

## **NEGATIVE EXPERIMENTAL RESULTS**

### **Cortical excitability in patients with focal epilepsy: a study with high frequency repetitive transcranial magnetic stimulation (rTMS)**

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*Epileptogenesis involves an increase in excitatory synaptic strength in the brain in a manner similar to synaptic potentiation. In the present study we investigated the mechanisms of short-term synaptic potentiation in patients with focal epilepsy by using 5 Hz repetitive transcranial magnetic stimulation (rTMS), a non invasive neurophysiological technique able to investigate the mechanisms of synaptic plasticity in humans.*

*Ten patients with focal idiopathic cortical epilepsy were studied. 5 Hz-rTMS (10 stimuli-trains, 120% of motor threshold, RMT) was delivered over the first dorsal interosseus (FDI) motor area of both (affected and unaffected) hemispheres. Changes in the motor evoked potential (MEP) size in the FDI muscle during the trains and the RMT were measured and compared between the hemispheres. 5 Hz-rTMS was also delivered in a group of healthy subjects over both hemispheres.*

*5 Hz-rTMS in patients elicited a reduced MEP facilitation compared to normal subjects. The reduced MEP amplitude was more evident in the affected hemisphere than in the unaffected hemisphere. RMT in the affected hemisphere was higher than in the unaffected hemisphere and in healthy subjects.*

*Our findings showing a decreased response to 5 Hz-rTMS over the affected hemisphere, differently from the expected results suggest a reduced cortical excitability in epileptic patients. We hypothesize an altered balance between excitatory and inhibitory circuits in epileptic patients under chronic therapy.*

**Key words:** cortical excitability, repetitive transcranial magnetic stimulation, epilepsy

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## **INTRODUCTION**

The epilepsies are one of the most common serious brain disorders that manifest in up to 1% of the population. Different biochemical

abnormalities modifying the intrinsic properties of neuronal membranes have been demonstrated, in particular alterations of voltage-dependent ionic

channels, deficit of ion transports, deficit of GABA-related inhibitory neurotransmission or increase of glutamate related excitatory neurotransmission. Nearly all of the genes thus far identified as causing inherited epilepsies encode ion channels, leading to the assumption that seizures are a consequence of changes in neuronal membrane excitability (Kullmann, 2002; Noebels, 2003) with an hyperexcitability of the cortical areas (Cantello et al., 2007; Macdonell et al., 2002; Tassinari et al., 2003). However studies targeting on cortical excitability in epileptic patients are still needed.

Transcranial magnetic stimulation (TMS) is a non invasive neurophysiologic technique able to stimulate the human brain through the intact scalp without causing pain at the surface (Berardelli et al., 1998; Siebner and Rothwell, 2003; Ridding and Rothwell, 2007). The stimulator produces a magnetic field that penetrates the scalp and skull and induces electrical currents in the area of the brain beneath the coil. TMS activates the axons of excitatory and inhibitory interneurons in the cortex and when applied over the primary motor cortex the final outcome of TMS in the target muscle in the opposite side of the body is a motor evoked response (MEP) in subjects at rest. Single pulse produces complex but short responses, conversely repetitive TMS (rTMS) can have more prolonged effects on the brain activity in cortical regions underneath the stimulation coil (local effect) and within functionally connected cortical or subcortical regions (remote effects) (Hallett, 2000). The effects on cortical activity induced by rTMS depend on several stimulus variables, such as the train length, the stimulation intensity and mostly the frequency of stimulation. Recent researches conducted in our laboratory have shown that rTMS delivered in short trains at 5 Hz frequency and suprathreshold intensity over the primary motor cortex in healthy subjects facilitates cortical excitability (Berardelli et al., 1998; Inghilleri et al., 2004; Gilio et al., 2007). Short trains at 5 Hz increase the MEP amplitude during the train and the MEP facilitation outlasts the train by about 1 s. Studies using 5 Hz-rTMS to test changes in cortical

excitability induced by antiepileptic drugs mainly acting on glutamate receptor and sodium channels suggests that rTMS acts through a mechanism involving glutamatergic neurotransmission, resembling short-term synaptic plasticity (Inghilleri et al., 2004, 2005). TMS has already provided useful information on short-term synaptic plasticity of the motor cortex in humans under pathological conditions (Gilio et al., 2002; Inghilleri et al., 2006a; Conte et al., 2007, Gilio et al., 2007).

To see whether in patients with focal epilepsy there is a hyperexcitability of the cortical areas we investigated the 5 Hz-rTMS induced MEP facilitation in both the affected and unaffected hemispheres. Data were compared with a group of healthy subjects. To minimize the potential risk of rTMS-induced adverse effects, we followed published safety guidelines (Pascual-Leone et al., 1993, 1994; Wassermann, 1998).

## MATERIAL AND METHODS

### Subjects

We studied 10 patients with focal idiopathic cortical epilepsy (6 men and 4 women; mean age  $30\pm 0.1$  years). Only patients with electrical sources of paroxysmal activity in neocortical regions were admitted in the study. They received standard pharmacological treatment without modification of antiepileptic drugs for at least 1 month before the study started; medication changes were not allowed. A group of 10 healthy control subjects was also studied (5 men and 5 women; mean age  $29\pm 0.2$  years). Informed consent was signed by all participants to the study and the experimental procedures were approved by the local ethical committee. All subjects were asked to report adverse effects experienced during or after rTMS. Stimulation was also immediately interrupted if afterdischarges were recorded at the end of the train.

The study was conducted by neurologists who were familiar with the rTMS technique and with the treatment of seizures.

### Stimulation and measurements

rTMS was delivered with a Magstim repetitive magnetic stimulator in subjects at rest. A figure-

of-eight coil was placed over the primary motor cortex (M1) to determine the optimal position for activating the contralateral first dorsal interosseus (FDI) muscle. In patient and in the healthy subjects rTMS was applied over the left and right M1 using trains of 10 stimuli at 5 Hz frequency and 120% resting motor threshold (RMT) intensity. The RMT was calculated at rest using the lowest intensity able to evoke a MEP of more than 50  $\mu$ V in at least five of ten consecutive trials in FDI muscle. Eight trains were delivered over the left and right M1 at 2 min intervals with the subjects fully relaxed and with the eyes closed (Conte et al., 2007). To avoid interhemispheric interactions affecting the results we investigated the right and left hemispheres in the same experimental session with an interval of about 2 h between each test. To avoid prolonging the study for more than three hours we decided to not investigate other measures of cortical excitability such as intracortical inhibition and facilitation and the input-output curve. In both hemispheres RMT was calculated at rest (Inghilleri et al., 2004) and eight rTMS trains were collected and averaged. The electromyographic (EMG) activity was recorded through a pair of surface disk electrodes placed over the FDI muscle of both sides. The size of MEPs evoked by each stimulus in the rTMS trains was measured peak-to-peak.

### Statistical analysis

The RMT values and the first MEP amplitude in patients and healthy subjects in the left and right hemispheres were analysed by using a between group one-way ANOVA with factor “side” (affected vs unaffected hemisphere in patients; left vs right hemisphere in healthy subjects) as main factor. Changes in the MEP amplitude during the course of the trains in both the hemispheres were analysed by a repeated measures between groups two-ways ANOVA with factors “number of stimuli” and “side”. All results were expressed as mean $\pm$ SE; p values <0.05 were considered to indicate statistical significance.

## RESULTS

None of the rTMS variables used caused any of the participants to experience adverse effects.

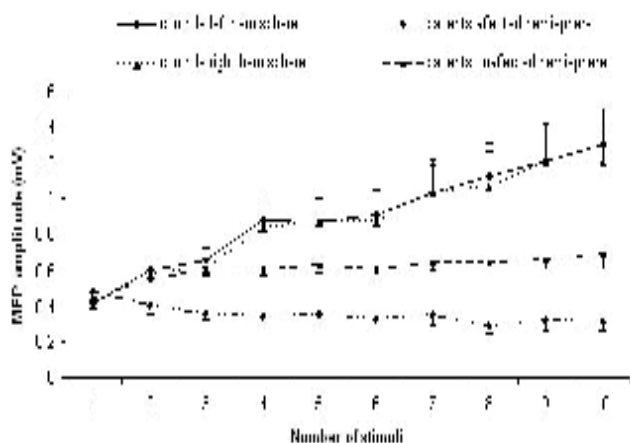
The RMT was significantly higher in the affected hemisphere in patients than in the unaffected hemisphere and in healthy subjects (patients affected hemisphere: 69 $\pm$ 4% maximal stimulator output; unaffected hemisphere: 55 $\pm$ 5%; healthy subjects left hemisphere: 45 $\pm$ 3%, right hemisphere: 44 $\pm$ 2%,  $p=0.04$ ).

The first MEP in the trains was similar in patients and healthy subjects in both the studied hemispheres ( $p=0.1$ ) (fig. 1).

ANOVA of the MEP size during the course of the trains showed a significant effect of factors “side”, “number of stimuli”, with significant interactions for factors “group”, “side” and “number of stimuli” ( $p=0.03$ ) (fig. 1). Post-hoc analysis showed that in healthy subjects during rTMS delivered over the left and right hemisphere MEPs progressively increased in size and did so to a similar extent. In patients during rTMS delivered over the unaffected hemisphere MEPs progressively increased in size with an extent lower than in healthy subjects. Conversely, in the affected hemisphere 5 Hz-rTMS left the MEP amplitude unchanged during the course of the trains.

## DISCUSSION

In the present study we describe an asymmetric excitability of the affected and unaffected hemispheres assessed by focal suprathreshold 5 Hz-rTMS in patients with focal idiopathic cortical epilepsy. rTMS also elicited significantly different responses (MEP facilitation) between the patients and healthy subjects. In healthy subjects during 5 Hz-rTMS delivered over the right and left hemisphere MEP size increased significantly and did so to a similar extent in both hemispheres. 5 Hz-rTMS in patients elicited a reduced MEP facilitation compared to normal subjects. The reduced MEP amplitude was more evident in the affected hemisphere than in the unaffected hemisphere. RMT in the affected hemisphere was higher than in the unaffected hemisphere and in healthy subjects.



**Fig. 1:** Effects of 5 Hz repetitive transcranial magnetic stimulation (rTMS) at 120% resting motor threshold intensity on the amplitude of motor evoked potentials (MEPs) in the first dorsal interosseus (FDI) muscle in healthy subjects (left and right hemispheres) and patients (affected and unaffected hemispheres). Data correspond to means±SE.

We took several precautions to ensure that technical problems had no influence on our rTMS findings. To exclude an involuntary background contraction we carefully checked the EMG activity and excluded trials with background EMG activity. Moreover, the first MEP in the trains was similar in size in the affected and unaffected hemisphere, excluding the possible effects of saturation. The results obtained in the group of healthy subjects make it unlikely that changes in the MEP size during 5 Hz-rTMS depended on the hemisphere stimulated or on hemispheric dominance. Finally, during rTMS we asked the patients and healthy controls to remain fully relaxed and keep the eyes closed to avoid attentional tasks or environmental distraction during stimulation (Conte et al., 2007). We therefore consider it unlikely that the altered response to 5 Hz-rTMS in patients depended on the different attentional levels (Conte et al., 2007; Stefan et al., 2004) exerted by the patients between trials.

The reduced 5 Hz-rTMS induced MEP facilitation observed in patients in the unaffected hemisphere and the lack of effects on the affected hemisphere seems in contrast with the current assumption that seizures are a consequence of changes in neuronal membrane excitability (Kullmann, 2002; Noebles, 2003)

with an hyperexcitability of the cortical areas (Cantello et al., 2007; Macdonell et al., 2002; Tassinari et al., 2003). However, for ethical reason, the patients recruited in the study were receiving standard chronic pharmacological treatment with antiepileptic drugs and they were studied during the interictal phases. In previous studies performed in our laboratory testing the effects of antiepileptic drugs on cortical excitability tested by 5 Hz-rTMS we demonstrated that all the antiepileptic drugs decrease the MEP facilitation with a dose-related effect by acting on cortical excitatory interneurons thereby altering rTMS-induced synaptic potentiation (Inghilleri et al., 2004, 2006b). Therefore our findings on the unaffected hemisphere may be due to a direct effect of antiepileptic drugs on cortical excitability. The new neurophysiologically interesting finding in patients is that 5 Hz-rTMS disclosed asymmetric MEP amplitude responses in the affected hemisphere. Previous studies have hypothesized that the MEP facilitation induced by 5 Hz-rTMS resembles short-term forms of enhancement in synaptic activity (Inghilleri et al., 2004, 2005; Gilio et al., 2007). These phenomena, largely confined to the pre-synaptic site (Castro-Alamancos and Connors, 1996; Fisher et al., 1997) also depend upon NMDA receptors at post-synaptic level. We therefore conjecture that the lack of MEP facilitation during the train of stimuli delivered at high frequency over the primary motor cortex reflects abnormal short-term synaptic enhancement as we have already reported in patients with central nervous system disorders (Inghilleri et al., 2006a; Gilio et al., 2002). The RMT was higher in the affected than in the unaffected hemisphere and healthy subjects also suggesting a decreased excitability within the studied cortical area.

Even though the present results seem in contrast with the hypothesis that epilepsy arises from a hyperexcitability of the cortical areas we conjecture that our findings probably reflect an altered balance between the excitatory and inhibitory circuits within the primary motor cortex and from distant areas in patients with focal epilepsy during the interictal phases.

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