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NEUROPHYSIOLOGICAL STUDY OF EPILEPTOGENIC NETWORKS IN EPILEPSY

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ABSTRACT

Introduction

Epilepsy is one of the most common serious brain disorders characterized by recurrent seizures. The current understanding of its pathophysiology is based on the "system hypothesis" that goes beyond the classical dichotomy between focal and generalized epilepsy: some types of epilepsy may depend on the susceptibility to epileptogenic factors within a specific brain system. The mechanism underlying these processes are still obscure.

To increase understanding into the specific networks involved in the pathophysiology of different types of epilepsy, we proposed extensive neurophysiological studies on patients with epilepsy. First, we studied patients with photosensitive epilepsy since they represent a "model" of system epilepsy. Then, we focused on patients with focal (FE) and generalized epilepsies (GE) to unravel the neurophysiological basis of seizure generalization. Finally, we explored the motor cortex plasticity in juvenile myoclonic epilepsy (JME), the most common subtype of GE in adults.

Methods

We used the paired transcranial magnetic stimulation (paired-TMS), i.e. a safe, painless and non-expensive neurophysiologic tool to investigate the time related changes in functional connectivity between primary motor cortex and other distant brain areas. We developed a novel methods to examine the functional connection linking visual to the motor areas in healthy subjects and in patients with photosensitivity to study the visuomotor integration. We also studied the interhemispheric connection involved in seizure generalization in FE and GE; to explore the motor cortex synaptic plasticity in patients with JME we used the paired associative stimulation.

Results

The findings support a physiologically relevant visuomotor functional connectivity, which likely contributes to visuomotor integration. Substantial physiologic changes in this network likely underlies the photosensitivity, which may finally justify the origin of epileptic motor phenomena, such as myoclonus.

We found significant differences in the interhemispheric connection of drug-treated patients with FE and those with IGE. Whilst interhemispheric inhibition changes would not be

crucial for the IGE pathophysiology, they may represent one key factor for the contralateral spread of focal discharges, and seizure generalization.

As to the patients with JME, we provided evidence of a defective long term potentiation-like plasticity, which may be primarily involved in the pathogenesis of myoclonus.

Conclusions

To our knowledge, these are the first studies evaluating the excitability of various epileptogenic networks using paired-TMS in patients with focal and generalized epilepsy. We documented substantial changes in the epileptogenic networks involved in different types of epilepsy. Further investigation into the pathophysiology of these diseases would increase understanding into the ictogenesis of human epilepsies and the neural networks involved and eventually open new therapeutic targets.

INTRODUCTION

Epilepsy is one of the most common serious brain disorders characterized by recurrent seizures. In Italy 30000 new cases are diagnosed each year, with an incidence higher in infants and elderly people. Around 500000 people are affected. Epilepsy has negative effects on quality of life, function, and increases risk of mortality despite available treatments (Duncan *et al.*, 2006). It is refractory to the treatment in about one-third of cases and the mechanisms underlying this drug resistance are not understood (Duncan *et al.*, 2006).

The pathophysiology of epilepsy rely on the epileptogenic process in which changes at the molecular level ultimately translate into an unbalance between excitatory and inhibitory neural circuits leading to cortical hyperexcitability in specific networks and maladaptive plasticity. The mechanism underlying these processes are still obscure.

The current understanding of the pathophysiology of epilepsies is based on the "system hypothesis" that goes beyond the classical dichotomy between focal and generalized epilepsy: some types of epilepsy may depend on the susceptibility to epileptogenic factors within a specific brain system (Avanzini *et al.*, 2012).

Photosensitive epilepsy (Verrotti *et al.*, 2005) represents an ideal model of system epilepsy because the flickering light stimuli consistently induce an epileptic response. In this syndrome, the involvement of a widespread epileptogenic visuomotor network has been recently suggested (Koepp *et al.*, 2015).

Unravelling how the epileptogenic process is affecting the brain networks will invaluably advance our understanding of epilepsy and will lead to development of improved therapeutic perspectives.

In the following parts we aim to introduce the photosensitivity and then the neurophysiological methods that proved to be the most useful to unravel the pathophysiology of epilepsy. Later, we aim to discuss the most recent evidences coming from non-invasive brain stimulation studies in photosensitivity.

PHOTOSENSITIVE EPILEPSY

Photosensitivity is a condition in which epileptic seizures are triggered by natural or artificial intermittent lights, such as flickering sunlight (Gowers, 1885), flashes of television programs (Livingston, 1952), or computer games (Takada *et al.*, 1999; Hughes, 2008).

The first scientific description of a "photoconvulsive" response dates back to 1946 by Grey Walter and colleagues (Walter *et al.*, 1946). Since then, in EEG laboratories different techniques have been used to detect the response to the intermittent photic stimulation (IPS) (Harding, 1994). Usually, the sensitivity of the technique increases between 15 and 20 flashes per second, with a binocular stimulation of the central visual field and patterned stimuli (Harding, 1994). In most cases, a diagnosis of photosensitivity is made in the EEG laboratory. However, some patients experience their first seizure while watching TV programs or playing video games (Harding, 1994).

Photosensitive epilepsies came to public attention after December 16, 1997, when approximately 700 children were transferred to hospital in Tokyo, Japan, after watching a made-for television cartoon called "Pocket Monster". Immediately after watching the program, children experienced the sudden onset of convulsions, headache, nausea and blurred vision (Ishida *et al.*, 1998). An official governmental report concluded that low-luminance/red-blue stimuli alternating at 12 Hz triggered seizures in Japanese children (Ishida *et al.*, 1998).

Photosensitivity is part of the reflex epilepsy spectrum in which seizures can be triggered by external stimuli (Berg *et al.*, 2010). Its most elementary and common form is the photoparoxysmal response (PPR) to IPS.

Epidemiology

A PPR in patients with epilepsy is a relatively common phenomenon, occurring in up to 10% of patients (Buchthal & Lennox, 1953; Wolf & Goosses, 1986; Obeid *et al.*, 1991; Gregory *et al.*, 1993). It is more common in children (Hughes, 2008), female (Zifkin & Kasteleijn-Nolst Trenite, 2000), and Caucasians (Hughes, 2008). Quirk et al. (1995) reported that approximately 2% of new diagnosis of epilepsy show a PPRs on their first EEG (Quirk *et al.*, 1995). Besides, a PPR may be detected in healthy subjects between 0,5% to 8,9% of cases (Kooi *et al.*, 1960; Verrotti *et al.*, 2002).

A genetic component in photosensitivity has become clear in the last few decades (Italiano *et al.*, 2016). There is an higher occurrence in the same families, in genetic disorders and in siblings (Covanis, 2005). The risk of having PPRs in the general population is about 3%, but it increases at about 20% in siblings of children with generalized PPR (Waltz & Stephani, 2000). This phenomenon is characterized by genetic heterogeneity and complexity.

The highest rate is observed during puberty until the age of 15 years and in female, likely because of hormonal differences (Wolf & Goosses, 1986; Kasteleijn-Nolst Trenite, 1989; Clement & Wallace, 1990). According to some authors (So *et al.*, 1993; Verrotti *et al.*, 2002), the presence of PPRs is not a risk factor for the development of epilepsy: in fact none of the subjects who showed PPRs without any other EEG abnormalities suffered from epileptic seizure during the follow-up.

Diagnosis

The PPR is routinely assessed during standard EEG registration. After the Consensus Meeting in Heemstede in 1996 and in Aix-en-Provence in 1999, some guidelines were elaborated (Kasteleijn-Nolst Trenite *et al.*, 1999; Rubboli *et al.*, 2004; Kasteleijn-Nolst Trenite *et al.*, 2012). Table 1 shows the most recent practical recommendation on IPS (Kasteleijn-Nolst Trenite *et al.*, 2012).

The importance of the eyes closure state is likely related to the interposition of the eyelid red filter that has a provocative effect on PPR (Kasteleijn-Nolst Trenite *et al.*, 2012). In some cases a PPR can be observed only when the eyes are closed because the light is spread over the entire retina and the excitability of the brain increases (Wilkins *et al.*, 1980; Kasteleijn-Nolst Trenite, 1989). An important recommendation is to turn off the stimulator immediately at the appearance of generalized epileptiform discharges on EEG, to avoid the development of a seizure. Subsequently, it is possible to restart the IPS again with a frequency of 60 Hz and go down in frequencies to find the upper PPR threshold (Kasteleijn-Nolst Trenite *et al.*, 2012).

Table 1

Photostimulator	Grass PS22 stimulator
Lamp	Xenon lamp, with circular reflector (diameter 13 cm)
Flashes intensity	At least 0.70 Joule
Viewing distance	30 cm
Patient position	Upright
EEG montage	16 channels
Flash frequencies required	1 - 2 - 8 - 10 - 15 - 18 - 20 - 25 - 40 - 50 - 60 Hz
Eye conditions	Eye closure, eye closed, eye open
Frequency duration	Trains of flashes of 5 s + 5 s rest, each eye condition (30s)
Total IPS duration	5 ½ min
Additional requirements	Electrodes for recording eye movements and surface electrodes to detect myoclonus

Summary of the practical recommendation on the photic stimulation technique (Kasteleijn-Nolst Trenite et al., 2012).

PPR classification

The type of PPR are commonly divided into 4 types (Waltz *et al.*, 1992; Doose & Waltz, 1993) (Figure 1):

- type I: occipital spikes within the occipital alpha rhythm;
- type II: parieto-occipital spikes with a biphasic slow wave;
- type III: parieto-occipital spikes with a biphasic slow wave and spread to the frontal region;
- type IV: generalised spikes and waves or polyspikes and waves.

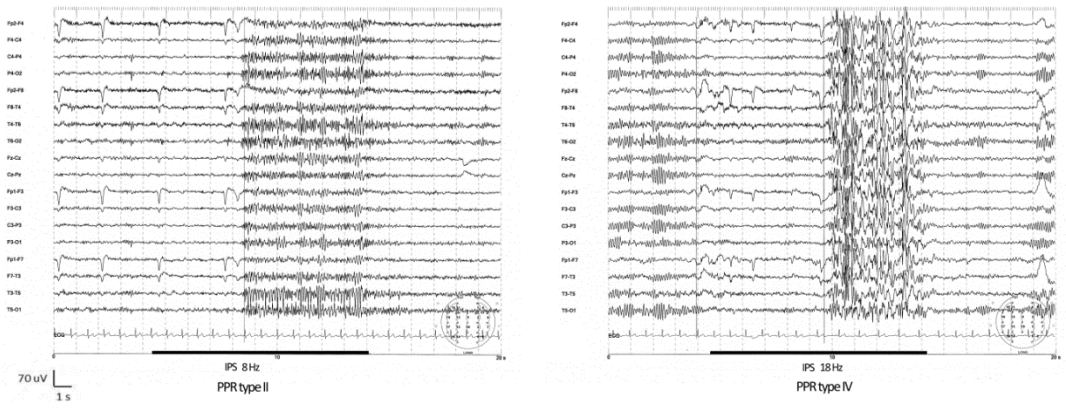

Figure 1

Example of PPR type II and type IV according to the classification of Waltz *et al.*, 1992. IPS, intermittent photic stimulation.

Epileptic syndromes with photosensitivity

In patients with epilepsy, a PPR can be found in different cases (Covanis, 2005; Verrotti *et al.*, 2012): in epilepsies without seizures induced by IPS, in epilepsies with and without seizures induced by IPS and in pure photosensitive epilepsies.

In general, it is known that PPR rates are higher in patients with generalized epilepsy than in focal epilepsy (Wolf & Goosses, 1986; Lu *et al.*, 2008). Seizures elicited by IPS are usually generalized tonic-clonic, myoclonic and absences (Covanis, 2005). The group of epilepsies without seizures induced by IPS is a miscellanea of different epileptic syndromes with no importance on the aetiology. The second group, in which seizures both induced and not induced by IPS coexists, contains: juvenile myoclonic epilepsy (JME), epilepsy with grand mal on awakening, eyelid myoclonia with absences (EMA), progressive myoclonic epilepsy (PME), Dravet syndrome, childhood absence epilepsy (CAE) (Verrotti *et al.*, 2012). In JME, the most common subtype of idiopathic generalized epilepsy (IGE) in youngsters between 8 and 36 years (Banerjee *et al.*, 2009; Camfield *et al.*, 2013), the incidence of photosensitivity ranges from 30% (Wolf & Goosses, 1986) to 90% (Appleton *et al.*, 2000). In EMA, the presence of photosensitivity, together with eyelid myoclonias with and without absences and eye closure induced seizures, is a diagnostic criteria of this syndrome (Covanis, 2005). In CAE the incidence of photosensitivity is between 17% (Wolf & Goosses, 1986) and 44% (Lu *et al.*, 2008). In Dravet syndrome, also known as severe myoclonic epilepsy of infancy, the PPR is observed in 40-60% of patients (Dravet, 2012; Specchio *et al.*, 2014).

The group of pure photosensitive epilepsies includes epilepsies with generalized seizures only provoked by IPS or flickering lights. According to Jeavons (Jeavons *et al.*, 1986), 40% of photosensitive patients suffer from this kind of epilepsy; a wide range of IPS frequencies (5-60 Hz) are effective (Covanis, 2005).

Most patients are sensitive at IPS between 10 and 30 Hz (Harding, 1994) and patients sensitive to higher frequencies are at risk of having seizures during fluorescent light stimulation or during TV programs (Kasteleijn-Nolst Trenite *et al.*, 2012). A PPR at 1-2 Hz, is a typical detection in progressive myoclonic epilepsies (Rubboli *et al.*, 1999). In adult patients without epilepsy, a late onset PPR at low frequencies (<5 Hz) can be found in the context of a severe progressive neurologic deterioration due to encephalopathies (Creutzfeldt-Jakob disease, MELAS) or neurodegenerative disorders (Lewy body disease) (Guellerin *et al.*, 2012).

Pathophysiology of photosensitivity

Photosensitivity has always attracted the attention of epileptologists. Indeed, it represents an ideal model of network epilepsy (Avanzini *et al.*, 2012), in which adequate visual stimuli trigger an epileptic event in the brain. The epileptogenic network underlying the PPR is primarily based on a hyperexcitable visual cortex in response to flickering lights (Strigaro *et al.*, 2012) and defective cortical mechanisms of contrast gain control (Porciatti *et al.*, 2000). The stimuli have two main salient features, the quantity of light (luminance) (Harding & Fylan, 1999) and the wavelength (Takahashi *et al.*, 1999), that contribute in evoking a PPR. Therefore, the maximal provocative effects can be obtained by either high luminance stimuli, like the IPS easily practiced in the EEG laboratories around the world, or deep-red colours whose epileptogenicity was highlighted in the Pokémon incident (Ishida *et al.*, 1998). However, hyperexcitability of the visual cortex do not explain how the PPR discharge propagates from posterior to anterior regions of the brain to generate the allied myoclonic jerks (Koepp *et al.*, 2015). Although the involvement of the peri-rolandic area in the PPR was showed in the Papio papio baboon, a model of generalized epilepsy with photosensitivity (Naquet *et al.*, 1995), human evidence have been scarce until recently.

TRANSCRANIAL MAGNETIC STIMULATION (TMS) IN EPILEPSY

Epilepsies are a complex group of syndromes characterized by episodic brain dysfunction manifesting as recurrent seizures (Engel, 2006). Admittedly, the underlying process is mediated by changes in both excitatory and inhibitory neural circuits leading to hyperexcitable seizure networks (Clark & Wilson, 1999). Sometimes, the primary motor cortex is a crucial part of these networks. More often, it can be influenced at a distance by non-motor epileptogenic areas (Hamer *et al.*, 2005). Besides, cortical area 4 (M1) is easily studied by transcranial magnetic stimulation (TMS), i.e. a safe, painless and non-expensive neurophysiologic tool (Macdonell *et al.*, 2002; Tassinari *et al.*, 2003; Schrader *et al.*, 2004). TMS was initially used to evaluate the integrity of the cortico-spinal tract through conduction studies (Barker *et al.*, 1985). Then, it was applied to measuring the excitatory and inhibitory properties of the cortex itself. Several TMS protocols were developed to study different properties of M1, such as the resting motor threshold (RMT) (Rossini *et al.*, 1999), the cortical silent period (CSP) (Cantello *et al.*, 1992) and paired-pulse measures such as short intracortical inhibition (SICI) and intracortical facilitation (ICF) (Kujirai *et al.*, 1993). These physiologic variables, over the past 25 years, proved much informative in terms of both physiology and disease, particularly movement disorders and epilepsy (Cantello *et al.*, 1992; Valls-Sole *et al.*, 1992; Kujirai *et al.*, 1993; Wilson *et al.*, 1993; Hallett, 1995; Ziemann *et al.*, 1998; Macdonell *et al.*, 2002).

The most robust findings across epilepsy studies came from one of these testing protocols, i.e. paired-pulse TMS (Brodtmann *et al.*, 1999; Cantello *et al.*, 2000a; Manganotti *et al.*, 2000; Werhahn *et al.*, 2000; Manganotti *et al.*, 2001; Hamer *et al.*, 2005; Badawy *et al.*, 2007; Badawy *et al.*, 2014). TMS has at a later stage evolved in a tool to study cortical plasticity (Ziemann *et al.*, 2008) and functional connectivity (Rothwell, 2011).

Single pulse

In the context of assessment of excitability of the primary motor area (M1), single pulse TMS applied over the cortical representation of a given muscle (usually the first dorsal interossesous, FDI) can be used to measure RMT, active motor threshold (AMT) and CSP. RMT is defined as the minimum stimulation intensity required to obtain a motor evoked potential (MEP) of at least 50 μV in approximately 5 out of 10 trials while the target muscle is

at rest (Rossini *et al.*, 1999). RMT is probably dependent on the intrinsic excitability of neural elements directly activated by TMS, i.e. cortico-cortical axons, their excitatory synaptic contacts with the corticospinal neurons and the initial axon segments of the corticospinal neurons (Amassian *et al.*, 1987; Di Lazzaro & Ziemann, 2013). RMT is thus mostly dependent on the state of voltage-gated sodium channels (VGSC), which directly regulate axon excitability (Hodgkin & Huxley, 1952) and of a-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid receptors (AMPA-R), which are responsible for fast excitatory synaptic transmission in the neocortex (Ozawa *et al.*, 1998). This is suggested by the observation that VGSC blocking agents increase RMT (Ziemann *et al.*, 2015) whereas enhancement of AMPA-R transmission reduces RMT (Di Lazzaro *et al.*, 2003). Single pulse can also be used to evaluate M1 inhibitory circuitry by measuring the CSP, which is defined as a TMS-induced interruption of activity in the EMG of voluntarily contracting muscles. It has been hypothesized that the late part of the CSP is caused by a long-lasting cortical inhibition mediated by GABAb receptors and thus it can be used to probe inhibitory mechanisms within M1 (Nakamura *et al.*, 1997; Siebner *et al.*, 1998). Single pulse TMS can also be delivered to the primary visual cortex (V1) to elicit perceptual excitatory phenomena called phosphenes (Meyer *et al.*, 1991; Merabet *et al.*, 2003) and to suppress visual perception (Amassian *et al.*, 1989); both are used as measures of V1 excitability.

Paired pulse

It is possible to use TMS to probe M1 intracortical circuitry with paired pulse paradigms. Among them, SICI measures the suppression of a suprathreshold TMS stimulus by a preceding subthreshold pulse, with an interstimulus interval (ISI) of 1-5 ms (Kujirai *et al.*, 1993). It has been hypothesized that the first pulse activates low-threshold inhibitory interneurons, which in turn suppress activity in corticospinal neurons through inhibitory post-synaptic potentials (IPSPs) mediated by GABAa receptors (Ilic *et al.*, 2002; Di Lazzaro & Ziemann, 2013). When the two pulses are applied with the same intensity but with an ISI of 10-15 ms inhibition turns to facilitation. This phenomenon is called ICF and mostly relies on glutamatergic neurotransmission (Ziemann *et al.*, 1996b). By contrast, long intracortical inhibition (LICI) is tested by applying two suprathreshold stimuli at an ISI between 50 and 200 ms (Valls-Sole *et al.*, 1992) and is thought to reflect slow IPSPs mediated by GABA-B receptors (Werhahn *et al.*, 1999).

Functional connectivity

The mentioned studies using single- or paired-pulse TMS paradigms (delivered with a one coil) investigated the excitability of a single brain area, mostly M1 and V1. More recently, two coils ("twin coil" approach) have been used to investigate the time related changes in functional connectivity between primary motor cortex and other distant brain areas. A conditioning stimulus (CS) is first used to activate the interested area, while the test stimulus (TS), given at different times afterward, is used to probe the excitability of motor cortical projections to contralateral hand muscles. This method has been successfully conducted and proved the existence of various pathways in humans (Rothwell, 2011), especially the functional connectivity between primary motor cortex and the controlateral M1 (Ferbert *et al.*, 1992), the cerebellum (Ugawa *et al.*, 1995), the premotor cortex (Civardi *et al.*, 2001) and the posteriorparietal cortex (Koch *et al.*, 2007a) with a milliseconds time resolution.

Cortical plasticity

Abnormal cortical plasticity has been frequently hypothesized to play a crucial role in the pathogenesis of epilepsy (Sutula, 2004; Lopantsev *et al.*, 2009). However, direct evidences supporting these hypothesis have been scarce so far in human epilepsy, possibly for experimental difficulties. TMS offers the unique opportunity to study non-invasively cortical plasticity. A variety of TMS protocols have been developed to probe mechanisms of synaptic plasticity in the intact human brain. Between these, paired associative stimulation (PAS) (Stefan *et al.*, 2000; Wolters *et al.*, 2003) involves repeated pairing of an electrical stimulus to the median nerve with a later TMS stimulus over the contralateral motor cortex inducing changes in cortical excitability. The effect depends on the interval between median nerve and TMS stimuli: intervals of 25 ms (PAS25) increase excitability whereas intervals of around 10 ms (PAS10) reduce excitability (Stefan *et al.*, 2000; Wolters *et al.*, 2003; Weise *et al.*, 2006). Pharmacological manipulations suggest that the effects involve temporary changes in synaptic efficacy that are equivalent to long term potentiation (LTP) and long term depression (LTD) described in animal preparations (Muller-Dahlhaus *et al.*, 2010). For example, sensorimotor cortex synaptic plasticity is abnormal in patients with progressive myoclonic epilepsy (Danner *et al.*, 2011).

TMS AND PHOTOSENSITIVITY

TMS proved to be an excellent method to study the cortical excitability in patients with epilepsy (Macdonell *et al.*, 2002; Tassinari *et al.*, 2003; Badawy *et al.*, 2014), but it has received relatively little attention in the study of photosensitivity in particular.

Single pulse TMS

Single pulse TMS measures were used by several authors to investigate M1 excitability in photosensitive patients at rest. Manganotti and coworkers found no difference in MEP amplitude and CSP between patients with juvenile myoclonic epilepsy, and healthy subjects (Manganotti *et al.*, 2000). However not all the examined patients had photosensitivity. Similarly, Groppa and coworkers (Groppa *et al.*, 2008) found no difference in MEP amplitude, and CSP duration between PPR-positive patients with idiopathic generalized epilepsy (IGE), PPR-negative patients with IGE, and PPR-negative healthy controls under resting conditions. This was confirmed by other investigators (Siniatchkin *et al.*, 2007) comparing PPR-negative and PPR-positive healthy subjects. While an increase in RMT was found in IGE patients with PPR compared with IGE patients without PPR (Groppa *et al.*, 2008) and with healthy subjects (Strigaro *et al.*, 2013), this has been attributed to the effect of antiepileptic medications on RMT (Ziemann *et al.*, 2015).

The same single pulse TMS measures were investigated in relation to IPS. IPS at frequencies of 50 and 60 Hz was able to decrease CSP duration in healthy subjects, while IPS at 5 and 30 Hz was not effective (Entezari-Taher & Dean, 2000). However, IPS at 50 Hz was not able to shorten CSP in PPR-positive and PPR-negative patients with IGE (Groppa *et al.*, 2008); the authors speculated that patients with IGE had an altered responsiveness of GABAergic inhibitory circuits in M1. This result argue against a specific increase in M1 excitability of M1 due to PPR. Other authors suggested that PPR might be linked to excitability alterations in V1. It was found that healthy individuals with PPR propagating to frontal regions had lower phosphene threshold, steeper stimulus-response curves and showed a stronger suppression of visual perception following TMS pulses compared with healthy individuals with PPR with occipital spikes only (Siniatchkin *et al.*, 2007). Subjects with propagating PPR also showed no CSP change during IPS, while in subjects without PPR CSP was shortened under the same conditions. This last finding was confirmed by our group in a

later study, where IPS at 20 Hz failed to modulate CSP only in patients showing type III or IV PPR (i.e. with frontal or generalized diffusion) (Strigaro *et al.*, 2013).

Paired pulse

SICI was smaller in patient with JME treated with antiepileptic drugs (AEDs), while the ICF and LICI were normal (Manganotti *et al.*, 2000); unfortunately the patient were not stratified according to the presence of PPR. Groppa and coworkers found no difference in SICI and ICF between PPR-positive patients with IGE, PPR-negative patients with IGE and PPR-negative healthy controls, both at rest and during IPS (Groppa *et al.*, 2008). In our study SICI and ICF were normal both at rest and during IPS, and this was true for patients with a PPR limited to occipital regions as well as for those having an anterior spread of PPR (Strigaro *et al.*, 2013).

Connectivity and plasticity

The mentioned studies using single- or paired-pulse TMS paradigms mostly investigated the excitability of M1 in patients with PPR; however, there are evidences suggesting hyperexcitability in the visual cortex of these patients (Porciatti *et al.*, 2000; Cantello *et al.*, 2011; Strigaro *et al.*, 2012). How the discharge propagates from posterior to frontal cortical regions, to generate the allied myoclonic jerks? We believe that valid answers may come from our recent electrophysiological studies on the mechanisms of visuo-motor integration presented in this thesis.

Some other evidences come from recent studies on physiological visuomotor integration with visual paired associative stimulation (V-PAS) (Suppa *et al.*, 2015a). This novel protocol induces long-term changes in the M1 excitability, which reflect long-term potentiation (LTP) and long-term depression (LTD) due to early visuomotor integration processes (Suppa *et al.*, 2015a). In patients with IGE and PPR, but not in PPR-negative patients, the V-PAS-induced plasticity was abnormal in M1. This may suggest that PPR arises from abnormal activity in a complex cortical network physiologically responsible for visuomotor integration (Suppa *et al.*, 2015b).

AIM OF THE THESIS

The aim of the present thesis was to increase understanding into the specific networks involved in the pathophysiology of different types of epilepsy with non-invasive brain stimulation (TMS). From this, we aimed to define specific neurophysiological phenotypes and translate the findings into clinically useful parameters.

First, we studied patients with photosensitive epilepsy since they represent a “model” of system epilepsy. Then, we focused on patients with focal and generalized epilepsies to unravel the neurophysiological basis of seizure generalization. Finally, we explored the motor cortex plasticity in juvenile myoclonic epilepsy, the most common subtype of generalized epilepsy in adults.

Further investigation into the pathophysiology of these diseases would increase understanding into the ictogenesis of human epilepsies and the neural networks involved and eventually open new therapeutic targets.

SELECTED PUBLICATIONS:

INTERACTION BETWEEN VISUAL AND MOTOR CORTEX: A TMS STUDY

Strigaro G, Ruge D, Chen JC, Marshall L, Desikan M, Cantello R, Rothwell JC.
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Abstract

The major link between the visual and motor systems is via the dorsal stream pathways from visual to parietal and frontal areas of cortex. Although the pathway appears indirect, there is evidence that visual input can reach the motor cortex at relatively short latency. To shed some light on its neural bases, we studied the visuo-motor interaction using paired transcranial magnetic stimulation (paired-TMS).

Motor evoked potentials (MEPs) were recorded from the right FDI in sixteen healthy volunteers. A conditioning stimulus (CS) was applied over the phosphene hotspot of visual cortex, followed by a test stimulus (TS) over left M1 at random interstimulus intervals (ISIs, 12-40 ms). The effects of paired stimulation were re-tested during visual and auditory reaction-time tasks (RT). Finally, we measured the effects of a CS on short-interval intracortical inhibition (SICI).

At rest, a CS over the occiput significantly (p<0.001) suppressed test MEPs at ISIs 18-40ms. In the visual RT, inhibition at ISI=40ms (but not 18ms) was replaced by a time-specific facilitation (p<0.001) whereas in the auditory RT the CS no longer had any effect on MEPs. Finally, an occipital CS facilitated SICI with an ISI=40ms (p<0.01).

We conclude that it is possible to study separate functional connections from visual to motor cortices using paired-TMS at ISI=18-40ms. The connections are inhibitory at rest and possibly mediated by inhibitory interneurones in motor cortex. The effect at ISI=40ms reverses into facilitation during a visuomotor, but not audiomotor RT. This suggests that it plays a role in visuomotor integration.

Introduction

Corticospinal excitability is modulated by a variety of sensory inputs, including auditory (Furubayashi *et al.*, 2000), somatosensory (Tokimura *et al.*, 2000), visual (Cantello *et al.*, 2000b), and even gustatory (Mistry *et al.*, 2006). This likely contributes to the sensorimotor integration underlying hand/limb movements (Goodale, 2011). In particular, somatosensory input has often been given special prominence, in view of its direct and short latency inputs. A large proportion of motor cortex neurones recorded in non-human primates respond at short latency to somatosensory inputs (Cheney & Fetz, 1984), and such responses are likely to be involved in long-latency transcortical stretch and cutaneous reflexes in humans (Macefield *et al.*, 1996). In contrast, visual inputs are classically viewed as relatively indirect and weak, with only about 3% neurones in primate motor cortex responding to visual stimulation (Lamarre *et al.*, 1983). However, later studies found visually responsive neurones in many areas of the cerebral cortex not directly involved in vision (i.e. premotor cortex, supplementary motor area, prefrontal cortex, frontal ocular fields) (Fadiga *et al.*, 2000). How these areas are involved in visuomotor integration is still largely unknown.

In humans there have been relatively few direct investigations of the effects of visual input on motor cortex (M1), but those that have been done suggest that moderately strong effects can be observed at a relatively short latency. The earliest studies were conducted in patients with photic reflex myoclonus in whom flashes of light can evoke a generalised myoclonic jerk (Shibasaki & Neshige, 1987; Artieda & Obeso, 1993). In a series of investigations on 6 patients, Artieda and Obeso (Artieda & Obeso, 1993) suggested that visual input was reaching the motor cortex rapidly from primary visual areas since transcranial magnetic stimulation (TMS) over the occiput during 1 Hz flash stimuli (to increase visuo-motor excitability) provoked a muscle twitch some 7 ms later than direct TMS over M1. A later study by Cantello et al (2000) in healthy volunteers followed up on these observations by using single pulses of TMS to assess the excitability of the motor cortex after a light flash. They found that excitability was reduced some 55-70 ms after the flash and pointed out that the response to a flash reaches visual cortex at about 40 ms, so that if a cortico-cortical pathway was involved from visual (V1) to motor cortex (M1), the transit time would be of the order of 15 ms, at least in normal subjects. These effects might be interpreted as the physiological counterpart of a pathological visuomotor connectivity seen earlier in patients with photic reflex myoclonus (Artieda & Obeso, 1993). Rapid access of visual input to motor areas of cortex is also evident from reaction time studies (Thut *et al.*, 2000; Makin *et al.*, 2009) and many event-related potential (ERP) studies (Saron *et al.*, 2001; Foxe & Simpson, 2002; Ledberg *et al.*, 2007). Yet

the precise neural basis of these phenomena is still largely obscure. Intuitively, the primary visual area would be the first cortical relay of the circuit and the primary motor cortex would represent the final output.

The aim of the present study was to devise a method to examine visuo-motor interaction in healthy participants. We used a "twin coil" TMS approach to test whether a conditioning pulse over the occiput influences the amplitude of the muscle twitches evoked from a later TMS pulse applied over M1. Connectivity was tested at rest as well as during the warning period prior to a simple visual reaction time task in order to examine whether it showed any task-related changes in excitability.

Materials and Methods

Subjects

A total of 16 healthy volunteers (8 women, 21–51 years old) were recruited. One subject was excluded since he reported no phosphenes. All of the remaining 15 participated in Exp. 1; 10 of these then participated in Experiments 3-5 (same individuals in all 3 experiments). All subjects were right-handed based on the Edinburgh Handedness Inventory and gave written informed consent. Experiments were approved by the Ethical Committee of University College London and were performed in accordance with the Declaration of Helsinki.

TMS

For paired-TMS we used two high-power Magstim 200 machines (Magstim, Whitland, UK). The magnetic stimulus had a nearly monophasic pulse configuration with a rise time of ~ 100 μs, decaying back to zero over ~ 0.8 μs. The stimulators were connected to a figure-of-eight coil (outer winding diameter 70 mm).

Test stimuli

MEPs were recorded from the first dorsal interosseous (FDI) muscles using 9 mm diameter Ag-AgCl surface-cup electrodes, in a typical belly-tendon montage. Responses were amplified by a Digitimer D360 device (Digitimer, Welwyn Garden City, UK). Filters were 20 Hz - 3 kHz, and the sampling rate was 10 kHz. The signal was then recorded by a PC using Signal software ver. 4.08 (Cambridge Electronic Devices, Cambridge, UK). The test coil was placed tangentially to the scalp at a 45° angle to the midline, to induce a posterior-anterior

(PA) current flow across the central sulcus. The hand motor area of the left M1 was defined as the point where stimulation consistently evoked the largest MEP. We defined the resting motor threshold (RMT) as the lowest intensity that evoked 5 small responses (~50 μV) in the relaxed FDI muscle in a series of 10 stimuli (Rossini *et al.*, 1994). The intensity of the TS was finally adjusted to evoke an MEP of ~ 1 mV peak-to-peak amplitude in the relaxed right FDI.

Experiment 1 (*n* =15).

Paired-TMS stimulation was conducted as in the pilot trial. The TS alone and CS plus TS were randomly intermixed at each ISI. Fifteen responses were collected for TS and 12 responses for CS plus TS. There was a 5 s (±20%) intertrial interval. For each trial we measured the average peak-to-peak MEP amplitude. The conditioned MEP was expressed as a percentage of the unconditioned MEP size. The centre of the conditioning coil was placed over the phosphene hot spot. This was located and the phosphene threshold (PT) determined according to the method of Stewart et al. (Stewart *et al.*, 2001). Subjects wore a blindfold and a cap whilst seated in a comfortable chair in a dimly lit room. Three points were marked over the occipital midline 2, 3 and 4 cm above the inion. The coil handle pointed upwards and was parallel to the subject's spine. The coil centre was first positioned 2 cm above the inion, then moved anteriorly across the marks, to determine the best site to elicit phosphenes ("hot spot"). Stimuli were initially applied at 60% of the stimulator output and at a maximum frequency of 0.2 Hz. The subject was asked about the presence of phosphenes immediately after each pulse. If a phosphene was reported 5 or more times out of 10, the pulse intensity was reduced by steps of 5%, then stimuli were repeated another 10 times. This protocol progressed until no phosphene was reported. The minimum intensity at which the subject perceived a phosphene 5 times out of 10 was the PT. If the initial intensity of 60% was ineffective, it was increased by steps of 5% maximum power, till phosphenes appeared. If the subject still failed to perceive a phosphene on the midline, the coil was shifted to a lateral position and the procedure was repeated at this location. One subject was excluded since he reported no phosphenes. The intensity of the CS was adjusted to be 80% PT or 90% PT. ISIs were 12, 15, 18, 21, 24, 27, 30, 35 and 40 ms. There were two sessions: one with eyes open and another with eyes closed.

Experiment 2 (n =8).

From Exp.1, 8 subjects were selected because they showed the strongest inhibition at ISI 18 and 40 ms. We then studied the effects of changing the CS site, in a setting otherwise

identical to Exp. 1. There were two sessions: conditioning stimuli with an intensity of 80% PT were applied to the phosphene hot spot or to a site 3 cm lateral to Pz (according to the 10-20 international system) on the right side. The subjects' eyes were open.

Experiment 3 (n=10).

The protocol described in Exp. 1 was then repeated during a visuo-motor RT task. We hypothesized that a physiologically relevant connectivity would show time-specific changes in such a context. We used a task similar to that of Touge et al (1998). Subjects sat relaxed in a chair with their right forearm lying comfortably on a pillow and their right hand on a button box. Eyes were open. Surface EMG was recorded from the FDI, APB (abductor pollicis brevis) and ADM (abductor digiti minimi) muscles. We ensured that there was no EMG activity at baseline. A black screen was placed in front of the subjects at a distance of 50 cm, which carried two light-emitting diodes (LEDs) separated by 1.5 cm. The red LED was the warning signal (WS) and the green LED was the response signal (RS). Subjects were instructed to use the WS to prepare for the upcoming response and to contract their right FDI muscle as quickly as possible in order to press the button with their right index finger as soon as they saw the RS. Each trial began with a WS followed by a RS given randomly 600±50 ms later. The intertrial interval was 5 s (±20%). We had two randomized sessions separated by at least one week. In each session we measured the effects of the CS on TS while subjects were at rest, outside of the reaction time task. CS was 90% PT. ISIs of 18 and 40 ms (the most effective in previous experiments) were randomly intermingled. Subjects also performed 4 blocks of the RT task. Each block had 4 conditions that were randomised within the block. Condition 1: subjects received a WS, followed 600±50 ms later by a RS, to which they had to react as quickly as possible. Condition 2: a TS alone given at -300, -150, -50 or +50 ms relative to RS (depending on the block, see below). Condition 3: same as condition 2, but the TS was preceded by a CS with an ISI of either 18 or 40 ms (depending on the block, see below). Condition 4: a TS alone was given in the intertrial interval (Figure 1). Thirty trials were recorded for each condition for a total of 120 trials. In one of the experimental sessions, the four trial blocks were: (1) TS at -300 ms, CS 18 ms before test; (2) TS at -150 ms, CS at 18 ms; (3) TS at -300 ms, CS at 40 ms before test; (4) TS at -150 ms, CS at 40 ms. The other experimental session contained TS at -50 ms and +50 ms. Before each session, at least 50 practice trials were given.

The responses to each single trial were stored on a computer and analysed off-line at the end of the experiment. Rejection criteria were: 1) baseline EMG levels $\geq$ 50 μV; 2)

reaction time < 100 ms and > 1000 ms; 3) failure to react. Later we analysed the RMS values of baseline EMG in the 100 ms before the TMS pulses in each trial to ensure the task specific conditioned MEP data were not contaminated by background EMG activity.

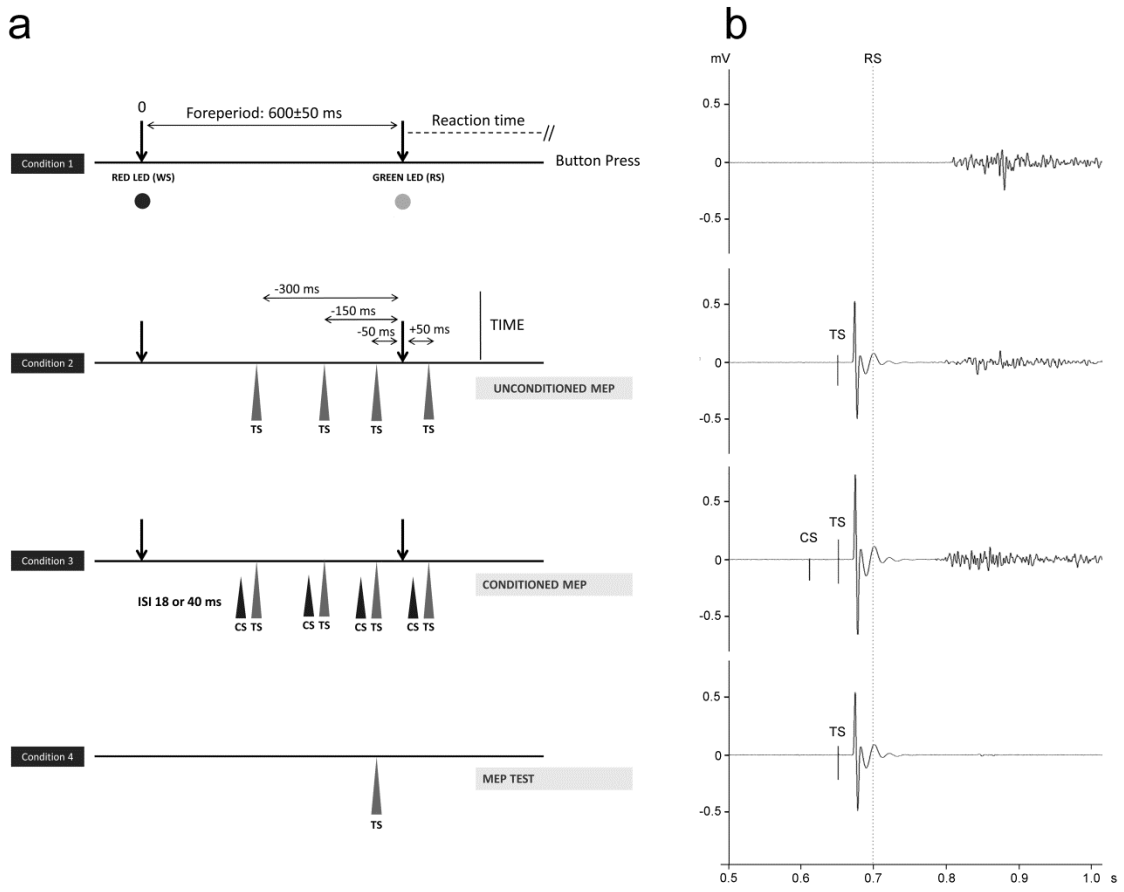

Figure 1

A, the setting of Experiment 3. In condition 1, subjects received a warning signal (WS), followed 600±50 ms later by a response signal (RS), after which they had to react as fast as possible. In condition 2, a test stimulus (TS) alone given at one of four different "times" (-300, -150, -50 or +50 ms). In condition 3, same as condition 2, but the TS was preceded by a conditioning stimulus (CS) with an ISI of either 18 or 40 ms. In condition 4 a TS alone was given in the intertrial interval.
B, typical example of changes in the MEP (grandaverage of the recorded trials) during the reaction time task. In this particular subject, a clear MEP increase can be seen 50 ms before the RS at an ISI of 40 ms (condition 3).

Experiment 4 (n=10)

We tested the paired-TMS protocol during an auditory RT task. The subjects, settings and conditions were the same as in Exp. 3. First, we measured the effects of the CS on TS with an ISI of 40 ms while subjects were at rest outside of the reaction time task. There followed two sessions: in one we used an auditory RT task, where the first tone (500 Hz, 50 ms) was the WS and the second tone (1000 Hz, 50 ms) was the RS; in the other, we re-tested the visual RT task. We also restricted our timings to TS at -50 ms (i.e. just prior to the RS) using an ISI between CS and TS of 40 ms since these parameters had produced large effects in Exp. 3.

Experiment 5 (n=10)

This experiment investigated the effects of a CS over the visual cortex on short interval intracortical inhibition (SICI) in the left M1 (Kujirai *et al.*, 1993). We used three high-power Magstim 200 machines. The first conditioning stimulus (CS1) was delivered with an intensity of 90% PT over the phosphene hot spot and the second one (CS2) over the left M1. Finally, the TS was applied over the left M1 with an intensity to elicit a MEP of ~ 1 mV. The intensity of CS2 was set to the relatively low value of 70% active motor threshold (AMT), to avoid floor effects on the percentage SICI. AMT was defined as the lowest intensity that evoked five small responses (about 100 μV) in a series of ten stimuli when the subject made a 10% of the maximum voluntary contraction of the right FDI. The ISIs between CS1 and CS2 were 18 and 40 ms, whilst the ISI between CS2 and TS was 2.2 ms. A randomized conditioning-test design was used. First we tested the effects on the test MEP (MEP1) of giving CS1 alone (with an ISI of 40, $CS1_{40ms}$; or ISI of 18 ms, $CS1_{18ms}$) or CS2 alone (MEP2). Then, the intensity of the TS was re-adjusted so that when $CS1_{18ms}$ +TS or $CS1_{40ms}$ +TS were applied the combined effect would elicit a MEP of ~ 1 mV ($MEP3_{1mV}$). Finally, two conditions were randomly intermingled: $CS1_{(18ms\ or\ 40ms)}$ + TS ($MEP3_{1mV}$) and $CS1_{(18ms\ or\ 40ms)}$ + CS2 + TS (MEP4). Fifteen trials were recorded for each condition. The ratio of MEP4/$MEP3_{1mV}$ was the amount of SICI in the presence of $CS1_{(18ms\ or\ 40ms)}$, whereas the ratio MEP2/MEP1 was the baseline SICI.

Data analysis.

All data were expressed as mean ± standard error of the mean (SEM). Student's paired *t* tests (two-tailed) were used to compare mean RMT with eyes open and closed obtained from all the participants. Spearman's rho was applied to study the correlation between motor and

phosphene threshold. In general, the effects of the CS on MEP amplitude were analysed with separate one-way ANOVAs for any given stimulation intensity and eyes state, with "ISI" (TS alone, CS plus TS at various ISIs) as the main factor. A significant main effect in these ANOVAs was followed by *post hoc* tests with Bonferroni corrections. Based on the conditions of the various experiments, we performed preliminary two or three-ways repeated-measures (rm) ANOVAs that accounted for the various factors to be analysed. Supplementary ANOVAs or rmANOVAs were finally carried out as dictated by the specific experiment, to assess the effects of additional confounders, e.g. in Exp. 3, a two-way rmANOVA explored the "time" (Figure 1) x "ISI" interactions. Mauchley's test was used to examine for sphericity. The Greenhouse-Geisser correction was used for nonspherical data. Occasionally, two-tailed paired Student *t* tests were used (Exp. 5). A p value < 0.05 was considered significant. Data were analysed using software (SPSS v. 19.0 for Windows; SPSS Inc.).

Results

Baseline physiological data are shown in Table 1. No differences were found between each experimental session. All subjects completed the experiments without complications.

Mean RMT with eyes open was 41.4% (range, 30–52%), the same as with eyes closed (40.6%; range 30-53%) (Student $t = 0.50$, $p = 0.63$). The phosphene hotspot was located in the midline in all subjects: it was 3 cm above the inion in 10/15 subjects, 2 cm in 4/15 and 4 cm in 1 subject (Figure 2, phosphene hotspot in a representative subject). Phosphenes were reported across both sides of the visual field. Mean PT was 62.8% (range, 40–76%). Motor and phosphene thresholds did not correlate (Spearman's rho = -0.15, $p = 0.62$ with eyes open; rho = 0.07 $p = 0.82$ with eyes closed).

Table 1. Physiological data (mean±SEM)

		RMT (%)	**PT (%)**	**UC MEP (mV)**
Experiment 1 (n = 15)				
EO	80% PT	41.4±1.9	62.8±2.6	1.07±0.08
	90% PT			1.13±0.09
EC	80% PT	40.6±1.9		0.98±0.08
	90% PT			0.96±0.05

Experiment 2 (n = 8)			
CS over control site	36.3±1.6	65.7±2.5	1.11±0.14
CS over phosphene hotspot			1.06±0.05
Experiment 3 (n = 10)			
TS at rest	39.1±1.6	62.3±2.7	1.09±0.07
TS -300 ms			1.15±0.09
TS -150 ms			1.04±0.07
TS at rest	40.8±2.1	65.2±2.3	1.13±0.10
TS -50 ms			1.10±0.12
TS +50 ms			1.17±0.06
Experiment 4 (n = 10)			
TS at rest	39.8±1.5	63.5±2.1	1.11±0.06
Auditory task			0.98±0.08
Visual task			1.08±0.05
Experiment 5 (n = 10)	**AMT (%)**		
TS	35.8±1.4	66.5±3.4	1.15±0.08
ISI 18 ms			1.14±0.08
ISI 40 ms			1.01±0.06

AMT, active motor threshold; CS, conditioning stimulus; EO, eyes open; EC, eyes closed; ISI; interstimulus interval; MEP, motor evoked potential; PT, phosphene threshold; RMS, root mean square; RMT, resting motor threshold; TS, test stimulus.

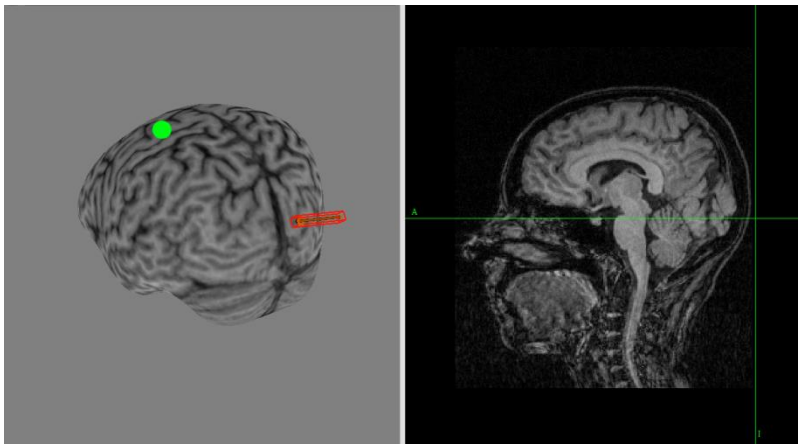

Figure 2

MRI reconstruction of a single subject. The red mark indicates the orientation of the magnetic field at the phosphene hot spot (striate cortex). The anterior green dot is at the hand area of the left motor cortex.

Experiment 1 (Conditioning MEPs with stimuli over the phosphene hot spot at rest).

In this experiment, the CS was placed over the phosphene hot spot. The effect of two different intensities of CS was measured on MEPs evoked from the left M1 with eyes open or closed throughout the testing (Figure 3a-d). A preliminary three-way rmANOVA showed a significant main effect of "ISI" ($F(5, 67) = 10.93$, $p < 0.001$), but no effect of "eye state" ($F(1, 14) = 1.50$, $p = 0.24$) or "intensity" ($F(1, 14) = 0.32$, $p = 0.58$) and no significant interactions ($p > 0.05$). Thus the time course of MEP suppression was the same at each intensity of CS and was unaffected by eye closure. The graphs also indicate the ISIs in each state where *post hoc* testing revealed significant ($p < 0.05$) effects compared to control (Figure 3a-d). Because ISIs of 18 and 40 ms were effective in all states these two intervals were then used in experiments 2-5.

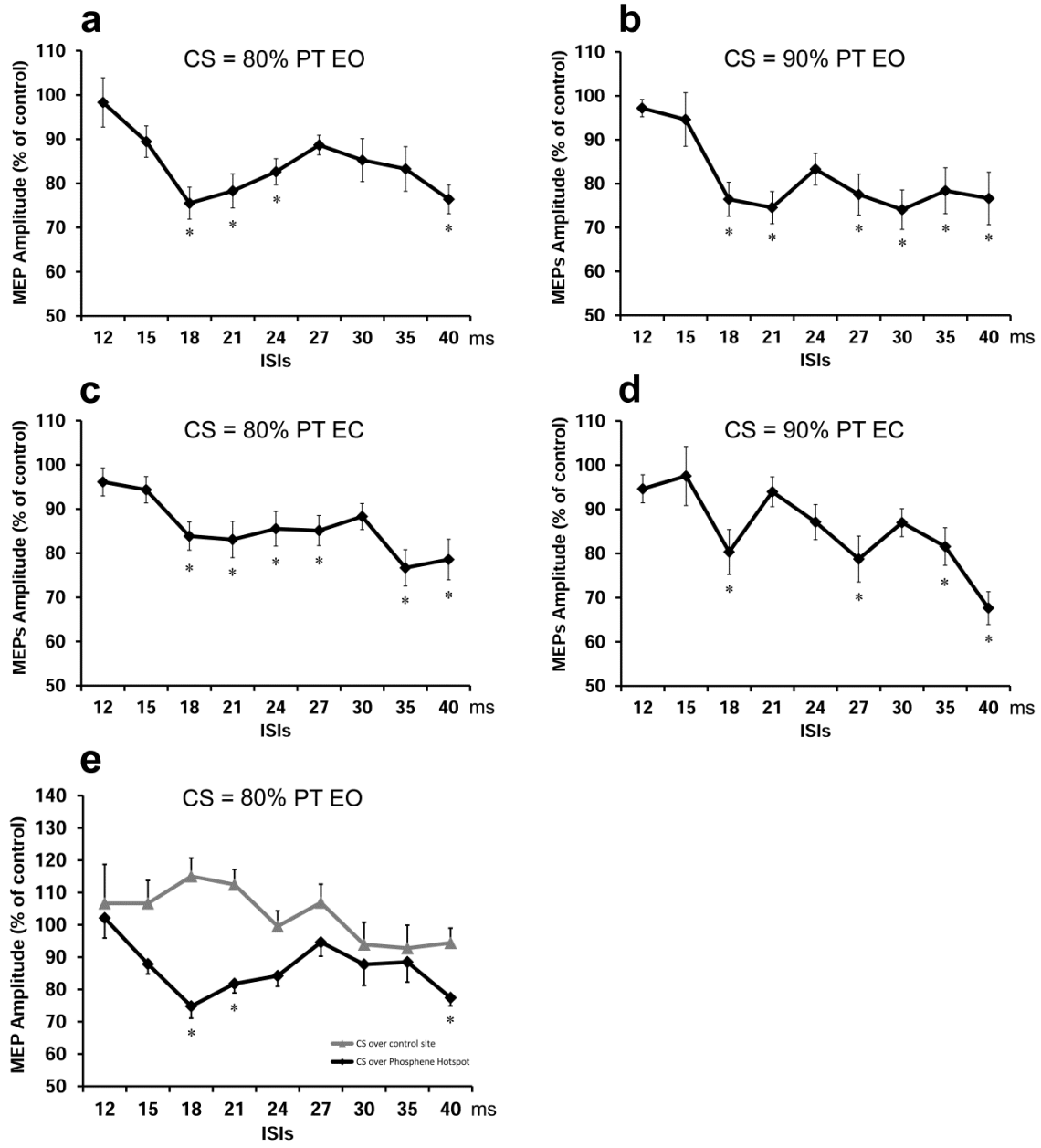

Figure 3

A-D, effects of a conditioning stimulus (CS) applied over the phosphene hot spot at different intensities (80% or 90% PT) and eye states (eyes open or closed) on the test MEPs with subjects at rest. Amplitude of MEPs (mV) is normalized and expressed as a percentage of control. Errors bars indicate SEM. Asterisks indicate a *p* value < 0.05 on *post hoc* analysis.

E, effects of changing the location of the conditioning stimulus (CS, 90% of the phosphene threshold, PT) on the test MEPs with subjects at rest. Grey line: CS applied to a scalp site 3 cm lateral to Pz on the right side. Black line: CS applied to the phosphene hot spot. Amplitude of MEPs (mV) is normalized and expressed as percentages of control. Errors bars indicate SEM. Asterisks indicate a *p* value < 0.05 on *post hoc* analysis.

Experiment 2 (Changing the site of the conditioning stimulus).

To confirm that the effect of the CS was spatially specific, we compared the effect of conditioning over the phosphene hot spot with conditioning over a point 3 cm lateral to Pz. Figure 3e shows that stimulation over the parietal site at this intensity had no effect whereas there was clear MEP suppression if the CS was over V1. A two-way rmANOVA showed a significant main effect of "stimulation site" ($F(1, 7) = 37.52$, $p < 0.001$), as well as a significant interaction between "stimulation site" and "ISI" ($F(8, 56) = 2.475$, $p = 0.02$), indicating that the time course of the effect on MEPs differed between sites. Follow up one-way ANOVAs revealed a significant main effect of ISI ($F(9, 63) = 4.73$, $p < 0.001$) at the phosphene hot spot but no effect of ISI over the parietal site ($F(9, 63) = 1.65$, $p = 0.12$). On *post hoc* analysis, the size of the MEP conditioned from V1 was significantly reduced at ISI 18 ms ($p = 0.001$), 21 ms ($p = 0.014$) and 40 ms ($p = 0.002$). No subject reported phosphenes after the control (parietal) stimulus.

Experiment 3 (Visuomotor functional connectivity during a visual RT task)

We next tested whether the effect of the CS varied during the course of a warned simple visual reaction time task. MEPs were conditioned by stimulation over the phosphene hot spot during the warning interval prior to the onset of the RS and at 50 ms following the RS prior to onset of movement. The effects were compared with those seen at complete rest outside the reaction task. MEPs to the M1 stimulus given alone were the same at rest at all intervals tested during the task (one-way rmANOVA, first session of task: ($F(3, 27) = 0.62$, $p = 0.61$); second session ($F(3, 27) = 0.24$, $p = 0.87$).

Figure 4a plots the size of the conditioned MEP as a percent of the test MEP alone for the two ISIs between CS and TS (18 and 40 ms). There are five bars for each ISI corresponding to suppression at rest and at -300, -150, -50 and +50 (with respect to the time of the RS) during the reaction task. The percentage suppression of MEP at an ISI of 18 ms was unchanged during the task whereas suppression at ISI = 40 ms gradually shifted to facilitation around the time of the RS.

This was confirmed by a two-way rmANOVA showing a significant main effect of "time" ($F(4, 36) = 39.64$, $p < 0.001$), "ISI" ($F(1, 9) = 25.40$, $p = 0.001$) and a significant "time" x "ISI" interaction ($F(2, 18) = 12.20$, $p < 0.001$). Follow up one-way ANOVAs showed no effect of "time" with an ISI = 18 ms ($F(3, 25) = 0.44$, $p = 0.73$) and no effects of "background EMG" both on the unconditioned ($F(1, 25) = 0.017$, $p = 0.90$) and conditioned

MEPs (F (1, 25) = 0.007, $p = 0.93$) (Table 2). On the contrary, there was a significant effect at ISI = 40 ms (F (3, 25) = 9.44, $p < 0.001$) and no effects of "background EMG" on the unconditioned (F (1, 25) = 0.28, $p = 0.60$) and conditioned trials (F (1, 25) = 0.32, $p = 0.574$) (Table 2). *Post hoc* analysis showed that the conditioned MEP was significantly larger 300 ms ($p = 0.034$), 150 ms, and 50 ms before and after the RS ($p < 0.001$).

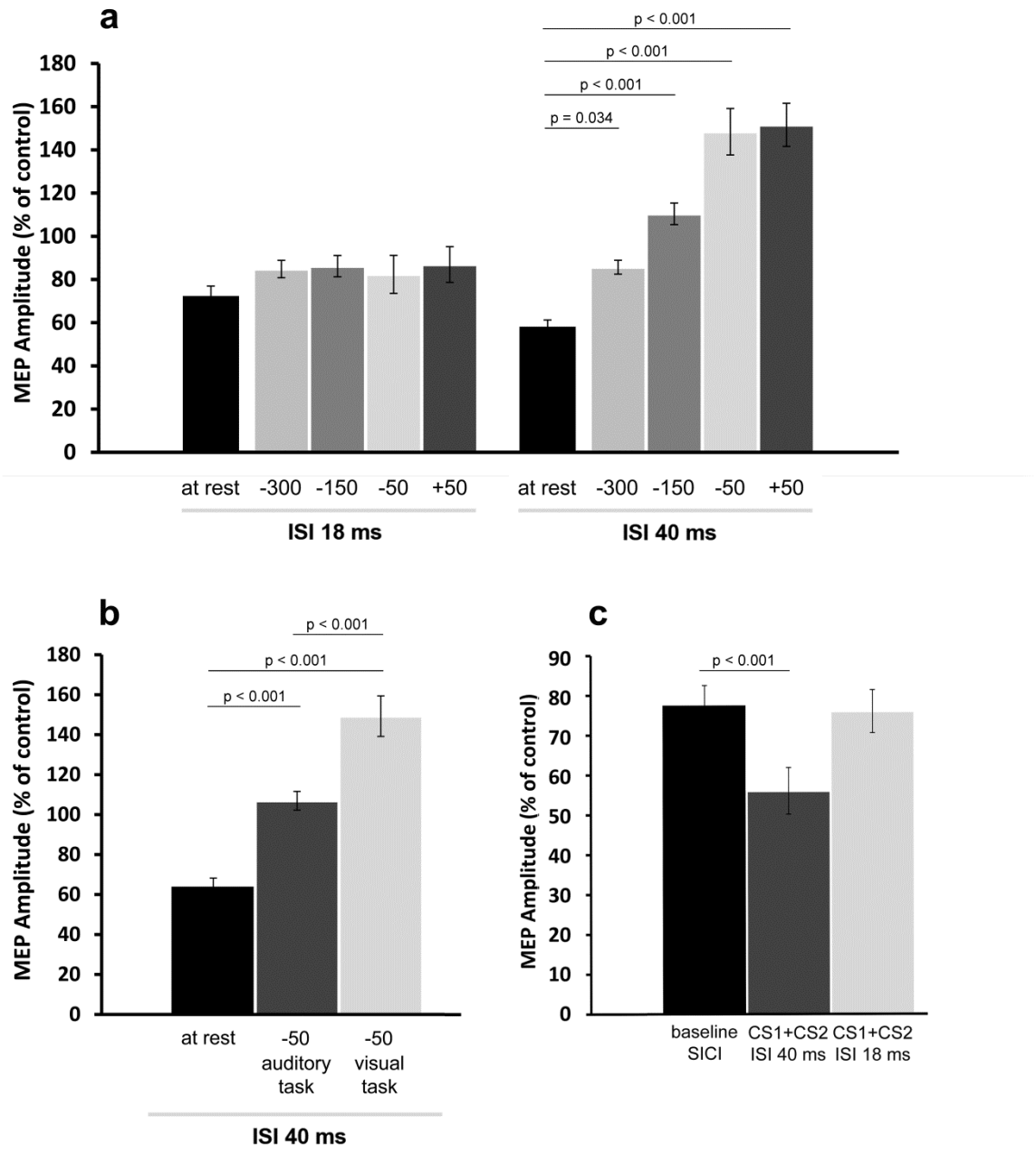

Figure 4

A, effects of the conditioning stimulus (CS, phosphene hot spot) on the test MEP amplitude at rest and at different times during the behavioural task (-300, -150, - 50 and +50 ms). Left, ISI 18 ms. Right, ISI 40 ms. Amplitude of MEPs (mV) is normalized and expressed as percentage of control. Errors bars indicate SEM.

B, effects of the conditioning stimulus (CS, phosphene hot spot) with an ISI of 40 ms on the MEP amplitude at rest and during a visual and an auditory reaction task 50 ms before the response signal

(RS). Amplitude of MEPs (mV) is normalized and expressed as a percentage of control. Errors bars indicate SEM.

C, comparison of the effects on short-interval intracortical inhibition (SICI) of conditioning stimuli applied over the visual cortex with an ISI of 18 and 40 ms. Errors bars indicate SEM.

Experiment 4 (Visuomotor functional connectivity during an auditory RT task)

In the visual task the CS (ISI = 40 ms) facilitated the conditioned MEP 50 ms prior to the RS. In the same subjects, we compared this with the effect when using the same timing in an auditory reaction task. The unconditioned MEP at rest was the same as during the visual and auditory task (50 ms before the RS) ($F(2,18) = 1.20$, $p = 0.323$). Figure 4b shows that the CS suppressed the MEP when subjects were tested at rest. However, during performance of the auditory task (-50 ms) there was no longer any effect of the CS on the TS whereas in the visual task it was facilitated. A one-way rmANOVA on the data confirmed that the effect of the CS differed between the three conditions ($F(2, 18) = 49.26$, $p < 0.001$). Follow-up analysis showed that although there was a significant difference between the effect at rest and at the -50 ms time points in both tasks (visual, $p < 0.001$; auditory, $p < 0.001$), the effect was larger in the visual task compared with the auditory task ($p < 0.001$).

Experiment 5 (Effects on SICI)

A CS over the phosphene hot spot increased the amount of SICI compared to baseline (baseline SICI, 77.5%; SICI in the presence of CS, 56%) (Student $t = 6.86$, $p < 0.001$) at an ISI of 40 ms, but not at 18 ms ($t = 0.254$, $p = 0.80$) (Figure 4c). As a result of intensity re-adjustment, the $MEP3_{1mV}$ size was 1.01 ± 0.1 mV (ISI 40 ms) and 1.14 ± 0.1 (ISI 18 ms), i.e. it was not statistically different from the MEP1 (1.15 ± 0.1 mV) ($F(2, 18) = 1.11$, $p = 0.35$).

Discussion

The present data show that TMS over the occipital region affects excitability of M1 when tested 18-40 ms later. Since the TMS coil was located over the optimal point to elicit stationary phosphenes (Afra *et al.*, 1998; Stewart *et al.*, 2001; Franca *et al.*, 2006), and an intensity below phosphene threshold was used, we suggest that the effect depends on activation of primary visual cortex (V1). We assumed that both hemispheres were activated since the coil position was on the midline in all the subjects and phosphenes were reported

across both sides of the visual field. The effect was present at both 80% and 90% phosphene threshold (PT) but was not significantly influenced by whether the eyes were open or closed. It was not caused by the auditory click made by the coil when discharged (Furubayashi *et al.*, 2000), as it was no longer present when the site of stimulation was moved 3 cm lateral to Pz.

Our results confirm the evidence reviewed in the Introduction that activity in visual cortex can modulate corticospinal excitability at short latency in subjects at rest. One of the limits of previous approaches is that they used natural visual stimuli and there is some uncertainty about the precise time at which these arrive in visual cortex. Most studies indicate that the first occipital visual evoked potentials begin around 35-40 ms (ffytche *et al.*, 1995), while intracranial electrodes recorded a latency of about 31-33 ms (Ducati *et al.*, 1988). Using these figures, the earliest TMS effect at ISI = 18 ms is compatible with the data on flash evoked suppression of MEPs noted by Cantello and colleagues at 55-70 ms after a flash (Cantello *et al.*, 2000b; Makin *et al.*, 2009) but later than the very rapid (7 ms) visuo-motor connectivity described in photic reflex myoclonus (Nakashima *et al.*, 1985; Shibasaki & Neshige, 1987; Artieda & Obeso, 1993; Kanouchi *et al.*, 1997). The shorter occipitomotor conduction time in the patients might well be explained by a pathological exaggeration of the normal physiological mechanism, resulting in a shorter latency response and a shift from inhibition to excitation of the motor cortex. A similar connection might explain the spread of the epileptic discharge from the hyperexcitable visual cortex to the motor cortex in photosensitive idiopathic epilepsies (Strigaro *et al.*, 2012; Strigaro *et al.*, 2013).

The later phase of interaction at ISI = 40 ms is compatible with the earliest signs of visual effect on motor cortex excitability described in a number of behavioural studies (e.g. 70 ms in Makin *et al.*, 2009). Longer latency visuo-motor effects have also been described by Suppa et al (2013) who showed that it was possible to induce long-term potentiation (LTP) and depression (LTD)-like plasticity in the primary motor cortex in healthy humans after repetitive pairing of a patterned visual stimulus and a TMS stimulus at specific time intervals around the latency of the P100 evoked potential. These varied between 40 and 140 ms after the individual P100 latency (i.e. between 140 and 240 ms after onset of the visual stimulus) (Suppa *et al.*, 2013) and are therefore longer than the ISIs we deal with in the present paper.

Apart from estimates of transit time, our data do not provide any information about the possible anatomical pathways that might mediate these functional effects. Connections in the dorsal visual stream via parietal and premotor cortex could provide one route. In addition, diffusion tensor imaging (DTI) techniques (Catani *et al.*, 2002) and anatomical dissection studies (Martino *et al.*, 2010; Sarubbo *et al.*, 2011) demonstrated the existence in humans of

the inferior fronto-occipital fascicle (IFOF), a long associative bundle connecting the occipital cortex and other posterior areas to the frontal lobe (Martino *et al.*, 2010). Although often seen as playing a role in transmitting information from frontal cortex to occiput for the purposes of "top down" control, the IFOF might also contain a direct efferent pathway from the occipital cortex, which can rapidly transmit visual information to the frontal regions (Martino *et al.*, 2010).

Most long range cortico-cortical connections are thought to be excitatory, as in the transcallosal pathway (Asanuma & Okuda, 1962; Ferbert *et al.*, 1992). The fact that we obtained an overall inhibitory effect in the present experiments would therefore be compatible with the idea that these excitatory projections synapse onto inhibitory interneurones in M1 that suppress corticospinal excitability. This is supported by our findings that a CS over the visual cortex increased SICI in the left M1, at least for ISI = 40 ms (not 18 ms). SICI is thought to test a GABAa-ergic form of intracortical inhibition in motor cortex (Ziemann *et al.*, 1996a). Thus the fact that SICI is made more effective by stimulation over visual cortex suggests that occipital input has access to inhibitory circuits in M1 and that this may contribute to the MEP suppression we have described. Visuo-motor suppression at 18 ms presumably does not depend on activity in the same set of interneurones since it has no effect on SICI. However, there are a number of possibilities that can be tested with TMS methods, including a GABAb-ergic system (tested with the long interval intracortical inhibition paradigm) (Valls-Sole *et al.*, 1992; Werhahn *et al.*, 1999) and a further pathway modulated by cholinergic input (tested with short afferent inhibition) (Tokimura *et al.*, 2000). Further work could tease apart these possibilities. At the present time, we conclude that the two phases of inhibition are caused by activity in two distinct pathways.

To assess the potential physiological role of this visuo-motor pathway, we examined connectivity during a visual RT task using ISIs of 18 and 40 ms since they produced the most consistent inhibitory effects. The task had no effect on MEP suppression at ISI = 18 ms at any of the time points studied during the task. This was not true for ISI = 40 ms. The inhibitory effect at rest (MEP reduced by 30-40%) gradually reversed into facilitation during movement preparation. Facilitation appeared to begin about 150 ms prior to the RS and was very clear at +50 ms (MEP increased by 40-50%). This contrasts with the results in an equivalent auditory reaction task. The usual visuo-motor suppression observed at rest was absent 50 ms prior to the RS, but there was no clear facilitation of the MEP as in the visual task. We suggest that rapid visuomotor connectivity is suppressed during an auditory task but becomes facilitatory during a visual task, perhaps improving access of visual input to motor areas. It is unclear

why connectivity at ISI = 18 ms was unaffected in the visual reaction task. Nevertheless, the finding does confirm the conclusion that these two effects are mediated by quite separate pathways.

During the RT tasks, we saw no significant changes in the unconditioned MEP at the time intervals we studied. In some previous studies, the MEP has been suppressed in the interval between the WS and RS (Hasbroucq *et al.*, 1997; Touge *et al.*, 1998; Davranche *et al.*, 2007). However, suppression is best observed when the WS-RS interval is constant and subjects can anticipate precisely when the RS is about to be delivered (Touge et al., 1998). In the present task the timing of the RS was not predictable since it was randomised to come 550-650 ms after the WS. MEPs also are known to increase following the RS prior to onset of EMG. However the effect usually starts more than 50ms after the RS which was beyond the time range studied in the present experiments.

There was one slightly unexpected feature of the present results: the excitability of the occipito-motor connection was the same when it was tested with the eyes open or closed. Previous work had shown that transient removal of vision increases the amplitude of early components of the flash-evoked EEG potential (Cantello *et al.*, 2011), and we had initially anticipated that it might also increase the size of any effects we observed. However, the amplitude of the VEP may well be influenced by subcortical rather than cortical changes. For example, eye closure produces effects on retinal sensitivity which could affect the flash-evoked input without affecting the excitability of V1 to TMS. We propose that although ambient light levels may affect the excitability of inputs to visual cortex, they do not influence the excitability of the output elements activated by TMS. One study noted that blindfolding increases excitability of M1, as tested by its effect on the amplitude of TMS-evoked muscle twitches (Leon-Sarmiento *et al.*, 2005). The effect was larger after 30 min of blindfolding than immediately after eye closure. In the present experiments the eyes were only closed for a short period and we did not detect any change of RMT or baseline MEPs between open and closed eyes. We are less certain why the responses to conditioning stimuli of 80% and 90% PT were similar. It seems possible that this was due to a lack of statistical power, given the tendency for more inhibition to occur at 90% PT whether the eyes were open or closed.

Conclusions

Our findings support the existence of physiologically relevant occipitomotor connections, which can be activated by means of TMS. They may contribute to rapid integration of visual input into motor tasks as well as being involved in the spread of a seizure from visual to motor areas in certain types of visual epilepsy.

OVERACTIVE VISUOMOTOR CONNECTIONS UNDERLIE THE PHOTOPAROXYSMAL RESPONSE

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Abstract

Objective. The photoparoxysmal response (PPR) involves rapid spread of epileptic activity from visual to parietal and frontal areas. We used a transcranial magnetic stimulation (TMS) technique to assess the physiological connections between primary visual (V1) and motor (M1) areas in patients with idiopathic generalized epilepsy (IGE). We hypothesized that in PPR-positive patients, M1 would respond excessively to inputs from V1.

Methods. Eleven photosensitive patients with IGE who had a PPR at the time of the study were compared with 10 similar patients without a PPR, and with 11 healthy subjects of similar age and sex. The connection between V1 and M1 was assessed in resting participants by delivering a conditioning stimulus (CS) over the phosphene hotspot of the visual cortex (intensity 90% phosphene threshold, PT) followed at random interstimulus intervals (ISIs) (15, 18, 21, 24, 27, 30, 35 and 40 ms) by a test stimulus (TS) over the left motor cortex to elicit a motor evoked potential (MEP) of ~ 1 mV from the right FDI.

Results. In healthy subjects, a CS over V1 suppressed M1 at ISIs between 18 and 40 ms. Similar effects occurred in IGE patients without a PPR. This was not true in PPR-positive IGE patients, in whom this type of physiological inhibition was significantly ($p<0.05$) reduced.

Significance. IGE patients with a PPR have an overactive functional response of M1 to inputs travelling from V1. This may represent one core factor for the anterior spread of the PPR itself and for the origin of the abnormal epileptic motor phenomenon, such as myoclonus.

Introduction

Epileptic photosensitivity is an exaggerated neural response to visual stimuli. Its most elementary and common form is the photoparoxysmal response (PPR) to intermittent light stimulation (ILS). Among patients with epilepsy, a PPR is found in about 10–20% of children and 5–10% of adults, and it is more common among female patients at any age (Verrotti *et al.*, 2005). It is usually associated with idiopathic generalized epilepsies (IGE), notably juvenile myoclonic epilepsy (JME), and it is a diagnostic criterion of the syndrome of eyelid myoclonia with absences (EMA) (Striano *et al.*, 2009).

There are few studies of the pathophysiology of PPR. Defective inhibition in the visual system has been recently described as one background factor contributing to the PPR (Strigaro *et al.*, 2012). A second factor may be exaggerated transmission of visual inputs to other brain areas. For example, previous authors have described an exaggerated response of M1 to visual inputs that might have a pivotal role in spread of activity during the PPR (Artieda & Obeso, 1993; Verrotti *et al.*, 2005). We recently proposed a paired transcranial magnetic stimulation (TMS) method to study the time course of visual inputs on M1. Conditioning stimuli delivered to the occipital region suppressed M1 activity while the subject was at rest, while it reversed into facilitation in the context of a visuomotor reaction task (Strigaro *et al.*, 2015c).

The present study was designed to examine the excitability of these connections in epileptic patients with a PPR.

Materials and Methods

Methods were fully described in a previous paper (Strigaro *et al.*, 2015c). Briefly, all neurophysiologic studies took place between 2:00 and 6.30 p.m. in a quiet room, at a standard temperature of 22°C. Subjects lay comfortably in a dimly illuminated (=ca. 30 Lux) room.

Subjects

We studied a total of 21 adult patients with IGE referred to the Epilepsy Clinic of the University Department of Neurology, Novara, Italy: 11 had IGE with PPR (9 women, mean age 36.5 years, standard deviation [SD] 14.3) and 10 had IGE without PPR (7 women, mean age 35.1 years, SD 11.4). They were selected because their updated profile, in the Clinic database, included the terms “idiopathic generalized epilepsy” with or without “photosensitivity” and “PPR”. Eleven normal subjects of similar age and sex acted as controls

(6 women, mean age 34.2 years; SD 8.5). They had no family or personal history of neurologic disease or epilepsy. Reportedly, both patients and controls had not been taking neuroactive drugs (alcohol and caffeine included) for 72 h prior to the study, except for the patient antiepileptic treatment. Their general, neurological and ophthalmological examinations were normal. All subjects were right-handed based on the Edinburgh Handedness Inventory and gave written informed consent. Experiments were approved by the local Ethics Committee and were performed in accordance with the Declaration of Helsinki.

Patient features

These are reported in Table 1. Eleven of the 21 patients were classified as photosensitive (IGE+PPR) because they showed a PPR to ILS, which did never entail clinical phenomena. Of this group, seven patients had a definite diagnosis of juvenile myoclonic epilepsy (JME) and one had a diagnosis of EMA, whereas we labelled the remaining three as (undefined) "photosensitive IGE". Ten of the 21 patients were not photosensitive (IGE-PPR), and just three of them had a definite diagnosis of JME. In general, their clinical course was favourable, since 19 of the 21 patients reported being seizure-free, whereas one patient from each group still had isolated myoclonia exclusively upon awakening (n. 10 and 17). All were on a standard antiepileptic treatment. Valproate, alone or in combination with levetiracetam, was the most frequent choice.

Table 1

MAIN FEATURES OF THE PATIENTS								
						PPR		
Patient #	**Age**	**Sex**	**Diagnosis**	**Seizure frequency**	**Current Treatment (mg/die)**	**PPR type (1-4)**	**Eye state**	**Range (Hz)**
1	42	F	EMA	Free	VPA 1000 PB 100	3	EC	1-60
2	26	F	JME	Free	LTG 400	2-3	EC	12-25
3	71	F	Photosensitive IGE	Free	LEV 1000	3	EC	8-20
4	28	M	JME	Free	VPA 300	2	EC	18
5	48	F	JME	Free	VPA 1300 LEV 1000	2	EC	8-20
6	38	F	Photosensitive IGE	Free	PB 115	4	EC>EO	1-25
7	21	F	JME	Free	VPA 800	2	EO>EC	6-25
8	22	F	JME	Free	LTG 100	4	EC	8-20
9	37	F	JME	Free	VPA 600	2	EC	16-20
10	30	M	JME	Daily M	TPM 100	4	EC>EO	12-25
11	38	F	Photosensitive IGE	Free	TPM 200	4	EC>EO	3-25
12	26	M	IGE	Free	OXC 1200	No		
13	29	M	IGE	Free	VPA 900	“		
14	49	F	IGE	Free	OXC 800	“		
15	39	M	JME	Free	LTG 300 PB 50 ESM 500	“		
16	54	F	IGE	Free	VPA 1000	“		
17	24	F	JME	Occasional M	LTG 400	“		
18	29	F	JME	Free	LEV 2000 VPA 1000	“		
19	36	F	IGE	Free	LEV 1000 LTG 300 PB 25	“		
20	45	F	IGE	Free	VPA 800	“		
21	20	F	IGE	Free	LEV 2500	“		

EC: eyes closed; EMA: eyelid myoclonia with absences; EO: eyes open; IGE: idiopathic generalized epilepsy; JME: juvenile myoclonic epilepsy; M, myoclonic seizure; ESM: ethosuximide; LEV: levetiracetam; LTG: lamotrigine; OXC: oxcarbazepine; PB: phenobarbital; PPR: photoparoxysmal response; TPM: topiramate; VPA: valproic acid.

Video-EEG recording

Prior to all experiments, the candidate subjects underwent a 30-min, 24-channel routine video-EEG recording without sleep deprivation. ILS was performed according to standardized methodology (Rubboli *et al.*, 2004) using a Nihon-Kohden 4421K flash stimulator (Nihon-Kohden Co., Tokyo, Japan). The distance between the stimulator and the patient nasion was 30 cm. Ten-second trains of flashes were delivered for each frequency, at intervals of ≥ 7 s. Eyes were open for the first 5 s, fixating at the centre of the lamp. Then the subject was asked to close his or her eyes and remain in the eyes-closed condition for the subsequent 5 s of stimulation. We delivered ILS at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, and 20 Hz. If a generalized epileptiform discharge occurred, the procedure was stopped. A second sequence with frequencies of 60, 50, 40, 30, and 20 Hz was then delivered with the same precaution. A subsequent analysis was carried out independently by two routine EEG readers, to assess the PPR subtype (Waltz *et al.*, 1992) and any clinical correlate of the PPR itself (Table 1).

TMS

For paired-TMS we used two high-power Magstim 200^2 machines (Magstim, Whitland, UK). The magnetic stimulus had a nearly monophasic pulse configuration with a rise time of ~ 100 μs, decaying back to zero over ~ 0.8 μs. The stimulators were connected to a figure-of-eight coil (outer winding diameter 70 mm).

Test stimuli

Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous (FDI) muscle using 9 mm-diameter Ag-AgCl surface cup electrodes, in a typical belly-tendon montage. Responses were amplified by a CED 1402 isolated amplifier (CED, Cambridge, UK). Filters were 20 Hz - 3 kHz, and the sampling rate was 10 kHz. The signal was then recorded by a PC using Signal software ver. 4.08 (Cambridge Electronic Devices, Cambridge,

UK). The test coil was placed tangentially to the scalp at a 45° angle to the midline, to induce a posterior-anterior (PA) current flow across the central sulcus (Figure 1). The hand motor area of the left primary motor cortex (M1) was defined as the point where stimulation consistently evoked the largest MEP. We defined the resting motor threshold (RMT) as the lowest intensity that evoked 5 small responses (~50 μV) in the relaxed FDI muscle in a series of 10 stimuli (Rossini *et al.*, 1994). The intensity of the test stimulus (TS) was finally adjusted to evoke a MEP of ~ 1 mV peak-to-peak amplitude in the relaxed right FDI.

Experimental procedure

Paired-TMS stimulation was conducted as follows (Figure 1). The TS was preceded at random interstimulus intervals (ISIs) by a conditioning stimulus (CS). Fifteen responses were collected for TS and 12 responses for CS plus TS. There was a 5 s (±20%) intertrial interval. For each trial we measured the average peak-to-peak MEP amplitude. The conditioned MEP was expressed as a percentage of the unconditioned MEP size. The centre of the conditioning coil was placed over the phosphene hot spot, which was located according to the method of Stewart et al. (2001). Likewise, the phosphene threshold (PT) was determined (Stewart *et al.*, 2001). Subjects wore a blindfold and a cap whilst seated in a chair. Three points were marked over the occipital midline 2, 3 and 4 cm above the inion. The coil handle pointed upwards and was parallel to the subject's spine. The coil centre was first positioned 2 cm above the inion, then moved anteriorly across the marks, to determine the best site to elicit phosphenes ("hot spot") (Figure 1). Stimuli were initially applied at 60% of the stimulator output and at a maximum frequency of 0.2 Hz. The subject was asked about the presence of phosphenes immediately after each pulse. If a phosphene was reported 5 or more times out of 10, the pulse intensity was reduced by steps of 5%, then stimuli were repeated another 10 times. This protocol progressed until no phosphene was reported. The minimum intensity at which the subject perceived a phosphene 5 times out of 10 was the PT. If the initial intensity of 60% was ineffective, it was increased by steps of 5% maximum power, till phosphenes appeared.

The intensity of the CS was adjusted to be 90% PT. ISIs were 15, 18, 21, 24, 27, 30, 35 and 40 ms. There were two sessions: one with eyes open and another with eyes closed.

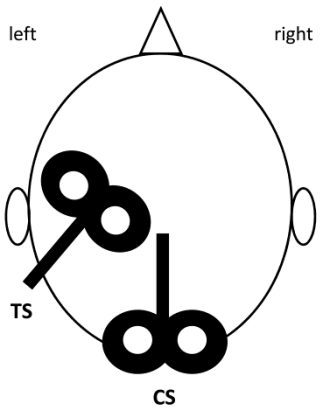

Figure 1

Experimental setting of the study. Coil positions over a skull sketch. TS, test stimulus, delivered over the hand motor area of the left M1. CS, conditioning stimulus, delivered over the phosphene hotspot of the visual cortex.

Data analysis

All data were expressed as mean ± standard error of the mean (SEM). Student's paired *t* tests (two-tailed) were used to compare mean RMT with eyes open and closed obtained from all the participants. Spearman's rho was applied to study the correlation between motor and phosphene threshold.

A preliminary three-way repeated-measures (rm) ANOVA analysed the effects of "ISI", "eye state" (within-subjects factors) and "group" (between-subjects factor). Separate one-way ANOVAs were used for further analyses where necessary. A significant main effect in these ANOVAs was followed by Bonferroni's *post hoc* tests. Mauchley's test was used to examine for sphericity. The Greenhouse-Geisser correction was used for nonspherical data. A p value < 0.05 was considered significant. Data were analysed using software (SPSS v. 19.0 for Windows; SPSS Inc.).

Results

Baseline physiological measures and *p* values are shown in Table 2. Briefly, no significant differences in the test MEP amplitude and PT were detected between the groups. A significantly higher RMT was detected in both groups of patients compared to HS ($p < 0.05$).

Table 2

DEMOGRAPHIC AND BASELINE TMS FEATURES				
	HS	**IGE-PPR**	**IGE+PPR**	**Differences among groups**
#	11	10	11	
Age	34.2 ± 8.5	35.1 ± 11.4	36.5 ± 14.3	n.s.
Sex (women)	6	7	9	n.s.
RMT	39.5 ± 6.7	57.4 ± 9.4	51.2 ± 12.5	IGE+PPR > HS (p = 0.018) IGE-PPR > HS (p = 0.001)
SI_{1mV}	49.2 ± 11.0	69.2 ± 12.6	62.6 ± 15.2	n.s.
Test MEP (mV) (EO)	1.19 ± 0.35	1.29 ± 0.53	1.27 ± 0.36	n.s.
Test MEP (mV) (EC)	1.04 ± 0.26	1.07 ± 0.42	1.27 ± 0.24	n.s.
PT	65.2 ± 8.0	71.3 ± 7.0	62.8 ± 13.5	n.s.
90% PT	58.5 ± 7.3	64.3 ± 6.5	56.6 ±12.2	n.s.

EO: eyes open; EC: eyes closed; HS: healthy subjects; IGE: idiopathic generalized epilepsy; ±PPR: ±Photoparoxysmal response; PT: phosphene threshold; RMT: resting motor threshold; SI_{1mV}: intensity required to elicit a 1 mV MEP; n.s.: non-significant.

A preliminary three-way rmANOVA using absolute values revealed significant effects of "ISI" ($F(8, 232) = 6.973$, $p < 0.001$), "group" ($F(2, 29) = 4.975$, $p = 0.014$) and "ISI" x "group" interaction ($F(16, 232) = 2.495$, $p = 0.002$). There was no "eye state" x "group" interaction ($F(2, 29) = 0.136$, $p = 0.874$) (Figure 2). Thus there was a difference in the effect of ISI between the groups, as well as overall differences between groups. The "group" effect was due to the fact that there was less suppression in the IGE+PPR than the other two groups (Bonferroni *post hoc* analysis gave a significant difference between the IGE+PPR group and both the IGE-PPR group ($p = 0.046$) and the healthy controls ($p = 0.024$)). To rule out a confounding effect of seizures, two patients (n. 10 and 17) have been excluded and a subsequent three-way rmANOVA confirmed significant effects of "ISI" ($F(8, 216) = 5.511$, p

< 0.001), “group” (F (2, 27) = 5.209, p = 0.012), “ISI” x “group” interaction (F (16, 216) = 2.513, p = 0.001) and no “eye state” x “group” interaction (F (2, 27) = 0.261, p = 0.772).

Analysis of the “ISI” x “group” interaction was performed after normalising the data to baseline values for all the participants. In general there was less suppression at around 18 and 40 ms in the IGE+PPR group than in the other groups. In the eyes open state, a *post hoc* Bonferroni analysis confirmed a significant difference at ISI 18 and ISI 40 ms between the IGE+PPR and the other two groups (IGE+PPR vs HS: $p = 0.002$ at ISI 18 and $p = 0.015$ at ISI 40 ms; IGE+PPR vs IGE-PPR: $p = 0.008$ at ISI 18 and $p = 0.001$ at ISI 40 ms) (Figure 2-3). Moreover, significant differences were detected at ISI 21 ms between the IGE+PPR and HS groups ($p = 0.033$) and at ISI 35 ms between the IGE+PPR and IGE-PPR patients ($p = 0.040$).

In the “eyes closed” state, there was a significant difference at ISI 21 ms between the IGE+PPR and IGE-PPR patients ($p = 0.011$) and at ISI 40 ms between the IGE+PPR and HS groups ($p = 0.002$) (Figure 2).

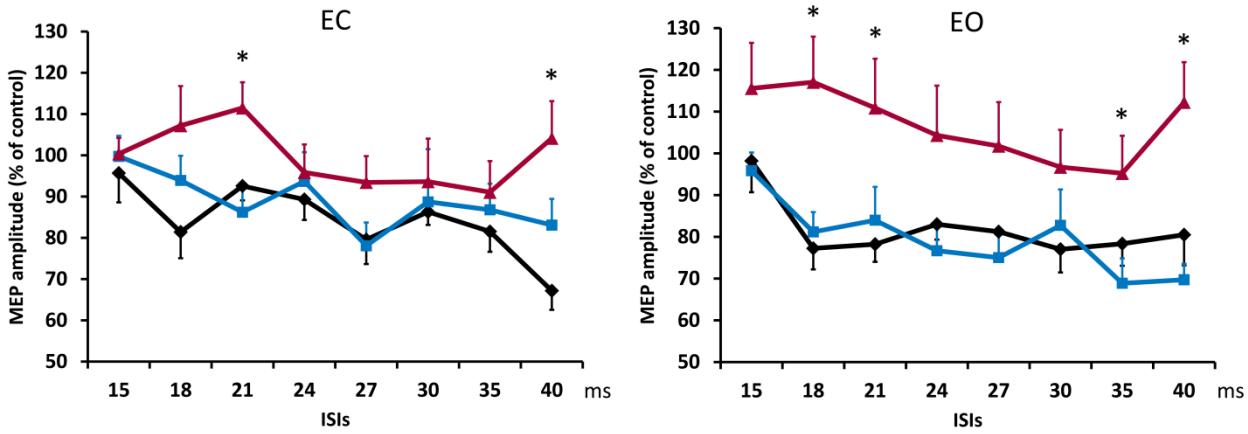

Figure 2

Effects of a conditioning stimulus (CS, 90% phosphene threshold), at different eye states (EC, eyes closed; EO, eyes open) on the test MEP, in subjects at rest. Red line: IGE+PPR patients. Blue line: IGE-PPR patients. Black line: healthy subjects. Amplitude of MEPs (mV) is normalized and expressed as a percentage of control. Errors bars indicate SEM. Asterisks indicate a p value < 0.05 on separate ANOVAs exploring ISIs.

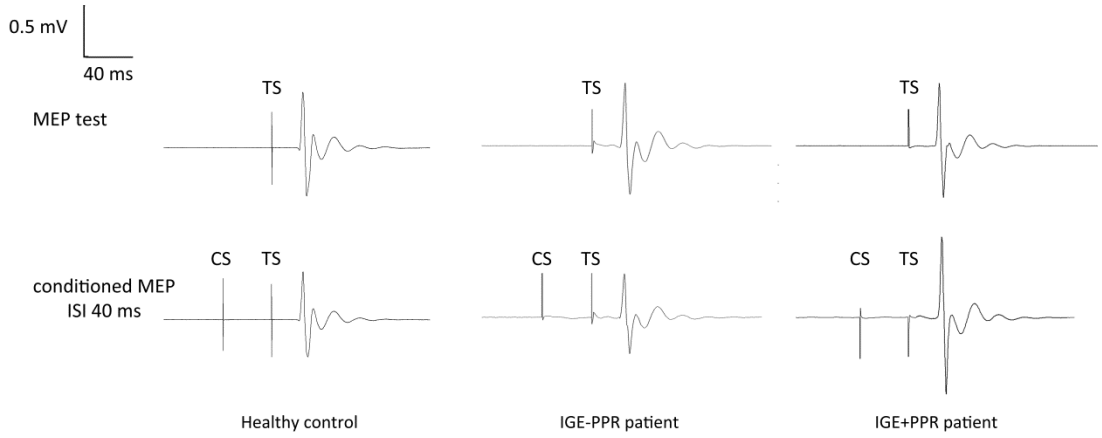

Figure 3

Typical example of changes in the MEP (grand-average of the recorded trials) with an ISI = 40 ms and with eyes open. A representative healthy control, a patient with IGE-PPR and a patient with IGE+PPR are depicted. In the IGE+PPR patient, a clear MEP increase can be seen at ISI 40 ms.

Discussion

The present study examined the functional connection linking visual to the motor areas using a paired pulse TMS method (Strigaro *et al.*, 2015c). We found that patients suffering from IGE who had a PPR at the time of the study did not show the usual suppression from V1 to M1 at rest, even though this was present in healthy individuals and in patients with IGE but without a PPR.

Paired pulse testing of V1-M1 connectivity

The paired TMS technique allows investigation of time-related changes in functional connectivity between M1 and other cortical areas (Ferbert *et al.*, 1992; Ugawa *et al.*, 1995; Civardi *et al.*, 2001; Koch *et al.*, 2007a; Rothwell, 2011). A CS is first used to activate the area of interest, while a TS, given at different times afterward, evokes a test MEP in contralateral hand muscles to probe the excitability of motor cortical projections. If the CS changes the amplitude of the test MEP, then there is an influence of the conditioning site on the motor cortex (Rothwell, 2011).

Paired TMS was recently employed to study the interaction between visual and motor cortex (Strigaro *et al.*, 2015c). The CS was applied over the optimal point to elicit stationary

phosphenes (Afra *et al.*, 1998; Stewart *et al.*, 2001; Franca *et al.*, 2006) and an intensity below PT was used. In healthy individuals this suppresses excitability of M1, particularly at ISIs of 18 and 40 ms. The inhibition is present at both 80% and 90% PT but is not influenced by whether the eyes are open or closed (Strigaro *et al.*, 2015c).The effect at ISI 40 ms (but not at 18 ms), is abolished just prior to movement in auditory or visual reaction time tasks; it even reverses into facilitation in a visuomotor reaction task (Strigaro *et al.*, 2015c). Thus the functional connection between V1 and M1 appears to be controllable: at rest when individuals are explicitly instructed to refrain from movement, the effect is inhibitory and could therefore reinforce a state of rest; when movement occurs, the connectivity can become excitatory, particularly if vision is actively being used to control the movement. The earliest TMS effect at rest (ISI 18 ms) is compatible with the data on flash evoked suppression of MEPs noted at 55–70 ms after a flash (Cantello *et al.*, 2000b) whereas the later phase (ISI 40 ms) with the earliest signs of visual effect on motor cortex excitability described in behavioural studies (Makin *et al.*, 2009).

In the present experiments, the CS intensity was set at 90% PT, a value which was previously shown to be the most effective (Strigaro *et al.*, 2015c). We hypothesized that, in IGE patients with a PPR, this form of neural inhibition might be deficient in the resting state. The consequence would be that activity in V1 would be much more likely to influence distant areas than in healthy individuals, and could contribute to the spread of the PPR. We do not know whether the abnormal excitability of V1-M1 connections was representative of all visuo-frontal connections. Indeed most of the patients with PPR in the present study had a diagnosis of JME, which may have a particular involvement of M1. However, it may be that V1 access to all frontal areas is controlled in ways that depend on the function of the linked areas. Failure to control connectivity in the resting state would increase the probability that V1 activity could propagate widely to frontal cortex as in the PPR.

Abnormal visuomotor connectivity in photosensitivity

The fact that there was significantly less visuo-motor suppression in the PPR-positive group than in the healthy controls or the PPR-negative group implies that the defect was associated with the photosensitivity. The question is where does the deficit lie, is it in the visual cortex or in the connections between V1 and other cortical areas? We adjusted the intensity of the CS to be 90% PT to try to ensure that it engaged the same amount of V1 output to networks involved in perception in all groups. Thus we think that the increased effect that it had on M1 in patients with PPR was due to increased access of input from V1 to

motor networks in M1. This may involve mechanisms similar to those responsible for the task-related changes in processing of visual input to motor cortex described in healthy individuals (Strigaro *et al.*, 2015c). In this case, interactions of visual stimuli with the GABAa-dependent phenomenon of short-interval intracortical inhibition (SICI) (Ziemann *et al.*, 1996a) suggest that the effect of V1 input on M1 is controlled by local circuits in M1. It is possible therefore that such control mechanisms are inadequate in patients with PPR and contribute to the abnormal spread of activity from V1.

It should be noted that in healthy individuals, task dependent control of V1-M1 interactions has only been demonstrated for the functional connection at ISI = 40ms but not at ISI = 18ms. This could mean that in patients, spread of the PPR from V1 depends on the particular connection studied at ISI = 40ms. However, when we probed the effectiveness of V1-M1 connections, we observed reduced inhibition at all ISIs including ISI = 18ms which implies a more widespread abnormality in several functional pathways. One possibility is that the V1-M1 input at 18ms is controlled by similar mechanisms to that at ISI = 40ms, but that we have yet to probe the connection in an appropriate task. If so then a general deficit in such systems, may underlie the reduced inhibition we observed. Indeed, in patients with photic reflex myoclonus in whom light flashes can evoke a generalised myoclonic jerk, Artieda and Obeso (1993) (Artieda & Obeso, 1993) found that input from V1 could reach motor cortex very rapidly: TMS over the occiput during 1 Hz flash stimuli (to increase visuo-motor excitability) provoked a muscle twitch some 7 ms later than direct TMS over M1.

The anatomic pathways mediating visuomotor connectivity are unknown. The dorsal visual stream, via parietal and premotor cortex, could provide one indirect connection. However, a rapid transmission of visual information to the frontal regions might be provided by the inferior fronto-occipital fascicle (IFOF), a long associative bundle connecting the occipital to the frontal lobe (Martino *et al.*, 2010). This tallies the current understanding of the pathophysiology of epilepsy, which relies on the "system hypothesis". This concept goes somewhat beyond the classical dichotomy between focal and generalized epilepsy, implying that some types of epilepsy may depend on the susceptibility to epileptogenic factors within any of the interconnected structures of a specific brain system (Avanzini *et al.*, 2012).

Combining pathological connectivity with pathophysiology in V1

Altered excitability in V1-frontal connections at rest is not the only factor that contributes to the PPR. A second factor is likely to be hyperexcitability of the visual cortex in response to the flickering light (Harding & Fylan, 1999; Porciatti *et al.*, 2000; Wilkins *et al.*,

2004; Verrotti *et al.*, 2005). Paired VEP studies (Cantello *et al.*, 2011) show that this relies on a mechanism of defective inhibition in the visual system, whose time course would fit some of the most "activating" ILS frequencies, such as 16 and 20 Hz (Strigaro *et al.*, 2012). We suggest that this abnormal V1 excitability, coupled with defective control over V1 inputs to frontal cortex produces the PPR. Thus the paradoxical facilitation of EEG activity evoked over central area which is seen in PPR-positive patients with the paired VEP technique would be due to a combination of reduced inhibition between stimuli in V1 coupled with excess spread of activation to frontal central areas (Strigaro *et al.*, 2012). Finally it should be noted that compensatory mechanisms could also co-exist that attempt to offset spread of activation. For example, in healthy volunteers, ILS shortens the duration of the cortical silent period (Cantello *et al.*, 1992) whereas there is no effect in epileptic patients with PPR (Groppa *et al.*, 2008; Strigaro *et al.*, 2013). This may imply an enhancement of the GABAb-ergic inhibition in the motor cortex of patients (Ziemann *et al.*, 1996a).

Effect of antiepileptic medication

Medication was a main limitation of the present study because most of the patients were undergoing a successful antiepileptic treatment. Only in two patients, equally distributed in the two groups, there was a suboptimal control of the seizures with residual isolated myoclonia. To rule out a confounding effect of seizures, these patients have been later excluded from the statistical analysis and no significant interference with the results has been revealed.

Both patient groups had a significantly higher baseline RMT which presumably results from concurrent antiepileptic medication (Reutens *et al.*, 1993; Cantello *et al.*, 2006; Strigaro *et al.*, 2011; Badawy *et al.*, 2014). However, in contrast to previous results (Brigo *et al.*, 2013) showing higher PT thresholds in treated epileptic patients, we found similar PT in patients and controls. It is likely that this apparent difference is due to a statistical sampling problem: indeed in the present study there was a tendency for PPR-negative patients to have a higher PT threshold than healthy volunteers whereas the PPR-positive patients had the same or lower PTs. Since we found similar unconditioned MEP amplitudes and CS intensity in controls, PPR-positive and PPR-negative patients, the altered connectivity in PPR-positive patients unlikely reflect the influence of chronic antiepileptic treatment. Most importantly, we studied the PPR that remained under treatment and we favour the view that our findings were residual to the depressing action of the drugs. A group of drug naïve patients would possibly have

expressed even more evident changes. However, obvious ethical constraints hindered the recruitment of an adequate patient sample.

Conclusions

An overactive functional connection between the primary visual and primary motor cortex, as studied by TMS, may contribute to the pathogenesis of PPR, a key feature of photosensitive idiopathic epilepsies. An excess response of M1 to visual inputs may underlie the fast spread of epileptic activity from posterior to anterior areas of the brain and the origin of the abnormal epileptic motor phenomenon, such as myoclonus.

DEFECTIVE INTERHEMISPHERIC INHIBITION IN DRUG-TREATED FOCAL EPILEPSIES

Strigaro G, Matino E, Falletta L, Pizzamiglio C, Tondo G, Badawy R, Cantello R.
Brain Stimul. 2016 Dec 9. pii: S1935-861X(16)30384-9.

Abstract

Background. Focal epilepsies (FEs) arise from a lateralized network, while in generalized epilepsies (GEs) there is a bilateral involvement from the outset. Intuitively, the corpus callosum is the anatomical substrate for interhemispheric spread.

Objective. We used transcranial magnetic stimulation (TMS) to explore whether there are any physiological differences in the corpus callosum of drug-treated patients with FE and those with genetic GE (GGE), compared to healthy subjects (HS).

Methods. TMS was used to measure the interhemispheric inhibition (IHI) from right-to-left primary motor cortex (M1) and viceversa in 16 patients with FE, 17 patients with GGE and 17 HS. A conditioning stimulus (CS) was given to one M1 10 and 50 ms before a test stimulus delivered to the contralateral M1. Motor evoked potentials (MEPs) were analysed both as a function of the side of stimulation and of the epileptic focus (left-right).

Results. In HS, IHI was reproducible with suppression of MEPs at ISIs of 10 and 50 ms. Similar effects occurred in GGE patients. FE patients behaved differently, since IHI was significantly reduced bilaterally. When FE patients were stratified according to the side of their epileptic focus, the long-ISI IHI (=50 ms) appeared to be defective only when the CS was applied over the "focal" hemisphere.

Conclusions. FE patients had a defective inhibitory response of contralateral M1 to inputs travelling from the "focal" hemisphere that was residual to the drug action. Whilst IHI changes would not be crucial for the GGE pathophysiology, they may represent one key factor for the contralateral spread of focal discharges, and seizure generalization.

Introduction

Epilepsy is a common neurological disorder characterized by an enduring predisposition to generate epileptic seizures (Fisher *et al.*, 2014). Its pathophysiology is complex and largely related to hyperexcitable neural networks resulting from the imbalance between excitatory and inhibitory circuits (Badawy *et al.*, 2014). The classical dichotomy in focal (FE) and generalized epilepsy (GE) reflects the origin of the epileptic discharge, whether it arises in a lateralized network or it rapidly involves bilateral structures.

Abnormalities in both excitatory and inhibitory neural circuits not only affect the seizure focus, but may also involve distant areas such as the primary motor cortex (M1) (Cantello *et al.*, 2000a; Varrasi *et al.*, 2004; Hamer *et al.*, 2005). White-matter bundles connecting distant cortical areas are the likely anatomical substrate of seizure propagation (Miro *et al.*, 2015). Of these, the corpus callosum represents the largest commissure connecting the two hemispheres (Wahl *et al.*, 2007). Its major role in seizure propagation is suggested by the efficacy of the palliative corpus callosotomy procedure in severe drug-resistant epilepsies (Asadi-Pooya *et al.*, 2008). Previous neuroimaging and anatomical studies have explored the role of corpus callosum in interhemispheric propagation (O'Dwyer *et al.*, 2010; Walker *et al.*, 2012). However, its physiological role in FE and GE is still a matter of debate (Lappchen *et al.*, 2011; Miro *et al.*, 2015). Changes in cortical excitability in the hemisphere ipsilateral and contralateral to the seizure focus (i.e. “focal” and “non-focal” hemisphere respectively) may well be a background factor for the propagation of the epileptic discharge, and may distinguish FE from GE (Badawy *et al.*, 2007). A second factor may be an exaggerated interhemispheric transmission/defective inhibition through the corpus callosum. Interhemispheric inhibition (IHI) by means of paired pulse transcranial magnetic stimulation (TMS) was first described by Ferbert et al. (Ferbert *et al.*, 1992). This paradigm employs a standard single TMS stimulus over the hand area of M1 that evokes a test motor evoked potential (MEP) in a muscle of interest. This stimulus can be preceded at different intervals by a conditioning stimulus (CS) over the hand area of the opposite hemisphere (Rothwell, 2011). The CS changes the amplitude of the test MEP at critical intervals with an “inter-hemispheric” inhibition with a latency of 6–50 ms (Ferbert *et al.*, 1992; Di Lazzaro *et al.*, 1999; Daskalakis *et al.*, 2002; Chen, 2004). IHI is mediated by transcallosal fibers since the effects were absent in patients with no corpus callosum (Meyer *et al.*, 1995). This method was subsequently validated by several studies in the normal subject (Meyer *et al.*, 1998; Di Lazzaro *et al.*, 1999; Lee *et al.*, 2007) and patients with different neurological abnormalities (Boroojerdi *et al.*, 1996; Hanajima *et al.*, 2001; Murase *et al.*, 2004; Li *et al.*, 2007; Lappchen

et al., 2011), including one describing the changes between the M1s following the removal of the epileptic focus in FE (Lappchen *et al.*, 2011).

The present study was designed to examine the excitability of bilateral M1-to-M1 interhemispheric connections in patients with FE and genetic GE (GGE) compared to healthy subjects (HS). In principle, we hypothesized that IHI would be defective in FE patients, particularly that the "focal" hemisphere would respond excessively to inputs from the "non-focal hemisphere".

Materials and Methods

All neurophysiologic studies took place between 2:00 and 6.30 p.m. in a quiet room, at a standard temperature of 22°C.

Subjects

We studied a total of 33 adult patients with epilepsy referred to the Epilepsy Clinic of the University Department of Neurology, Novara, Italy. Sixteen had FE (10 women, mean age 36.4 years ± 9.3) and 17 had GGE (11 women, mean age 34.2 years ± 12.5). Seventeen normal subjects of similar age and sex acted as controls (11 women, mean age 30.1 years ± 7.9). They had no family or personal history of neurologic disease or epilepsy. Apart from the regular antiepileptic medication taken by the patients, both patients and controls had not been on neuroactive drugs (alcohol and caffeine included) for 72 h prior to the study. Their general and neurological examinations were normal. All subjects were right-handed based on the Edinburgh Handedness Inventory. They gave written informed consent to the experimental procedures, which were approved by the local Ethics Committee and were performed in accordance with the Declaration of Helsinki.

Patient features

These are reported in Table 1. Thirteen of the 16 patients with FE had temporal lobe epilepsy (TLE) and 3 frontal lobe epilepsy (FLE). Magnetic resonance imaging (MRI) revealed a brain lesion in 12 out of the 16 patients (Table 1). No abnormalities of the corpus callosum have been detected. On the basis of ictal Video-EEG recordings, 8 out of the 16 patients had a definite left epileptic focus, whilst the remaining 8 were diagnosed with a right focus. Nine were seizure-free and the remainder had only focal seizures without secondary generalization in the last one year of observation. Eight patients have experienced secondary

generalization in the past, 7 became seizure-free and 1 had residual focal motor seizures. All of them were taking one or multiple antiepileptic drugs (AEDs); carbamazepine and levetiracetam were the most commonly used drugs.

In the GGE group, the most common subtypes were juvenile myoclonic epilepsy (JME) (6/17 patients), epilepsy with tonic-clonic seizures (TCE) (6/17 patients) and juvenile absence epilepsy (JAE) (4/17 patients). One patient had eyelid myoclonia with absences (EMA). All of them were seizure-free on AEDs, the most common of which were valproate and lamotrigine.

Table 1

MAIN FEATURES OF THE PATIENTS WITH FOCAL EPILEPSY										
Patient #	**Age**	**Sex**	**Diagnosis**	**Epileptogenic focus**	**Seizure type**	**Seizure frequency***	**Current Treatment (mg/die)**	**Serum levels**	**Ictal onset location/side**	**Neuroimaging**
1	46	M	TLE	R	CF	3-4/month	CBZ 600 LTG 250 LEV 1000	CBZ 7,9 ug/L LTG 1,8 mg/L LEV 3,5 mg/L	Temporal/right	Previous right temporo-parietal hematoma
2	36	F	TLE	R	CF, SG	Free	PB 100 VPA 1250	PB 16,5 ug/L VPA 53 ug/ml	Temporal/right	-
3	56	M	TLE	R	CF, SG	Free	CBZ 400	CBZ 4,2 ug/L	Frontotemporal /right	Right temporo-parietal ischaemia
4	47	F	FLE	L	SF (jerks right upper limb)	3-4/month	LTG 450 CBZ 800	LTG 5,3 mg/L CBZ 8 ug/ml	Frontotemporal /left	Left frontal cortical dysplasia
5	27	M	FLE	R	SF (jerks left upper limb), SG	2-3 SF/day	CBZ 800 LEV 3000	CBZ 6 ug/ml	Frontal/right	Right frontal cortical dysplasia
6	30	F	TLE	L	CF	1-2/month	LEV 2000 TPM 300 CLB 15	LEV 26 mg/L TPM 12,8 ug/ml	Frontotemporal /left	Diffuse cortical heterotopias
7	26	F	FLE	L	CF, vocalization	Free	CBZ 700	CBZ 9,7ug/ml	Frontotemporal /left	Cortical heterotopias
8	33	M	TLE	R	CF, SG	Free	LEV 1500 CBZ 400	CBZ 6,8 ug/ml LEV 24,6 mg/L	Frontotemporal /right	-
9	44	M	TLE	L	CF, automatisms	3/month	CBZ 1000 LEV 2500	CBZ= 11 ug/ml	Temporal/left	Left parahippocampal gangliocytoma

							TPM 200			
10	43	F	TLE	R	CF, automatisms, SG	Free	LEV 3000 LTG 300 PB 100	PB 20,6 ug/ml LTG 3,6 mg/L LEV 47,4 mg/L	Frontotemporal /right	-
11	23	F	TLE	L	CF	Free	CBZ 500	CBZ 7 ug/ml	Frontotemporal /left	Previous left fronto-temporal *encephalitis*
12	43	M	TLE	R	GTC	Free	CBZ 800	CBZ 6,5 ug/ml	Frontotemporal /right	Right temporal cyst
13	28	F	TLE	L	CF, SG	Free	CBZ 600	CBZ 5.8 ug/ml	Temporal/left	Left temporal cortical dysplasia
14	35	F	TLE	R	CF	2-3/month	CBZ 1200 TPM 300	CBZ 8.7 ug/ml TPM 3.9 ug/ml	Temporal/right	Abnormal gyration of the right upper temporal gyrus
15	29	F	TLE	L	CF	1-2/day	LEV 3000 LCM 200	-	Temporal/left	-
16	37	F	TLE	L	CF, SG	Free	LTG 200 LEV 1000 LCM 400	LTG 3.1 mg/L LEV 11.5 mg/L LCM 10.6	Temporal/left	Left mesial hippocampal sclerosis

MAIN FEATURES OF THE PATIENTS WITH GENETIC GENERALIZED EPILEPSY										
Patient #	**Age**	**Sex**	**Diagnosis**	**Epileptogenic focus**	**Seizure type**	**Seizure frequency**	**Current Treatment (mg/die)**	**Serum levels**	**EEG**	**Neuroimaging**
1	21	M	JAE	-	GTC, absences	Free	VPA 800	VPA 88 ug/ml	Normal	Normal
2	45	F	JME	-	GTC, absences, My	Free	VPA 1600 TPM 200	VPA 106 ug/ml TPM 6,4 ug/ml	-	-
3	41	M	TCE	-	GTC	Free	VPA 1500	VPA 80 ug/ml	-	-
4	55	F	JAE	-	GTC, absences	Free	LTG 400 LEV 1500	LEV=24,3 mg/L LTG=11,4 ug/ml	-	-

							ESM 500			
5	28	F	JME	-	GTC, My	Free	LTG 200	LTG 7,2 ug/ml	-	-
6	50	F	JAE	-	GTC, absences	Free	CBZ 800	CBZ 8,4 ug/ml	-	-
7	31	M	JAE	-	GTC, absences	Free	VPA 1500	VPA 77 ug/ml	-	-
8	44	F	EMA	-	GTC, absences, palpebral myoclonus	Free	LEV 2000	LEV 14,6 mg/L	-	-
9	24	F	JME	-	GTC, My	Free	LTG 100	-	-	-
10	55	F	JME	-	GTC, My	Free	PB 200 LEV 1000	PB 28 ug/ml LEV 17,7 mg/L	-	-
11	35	F	TCE	-	GTC	Free	CBZ 600	CBZ 6,5 ug/ml	-	-
12	24	F	TCE	-	GTC	Free	LTG 150	LTG 4,3 ug/ml	-	-
13	21	F	TCE	-	GTC	Free	VPA 800	-	-	-
14	21	M	TCE	-	GTC	Free	VPA 750	VPA 70 ug/ml	-	-
15	42	M	JME	-	My, absences	Free	LTG 300 ESM 500	LTG 4,1 ug/ml ESM 28 ug/ml	-	-
16	25	F	JME	-	GTC, My	Free	VPA 800	VPA 57 ug/ml	-	-
17	20	M	TCE	-	GTC	Free	VPA 1000	VPA 77 ug/ml	-	-

CF: complex focal seizure; CBZ: carbamazepine; CLB: clobazam; EMA: eyelid myoclonia with absences; ESM: ethosuximide; FLE: frontal lobe epilepsy; GTC: generalized tonic–clonic seizure; JAE: juvenile absence epilepsy; JME: juvenile myoclonic epilepsy; LCM: lacosamide; L: left; LEV: levetiracetam; LTG: lamotrigine; My, myoclonic seizure; PB: phenobarbital; R: right; SF: simple focal seizure; SG: secondary generalized tonic–clonic seizure; TCE: epilepsy with tonic-clonic seizures; TLE: temporal lobe epilepsy; TPM: topiramate; VPA: valproic acid.

*Average number of seizures per month in the 3 months preceding and the 3 months subsequent to the experimental session

TMS

For paired-TMS we used two high-power Magstim 200^2 machines (Magstim, Whitland, UK). The magnetic stimulus had a nearly monophasic pulse configuration with a rise time of ~ 100 µs, decaying back to zero over ~ 0.8 ms (Koch *et al.*, 2007b). The stimulators were connected to a figure-of-eight coil (outer winding diameter 70 mm).

Test stimuli

Motor evoked potentials (MEPs) were recorded from the left and right first dorsal interosseous (FDI) muscles (Ferbert *et al.*, 1992; Avanzino *et al.*, 2007), using 9 mm-diameter Ag-AgCl surface cup electrodes, in a typical belly-tendon montage. Responses were amplified by a CED 1402 isolated amplifier (CED, Cambridge, UK). Filters were 20 Hz - 3 kHz, and the sampling rate was 10 kHz. The signal was recorded by a PC using Signal software ver. 4.08 (Cambridge Electronic Devices, Cambridge, UK). The test coil was placed tangentially to the scalp at a 45° angle to the midline, to induce a posterior-anterior (PA) current flow across the central sulcus (Figure 1C). The hand motor area of the left and right M1 was defined as the point where stimulation consistently evoked the largest MEP. We defined the resting motor threshold (rMT) as the lowest intensity that evoked 5 small responses (~50 µV) in the relaxed FDI muscle in a series of 10 stimuli (Rossini *et al.*, 1994). The intensity of the test stimulus (TS) was finally adjusted to evoke a MEP of ~ 1 mV peak-to-peak amplitude in the relaxed FDI.

Interhemispheric inhibition

Interhemispheric inhibition (IHI) was measured with a paired-pulse paradigm previously described (Ferbert *et al.*, 1992; Avanzino *et al.*, 2014) both from left-to-right

(LtoR) and from right-to-left (RtoL) M1s in a randomized order (Figure 1C). Coils were positioned at an angle of 45° from the midline with the handles pointing backward and laterally. The coils were adjusted over both hemispheres to the *hotspot* of the contralateral FDI and the positions were marked on the scalp so that the angle and coil position was the same throughout the investigation (Lappchen *et al.*, 2011). In all patients, the coil position was not limited by the shape of the skull.

A CS was given to one hemisphere 10 (short latency IHI, SIHI) or 50 ms (long latency IHI, LIHI) before a TS delivered to the other side. The TS and the CS were adjusted to produce a MEP with a peak-to-peak amplitude of ~1 mV (CS_{1mV}; TS_{1mV}) (Ni *et al.*, 2009). There were two randomized blocks, i.e. IHI from right-to-left and viceversa. Each block had three conditions that were randomized within the block. Condition 1: TS alone (MEP test). Condition 2 and 3: the same as condition 1, except that the TS was preceded by a CS with an ISI of 10 or 50 ms (conditioned MEP). Fifteen trials for each condition were recorded (total of 45 trials) in random order for each subject with a 5 s (±20%) intertrial interval. The responses to each single trial were stored on a PC and analysed offline at the end of the experiment. For each condition, we calculated the average of the single trial peak-to-peak MEP amplitude. The conditioned MEP was expressed as a percentage of the MEP test size.

Data analysis

All data were expressed as mean ± standard error of the mean (SEM). The normality of the dataset was proved using the Kolmogorov–Smirnov test. One-way ANOVAs were used to compare demographic features and baseline physiological measures between groups (Table 2). Student's paired *t* tests (two-tailed) were used for interhemispheric comparisons.

A preliminary two-way repeated-measures (rm) ANOVA was used to analyse the effects of "ISI" (within-subjects factors) and "group" (between-subjects factor). Separate one-way ANOVAs were used for further analyses where necessary. A significant main effect in these ANOVAs was followed by Bonferroni's *post hoc* tests. Mauchley's test was used to examine for sphericity. The Greenhouse-Geisser correction was used for nonspherical data. A p value < 0.05 was considered significant. Data were analysed using software (SPSS v. 19.0 for Windows; SPSS Inc.).

Results

Table 2 shows the demographic features, baseline physiological measures and *p* values. Briefly, all the participants were in their adulthood and the groups were similar for age and sex with no statistical differences ($p > 0.05$). Patients with GGE and FE showed a significantly higher baseline rMT compared to controls, for both the left ($F(2, 47) = 4.707$, $p = 0.014$) and the right hemisphere ($F(2, 47) = 4.701$, $p = 0.014$). When considering interhemispheric differences, significantly higher rMT in the non-dominant right hemisphere compared to the dominant side was detected in both groups of patients as well as in HS ($p < 0.05$). Finally, no significant differences in test MEP amplitude were detected between the groups.

Table 2

DEMOGRAPHIC AND BASELINE TMS FEATURES				
	HS	**FE**	**GGE**	**Differences among groups**
#	17	16	17	
Age	30.1 ± 7.9	36.4 ± 9.3	34.2 ± 12.5	n.s.
Sex (women)	11	10	11	n.s.
RMT sx	38.0 ± 5.8*	45.6 ± 7.9*	45.0 ± 9.6*	p = 0.014
SI_{1mV} sx	53.2 ± 12.3	59.1 ± 14.0	54.0 ± 15.5	n.s.
Test MEP FDI dx (mV)	1.06 ± 0.26	0.92 ± 0.32	1.02 ± 0.37	n.s
RMT dx	40.7 ± 4.6	50.9 ± 9.7	49.8 ± 14.9	p = 0.014
SI_{1mV} dx	57.2 ± 13.9	65.4 ± 15.5	58.4 ± 18.3	n.s.
Test MEP FDI sx (mV)	1.25 ± 0.33	1.06 ± 0.47	1.08 ±0.44	n.s

FDI: first distal interosseous; FE: focal epilepsy; HS: healthy subjects; GGE: genetic generalized epilepsy; n.s.: non-significant; RMT: resting motor threshold; SI_{1mV}, intensity required to elicit a 1 mV MEP. * The within-group comparison left versus right hemisphere is significant ($p < 0.05$).

IHI from right to left

A preliminary two-way rmANOVA, using absolute values, revealed significant effects of "ISI" ($F(2, 94) = 99.358$, $p < 0.001$) and "ISI" x "group" interaction ($F(4, 94) = 5.377$, $p = 0.001$). There was no "group" effect ($F(2, 47) = 0.287$, $p = 0.752$). Analysis of the "ISI" x "group" interaction was performed after normalising the data to baseline values. In general,

there was less suppression at ISI 10 and 50 ms in the FE group than in the other groups. A *post hoc* Bonferroni analysis confirmed a significant difference at ISI 10 and ISI 50 ms between the FE and the other two groups (FE vs HS: $p = 0.001$ at ISI 10 and $p = 0.029$ at ISI 50 ms; FE vs GGE: $p = 0.017$ at ISI 10 and $p = 0.024$ at ISI 50 ms) (Figure 1A).

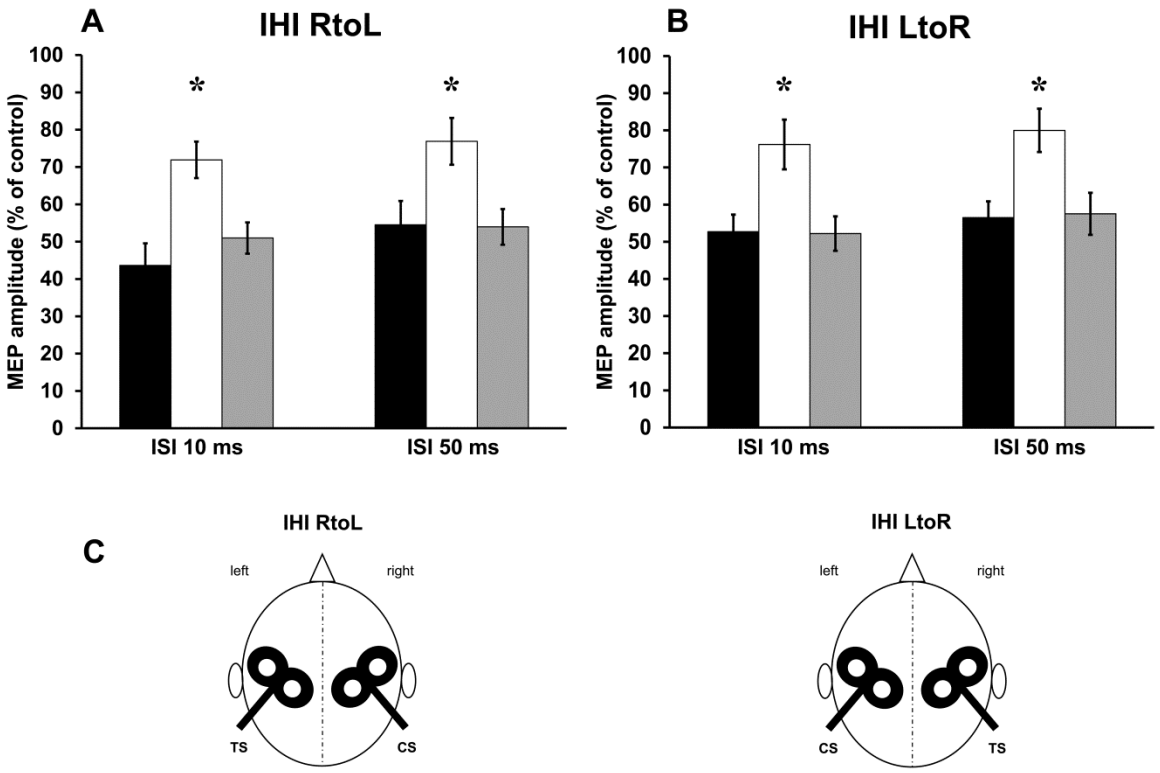

Figure 1

Interhemispheric inhibition (IHI) in subjects at rest. *Panel A*, IHI from right to left (RtoL). *Panel B*, IHI from left to right (LtoR). Black columns: healthy subjects. White columns: patients with FE. Grey columns: patients with GGE. Amplitude of MEPs (mV) is normalized and expressed as a percentage of control. Errors bars indicate SEM. Asterisks indicate a p value < 0.05 between the FE and the other two groups. *Panel C*, experimental settings. Coil positions over a skull sketch. TS, test stimulus, delivered over the hand motor area of one hemisphere. CS, conditioning stimulus, delivered over the homologous contralateral area.

IHI from left to right

In general, results were similar to IHI from right to left. A preliminary two-way rmANOVA using absolute values revealed significant effects of "ISI" ($F(2, 94) = 50.960$, $p <$

0.001), “ISI” x “group” interaction (F (4, 94) = 2.551, p = 0.044) and no “group” effect (F (2, 47) = 0.890, p = 0.417). A *post hoc* Bonferroni analysis confirmed a significant difference at ISI 10 and ISI 50 ms between the FE and the other two groups (FE vs HS: p = 0.010 at ISI 10 and p = 0.009 at ISI 50 ms; FE vs GGE: p = 0.008 at ISI 10 and p = 0.014 at ISI 50 ms) (Figure 1B).

Focus side

The FE group was stratified according to the side of the epileptogenic focus in two groups, i.e. right (n = 8) and left FE (n = 8). Both where then compared to the HS group.

IHI from right to left. A preliminary two-way rmANOVA using absolute values revealed significant effects of “ISI” (F (2, 60) = 35.401, p = 0.001) and “ISI” x “group” interaction (F (4, 60) = 5.589, p = 0.001). There was no “group” effect (F (2, 30) = 1.072, p = 0.355) (Figure 2A). Analysis of the “ISI” x “group” interaction was performed after normalising the data to baseline values. At ISI 50 ms, but not at 10 ms, there was less suppression in the right FE than in left FE group, which was similar to HS (Figure 2A, 3). A *post hoc* Bonferroni analysis confirmed a significant difference at ISI 50 ms between the right FE and the other two groups (right FE vs HS: p = 0.002; right FE vs left FE: p = 0.027) (Figure 2A, 3).

IHI from left to right. A preliminary two-way rmANOVA using absolute values revealed significant effects of “ISI” (F (2, 60) = 18.345, p = 0.001), “ISI” x “group” interaction (F (4, 60) = 3.382, p = 0.015) and no “group” effect (F (2, 30) = 0.500, p = 0.611) (Figure 2B). At ISI 50 ms, but not at 10 ms, there was less suppression in the left FE than in right FE group which was similar to HS. A post hoc Bonferroni analysis confirmed a significant difference at ISI 50 ms between the left FE and the other two groups (left FE vs HS: p < 0.001; left FE vs right FE: p = 0.023) (Figure 2B).

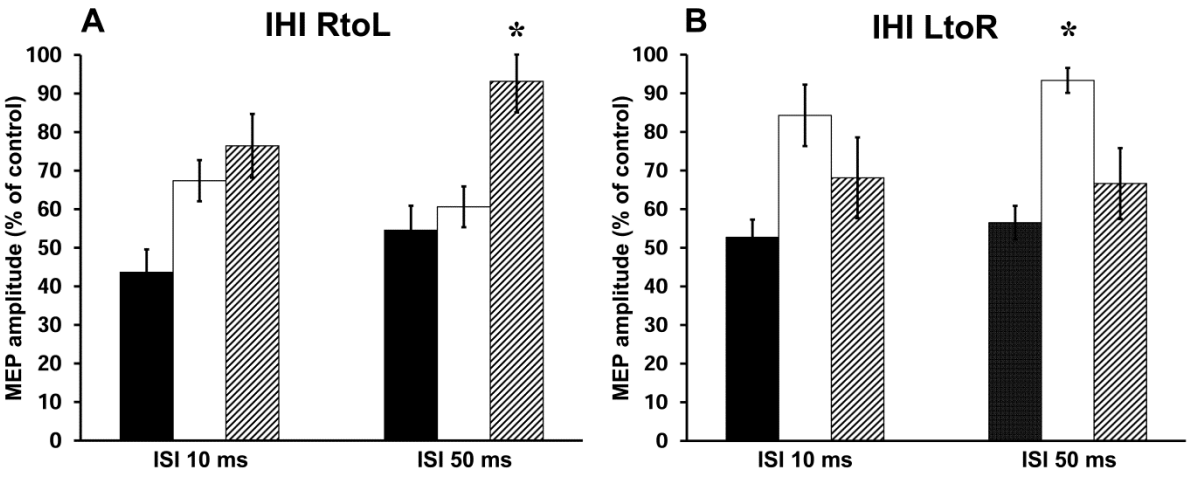

Figure 2

Interhemispheric inhibition as a function of the side of the epileptogenic focus (right FE versus left FE). Black columns: healthy subjects. White columns: patients with left FE. Dashed columns: patients with right FE. Asterisks indicate a *p* value < 0.05 between right or left FE and the other two groups.

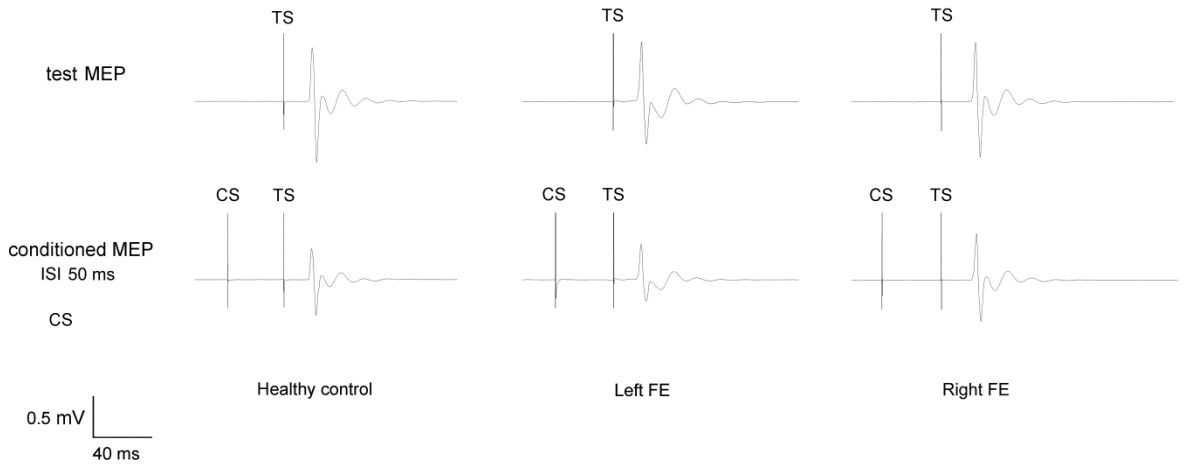

Figure 3

IHI from right to left: typical example of changes in the MEP (grand-average of the recorded trials) with an ISI = 50 msec. A representative healthy control, a patient with left FE and a patient with right FE are depicted. In the right FE patient, a clear MEP increase can be seen at ISI 50 msec.

Secondary generalization

The FE group was then stratified based on whether the seizures became secondarily generalized or not into two homogeneous groups, i.e. patients with (n = 6, right focus) and without secondary generalization (n = 6, left focus). Then, IHI from the focal hemisphere to the non-focal hemisphere was compared between these two groups and with HS.

A one-way ANOVA using normalized values confirmed a significant "group" effect ($p < 0.001$) related to a significant difference at ISI 10 and ISI 50 ms between the FE groups and HS ($p < 0.02$) as showed by *post hoc* Bonferroni analysis. However, there was no difference between patients with and those without secondary generalization ($p > 0.05$).

Discussion

To our knowledge, this is the first TMS study evaluating IHI in patients with drug-treated focal and generalized epilepsy. We found that patients with FE had a bilateral defective IHI at short and long ISIs (i.e. 10 and 50 ms), whilst IHI in patients with GGE was similar to healthy individuals. We selected these two intervals (i.e. 10 and 50 ms) from a wide range of ISIs (6–50 ms) (Ferbert *et al.*, 1992; Daskalakis *et al.*, 2002) because they showed the maximum inhibitory effects in previous studies (Ni *et al.*, 2009). IHI is deficient in patients with abnormalities of the corpus callosum, therefore it is likely transmitted through this large structure (Meyer *et al.*, 1995). IHI is absent in children (Heinen *et al.*, 1998) and develops during later childhood and adolescence (Sommer *et al.*, 2012) because myelination of callosal fibers is completed at around 18-20 years of age (Pujol *et al.*, 1993). This is why we only included adults (>30 years) in the present study (Table 2). If so, our main finding of a defective IHI in FE might be primarily explained by some abnormalities in this commissure, tackling the classical view of epilepsy as a grey matter disorder (O'Dwyer *et al.*, 2010; Miro *et al.*, 2015). Furthermore, when FE patients were stratified according to the side of their epileptic focus, the long-ISI (= 50 ms), but not the short-ISI (= 10 ms) IHI, appeared to be defective only when the CS was applied over the "focal" hemisphere. Namely, inputs arising from the M1 ipsilateral to the seizure onset zone elicited an excess excitatory response in the contralateral M1 in this group, whether seizures secondarily generalized or remained focal.

SIHI and LIHI are likely mediated through transcallosal neurons with excitatory properties (Lee *et al.*, 2007). The projecting callosal neurons activate inhibitory interneurons in the contralateral target M1, which are responsible for the overall effect of IHI (Daskalakis *et al.*, 2002; Chen, 2004; Lee *et al.*, 2007) (Figure 4A).

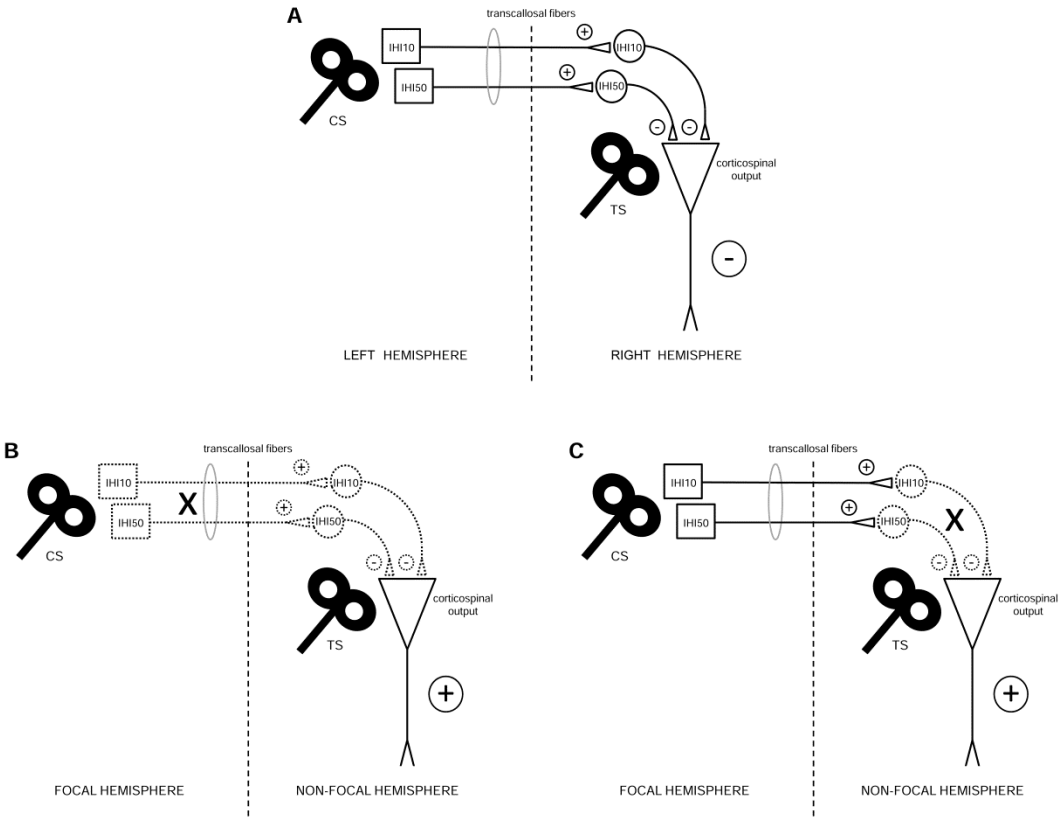

Figure 4

Model of interactions between intracortical and interhemispheric circuits in healthy subjects (*Panel A*) and in patients with FE (*Panel B, C*).

Panel A, the projecting callosal neurons activate inhibitory interneurons in the contralateral target M1 in HS. In patients with FE, inputs arising from the M1 ipsilateral to the focal-hemisphere elicited an excess excitatory response in the contralateral M1. Two possible mechanisms are proposed: *Panel B,* callosal degeneration; *Panel C,* inhibitory circuits activated by the callosal fibers in the target hemisphere are less excitable.

CS, conditioning stimulus; IHI, interhemispheric inhibition; –, inhibitory connections; +, excitatory connections; TS, test stimulus.

These connections may differ for SIHI and LIHI (Chen *et al.*, 2003; Kukaswadia *et al.*, 2005). If so, our main finding of defective IHI in FE might be primarily explained by two possible mechanisms (Figure 4). One explanation is the damage of the involved pathways (Figure 4B). Neuroimaging studies on animal models of epilepsy (Otte *et al.*, 2012a), showed that white matter damage and decreased connectivity within the corpus callosum was not

restricted to bundles close to the epileptogenic focus (Otte *et al.*, 2012a). Similarly, several studies showed that patients with TLE had diffuse white matter abnormalities more severely involving the focal hemisphere and the tracts closely connected with the affected temporal lobe (Otte *et al.*, 2012b). Structural changes in the corpus callosum have also been described in patients with bilateral refractory TLE (Miro *et al.*, 2015) and FLE (O'Dwyer *et al.*, 2010). The hypothesis that callosal degeneration is the cause of our finding (Figure 4B), is supported by the fact that the effect involves both short and long-ISIs. Another possibility is that the inhibitory circuits activated by the callosal fibers in the target hemisphere are less excitable (Figure 4C). However, this is a remote possibility because there are no studies showing reductions in cortical inhibition in the non-focal hemisphere. For example, some previous TMS studies evaluated short and long intracortical inhibition (SICI, LICI) in both hemispheres in patients with FE and GGE (Cantello *et al.*, 2000a; Badawy *et al.*, 2007; Badawy *et al.*, 2014; Badawy *et al.*, 2015). Patients with GGE showed widespread and bilateral abnormalities, i.e. decreased intracortical inhibition, whereas alterations were lateralized to the affected hemisphere in patients with FE, at least in a cohort of patients with newly diagnosed epilepsy (Badawy *et al.*, 2007). However, considering different FE syndromes, such lateralization was consistent just in TLE, and if patients were at disease onset and drug-naïve (Badawy *et al.*, 2015). In mixed FE syndromes of chronic nature, these hemispheric differences got lost (Cantello *et al.*, 2000a; Badawy *et al.*, 2015).

Finally, it should be noted that we cannot exclude the minor role of subcortical and/or spinal structures in the pathogenesis of defective IHI (Gerloff *et al.*, 1998).

The corpus callosum is involved in the propagation of epileptic activity in animal models (Kusske & Rush, 1978; Musgrave & Gloor, 1980; Walker *et al.*, 2012) and in patients with drug-resistant epilepsy with a significant reduction in generalized seizures after callosotomy (Asadi-Pooya *et al.*, 2008). Thus, a defective inhibitory response of contralateral M1 to inputs travelling from the focal hemisphere may well represent one key factor for the contralateral spread of focal discharges, and seizure generalization. On the contrary, IHI turned out to be normal in the group of patients with GGE. Indeed, the simultaneous involvement of bilateral networks from the seizure outset in GGE (Avanzini *et al.*, 2012) might suggest a callosal over-activity. This is further corroborated by the reported seizure frequency reduction in patients with refractory GGE following corpus callosotomy (Jenssen *et al.*, 2006; Cukiert *et al.*, 2009). However, this was not the case in our study, since we considered seizure-free patients with GGEs. Thus, it appears that abnormalities of the corpus callosum might be involved more in the pathogenesis of drug refractoriness in GGE, rather

than GGE itself. The findings also extend the TMS evidence of different pathophysiological mechanisms underlying focal and generalized epilepsies (Badawy *et al.*, 2014). Interestingly, recent studies have raised the possibility that white matter disruption may be the underlying mechanism responsible for myoclonus in JME (Liu *et al.*, 2011), supporting the idea that GE subsyndromes have unique anatomic substrates. Our group of GE was heterogeneous and the number of patients included is insufficient to make a reliable distinction between subsyndromes; therefore, we cannot exclude specific IHI changes in JME compared to other patients with GE.

To our knowledge, the only study addressing IHI in focal epilepsy considered a cohort of patients with symptomatic FE, following successful surgical removal of an epileptic focus (Lappchen *et al.*, 2011). These authors tested the SIHI at ISI = 8 ms before and after the epilepsy surgery and found a stronger inhibitory effect of the non-focal hemisphere after surgery (Lappchen *et al.*, 2011). In our study there was a bilateral defective SIHI (at ISI = 10 ms), which was not correlated with the lateralization of the epileptic focus. These apparently conflicting results might be explained by major differences in the patient clinical features. Lappchen et al. (2011) indeed studied the effects of the surgical interruption of the epileptogenic network in patients with drug-resistant seizures. By contrast, we studied a cohort of patients mostly seizure-free, or with a very low seizure frequency, in whom the epileptogenic network was still intact.

Generally, TMS proved a valid method to study the epileptogenic network in patients (Strigaro *et al.*, 2013; Badawy *et al.*, 2014; Strigaro *et al.*, 2015b). Primary motor cortex (M1) is the main target, because it is the most accessible cortical area to TMS. If not directly involved in the pathogenesis of epilepsy (e.g. juvenile myoclonic epilepsy (Strigaro *et al.*, 2015a), it can be influenced at a distance by non-motor epileptogenic areas (Hamer *et al.*, 2005). Indeed, our cohort of patients had chronic epilepsy, therefore we suggest that the defective IHI may be related to a widespread epileptogenic network involving bilateral circuits beyond the seizure onset zone.

Interhemispheric inhibition can also be evaluated with single-pulse TMS on M1 and recoding the short attenuation or interruption of ongoing voluntary EMG activity in the ipsilateral hand muscle, i.e. ipsilateral silent period (ISP) (Wassermann *et al.*, 1991; Giovannelli *et al.*, 2009; Perez & Cohen, 2009). Although both IHI and ISP provide information on interhemispheric inhibition, they are due to different neuronal mechanisms (Gerloff *et al.*, 1998; Chen *et al.*, 2003). In the present study, we were primarily interested on

cortical excitability changes in epilepsy, therefore inhibition of MEPs measured with IHI appeared a more useful parameter than inhibition of volitional motor activity measured with ISP (Giovannelli et al., 2009).

AEDs may reduce brain excitability and therefore influence several TMS measures (Ziemann *et al.*, 1996a; Strigaro *et al.*, 2011; Sommer *et al.*, 2012). As expected, patients with epilepsy, both FE and GGE, showed a higher baseline rMT than controls. This is likely related to the well-known effects of some AEDs (i.e. carbamazepine, valproate) on voltage-gated sodium channels, whose function is reflected by the motor threshold (Ziemann *et al.*, 1996a; Kazis *et al.*, 2006). More interestingly, we detected a higher rMT in the non-dominant compared to the left dominant hemisphere, both in patients and controls, as previously reported by some (Macdonell *et al.*, 1991) but not all (Civardi *et al.*, 2000) researchers. This possibly relates to handedness (De Gennaro *et al.*, 2004) and physiological asymmetries in the corticospinal system with a greater number of motoneurons projecting to the right-hand muscles (Macdonell *et al.*, 1991).

The present study has some limitations. First, the sample size of the FE group prevented any correlation between IHI and important clinical features such as seizure type, aetiology, etc... Secondly, all patients were taking AEDs, whose action might have confounded the findings of ours. However, to our knowledge, there is only one study on the effects of AEDs on IHI showing that a single dose of CBZ reduced IHI at ISI = 8 ms (but not at 10 and 50 ms) in HS (Sommer *et al.*, 2012). CBZ is a sodium channel blocker and this effect is possibly related to reduced firing rate of inhibitory interneurons mediating inhibition (Sommer *et al.*, 2012). We believe, however, that a group of drug naïve patients would possibly have ruled out any confounding effect mediated by drugs acting on the IHI neural circuits. Obvious ethical constraints hindered the recruitment of an adequate patient sample. Finally, ISI 10 ms was bilaterally defective but when we stratified the patients according to the focus lateralization, the significant effect disappeared. This is likely related to the small sample size and low statistical power.

Conclusions

A disrupted transcallosal inhibition between the focal and the non-focal hemisphere, as studied by TMS, may contribute to the pathogenesis of FEs. Particularly, this may represent one key factor for the contralateral spread of the epileptic discharge and seizure generalization.

ABNORMAL MOTOR CORTEX PLASTICITY IN JUVENILE MYOCLONIC EPILEPSY

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Abstract

Purpose. Abnormal cortical plasticity has been hypothesized to play a crucial role in the pathogenesis of juvenile myoclonic epilepsy (JME). To study the motor cortical plasticity we used paired associative stimulation (PAS). When a repetitive electrical stimulus to the median nerve is paired with a transcranial magnetic stimulus (TMS) pulse over the controlateral motor cortex with at an interstimulus interval (ISI) of 21.5-25 ms, a long term potentiation (LTP)-like synaptic plasticity is induced in the corticospinal system.
Aim of this study was to investigate the motor cortex LTP-like synaptic plasticity by means of PAS in patients with JME.

Methods. Twelve adult patients with JME were compared with 13 healthy subjects of similar age and sex. PAS consisted of 180 electrical stimuli of the right median nerve paired with a single TMS over the hotspot of right abductor pollicis brevis (APB) at an ISI of 25 ms (PAS25). We measured motor evoked potentials (MEPs) before and after each intervention for up to 30 min.

Results. In healthy subjects the PAS25 protocol was followed by a significant increase of the MEP amplitude ($p < 0.001$). On the contrary, in patients with JME, the MEP amplitude did not change.

Conclusion. Defective motor cortex plasticity is likely involved in the pathogenesis of JME.

Introduction

Juvenile myoclonic epilepsy (JME) is the most common idiopathic generalized epilepsy (IGE), with a presumed genetic aetiology (Camfield *et al.*, 2013). Myoclonic jerks, absences and generalized tonic-clonic seizures are the core findings in this syndrome (Serafini *et al.*, 2013). So far, motor cortex hyperexcitability (Badawy *et al.*, 2013) and abnormal function of fronto-thalamic networks have been involved in the pathophysiology of JME (Bagshaw & Cavanna, 2013; Kim *et al.*, 2014; Caeyenberghs *et al.*, 2015). Hyperexcitability of primary visual areas and excessive response of the primary motor cortex to visual inputs would be another important factor (Strigaro *et al.*, 2012; Strigaro *et al.*, 2013) since the presence of a photoparoxysmal response is common (Serafini *et al.*, 2013).

Abnormal cortical plasticity has been frequently hypothesized to play a crucial role in the pathogenesis of epilepsies (Sutula, 2004; Lopantsev *et al.*, 2009), at least in experimental models of temporal lobe epilepsy (Artinian *et al.*, 2015). However, considering the clinical context, there are no direct evidences to support this hypothesis, possibly because of experimental difficulties. Transcranial magnetic stimulation (TMS) is a well-established, safe, painless and non-expensive neurophysiologic method for non-invasive measurement of cortical excitability (Badawy *et al.*, 2014). It also offers a unique opportunity to study cortical plasticity in a non-invasive fashion. In the last few years, a variety of TMS protocols have been developed to probe mechanisms of synaptic plasticity in the intact human brain (Ziemann, 2004). Among these, paired associative stimulation (PAS) involves repeated pairing of an electrical stimulus to the median nerve with a later transcranial magnetic stimulus (TMS) over the contralateral motor cortex (Stefan *et al.*, 2000; Wolters *et al.*, 2003). This induces changes in cortical excitability, whose sign depends on the interval between the median nerve and the TMS stimuli. Intervals of 25 ms (PAS25) have an enhancing effect, whereas intervals of around 10 ms (PAS10) reduce excitability (Stefan *et al.*, 2000; Wolters *et al.*, 2003; Weise *et al.*, 2006). Pharmacological studies suggest that such changes involve temporary modifications in synaptic efficacy, equivalent to long-term potentiation (LTP) and long-term depression (LTD), as described in animal preparations (Muller-Dahlhaus *et al.*, 2010).

Aim of the present study was to test the effects of PAS25 in patients with JME compared to healthy controls. We wanted to explore if, in the complex framework of the JME pathophysiology, an abnormal motor cortical plasticity could play a given role.

Materials and Methods

Subjects

We studied 12 consecutive adult patients with JME (10 female, mean age 32.8 years, SD 10.7) referring to the Epilepsy Clinic of the University Department of Neurology, Novara, Italy. Diagnoses were made by two experienced epileptologists not involved in the present study on the basis of the clinical history, seizure type and electroencephalography (EEG) findings according to the established diagnostic criteria (1989).

Thirteen normal subjects of similar age and sex acted as controls (10 female, mean age 27.9 years; SD 5.6). They had no family or personal history of neurologic disease or epilepsy. Reportedly, both patients and controls had not been taking neuroactive drugs (alcohol and caffeine included) for 72 h prior to the study, except for the patient antiepileptic treatment. Their general and neurological examinations were normal. All subjects were right-handed based on the Edinburgh Handedness Inventory and gave written informed consent. Experiments were approved by the local Ethics Committee and were performed in accordance with the Declaration of Helsinki.

Patient features

The clinical features of patients are reported in Table 1. Eight of the 12 patients were classified as photosensitive because they showed a photoparoxysmal response (PPR) to intermittent light stimulation (ILS), which did never entail clinical phenomena. ILS was performed according to the international standards (Rubboli *et al.*, 2004). In general, the clinical course of the patients was favourable, and all of them reported being seizure-free. All patients were on a standard antiepileptic treatment. Valproate, alone or in combination with levetiracetam, was the most frequent choice.

Table 1

Table 1. MAIN FEATURES OF THE PATIENTS				
Patient #	**Age**	**Sex**	**Current Treatment (mg/die)**	**Photosensitivity**
1	26	F	400 LTG	Yes
2	48	F	1300 VPA + 1000 LEV	Yes
3	22	F	100 LTG	Yes
4	49	F	800 VPA	No
5	45	F	800 VPA	No
6	25	M	900 VPA	No
7	42	F	1000 VPA + 100 PB	Yes
8	24	F	400 LTG	Yes
9	28	M	300 VPA	Yes
10	26	F	1000 LEV	Yes
11	38	F	115 PB	Yes
12	21	F	800 VPA	No

JME: juvenile myoclonic epilepsy; LEV: levetiracetam; LTG: lamotrigine; PB: phenobarbital; VPA: valproic acid.

TMS and EMG recordings

All neurophysiologic studies took place between 2:00 and 6.30 p.m. in a quiet laboratory, at a standard temperature of 22°C.

Subjects sat comfortably in a chair with both arms resting on a pillow placed on their lap. Motor-evoked potentials (MEPs) were recorded from the abductor pollicis brevis (APB) muscle using 9 mm-diameter Ag-AgCl surface-cup electrodes, in a typical belly-tendon montage. Data were collected, amplified (gain, 1000x), and filtered (20 Hz to 3 kHz) through a CED 1902 isolated amplifier (CED, Cambridge, UK) that fed signals to an A/D converter (CED Micro 1401 Mk II). The sampling rate was 10 kHz. The signal was then recorded by a PC using Signal software ver. 4.08 (Cambridge Electronic Devices, Cambridge, UK).
TMS was delivered through a Magstim 200^2 stimulator (Magstim) every 4.5–5.5 s. A figure-of-eight coil (outer winding diameter 70 mm) was held tangentially on the scalp at an angle of 45 deg to the midsagittal plane with the handle pointing laterally and posteriorly. Stimuli were applied to the motor cortex representation of the right APB. The motor hot spot was defined

as the point where a magnetic stimulus of constant, slightly suprathreshold intensity consistently elicited an MEP of the highest amplitude. Motor cortex excitability was measured as the peak-to-peak amplitude of the MEP generated by single pulse TMS.

Paired associative stimulation (PAS)

PAS consisted of 180 electrical stimuli of the right median nerve at the wrist paired with a single TMS shock over the hotspot of right APB muscle at a rate of 0.2 Hz (Stefan *et al.*, 2000; Hamada *et al.*, 2012). Electrical stimulation (square wave pulse; stimulus duration, 0.2 ms) was applied at an intensity of three times the perceptual threshold using a constant current generator (Digitimer, Welwyn Garden City, UK). TMS was applied at an intensity required to elicit a 1 mV MEP (SI_{1mV}). The effects of PAS given with an interstimulus interval of 25 ms between peripheral and TMS stimuli were tested (PAS25). Subjects were instructed to look at their stimulated hand and count the peripheral electrical stimuli they perceived. The MEPs evoked in the APB were displayed online during the intervention to control for the correct coil position and stored for off-line analysis.

Experimental procedures

The resting motor threshold (RMT) and MEP size were measured. RMT was defined as the lowest intensity that evoked a response of about 50 μV in the relaxed APB in at least 5 of 10 consecutive trials (Rossini *et al.*, 1994). The stimulus intensity was changed in steps of 1% of the maximum stimulator output (MSO). Thirty MEPs were recorded with a stimulus intensity of SI_{1mV} before (baseline) and for up to 30 min (T0, T15 and T30) after PAS25. SI_{1mV} was kept constant throughout the experiment. The mean peak-to-peak amplitude was calculated for the data obtained before and after PAS in each single subject.

Data analysis

The baseline physiological parameters are given in Table 2. The between-group comparability of these variables was tested by a Student's paired *t* test (two-tailed). MEP amplitudes at each time point were averaged and normalized to baseline. Then they entered a two-way repeated measures (rm) ANOVA with factors “GROUP” (patients, controls) and “TIME” (T0, T15 and T30). In order to evaluate the effects of PAS in each group, a one-way ANOVA was employed with a main factor of “TIME” (baseline, T0, T15 and T30), using absolute MEP values in each experimental session. The Greenhouse–Geisser correction was used if necessary to correct for non-sphericity; *P* values <0.05 were considered

significant. Bonferroni's post hoc tests or paired *t* tests (two-tailed) were used for further analyses. Data were analysed using software (SPSS v. 19.0 for Windows; SPSS Inc.). All data are given as mean ± standard error of the mean (SEM).

Table 2

PHYSIOLOGIC FEATURES			
	HS	**JME**	**Differences among groups**
#	13	12	
Age	27.9 ± 1.5	32.8 ± 3.1	n.s.
Sex (female)	10	10	n.s.
RMT (%)	40.2 ± 1.0	44.7 ± 2.5	n.s.
PsT (mA)	2.2 ± 0.2	2.6 ± 0.2	n.s.
SI_{1mV} (%)	51.8 ± 2.5	55.3 ± 3.2	n.s.
Baseline MEP (mV)	0.94 ± 0.07	1.08 ± 0.08	n.s.

HS: healthy subjects; JME: juvenile myoclonic epilepsy patients; MEP, motor evoked potential; psT: peripheral sensory threshold; RMT: resting motor threshold; SI_{1mV}: intensity required to elicit a 1 mV MEP; n.s.: non-significant.

Results

The procedure was well tolerated and no subjects experienced adverse events or seizures during and after the experimental session. Baseline physiological measures and *p* values are shown in Table 2. In brief, no significant differences in the RMT and baseline MEP amplitude were detected between the two groups.

A preliminary two-way rmANOVA on normalized to baseline values revealed a significant effect of GROUP ($F(1, 23) = 14.244$, $p = 0.001$) but no effects of TIME ($F(2, 46) = 0.251$, $p = 0.779$) nor a GROUP x TIME interaction ($F(2, 46) = 0.157$, $p = 0.855$). Post hoc paired *t* tests revealed significant group differences at T0 ($p = 0.001$), T15 ($p = 0.003$) and T30 ($p = 0.013$) (Figure 1).

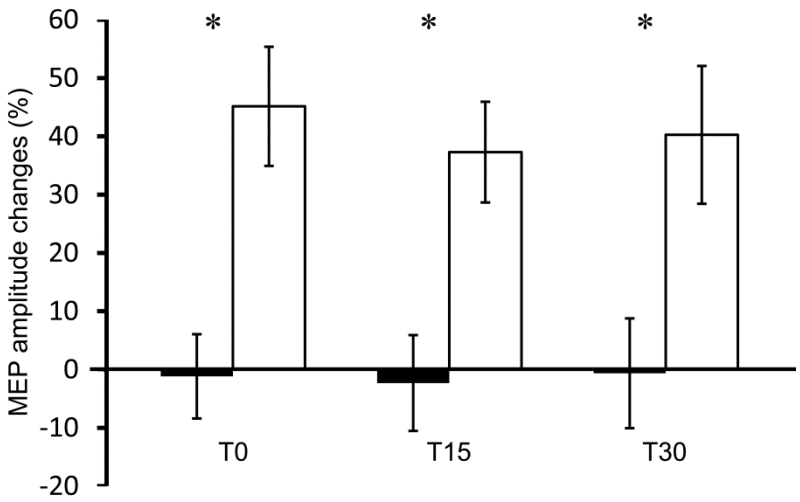

Figure 1

Grand average of normalized MEPs at T0, T15 and T30 to baseline in patients (black) and controls (white). Asterisks indicate a significant difference ($p < 0.05$).

One-way ANOVAs separately showed that following PAS25, MEP sizes were significantly increased at T0, T15 and T30 compared to baseline MEP values (effects of 'TIME', $F(3, 36) = 10.315$, $p < 0.001$) in the control group but not in the patients (effects of 'TIME', $F(3, 33) = 0.158$, $p = 0.924$). Post hoc analysis with Bonferroni's correction showed a significant increase of MEP sizes compared with baseline at T0 ($p < 0.001$), T15 ($p = 0.003$) and T30 ($p < 0.001$).

Discussion

As in the case of ours, the PAS protocol is usually followed - in healthy subjects - by a sustained increase of the MEP size. Admittedly this represents a LTP-like plasticity phenomenon (Stefan *et al.*, 2000). On the contrary, in our JME patients, the MEP amplitude did not change, suggesting that LTP-plasticity may be definitely altered in this disease.

PAS is thought to explore the motor cortex synaptic plasticity (Stefan *et al.*, 2000; Hamada *et al.*, 2012; Strigaro *et al.*, 2014), that is the ability of neurons to change the efficacy of their synaptic transmission (Dan & Poo, 2006). A long-lasting enhancement is called LTP, and it has been involved in motor learning (Ziemann *et al.*, 2004; Rosenkranz *et al.*, 2007; Jung & Ziemann, 2009; Rajji *et al.*, 2011) whereas its impairment is crucial in the pathophysiology of a number of movement disorders (Quartarone *et al.*, 2003; Morgante *et*

al., 2006; Brandt *et al.*, 2014). In contrast, in patients with epilepsy, studies with LTP-like plasticity protocols were very limited so far, possibly because epilepsy itself represents a relative contraindication to TMS, due to the theoretical risk of seizure induction. However, this is a rare event, associated with a crude risk of 1.4% (Bae *et al.*, 2007) and accordingly, the most recent guidelines considered repetitive TMS safe in this context (Lefaucheur *et al.*, 2014). On the contrary, LTD-like plasticity protocols, i.e. low-frequency repetitive TMS, have been frequently applied for therapeutic purposes (Cantello *et al.*, 2007; Lefaucheur *et al.*, 2014).

We studied patients with JME because the primary motor cortex is both involved in the pathophysiology of cortical myoclonus (Serafini *et al.*, 2013) and the most accessible cortical area to TMS (Badawy *et al.*, 2014). Furthermore, when the motor cortex is not directly involved, it can be influenced at a distance by non-motor epileptogenic areas (Hamer *et al.*, 2005). Admittedly, a number of excitability measures have already been studied in patients with JME (Badawy *et al.*, 2014). Overall, the most consistent finding is related to short-interval intracortical inhibition (SICI) substantially reduced in patients with JME compared to healthy controls (Manganotti *et al.*, 2000; Manganotti *et al.*, 2004; Badawy *et al.*, 2010) and further decreased after sleep deprivation (Manganotti *et al.*, 2006). SICI reduction is thought to reflect a defective gabaergic inhibition in the motor cortex and particularly of GABAA receptor-mediated effects (Ziemann *et al.*, 1996a; Badawy *et al.*, 2014). Besides, the PAS protocol was first applied in a recent study on a small cohort of patients with Unverricht–Lundborg disease, i.e. the most common form of progressive myoclonic epilepsy (PME). The response to PAS25 was found defective, which was interpreted as disturbed motor cortical functions underlying the motor symptoms (Danner *et al.*, 2011). Interestingly, our results are in line with these findings and highlight the importance of abnormal motor cortical excitability in both PME and JME, although the underlying pathophysiology is most likely different (Badawy *et al.*, 2010).

We suggest three possible mechanisms involved in the disruption of the motor cortical plasticity in patients with JME: (1) A pathological form of plasticity may occur during epileptogenesis leading into an unbalance between excitatory and inhibitory neural circuits in specific networks, i.e. the motor cortex (Pitkanen & Lukasiuk, 2011). In fact, a close relation between LTP and epileptogenesis was recently demonstrated in models of hippocampal epilepsy (Lopantsev *et al.*, 2009). Additionally, kindling protocols trigger a large number of effects, some of which appear similar to LTP (Albensi *et al.*, 2007). In this view, abnormal cortical plasticity may be the neurophysiological background for the development of

myoclonus both in JME and PME (Danner *et al.*, 2011). (2) Seizures themselves have a significant and lasting impact on the brain in animal models of epilepsy (Lopantsev *et al.*, 2009), leading to structural and functional alterations of neuronal circuits which may be accompanied by declining cognitive and behavioural functions (Sutula, 2004), as already recognised in JME (Schmitz *et al.*, 2013). The background of these manifestations might include an impairment of cortical plasticity. (3) The antiepileptic treatment itself may induce long lasting changes in cortical plasticity. Indeed, AEDs may affect cortical excitability (Ziemann *et al.*, 1996a) and recent evidences suggest that a single dose of lamotrigine (Delvendahl *et al.*, 2013) and levetiracetam (Heidegger *et al.*, 2010) resulted in a significant reduction of the LTP-like MEP increase in healthy subjects. It has been suggested that this action may contribute to its antiepileptic effects and a successful antiepileptic treatment may have to reduce plasticity to be effective (Heidegger *et al.*, 2010).

Any combination of these mechanisms might be possible, although we favour the hypothesis on the role of a defective plasticity as the background for the development of the motor features in JME (and PME) such as the epileptic myoclonus. Indeed, the motor cortex hereby studied is one of the fundamental elements of a complex fronto-thalamic network (Bagshaw & Cavanna, 2013; Kim *et al.*, 2014; Caeyenberghs *et al.*, 2015) which is affected by multi-focal disease mechanisms in JME (Koepp *et al.*, 2013) and possibly explains the peculiar seizure types, i.e. myoclonic jerks and absences (Bagshaw & Cavanna, 2013). Besides, motor cortex itself is part of a visuo-motor network (Strigaro *et al.*, 2015c) which is most likely involved in the pathophysiology of the common PPR (Strigaro *et al.*, 2012; Strigaro *et al.*, 2013).

Pharmacological manipulation of PAS-induced LTP-like plasticity in healthy volunteers revealed suppressive effects of the antagonists of major neuromodulatory neurotransmitters dopamine, norepinephrine and acetylcholine (Korchounov & Ziemann, 2011). Of these, the dopaminergic signalling appears of greater importance because it is necessary for normal motor skill learning and synaptic plasticity within the primary motor cortex of animal models (Molina-Luna *et al.*, 2009). Interestingly, a specific alteration of the dopaminergic system (Landvogt *et al.*, 2010) and impaired dopamine uptake in the midbrain of JME patients (Ciumas *et al.*, 2008; Ciumas *et al.*, 2010; Odano *et al.*, 2012) has been revealed with positron emission tomography (PET). Additionally, dopamine itself is neuroprotective and have inhibitory properties on seizures (Bozzi *et al.*, 2000; Bozzi & Borrelli, 2006), particularly myoclonic seizures (Greer & Alpern, 1977). Therefore, we speculate that the disruption of motor cortical plasticity in patients with JME may be the

neurophysiological counterpart of a defective dopaminergic signalling. Further studies correlating dopaminergic signalling and TMS measures are needed to corroborate this hypothesis.

This study has few limitations. First, the sample size is small and general conclusions should be inferred with caution. It prevented useful correlations with the clinical features. Secondly, a control group of drug naïve patients with myoclonic seizures would have possibly disentangled the confounding role of AEDs on cortical plasticity. However, ethical constraints hindered the recruitment of these patients.

Conclusions

The present data provide evidence of a defective LTP-like plasticity in a cohort of patients with JME, which may be primarily involved in the pathogenesis of myoclonus in this frequent form of epilepsy.

CONCLUSIONS

To our knowledge, these are the first studies evaluating the excitability of various epileptogenic networks using paired-TMS in patients with focal and generalized epilepsy. We developed a novel methods to examine the functional connection linking visual to the motor areas in healthy subjects. The latter was applied to study patients with photosensitivity since they represent a "model" of system epilepsy. Moreover, we explored the connection involved in seizure generalization. Finally, we explored the motor cortex plasticity in JME, the most common subtype of IGE in adults.

Here are summarised the most important results of the works selected for this thesis:

1) We developed a novel paired-TMS method to study the physiological connections between primary visual (V1) and motor areas (M1). Conditioning stimuli delivered to V1 suppressed M1 excitability while the subject was at rest, whereas M1 excitability turned from inhibition to facilitation in the context of a visuomotor reaction task (Strigaro *et al.*, 2015c). These findings support a physiologically relevant visuomotor functional connectivity, which likely contributes to visuomotor integration. When studied in patients with IGE showing a PPR, the usual suppression at rest was replaced by an overactive functional connection between V1 and M1. This excessive response of M1 to visual inputs may underlie the fast spread of epileptic activity from posterior to frontal cortical areas and the origin of epileptic motor phenomena, such as myoclonus (Strigaro *et al.*, 2015b). We propose that abnormal V1 excitability (Strigaro *et al.*, 2012), coupled with some substantial physiologic changes in the visuomotor network (Strigaro *et al.*, 2013; Suppa *et al.*, 2015b) likely underlies the PPR, as well as the fast spread of paroxysmal activity from posterior to anterior areas of the brain, which may finally justify the origin of epileptic motor phenomena, such as myoclonus.

2) We explored whether there are any physiological differences in the interhemispheric connection of drug-treated patients with FE and those with IGE. FE patients had a defective inhibitory response of contralateral M1 to inputs travelling from the "focal" hemisphere that was residual to the drug action. Whilst IHI changes would not be crucial

for the IGE pathophysiology, they may represent one key factor for the contralateral spread of focal discharges, and seizure generalization (Strigaro et al., 2016).

3) Abnormal cortical plasticity has been hypothesized to play a crucial role in the pathogenesis of JME. Therefore, the motor cortex LTP-like synaptic plasticity was explored by means of PAS in a cohort of patients with JME with and without PPR. The present data provided evidence of a defective LTP-like plasticity in patients with JME, which may be primarily involved in the pathogenesis of myoclonus (Strigaro *et al.*, 2015a).

In the future, we aim to further increase understanding into the specific networks involved in the pathophysiology of different types of epilepsy. We aim to define specific neurophysiological phenotypes which may be the expression of unique molecular alterations and translate the findings into clinically useful parameters. Further investigation into the pathophysiology of these diseases would increase understanding into the ictogenesis of human epilepsies and the neural networks involved and eventually open new therapeutic targets.

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