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Cyclical changes of cortical excitability and metaplasticity in migraine: Evidence from a repetitive transcranial magnetic stimulation study

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ABSTRACT

The primary brain dysfunctions leading to the onset of a migraine attack remain largely unknown. Other important open questions concern the mechanisms of initiation, continuation, and termination of migraine pain, and the changes in brain function underlying migraine transformation. Brief trains of high-frequency repetitive transcranial magnetic stimulation (rTMS), when applied to the primary motor cortex at suprathreshold intensity ($\geqslant$120% of resting motor threshold [RMT]), elicit in healthy subjects a progressive, glutamate-dependent facilitation of the motor evoked potentials (MEP). Conversely, in conditions of increased cortical excitability, the rTMS trains induce inhibitory MEP responses likely mediated by cortical homeostatic mechanisms. We enrolled 66 migraine-without-aura patients, 48 migraine-with-aura patients, 14 patients affected by chronic migraine (CM), and 20 healthy controls. We assessed motor cortical response to 5-Hz rTMS trains of 10 stimuli given at 120% RMT. Patients with episodic migraine were studied in different phases of the migraine cycle: interictal, preictal, ictal, and postictal states. Results showed a facilitatory MEP response during the trains in patients evaluated in the preictal phase, whereas inhibitory responses were observed during and after a migraine attack, as well as in CM patients. In the interictal phase, different responses were observed, depending on attack frequency: facilitation in patients with low and inhibition in those with high attack recurrence. Our findings suggest that changes in cortical excitability and fluctuations in the threshold for inhibitory metaplasticity underlie the migraine attack recurrence, and could be involved in the process of migraine transformation.

1. Introduction

Migraine is a neurological disorder with complex and poorly understood underlying mechanisms. Most current models of migraine pathogenesis claim that a condition of brain hyperresponsivity to several exogenous and endogenous stimuli may underlie the susceptibility to migraine attacks [8,23,50,62]. However, the exact pathophysiological mechanisms leading to the attack onset remain under debate. Some authors have pointed to the brainstem as "the generator" of the attacks [1,18,61], whilst others have provided evidence that the migraine attacks may start at the cortical level [15,50,63].

The process of migraine "transformation" has become another hot topic of research in the field of migraine pathophysiology. It refers to the progression over time from episodic migraine (EM) to chronic migraine (CM), a condition associated with more severe disability and possibly higher risk of brain damage [12,14,53]. Though many risk factors, such as obesity and medication overuse, have been identified, the mechanisms of disease evolution are still unknown [11].

In recent decades, transcranial magnetic stimulation (TMS) has evolved as an excellent tool to noninvasively investigate the cortical excitability state in vivo in various neurologic disorders [52]. Very few studies, however, have been performed in EM patients in different phases of the migraine cycle, and conflicting findings have been reported in CM patients.

Aims of the present work were: 1) to investigate changes in motor cortical excitability throughout the migraine cycle (ie, interictal, preictal, ictal, and postictal periods) in patients suffering from episodic migraine with (MwA) and without aura (MwoA); 2) to

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compare motor cortical excitability among EM and CM patients, and healthy subjects; 3) to evaluate whether different patterns of cortical excitability underlie different clinical phenotypes.

The TMS paradigm used in the study consists of brief trains of repetitive TMS (rTMS) applied over the hand primary motor cortex at 5-Hz frequency and intensity of 120% of resting motor threshold (RMT). In normal subjects, the rTMS trains induce a progressive potentiation of the motor evoked potentials (MEPs) elicited at each train stimulus [48] that is thought to be mediated by presynaptic facilitatory mechanisms of glutamate release [30,37]. The presynaptic glutamatergic terminal also represents a crucial site for the homeostatic regulation of cortical excitability, that is, cortical homeostatic plasticity, or metaplasticity [43,46,47]. Accordingly, 5-Hz rTMS trains given at 120% RMT have been shown to induce, in condition of experimentally enhanced cortical activity, inhibitory homeostatic MEP responses in normal subjects [26].

On these bases, in the present work, 5-Hz rTMS trains were applied at 120% RMT to the migraine motor cortex to focus on the interplay between abnormal cortical excitability and mechanisms of cortical metaplasticity in different migraine subtypes. Metaplasticity refers to those mechanisms that stabilize cortical excitability by keeping neuronal firing rates within a physiological dynamic range [10,58]. Recently, it has been suggested that metaplasticity could play a role in migraine pathogenesis [4,25,57].

Our study might provide useful clues as to how changes in cortical excitability and homeostatic plasticity could contribute to the paroxysmal nature of migraine and its tendency to evolve over time.

2. Methods

2.1. Subjects

One hundred forty-eight right-handed subjects were eligible to participate in this study: 66 patients with MwoA (51 F/15 M, mean age 37.9 ± 9.6 years), 48 patients with MwA (34 F/14 M, mean age 38.3 ± 12.4 years), 14 patients with CM (12 F/2 M, mean age 38.3 ± 14.5 years), and 20 healthy controls (15 F/5 M, mean age 33.8 ± 7.5 years) without past medical history or familiarity for migraine. Patients were recruited from the Headache Outpatient Service of the Neurology Department at the University of Palermo, Italy.

Diagnoses of EM and CM were made according to the International Classification of Headache Disorders, 2nd edition [21] and the revised criteria [35], respectively. Additionally, a daily headache diary was used to assess headache characteristics for a minimum of 3 months before the patients were enrolled in the study. All patients suffering with MwA experienced visual aura in at least 50% of their attacks. EM patients with or without aura had a mean attacks frequency ranging from 0.5 to 8 attacks per month (1–12 headache days), while CM patients had monthly migraine days $\geqslant$ 8 and headache days $\geqslant$ 15 for at least 3 months. All CM patients had past history of MwoA meeting International Headache Society criteria. None of the participants was taking prophylactic drugs at least 3 months prior to the study. CM patients were excluded if their headaches followed head trauma, if they had a prominent psychological illness, or if their headaches occurred in the presence of symptomatic medication overuse. All patients denied any history of systemic or other neurological diseases, and presented normal physical and neurological examinations.

Different subgroups of patients with EM were evaluated in different phases of the migraine cycle. The subjects who did not have migrainous headache within a period of 2 days before and after the experimental evaluation were classified as interictal. Patients suffering from a migraine attack at the time of the experiment were classified as ictal, whereas those evaluated within the 48 hours preceding or following the headache were respectively classified as preictal and postictal. Based on previous work [19], recordings for CM patients were performed as in interictal EM patients (no acute migraine within the 48 hours preceding or following the electrophysiological evaluation) but present background (or interval) headache during evaluation was allowed. Occurrence of attack after recording was verified by means of a telephone call. Selection of the time window for the peri-ictal period was based on earlier studies [31,40].

To avoid possible unspecific effects related to pharmacological activity, patients underwent the electrophysiological assessment only when they had not taken symptomatic medications in the 24 hours preceding the evaluation. To minimize any hormonal effect, female patients and controls were not examined during the menstrual phase.

Before enrollment, all the subjects were checked for contraindications to TMS [41], and gave their written informed consent to participate. The study conformed to the Declaration of Helsinki, and the experimental procedures were approved by the local ethics committee. The demographic and clinical data of subjects are summarized in Table 1.

2.2. Stimulation procedures

All subjects were comfortably seated on a chair and told to be as relaxed as possible. They wore a tight-fitting plastic swimming cap to mark the optimum stimulation site and ensure optimum coil placement. Electromyography (EMG) signals were recorded from the right abductor pollicis brevis muscle using 0.9-cm-diameter Ag–AgCl surface electrodes placed 3 cm apart over the belly and tendon of the muscle. The EMG activity was recorded with a bandpass of 10 to 1000 Hz and a display gain ranging from 50 to 1000 μV/cm. EMG signals were collected, averaged, and analyzed off-line. Focal TMS was delivered over the hand motor cortex of the left hemisphere using a figure-of-8 coil connected to a monophasic Cadwell High Speed Magnetic Stimulator (Cadwell Laboratories, Kennewick, WA, USA). The stimulating coil with posteroanterior orientation was placed over the optimal site for eliciting responses in the contralateral target muscle [3]. The RMT for eliciting responses in the relaxed abductor pollicis brevis muscle was defined as the minimum intensity of stimulation needed to produce responses of 50 μV in at least 50% of 10 trials. The subjects were given audiovisual feedback of EMG activity to help maintain complete muscle relaxation. The coil position was continuously monitored throughout the experiment in order to keep it constant. Stimulation was performed following safety guidelines [51].

2.3. Experimental paradigm and measurements

All subjects underwent an experimental evaluation consisting of 6 trains of 10 stimuli delivered at 5-Hz frequency to the left primary motor hand area. The rTMS trains were applied with a 2-minute intertrain interval on subjects at rest at an intensity of the stimulator output equal to 120% of the RMT. To evaluate changes in MEP size during the rTMS trains, for each subject, MEP amplitudes were calculated peak-to-peak from single traces and then averaged according to their position in the train. In addition, since different, even opposite (facilitatory or inhibitory) MEP responses may be elicited by the rTMS trains [16,25,26], individual analyses were made and the response pattern in each subject was classified as "facilitatory," "inhibitory," or "flat." We classified as "facilitatory" the responses in which at least 6 of the MEPs following the first in the train were larger in amplitude as compared to the first MEP, with a ratio between the largest and the first MEP size $\geqslant$ 1.3.

Table 1
Demographic and clinical characteristics of the enrolled subjects: mean ± SD and range interval.

	RMT	Age (years)	Sex (F/M)	Attack frequency (attacks/month)	Headache history (years)	Headache severity (1–3)	Attack duration (hours)
Migraine with aura (n = 48)							
Interictal (n = 27)	64.2 ± 7.5 (44–78)	38.8 ± 13.7 (18–64)	20/7	2.9 ± 2.1 (0.5–8)	19.1 ± 9.6 (5–43)	1.8 ± 0.7 (1–3)	27.1 ± 13 (6–72)
Preictal (n = 7)	67 ± 6.5 (60–80)	33.3 ± 5.3 (27–41)	5/2	3.9 ± 2 (2–8)	17.6 ± 2.5 (15–20)	2 ± 0.8 (1–3)	32 ± 13 (12–48)
Ictal (n = 7)	68 ± 9.7 (56–83)	40.3 ± 14.4 (18–67)	5/2	4.1 ± 2.8 (1–8)	18.6 ± 7.1 (15–30)	1.7 ± 0.8 (1–3)	34 ± 20.3 (12–72)
Postictal (n = 7)	64.3 ± 11.4 (43–78)	37.7 ± 10.4 (25–58)	5/2	3.6 ± 1.3 (2–6)	18.6 ± 7.5 (10–30)	1.7 ± 0.8 (1–3)	27.3 ± 17.8 (12–48)
Migraine without aura (n = 66)							
Interictal (n = 36)	65.6 ± 9.2 (48–82)	39.2 ± 9.5 (20–53)	29/7	4.1 ± 2.3 (0.5–8)	19.2 ± 9.2 (2–35)	1.9 ± 0.7 (1–3)	32.1 ± 18.3 (6–72)
Preictal (n = 10)	65.2 ± 12.3 (42–82)	33.1 ± 8.6 (25–49)	8/2	4.4 ± 2.3 (0.5–8)	15.5 ± 6.8 (10–30)	1.9 ± 0.6 (1–3)	31.2 ± 18.1 (12–72)
Ictal (n = 10)	63.4 ± 9.47 (45–75)	39.4 ± 10.9 (23–54)	8/2	4.6 ± 2.2 (1–8)	20.7 ± 9.3 (8–34)	1.9 ± 0.9 (1–3)	27.2 ± 23.8 (12–72)
Post-ictal (n = 10)	61.7 ± 10.4 (42–80)	36.1 ± 10.3 (24–50)	8/2	3.6 ± 2.1 (0.5–8)	15.4 ± 6.9 (6–25)	2.1 ± 0.7 (1–3)	25.5 ± 17.3 (12–48)
Chronic migraine (n = 14)	69 ± 5.7 (58–80)	38.3 ± 14.6 (21–63)	12/2	13.9 ± 3.1 (10–18)	21.7 ± 10.4 (10–45)	1.5 ± 0.6 (1–3)	25.6 ± 14.4 (6–48)
Healthy subjects (n = 20)	60 ± 5.9 (48–70)	33.8 ± 7.5 (25–50)	15/5	–	–	–	–

RMT, resting motor threshold.

Conversely, responses were classified as "inhibitory" when at least 6 of the MEPs following the first were smaller, with a ratio between the smallest and the first MEP size $\leqslant 0.7$. Responses that did not fit into either of the 2 aforesaid patterns were classified as "flat." These criteria were chosen based on our previous observation that, although in most subjects all MEPs elicited by the rTMS train are higher or lower in size as compared to the first MEP, it may also occur that: 1) in some cases, a clear MEP facilitation is observed only from the third or fourth response in the train; 2) in subjects presenting a clear inhibitory MEP response, MEPs can slightly increase after the initial decrement so that the last 2 or 3 MEPs in the train can reach an amplitude similar to that of the first MEP [16,25,26]. As above, MEP ratios of $\geqslant 1.3$ and $\leqslant 0.7$ were arbitrarily chosen to classify an MEP response pattern as facilitatory or inhibitory, respectively, as we considered adequate an amplitude difference of at least 30% between the first response and the higher or lower MEP in the train.

2.4. Statistical analysis

Between-group repeated-measures analysis of variance (ANOVA) with "number of stimuli" (10 levels) as within-subjects factor was used to assess statistical significance in comparing changes in MEP amplitude during the rTMS trains in the 2 groups of patients with EM (MwA and MwoA) evaluated interictally, in CM patients, and healthy subjects.

To evaluate, in patients with EM, possible changes of the motor cortical responses throughout the migraine cycle, we performed a 3-way ANOVA with "group" (2 levels: MwA and MwoA) and "cycle phase" (four levels: interictal, preictal, ictal, and postictal phase) as between-subjects factors, and "number of stimuli" (10 levels) as within-subjects factor.

After individual analysis (see above), if distinct subgroups of patients presenting different response patterns (ie, facilitatory, flat, or inhibitory) were identified within a group (ie, interictal, preictal, ictal, postictal, or CM patients), a 2-way ANOVA with "subgroup" as between-subjects factor and "number of stimuli" (10 levels) as within-subjects factor was performed. In addition, possible differences for demographic and clinical parameters between patient subgroups were evaluated by using Student's *t*-test.

One-way ANOVA was used to compare the average age, first MEP size, and RMT between MwA and MwoA patients evaluated in the various phases of the migraine cycle, CM patients, and healthy subjects. One-way ANOVA was also performed to compare clinical parameters (attack frequency, duration of the disease, headache duration and severity) between different groups of migraine patients who underwent the rTMS trains in different cycle phases.

The sphericity assumption was checked by using Mauchly's test, and Huynh-Feldt's correction was adopted, if necessary, for the degrees of freedom. Statistical analyses were done with Statistica 7.0 software (StatSoft, Tulsa, OK). Duncan's test was used for post hoc analysis. For all analyses, the level of statistical significance was set at $P < 0.05$.

3. Results

The experimental procedures were well tolerated in all subjects, and no adverse effects were reported. No significant differences in the average age, first MEP size, and RMT were found between MwA and MwoA patients evaluated interictally, CM patients, and healthy subjects. No significant differences in the first MEP size and RMT, as well as in the average age and clinical parameters, were found between MwA and MwoA patients evaluated in different phases of the migraine cycle (Table 1).

Between-group ANOVA for the MEP values during the rTMS trains in MwA and MwoA patients evaluated interictally, CM patients, and healthy subjects (Fig. 1) showed a significant effect of factor "number of stimuli" [$F(3, 313) = 8.08$; $P = 0.00001$] and a significant interaction between "group" and "number of stimuli" [$F(10, 313) = 2.29$; $P = 0.01$]. Post hoc analysis showed that MEP amplitudes significantly increased and decreased throughout the rTMS trains, respectively, in the healthy subjects and in patients with CM. Conversely, no significant MEP changes were shown in the MwA and MwoA patients.

ANOVA performed to compare responses during the rTMS trains in patients with EM (with and without aura) evaluated in the different phases of the migraine cycle (Fig. 2A, B) showed a significant effect of factors "number of stimuli" [$F(9, 954) = 5.7268$, $P = 0.00001$] and "cycle phase" [$F(3, 106) = 6.2573$, $P = 0.0006$], and a significant interaction between "number of stimuli" and "cycle phase" [$F(27, 954) = 5.9799$, $P = 0.00001$]. No significant effect of factor "group" or significant interaction between "number of stimuli," "cycle phase," and "group" were observed. Post hoc analysis showed increased MEP amplitudes throughout the trains in MwA and MwoA patients evaluated in the preictal period, whilst inhibitory responses were observed during the ictal and postictal period. No significant MEP changes during the course of the trains were shown in both MwA and MwoA patients evaluated interictally.

Individual analysis of the motor cortical responses during the trains (Table 2) showed that all the healthy subjects and the migraine patients who underwent the electrophysiological measures in the preictal phase had facilitatory or flat responses. Conversely, all patients evaluated in the ictal and postictal period, as well as the CM patients, showed inhibitory MEP responses. Differently, in MwA and MwoA patients evaluated in the interictal state, 2 different response patterns were observed: a subgroup of patients presented facilitatory or flat responses during the rTMS trains,

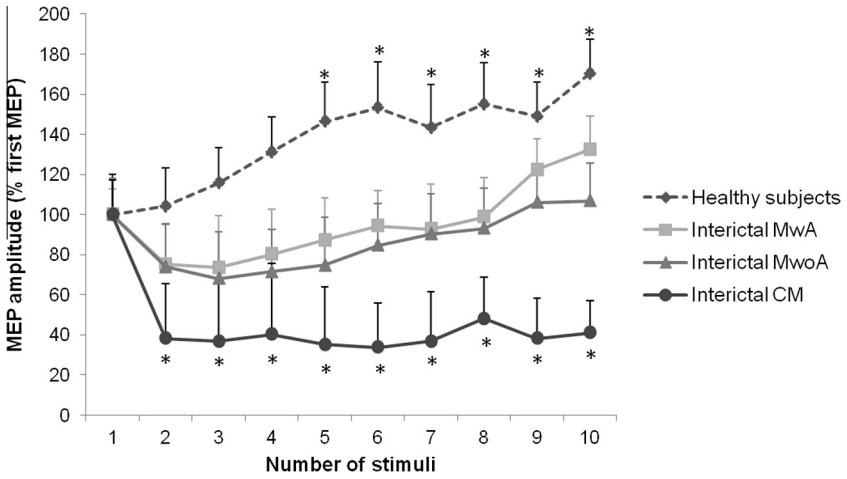

Fig. 1. Motor evoked potentials (MEP) elicited by repetitive transcranial magnetic stimulation (rTMS) trains delivered at an intensity of 120% of the resting motor threshold in patients suffering from episodic migraine (with or without aura) evaluated in the interictal phase, in patients with chronic migraine, and in healthy subjects. Mean MEP amplitudes are expressed as percentage of the first MEP size in the train. Error bars indicate SEM. Asterisks (∗) indicate significant variations of MEP amplitudes during the rTMS trains as compared to the first MEP response. MwA, migraine with aura; MwoA, migraine without aura; CM, chronic migraine.

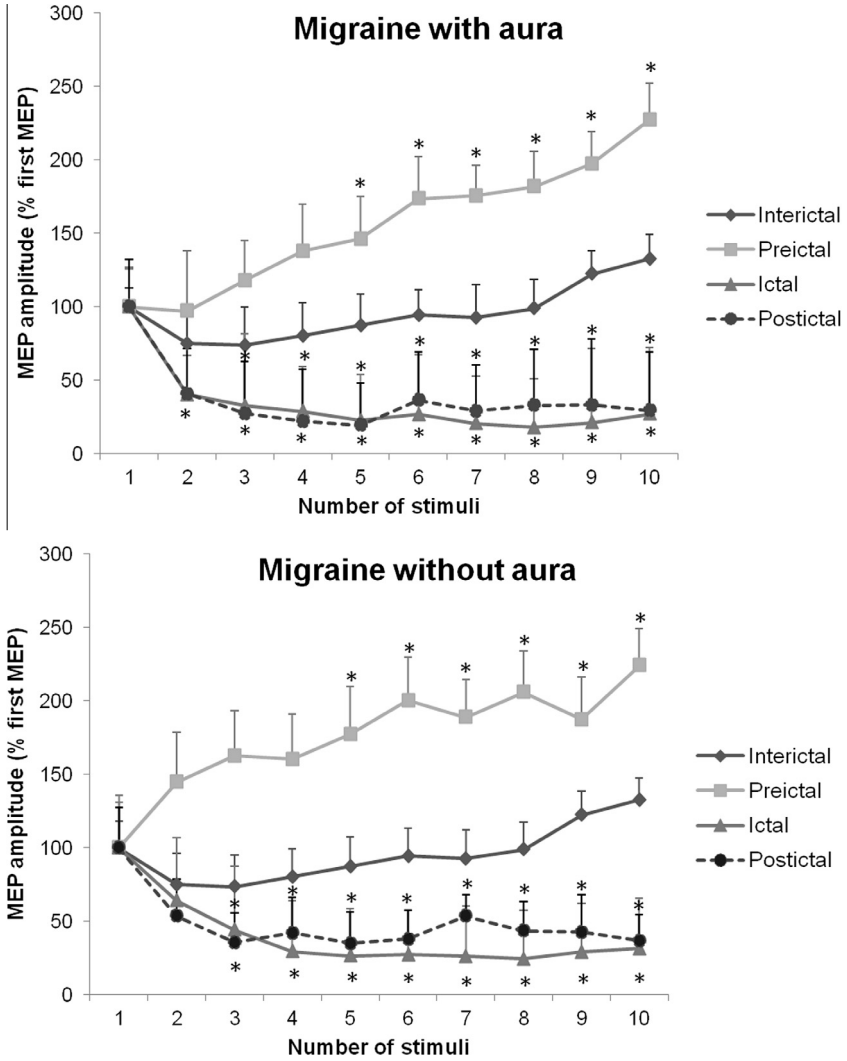

Fig. 2. Motor evoked potentials (MEP) elicited by repetitive transcranial magnetic stimulation (rTMS) trains delivered at an intensity of 120% of the resting motor threshold in patients suffering from migraine with aura (above) and migraine without aura (below) evaluated in different phases of the migraine cycle: interictal, preictal, ictal, and postictal period. Mean MEP amplitudes are expressed as percentage of the first MEP size in the train. Error bars indicate SEM. Asterisks (∗) indicate significant variations of MEP amplitudes during the rTMS trains as compared to the first MEP response.

Table 2
Number of subjects presenting different MEP response patterns during the rTMS trains in MwA and MwoA patients evaluated in the different phases of the migraine cycle, in CM patients, and in healthy subjects.

	MEP response pattern		
	Facilitatory	Flat	Inhibitory
Migraine with aura (n = 48)			
Interictal (n = 27)	12	1	14
Preictal (n = 7)	5	2	–
Ictal (n = 7)	–	–	7
Postictal (n = 7)	–	–	7
Migraine without aura (n = 66)			
Interictal (n = 36)	9	5	22
Preictal (n = 10)	7	3	–
Ictal (n = 10)	–	–	10
Postictal (n = 10)	–	–	10
Chronic migraine (n = 14)	–	–	14
Healthy subjects (n = 20)	15	5	–

MEP, motor evoked potentials; rTMS, repetitive transcranial magnetic stimulation; MwA, migraine with aura; MwoA, migraine without aura; CM, chronic migraine.

whilst another exhibited inhibitory responses. These subgroups of MwA and MwoA patients presenting different facilitatory/flat or inhibitory response patterns were compared with each other and with controls by means of a 2-way ANOVA (Fig. 3). Patients with facilitatory or flat responses were collapsed together, given that both these 2 response patterns were observed in the control subjects. Results showed a significant effect of factor "number of stimuli" [$F(4, 286) = 17.36$, $P = 0.00001$] and a significant interaction between "group" and "number of stimuli" [$F(15, 286) = 6.18$, $P = 0.00001$]. Post hoc analysis showed that MEP amplitudes significantly increased in the healthy subjects and, to a greater extent, in the MwA and MwoA patients who presented a facilitatory/flat response pattern during the rTMS trains. Conversely, a significant decrease of MEP size during the trains was observed in both MwA and MwoA patients showing an inhibitory response pattern during the trains. Student's *t*-test used to compare interictal MwA and MwoA subgroups of patients presenting opposite response patterns (Fig. 4) showed a significant difference for the mean attack frequency, which was higher in the patients with inhibitory MEP responses than in those with facilitatory/flat response pattern (4.2 ± 1.7 vs 1.2 ± 0.9 attack/month in MwA patients, $P < 0.00001$; 4.7 ± 2.1 SD vs 1.7 ± 0.9 attack/month in MwoA patients, $P < 0.00001$). No differences for other clinical parameters were observed between subgroups (Fig. 4). Pearson's test was used to correlate the mean attack frequency with the extent of MEP changes during the rTMS trains, which was evaluated as the ratio between the largest or smallest MEP in the rTMS train (for facilitatory/flat and inhibitory responses, respectively) and the first MEP size (Fig. 5). Analysis showed a significant correlation in both subgroups of interictal MwA ($r = -0.5$, $P < 0.01$) and MwoA ($r = -0.41$, $P < 0.05$) patients.

4. Discussion

4.1. Motor cortical responses in interictal EM patients

In the present study, brief 5-Hz rTMS trains, when given at 120% RMT to the primary motor cortex, do not elicit a normal MEP potentiation in MwA and MwoA patients evaluated interictally (Fig. 1). The rapid and short-lasting MEP potentiation observed during suprathreshold rTMS trains in healthy subjects is thought to be mediated by mechanisms of short-term synaptic enhancement acting at the cortical level [9,37,38,48], which are mainly due to a calcium-dependent regulation of glutamate release [39,64]. Thus, our results are in line with other neurophysiological [22,27,55], neuroimaging [57], and biological [2,28,29,44] findings, indicating a possible glutamatergic dysfunction in migraine.

On an individual analysis, we observed opposite response patterns in patients evaluated interictally, as compared to healthy subjects (Fig. 3). In particular, increased MEP potentiation and paradoxical MEP inhibition were detected in different subgroups of patients who presented, respectively, a lower and higher mean attack frequency (Fig. 4). These findings are only apparently in contrast with those by Conte et al. [22], who showed different response patterns throughout 5-Hz rTMS trains between MwA and MwoA. Indeed, in line with the present results, the authors observed an increased MEP facilitation in a group of MwA patients presenting a low mean attack frequency. Conversely, lack of MEP potentiation was detected in MwoA patients with a higher frequency of attacks.

The increased MEP potentiation observed in patients with low attack frequency is in line with other TMS studies showing increased motor cortical responsivity [22,27,55] and reduced threshold for facilitatory responses (ie, MEP potentiation at 110% RMT) during 5-Hz rTMS trains in migraine [16]. Conversely, the inhibitory response pattern observed in patients presenting a higher attack frequency could be interpreted in the context of cortical

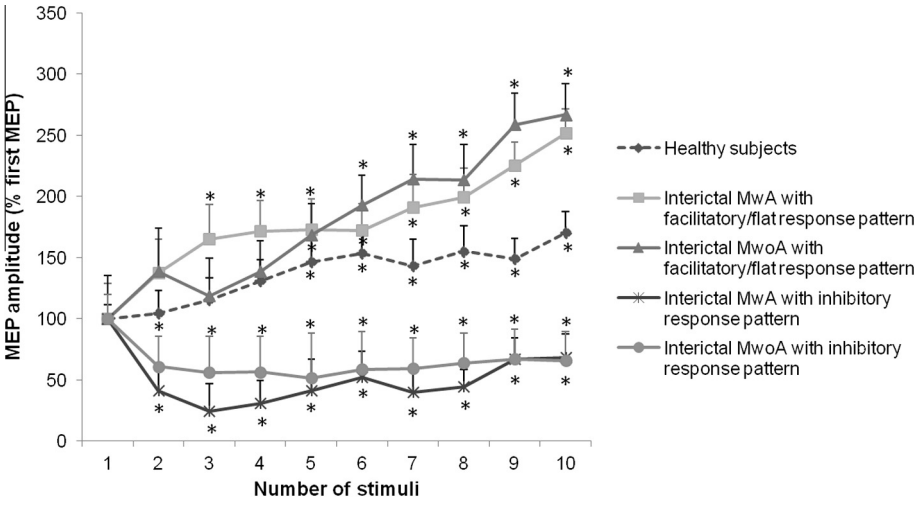

Fig. 3. Motor evoked potentials (MEP) elicited by repetitive transcranial magnetic stimulation (rTMS) trains delivered at an intensity of 120% of the resting motor threshold in different subgroups of migraine with aura (MwA) and migraine without aura (MwoA) patients presenting facilitatory/flat or inhibitory MEP response pattern in the interictal phase. Mean MEP amplitudes are expressed as percentage of the first MEP size in the train. Error bars indicate SEM. Asterisks (*) indicate significant variations of MEP amplitudes during the rTMS trains as compared to the first MEP response.

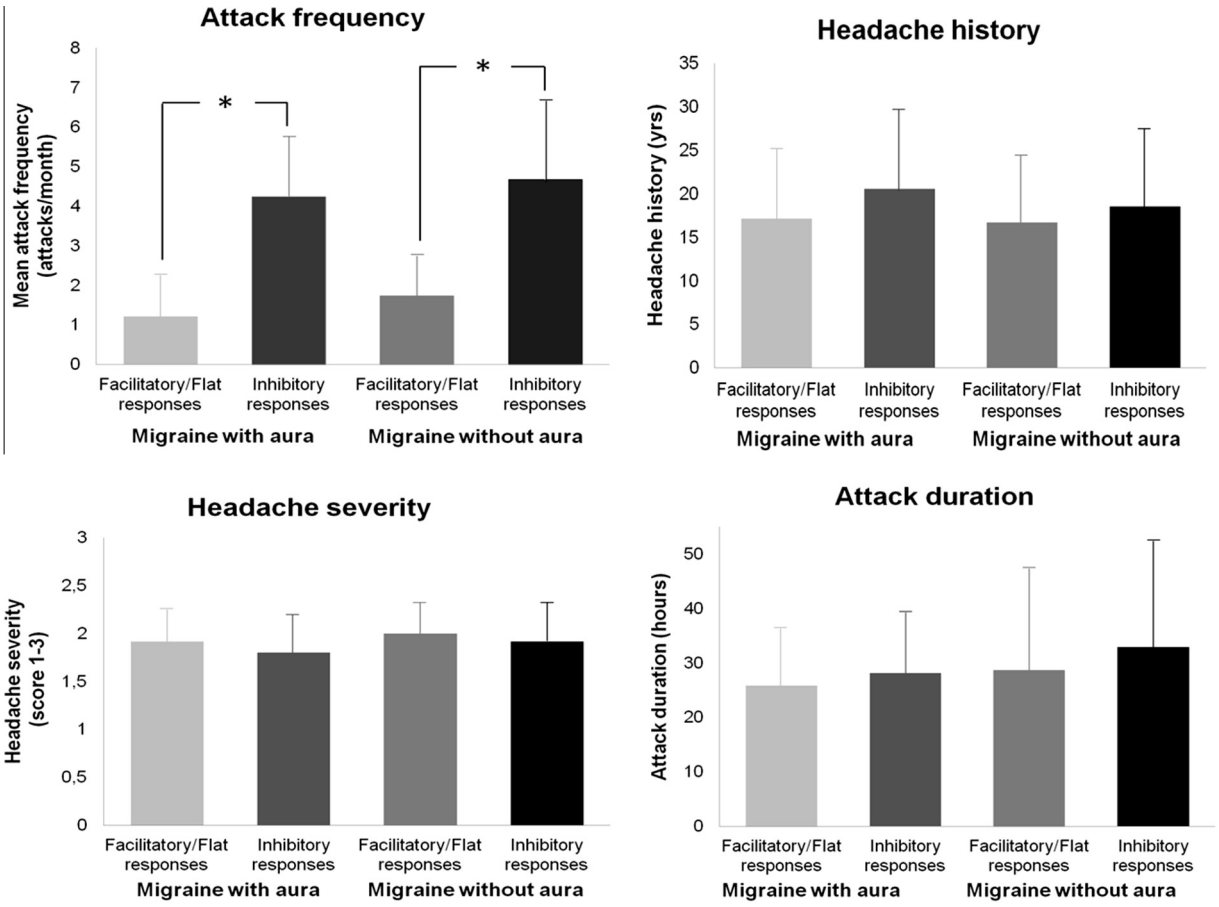

Fig. 4. Clinical feature comparison between migraine with aura and migraine without aura patients evaluated in the migraine interval and presenting opposite facilitatory/flat or inhibitory response pattern during the repetitive transcranial magnetic stimulation trains. *$P < 0.00001$. Headache severity was scored on a 1–3 point scale, with 1 presenting no effect on daily activity, 2 for partial inhibition of daily activity, and 3 for loss of daily activities.

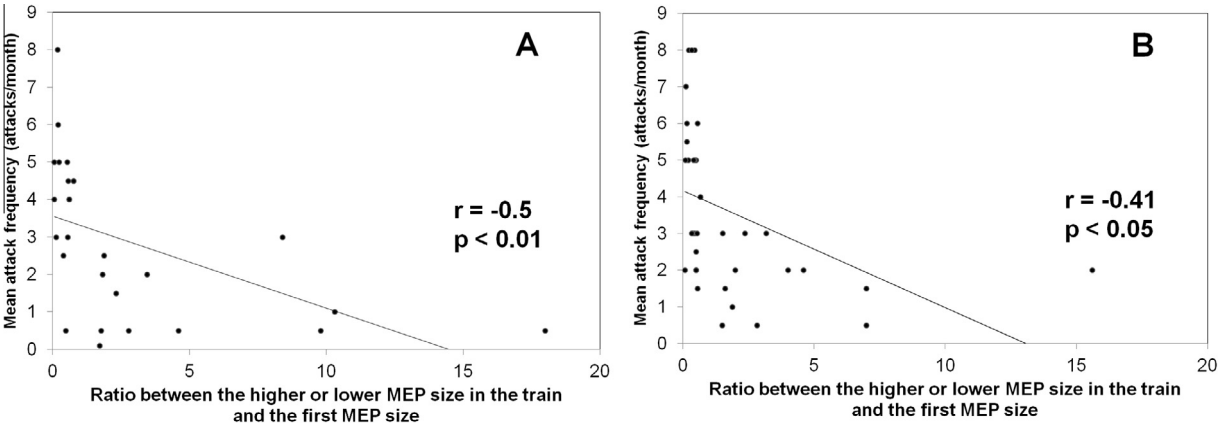

Fig. 5. Correlations between individual extent of motor evoked potentials (MEP) changes throughout the repetitive transcranial magnetic stimulation trains (size ratio between the largest or smallest MEP in the train and the first MEP response, for facilitatory/flat and inhibitory responses respectively) and mean attack frequency (attacks/months) in migraine with aura (A) and migraine without aura (B) patients evaluated in the migraine interval.

homeostatic plasticity. This explanation fits with our finding that in EM, parallel to the increase in attack frequency, MEP facilitation progressively decreases up to being replaced by an increasingly more pronounced inhibitory response (Fig. 5). Indeed, according to the rules of cortical metaplasticity, the enhancement in cortical excitability that occurs with increase in attack frequency [5,59,60] would reduce the threshold for inhibitory homeostatic responses. Such an interpretation agrees with experimental findings in healthy subjects [26], as well as with: 1) finding of reduced threshold for inhibitory metaplasticity (ie, MEP inhibition at 130% RMT) during 5-Hz rTMS trains in migraineurs as compared to normal subjects [16]; 2) evidence that preconditioning with cathodal transcranial direct-current stimulation, which reduces the cortical excitability level, restores a normal MEP facilitatory response during the rTMS trains in MwA and MwoA patients [25]. Inhibitory homeostatic mechanisms of cortical excitability prevent induction of exaggerated and potentially dangerous excitation in response to high-magnitude stimuli [34]. Accordingly, in migraine patients with higher

attack frequency, the shift in the threshold for inhibitory metaplasticity could be a protective mechanism avoiding excessive and runaway cortical activation in response to endogenous and exogenous stimuli. In addition, we are tempted to speculate that inhibitory metaplasticity could represent an attempt to prevent the occurrence of further attacks by reducing cortical responsivity to migraine triggers (Fig. 6A). This idea comes from evidence that the threshold for inhibitory homeostatic plasticity, which is not constant but physiologically fluctuates in relation to changes in cortical activity [10,58], rises just before the attack onset (see below).

4.2. Motor cortical excitability throughout the migraine cycle in EM patients

We observed noticeable changes in the MEP response during the rTMS trains in EM patients evaluated in different phases of the migraine cycle (Fig. 2). It is noteworthy that these different groups of patients did not significantly differ in any demographic or clinical feature (Table 1), thus, the observed cortical excitability changes are likely linked to the pathophysiological mechanisms underlying the recurrence of the attacks. To our knowledge, only another TMS study has been performed, in children suffering from MwoA, to evaluate systematically the cortical excitability changes during the migraine cycle [56]. The authors used TMS paradigms different from ours showing shifts in the visual cortex excitability, but not cyclical modification of the RMT as in our results.

In both MwA and MwoA patients evaluated in the 48 hours preceding the attack, we showed a restoration of the MEP facilitatory response. This may indicate that the threshold for inhibitory homeostatic responses to the rTMS trains rises in the preictal period. Such an interpretation is in accord with neurophysiological and neuropsychological evidence that hyperresponsivity, which characterizes migraine patients during the interval, tends to normalize, or even to switch to hypoactivity, before an attack [13,17,33,56]. Indeed, consistent with the rules of cortical metaplasticity, the threshold for inhibitory homeostatic plasticity rises

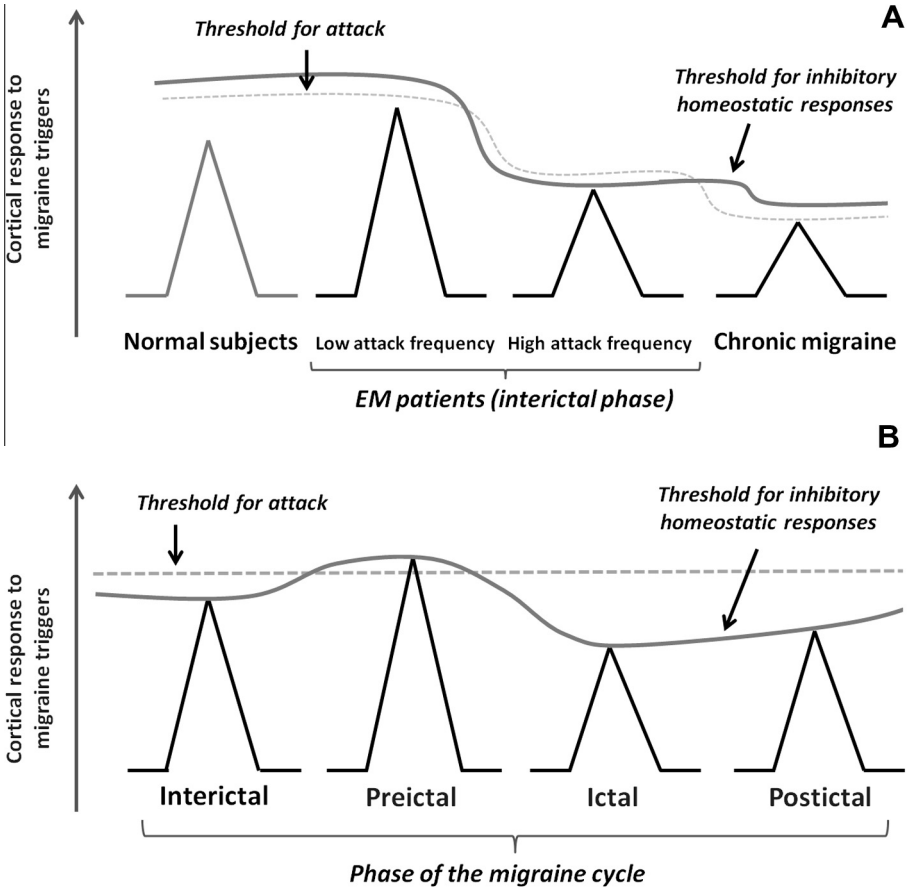

Fig. 6. Schematic representation of the hypothetical changes in the threshold for the attack and for inhibitory metaplasticity in migraine. The migraine threshold (dashed line) is thought of as a critical level of cortical activation that triggers the migraine attack. The threshold for inhibitory metaplasticity (solid line) corresponds to the cortical activation level needed to induce homeostatic inhibitory responses to excitatory stimuli acting as migraine triggers. The height of the triangles represents the extent of the cortical response to endogenous or exogenous stimuli (migraine triggers) able to increase cortical activity in different subgroups of patients and in healthy subjects. (A) In normal subjects, exposure to factors capable to potentially precipitate an attack does not trigger migraine symptoms because cortical activation does not reach the migraine threshold. Instead, in episodic migraineurs with sporadic attacks, hyperresponsivity to several sensory stimuli could account for the easier achievement of the migraine threshold. The repetition of migraine attacks induces a decrease in the threshold for further attacks, so leading to increased trigger sensitivity and higher frequency of attacks. In patients with episodic migraine (EM) with high attack frequency, the threshold for inhibitory metaplasticity could be lower, in the interictal period, as a compensatory mechanism aiming to prevent occurrence of further attacks. In chronic migraineurs, a further decrease in the migraine threshold could explain why inhibitory metaplasticity, although normally activated, is no longer capable to prevent cortical activation from reaching the migraine threshold. (B) As previously shown, recurrence of attacks at short time intervals could reduce the threshold for inhibitory metaplasticity during the interictal phase, possibly to compensate the progressive lowering in the migraine threshold. However, according to the metaplasticity rules, the threshold for activating inhibitory homeostatic mechanisms will tend to rise up to normal level when the cortical activity transiently decreases. Such a condition may be responsible, in the preictal phase, for increased trigger sensitivity and vulnerability to the onset of a migraine attack. Indeed, in such a condition, homeostatic mechanisms could not be able to prevent oncoming trigger factors from inducing excessive cortical activation reaching the migraine threshold. Finally, cortical overactivation that triggers the attack could abruptly induce a decrease in the threshold for inhibitory metaplasticity, as seen in the ictal and postictal period. The homeostatic inhibition of the cortical response to several sensory stimuli could be important to terminate the migraine attack and prevent the early recurrence of migraine symptoms.

when cortical activity reduces. One could suppose that the increase in the threshold for activation of inhibitory metaplasticity could play a role in the neurophysiological disposition to a migraine attack. Indeed, it could allow different migraine-precipitating agents to induce an uncontrolled cortical hyperactivation, culminating in a migraine attack (Fig. 6B).

In the MwA and MwoA patients who underwent the rTMS trains during a spontaneous attack, a significant decrease of MEP size during the rTMS trains was observed. This result does not conflict with neurophysiological evidence that the cortical preactivation level increases during the ictal period [40], and with findings of increased glutamate levels in plasma and cerebrospinal fluid during the migraine attack [2,44]. In fact the abrupt increase in cortical activation leading to the migraine attack would contextually lower the threshold for inhibitory homeostatic responses, in line with evidence in healthy subjects that inhibitory MEP responses may be elicited by the rTMS trains after experimental increase of cortical excitability [26].

Further studies are needed to clarify whether inhibitory metaplasticity simply represents, during the attack, a protective mechanism preventing excitotoxicity, or can be directly involved in migraine pathophysiology. It is conceivable, indeed, that inhibitory homeostatic mechanisms reducing brain responsivity to different migraine triggers, could be involved in the relief of the migraine attack. Inhibitory metaplasticity remains active, for a certain period, also after the end of the attack, as shown by the inhibitory response pattern seen during the rTMS trains in patients evaluated in the postictal period. At least theoretically, in this phase of the migraine cycle, inhibitory homeostatic mechanisms could play a role in preventing the early recurrence of the migraine symptoms (Fig. 6B).

4.3. Motor cortical responses in CM patients

The mechanisms by which EM evolves to CM remain a somewhat controversial subject. Evidence has been accumulated that a progressive increase in cortical excitability could underlie the process of migraine transformation [7,24,49]. A dysfunction of the brainstem [6,42] and the development of central sensitization [32,45] are also supposed to play a central pathogenic role. Even jointly, all these mechanisms could lead to a vicious cycle in which recurrence of the attacks progressively lowers the migraine threshold, thus making CM patients unusually susceptible to migraine attacks.

In our study, we observed in CM patients an inhibitory MEP response pattern during the 5-Hz rTMS trains (Fig. 1), resembling that observed in EM patients with high attack frequency evaluated interictally, and in patients in the ictal state. As in EM, in CM patients the inhibitory response pattern also may be expression of reduced threshold for inhibitory homeostatic responses, with a possible protective meaning with respect to the increased cortical excitability. In addition, we could suppose that due to an excessive decrease of the migraine threshold, inhibitory homeostatic mechanisms could become ineffective both in preventing the emergence of a migraine attack and in stopping it (Fig. 6B) [20,54]. This hypothesis agrees with other neurophysiological findings supporting the concept of a persistent ictal-like state of cortical excitability in CM [20,54].

4.4. Conclusions

Some methodological considerations and limitations of the current study are worth mentioning. First, interictal and peri-ictal recordings in the same subject were not obtained. Theoretically, a longitudinal study would be more sensitive in determining cortical excitability changes throughout the migraine cycle, although homogenization of the menstrual phase may be technically challenging for longitudinal neurophysiological studies in migraine [19]. Another mention should be made that the number of patients evaluated in the peri-ictal state was relatively small. Thus, though we observed the same MEP response pattern in each subgroup of patients evaluated in the preictal, ictal, and postictal period, we cannot exclude that various clinical aspects could differently affect the cortical response to the rTMS trains in the peri-ictal migraine cycle phases, and further, more focused studies are needed to address this issue. Further investigations will be needed also to assess to what extent our results on the motor area could be generalizable to the whole migraine cortex.

In conclusion, our findings support the concept that cortical hyperresponsivity could account for the increased susceptibility to migraine. Moreover, consistent with the emerging role of metaplasticity in several brain disorders [36], we provide evidence that fluctuations in the threshold for inhibitory metaplasticity could explain why the susceptibility to the migraine triggers and the neurophysiological readiness to generate a migraine attack is not constant but significantly changes over time. If confirmed by future research, the present results could open new interesting therapeutic perspectives in migraine disorders.

Conflict of interest statement

None of the authors of this manuscript declare any conflict of interest.

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