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Short-term synaptic plasticity in chronic migraine with medication overuse

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1. INTRODUCTION

1.1. Migraine: epidemiology and pathophysiology

Headaches are the most common painful syndromes in the young-middle age, affecting people's quality of life and causing a significant economic impact.

Among primary headaches, migraine is the most common and disabling one, affecting about 15% of the population of North America and Western Europe.

Since migraine was first included in the Global Burden of Disease study (GBD), which represents the most comprehensive worldwide observational epidemiological study to date, it has ascended the ranks of top causes of disability worldwide.

In the recent publication of 2016, in the age group of 15–49 years, migraine is the top cause of disability.

Even though a comprehensive knowledge of migraine pathophysiological mechanism is still lacking, many efforts have been done, in the last decades, to disentangle the complicated puzzle of migraine pathophysiology with the help of new research tools, as neurophysiological and modern neuroimaging technologies.

Clinical neurophysiology methods are non-invasive techniques that allow in vivo measurements of cortical excitability and electrocortical responses to various sensory stimuli and to deepen our knowledge on cortical plasticity in healthy subjects and in migraine patients.

Progresses in headache research were also favoured by the introduction of the International Classification of Headache Disorders (ICHD) and its revisions, because detailed diagnostic criteria allowed to perform better comparison of clinical and neurophysiological data between headache centres.

Migraine is considered a neurovascular disorder, where the trigemino-vascular system plays a central role. Neurogenic inflammation in meningeal trigeminal afferents that innervate the dural vasculature is characterized by the release of neuropeptides (substance P, calcitonin gene related peptide), leading to vasodilation, plasma extravasation and mast cell degranulation. These sensitized trigeminal

afferents, with their cell bodies located in the trigeminal ganglion, project to the trigeminal nucleus caudalis in the brainstem, which in turn projects to higher brain centers.

Indeed, together with the trigemino-vascular system, the brainstem, the thalamus and the cerebral cortex are also involved in migraine pathophysiology as main actors. In particular, abnormal thalamic pacemaker rhythmic activity, namely "thalamo-cortical dysrhythmia" could be responsible for a low level of cortical preactivation in the sensory cortices.

Since episodic migraine is characterized by recurrent clinical attacks separated by variable-length headache-free intervals, several studies have focused on ictal versus interictal electrophysiological abnormalities. They showed that migraine brain exhibits, if compared to healthy subjects, interictal dysfunctions of the central nervous system. Such dysfunctions probably represent the neurophysiological substrate of the clinical predisposition to attack recurrence.

This predisposition is the "core" of migraine disease and is genetically determined, with environmental factors that may act as triggers.

Furthermore, chronic migraine, defined as headache occurring on at least 15 days per month for more than 3 months, behaves from a neurophysiological point of view, like a "never ending migraine attack". Consequently, the exploration of neurophysiological disfunctions in migraine could help defining and understanding the mechanisms involved in migraine pathophysiology and chronification.

1.2. Habituation and sensitization in migraine

Since migraine is characterized by a dysfunction in sensorial information processing, studying the cortical responses to sensory stimulation, particularly in interictal phase, could help defining the neurophysiological markers of migraine brain, and probably understanding the mechanisms underlying predisposition to attack recurrence and chronification.

Habituation is defined as a decremental response to repeated sensory stimulations (Harris, 1943) and represents a physiological response observed in a wide range of neuronal circuits, allowing to control the signal-to-noise ratio generated by sensory stimuli and to orientate human response to environmental modifications.

According to the "dual-process" theory (proposed in the 70's by Groves and Thompson), during a sequence of repetitive stimuli, two opposing processes compete to define the final response: sensitization and habituation. The first one is prevalent in the initial part of the stimulus session, causing a transitory increase in response amplitude, while the second one occurs in the following phase and accounts for the delayed response decrement.

Habituation is a physiological response that protects the cortex against the risk of inward information overflow, while preparing brain networks to adequately respond to subsequent relevant stimuli.

Since habituation may be considered a basic form of learning (Thomson et al, 1966; Chung et al, 2002), the phenomenon of habituation is useful for studying the mechanisms of information processing and learning within the central nervous system and, ultimately, the neuronal substrates of behaviour.

Habituation of the evoked potentials can be assessed by averaging successive blocks of responses. Migraineurs are interictally characterized by a "deficient habituation", meaning that they show, instead of a physiological decrease (habituation), an amplitude increase of scalp-evoked potentials to repeated stereotyped stimuli.

This phenomenon was reported in migraine patients for almost all sensory modalities: visual (Schoenen et al, 1995), auditory (Ambrosini et al, 2009),

somatosensory (Ozkul et al, 2002) and painful stimuli (Valeriani et al, 2003; de Tommaso et al, 2005). Nevertheless, it was also reported for cognitive stimulations (Kropp et al, 1995).

Interestingly, the habituation deficit may have a familiar character and was proposed as a neurophysiological marker for migraineurs (Siniatchkin et al, 2001) and asymptomatic subjects at risk of developing the disorder (Di Clemente et al, 2007).

Besides the lack of habituation, evoked potentials in migraineurs are characterized by a low amplitude of the first block of averagings (for a review see Ambrosini et al, 2003), ruling out the theory that the habituation deficit in migraine could be due to cortex hyperexcitability. These data support the hypothesis of an interictal reduced pre-activation level of sensory cortices, possibly due to insufficient activation by aminergic projections from the upper brainstem (Schoenen et al, 1996) and, consequently, a dysfunction in thalamo-cortical drive, known as “talamo-cortical dysrhythmia” (Llinas et al, 1999; Coppola et al, 2007).

Therefore, the heightened response to repeated stimuli (or habituation deficit) in migraineurs is the consequence of sensory cortices “hyperresponsivity”, which results in an exaggerated energy demand and, possibly, in subtle cognitive dysfunctions (Magis et al, 2007).

However, habituation is not a static phenomenon but fluctuates over time in relation to the migraine cycle. In particular, the habituation deficit reaches its maximum during the days preceding the attack, while increases and normalizes immediately before and during the attack, when the thalamo-cortical drive also increases (Coppola et al, 2005). Such fluctuation is probably related to changes in serotonin transmission, which is low interictally (Panconesi et al, 2008) and may increase ictally.

On the other side, sensitization, defined as facilitation occurring at the beginning of the stimulus presentation, was evidenced during the attack, especially with somatosensory stimuli (as reflected by a significant increase in SSEP 1st N20-P25 block amplitude), while disappeared between attacks (Coppola et al, 2010).

Sensitization was evidenced also in chronic migraine with and without medication overuse. However, in chronic migraine without drug overuse a normal habituation

was found for visual evoked responses (Chen et al, 2011; Schoenen et al, 2011), while in medication overuse headache (MOH) a deficient habituation was found for somatosensory evoked potentials (Coppola et al, 2010).

Therefore, cortical sensitization and hyperresponsiveness (Coppola et al, 2010; Currà et al, 2011) could be considered neurophysiological markers of MOH.

The neural network underlying habituation is poorly understood. Consequently, the deficient habituation, even representing the most reproducible abnormality of evoked potentials detectable during the pain-free interval in migraineurs, still lacks a conclusive interpretation.

Relevant information on the pathophysiology of the interictal dysfunction in migraine came from studies of the high-frequency oscillations (HFOs) embedded in somatosensory and visual evoked potentials. Early somatosensory HFOs, reflecting spike activity in thalamo-cortical drives, were shown to decrease in interictal migraineurs and to normalize during the attack, while a significant habituation deficit of the late visual HFOs was observed in the interictal phase, demonstrating a dysfunction in cortical oscillatory networks, reflected by an abnormal thalamic rhythmic activity, named “thalamo-cortical dysrhythmia” (Coppola et al, 2007).

Several biochemical and neuroimaging studies suggested that the habituation deficit could be related to modifications in serotonin transmission, which fluctuate during the migraine cycle (Ferrari et al, 1993, Evers et al, 1999), reflecting dysfunctions in monoaminergic nuclei activity and activation of the pontomesencephalic areas of the brainstem during migraine attacks (Weiller et al, 1995; Bahra et al, 2001).

Futhermore, the serotonergic system is presumably affected by the chronic use of medications, determining neuronal hyperexcitability and trigeminal activation in patients affected by MOH (Srikiatkhachorn et al, 2000; Dobson et al, 2004).

1.3. Motor cortex excitability in migraine

Impairment of mechanisms regulating the responsivity to various stimuli is a hallmark of migraine brain. Although the mechanisms underpinning brain "dysexcitability" are still debated, there is general agreement that such abnormalities widely affect subcortical areas and likely the whole cerebral cortex.

Therefore, besides studying sensory processing in migraine patients, great effort was also made to characterize motor cortex excitability in migraine.

Cortical excitability of the motor cortex can be examined using transcranial magnetic stimulation (TMS) over the motor cortex and then recording the evoked peripheral activity from a muscle, namely the motor-evoked potentials (MEPs). TMS is a non-invasive technique that is used worldwide both in clinical practise, to assess the conduction of the descending cortico-nuclear and cortico-spinal pathways, and for neuroscientific purposes. Indeed, changes in motor activation and excitability can be easily assessed by recording MEPs (for a review see Rossini et al, 2015). Neurophysiological measures, such as corticomotor threshold (MT), MEP amplitude and latency, Cortical Silent Period (CSP) duration, Central Motor Conduction Time (CMCT) can be used to provide evidence of pathological changes in motor cortical control or corticospinal output in patients.

Corticospinal excitability can be estimated by measuring the cortical motor threshold (or resting motor threshold, RMT), which is the minimal intensity of motor cortex stimulation required to elicit a MEP of minimal amplitude in a relaxed target muscle. The MEP size can be estimated by measuring the peak-to-peak amplitude after setting the stimulation intensity at 115-125% of the individual's RMT.

Intestingly, studies regarding motor cortex excitability in migraine patients reported controversial findings. In particular, resting motor threshold in interictal migraine were reported to be normal (Werhahn et al, 2000), increased (Afra et al, 1998) or reduced (van der Kamp et al, 1996).

A recent neurophysiological study didn't find any difference in RMT between interictal migraineurs and controls but, by exploring the effect of a first conditioning stimulus on the motor evoked potential (MEP) elicited by a second test stimulus, modifications in short-term intracortical inhibition and facilitation mechanisms

according to the migraine cycle (ictal, interictal, pre-ictal) were disclosed. Indeed, they found decreased short-interval intracortical inhibition (SICI) in interictal migraineurs when compared to healthy controls, a shortened CSP only in female interictal migraineurs and a decreased ICF in pre-ictal compared to interictal migraineurs (Neverdahl et al, 2017).

Furthermore, intracortical excitability was found to be variable in relation to the intensity of stimulation, indicating that different neuronal circuits can show different activation and inhibition thresholds: an increased ICF was found in migraineurs, as compared to the healthy subjects, only by using a 110% intensity of the test stimulus (Cosentino, 2018). Anyway, neither in this case, the authors found any differences between interictal migraineurs and controls as regards RMT (Cosentino et al, 2018).

That probably happened because motor cortex excitability is not a static parameter. As happens for habituation in sensory cortices, motor cortex excitability may fluctuate according to the migraine cycle and, within interictal phase, according to the time interval from the last ictal phase.

Indeed, we recently found that motor cortex excitability (MEP threshold and amplitude) in interictal migraineurs varies on the basis of the time elapsed since the last attack: RMT is lower when long time interval has passed after an attack and is higher when measured close to an attack (Cortese et al, 2017). Such dynamic RMT variations in relation to the migraine cycle represent time-dependent plastic changes in brain excitability that resemble those occurring for visual and somatosensory evoked potentials.

Several neurophysiological studies failed to disclose significant differences in motor cortex excitability (in terms of RMT, latency and first MEP size) between chronic migraine patients, healthy subjects and episodic migraineurs (Cosentino et al, 2014; Cortese et al, 2018; Ozturk et al, 2002). That means that basal MEP amplitude in chronic migraine is not different from healthy subjects, but it doesn't exclude dysfunctions in motor cortex plasticity, as will be discussed later.

2. CHRONIC MIGRAINE

2.1. Clinical aspects of chronic migraine

Chronic migraine is a disease characterized by a deep impact on patients' life (see May et al, 2016), with considerable disability rates and burden of disease. Chronic migraine, if compared to episodic migraine, has a more profound impact on socioeconomic functioning and quality of life (Buse et al, 2012; Blumenfeld et al, 2011).

Impressively, about 25% of patients with chronic migraine report a very severe headache-related disability, as defined by the Migraine Disability Assessment Scale (also known as MIDAS). That brings to reduced household and family activities and high direct costs (related to healthcare and therapies) and indirect costs (due to absenteeism from work and reduced productivity) (Bigal et al, 2008; Munakata et al, 2009).

According to the current diagnostic criteria of the International Classification of Headache Disorders (ICHD-3 beta), chronic migraine is defined as headache occurring on at least 15 days per month for more than 3 months, fulfilling, at least 8 headache days per month, the criteria for migraine headache (Headache Classification Committee of the HIS, 2013). Noticeably, in contrast to earlier classification editions, analgesic overuse is no longer an exclusion criterion for the diagnosis of chronic migraine. Consequently, according the new criteria, patients with medication overuse should be considered as affected by both chronic migraine and medication overuse headache (MOH).

The prevalence of chronic migraine is about 1–2% in the general population, with three times higher prevalence in women than in men (Buse et al, 2012).

Primary chronic migraine is rare; usually chronic migraine usually evolves from episodic migraine with an annual progression rate of about 3% (Scher et al, 2003). Risk factors for migraine chronification are: age, female sex and low educational status (among the non-modifiable risk factors) and overuse of acute migraine

medication (Bigal et al, 2008), ineffective acute treatments (Lipton et al, 2015), obesity and insulin-resistance (Peterlin et al, 2010; Fava et al, 2014), depression (Ashina et al, 2012), and stressful life events (Scher et al, 2003) (among the potentially modifiable factors).

Probably the most important risk factor for migraine chronification is the overuse of acute migraine medication. Medication overuse headache (MOH) represents a relevant social burden, affecting around 63 million people worldwide (Kristoffersen et al, 2014). The prevalence of MOH in general population is between 1 and 2% (ranging from 0,5% and 7,2% in different countries), affecting mostly middle-aged adults from age of 30 to 50 years, with higher prevalence in studies from headache specialist centers, ranging from 30% to 50% of patients (Westergaard et al, 2014; Munksgaard et al, 2014). The majority of studies reports higher incidence in females with a male-to-female ratio of around 1 to 3–4 (Kristoffersen et al, 2014).

MOH consists of a complication of a pre-existing headache syndrome and is characterized by overuse of one or several types of acute painkilling medications as simple analgesics, combination-analgesics, ergots, triptans and opioids. The diagnosis is based on headache frequency (equal to or greater than 15 days/a month) and overuse of headache medications on more than 10 or 15 days per month, depending on the drug class, for more than 3 months. Noticeably, migraine is the most common pre-existing headache disorder.

Medication overuse discontinuation leads to reduction of headache frequency, facilitating prophylactic therapy effectiveness.

Even though MOH usually resolves once the overuse is stopped (Manzoni et al, 2015), it is no longer a requirement for the diagnosis to be made.

The risk of chronification depends on the type of used drug, with lower risk for triptans and ergotamine, if compared to analgesics and opioids (Thorlund et al, 2016).

The most important risk factors associated to the development of MOH are: regular use of benzodiazepines, depression, physical inactivity, smoking, age younger than 50, female gender and low level of education (Hagen et al, 2011). Furthermore, an increased risk of developing MOH was detected if a family history of MOH or other substance abuse was present (Cevoli et al, 2009).

Indeed, genetic polymorphic variants in genes of the dopaminergic system and genes related to drug-dependence pathways have been described as potential risk factors for excessive use of acute medications and consequent development of MOH (Cargnin et al, 2017).

Patients affected by MOH often show multiple psychiatric comorbidities. Anxiety and depression are the most frequently described (Lampl et al, 2016). An association was also found between MOH and greater susceptibility to drug dependency and with clinically relevant obsessive-compulsive disorder (Sarchielli et al, 2016). A high prevalence of sleep complaints, including insomnia, daytime sleepiness, and snoring was also reported (Sancisi et al, 2010) in MOH patients.

Various respiratory and cardiovascular conditions more likely coexist with chronic migraine than with episodic migraine (Buse et al, 2010).

Anyway, protective factors, such as physical exercise, stress management, and preventive medications, may help reducing the frequency of migraine attacks, thereby reducing the risk of migraine chronification.

Withdrawal of acute painkilling drugs is the first-line approach for the management of MOH patients.

A recent randomized clinical trial showed that complete discontinuation of acute medications is the most effective strategy (if compared to restricted medications intake) (Carlsen et al, 2018).

Withdrawal can be quite difficult for some patients because of the frequent appearance of withdrawal symptoms as headache, nausea, vomiting, anxiety, sleep disturbances, that usually last for 2–10 days and can be very disturbing.

The choice of the setting for withdrawal (inpatient or outpatient withdrawal) should consider several factors, including the type of overused medications, the duration of the overuse, the possible history of previous detoxification failures or psychiatric comorbidities.

In clinics, a standardized therapeutic protocol for medication withdrawal is lacking.

Several strategies are commonly used such as intravenous hydration, rescue medications (different from overused drugs), antiemetics, benzodiazepines, and sometimes corticosteroids.

Special reference needs to be made about prophylactic treatment.

Discussion between the three main options is still going on: some authors advocate withdrawal of acute medication alone, others suggest early prophylaxis alone, and a third group stands for withdrawal in combination with early prophylaxis (Rossi et al, 2009).

Although evidence-based recommendation for MOH treatment is not possible for the lack of randomized controlled trials, there is currently more evidence for discontinuation of acute medication overuse, or tapering plus early prophylaxis, than for withdrawal alone (Chiang et al, 2016).

Anyway, what we know for sure is that a successful detoxification leads to a better response for preventive treatments, even in patients with little improvement in headache frequency after withdrawal (Zeeberg et al, 2006).

There are various prophylactic treatment options for chronic migraine. Standard pharmacological treatment includes topiramate, which has been investigated in more than one double-blinded RCTs (Diener, 2007; Silberstein, 2009), but also candesartan (Stovner et al, 2013), amitriptyline (Magalhães et al, 2010), sodium valproate (Yurekli et al, 2008), gabapentin (Spira et al, 2003) and tizanidine (Saper et al, 2002).

Botulinum neurotoxin A (BoNT-A) is specifically approved for chronic migraine. In two large-scale phase III RCTs, called Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) 1 and 2, BoNT-A at a dose of 155-195 U, was shown to reduce the number of headache days in chronic migraine patients with or without acute medication overuse (Aurora et al, 2010; Diener et al, 2010).

Non-pharmacological therapies include biofeedback, manual therapy, stress management, neuromodulatory techniques. Neuromodulatory methods can target peripheral nerves or specific areas in the central nervous system. Among peripheral neuromodulation methods, the most frequently used are: pharmacological blockade of the greater occipital nerve (Saracco et al, 2010) and electrical stimulation of

occipital nerves (Dodick et al, 2015), supraorbital nerves (Schoenen et al, 2013; Hann et al, 2013); or the vagal nerve (Straube et al, 2015; Kinfe et al, 2015). Transcranial magnetic stimulation (TMS) (Shehata et al, 2016) and transcranial direct current stimulation (tDCS) (Antal et al, 2011; Andrade et al, 2017) are used to achieve central neuromodulation.

2.2. Cortical plasticity in chronic migraine

Animal studies, genetic studies, structural and functional neuroimaging, and neurophysiological examinations have been carried on to disclose some aspects of chronic migraine pathophysiology, which is far from being completely understood.

Dysfunction of the descending pain-modulating network (Bigal et al, 2008) and central sensitization probably play a central role in migraine chronification.

Some evidences support the hypothesis of a persistent dysfunction in the periacqueductal gray (PAG) in chronic migraine, likely caused by repeated migraine attacks (Welch et al, 2001).

Accordingly, disrupted functional connectivity between the PAG and brain regions primarily involved in nociception, somatosensory processing, emotion processing, and pain modulation was shown in animal models (Zihihua et al, 2017). Furthermore, atypical resting state functional connectivity of affective pain processing brain regions was evidenced in patients affected by chronic migraine (Todd et al, 2013).

Overall, neurophysiological and imaging studies seem to indicate that chronic migraine behaves like a "never ending migraine attack". Indeed, the pattern of brainstem activation was found to be similar to that observed during an attack in episodic migraine (Aurora et al, 2007) and evoked potentials studies indicate an increased cortical excitability of somatosensory and visual cortex (Coppola et al, 2010; Chen et al, 2011). Accordingly, using a method called magnetic suppression of perceptual accuracy (MSPA), decreased activity of inhibitory cortical interneurons, reflected in the smallest suppression index, was found in chronic migraine patients, if compared to episodic migraineurs and healthy controls (Aurora et al, 2007).

Increased evoked responses were found also after noxious stimulations: increased laser-evoked potential (LEP) amplitude (de Tommaso et al, 2003) and pain-related evoked potentials (PREPs) amplitude after electrical cephalic and extracephalic stimulation (Ayzenberg et al, 2006) were observed in chronic migraine patients.

Hence, chronic migraine is characterized by sensitization of sensory cortices, as reflected by an increased response amplitude to single or low numbers of non-noxious and noxious stimuli.

The clinical manifestation of central sensitization is probably represented by cutaneous allodynia, that occurs transiently during migraine attacks in episodic migraine, while is more persistent in chronic migraine (Lovati et al, 2008).

Interestingly, habituation (defined as a decrease in average response amplitude after high numbers of stimuli) was found to be normal in chronic migraine in the visual cortex (Chen et al, 2011), while a deficient habituation was shown in patients with MOH, at least in the somatosensory cortex (Coppola et al, 2010).

The differences in habituation between the two groups of chronic migraineurs (with and without medication overuse) are probably related to the different mechanism of migraine chronification.

In MOH patients, migraine chronification is the consequence of the effects of the prolonged overuse of drugs on the brain. Indeed, the increased SSEP amplitude in MOH was found to be proportional to the duration of headache chronification (Coppola et al, 2010).

The serotonergic system is presumably affected by the chronic use of medications, resulting in neuronal hyperexcitability, enhanced cortical spreading depression and trigeminal activation (Srikiatkhachorn et al, 2000; Dobson et al, 2004).

Interestingly, chronic migraine and MOH patients exhibit similar phenotype, similar response to single or low number of stimuli (cortical sensitization), but different adaptation to repetitive stimuli (normal habituation for CM and deficient habituation for MOH).

Furthermore, a progressive normalization of sensory processing after detoxification during follow-up was demonstrated (Munksgaard et al, 2013). This adds to the importance of detoxification to favour not only a clinical improvement but also the reversal of electrophysiological abnormalities of MOH.

Since sensory cortices are strictly interconnected with other cortical and subcortical structures, several neurophysiological and neurofunctional imaging studies tried to shed light on the complex balance between excitatory and inhibitory networks in the whole brain in chronic migraineurs.

A magnetoencephalography (MEG) study on a pediatric population showed an aberrant brain activation during a simple motor task (Leiken et al, 2016). The authors

found significantly prolonged latencies of movement-elicited magnetic fields in chronic migraine and relevant spatio-temporal and spectral differences between chronic and acute migraine, with a significant increase of brain activation in chronic migraine also in the ipsilateral sensori-motor cortices and deep brain areas. This finding indicate that chronic migraine is characterized by the recruitment of an abnormally large neural network for a basic motor task, indicating aberrant neural activation in both cortical and subcortical structures.

A PET study showed that MOH patients exhibit significant metabolic reductions in thalamus and an increased metabolism in middle temporal gyrus and insula relative to chronic migraineurs without medication overuse (Di et al, 2013).

Accordingly, a hypometabolism of the bilateral thalamus, orbitofrontal cortex (OFC), anterior cingulate gyrus, insula/ventral striatum and right inferior parietal lobule was found in MOH, with a following recovery to normal metabolism after withdrawal of analgesics, for all dysmetabolic areas except the OFC (Fumal et al, 2006).

Hence, several regions involved in pain processing networks were hypometabolic during medication overuse but recovered to normal metabolism after painkilling medications withdrawal, except for the OFC, whose dysfunction is linked with drug dependence and addiction. This region remained hypometabolic after successful detoxification, thus implying a potential causal role (Fumal et al, 2006).

Exploring motor cortex excitability Ozturk and coworkers found no differences in thresholds, latencies and amplitudes of motor evoked potentials between chronic, episodic migraine and controls, while, exploring cortical inhibitory circuits by measuring the TMS-induced cortical silent period (CSP), they observed longer duration of the cortical silent period (CSP) in CM patients, being significantly different from both other groups (Ozturk et al, 2002).

Another neurophysiological study on MOH patients revealed a normal CSP duration of the facial muscles in the whole group of MOH patients. Nevertheless, a subgroup analysis revealed that CSP duration was different according to the headache medication overused, with longer duration for patients overusing NSAIDs (Currà et al, 2011).

This finding of different neurophysiological effects depending on the overused drug, support the hypothesis of a direct effect of the overused medication in promoting plastic modifications in brain networks that may facilitate migraine chronification.

Taken together, the previous evidences reveal that chronic migraine and MOH patients, even similar from a clinical point of view, exhibit metabolic and neurophysiological differences that may suggest a different mechanism of migraine chronification.

Furthermore, even though motor cortex excitability (in terms of RMT and MEP amplitude) seems to be within normal limits, some evidences point toward dysfunctional plastic responses.

Indeed, studying motor cortex plasticity, a paradoxical inhibitory response was found after facilitatory high-frequency repetitive transcranial magnetic stimulation of the motor cortex in chronic migraine (Cosentino et al, 2014). The author hypothesized that in conditions of increased cortical excitability the rTMS trains induce paradoxical responses, mediated by cortical homeostatic mechanisms.

Hypothesizing that MOH and CM, despite exhibiting a similar clinical phenotype, could show different plastic behaviour, probably related to different pathophysiological mechanisms of migraine chronification, we recently performed a detailed examination of short-term plasticity mechanisms of the primary motor cortex in CM and MOH patients, using both low- and high- frequency rTMS over the motor cortex. We evidenced a dysfunction in brain plasticity in patients affected by MOH, showing a paradoxical inhibitory response to facilitatory trains of rTMS (Cortese et al, 2018), thus identifying distinctive neurophysiological mechanisms underpinning learning and plasticity in patients with CM or MOH.

3. SHORT-TERM SYNAPTIC PLASTICITY IN MEDICATION OVERUSE HEADACHE

3.1. Rationale of the study

Withdrawal from acute medication is the first-choice strategy in the management of MOH patients, but the mechanisms involved in clinical improvement after detoxification are not clear, even though numerous structural and functional neuroimaging studies showed that detoxification is associated to normalization of gray matter volume and connectivity of several brain areas involved in pain processing, cognition and planning strategies.

Since we previously found that patients affected by chronic migraine with medication overuse show a maladaptive plasticity of the motor cortex, with a paradoxical inhibitory response to facilitatory trains of rTMS (Cortese et al, 2018), we carried on a neurophysiological study to understand the effects of detoxication on motor cortex plasticity.

In particular, we performed an rTMS study to compare short-term plasticity mechanisms in MOH patients before and after withdrawal from acute medications. We found that the dysfunctions in short term potentiation mechanisms in MOH are fully reversible after withdrawal, indicating that this strategy may achieve clinical improvement by restoring the physiological brain plasticity. This finding adds to the importance of starting a withdrawal treatment as early as possible in patients with MOH in order to facilitate normalisation of brain plasticity mechanisms.

This study has been recently submitted for possible publication to “Neurological Sciences”.

3.2. Withdrawal from acute medication normalises short-term cortical synaptic potentiation in medication overuse headache

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Introduction

The *International Classification of Headache Disorders* (ICHD 3) [1] defines medication overuse headache (MOH) as headaches occurring ≥ 15 days per month for a period of at least 3 months as the result of excessive intake of acute medications such as non-steroidal analgesic drugs (NSAIDs) and triptans. Several electrophysiological studies have investigated the pathophysiology of MOH and demonstrated that patients with MOH exhibit characteristic neurophysiological abnormalities. For example, patients with MOH show response sensitisation of the somatosensory cortex in response to different repetitive sensorial stimulations, demonstrated by an initial increase in the amplitude of evoked potentials [2]. Patients with MOH also exhibit impaired amplitude habituation, defined as the absence of a decrease in amplitude in response to repeated stimulation [2–4]. Since habituation is a basic form of learning [5], these findings suggested that patients with MOH experience alterations in neural plasticity and learning processes.

We recently assessed neural plasticity in the motor cortex of chronic migraineurs with and without medication overuse using low- and high-frequency repetitive transcranial magnetic stimulation (rTMS). We found that, depending on the duration of overuse headache, patients did not show short-term potentiation of motor evoked

potentials in response to facilitatory trains of rTMS [6]. In contrast, chronic migraineurs without medication overuse showed normal responses to inhibitory/facilitatory trains of rTMS. These observations led us to hypothesise that medication overuse induces a dysfunctional state of brain plasticity. On this premise, we further speculated that medication-induced alterations in short-term plasticity would normalise after the discontinuation of medication overuse.

The aim of this study was to examine responses of patients with MOH to both low- and high-frequency rTMS over the motor cortex before and after drug withdrawal in comparison to normal subjects in order to understand the characteristics of short-term plasticity dysfunction in MOH.

Material and Methods

Subjects

We recruited 16 patients with *de novo* MOH (according to the International Classification of Headache Disorders III [1]) from our headache clinic. Of these, 3 patients were excluded because they did not meet the primary inclusion criteria (see below). We previously published the results of rTMS studies performed on the initial 8 patients [6] and have combined these data with data from 5 additional patients in order to verify the observed effect of acute medication withdrawal. Participants were included if they were between 18 and 65 years of age, had at least a 1-year clinical history of migraine, and had never completed a detoxification program before their first screening visit. The inclusion criteria were restricted to patients with MOH as a result of NSAID use only (IHCD-III code 8.2.3.2) based on a previous study demonstrating that these patients exhibit more pronounced sensorimotor abnormalities than patients overusing acute migraine medications such as triptans [2, 7]. Participants were excluded from the study if they had been taking regular medications in the previous 3 months (e.g., antibiotics, corticosteroids, antidepressants, benzodiazepines, or prophylactic migraine medication; contraceptive pills were allowed) or if they had a history of other neurological disorders, systemic hypertension, diabetes or other metabolic or autoimmune disease, or any other type of primary or secondary headache. All participants

received a comprehensive description of the study and provided written informed consent prior to participation. The study was approved by the local ethics review board and was conducted in accordance with the Declaration of Helsinki.

After application of the inclusion/exclusion criteria, the final dataset comprised 13 patients. All patients had a clear history of episodic migraine without aura (ICHD-III code 1.1) prior to the development of MOH. With the exception of 2 patients who indicated the presence of mild headache (mean visual analogue scale score, 4/10), all patients underwent MEP recordings in a pain-free state. Recordings were performed at least 3 hours after the last dose of medication; because patients with MOH self-administer acute medication in a compulsive manner, we were unable to prevent patients from taking medication on the day of recording. For comparison, we also recruited 16 healthy volunteers (HVs) with comparable distributions of age and sex and no personal or familial history (first- or second-degree relatives) of migraine or other health conditions. Neurophysiological data for these HVs were previously published elsewhere [6]. To avoid hormonal interference, female participants completed the experimental protocol between menstrual periods. All participants were right-handed.

Patients with MOH underwent a 3-week standard acute medication withdrawal program without any prophylactic medication. After the withdrawal period, patients were re-evaluated using the same experimental TMS protocol. We ensured that the post-withdrawal recording session occurred at least 3 days before and after a migraine attack, as verified by telephone or email interview.

TMS procedures

During the TMS procedure, patients were seated in a comfortable armchair and instructed to relax with their eyes closed. TMS was delivered through a high-frequency biphasic magnetic stimulator (MagstimRapid, The Magstim Company Ltd., Whitland, South West Wales, United Kingdom) connected to a figure-of-eight coil with a maximal output of 1.2 T. We first determined the optimal orientation and position of the coil (i.e., “hot spot”) over the left motor area for stimulating the first right dorsal interosseous (FDI) muscle. Thereafter, the resting motor threshold (RMT)

was identified using single TMS pulses. The RMT was defined as the minimal intensity required to elicit an electromyographic (EMG) response of at least 50 μV with 50% probability in a fully relaxed muscle. Complete relaxation of the FDI muscle was verified by the absence of EMG signals as determined by visual (on a monitor) and acoustic feedback. Because all participants were right-handed and because patients did not always experience the headaches on the same side, rTMS trains were delivered exclusively over the left motor cortex. EMG activity in the right FDI muscle was recorded with surface electrodes. Thereafter, 10 consecutive trains of 10 single pulses of TMS (stimulus intensity, 120% of the RMT; inter-train interval, 1 min) were delivered at a frequency of 1 or 5 Hz in 2 separate sessions (with an intersession interval of at least 1 week) in a randomised order. The resulting EMG signal was filtered (20 Hz–1 kHz) and stored on a personal computer. All recordings were collected during a 3-hour period in the morning between 09:00 and 12:00 by 2 investigators (C.L. and C.C.). The 10 trains of 10 stimuli were averaged and analysed off-line in a blind manner by a single investigator (F.C.). Peak-to-peak MEP amplitudes (μV) were measured for each of the 10 responses within the train of 10 stimuli.

Statistical analysis

All data were analysed in a blinded manner by a single investigator (G.C.) using Statistica version 8.0 (StatSoft Inc., Tulsa, USA) for Windows (Microsoft Corporation, Redmond, WA, USA).

We first checked the normality of the data distribution using the Kolmogorov-Smirnov test. A preliminary descriptive analysis revealed that some the peak-to-peak MEP amplitudes within individual rTMS trains had non-normal distributions. After log transformation ($\log_{10}[x]$), all data satisfied a normal distribution (Kolmogorov-Smirnov test, $p > 0.05$).

In order to compare the baseline findings in patients with MOH (MOH-b) with those of HVs, we performed a repeated measures analysis of variance (rm-ANOVA) with “group” as the between-subject factor (HV, MOH-b) and “stimuli” as the within-subject factor (n = 10). Moreover, as previously described [6], we calculated the slope of the linear regression line for all 10 stimuli using normalised data in order to quickly

evaluate MEP amplitude trends within trains of rTMS stimuli. Baseline slope values were compared using independent Student's t-tests. Relative changes (RC) in mean monthly headache days and in the slope of the linear regression were assessed using the following formula: RC = 100 − ([MOH-a × 100] ÷ MOH-b), where MOH-a represents findings obtained after medication withdrawal. Electrophysiological and clinical variables before and after the 3-week acute medication withdrawal program were compared using paired Student's t-tests. The threshold for statistical significance was $P < 0.05$.

Results

Basic clinical and neurophysiological parameters

Complete rTMS trains of MEPs were obtained for all study participants. Baseline neurophysiological parameters (RMT and the 1st MEP amplitude) were not significantly different between groups for either condition (1 and 5 Hz rTMS) or after 3-week withdrawal from acute medication in patients with MOH.

Effects of rTMS on baseline neurophysiological parameters

In a rm-ANOVA model using the rTMS 1 Hz MEP peak-to-peak amplitude as the dependent variable, there was a borderline significant main effect of stimuli ($F_{9,243}$ = 1.867, p = 0.057), but not of group ($F_{1,27}$ = 0.0255, p = 0.874) or the group × stimuli interaction effect ($F_{9,243}$ = 0.340, p = 0.961) (Figure 1, left panel). The slope of the linear regression of MEP amplitudes over all stimuli was not significantly different between groups (t = 0.490, p = 0.628) (Figure 2, left panel).

In a rm-ANOVA model using the rTMS 5 Hz MEP peak-to-peak amplitude as the dependent variable, there was a significant main effect of stimuli ($F_{9,243}$ = 2.367, p = 0.014) and the group × stimuli interaction ($F_{9,243}$ = 3.714, p = 0.0002) but not group ($F_{1,27}$ = 1.029, p = 0.319) (Figure 1, right panel). The slope of the linear regression of MEP amplitudes over all stimuli was significantly different between groups (t = 3.803, p = 0.0007) (Figure 2, right panel).

Effects of drug withdrawal on neurophysiological and clinical parameters

There was no significant difference in the mean slope of the linear regression of MEP amplitudes over all stimuli obtained in response to 1Hz rTMS before and after the 3-week drug withdrawal period in patients with MOH (t = −0.810, p = 0.937) (Table 2). In contrast, there was a significant difference in the mean slope of the linear regression of MEP amplitudes recorded in response to 5Hz rTMS between before and after drug withdrawal (t = -2,831, p = 0.015). Of note, the mean slope of MOH-a data was not significantly different from that for HVs (t = 0.854, p = 0.400).

Mean days with headache per month and the mean number of tablets taken per month were also significantly decreased 1 month after withdrawal compared to baseline in patients with MOH (t = 12.338, p < 0.001; t = 5.252, p < 0.001 respectively) (Table 1). Moreover, there was significant negative correlation between the percentage reduction of days with headache at 1-month after withdrawal and the relative variation of the slope of the linear regression of MEP amplitudes recorded in response to 5 Hz rTMS (r = −0.637, p = 0.019) (Figure 3).

Table 1. Demographics characteristics of study participants and headache profiles of patients. Data expressed as mean ± SD. HV healthy volunteers; MOH medication overuse headache patients before (MOH-b) and after (MOH-a) acute medication withdrawal; N number of subjects.

	HV (n = 16)	MOH-b (n = 13)	MOH-a (n = 13)
Women (n)	12	11	11
Age (years)	32.1 ± 10.2	34.5 ± 9.8	
Duration of history of migraine (years)		13.5 ± 10.3	
Days with headache/month (n)		24.4 ± 6.2	5.0 ± 4.8 *
Severity of headache attacks (0–10)		8.7 ± 1.8	7.3 ± 2.6
Nausea/vomiting (n)		12	
Photophobia (n)		11	
Phonophobia (n)		13	
Pulsating (n)		12	
Duration of the chronic headache (month)		41.9 ± 24.6	
Tablet intake/month (n)		42.5 ± 43.3	0.7 ± 1.2 *

**p < 0.001 vs. MOH before withdrawal*

Table 2. Transcranial magnetic stimulation (TMS) resting motor thresholds (RMT) and motor evoked potential (MEP) 1st amplitude (Log transformed) and slope of the linear regression line from the 1st to the 10th stimulus of the train in MOH subgroup (n = 13) before and after 3 weeks of drug withdrawal. Data expressed as mean ± SD. HV healthy volunteers; CM chronic migraine patients; MOH medication overuse headache patients; N number of subjects; § $p < 0.05$ v. HV.

	HV (n = 16)	MOH-b (n = 13)	MOH-a (n = 13)
1 Hz repetitive TMS train			
RMT (%)	54.9 ± 11.3	60.0 ± 11.4	61.2 ± 8.8
1st MEP amplitude	2.2 ± 0.3	2.3 ± 0.5	2.2 ± 0.5
MEP slope	- 0.0020 ± 0.0151	- 0.0056 ± 0.0286	- 0.0050 ± 0.0320
5 Hz repetitive TMS train			
RMT (%)	54.6 ± 11.4	59.2 ± 9.0	58.5 ± 6.6
1st MEP amplitude	2.3 ± 0.3	2.4 ± 0.3	2.2 ± 0.5
MEP slope	0.0104 ± 0.0309	- 0.0303 ± 0.0255 §	0.0006 ± 0.0300 *

**p < 0.05 vs. MOH before withdrawal*

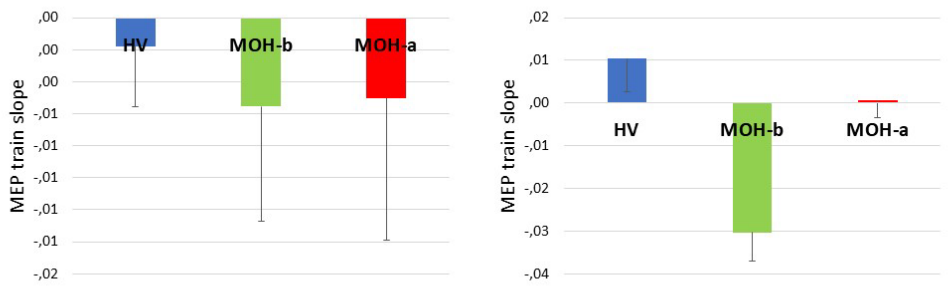

Figure 1. Motor evoked potentials (MEP) elicited by repetitive transcranial magnetic stimulation trains delivered at 1 Hz [left panel] and 5 Hz [right panel] at 120% resting motor threshold in healthy volunteers (HV), in medication overuse headache (MOH) patients before (MOH-b) and after (MOH-a) 3-week drug withdrawal.

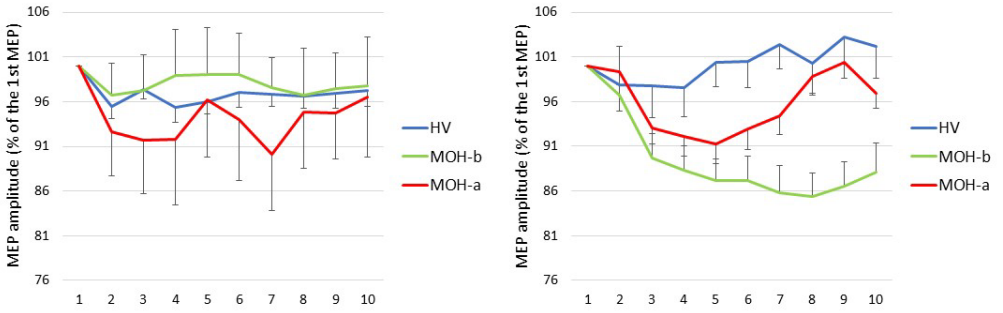

Figure 2. Bar charts representing the motor evoked potential (MEP) amplitude slope of the linear regression line from the 1st to the 10th stimulus of the 1 Hz [left panel] and 5 Hz [right panel] train of transcranial magnetic stimulations in healthy volunteers (HV) and medication overuse headache patients before (MOH-b) and after (MOH-a) 3-week drug withdrawal.

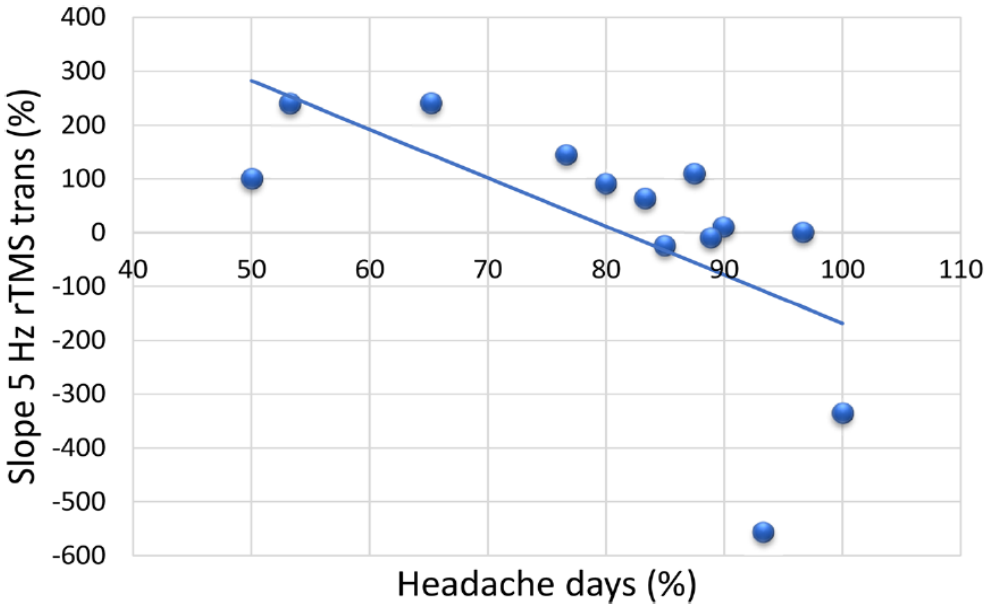

Figure 3. Percentage changes in the mean monthly headache days and the slope of the linear regression after patients with medication overuse headache completed a 3-week medication withdrawal program.

Discussion

The main finding of the present study was that a standard withdrawal program for patients overusing medication restored normal short-term synaptic potentiation in

the primary motor cortex of patients with MOH. Several neurobiological factors can account for these results.

In healthy subjects, trains of rTMS alter MEP amplitudes during and immediately after stimulation depending on the frequency and intensity of stimulation. When applied over the motor cortex at suprathreshold intensity (120% RTM), high frequency (5 Hz) rTMS increases MEP amplitudes [8], whereas low frequency stimulation (1 Hz) diminishes MEP amplitudes. Therefore, rTMS produces plastic changes in motor cortex excitability that outlast the period of stimulation for a period of minutes to hours [8–10].

The results of this study confirm our previous finding of dysfunctional short-term synaptic potentiation in patients with MOH [6]; in this study, trains of high-frequency rTMS induced a paradoxical decrease in amplitude in patients with MOH prior to medication withdrawal. This neurophysiological dysfunction may reflect a general alteration in plasticity and learning processes in the MOH brain. Moreover, the absence of these abnormalities in another group of patients with chronic migraine patients without MOH suggests that these findings are specifically related to medication overuse. In our previous study, this conclusion was underscored by the observation that longer durations of medication overuse were associated with more pronounced dysfunction of short term potentiation in the motor cortex [6], as previously demonstrated for the somatosensory cortex [2].

The present results expand on our previous findings by demonstrating that complete medication withdrawal restores normal short-term potentiation mechanisms within the motor cortex of patients with MOH. Moreover, withdrawal-related normalisation of rTMS responses corresponded to a change from a chronic migraine to episodic migraine as indicated by a relative reduction in the number of monthly headache days.

Since the primary motor cortex is involved in several aspects of pain integration and modulation, likely influencing affective or sensory components of pain or by top-down activation of descending antinociceptive systems [11, 12], drug withdrawal may induce the normalisation of a complex network involving brain areas that participate in pain modulation and control such as M1. Consistent with this idea,

previous studies have associated the discontinuation of medication overuse with the normalisation of several neurophysiological parameters and morphological features in brain areas of the salient network (also known as the “pain matrix”) [13].

Pain-related cortical potentials [4, 14] and spinal noxious flexion reflex responses [15] are sensitised in patients with MOH. These abnormal responses normalise after withdrawal treatment [4, 14, 15]. Perrotta and colleagues found that at the spinal level, the sensitisation process in MOH was related at least in part to insufficient descending inhibition from the brainstem, subserving the counterirritation phenomenon activated by heterotopic pain stimulation to suppress incoming nociceptive information [15]. The supraspinal antinociceptive structures include the periaqueductal grey, rostral ventromedial medulla, thalamus, nucleus raphe magnus, and nucleus reticularis gigantocellularis [16]. Altered structural integrity and functional connectivity of descending pain modulatory areas such as the periaqueductal grey [17–20] and thalamic nuclei [21] has been repeatedly identified in patients with MOH. These structures are all interconnected with areas belonging to the salient network such as the sensorimotor cortex and orbitofrontal and anterior cingulate cortices [13].

A voxel-based morphometry study identified significant increases in grey matter volume in the midbrain (including periaqueductal grey matter) of patients with MOH and subsequent decreases in volume after the discontinuation of medication overuse. Of note, low grey matter volume in the orbitofrontal cortex before withdrawal was associated with a poor response to drug discontinuation in a previous study [17]. In another study, the orbitofrontal cortex was less connected both metabolically [22] and functionally to nociceptive input regions such as spinal trigeminal nucleus and cerebellum [23] in patients with MOH before drug withdrawal, whereas these connections were normalized after drug withdrawal [22, 23]. Taken together, these data support the hypothesis that medication overuse promotes maladaptive neurophysiological and morphological changes in the brain.

Conclusion

In conclusion, we demonstrate that the dysfunction of short-term plasticity mechanisms in patients with MOH are alleviated by the discontinuation of medication overuse. On this premise, clinical improvements associated with withdrawal treatment may be related to the restoration of physiological brain plasticity. Our findings underscore the importance of initiating withdrawal treatment as early as possible in patients with MOH in order to facilitate normalisation of brain plasticity mechanisms. Future studies in a larger cohort of patients are necessary to determine the exact relationships between neurophysiological changes and clinical variables in patients with MOH, and whether the normalisation of such brain processes allow patients to regain clinical efficacy from acute and prophylactic migraine medications.

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4. DISCUSSION AND FUTURE PERSPECTIVES

Migraine pathophysiology represents a complicated puzzle, which has not been completely disentangled.

Overall, the scientific evidences highlight the concept that both episodic and chronic migraine are characterized by neurophysiological dysfunctions in sensory processing and motor cortex plasticity, that probably represent permissive factors predisposing the brain to migraine attacks and pain chronification. The understanding of such dysfunctions can be useful not only to shed light on the complex mosaic of migraine pathophysiology, but also to set targets for neuromodulatory therapeutic strategies to prevent migraine attacks and interfere with the mechanisms involved in migraine chronification.

Chronic migraine is characterized by a maladaptive plasticity. From an electrophysiological point of view, sensory cortices in chronic migraine show abnormalities that have also been reported in episodic migraineurs during attacks, as if chronic migraine was a “never ending migraine attack”. Indeed, sensory cortices in chronic migraine are sensitized and exhibit normal habituation. Contrarily, in medication overuse headache (MOH) patients, sensitization and deficient habituation were demonstrated in the sensory cortices (for a review see Coppola et al, 2013).

Even though no differences were found in motor cortex excitability between chronic migraine, episodic migraine and healthy subjects, some studies revealed alteration in motor cortex plasticity.

Indeed, a paradoxical inhibitory response was found after facilitatory high-frequency repetitive transcranial magnetic stimulation of the motor cortex in chronic migraine (Cosentino et al, 2014)

The interest in studying motor cortex plasticity in chronic pain syndrome, came from the results of several neurostimulation studies showing that both invasive and non-invasive neuromodulatory techniques applied on the motor cortex could achieve an analgesic effect in various kinds of chronic pain (Leufaucheur et al, 2001; Fregni et al, 2006), probably through the activation of a top-down control of the

thalamocortical pathways or favouring opioids release (see Dos Santos et al, 2016). Furthermore, chronic pain can induce a reorganization of the motor cortex, whose extension is positively associated to pain intensity (Lotze et al, 1999).

Comparing motor cortex responses to trains of facilitating (high-frequency) and inhibiting (low-frequency) TMS in patients affected by MOH with those affected by chronic migraine (without medication overuse) and with healthy subjects, we showed that in MOH patients, rTMS-5 Hz depressed instead of potentiating MEP amplitudes with a significantly different response from that in HVs and CM patients (Cortese et al, 2018).

This finding suggests that CM and MOH patients, although exhibiting a similar phenotypic expression, represent distinct pathological conditions, characterized by different pathophysiological mechanisms of migraine chronification.

Furthermore, we found that the slope of the linear regression of MEP amplitudes was negatively correlated with the duration of overuse headache in MOH patients.

That means that medication overuse itself may probably promote plastic modifications in the motor cortex. This hypothesis is also supported by the finding of different CSP duration in MOH patients according to the different overused drug.

Studies about the relationship between chronic migraine and motor cortex plasticity could be interesting, not only to disclose the neurophysiological mechanisms underpinning learning processes and plastic behaviour in chronic migraine, but also to develop future therapeutic targets and interventions.

Interestingly we found that a 3-week pharmacological wash-out program restored a normal short-term synaptic potentiation in the primary motor cortex of patients with medication overuse headache. This finding has important pathophysiological implications. Firstly, a direct effect of medication overuse on the brain, causing short-term plasticity dysfunctions, may be hypothesized. Secondly, since such dysfunctions are reversible after drug discontinuation, it's conceivable that the restoration of physiological brain plasticity could be the neurophysiological underpinning of the clinical improvement.

The presence of brain dysfunctions in MOH patients that can be reverted after detoxification was also described using metabolic and functional neuroimaging

techniques. These findings support the importance of early medication withdrawal in MOH patients also to prevent the development of more pronounced alterations in brain plasticity.

Probably drug withdrawal is able to induce the normalization of a complex network involving areas participating in pain modulation, including the primary motor cortex. Indeed, this area is known to be involved in several aspects of pain integration and modulation, likely influencing affective or sensory components of pain or by top-down activation of descending antinociceptive systems.

Our findings have important implications in neurorehabilitation.

Since neurorehabilitation includes all the approaches aimed to aid recovery from a nervous system injury or dysfunction and reduce disability, drug withdrawal in medication overuse headache could be completely considered a neurorehabilitation strategy. Indeed, its objective is brain recovery both from an electrophysiological point of view, with the restoration of physiological cortical plasticity, and from a clinical point of view, reducing the disability caused by chronic migraine and inducing the conversion from chronic to episodic migraine.

Even though, sometimes, simple information and advice may be enough to achieve headache improvement, for several patients drug discontinuation could be quite hard. Patients need to be guided by the physician during the process since withdrawal symptoms (headache, nausea, vomiting, arterial hypotension, tachycardia, sleep disturbances), lasting generally for 2–10 days, could complicate the discontinuation phase and induce patients to fall back into medication overuse.

The normalization of brain plasticity after medication discontinuation underscores the importance of initiating withdrawal treatment as early as possible in patients with medication overuse headache in order to induce the restoration of physiological brain plasticity and prevent the development of more pronounced alterations in brain plasticity and learning processes.

An interesting future perspective could be to use neuromodulatory strategies in order to normalize brain plasticity in medication overuse headache patients, thus helping them in the withdrawal treatment.

Remarkably, the response to high frequency stimulation could be used as a biomarker during the discontinuation process and to distinguish between chronic migraine patients with or without medication overuse.

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6. APPENDIX: OTHER SCIENTIFIC WORKS ON MIGRAINE

This appendix collects three research articles on episodic and chronic migraine that I have published during my PhD program, in collaboration with my research team.

Patients were recruited from our headache clinic and underwent neurophysiological examinations in our laboratories. The results of these studies represent an important tile in the complex puzzle of migraine pathophysiology, and particularly in the understanding of cortical excitability and plastic mechanisms in both somatosensory and motor cortex.

The last work, regarding “Short-term cortical synaptic depression/potentiation mechanisms in chronic migraine patients with or without medication overuse”, showed a maladaptive plasticity of the motor cortex in chronic migraine with medication overuse, giving us the hint to deepen our understanding about this field, conceiving a study about the effect of detoxication on short-term synaptic plasticity of the motor cortex in MOH patients, which has been chosen as the topic of my PhD final work.

A. Anodal transcranial direct current stimulation over the left temporal pole restores normal visual evoked potential habituation in interictal migraineurs

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Background

Migraine is a neurological disorder that is characterized by recurrent clinical attacks separated by variable-length headache-free intervals. Although the pathogenesis of migraine is far from completely understood, clinical neurophysiology and neuroimaging studies in recent decades have disclosed subtle functional and morphological abnormalities that manifest during the interictal phase and distinguish migraineurs from normal healthy subjects [1–3]. Among the various subcortical and cortical areas implicated in migraine pathophysiology, emerging evidence highlights the temporal pole (TP) as a key neural substrate. In humans, the TP serves as a multimodal neural hub that receives and integrates various sensory modalities including olfactory, auditory, taste, and visual inputs. Moreover, the TP participates in the ventral visual stream (VVS) for visual information processing [4–6]. During an olfactory task, interictal migraineurs exhibited significantly higher brain glucose metabolism in the left TP compared to control subjects [7]. Moreover, BOLD signal in the TP in response to noxious stimulation was reduced in interictal patients compared to patients who were actively experiencing a migraine [8, 9]. In resting-state MRI studies comparing interictal migraineurs to healthy control subjects, decreased grey matter density was observed in the left TP [10] and the left TP exhibited decreased connectivity with components of the default-mode network [11]. Finally, the TP was implicated as an important area for differentiating patients with migraine from healthy control subjects in a cross-sectional brain MRI investigation [12]. Taken

together, these findings suggest that the TP is both intricately related to the pathophysiology of migraine and sensitive to the cyclical recurrence of migraine attacks.

Transcranial direct current stimulation (tDCS) is a non-invasive technique for neuromodulation in humans that affects cortical excitability in a polarity-specific manner [13, 14]. Anodal polarization increases the excitability of cortical areas below electrodes, whereas cathodal polarization typically decreases cortical excitability [15]. A number of tDCS studies in different pain disorders [16, 17] have demonstrated that tDCS is well-tolerated by patients [18]. Anodal tDCS proved effective over either the motor cortex or the dorsolateral prefrontal cortex when used as prophylactic strategy both in episodic [19] and chronic [20, 21] migraine. Moreover, some studies reported that, in addition to the therapeutic effects, tDCS over the visual cortex also normalized interictal cortical hyperresponsivity in episodic migraine [22].

Nonetheless, to our knowledge, no study to date has targeted the TP for anodal tDCS in migraine, to enhance interictal temporal lobe activity and thereby interfere with an aspect of migraine pathophysiology. Thus, we examined whether anodal stimulation of the TP could restore normal function of the TP and thus physiological information processing in migraine. Moreover, given that the TP processes all kinds of sensorial information except for somatosensory information, we examined the habituation responses of evoked potentials to somatosensory stimuli (as a negative control) as well as visual stimuli.

Methods

Participants

Forty patients with migraine without aura (diagnosed in accordance with the International Classification of Headache Disorders III beta edition) were recruited from our headache clinic (Table 1). Of these, 4 patients were excluded because they did not meet the primary inclusion criteria (see below). Subjects were included if they were between 18 and 65 years of age and had at least a 1-year clinical history of migraine with 2–8 attacks per month. The use of preventive anti-migraine medication was not permitted during the 3 months preceding the study. The primary inclusion

criterion was being attack-free for at least 3 days before and after each recording sessions, and was verified by headache diary and telephone or e-mail interview. Subjects were excluded from the study if they were regularly taking medication (e.g., antibiotics, corticosteroids, antidepressants, benzodiazepines, or prophylactic migraine medication) except for contraceptive pills; if they did not have a best-corrected visual acuity of > 8/10; and if they had a history of other neurological disease, systemic hypertension, diabetes or other metabolic disease, autoimmune disease, or any other type of primary or secondary headache. Female participants were always recorded mid-menstrual cycle. All participants received a complete description of the study and provided written informed consent. The study was approved by a local ethical review board and was conducted in accordance with the Helsinki Declaration.

Table 1 *Descriptive statistics of clinical and demographic characteristics of migraine patients between attacks in the sham and real group*

	Real (*n* = 18)	Sham (*n* = 18)	*p* value
Women (n)	13	11	0.495
Age (years)	28.6 ± 7.6	26.9 ± 4.9	0.430
Duration of migraine history (years)	15.6 ± 8.3	12.4 ± 7.0	0.220
Attack frequency/month (n)	5.0 ± 3.2	3.9 ± 2.1	0.231
Attack duration (hours)	17.1 ± 17.4	18.1 ± 14.8	0.854
Visual analogue scale (n)	7.0 ± 0.7	6.6 ± 1.2	0.230
Days from the last migraine attack (n)	8.5 ± 8.5	11.7 ± 13.0	0.388
Family history of migraine (%)	51.4	48.6	0.210
Acute medication intake/month (n)	2.0 ± 1.9	2.0 ± 1.8	0.996

Data are expressed as means ± SD

Experimental procedure

The 36 enrolled patients were equally randomized to receive anodal tDCS (N = 18) or sham tDCS (N = 18). Randomization was conducted using a secure web-based database. For all patients, visual evoked potential (VEP) and somatosensory evoked potential (SSEP) recordings were performed in a random order during a single session before and immediately after real or sham tDCS. All recordings were performed in the afternoon (between 14:00 and 18:00) by the same investigators (F.C. and I.B.); these investigators were not involved in recruitment, inclusion, or randomization of subjects, and had no interactions with participants prior to the examination. All

recordings were numbered anonymously and analysed offline in a blinded fashion by a single investigator (G.C.), who was not blinded to the order of the blocks.

tDCS

tDCS (2 mA, 20min) was delivered using a constant current electrical stimulator (Brainstim®, EMSmedical) through a pair of surface electrodes: the anode was placed over the left temporal pole and the cathode was placed above the right shoulder. The electrodes were square in shape (25 cm^2), 6-mm thick, and covered in a saline-soaked sponge. Current was delivered at a density of 0.08 mA/cm^2, resulting in a total charge of 96 mC/cm^2. These parameters are below the threshold for possible tissue damage [14]. During stimulation, tDCS is not usually perceived except for occasional short-lasting itching sensations below the electrodes.

The stimulation site over the left temporal pole was determined by moving laterally 40% of the intra-auricular distance from the vertex and anteriorly 5% of the distance from inion to nasion [23, 24]. The target site was located approximately halfway between the T7 and FT7 EEG positions of the international 10–20 system. This positioning method, although less accurate than neuronavigation-based techniques, adequately correlates with MRI-guided stereotactic approaches [25, 26].

For sham tDCS, the electrode positions and stimulation intensity were the same as that used for anodal stimulation, but current was only applied for the first and last 30 seconds of the 20-min period. This was done so that patients would not easily be able to distinguish between real tDCS and sham tDCS sessions. Participants in the sham and real arms guessed the type of stimulation in 5 and 6 cases out of 18 respectively ($chi^2 = 0.717$, $p = 1.0$). The experimenters who applied tDCS (F.C. and I.B.) were also blind to the nature of the procedure (real versus sham tDCS); rather, a third experimenter (C.D.L.) pre-programmed the stimulator and ensured the randomization order.

VEP study

Subjects were seated in a semi-dark, acoustically isolated room in front of a TV monitor surrounded by a uniform luminance field of 5 cd/m^2. VEPs were elicited by

monocular stimulation of the right eye. Visual stimuli were full-field checkerboard patterns (contrast, 80%; mean luminance, 50 cd/m^2) generated on the TV monitor and reversed in contrast at a reversal rate of 3.1 reversals per second. The viewing distance was 114 cm and single check edges subtended a visual angle of 15 min. Subjects were instructed to fixate with their right eye on a red dot in the middle of the screen while the contralateral eye was covered with a patch. VEPs were recorded from the scalp through silver cup electrodes positioned at Oz (active electrode) and at Fz (reference electrode as per the international 10–20 system). A ground electrode was placed on the right forearm. Signals were amplified by Digitimer™ D360 pre-amplifiers (band-pass, 0.05–2,000 Hz; gain, 1,000) and recorded on a CED™ power 1401 device (Cambridge Electronic Design Ltd, Cambridge, UK). A total of 600 consecutive sweeps (sweep duration, 200 ms) were collected and sampled at 4,000 Hz. After offline application of a 100-Hz low-pass digital filter, cortical responses were partitioned into 6 sequential blocks of 100 (including at least 95 artefact-free sweeps). Responses in each block were averaged offline (block averages) using the Signal™ software package version 3.10 (CED Ltd). VEP latencies (N1, P1, and N2) and amplitudes (N1-P1 and P1-N2) were identified. Habituation was defined as the slope of the linear regression line for the 6 blocks.

SSEP study

SSEPs were elicited by electrical stimulation of the right median nerve at the wrist using a constant current square wave pulse (width, 0.1 ms; cathode proximal) with a stimulus intensity of 1.5-times the motor threshold and a repetition rate of 4.4 Hz. The active electrodes were placed over the contralateral parietal area (C3', 2 cm posterior to C3 as per the international 10–20 system; referenced to Fz), over the fifth cervical spinous process (Cv5; referenced to Fz), and over Erb's point ipsilateral to the stimulus (referenced to the contralateral side). The ground electrode was placed on the right arm. SEP signals were amplified and recorded with the same hardware/software equipment described above for VEP recording.

Subjects were seated in a comfortable chair in a well-lit room with their eyes open. Subjects were asked to fix their attention on the stimulus-induced thumb

movement. During continuous median-nerve stimulation at the wrist, 500 sweeps (sweep duration, 50 ms) were collected and sampled at 5000 Hz. A total of 500 artefact-free evoked responses were recorded and averaged for each subject (grand average). After digital filtering of the signal between 0–450 Hz, various SEP components (N9, N13, N20, P25, and N33) and their respective peak-to-peak amplitudes (N9-p, N13-p, N20-P25, and P25-N33) were identified. Thereafter, based on the observation of a habituation effect from the 2nd block of 100 averaged responses onwards in previous studies [27, 28], the first 200 evoked responses were partitioned into 2 sequential blocks of 100 (including at least 95 artefact-free sweeps). Each block was averaged offline (block averages) and analysed for N20–P25 amplitudes. Habituation was expressed as the slope of the linear regression line for the 2 blocks [28].

For both VEPs and SSEPs, artefacts were automatically rejected using the Signal^{TM} artefact rejection tool if the signal amplitude exceeded 90% of the analogue-to-digital converter (ADC) range. Signal was corrected offline for DC drift.

Statistical analysis

Data were collected and analysed in a blinded fashion by a single investigator (V.P.) using Statistica for Windows (StatSoft Inc., Tulsa, USA) version 8.0 software. Sample size calculations were based on a ketogenic diet clinical trial that examined the same evoked potentials [29] with a desired power of 0.80 and an α error of 0.05. Since our primary endpoint was to discover differences between the effects of real and sham tDCS on habituation, we used the amplitude habituations of the N1–P1 VEP and N20–P25 SSEP cortical components in the 2 conditions (before versus after ketogenic diet) to compute the sample size. The minimal required sample size was calculated to be 16 subjects for VEP habituation and 9 subjects for SSEP habituation.

A Kolmogorov-Smirnov test showed that VEP and SSEP component latencies and amplitudes had a normal distribution. General linear models approach was used to analyse the ‘between-factor’ × ‘within-factors’ interaction effect. The between-subject factor was ‘group’ (real tDCS versus sham tDCS) or ‘time’ (before stimulation versus after stimulation) and the within-subject factor was ‘block’. Three models of

repeated measures analysis of variance (ANOVA), two for VEPs (N1-P1 and P1-N2) and another for SSEPs, followed by univariate ANOVA, were used to investigate the interaction effect. Moreover, in order to analyse the slope of the linear regression (as a measure of habituation), we used a rm-ANOVA with the between-subject factor 'group' (real tDCS versus sham tDCS) and the within-subject factor 'time' (before stimulation versus after stimulation). Univariate results were analysed only if Wilk's Lambda multivariate significance criterion was achieved. The sphericity of the covariance matrix was verified with the Mauchly Sphericity Test; in the case of violation of the sphericity assumption, the Greenhouse-Geisser epsilon adjustment was used.

In the rm-ANOVA and ANOVA models, partial eta2 (η_p^2) and observed power (op) were used as measures of effect size and power, respectively. To identify the comparison(s) contributing to major effects, we performed post hoc Tukey Honest Significant Difference (HSD) tests.

One-way ANOVA tests were used to compare the baseline grand averaged VEP and SSEP latencies and amplitudes between sham and real tDCS. Paired-sample t tests were used to compare the grand averaged VEP and SSEP latencies and amplitudes before vs. after both sham and real tDCS. P values less than 0.05 were considered statistically significant.

Results

Basic neurophysiological parameters

VEP and SSEP recordings were obtained from all participants. The grand averaged VEP latencies (N1, P1, and N2; Table 2) and SEP latencies (N9, N13, N20, P25, and N33; Table 3) as well as their corresponding amplitudes (VEP: N1–P1 and P1–N2; SEP: N9, N13, N20–P25, and P25–N33) were not significantly different between real and sham tDCS groups ($P > 0.05$). Before stimulation, both groups showed positive slope values indicating a lack of habituation in response to visual (N1–P1: real tDCS = +0.112, sham tDCS = +0.059; P1–N2: real tDCS = +0.055, sham tDCS = +0.039) and somatosensory (real tDCS = +0.448, sham tDCS = +0.234) repetitive stimulations.

Effects of tDCS on neurophysiological parameters

The grand averaged VEP latencies (N1, P1, and N2; Table 2) and SSEP latencies (N9, N13, N20, P25, and N33; Table 3) as well as their corresponding amplitudes (VEP: N1–P1 and P1–N2; SSEP: N9, N13, N20–P25, and P25–N33) were not significantly different before and after stimulation in both the real and sham tDCS groups ($P > 0.05$).

Table 2 Latencies (in milliseconds) and amplitudes (μV) of VEPs in migraine patients' groups undergoing real or sham transcranial direct current stimulation (tDCS) before and after intervention

Electrophysiological parameters	Real (*n* = 18)		Sham (*n* = 18)	
	Before	After	Before	After
N1	80.3 ± 5.7	78.9 ± 6.4	78.4 ± 2.0	78.5 ± 3.1
P1	105.5 ± 6.1	105.2 ± 5.8	105.1 ± 4.3	106.7 ± 4.7
N2	146.1 ± 8.9	146.9 ± 9.7	150.7 ± 6.7	151.1 ± 6.8
N1-P1 1st amplitude block (μV)	8.3 ± 3.1	8.9 ± 3.6	7.2 ± 2.7	6.7 ± 2.4
N1-P1 amplitude slope	0.112 ± 0.315	- 0.236 ± 0.339 **	0.059 ± 0.241	0.038 ± 0.182
P1-N2 1st amplitude block (μV)	8.3 ± 3.1	8.9 ± 4.2	6.4 ± 3.4	6.3 ± 2.9
P1-N2 amplitude slope	0.055 ± 0.507	- 0.345 ± 0.569	0.039 ± 0.272	- 0.001 ±0.269

*Data are expressed as means ± SD. ** = p < 0.01 before vs. after the intervention*

Table 3 Grand-average somatosensory evoked potentials (SSEPs) latencies and amplitudes in migraine patients' groups undergoing real or sham transcranial direct current stimulation (tDCS) before and after intervention

Electrophysiological parameters	Real (*n* = 18)		Sham (*n* = 18)	
	Before	After	Before	After
N9 (ms)	9.5 ± 0.6	9.7 ± 0.8	9.5 ± 0.6	9.6 ± 0.6
N13 (ms)	13.2 ± 0.8	13.3 ± 0.8	13.1 ± 0.7	13.2 ± 0.7
N20 (ms)	18.8 ± 0.9	19.0 ± 0.8	18.6 ± 1.1	18.8 ± 1.1
P25 (ms)	23.6 ± 2.2	23.9 ± 2.1	22.9 ± 2.2	23.2 ± 2.2
N33 (ms)	31.5 ± 2.6	31.5 ± 1.6	31.9 ± 2.1	31.5 ± 1.3
N9-p (μV)	4.1 ± 1.6	3.8 ± 1.4	3.5 ± 1.4	3.5 ± 1.9
N13-p (μV)	2.0 ± 0.8	2.0 ± 0.6	2.0 ± 0.6	1.8 ± 0.7
N20-P25 (μV)	2.3 ± 1.3	2.4 ± 1.5	2.3 ± 0.7	2.1 ± 0.9
P25-N33 (μV)	1.3 ± 0.5	1.3 ± 0.9	1.2 ± 0.5	1.0 ± 0.5
N20-P25 1st amplitude (μV)	2.4 ± 1.1	2.2 ± 1.2	2.3 ± 0.7	2.2 ± 0.6
N20-P25 amplitude slope	0.448 ± 0.710	0.315 ± 0.543	0.234 ± 0.406	0.213 ±0.481

Data are expressed as means ± SD

In the rm-ANOVA model using the VEP N1–P1 peak-to-peak block amplitude as the dependent variable, the multivariate test was significant for the 'group' × 'time' × 'block' interaction effect ($F_{5,340} = 3.290$, $p = 0.006$). The univariate rm-ANOVA for

N1–P1 peak-to-peak amplitudes confirmed a significant interaction factor effect (Greenhouse-Geisser epsilon adjustment applied, $F_{4.1,282.1} = 3.29$, $\varepsilon = 0.83$, $p = 0.01$, partial $\eta 2 = 0.05$, op = 0.89) in the multivariate test. At the post-hoc analysis 1st N1-P1 VEP amplitude block did not differ between before and after both stimulations. The linear regression N1–P1 slope of VEP amplitudes over all blocks was significantly different between before and after stimulation ($F_{1,34} = 5.21$, $p = 0.029$, partial $\eta 2 = 0.133$, op = 0.60; raw data are shown in Figure 1).

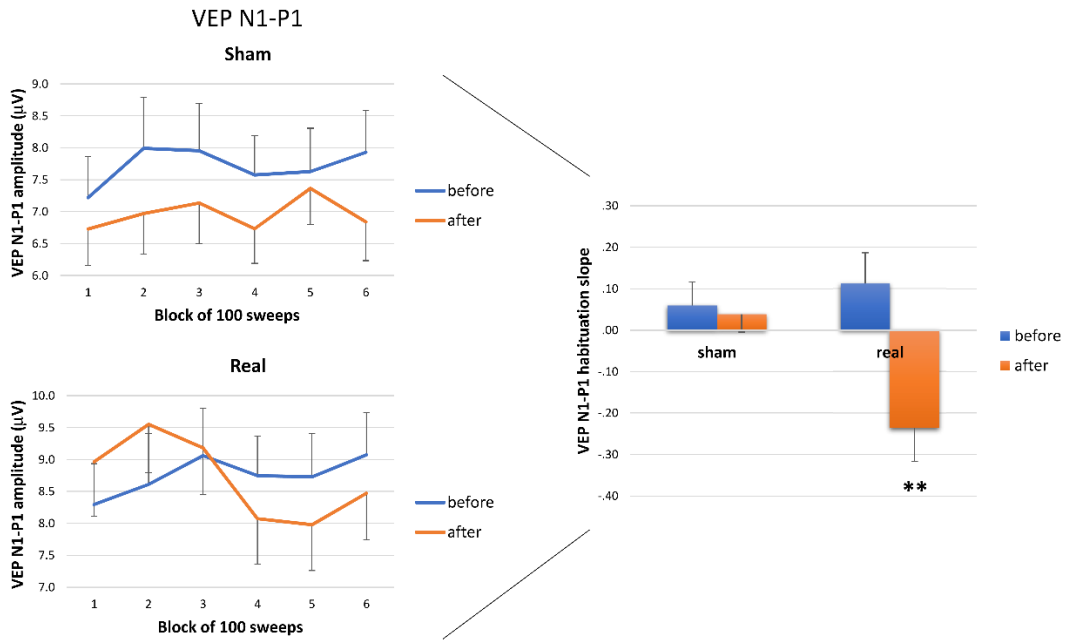

Fig. 1 Left panel: Amplitudes (mean ± standard error of the mean) of the N1–P1 visual evoked potential (VEP) component in 6 sequential blocks of 100 recordings are shown before and after sham tDCS (upper panel) and anodal tDCS (lower panel). Right panel: The bar graph represents the habituation slope of VEP N1–P1 peak-to-peak amplitudes (mean ± standard error of the mean) before and after sham tDCS and real tDCS. The arrow highlights interictal VEP habituation that was reduced before real tDCS but normalized after. ** = $p < 0.01$ before vs. after the intervention

A post-hoc analysis showed that the slope of VEP amplitudes from block 1 to block 6 was positive before the intervention in both the real tDCS (+0.112) and sham tDCS (+0.059) groups, whereas after the intervention these values were negative in the

real tDCS group (−0.236, p = 0.003 versus before stimulation) but positive in the sham tDCS group (+0.038, p > 0.05 versus before stimulation) (Figure 1, right panel).

In the rm-ANOVA model using the VEP P1–N2 peak-to-peak block amplitude as the dependent variable, the 'group' × 'time' × 'block' interaction effect was not significant ($F_{5,340}$ = 1.55, p = 0.171) in the multivariate test (Figure 2).

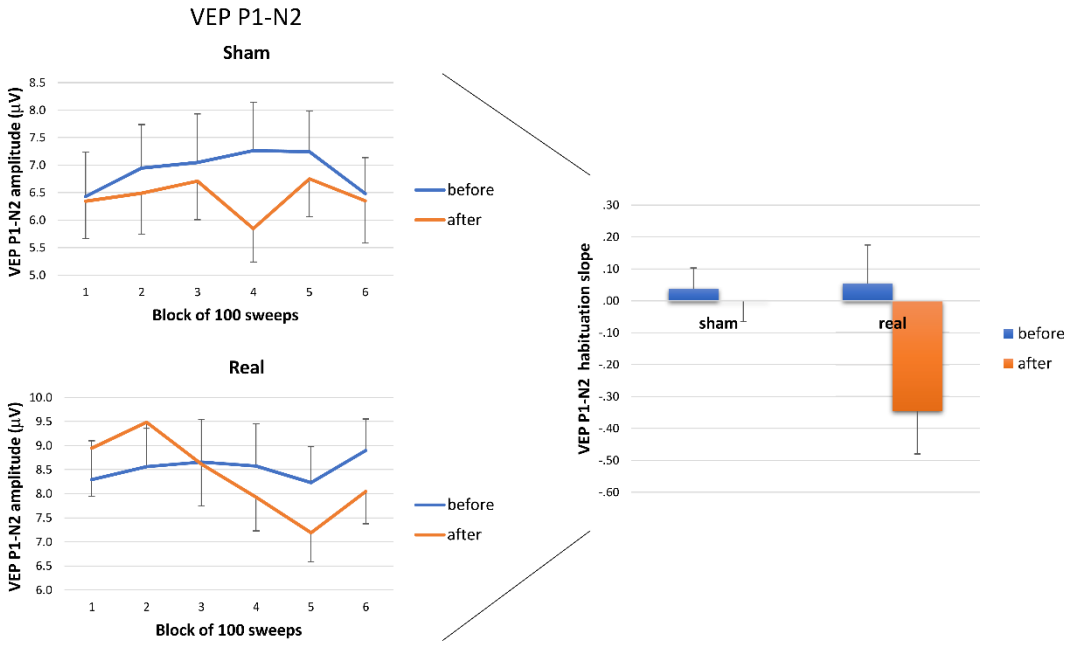

Fig. 2 Left panel: Amplitudes (mean ± standard error of the mean) of the P1–N2 visual evoked potential (VEP) component in 6 sequential blocks of 100 recordings are shown before and after sham tDCS (upper panel) and real tDCS (lower panel). Right panel: The bar graph represents the habituation slope of VEP P1–N2 peak-to-peak amplitudes (mean ± standard error of the mean) before and after sham tDCS and real tDCS.

In the rm-ANOVA model using the SSEP N20–P25 peak-to-peak block amplitude as the dependent variable, the 'group' × 'time' × 'block' interaction effect was not significant ($F_{1,68}$ = 0.19, p = 0.659) in the multivariate test (Figure 3).

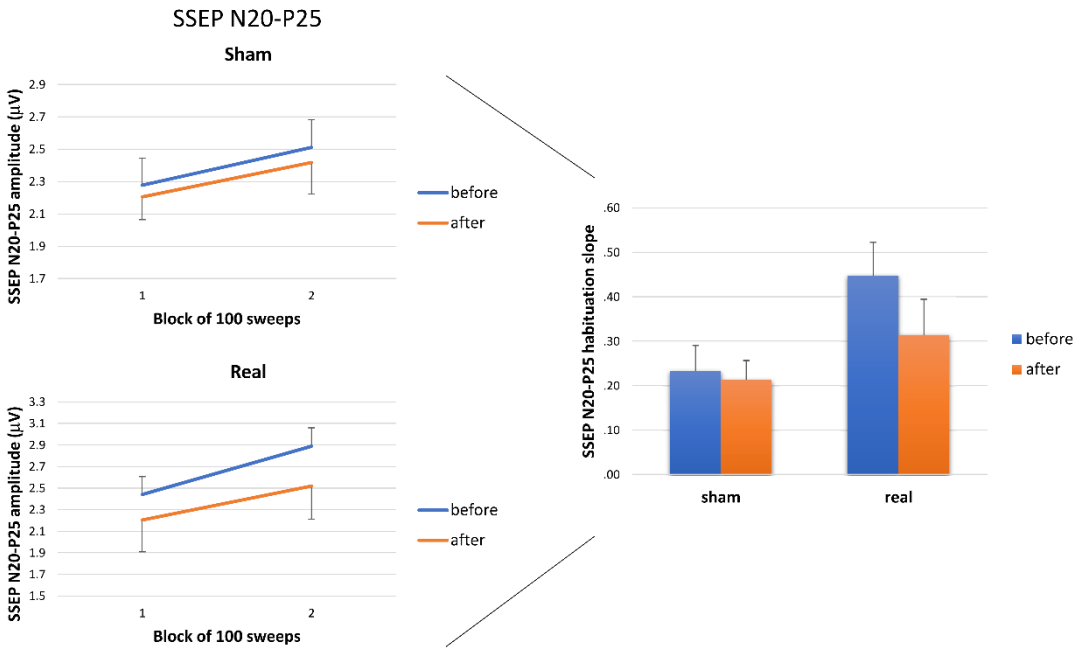

Fig. 3 Left panel: Amplitudes (mean ± standard error of the mean) of the N20–P25 somatosensory evoked potential (SSEP) component in 2 sequential blocks of 100 recordings are shown before and after sham tDCS (upper panel) and real tDCS (lower panel). Right panel: The bar graph represents the habituation slope of SSEP N20–P25 peak-to-peak amplitudes (mean ± standard error of the mean) before and after sham tDCS and real tDCS.

Discussion

The present study mainly revealed that a single session of anodal tDCS over the left temporal pole restored normal visual but not somatosensory habituation in interictal migraineurs.

Neurophysiological studies have shown that interictal migraineurs exhibit dysfunctional sensory information processing in the form of habituation deficits in response to various sensory inputs, including visual and somatosensory inputs [2]. Recent neuroimaging studies have revealed subtle microstructural alterations in the brains of patients with migraine in areas associated with the ictal-interictal cycle. Among these studies, some evidence highlights a pathophysiological role for the TP in migraine [7–12].

The TP region encompasses the most anterior segment of the temporal lobe and receives extensive inputs from visual regions of the thalamus [30, 31]. Additionally, the TP is highly interconnected with the amygdala, hippocampus, superior temporal

gyrus, hypothalamus, occipitobasal cortex, prefrontal regions, and insula, suggesting its participation in autonomic regulation, memory, and emotional processing [32, 33]. The TP is considered a multisensory associative cortex because it is also connected to the main sensory systems of the temporal lobe, including the visual, auditory, olfactory, and gustative systems, but not the somatosensory system [32, 34]. Indeed, neuroimaging studies have demonstrated subregional activation of the TP in response to specific sensory stimuli, with the ventromedial aspect of the TP having a predominant role in higher order visual information processing [34] as part of the VVS.

Our finding that anodal (excitatory) stimulation of the left TP restored physiological visual information processing but not somatosensory processing in interictal migraineurs is largely consistent with the abovementioned roles of the left TP in high-level multimodal perceptual processing. A selective effect of tDCS over the TP on visual information processing is probably related to the role of the TP in the VVS and its lack of participation in somatosensory elaboration. Interestingly, another study observed similar normalization of abnormal interictal VEP habituation in response to the application of tDCS over the occipital cortex in migraineurs [22]. This can be explained either by a direct interconnection between the TP and occipital cortex along the VVS or an indirect effect of the tDCS on brain structures that positively modulate both cortices.

The VVS is involved in visual recognition and in the assignment or retrieval of a given meaning for visual information [35]. After early activation of the occipital area, the complexity of representation of visual information increases as information flows to the anterior regions of the VVS, with the TP located at the end of the stream and sending backward facilitatory projections to the occipital cortex to optimize sensory processing (e.g., improve perception and learning) [35, 36]. Consistent with this evidence, we observed that the enhancement of TP activity with anodal tDCS improved VEP amplitude habituation, a basic form of learning [37], without affecting initial baseline excitability (reflected by non-significant changes in 1st block VEP amplitudes). In habituation paradigms, early and late responses can behave differently as a result of regulation by different mechanisms; according to the dual-

process theory, increasing responsiveness (sensitization) competes with decreasing responsiveness (habituation) to determine final behavioural outcomes. Facilitation occurs at the beginning of the stimulus session and accounts for an initial temporary increase in response amplitude, whereas habituation occurs throughout the recording session and accounts for delayed decreases in responsiveness [38]. Therefore, our results regarding the selective effect of anodal tDCS on delayed habituation in migraineurs appear to be in line with the putative mechanism of tDCS; that is, the ability of tDCS to affect the potentiation of long-term learning processes and synaptic plasticity underlying learning and memory [39]. Alternatively, it has been shown that anodal tDCS exerts modulatory effects on thalamo-cortical circuits by increasing functional coupling between the thalamus and cortex [17, 40]. These experimental observations are of particular interest in migraine because independent research groups have previously reported reduced functional [41, 42] and morphological [43, 44] thalamic integrity coupled with decreased intracortical inhibition during visual stimulation in migraineurs [45, 46]. We thus can hypothesize that an alternative mechanism of action for anodal tDCS in the present study is increased thalamo-cortical activity, which in turn increased delayed inhibitory mechanisms to restore normal VEP habituation.

Irrespective of the mechanism, the observation that tDCS over the left TP is able to restore normal VEP habituation in interictal migraineurs leads to hypothesize that together with the visual, motor, and dorsolateral prefrontal cortices [19, 20], the TP could represent a novel target for tDCS as a prophylactic strategy for treating migraine [47].

This study had some limitations. For example, we only stimulated the left TP, such that we cannot know whether anodal tDCS of the right TP would have yielded similar results. Several studies have shown divergent functional roles of the left and right TP, where the right TP is more involved in elaborating socio-emotional implications of multisensory perceptual stimuli [48] while the left TP is mostly implicated in perceptual decoding, semantic processing, and conceptualization [34]. Nonetheless, both the left and right TPs are joined via the anterior white commissure to advance multimodal perceptual analysis [32], such that the relevance of the right TP cannot

be discounted. Furthermore, the positioning method we used is accurate, although not as accurate as neuronavigation-based techniques, which are unfortunately only available for neurosurgical procedures in our clinic. Another shortcoming of the present study is the lack of inclusion of a healthy control group undergoing the same stimulations, although this would not add anything to the results of the study because the healthy subjects usually already habituate normally at the baseline, i.e. we cannot normalize the already normal information processing.

Conclusions

In conclusion, anodal but not sham tDCS selectively enhanced visual but not somatosensory habituation in interictal migraineurs probably by restoring normal inhibitory activity of the left TP. We propose that this effect can be explained by either a direct interference with short- and long-term synaptic plasticity mechanisms or an indirect potentiation of the thalamo-cortical circuit. Further studies are needed to determine whether TP stimulation also normalizes the habituation response to other sensory inputs, such as auditory and nociceptive inputs. Regardless of the underlying cellular and molecular mechanisms of our observed effect, we propose that the TP should be considered as a key site of involvement in the pathophysiology of migraine and as a potential therapeutic target. Clinical studies are needed to clarify whether repeated sessions of anodal tDCS improve TP function and connectivity in patients with migraine to ultimately reduce the number and severity of migraine attacks.

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B. Excitability of the motor cortex in patients with migraine changes with the time elapsed from the last attack

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Background

Although the pathophysiology of migraine remains unclear, neurophysiological studies performed over the last few decades have shown that patients affected by migraine exhibit interictal abnormalities in their cortical information processing system [1, 2]. These functional brain abnormalities are not constant; rather, they cyclically change until an attack occurs, whereupon the cortical responsiveness normalises [3]. The latter was demonstrated when information processing was assessed by cortical evoked potentials (EPs). In fact, the migraineur brain is frequently characterised by abnormal EP amplitude habituation in response to any kind of sensory stimulation [3]. We recently found that in migraineurs, the degree of EP abnormalities fluctuates over time, particularly in relation to the occurrence of migraine attacks (i.e. the degree of abnormalities is higher at long time intervals after an attack while it is minimal and within the normal range during an attack) [4–6]. Cortical excitability can also be examined noninvasively by applying transcranial magnetic stimulation (TMS) pulses over different areas of the cortex and then recording the evoked peripheral activity. TMS studies of the motor cortex rely on an objective measure, namely the motor-evoked potentials (MEPs) recorded from the peripheral muscles. In clinical practice and in scientific studies, corticospinal excitability is estimated objectively by examining the cortical motor threshold (or resting motor threshold, RMT), which is the minimal intensity of motor cortex stimulation required to elicit a MEP of minimal amplitude in the relaxed target muscle. The MEP size or amplitude can then be measured by setting the TMS intensity

to 115–125% of the individual's RMT [7]. Lower MEP thresholds and larger MEP amplitudes suggest higher cortical excitability. In patients with migraine, controversial findings have been reported regarding the degree of motor cortex excitability. Globally, thresholds for MEPs were found to be normal [8–13], increased [14–16], or reduced [17–19] in migraineurs.

However, whether these inconsistent findings result from variation in the cortical excitability related to the time interval between the ictal and interictal state remains unknown.

Here, we sought to understand whether the actual MEP threshold and amplitude in patients with migraine varies on the basis of the time elapsed since the last attack and in comparison to healthy volunteers (HVs). Consistent with the abovementioned changes in EP according to the time elapsed from the last attack [4, 6], we hypothesised that motor cortex excitability would also become increasingly abnormal in patients with migraine as the time from the last migraine attack increased.

Methods

Participants

Thirty-one patients affected by migraine without aura (MO) who consecutively attended the Headache Clinic of the 'Sapienza' University of Rome Polo Pontino, Italy, were enrolled in this study. Only the data from patients who had an interval of at least 3 days between the recording and their last or next migraine attack (checked by email or telephone) were included. We also excluded those participants who were taking any type of medication on a regular basis, except contraceptive pills.

We evaluated the following clinical characteristics of the patients: duration of migraine disease (years), attack frequency (number/month), attack duration (hours), severity of headache attacks (0–10), and number of days elapsed since the last migraine attack (Table 1). This information was collected from participants' 1-month headache diaries, which were obtained either during the screening visit or on the day of the recording session.

Table 1 Clinical and demographic characteristics of HVs and MO patients. Data are expressed as means ± SD

	HV (*n* = 24)	MO (*n* = 26)
Women (n)	16	18
Age (years)	30.4 ± 10.2	29.4 ± 6.8
Duration of migraine history (years)		13.9 ± 6.9
Attack frequency/month (n)		3.1 ± 2.7
Attack duration (hours)		22.3 ± 18.8
Visual analogue scale (n)		7.4 ± 1.5
Days from last migraine attack (n)		10.6 ± 8.4

Twenty-four HVs with a similar age and sex distribution as the patients with MO (mean age ± standard deviation: 30.4 ± 10.2 years, 16 women) and without a personal or familial history of migraine or any detectable medical condition were used for comparison. All participants were right-handed.

The physicians and neurophysiologists involved in the study were blinded to the electrophysiology and clinical history of the participants, respectively. This study was conducted in accordance with the Declaration of Helsinki and the study was approved by the Ethical Committee of the 'Sapienza' University of Rome Polo Pontino. All individuals provided written informed consent to participate in the study.

Transcranial magnetic stimulation procedures

TMS was delivered through a high-frequency biphasic magnetic stimulator (MagstimRapid, The Magstim Company Ltd., Whitland, South West Wales, UK), which wasconnected to a figure-of-eight coil with a maximal output of 1.2 Tesla. Firstly, we determined the optimal orientation and position of the coil (i.e. 'hot spot') over the left motor area for stimulating the first dorsal interosseous muscle. After that, we identified the RMT by using single TMS pulses; complete relaxation of the first dorsal interosseous muscle was checked by verifying the absence of electromyographic signals, both visually (on a monitor) and by acoustic feedback. The RMT was defined as the minimal intensity required to elicit an electromyographic response of at least 50 μV with 50% probability in a fully relaxed muscle [7, 20–23].

During TMS, patients were seated in a comfortable armchair and asked to remain fully relaxed with their eyes closed to ensure similar attention levels. We delivered 10 single pulses of TMS (stimulus intensity: 120% of the RMT, rate: 0.1 Hz) and averaged the resulting MEPs.

Statistical analysis

All analyses were conducted with the Statistical Package for the Social Sciences (SPSS) for Windows, version 21.0. The normality of the data for each group of participants was tested with the Shapiro–Wilk test. Since the MEP amplitude showed a non-Gaussian distribution, it was analysed with the non-parametric Mann-Whitney U-test. As the RMT was normally distributed, it was analysed using independent-samples t-tests. Spearman's rho correlation test was used to search for correlations between the neurophysiological parameters and clinical variables mentioned above. Differences were considered statistically significant when the p value was <0.05.

Results

Among the 31 enrolled patients, five were excluded from the subsequent analyses because they had an attack during the hours after the recording session. Therefore, the final dataset consisted of 26 patients (Fig. 1).

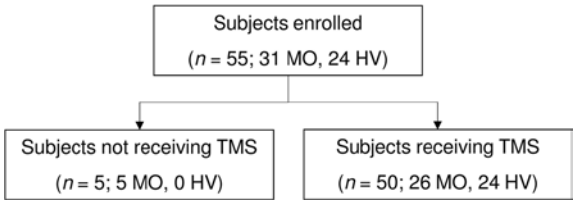

Fig. 1 Flow chart showing the number of included/excluded participants in the various stages of the study

The participant demographics and clinical characteristics of the MO group are listed in Table 1. Assessable MEP recordings were obtained from all participants. Examples of MEP recordings from participants in the HV and MO groups are shown in Fig. 2.

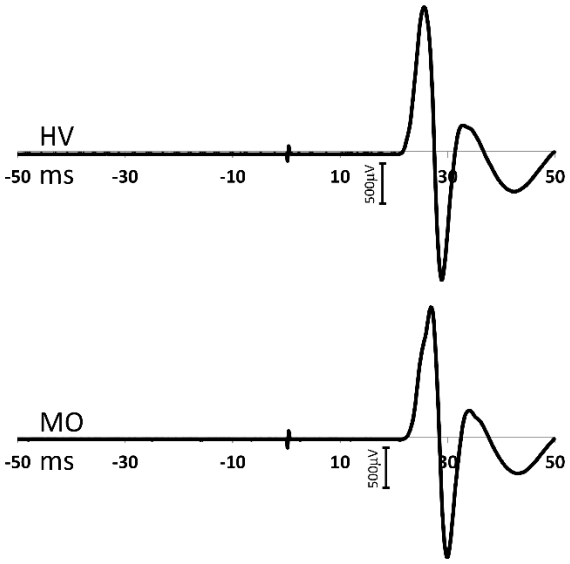

Fig. 2 Trace illustrations of motor-evoked potentials (MEPs) from healthy volunteers (HVs) and patients with migraine without aura (MO)

No differences in interictal RMTs or MEP amplitudes were noted between the two participant groups (t = 0.536, p = 0.594 and U = 305.0, p = 0.892, respectively; Fig. 3).

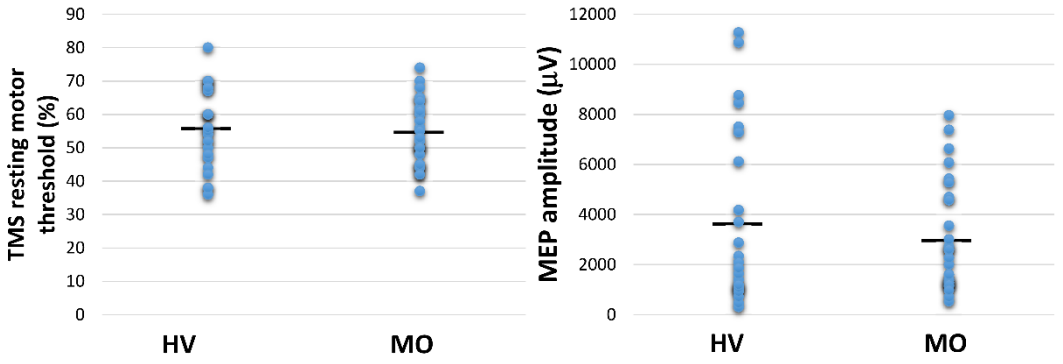

Fig. 3 Grouped scatter-plot showing the resting motor thresholds (RMT [%]; left panel) and motor-evoked potential (MEP) amplitudes (right panel) in healthy volunteers (HVs) and patients with migraine without aura (MO)

Spearman's test revealed correlations between the neurophysiological parameters and clinical variables. In the MO group, the RMT was negatively correlated with the number of days since the last migraine attack (rho = -0.404, p = 0.04; Fig. 4). No other significant correlations were identified between the neurophysiological and clinical data in patients with MO.

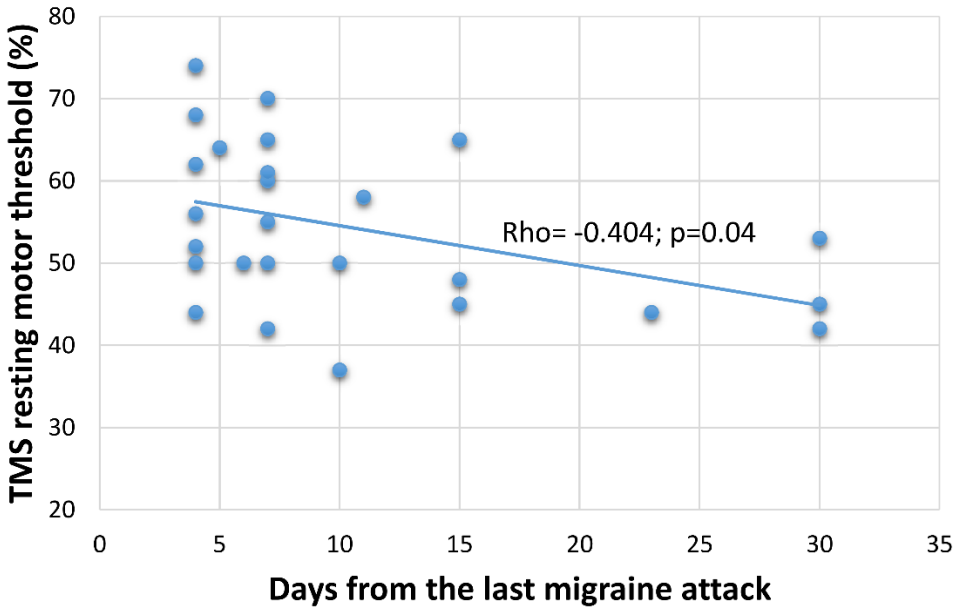

Fig. 4 Correlation between the number of days since the last migraine attack and the resting motor threshold (RMT [%]) in patients with migraine who were between attacks

Discussion

Many clinical neurophysiology studies have shown that when patients with migraine are between attacks, their cortical responsiveness during the repetition of a series of stereotyped stimuli is enhanced when compared to controls. This functional brain abnormality has been detected in EPs for virtually all sensory modalities [3]. As mentioned earlier, previous single-pulse TMS studies examining motor cortex excitability in patients with migraine reported conflicting results. Overall, the results of the present study are concurrent with those of previous studies showing that the interictal RMTs and MEP amplitudes of patients with migraine do not differ from those of HVs [8–13].

To our knowledge, our study is the first to report a negative correlation between the RMT and time elapsed from the last migraine attack in patients with MO. This findings is consistent with previous evidence obtained with psychophysiological tests [24], neuroimaging techniques [25, 26], and cortical EPs [4–6] showing that during

the variable pain-free period between two migraine attacks, the brain of an individual with migraine is exposed to subtle cyclic functional changes. Indeed, at the cortical level, we previously observed that patients with MO and a subgroup of patients with migraine with visual aura associated with paraesthesia and/or dysphasia exhibited a strong decrease in EP amplitude habituation during the stereotyped presentation of visual stimuli with the passing of time from the last attack [4, 5]. The results of the present study revealed that the same correlation is valid for the resting excitability of the motor cortex in response to single-pulse TMS. This finding indicates that motor cortex excitability fluctuates during interictal phases; specifically, as the time elapsed from the last attack increases so does the motor cortex disexcitability. These results are in favour of a migraine cycle-dependent subtle imbalance between excitation and inhibition in the motor cortex. Below, we discuss the possible neurophysiological underpinnings of these TMS results and their relevance to migraine pathophysiology.

TMS is a non-invasive technique that permits researchers to objectively evaluate the RMT and estimate motor cortex excitability [7]. At the RMT, TMS indirectly activates the pyramidal tracts by eliciting so-called indirect waves (I-waves), which result from the complex interactions among different types of cortical cells that discharge at a high frequency [27–29]. Modelling studies have shown that when the coil is placed tangentially on the scalp—as was the case here—the majority of the induced current flows parallel to the surface of the brain rather than perpendicular to the grey matter [30]. Consequently, TMS-induced horizontal current flow preferentially activates the horizontally oriented axons of cortical interneurons or cortico-cortical fibres that activate pyramidal neurons trans-synaptically (I-waves) instead of activating pyramidal neurons directly (D-waves). Therefore, the excitation threshold depends on the orientation and membrane properties of the axons activated by the TMS-induced electrical field, including axons of the tangentially oriented cortico-cortical loop fibres that modulate the excitability of the corticospinal output neurons.

Among the cortico-cortical fibre systems, it is important to consider the influence that collateral gamma-aminobutyric acid (GABA)-ergic axons, which project from the somatosensory cortex, have on motor cortex excitability, as shown in animal studies

[31, 32] and in human studies using paired associative stimulation [33]. Moreover, it is well known that cortico-cortical loops, particularly in the general and somatosensory cortices, are strongly modulated by thalamocortical afferent fibres [32]. Interestingly, somatosensory lateral inhibition and thalamocortical drives are both involved in the pathophysiology of interictal migraine. Early somatosensory high-frequency oscillation bursts (detected by the appropriate filtration of common somatosensory evoked potentials), which reflect thalamocortical spike activity, are reduced in episodic migraine interictally; however, they normalise during an attack [34]. The microstructural correlates of these thalamic functional fluctuations were recently investigated in a diffusion tensor magnetic resonance study [26], which found that the interictal fractional anisotropy was significantly increased while the mean diffusivity was slightly decreased within the thalamus bilaterally. Interestingly, the right thalamic fractional anisotropy was positively correlated with the number of days since the last migraine attack, which is consistent with the results of the present study. Furthermore, a recent neurophysiological study [6] showed that patients with migraine have deficient lateral inhibition within the somatosensory cortex during the interictal phase; however, they show normal lateral inhibition during the attack. Nonetheless, the degree of somatosensory lateral inhibition is directly related to the somatosensory thalamocortical activity (evaluated as the amplitude of presynaptic high-frequency oscillations) and inversely related to the number of days elapsed since the last attack [6].

Owing to this interictal, morphofunctional thalamocortico-cortical evidence in patients with migraine, we postulate that the reduced thalamic control of the sensorimotor cortical activity and decreased degree of somatosensory lateral inhibition, which are both inversely correlated with the number of days since the last attack, could account for the observed subtle fluctuations in the RMT during the variable pain-free period between migraine attacks. However, whether these abnormalities in sensorimotor cortical activity are consequences of the ‘thalamocortical dysrhythmia’ [35, 36] (a model theory on cyclical functional abnormalities in migraine) remains unknown. Regardless, in a previous study on a group of mixed patients and HVs, we found that inhibitory TMS-induced plastic

changes were inversely related to the level of thalamocortical activation [37], supporting the hypothesis that anomalous thalamic control could underlie the abnormal TMS findings in patients with migraine who are between attacks.

Finally, we acknowledge as a possible limitation of the present study that some researchers observed that the RMT was not stable over days, which may have complicated the interpretation of values measured at one point in time [38]. However, this is not completely detrimental because it may further support our findings that cortex excitability is not stable between attacks but rather undergoes daily fluctuations during the so-called migraine cycle.

Conclusions

Here, in patients with MO who were between attacks, we detected a negative correlation between the RMT and the number of days since the last attack. Our results help explain the conflicting findings reported previously on the degree of motor cortex excitability in patients with migraine by showing that the RMT is strongly dependent on the phase of the migraine cycle. We propose that hypofunctioning of the thalamocortical loops and somatosensory lateral inhibition, beyond accounting for the dynamic variations in the sensory cortex habituation deficits, may contribute to the observed subtle fluctuations in motor cortex excitability in patients with migraine. We believe this occurs by influencing the cortico-cortical GABAergic inhibitory connections between the somatosensory and motor cortical areas. Further studies are needed to determine whether interactions among sensory and motor cortical activity under the control of thalamic nuclei are involved in the clinical and morphofunctional features of patients with migraine, including those experiencing aura or headache chronification.

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C. Short-term cortical synaptic depression/potentiation mechanisms in chronic migraine patients with or without medication overuse

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Introduction

Chronic migraine (CM) is characterized by headaches occurring ≥15 days per month, with ≥8 headache days fulfilling the criteria for migraine headaches, for at least 3 months [1]. Every year, approximately 3% of migraineurs progress to CM [2]. Different factors may favour migraine chronification, including overuse of analgesics, ineffective acute treatment(s), obesity, and psychological factors such as depression, stressful life events, and specific personality traits [3]. Medication overuse headache (MOH) is very prevalent among patients attending specialized headache clinics and is associated with excessive use of acute medication drugs, defined as intake of analgesics or triptans on more than 15 and 10 days per month, respectively [1].

According to the current diagnostic criteria from the *International Classification of Headache Disorders* (ICHD 3 beta), analgesic abuse is no longer an exclusion criterion for the diagnosis of CM. However, morphofunctional studies have shown that MOH patients exhibit peculiar cerebral morphological [4–6] and electrophysiological patterns when compared with pure CM patients (i.e., without medication overuse). In particular, while evidence for cortical sensitization (calculated as the initial amplitude increase of evoked potentials) has been observed in both pure CM and MOH patients in response to different sensorial stimulations [4–6], deficient habituation—or persistent sensitization—to repetitive somatosensorial stimulation is exhibited by patients with MOH [4, 7, 8], but not those with CM [6].

Because habituation can be considered a basic form of learning and memory [9], these findings suggest that the mechanisms underlying sensorimotor plasticity and learning processes could be dysfunctional in CM patients and depend on the co-occurrence of medication overuse.

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive tool used to modulate cortical excitability. When applied over the motor cortex, this neuromodulatory technique has been shown to induce pain relief in different types of chronic pain [10], mainly by effecting plastic changes in the motor area, whose extension is positively associated to pain intensity [11].

In normal subjects, rTMS is able to induce functional plastic changes depending on the number, intensity, and frequency of the stimulation pulses. In particular, high-frequency trains (5 Hz) of rTMS have been reported to increase cortical excitability in the short term, while low-frequency stimulations (0.1-1 Hz) have been reported to decrease it [12, 13]. Because chronic pain is characterized by maladaptive plasticity in the motor system, studying the relationship between CM and motor cortex excitability could be interesting, not only to reveal the mechanisms related to headache chronification, but also for future therapeutic targets and interventions.

In patients affected by episodic migraine with aura, low-frequency rTMS was shown to produce a paradoxical increase of intracortical facilitation in the motor cortex [14]. Studies investigating effects of high-frequency rTMS in patients affected by migraine with aura yielded different results depending on the TMS variables and experimental protocols. In patients, 5 Hz-rTMS on the motor cortex induced motor evoked potential (MEP) facilitation when the stimulation was delivered at an intensity of 110% resting motor threshold (RMT) and paradoxical MEP inhibition when delivered at 130% RMT [15]. In patients with episodic migraine without aura, Conte and co-workers [16] found that 5 Hz-rTMS, delivered at 120% RMT, induced abnormally high MEP facilitation. Moreover, in patients affected by migraine without aura, MEP response to trains of high-frequency rTMS yielded different effects depending on the phase within the migraine cycle, and on the frequency of migraine, with a physiological increasing response in the interictal phase and paradoxical

decremental response in both episodic migraineurs recorded ictally and in CM patients [17].

To the best of our knowledge, no studies to date have performed a detailed examination of short-term plasticity mechanisms of the primary motor cortex individually in CM and MOH patients. The goal of the current study, therefore, was to use both low- and high- frequency rTMS over the motor cortex to identify distinctive neurophysiological mechanisms underpinning learning and plasticity in individuals with CM or MOH compared with normal subjects.

Material and Methods

Subjects

Among consecutive patients attending the authors' headache clinic, 40 provided informed consent to participate in the study, of whom 8 were excluded because they did not fulfil the inclusion criteria. Participants were included if they were between 18 and 65 years of age and had at least a 1-year clinical history of migraine. Participants were excluded from the study if they were regularly taking medication (e.g., antibiotics, corticosteroids, antidepressants, benzodiazepines, or prophylactic migraine medication) during the 3 months preceding the study, except for contraceptive pills (taken by 3 HV, 2 CM, and 2 MOH). Individuals with a history of other neurological disorder(s), systemic hypertension, diabetes or other metabolic or autoimmune disease, or any other type of primary or secondary headache, were also excluded. Patients did not always experience the headaches on the same side. All participants received a complete description of the study and provided written informed consent. The study was approved by the local ethics review board and was conducted in accordance with the Helsinki Declaration.

According to the inclusion/exclusion criteria, the final dataset comprised 32 patients (Table 1), of whom 16 were diagnosed with *de novo* CM (IHCD-IIIb code 1.3), with no history of medication overuse, and 16, with *de novo* MOH (ICHD-IIIb code 8.2), who never underwent a detoxification program during their first screening visit. The inclusion criteria were restricted to MOH patients overusing non-steroidal anti-inflammatory drugs (NSAIDs) only (IHCD-IIIb code 8.2.3), because it has been

demonstrated in a previous study that these patients exhibit the most pronounced abnormalities at the sensorimotor system level compared with MOH patients overusing anti-migraine-specific (triptan) acute medication [4, 18]. Before progressing to MOH, all patients had a clear-cut history of episodic migraine without aura (ICHD-IIIb code 1.1). Except for 4 patients who had mild headache (mean visual analogue scale score 4/10), all patients underwent the MEP recordings in a pain-free state. Because MOH patients tend to take acute medications compulsively and frequently during the day, it was impossible to prevent them from taking medication on the day of recordings. It was managed, however, to perform the recordings at least 3 h after the last medication intake. For comparison, MEP trains were recorded in 16 healthy volunteers (HVs) with comparable age and sex distribution (Table 1), and no personal or familial history (first- or second-degree relatives) of migraine and no detectable medical condition. To avoid variability due to hormonal changes, female participants were examined outside their pre-menstrual or menstrual cycles.

Table 1. Demographic characteristics of study participants and headache profiles of patients. Data expressed as mean——————————SD. HV: healthy volunteers; CM: chronic migraneurs; MOH: medication overuse headache patients; n: number of subjects.

	HV (n = 16)	CM (n = 16)	MOH (n = 16)
Women (n)	12	12	13
Age (years)	32.1 ± 10.2	31.1 ± 10.2	34.4 ± 11.6
Duration of history of migraine (years)		13.5 ± 10.3	16.5 ± 9.2
Days wit headache/month (n)		22.6 ± 6.4	20.4 ± 6.9
Severity of headache attacks (0-10)		6.9 ± 2.2	8.1 ± 1.6
Nausea/vomiting (n)		13	16
Photophobia (n)		15	14
Phonophobia (n)		13	14
Pulsating (n)		13	14
Duration of chronic headache (years)		22.7 ± 24.6	18.1 ± 14.9
NSAID tablet intake/month (n)		32. ± 3.8	27.8 ± 13.7*

**p< 0.001 vs. CM.*

TMS procedures

During TMS, patients were seated in a comfortable armchair and asked to remain fully relaxed with their eyes closed to ensure similar attention levels. TMS was delivered through a high-frequency biphasic magnetic stimulator (MagstimRapid,

The Magstim Company Ltd., Whitland, South West Wales, United Kingdom), which was connected to a figure-of-eight coil with a maximal output of 1.2 T. First, the optimal orientation and position of the coil (i.e. 'hot spot') over the left motor area for stimulating the first dorsal interosseous muscle were determined. Thereafter, the RMT was identified using single TMS pulses; complete relaxation of the first dorsal interosseous (FDI) muscle was verified by the absence of electromyographic (EMG) signals, both visually (on a monitor) and by acoustic feedback. The RMT was defined as the minimal intensity required to elicit an EMG response of at least 50 μV with 50% probability in a fully relaxed muscle. Because all of the enrolled participants were right-handed, and because patients did not always experience the headaches on the same side, rTMS trains were only delivered over the left motor cortex. EMG activity in the right FDI muscle was recorded through surface electrodes placed over the right FDI muscle. Thereafter, 10 consecutive trains of 10 single pulses of TMS (stimulus intensity, 120% of the RMT; inter-train interval, 1 min) were delivered at a frequency of 1 or 5 Hz in two separate sessions (intersession interval of at least 1 week) performed in random order. The resulting EMG activity was filtered (bandwidth 20 Hz–1 kHz). All recordings were collected in 3 h period between 09.00 am and 12.00 pm by two investigators (C.L., C.C.). The 10 trains of 10 stimuli were averaged, then numbered anonymously and analysed off-line in a blind manner by one investigator (F.C.). The peak-to-peak MEP amplitudes (□V) of each of the 10 responses were measured within the train of 10 stimuli.

Statistical analysis

Data were statistically analysed in a blinded manner by a single investigator (G.C.) using Statistica version 8.0 (StatSoft Inc., Tulsa, USA) for Windows (Microsoft Corporation, Redmond, WA, USA).

Data were first analysed using the Kolmogorov-Smirnov to test for normal distribution. Preliminary descriptive analysis revealed that some the 10 MEP peak-to-peak amplitudes within the rTMS trains had a non-normal distribution. After log transformation (log10[x]), all data achieved normal distribution (Kolmogorov-Smirnov test, $p > 0.05$).

A repeated measures analysis of variance (rm-ANOVA) was performed using the between-subject factor 'group' (HV, CM, MOH) and the within-subject factor was 'stimuli'. To investigate the interaction effect, the two models of rm-ANOVA were followed by univariate ANOVA. Moreover, to quickly evaluate MEP amplitude trends within trains of rTMS stimuli, the slope of the linear regression line was calculated for the 10 stimuli for each participant on the normalized data. To analyse the slope of the linear regression, an ANOVA model with the between-subject factor 'group' (HV, CM, MOH) was used; post hoc Tukey honest significant difference tests were also performed.

A one-way ANOVA test was used to compare the clinical and neurophysiological (RMT, 1st amplitude MEP) variables at baseline. Pearson's coefficient was used to test for correlations between neurophysiological (1st MEP amplitude, MEP amplitude slope) and clinical variables (disease duration, days with headache, visual analogue scale score, monthly tablet intake, duration of the chronic phase, duration of the overuse phase). P values < 0.05 were considered statistically significant.

Results

Basic clinical and neurophysiological parameters

Assessable rTMS trains of MEPs were acquired from all study participants. The patient groups exhibited similar clinical features except for the mean monthly tablet intake (Table 1), which was clearly higher in MOH than in CM patients ($p < 0.001$). The RMT and the 1st MEP amplitude were not significantly different between groups at both 1 and 5 Hz rTMS (Table 2).

Effects of rTMS on neurophysiological parameters

In the rm-ANOVA model using the rTMS 1 Hz MEP peak-to-peak amplitude as the dependent variable, the multivariate test was significant for the factor 'stimuli' ($F_{9,405} = 5.220$, $p < 0.001$), but not for the factor 'group' ($F_{2,45} = 0.892$, $p = 0.417$) and for the 'group' × 'stimuli' interaction effect ($F_{18,405} = 0.589$, $p = 0.907$) (Figure 1 [left panel). As confirmation, the slope of the linear regression of MEP amplitudes over all stimuli

was not significantly different between groups ($F_{2,45}$ = 0.726, p = 0.489) (Figure 2 [left panel]).

In the rm-ANOVA model using the rTMS 5 Hz MEP peak-to-peak amplitude as the dependent variable, the multivariate test was not significant for the factors 'stimuli' ($F_{9,405}$ = 1.535, p = 0.133) and 'group' ($F_{2,45}$ = 0.085, p = 0.918), but it reached statistical significance for the 'group' × 'stimuli' interaction effect ($F_{18,405}$ = 2.846, p < 0.001) (Figure 1 [right panel]). The slope of the linear regression of MEP amplitudes over all stimuli was significantly different between groups ($F_{2,45}$ = 6.11, p = 0.004) (Figure 2 [right panel]). A post-hoc analysis revealed that the slope of MEP amplitudes from stimulus 1 to 10 calculated in the MOH patient group (- 0.021) was significantly different from that calculated in HVs (+ 0.010, p = 0.001) and in CM patients (- 0.003, p = 0.047) (Table 2 and Figure 2 [right panel]).

In CM patients, the mean severity of migraine assessed according to visual analogue scale correlated negatively with the slope of the linear regression of MEP amplitudes recorded in response both to 1 Hz (r = - 0.507, p = 0.045) and to 5 Hz (r = - 0.637, p = 0.008) rTMS trains. Whereas in MOH patients, the duration of the overuse phase correlated negatively with the slope of the linear regression of MEP amplitudes recorded in response to 5 Hz rTMS trains (r = - 0.506, p = 0.045). No other significant correlation between neurophysiological and clinical variables was observed in either group.

Table 2. Transcranial magnetic stimulation (TMS) resting motor thresholds (RMT) and motor evoked potential (MEP) first amplitude (Log transformed) and slope of the linear regression line from the first to the 10th stimulus of the train. Data expressed as mean——————————————SD. HV: healthy volunteers; CM: chronic migraine patients; MOH: medication overuse headache patients; n: number of subjects.

	HV (n = 16)	CM (n = 16)	MOH (n = 16)
1 Hz repetitive TMS train			
RMT (%)	54.9 ± 11.3	55.0 ± 12.6	53.6 ± 6.4
First MEP amplitude	2.2 ± 0.3	2.3 ± 0.5	2.5 ± 0.4
MEP slope	-0.002 ± 0.015	-0.005 ± 0.017	-0.009 ± 0.017
5 Hz repetitive TMS train			
RMT (%)	54.6 ± 11.4	54.0 ± 11.5	54.2 ± 6.4
First MEP amplitude	2.3 ± 0.3	2.3 ± 0.5	2.4 ± 0.4
MEP slope	0.010 ± 0.031	-0.003 ± 0.027	-0.021 ± 0.0.16*

**p< 0.05 vs. CM and HV.*

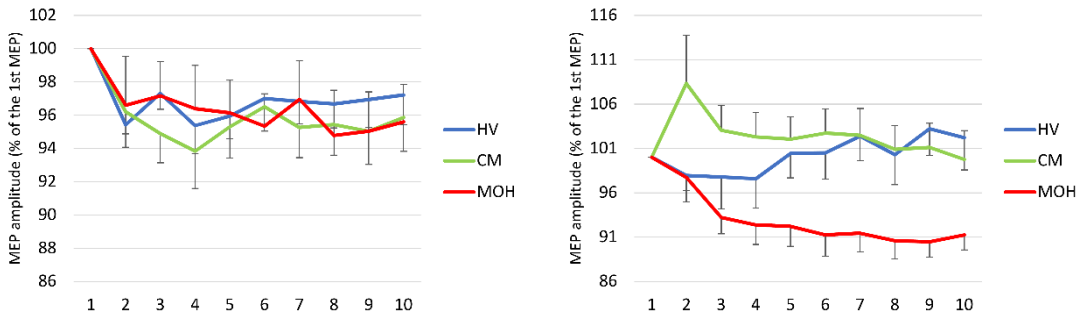

Figure 1. Motor evoked potentials (MEP) elicited by repetitive transcranial magnetic stimulation trains delivered at 1Hz (left panel) and 5Hz (right panel) at 120% resting motor threshold in healthy volunteers (HV), chronic migraine (CM), and medication overuse headache (MOH) patients.

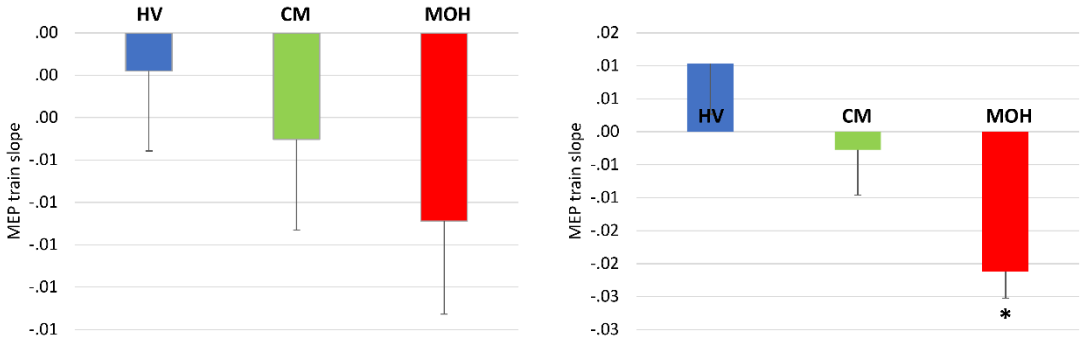

Figure 2. Bar charts representing the motor evoked potential (MEP) amplitude slope of the linear regression line from the first to the 10th stimulus of the 1Hz (left panel) and 5Hz (right panel) train of stimuli in healthy volunteers (HV), chronic migraine (CM), and medication overuse headache (MOH) patients (*p<0.05 MOH vs. HV and CM).

Discussion

The main finding of this study was that the mechanisms of short-term synaptic potentiation—but not depression—in the primary motor cortex of patients affected by MOH are different from those in HVs and pure CM patients. In fact, whereas 1 Hz-rTMS induced similar effects in the 3 groups, causing a decrease in M1 excitability, 5 Hz-rTMS led to MEP facilitation in normal subjects, while having a paradoxical inhibitory effect in MOH patients (with a significantly different slope of MEP amplitudes from that calculated in HVs and pure CM patients). We discuss the

possible neurobiological underpinnings of these data on motor cortex excitability in CM and MOH and their relevance to their pathophysiology.

In healthy subjects, rTMS at a frequency of 5 Hz with an intensity above RMT was shown to increase MEP magnitude and to induce a post-train facilitation up to 4 min [19]. This facilitation occurs at the cortical level and the mechanism involved is not completely clear because the output from corticospinal cells depends on the sum of all inhibitory and excitatory inputs to the pyramidal cells. Using 5 Hz-frequency rTMS at different stimulation intensities, several studies have reported an increase in cortical silent period duration within the stimulation train [20] and a decrease in intracortical inhibition both within train and post-train [21]. The latter finding is consistent with the reported effects of high-frequency rTMS in increasing MEP magnitude, because the down-regulation of inhibitory inputs is expected to result in increased excitability. Pharmacological studies performed to characterise the plasticity underlying this process reported that rTMS-induced facilitation is distinguished by a specific pharmacological profile suggesting a short-term potentiation mechanism and particularly a post-tetanic potentiation (PTP) [22]. PTP, which is a N-methyl-D-aspartate-receptor independent mechanism, was shown to be sustained by presynaptic processes including an increased spontaneous release of neurotransmitters and increased calcium influx [23]. This is consistent with studies reporting that short-lasting MEP facilitation, induced by 5 Hz rTMS, mainly depends on presynaptic mechanisms of glutamatergic neurotransmission [15, 16, 20].

In our MOH patients, we found a paradoxical decrease—instead of a normal increase—in MEP amplitude during 5 Hz rTMS trains despite a physiological decrease in response during 1 Hz rTMS trains. This paradoxical pattern may reflect either an increase in GABAergic or a reduction in presynaptic glutamatergic excitatory neurotransmissions. One possible explanation for this phenomenon could be the homeostatic plasticity of the human motor cortex. In a hyper-excited cortex high-frequency rTMS could facilitate the activation of homeostatic inhibitory mechanisms aimed to maintain cortical level of excitability within a physiological range and stabilize the properties of neural networks [24]. However, this homeostatic mechanism would be engaged only in presence of a hyper-excitable motor cortex.

The 1st MEP amplitude block in our MOH patients did not differ from that of HV and CM patients. Therefore, this mechanism cannot explain our results.

Interestingly, the MEP amplitude slope of the linear regression line in MOH patients was not only significantly different from that of healthy subjects, but also from that of pure CM patients, indicating that the mechanisms of short-term synaptic plasticity are different in the two groups of patients. We noticed a trend toward a decrease in cortical excitability during 5 Hz rTMS in CM patients, but we failed to show a significant difference in MEP amplitude slope between CM and HVs. In contrast to the present results, the results of the study by Cosentino et al [17] showed that MEP amplitudes significantly decreased during high-frequency trains in patients affected by CM when compared to those in healthy subjects. The difference in the reported results could be explained by the different experimental protocol and TMS apparatus we used and the clinical differences in the patients between the two studies. In fact, we used 10 trains of 10 stimuli with a 1 min inter-train interval, instead of 6 trains of 10 stimuli with a 2 min inter-train interval used in the study by Cosentino et al [20], and we considered CM patients with a shorter mean duration of history with the disease (13,5 years versus 21,7 years). Moreover, the different magnetic stimulator and coil used by Cosentino et al (Cadwell High Speed Magnetic Stimulator) could account for different effective stimulation intensities. Furthermore, our criteria for MEP behavioural assessment differed because we considered the slope of the linear regression of MEP amplitudes over all stimuli, while Cosentino et al [17] classified responses as ‘‘facilitatory’’ or “inhibitory”, in which at least 6 of the MEPs were larger or smaller in amplitude than the first MEP, respectively.

One possible explanation for the different outcomes in response to high-frequency rTMS trains between CM and MOH patients may be that they exhibit different habituation responses to repetitive stimulations. In fact previous studies have shown that pure CM patients exhibit a normal habituation pattern to sensorimotor stimulation(s) [6] (which is similar to healthy subjects), while MOH patients exhibit a habituation deficit [4, 25], although both groups of patients exhibit an initial response sensitization [4, 6, 25]. The latter evidence implies that the neurobiological mechanisms that may differentiate the brain response in CM and

MOH patients are not related to a central sensitization process because it is a general mechanism of pain chronification, but to a factor able to set delayed behavioural response plasticity. Habituation represents a basic form of learning and plasticity; therefore it is not surprising that mechanisms underlying neural plasticity and learning processes could be differentially modulated depending on the co-occurrence of external neurobiological factors such as the clinical features and behaviour of patients

This interpretation is supported by the correlation analysis. In CM patients, the mean severity of migraine was negatively correlated with the slope of the linear regression of MEP amplitudes recorded in response both to 1 Hz and to 5 Hz rTMS trains. This supports our argument that short-term plasticity of the motor cortex is positively influenced by the severity of chronic head pain, as already observed in other chronic painful conditions [11].

The same correlation was not observed in MOH patients. They showed a peculiar neurophysiological pattern that was proportional to the duration of the overuse phase, such that the greater the decreasing response during 5 Hz rTMS trains, the higher the duration of the overuse headache. Interestingly, previous studies have shown that the association between the duration of medication overuse and neurophysiological properties in the brain of MOH patients is influenced by genetic factors [25, 26]. Overall, these data reinforce the concept of MOH as a bio-behavioural disorder in which chronic headache is the result of a co-occurrence of biologically inherited, behavioural and environmental (i.e., medication overuse) factors.

A limitation of the present study was the lack of a detailed examination of short-term plasticity mechanisms in the primary motor cortex in CM and MOH patients. Furthermore, it would be interesting to compare motor cortex plasticity in chronic *vs.* episodic migraine patients; however, this study focused on chronic migraine. This is because our objective was to provide insights about modifications in motor cortex plasticity in relation to different chronification mechanisms. Another, methodological, limitation of the present study was that we only stimulated the right hemisphere in all subjects, as we assumed that, in patients with non-fixed side of

headache, the mechanisms of short term plasticity are shared between the right and left motor cortices. Finally we did not administer a specific questionnaire relating to depression, even though there is evidence that depression may affect neuroplasticity [27].

Conclusions

Our study demonstrates that the mechanisms of short-term plasticity induced by high-frequency rTMS are dysfunctional in MOH patients when compared with pure CM patients and HVs. The evidence of different plastic behaviour in the two groups of patients may indicate that MOH and CM—despite exhibiting a similar phenotype—exhibit different neurophysiological learning processes, probably related to different pathophysiological mechanisms of migraine chronification and that chronic exposure to non-steroidal anti-inflammatory drug use could cause modifications in short-term plasticity mechanisms.

Further studies are needed to understand whether pharmacological interventions or medication withdrawal are able to reverse the dysfunctional plasticity to a normal state and to reveal whether modifications of cortical excitability using non-invasive stimulation techniques are able to promote this process and induce clinical benefit. Finally, assessing brain excitability in migraine is limited by exploring only one of the aspects of a more complex picture of abnormal cortical excitability; therefore, future studies should combine different neurophysiological techniques to explore different pathophysiological aspects of migraine chronification.

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