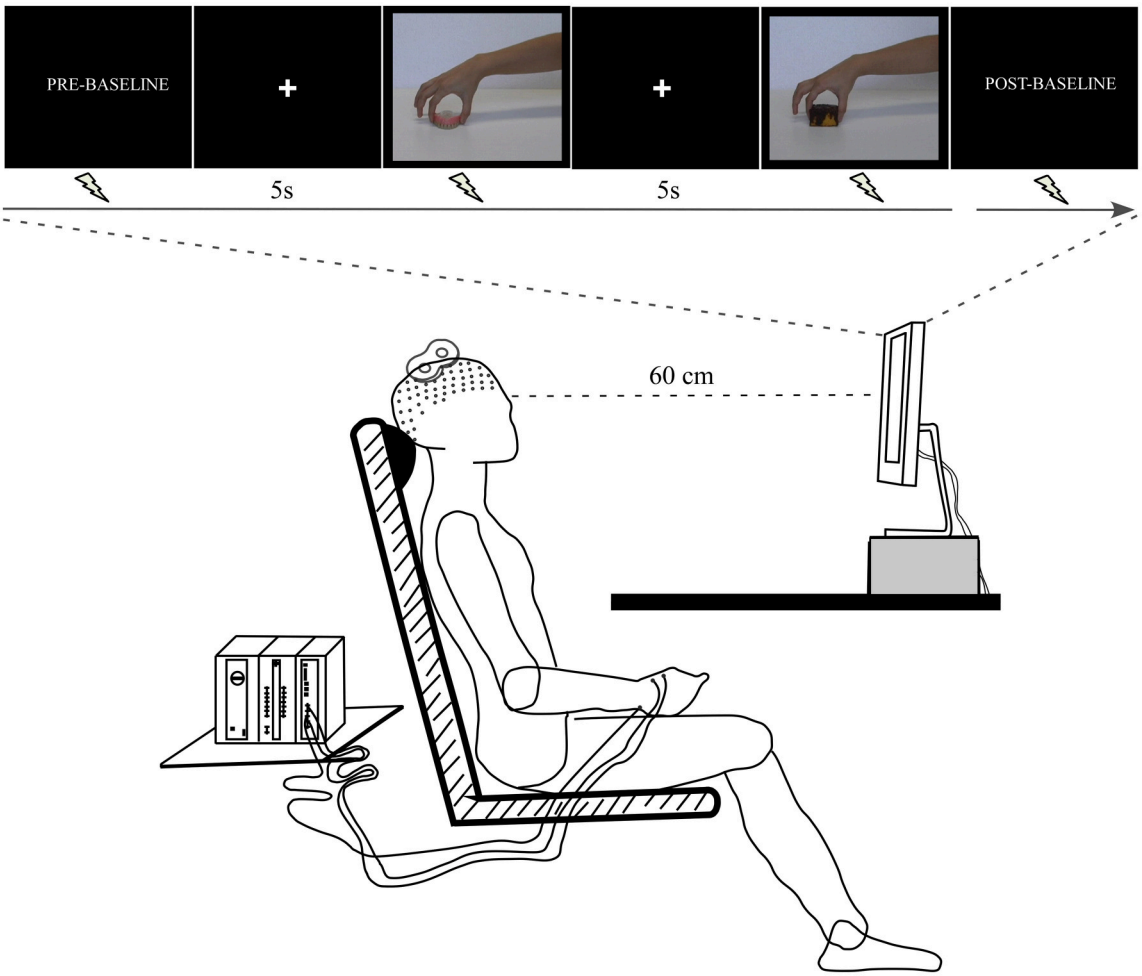

FIGURE 2 | Experimental procedure. The participant sat at a table where a computer screen was positioned. The arms remained at rest throughout the experimental session. The TMS coil was placed over the left motor cortex. The electromyographic (EMG) signal was recorded from *right first dorsal interosseus* (FDI) muscle.

to answer questions at the end of the experiment. Thank you for your participation." Then, a black screen that acted as a baseline was presented for 2 min (*Pre-Baseline*). Following this period, a white cross aligned with the center of the scene appeared on the black screen to focus the participant's gaze on this spot, and was followed by presentation of the video clips. This black screen

with a white centered cross was presented for 5 s between each individual clip. A total of 10 videos of each emotional category (pleasant and unpleasant) were randomly presented twice. At the end of this period, there was another baseline period (*Post-baseline*). The above sequence comprised an experimental block. A total of two blocks were carried out. TMS pulses were applied randomly during the video-clip presentation: an equal number either at maximum *grip aperture* or *contact time*. Thus, the total number of trials per condition (*maximum grip aperture* and *contact time*) and per emotional category was 20 per participant. The pulse was applied at these two different moments based on the grasping adjustments evolving through time; i.e., the phase when the hand is open to its maximum, followed by the phase of the hand touching the object (Jeannerod, 1984). The maximum grip aperture was considered as the time (≈70% of movement duration) when the hand reached the widest grip aperture value of the index-thumb distance. In addition, TMS pulses were delivered ten times at regular intervals during *Pre-baseline* and *Post-baseline* periods. The interval between TMS pulses was approximately 9–10 s, in order to avoid cumulative effects (Chen et al., 1997; Rothwell et al., 1999). Videos were presented using the Presentation software (Neurobehavioral System, Inc., Albany, CA). Blocks were separated by 5 min of rest. During this period, instructions concerning the upcoming block were repeated. **Figure 2** presents the experimental procedure.

Before the experiment started participants were exposed to a familiarization session during which they watched two video-clips from each emotional category that were not presented during the experimental session.

Video-Clip Rating

The 20 video-clips presented during the TMS session were evaluated at the end of the experiment in valence and arousal dimensions by 13 participants. Upon each video-clip presentation, participants had 10 s to classify how they had felt when they observed each emotional video-clip in the affective rating scale (SAM; Lang et al., 2008) using the same procedure previously described in de Oliveira et al. (2012). The duration of the entire experimental session was around 50 min.

Corticospinal Excitability (CSE)

CSE was measured by applying single pulses of Transcranial Magnetic Stimulation (TMS) by means of a double coil powered by a Magstim stimulator (Magstim 200; Magstim Co., Whitland, UK). A cap containing a 1 cm^2 spaced grid was positioned over the participant's skull to guide the TMS coil placement. Earplugs were provided to protect the participant's hearing. The coil was positioned tangentially over the optimal scalp location of the left primary motor cortex. First, the optimal position (hot spot) for eliciting motor-evoked potentials (MEPs) from the *right* first *dorsal interosseous* (FDI) muscle was identified. The resting motor threshold was then defined as the minimal intensity needed to evoke MEPs larger than 50 μV peak-to-peak amplitude in the FDI in at least three out of six pulses. The stimulation intensity was then set at 110% of the motor threshold to evoke MEPs.

Electromyographic Signal Acquisition

The electromyographic (EMG) signal was recorded using two pairs of Ag-AgCl electrodes, arranged in a bipolar montage over the belly of the *right* FDI. EMG activity was recorded using an EMG100 acquisition module coupled to an MP150 amplifier (BIOPAC Systems Inc., USA) and stored on a computer for offline analysis. Data were sampled at 20 KHz and band-pass filtered between 10 and 5 KHz with a 60 Hz notch filter.

Data Analysis

MEPs were quantified based on their latency and peak-to-peak amplitudes using a MATLAB routine (Mathworks, USA). This routine was designed to segment the EMG epochs corresponding to each trial. The beginning and the end of each MEP were marked manually on each trial. The latency was computed by counting the time elapsed between the TMS trigger and the beginning of the MEP response in the EMG signal. The MEP amplitude was calculated by measuring the peak-to-peak amplitude. The root-mean-square (RMS) of the EMG activity 200 ms prior to the TMS pulse was measured to ensure that the EMG baseline activity remained lower than 10 μV for all experimental conditions.

Outlier detection was computed by calculating the mean latency and mean MEP amplitude for each specific block and each participant. Latency and MEP amplitude values exceeding 2.5 standard deviations from the mean were marked as outliers and discarded. Based on this criterion, 10% of the trials were discarded from the analyses. The number of discarded trials did not differ between emotional categories ($p = 0.79$). Given that the CSE did not change between *Pre-baseline* (0.87 μV ± 0.64) and *Post-baseline* (0.92 μV ± 0.54; $p = 0.67$), these measures were collapsed into one baseline condition. The MEP amplitudes collected during emotional video-clips were normalized relative to this baseline for each participant within the block.

Statistical Analysis

Statistical analysis was performed with SPSS (SPSS; San Rafael, CA). A three-way repeated-measures Anova was used to compare CSE based on *valence* (pleasant and unpleasant), *conditions* (maximum grip aperture and contact time), and *blocks* (1 and 2). Tests of normality were performed to determine the probability that the sample came from a normally distributed population (Shapiro-Wilk's W test, $p \geq 0.05$). Data sphericity was verified before each test (for all tests: $p \geq 0.05$). The level of significance was set to 0.05. Tukey HSD *post-hoc* analysis was employed to test individual comparisons whenever a statistical significance was attained. *T*-test was used for comparing the video-clip ratings on valence and arousal dimensions. The effect size was computed based on the partial eta-squared (n^2_p). Also, the statistical power (β) was indicated whenever applicable.

Results

Video-Clip Rating

In terms of valence, the observation of unpleasant actions (2.85 ± 0.25) scored significantly lower than that of pleasant actions (6.81 ± 0.22; $p \leq 0.01$; $\beta = 0.99$). In addition, the observation of unpleasant (4.43 ± 0.45) and pleasant videos (3.78 ± 0.49) scored

similarly in terms of *arousal* ($p = 0.22$). These results can be seen in **Figure 3**.

Corticospinal Excitability (CSE)

A repeated-measures Anova revealed a main effect of valence [$F_{(1, 13)} = 102.57$, $p = 0.007$; $n^2_p = 0.44$; $\beta = 0.84$], indicating that CSE was higher during the observation of grasping unpleasant (0.95 ± 0.12) compared to pleasant (0.90 ± 0.12) objects (**Figure 4A**). This analysis also resulted in a significant condition vs. block interaction [$F_{(1, 13)} = 7{,}34$, $p = 0.02$; $n^2_p = 0.36$; $\beta = 0.71$]. *Post hoc* analysis showed that CSE was higher during the observation of maximum grip aperture (0.98 ± 0.12) compared to contact time (0.87 ± 0.12) during block 2 (**Figure 4B**). There was a tendency for condition [$F_{(1, 13)} = 4{,}23$, $p = 0.06$; $n^2_p = 0.25$; $\beta = 0.48$], but neither other main effects nor any significant interactions (see **Table 2**).

DISCUSSION

This aim of the study was to evaluate the effect of emotion on the motor system when the goal of the action was to interact with the source of the emotion. An ensemble of objects was selected and video-clips that mimic grasping actions in the real world were made. These videos were categorized using the Self-Assessment Manikin (Lang et al., 2008) and unpleasant and pleasant video-clips differing in *valence* but not in *arousal* were selected. To test the effect on CSE of passive observation of grasping actions directed to emotion-laden objects, TMS pulses were applied

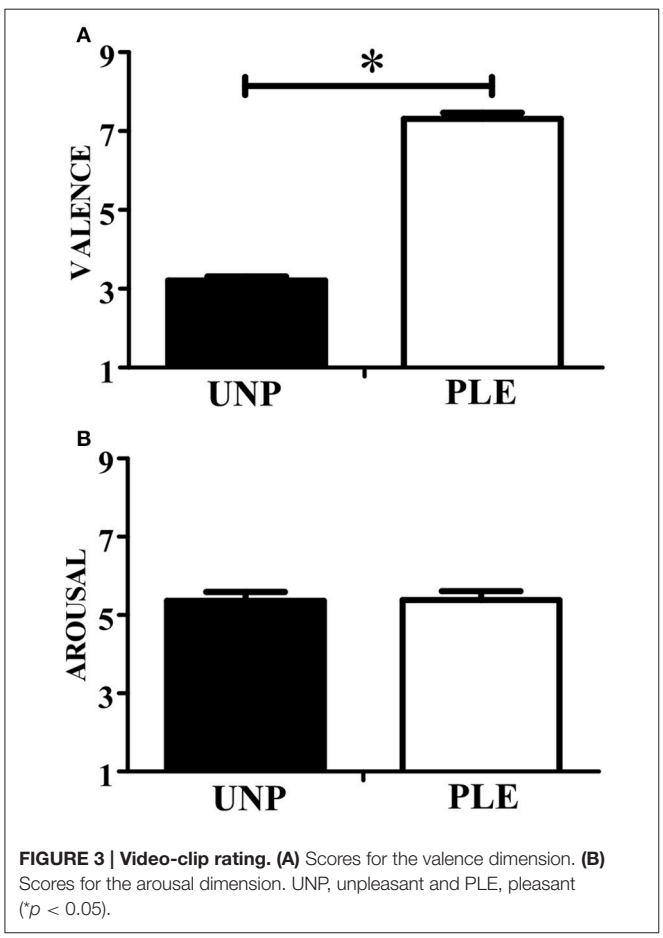

FIGURE 3 | Video-clip rating. (A) Scores for the valence dimension. **(B)** Scores for the arousal dimension. UNP, unpleasant and PLE, pleasant (*$p < 0.05$).

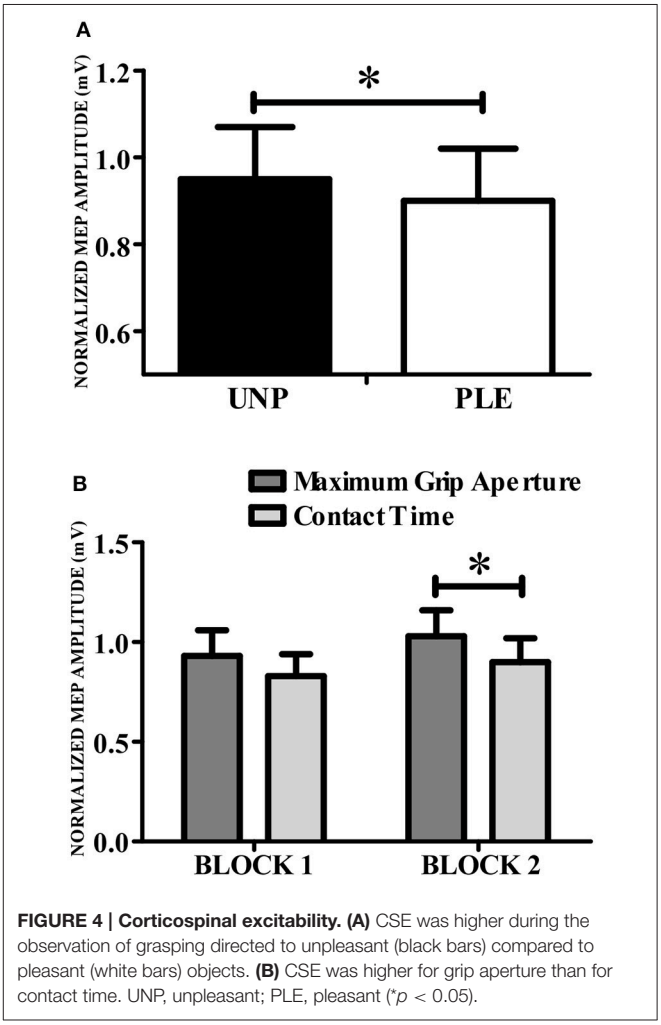

FIGURE 4 | Corticospinal excitability. (A) CSE was higher during the observation of grasping directed to unpleasant (black bars) compared to pleasant (white bars) objects. **(B)** CSE was higher for grip aperture than for contact time. UNP, unpleasant; PLE, pleasant (*$p < 0.05$).

TABLE 2 | MEP values (mV) per experimental condition.

	Mean	Standard Error
VALENCE		
Unpleasant	0.945	0.121
Pleasant	0.896	0.124
[$F_{(1. 13)} = 102.57$. $p = 0.007$; $n^2_P = 0.44$; $\beta = 0.84$]		
MAXIMUM GRIP APERTURE		
Block 1	0.926	0.126
Block 2	1.027	0.125
CONTACT TIME		
Block 1	0.826	0.113
Block 2	0.902	0.124
[$F_{(1. 13)} = 7.34$. $p = 0.02$; $n^2_p = 0.36$; $\beta = 0.71$]		

during presentation of the videos either at the moment of *maximum grip aperture* or *contact*. CSE was higher during the observation of grasping directed to unpleasant compared to pleasant objects. In addition, a larger CSE was found at the moment of maximum grip aperture compared to the moment of contact.

Unlike previous studies that investigated the effect of emotion over the motor system through the observation of emotional-laden pictures (Oliveri et al., 2003; Hajcak et al., 2007; Coelho et al., 2010; Enticott et al., 2012; Hill et al., 2013), in the present study videos depicting a goal-directed action were used. The observation of actions directed to an object provides a way to study the motor representations enrolled in the action itself (Koch et al., 2010). In addition, when observing an action, the target of the action seems to be taken into account (Fogassi et al., 2001; Umiltà et al., 2001; Cattaneo et al., 2005, 2009; Koch et al., 2010; Ocampo and Kritikos, 2011). This is in agreement with the basic idea that the motor system represents the transformations of goal-relevant sensory information to code motor outputs (Johansson and Cole, 1992). Herein, the higher CSE prompted by the observation of grasping directed to unpleasant compared to pleasant objects indicates that the valence implied in the actions' goal influences the observer's motor representations. Indeed, the observation of an action seems to automatically retrieve its motor representations (Rizzolatti et al., 1988, 2014; Rizzolatti and Craighero, 2004).

Notably, the CSE modulation during action observation matches the effects of valence found during motor preparation when actually grasping objects. In previous studies we examined the effects of preparing to grasp emotion-laden stimuli on readiness potential (RP; de Oliveira et al., 2012) and on CSE (Nogueira-Campos et al., 2014). RP is a marker of motor preparation and reflects the recruitment of the fronto-parietal areas preceding a voluntary movement (Shibasaki and Hallett, 2006). The CSE prompted by applying a TMS pulse over the primary motor cortex before the movement onset reflects preparatory activity as well (Hasbroucq et al., 1997). We found higher RP preceding grasping directed to unpleasant stimuli and lower RP directed to pleasant ones (de Oliveira et al., 2012). Likewise, we found a higher CSE for unpleasant stimuli and a lower CSE for pleasant ones when the TMS pulse was applied before the movement onset (Nogueira-Campos et al., 2014). The CSE seemed to reflect the higher recruitment of motor-related areas when the participants prepared to act in the unpleasant as compared to the pleasant category. Hence, when participants are asked to interact with emotion-laden stimuli they estimate the value embedded in the action's goal.

These changes not only occur when participants are preparing to grasp objects but are also triggered when participants observe others' actions, and unfold over time (Gangitano et al., 2004), giving support to the idea that during action observation the observer anticipates the outcome of others' actions (Kilner et al., 2004; Neal and Kilner, 2010; Rizzolatti et al., 2014). Thus, observing an action directed to emotion laden-objects may have triggered the motor representations in a predictive way, leading to a valence-laden modulation over the CSE in accordance with the effects that have previously been described during motor preparation (de Oliveira et al., 2012; Nogueira-Campos et al., 2014).

As expected, the observation of grasping directed to emotion-laden objects also prompted a higher CSE at the moment of maximum grip aperture compared to the moment of contact. Indeed, CSE is modulated based on the mechanical changes of the hand, i.e., higher for maximum grip aperture during the observation of reach-to-grasp actions (Gangitano et al., 2001, 2004). Herein, the coding of temporal hand adjustments was more pronounced in the second block, although there was a clear tendency in the same direction as the first block. The processing of motor cues imprinted in the observed action suggests the enrolling of the observers' motor system in coding such action, being more evident when the context is totally predictable (Kilner et al., 2004). Likewise, our results suggest that, beyond motor representations, the motor system also encodes the emotion content behind the observed action in order to guide the individuals' actions in interactive contexts. Crucially, the effect of emotion upon CSE was pervasive, possibly reflecting the core survival function of emotion (Mourão-Miranda et al., 2003; Lang and Bradley, 2010; Filmer and Monsell, 2013; Borgomaneri et al., 2014).

One could claim that the emotion-related effects on CSE are merely due to the observation of emotion-laden objects. Although there is evidence that arousal (Hajcak et al., 2007; Borgomaneri et al., 2012; Hill et al., 2013) and valence (Coelho et al., 2010; Enticott et al., 2012) of emotion-laden pictures modulate CSE, in our previous work, the observation of static graspable emotion laden-stimuli did not induce a specific modulation over the CSE (Nogueira-Campos et al., 2014). The divergent valence effect occurred only when the participants were engaged in preparing to make a movement. Such results strengthen the premise that the valence effect described here associates with the recruitment of motor representations enrolled in the preparation of the observed action itself. In addition, the present findings add to the previous one by showing a specific valence modulation over CSE during the observation of an action whose goal is to interact with the source of emotion.

On the other hand, we cannot preclude the possibility that the valence modulation over CSE is due to the recruitment of other brain regions besides the primary motor cortex. Indeed, the interactions between motor areas (putamen, pre-motor, and intraparietal cortex) and circuits coding emotion (insula, amygdala, and cingulate cortex) have been posed as fundamental in the processing of actions embedded in an emotional context (Grosbras and Paus, 2006; Pereira et al., 2010; Coombes et al., 2012). Interestingly, recent findings have proposed the insula, a region traditionally related to emotion expression (Bechara and Naqvi, 2004; Craig, 2009), as central for modulating motor system activity during the observation of arm movements (Di Cesare et al., 2015). Further studies should be conducted to broaden the investigation about the role of the motor system, including the action-perception

network, during the action directed to emotional-laden objects.

Finally, the present findings indicate that the valence implied in an observed action goal prevails over motor representations. Taken together, these results corroborate the proposal that both the temporal dynamics as well as the action goal are taken into account by the motor system during grasping directed to an emotion-laden object. The privileged influence of valence over CSE can reflect the capacity of the motor system to predict the consequences of actions in emotional interactive contexts. Further, this capacity may be crucial in correctly responding to other's actions.

AUTHOR CONTRIBUTIONS

Conception and design of the work: AN, GS, VD, LD, ER, and CV. Performing the experiments: AN and GS. Data Analyses: AN, VD, and CV. Writing the paper: AN and CV.

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