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Homeostatic effects of plasma valproate levels on corticospinal excitability changes induced by 1 Hz rTMS in patients with juvenile myoclonic epilepsy

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Abstract

Objective: The preliminary results of noninvasive brain stimulation for epilepsy treatment have been encouraging, but mixed. Two important factors may contribute to this heterogeneity: the altered brain physiology of patients with epilepsy and the variable presence of antiepileptic drugs. Therefore, we aimed to study the effects of 1 Hz rTMS on corticospinal excitability in patients with juvenile myoclonic epilepsy (JME) in two different conditions: low- or high-plasma valproate levels.

Methods: Fifteen patients with JME and 12 age-matched healthy subjects participated in this study. Corticospinal excitability before and after 1 Hz rTMS was assessed in JME patients with low- and high-plasma valproate levels; and these results were compared with those in healthy subjects.

Results: In patients with chronic use of valproate and low-plasma concentrations, 1 Hz rTMS had a similar significant inhibitory effect on corticospinal excitability as in healthy subjects. However, in the same patients when the serum valproate concentration was high, 1 Hz rTMS increased the corticospinal excitability significantly. In addition, there was a significant positive correlation between plasma valproate levels and the motor threshold changes after 1 Hz rTMS.

Conclusions: Our findings can be accounted for by mechanisms of homeostatic plasticity and illustrate the dependency of the modulatory effects of rTMS on the physiologic state of the targeted brain cortex.

Significance: The therapeutic use of rTMS in epilepsy should take into consideration the interaction between rTMS and drugs that change cortical excitability.

Keywords: Transcranial magnetic stimulation; Corticospinal excitability; Valproate; Homeostatic plasticity

1. Introduction

Low-frequency rTMS can decrease cortical excitability (Chen et al., 1997a), an application that might have a potential clinical use in a variety of disorders characterized

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by abnormally increase cortical excitability, such as epilepsy (Hoffman and Cavus, 2002; Pascual-Leone et al., 1998). Such modulatory effects of rTMS appear dependant on the stimulation intensity and particularly the stimulation frequency used (Maeda et al., 2000). Furthermore, there is substantial inter-individual variability for which the causes are not completely clear (Maeda et al., 2000). The stimulation parameters that suppress cortical excitability in some subjects appear capable of the opposite impact on cortical excitability in other subjects. Individual characteristics may play a critical role in such disparities (Gangitano et al., 2002) but the state of cortical activity at the time of TMS application is likely an important variable, since the effects of TMS may be best conceptualized as an interaction of the stimulation with the ongoing activity in the targeted brain area and its connected neural network.

Indeed, Siebner et al. (2004) showed that the effects of 1 Hz rTMS can be modified depending upon the baseline cortical excitability. These authors used transcranial direct current stimulation (tDCS) to change the baseline motor cortex excitability before the application of 1 Hz rTMS. tDCS is supposed to modify neuronal membrane properties (hyper- or depolarization depending on current orientation) (Bindman et al., 1964). The results of this study suggest a homeostatic effect: following cortical excitability increase ('facilitatory preconditioning'), induced by anodal tDCS, 1 Hz rTMS results in a reduction of corticospinal excitability, whereas after a decrease in cortical excitability ('inhibitory preconditioning'), induced by cathodal tDCS, 1 Hz rTMS has the opposite effects, increasing corticospinal excitability. Therefore, it appears that the initial state of cortical excitability determines the effects of rTMS on cortical excitability. This conclusion is particularly important in the clinical setting where patients with neuropsychiatric disorders have a priori altered cortical excitability.

In epilepsy, cortical excitability is abnormally increased, but antiepileptic medications do suppress cortical excitability and may reduce it to abnormally low levels (Ziemann et al., 1996). Therefore, it is possible, that similarly to the findings with tDCS discussed above (Siebner's study) the modulatory effects of rTMS is modified depending on plasma levels of antiepileptic medications in patients with epilepsy. Therefore, we aimed to study the effects of 1 Hz-rTMS on corticospinal excitability in patients with juvenile myoclonic epilepsy (JME) in comparison to healthy controls. We also evaluated whether the effects of 1 Hz rTMS depend on the valproate plasma concentration in the patient group. The results of this study might be important not only in epilepsy, but also to guide the use of rTMS in other neuropsychiatric disorders. Although JME patients might not need an alternative treatment—such as rTMS—as seizure control can be obtained in 85% of these patients using monotherapy with valproate (Renganathan and Delanty, 2003), we decided to study JME patients for the following reasons: (i) JME patients have a generalized epileptic syndrome, and, thus, the motor cortex—where we assessed the corticospinal excitability—is also affected; (ii) the presence of the myoclonic jerks confirms the involvement of the motor cortex in this disease; (iii) these patients are a relatively homogeneous group of patients regarding demographic and clinical characteristics; (iv) seizures are generally controlled with monotherapy with a single drug (valproate) (Renganathan and Delanty, 2003) and (v) no alterations on high resolution magnetic resonance imaging are found and there is strong evidence suggesting that JME is a functional disease of increased cortical excitability (Renganathan and Delanty, 2003). All these features together indicate that JME patients are a reliable group of patients to be further investigated.

2. Methods

2.1. Participants

2.1.1. Juvenile myoclonic epilepsy patients

Patients were prospectively and sequentially selected from a specialized epilepsy clinic if they fulfilled the following criteria: (1) diagnosis of juvenile myoclonic epilepsy (JME) based on clinical features and electroencephalogram (EEG) data (all these patients have been followed in the clinic for at least 1 year); (2) patients had experienced at least one generalized motor seizure (generalized tonic clonic seizure); (3) age of at least 12 years; (4) monotherapy with valproate for the past 6 months; (5) compliance with antiepileptic drug (AED) treatment for the preceding year (as measured by stable plasma valproate levels); (6) right-handed subjects (as assessed by the Edinburgh Handedness Inventory). We excluded patients with (1) any history of substance abuse or use of any other neuropsychotropic medication; (2) any neuropsychiatric comorbid; (3) history of closed head injury resulting in loss of consciousness; or (4) contraindications to TMS (Wassermann 1996). Fifteen patients (mean age 21.6 ± 7.4 years, mean $\pm$ SD) participated in the study.

2.1.2. Healthy controls

We recruited age-matched healthy subjects to control for the effects of 1 Hz rTMS on corticospinal excitability. Inclusion criteria consisted of (1) right-handedness as assessed by the Edinburgh Handedness Questionnaire; (2) absence of any neuropsychiatric disorder; (3) no psychotropic medications use for the past 6 months; (4) no closed head injury resulting in loss of consciousness; or (4) absence of contraindications to TMS (Wassermann, 1998). Twelve healthy human subjects participated in this study (mean age 20.8 ± 5.2 years, mean $\pm$ SD).

The study was performed in accordance with the declaration of Helsinki (1964) (http://www.wma.net/e/policy/b3.htm). Written informed consent was obtained from all participants (and parents for patients with age under 18) prior to inclusion in the study, which was approved by the local ethics committee (University of Sao Paulo).

2.2. Measurement of corticospinal (CS) excitability via single pulse transcranial magnetic stimulation

We used single-pulse transcranial magnetic stimulation to measure corticospinal excitability. Focal TMS was performed using a commercially available figure-of-8 coil (outside diameter of each wing 7 cm) and a Dantec stimulator (1.5 Tesla version; Medtronic, Minneapolis, US). The magnetic stimulus had a biphasic waveform. In order to study the effects of 1 Hz rTMS on corticospinal excitability, we measured the resting motor threshold (MT) of right first dorsal interosseous (FDI) and recorded motor evoked potentials (MEPs) from the FDI. These measurements were performed immediately before and after rTMS treatment.

For the motor threshold determination, patients were seated in a dental chair and electrodes were placed in a belly-tendon arrangement for the FDI muscle. Initially we assured that the muscle was completely relaxed by online monitoring with surface electromyographic activity at high gain (10–50 μV). Optimal scalp position for induction of MEPs were determined following published guidelines (Rossini et al., 1994). The coil was held tangentially to the skull with the handle pointing occipitally with an angle of 45° to the midline of the subject's head. To determine the threshold intensity, stimulation was initiated at 65% (or higher if needed) of the maximum stimulator output and decreased in 5% increments or 2% when near the threshold level. The motor threshold was defined as the lowest stimulation intensity to produce at least five MEPs with peak-to-peak amplitudes of 50 μV from 10 consecutive stimuli.

In addition, to the resting motor threshold, we also analyzed the characteristics of the motor evoked potentials. Stimulation intensity was adjusted to achieve a baseline MEP in the FDI of about 1 mV peak-to-peak amplitude before rTMS. Stimulation intensity and electrodes position were kept constant for each subject throughout the experiment. The MEPs were recorded using electrodes in the same belly-tendon arrangement described above and were stored in a PC computer using the program Keypoint (Medtronic, Minneapolis, US) for off-line analysis. The electromyographic activity was amplified with a bandpass filtering between 10 and 2000 Hz and the signal was digitized at a frequency of 5 KHz. We recorded 10 MEPs for each time point (immediately before and after the rTMS treatment) and averaged their peak-to-peak amplitude and area-under-the-curve.

For the corticospinal excitability measurement we chose to evaluate MT and MEP as MT might reflect (at least partially) changes in the neuronal membrane excitability and MEP amplitude and area may reflect a change of the global corticospinal excitability. We decided not to correct the post-rTMS intensity of stimulation for the study of MEP (according to the motor threshold) as this would decrease the sensitivity of this method (for instance, Fitzgerald et al. (2002), using this post-rTMS intensity adjustment, did not find significant changes in the MEP size after the 1 Hz rTMS stimulation in healthy subjects (Fitzgerald et al., 2002)). However, we acknowledge that not performing this adjustment could have confounded the results for the MEP changes, i.e. MEP changes might have been a result of the motor threshold change as the MEP size depends on the level of the intensity above the motor threshold; as we maintained the stimulation constant, it is conceivable that a change in the MEP is due to a difference in the resting motor threshold between pre and post-1 Hz stimulation. Therefore, the results of the MEP changes should be viewed with this limitation.

2.3. Modulation of cortical excitability via repetitive transcranial magnetic stimulation

We used rTMS to modulate cortical excitability. Repetitive TMS was applied in one continuous train of 15 min duration (similar duration as the study of (Chen et al., 1997a)) at 1 Hz over left M1 using an intensity that was set at 90% of the participant's MT (similar intensity as the study of (Gangitano et al., 2002)). This paradigm of stimulation is in accordance with current rTMS safety guidelines (Wassermann, 1998).

2.4. Valproate

We measured the effects of 1 Hz rTMS on corticospinal excitability in the patient group in two different conditions: at low plasma concentration of valproate (referred to as 'low-VPA') and high plasma concentration of valproate (referred to as 'high-VPA').

For the low-VPA measurement, patients were instructed not to take their medication (valproate) on the night before and morning of the experiment. Therefore, patients were off medication for approximately 12 h prior to the experiment, which was conducted at the same time of the day (morning) in all patients to avoid circadian influences. Because half-life for valproate ranges from 8 to 10 h (Katzung, 2004), 12 h represents 1.5 half-lives and, thus, is appropriate to measure the effects of 1 Hz rTMS in a low plasma concentration of this drug.

For the high-VPA measurement, patients were instructed to take their medication as usual on the night before the experiment, and to take 500 mg of valproate 1 h before the experiment in the TMS laboratory. In addition, they were instructed to have a light meal in the morning of the experiment to assure good absorption of the drug. Because the peak plasma valproate concentration is reached approximately 1 h after drug ingestion, we expect that, in all patients, testing was conducted at peak-dose time.

Plasma valproate levels were measured in all patients (on both days—in the low-VPA and high-VPA conditions) immediately after the completion of the TMS experiment. The serum levels were determined by the clinical laboratory

of our hospital and the results are expressed as microgram per millilitre (therapeutic range is determined as 30–100 μg/ml).

2.5. Experimental design

In experiment 1, we evaluated the effects of 1 Hz rTMS in JME patients in two different conditions: in the high—and the low—plasma concentration of valproate. We randomized and counterbalanced the order of these two conditions. Furthermore, the two experiments were performed with at least 1-week interval to avoid carry-over effect. The investigators collecting the data were blinded towards the valproate plasma level condition (i.e. low-vs. high-VPA).

2.5.1. Experiment 1a: effects of 1 Hz-rTMS on corticospinal excitability in JME patients in the low plasma concentration of valproate (low-VPA)

First we measured the corticospinal excitability (resting MT and MEP), then applied 1 Hz-rTMS for 15 min, and finally repeated the assessment of corticospinal excitability. After the completion of the after-treatment corticospinal excitability measurement, we drew blood from these patients to assess the plasma levels of valproate.

2.5.2. Experiment 1b: effects of 1 Hz-rTMS on the corticospinal excitability in JME patients in the high plasma concentration of valproate (high-VPA)

For this condition, 500 mg of valproate was given for patients 1 h before the rTMS session. The same procedure of experiment 1a was performed: measurement of corticospinal excitability (resting MT and MEP), 15 min of 1 Hz-rTMS, reassessment of corticospinal excitability and blood drawn to assess the plasma levels of valproate.

2.5.3. Experiment 2: effects of 1 Hz-rTMS on the corticospinal excitability in healthy subjects

In the healthy controls, the effects of 1 Hz rTMS on cortical excitability were measured using the exact same TMS-procedure as in the patient group. We decided not to assess the effects of 1 Hz rTMS after valproate ingestion in healthy subjects, as this would not mimic the chronic use of valproate by the JME patients. Furthermore, although 12 h of valproate washout decreases the serum levels of valproate it does not eliminate this drug completely. We felt that the alternative of a longer washout for JME patients or chronic use of valproate for healthy subjects would not be ethical. Therefore, the control group served as a reference group for the effects of rTMS in JME in patients with low and high plasma levels of valproate.

2.6. Data analysis

Analyses were done with Stata statistical software (version 8.0, College Station, Texas). We analyzed the effects of 1 Hz-rTMS treatment on corticospinal excitability performing a two-way analysis of variance (ANOVA) in which we included two factors: group (JME patients-low-VPA; JME patients-high-VPA and healthy controls) and time (pre- and post-stimulation) with repeated measurement on *time*. This analysis was performed on each primary outcome measure (MT, MEPamp, MEParea).

When appropriate, post-hoc comparisons were carried out using Scheffe correction for multiple comparisons. Furthermore, in an exploratory way, we analyzed whether the changes in the cortical excitability were correlated either with the plasma valproate levels or with some other characteristic such as valproate dosage and JME severity. We used Pearson correlation coefficient to analyze these relationships.

Unless stated otherwise, statistical significance refers to a two-tailed P value <0.05.

3. Results

3.1. Clinical characteristics

Seizures were well controlled in all patients. Most patients ($n=11$) were on low doses of valproate (250–1000 mg/day) and few ($n=4$) were on moderate to high doses (2500–3000 mg). One patient had relatively frequent myoclonic seizures, however, some of these episodes were attributed to triggering factors such as sleep deprivation and fatigue. In the other patients, episodes of myoclonic jerks were described particularly in association to fatigue. On average, these patients had had 24.8 ± 32.8 episodes of seizures until the control with valproate and, on average, they experienced 3.5 ± 6.5 seizures per year. None of the patients experienced myoclonic jerks or generalized seizures because of the medication withdrawal for 12 h in experiment 1a.

3.2. Overall comparisons: JME-low-VPA vs. JME-high-VPA vs. healthy controls

We initially performed a two-way ANOVA in which we included two factors: group (low-VPA, high-VPA and controls) and time (pre- and post-stimulation) for each of the main outcome measures (MT, MEPamp and MEParea). These analyses revealed a significant interaction term-group×time (MT: $F_{2,39}=12.52$, $P=0.001$, MEPamp: $F_{2,39}=6.42$, $P=0.004$ and MEParea: $F_{2,39}=8.19$, $P=0.001$), indicating that the effects of 1 Hz rTMS (pre- vs. post-stimulation) depended on the group of stimulation (patients with different valproate levels (low-VPA vs. high-VPA) and healthy controls). We then performed post-hoc comparisons (using Scheffe correction) to test for differences between pre- and post- values

for each group separately (low-VPA, high-VPA and controls).

3.3. JME patients with low plasma valproate levels (low-VPA)

The mean average of plasma levels of valproate after withholding VPA for 12 h was 21.0±15.2 μg/ml (range 0 to 44 μg/ml). The mean pre-stimulation motor threshold was 41.2% (±9.1) of the maximal output of the stimulator and was significantly increased after 1 Hz rTMS to 43.9% (±10.8) ($P=0.004$). The MEP analysis revealed similar significant changes: in the baseline, the mean MEPamp was 1.27 mV (±0.64) and MEParea was 4.59 mV ms (±3.11) and, after the stimulation, there was a significant decrease of these two measures (1.14±0.54 and 4.21±2.79, respectively, —$P=0.047$ and $P=0.016$). These results show that 1 Hz rTMS in this group of patients (low-VPA) induced a decrease in the cortical excitability (Fig. 1A–C).

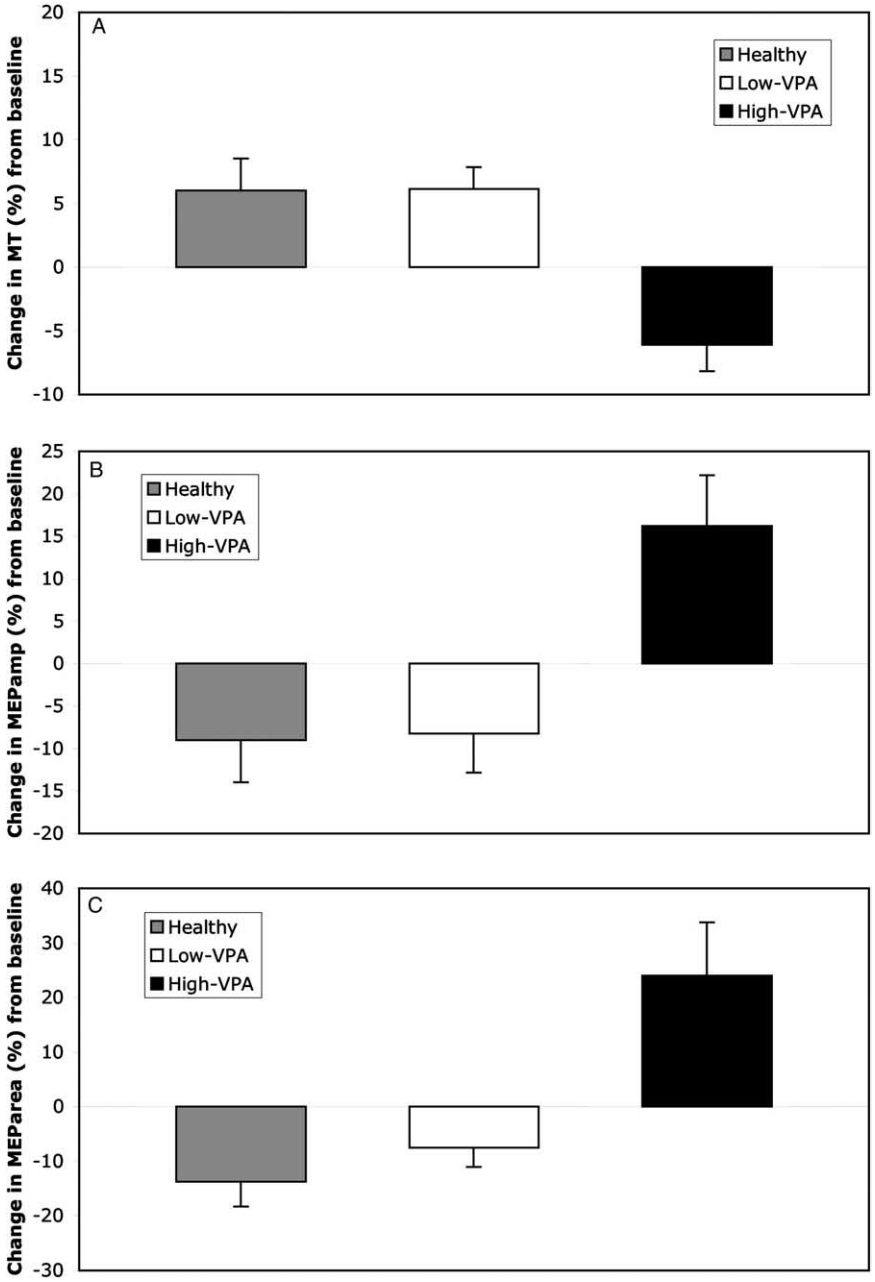

Fig. 1. Effects of 1 Hz rTMS on corticospinal excitability in healthy subjects (grey column) and patients with juvenile myoclonic epilepsy in two conditions: low plasma valproate levels (low-VPA—white column) and high plasma valproate levels (high-VPA—black column). (A) shows the mean motor threshold change (comparison between pre and post-treatment) after 1 Hz rTMS treatment. (B) shows the mean changes in the motor evoked potential (MEP) amplitude and (C) shows the mean changes in the MEP area (comparison between pre and post-treatment) after 1 Hz rTMS treatment. Error bars indicate SEM (standard error of the mean).

3.4. JME patients with high plasma valproate levels (high-VPA)

The mean average of plasma valproate levels 1 h after ingestion of 500 mg of valproate was 67.3±35.2 μg/ml (range 23–126)—significantly different from the low-plasma VPA concentration condition ($P=0.0001$). In contrast to the condition with low valproate plasma levels, high-VPA levels were associated with an increase in the cortical excitability after 1 Hz rTMS, indicating an opposite modulatory effect of inhibitory rTMS. The mean pre-stimulation motor threshold was 43.7% (±10.2) of the maximal output of the stimulator and was significantly decreased after 1 Hz rTMS to 40.9% (±9.0) ($P=0.008$). The MEP analysis revealed similar significant changes: in the baseline, the mean MEPamp was 1.11 mV (±0.61) and MEParea was 3.58 mV ms (±2.13) and, after the stimulation, there was a significant increase of these two measures (1.25±0.68 and 4.33±2.73, respectively, —$P=0.025$ and $P=0.031$) (Fig. 1A–C).

3.5. Healthy controls

In line with previous literature, 1 Hz rTMS induced a decrease in the cortical excitability in the 12 healthy subjects similarly to the patients with low-VPA. The mean pre-stimulation motor threshold was 38.1% (±7.2) of the maximal output of the stimulator and was significantly increased after 1 Hz rTMS to 40.4% (±8.8) ($P=0.03$). The MEP analysis revealed similar changes in the same direction: in the baseline, the MEPamp was 1.21 mV (±0.91) and MEParea was 5.8 mV ms (±5.2) and, after the stimulation, there was a trend toward a significant decrease of MEPamp (1.15±0.98, $P=0.08$) and a significant decrease of the MEParea (5.36±5.43, $P=0.008$) (Fig. 1A–C).

3.6. Effects of valproate on corticospinal excitability (baseline comparisons)

Because we showed a differential effect of 1 Hz rTMS on CS excitability between low-VPA and high-VPA conditions, we compared the baseline CS excitability between these two conditions. Therefore, we assessed the effects of valproate on the cortical excitability analyzing the corticospinal excitability measurements before each day of experiment (i.e. low-VPA and high-VPA conditions). This analysis showed that 500 mg of valproate, compared to 12 h VPA washout, resulted in a significant increase in the motor threshold (% of the maximum output stimulator) (low-VPA vs. high-VPA: 41.2±9.1 vs. 43.7±10.2, $P=0.013$), a significant decrease in the MEPamp (mV) (low-VPA vs. high-VPA: 1.27±0.64 vs. 1.11±0.61, $P=0.018$), and a trend toward a significant difference in the MEParea (mV ms) (low-VPA vs. high-VPA: 4.6±3.1 vs. 3.6±2.1, $P=0.09$).

3.7. Correlations

In order to analyze whether the effects of 1 Hz rTMS on corticospinal excitability were correlated to the plasma valproate levels or other clinical features, such as valproate dosage use and frequency of seizures, we performed correlation analyses using Pearson's correlation coefficient. We focused on the motor threshold, as this was the variable that gave the most stable and significant results. Firstly, we analyzed whether changes in the MT in the low-VPA and high-VPA conditions were correlated to the valproate levels. This analysis showed that there was a significant negative correlation between valproate levels and the motor threshold change in the low-VPA condition ($r=-0.61$, $P=0.017$) (Fig. 2A) and in the high-VPA condition ($r=-0.58$, $P=0.024$) (Fig. 2B), suggesting that higher plasma VPA levels were associated with an opposite effect of 1 Hz rTMS on CS excitability—a facilitatory rather than an inhibitory effect. We then performed the same analysis, but instead of the plasma valproate levels, we used valproate dosage and the mean number of seizures per year. These analyses revealed a trend

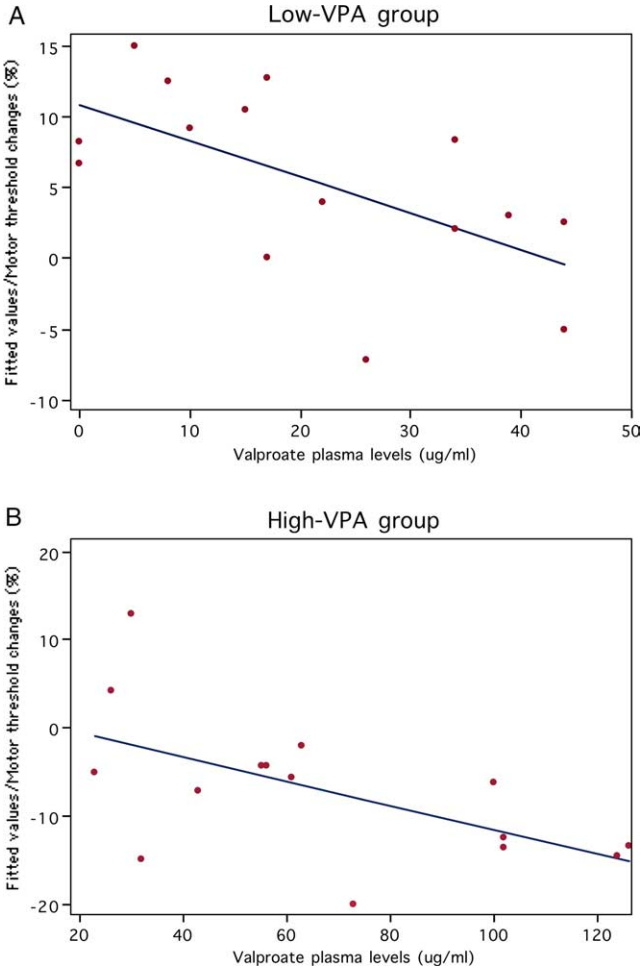

Fig. 2. Correlation between plasma valproate levels and motor threshold (MT) in JME patients with low plasma valproate levels (A) and high valproate levels (B).

for a significant positive correlation between valproate dosage and MT changes for the low-VPA ($r=0.50$, $P=0.055$) and high-VPA condition ($r=0.46$, $P=0.08$), and a trend for a significant negative correlation between seizures frequency and MT changes for high-VPA condition ($r=-0.46$, $P=0.08$), but not for low-VPA condition ($r=0.15$, $P=0.59$).

3.8. Data variability

Because it has been shown previously that some subjects can have a paradoxical effect to rTMS (i.e. the effects of rTMS on CS excitability can go in the opposite direction compared to what is found in most subjects), we plotted the data of MEPamp changes for each individual and for each different condition (Fig. 3A–C). Overall, 19% of the subjects had this paradoxical effect. We, therefore, performed a similar cluster analysis—as used in a previous study (Gangitano et al., 2002) and observed that, excluding the subjects with this paradoxical effect, the magnitude and the significance of our results became larger for each of the 3 groups (MEPamp analysis): 15.8% decrease and $P<0.0001$ for the JME-low-VPA; 25% increase and $P<0.0001$ for the JME-high-VPA; and 14.8% decrease and $P=0.0009$ for healthy subjects.

4. Discussion

Our findings show that the direction of the after-effects of 1 Hz rTMS on corticospinal excitability depends on plasma

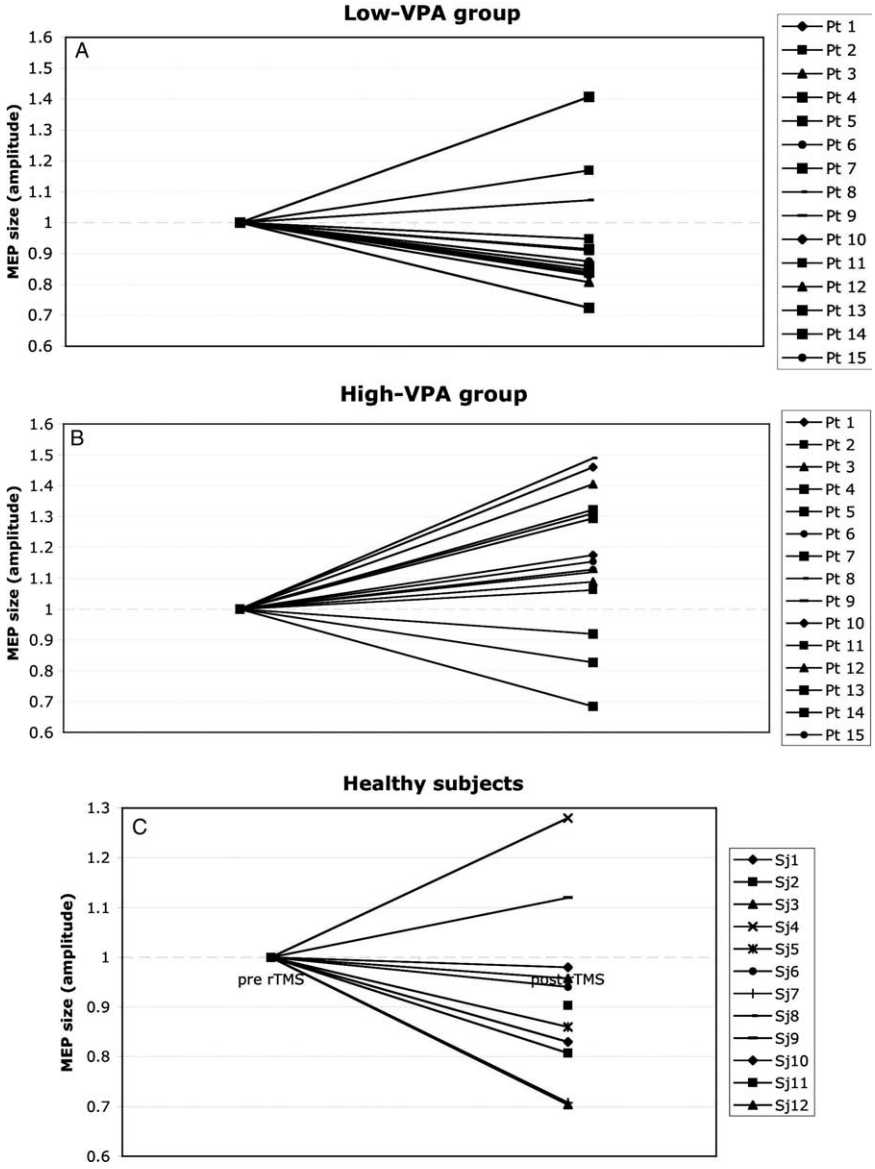

Fig. 3. Plot showing the variability of the modulatory effects of 1 Hz rTMS across subjects. These plots show the difference in the corticospinal excitability (as indexed by MEP) between pre- and post-rTMS treatment for MJE patients with low plasma valproate levels (A), MJE patients with high plasma valproate levels (B) and healthy controls (C).

valproate levels in patients with JME: whereas 1 Hz rTMS in patients with the low plasma concentration of valproate resulted in a reduction of the corticospinal excitability, 1 Hz rTMS in patients with high plasma levels of valproate resulted in the opposite effect, i.e. an increase in the corticospinal excitability. This reversed response to rTMS treatment might be a result of a different state of neuronal excitability at low vs. high valproate concentrations that we revealed by comparing CS excitability at baseline before rTMS treatment. Furthermore, the suppressive effects of 1 Hz rTMS on CS excitability in patients with low plasma concentration of valproate were similar to those found in healthy subjects on no medications. Moreover, we showed that the effects of 1 Hz-rTMS on CS excitability were positively correlated with plasma valproate levels. Finally, the data of our study showed similar variability than previous studies in regards to the direction of the rTMS effects, such as that 19% of the subjects, on average, behaved in an opposite way compared to the majority of the subjects.

4.1. Baseline differences in corticospinal excitability

One important point of our results is the difference in baseline CS excitability between low-vs. high-VPA plasma valproate levels. Several studies have demonstrated that patients with JME have an increase in baseline cortical excitability as indexed by a higher P25 and N33 amplitude (Salas-Puig et al., 1992) and by a loss of MEP inhibition (Caramia et al., 1996)—that can be reversed, to some extent, by valproate therapy (Erdem et al., 2001). Furthermore, valproate decreases cortical excitability in idiopathic generalized epilepsy (Reutens et al., 1993) and in patients with migraine with aura (Mulleners et al., 2002) as shown by an increase in the resting motor threshold. In line with these findings, we found reduced corticospinal excitability at baseline in terms of MT, MEP-amplitude and -area in patients with high as compared to low valproate levels. However, there was no significant increase in the baseline CS excitability in patients with low plasma valproate levels as compared to healthy controls suggesting that either the low plasma concentration of valproate was sufficient to normalize the CS excitability or the chronic use of valproate was responsible for this CS excitability normalization.

The important point here is that these differences in baseline CS excitability in JME patients with low vs. high valproate levels might alter the way the brain is responding to 1 Hz rTMS treatment, as it has previously been shown that modulation of cortical baseline activity by anodal or cathodal tDCS (facilitatory vs. inhibitory conditioning) leads to reversed effects of 1 Hz rTMS treatment on CS excitability (Siebner et al., 2004). Inhibitory effects of 1 Hz rTMS were observed when rTMS was applied on a background of increased CS excitability, while facilitatory effects of 1 Hz rTMS were observed when applied on an initial state of reduced baseline excitability (Siebner et al., 2004). Based on these results, one could have hypothesized that patients with high valproate levels and low CS excitability at baseline would have shown a facilitatory effect of 1 Hz rTMS, in contrast to patients with low valproate plasma concentration. Our results are partially in line with this hypothesis as we showed that the patients in the high valproate condition responded with an increase in cortical excitability after rTMS treatment as compared to patients in the low valproate condition.

4.2. Effects of 1 Hz rTMS on corticospinal excitability in JME patients with low vs. high plasma valproate levels

One Hertz rTMS in patients with low plasma valproate levels resulted in a similar inhibitory effect than in healthy controls and this effect was also similar to the inhibitory effect of 500 mg of valproate on the CS excitability. These results are important to support the use of rTMS as a potential alternative treatment for epilepsy.

However, we also found that if 1 Hz rTMS is administered in patients with high plasma valproate levels, there is no additive inhibitory effect. Instead, an opposite, facilitatory effect of 1 Hz rTMS is observed resulting in an increase of corticospinal excitability. It is conceivable that this increase in the CS excitability after 1 Hz-rTMS reflects a protective mechanism of the cortex to avoid a large change in the corticospinal excitability that could destabilize the properties of the neuronal networks (Abbott and Nelson, 2000). Indeed, it has been proposed previously that regulatory mechanisms exist to avoid drastic changes in brain plasticity (Sejnowski, 1977). In this context, a prolonged reduction in postsynaptic activity would be expected to favor an induction of long-term potentiation (LTP), whereas, a prolonged increase in postsynaptic activity should favor an induction of long-term depression (LTD) (Bienenstock et al., 1982). Therefore, and because valproate decreased CS excitability, 1 Hz rTMS might have favored protective LTP-mechanisms in patients with high valproate levels.

Evidence for such a homeostatic mechanism has been provided recently by Siebner et al. (2004). In this study, the authors showed that the effects of low-frequency rTMS change with modulation of the cortical baseline state before rTMS treatment through the technique of tDCS that can either hyper- or depolarize the neuron (Purpura and McMurtry, 1965). Siebner et al. (2004) used cathodal tDCS to decrease cortical excitability at baseline, which was associated with facilitatory effects of subsequent 1 Hz rTMS. Our results of facilitatory 1 Hz rTMS effects in patients with high valproate levels and reduced baseline CS excitability are in line with the results from Siebner's study. In addition, in JME patients with low plasma valproate concentration—who showed similar baseline CS excitability compared to the healthy subjects—1 Hz rTMS had inhibitory effects on corticospinal excitability that were comparable to those observed in the healthy control group.

We speculate that valproate might have had a similar preconditioning effect on the CS excitability than cathodal tDCS. Indeed previous research suggested that valproate can have an effect on sodium channels and, therefore, decreases the neuron firing due to a neuronal hyperpolarization (Taverna et al., 1998). This mechanism would be similar to the membrane mechanism that has been proposed for cathodal stimulation (Nitsche et al., 2005): cathodal stimulation results in a hyperpolarization of the neuron membrane. Therefore, valproate intake 1 h before 1 Hz rTMS might have primed the effects of this treatment. In addition, valproate may also act as a γ-aminobutyric acid (GABA) agonist and this may be associated to its inhibitory effect on cortical excitability (Loscher and Nordlund, 2002). Because the inhibitory effects of 1 Hz rTMS may also be related to a gabaergic activity as demonstrated by an increase in the intracortical inhibition (Tergau et al., 1997)—although this mechanism is still not certain (Fitzgerald et al., 2002); this might have resulted in an over stimulation of the gabaergic activity that triggered mechanisms of protection to avoid an excessive inhibition of the cortical activity (similar to other homeostatic mechanisms) that would have resulted in an increase of the cortical excitability.

Not only valproate, but other drugs affecting ion channels, can increase the motor threshold and consequently decrease the corticospinal excitability (Chen et al., 1997b; Goyal et al., 2004; Reis et al., 2004; Turazzini et al., 2004; Ziemann et al., 1996). Therefore, other antiepileptic drugs might have the same preconditioning effect as valproate and, thus, can interfere with the direction of the effects of rTMS treatment.

A question that might be raised from our results is whether a previous excitation of brain activity in these patients would increase the rTMS inhibitory effects—similarly to the preconditioning with anodal stimulation before 1 Hz rTMS (Siebner et al., 2004). One might assume that because JME patients without treatment have an increase in the CS excitability, the effects of rTMS in these patients would be greater compared to healthy controls. Although we measured the rTMS effects in these patients in the low plasma concentration of valproate, this washout period was not enough to eliminate the long-term effects of valproate and indeed these patients behaved similarly to healthy subjects. Therefore, one can speculate that a longer washout period might elevate the CS excitability and, therefore, enhance the effects of 1 Hz rTMS. However, a longer washout period would expose these patients to a higher risk of seizure.

4.3. Clinical implications

The results of our study have obvious clinical implications as we show for the first time that the effects of low-frequency rTMS on CS excitability in subjects with generalized idiopathic epilepsy are the same as those of healthy subjects if the antiepileptic drug is withheld for 12 h. Moreover, the results also show that the effects of 1 Hz rTMS on corticospinal excitability can be reversed if rTMS is administered after valproate intake (high plasma concentration of valproate). This might explain the mixed results of previous rTMS studies in epilepsy research as this factor (drug concentration at the time of rTMS treatment) was not taken into account. Therefore, the recommendation, based on our findings, would be to administer rTMS during low concentration of antiepileptic drugs. However, this recommendation would be based on the assumption that changes in CS excitability is a good surrogate for seizure activity. Although some studies have shown that the oral doses and plasma levels of Lamotrigine are correlated with its inhibitory effect on CS excitability (Tergau et al., 2003)—the correlation with the clinical outcome remains uncertain. Hence, further studies are needed to probe for the interaction of antiepileptic medication and rTMS treatment outcome.

Certainly a study evaluating the clinical effects, rather than the corticospinal excitability changes, in two groups of patients—one receiving rTMS in the high plasma concentration of antiepileptic drugs and the other in the low plasma concentration of these drugs—would be a good approach to assess the clinical implications of our results. However, such study would be difficult to perform as, in most of the cases, refractory epileptic patients (generally the population of patients that is targeted by rTMS treatment) use several types of antiepileptic drugs and, thus, posing another level of complexity as it would be difficult to control for the interaction effect between these drugs.

Another inference that can be made from our results and previous research is if the priming treatment (with valproate in our study) modified the effects of 1 Hz rTMS, this priming effect could be used to increase the inhibitory effects of 1 Hz-rTMS. Indeed as priming can be obtained with the simple technique of transcranial direct current stimulation (Lang et al., 2004; Siebner et al., 2004) or with rTMS (Iyer et al., 2003), one could use anodal tDCS or high-frequency rTMS before 1 Hz rTMS to increase the inhibitory potency of this treatment. However, this raises a safety issue as a temporary increase in the cortical excitability could trigger a seizure in epileptic patients.

5. Conclusion

Our findings open an avenue for future studies investigating the effects of rTMS in patients using medications that affect cortical excitability. These studies might focus either on the safety issue or the potentiation of the rTMS effect. For instance, a drug that inhibits corticospinal excitability, such as benzodiazepine, used in a patient that is undergoing rTMS treatment to increase CS excitability, such as for depression treatment, might enhance the effects of this treatment. Our findings show for the first time that the effects of 1 Hz rTMS depend on the plasma valproate levels. These results might be

applied to other clinical applications of rTMS, such as in stroke patients (Khedr et al., 2005; Mansur et al., 2005), Parkinson's disease (Fregni et al., in press), depression (Martin et al., 2003) and schizophrenia (Hoffman et al., 2005). Future rTMS clinical trials should account for the interaction between rTMS and baseline cortical excitability.

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References

Abbott LF, Nelson SB. Synaptic plasticity: taming the beast. Nat Neurosci 2000;3(Suppl):1178–83.

Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 1982;2(1):32–48.

Bindman LJ, Lippold OC, Redfearn JW. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. J Physiol 1964;172: 369–82.

Caramia MD, Gigli G, Iani C, Desiato MT, Diomedi M, Palmieri MG, Bernardi G. Distinguishing forms of generalized epilepsy using magnetic brain stimulation. Electroencephalogr Clin Neurophysiol 1996;98(1):14–19.

Chen RJ, Classen C, Gerloff P, Celnik EM, Wassermann M, Hallett M, Cohen LG. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997a;48(5):1398–403.

Chen R, Samii A, Canos M, Wassermann EM, Hallett M. Effects of phenytoin on cortical excitability in humans. Neurology 1997b;49(3): 881–3.

Erdem H, Yigit A, Culcuoglu A, Mutluer N. Effect of sodium valproate on somatosensory evoked potentials in juvenile myoclonic epilepsy. Ups J Med Sci 2001;106(3):197–203.

Fitzgerald PB, Brown TL, Daskalakis ZJ, Chen R, Kulkarni J. Intensity-dependent effects of 1 Hz rTMS on human corticospinal excitability. Clin Neurophysiol 2002;113(7):1136–41.

Fregni F, Simon DK, Wu A, Pascual A. Noninvasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. J Neurol Neurosurg Psychiatry 2005;76(12):1614–23.

Gangitano M, Valero-Cabre A, Tormos JM, Mottaghy FM, Romero JR, Pascual-Leone A. Modulation of input–output curves by low and high frequency repetitive transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol 2002;113(8):1249–57.

Goyal V, Bhatia M, Behari M. Increased depressant effect of phenytoin sodium as compared to carbamazepine on cortical excitability: a transcranial magnetic evaluation. Neurol India 2004;52(2):224–7.

Hoffman RE, Cavus I. Slow transcranial magnetic stimulation, long-term depotentiation, and brain hyperexcitability disorders. Am J Psychiatry 2002;159(7):1093–102.

Hoffman RE, Gueorguieva R, Hawkins KA, Varanko M, Boutros NN, Wu YT, Carroll K, Krystal JH. Temporoparietal transcranial magnetic stimulation for auditory hallucinations: safety, efficacy and moderators in a fifty patient sample. Biol Psychiatry 2005;58(2):97–104.

Iyer MB, Schleper N, Wassermann EM. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J Neurosci 2003;23(34):10867–72.

Katzung BG. Basic & clinical pharmacology. New York: Lange Medical Books/McGraw Hill; 2004.

Khedr EM, Kotb H, Kamel NF, Ahmed MA, Sadek R, Rothwell JC. Longlasting antalgic effects of daily sessions of repetitive transcranial magnetic stimulation in central and peripheral neuropathic pain. J Neurol Neurosurg Psychiatry 2005;76(6):833–8.

Lang N, Siebner HR, Ernst D, Nitsche MA, Paulus W, Lemon RN, Rothwell JC. Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects. Biol Psychiatry 2004;56(9):634–9.

Loscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs 2002;16(10):669–94.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. Exp Brain Res 2000;133(4):425–30.

Mansur CG, Fregni F, Boggio PS, Riberto M, Gallucci-Neto J, Santos CM, Wagner T, Rigonatti SP, Marcolin MA, Pascual-Leone A. A sham stimulation-controlled trial of rTMS of the unaffected hemisphere in stroke patients. Neurology 2005;64(10):1802–4.

Martin JL, Barbanoj MJ, Schlaepfer TE, Thompson E, Perez V, Kulisevsky J. Repetitive transcranial magnetic stimulation for the treatment of depression. Systematic review and meta-analysis. Br J Psychiatry 2003;182:480–91.

Mulleners WM, Chronicle EP, Vredeveld JW, Koehler PJ. Visual cortex excitability in migraine before and after valproate prophylaxis: a pilot study using TMS. Eur J Neurol 2002;9(1):35–40.

Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, Fricke K, Liebetanz D, Lang N, Antal A, Paulus W, Tergau F. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol 2005;568(pt 1): 291–303.

Pascual-Leone A, Tormos JM, Keenan J, Tarazona F, Canete C, Catala MD. Study and modulation of human cortical excitability with transcranial magnetic stimulation. J Clin Neurophysiol 1998;15(4): 333–43.

Purpura DP, McMurtry JG. Intracellular activities and evoked potential changes during polarization of motor cortex. J Neurophysiol 1965;28: 166–85.

Reis J, Wentrup A, Hamer HM, Mueller HH, Knake S, Tergau F, Oertel WH, Rosenow F. Levetiracetam influences human motor cortex excitability mainly by modulation of ion channel function—a TMS study. Epilepsy Res 2004;62(1):41–51.

Renganathan R, Delanty N. Juvenile myoclonic epilepsy: under-appreciated and under-diagnosed. Postgrad Med J 2003;79(928):78–80.

Reutens DC, Berkovic SF, Macdonell RA, Bladin PF. Magnetic stimulation of the brain in generalized epilepsy: reversal of cortical hyperexcitability by anticonvulsants. Ann Neurol 1993;34(3):351–5.

Rossini PM, Barker AT, Berardelli A. Non-invasive electrical and magnetic stimulation of the brain spinal cord and roots: basic principles and procedures for routine clinical application: report of an ICFN committee. Electroencephalogr Clin Neurophysiol 1994; 91:79–91.

Salas-Puig J, Tunon A, Diaz M, Lahoz CH. Somatosensory evoked potentials in juvenile myoclonic epilepsy. Epilepsia 1992;33(3): 527–30.

Sejnowski TJ. Statistical constraints on synaptic plasticity. J Theor Biol 1977;69(2):385–9.

Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, Rothwell JC. Preconditioning of low-frequency repetitive transcranial

magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. J Neurosci 2004;24(13):3379–85.

Taverna S, Mantegazza M, Franceschetti S, Avanzini G. Valproate selectively reduces the persistent fraction of Na+ current in neocortical neurons. Epilepsy Res 1998;32(1–2):304–8.

Tergau F, Tormos JM, Paulus W, Pascual AP, Ziemann U. Effects of repetitive transcranial magnetic stimulation (rTMS) on cortico-spinal and cortico-cortical excitability. Neurology 1997; 48:A107.

Tergau F, Wischer S, Somal HS, Nitsche MA, Mercer AJ, Paulus W, Steinhoff BJ. Relationship between lamotrigine oral dose, serum level and its inhibitory effect on CNS: insights from transcranial magnetic stimulation. Epilepsy Res 2003;56(1):67–77.

Turazzini M, Manganotti P, Del Colle R, Silvestri M, Fiaschi A. Serum levels of carbamazepine and cortical excitability by magnetic brain stimulation. Neurol Sci 2004;25(2):83–90.

Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. Electroencephalogr Clin Neurophysiol 1998;108(1):1–16.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol 1996;40(3):367–78.