# Capturing the epileptic trait: cortical excitability measures in patients and their unaffected siblings

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# Capturing the epileptic trait: cortical excitability measures in patients and their unaffected siblings

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**We used transcranial magnetic stimulation to investigate whether the cortical excitability changes observed amongst the different generalized and focal epilepsy syndromes are reflected in their asymptomatic siblings and if these changes depended on the clinical phenotype. We studied 157 patients with epilepsy (95 generalized and 62 focal) and their asymptomatic siblings (138 and 82, respectively). Motor threshold and paired pulse transcranial magnetic stimulation at short (2, 5, 10 and 15 ms) and long (100–300 ms) interstimulus intervals were measured. Results were compared to those of 12 control subjects and 20 of their siblings. There were no differences in cortical excitability between healthy control subjects and their siblings. Compared with control subjects, cortical excitability was higher in siblings of patients whether generalized ($P < 0.05$; short and long interstimulus intervals) or focal ($P < 0.05$; long interstimulus intervals). Compared with epilepsy, motor threshold was lower ($P < 0.05$) in patients with juvenile myoclonic epilepsy compared with their siblings only early at onset in the drug naïve state. In all groups (generalized and focal) cortical excitability was lower in siblings only at the long interstimulus intervals (250 and 300; $P < 0.05$). Cortical excitability is higher in asymptomatic siblings of patients with generalized and focal epilepsy in a similar manner. The disturbance seems to involve intracortical inhibitory circuits even in the siblings of patients with a structural abnormality (acquired epilepsy). This implies there are certain genetic factors that predispose to both generalized and focal epilepsies and a complex genetic/environmental interaction then determines the clinical phenotype.**

**Keywords:** asymptomatic siblings; cortical excitability; epilepsy; seizures; transcranial magnetic stimulation
**Abbreviation:** TMS = transcranial magnetic stimulation

## Introduction

The epilepsies are a diverse group of conditions characterized and defined by paroxysmal activity in populations of cortical neurons that result in recurrent seizures. There is considerable evidence that there is an underlying genetic contribution to this condition and indeed a substantial subgroup of the epilepsies are specifically classified as 'genetic' epilepsies (Berg *et al*., 2010). Furthermore at the other end of the spectrum, there is mounting evidence that even lesional and post-traumatic epilepsies are underpinned by a genetic predisposition (Scher *et al*., 2011; Kabat and Krol, 2012). While a complex interaction between genetic and possibly

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environmental factors is likely to influence the particular seizure phenotype seen in these syndromes (Berkovic *et al*., 2006), alteration of ion channels arising from genetic variation or functional modification, is a potential unifying theme for the hyperexcitability seen in individuals with epilepsy (Helbig *et al*., 2008).

Many large-scale epidemiological studies show that ~5% of patients have a first-degree relative with epilepsy (Beaussart and Loiseau, 1969; Bianchi *et al*., 2003). In fact, the degree of familial aggregation in the epilepsies is relatively high for a complex disorder and is consistent with strong familial risk factors (Burton *et al*., 2005). Because of that, family studies that compare epilepsy-affected and unaffected family members have provided a powerful tool in identifying other common and presumably genetically based characteristics including behavioural traits (Hesdorffer *et al*., 2012), cognitive abnormalities (Wandschneider *et al*., 2010), anatomy (Scanlon *et al*., 2013) and EEG characteristics (Rodin and Gonzalez, 1966; Doose *et al*., 1977; Atakli *et al*., 1999; Akgun *et al*., 2009) in epilepsy. The reports of abnormalities on up to 50% of the EEG recordings of asymptomatic first degree family members of patients with epilepsy (Rodin and Gonzalez, 1966; Atakli *et al*., 1999; Akgun *et al*., 2009) as well as high amplitudes in cortical somatosensory evoked potentials in asymptomatic siblings of patients with juvenile myoclonic epilepsy (Atakli *et al*., 1999) suggest a common tendency for a hyperexcitable cortex that may or may not present itself as epilepsy. It would thus be interesting to interrogate patterns of change in cortical excitability in affected and unaffected family members.

Transcranial magnetic stimulation (TMS) provides an excellent safe and sensitive *in vivo* means of measuring human cortical excitability (Rossini and Rossi, 2007). Measurements made using TMS are dependent on small excitatory and inhibitory networks of interneurons and their synaptic interactions with each other and with motor neurons (Rothwell, 1997). Hence TMS is uniquely able to obtain information about the state of excitability of local neuronal circuits *in vivo* in the human brain. Using different TMS testing paradigms, increased cortical excitability was found to be the characteristic feature of the interictal state in patients with a variety of epilepsy syndromes, both at onset and late in the course of the seizure disorders (Reutens and Berkovic, 1992; Cantello *et al*., 2000; Manganotti *et al*., 2000; Werhahn *et al*., 2000; Hamer *et al*., 2005; Badawy *et al*., 2007). The pattern of increase in cortical excitability has provided important insights into the pathophysiology of different forms of epilepsy. Employing TMS to study non-medicated/non-affected siblings of patients with epilepsy has the potential to provide further information on the underlying mechanisms of this complex disorder.

# Materials and methods

## Participant populations

The study protocol was approved by the St Vincent's Hospital Human Research Ethics Committee and written informed consent was obtained from each participant including parental consent from those participants under the age of 18 years. The participants were divided into different groups as follows.

### Non-epilepsy control subjects and their siblings

Twelve healthy participants (seven females) and 20 of their siblings (12 females) with a mean age of 22 years (range 14–45 years) without a personal or family history of seizures or any other neurological conditions. None of the participants were taking medication of any kind. To facilitate result analysis, the participant first recruited was included in the control group and his or her siblings were included in the siblings group. There was no consanguinity in any of the parents and there were no identical twins; however, this group included a non-identical twin couple.

### Patients with epilepsy and their siblings

We report the findings from a subgroup of patients with different generalized and focal epilepsy syndromes. These patients were consecutively recruited through (i) Outpatient Epilepsy Clinic; and (ii) screening the databases of the Epilepsy Clinic and Epilepsy Surgery Program at St Vincent's Hospital in Melbourne, Australia. These are tertiary referral centres; the first provides the management of patients with epilepsy and the latter aims for the characterization and pre-surgical evaluation of patients with refractory focal epilepsy.

To prevent any hypothetical effect of prolonged long standing epilepsy and to maintain homogeneity between the groups, only participants under the age of 45 years were included. In addition, participants under the age of 14 years were not included as normal single and paired pulse TMS values in children under this age are non-comparable to older participants and have not been established in children with epilepsy (Quintana, 2005; Garvey and Mall, 2008). Thus patients with childhood absence epilepsy and focal epilepsies of childhood unfortunately could not be included.

The diagnoses were made by at least two experienced epileptologists who were unaware of the study based on clinical history, EEG and imaging findings.

For the purposes of the current study, patients with neurologically normal asymptomatic siblings (range 1–4) within the same age range (14–45 years) who had never experienced any form of seizures were included in a separate analysis. There were no identical twins; however, there were discordant (one affected twin) non-identical twins (Table 1). There were also two consanguineous families as shown in Table 1.

The patients were categorized based on the type of syndrome and status at the time of testing (Table 1).

The syndrome category was subdivided into: (i) 'genetic' (previously named idiopathic) generalized epilepsy syndromes presenting during late childhood, adolescence and early adulthood. These included juvenile myoclonic epilepsy, juvenile absence epilepsy and generalized epilepsy with tonic-clonic seizures alone; and (ii) focal epilepsy syndromes, which were divided into temporal lobe epilepsy and extra temporal lobe epilepsy.

For status at the time of testing our previous studies showed that TMS measures differ depending on whether the patient cohorts are studied at onset prior to exposure to anti-epileptic drugs, become seizure free after medication or continue to have refractory seizures (Badawy *et al*., 2010, 2013). Consequently we further subdivided our groups into: (i) drug naïve new onset epilepsy, these groups comprised patients newly diagnosed with epilepsy. They were recruited on presentation to the clinic and were studied with TMS within the same week and prior to any exposure to anti-epileptic drugs; (ii) refractory seizures, patients were considered refractory if they continued to have ongoing seizures for at least 3 years despite trials of at least two different anti-epileptic drugs at therapeutic doses (Kwan and Brodie,

**Table 1 Demographics of participants included in each group**

| Epilepsy syndrome | Subgroup | Number (number of females) | Age in years (range) | Age of onset in years (range) | Seizure frequency; all types (range) | Anti-epileptic drugs | Number of siblings (number of females) | Mean age of siblings in years (range) |
|---|---|---|---|---|---|---|---|---|
| **Generalized epilepsy** | | | | | | | | |
| **Juvenile myoclonic epilepsy** | **Drug naïve new onset** | 7 (4) | 20 (14–26) | – | 4 (2–8) | None | 11 (7) | 21 (14–30) |
| | **Refractory seizures** | 12 (6) | 25 (15–40) | 18 (12–25) | 6/month (2–14) | VPA, LEV, LTG, TPM | 18 (11) | 25 (14–43) |
| | **Seizure free** | 14 (6) | 22 (15–43) | 18 (12–25) | 0 | VPA, LEV, LTG, TPM | 21 (11*) | 25 (15–45) |
| **Juvenile absence epilepsy** | **Drug naïve new onset** | 4 (2) | 18 (14–23) | – | 4 (2–8) | None | 9 (4) | 20 (15–26) |
| | **Refractory seizures** | 12 (7) | 24 (14–44) | 23 (11–26) | 8/month (2–16) | VPA, LEV, LTG, TPM | 13 (7) | 24 (16–39) |
| | **Seizure free** | 12 (7) | 24 (16–39) | 20 (12–22) | 0 | VPA, LEV, LTG, TPM | 16 (10)# | 22 (15–40) |
| **Generalized epilepsy with tonic-clonic seizures** | **Drug naïve new onset** | 7 (3) | 20 (15–31) | – | 1 (1–2) | None | 17 (10)* | 23 (16–44) |
| | **Refractory seizures** | 14 (9) | 25 (16–43) | 22 (10–25) | 4/year (2–12) | VPA, LEV, LTG, TPM | 20 (9) | 20 (14–45) |
| | **Seizure free** | 13 (7) | 26 (16–45) | 19 (11–26) | 0 | VPA, LEV, LTG, TPM | 13 (6) | 27 (15–40) |
| **Focal epilepsy** | | | | | | | | |
| **Temporal lobe epilepsy** | **Drug naïve new onset** | 6 (3) | 24 (14–32) | – | 4 (1–6) | None | 7 (4)# | 23 (14–40) |
| | **Refractory seizures** | 14 (8) | 27 (17–45) | 20 (13–29) | 7/month (2–14) | CBZ, GBP, LAC, LEV, LTG, OXC, TPM, VPA | 18 (9) | 25 (16–42) |
| | **Seizure free** | 14 (7) | 26 (17–45) | 22 (11–30) | 0 | CBZ, GBP, LAC, LEV, LTG, OXC, TPM, VPA | 16 (8) | 26 (14–45) |
| **Extra-temporal lobe epilepsy** | **Drug naïve new onset** | 5 (2) | 25 (14–32) | – | 3 (1–5) | None | 11 (4) | 22 (14–40) |
| | **Refractory seizures** | 12 (6) | 27 (19–43) | 22 (13–29) | 8/month (2–14) | CBZ, GBP, LAC, LEV, LTG, OXC, TPM, VPA | 15 (8)* | 23 (15–42) |
| | **Seizure free** | 11 (5) | 29 (17–40) | 24 (11–31) | 0 | CBZ, GBP, LAC, LEV, LTG, OXC, TPM, VPA | 15 (8) | 27 (15–45) |

*A discordant twin couple; #A family with consanguineous parents.
CBZ = carbamazepine; GBP = gabapentin; LAC = lacosamide; LEV = levetiracetam; LTG = lamotrigine; OXC = oxcarbazepine; TPM = topiramate; VPA = sodium valproate.

2000; Kwan *et al*., 2010). This included generalized or secondarily generalized tonic-clonic seizures, absences, myoclonic seizures, focal seizures with loss of awareness and unequivocal focal seizures comprising visual, auditory, motor, sensory or autonomic manifestations with retained awareness. Isolated infrequent non-specific vague feelings, uneasiness or brief déjà vu were not considered seizures; and (iii) seizure free, patients were included in the seizure free group if they did not experience any of the seizures described above for at least 12 months before the TMS test.

## Patient inclusion criteria

Patients were included in the study if they had: (i) generalized epilepsy, generalized epileptiform abnormalities (3.5–5 Hz spike-wave) on at least one EEG recording and a history of generalized tonic-clonic seizures, myoclonic and/or absence seizures; (ii) focal epilepsy, syndromic classification required that the seizure symptomatology (specifically characteristics of the aura when consistently present) and the EEG showed either a left or right-sided lateralization as well as localization to a certain lobe. The EEG was considered localizing only if definite and prominent sharp-slow discharges were seen consistently over one region either frontal (Fp1-Fz-F3/ Fp2-Fz,F4), temporal (T1-T3/ T2/T4), parietal (P3-C3/P4-C4) or occipital (O1/O2). Patients with temporal intermittent rhythmic delta activity were included in the temporal lobe epilepsy group only if the activity was consistently recorded over one hemisphere. Non-specific slowing or sharp waves were not considered lateralizing or localizing even if only recorded on one side. Further localizing signs were found on brain magnetic resonance images. Imaging was only routinely performed on patients thought to have focal epilepsy. The findings were available for all patients and are summarized in Table 2; and (iii) normal neurological examination.

## Patient exclusion criteria

Patients were excluded from the study for the following: (i) suspicion of non-epileptic events (psychogenic non-epileptic seizures, migraine, parasomnias etc.); (ii) had an undetermined epilepsy syndrome (not clear whether generalized or focal epilepsy); (iii) seizure foci originating in the vicinity of the motor area (seizure semiology or on imaging); (iv) bilateral seizure foci; (v) in the drug naïve new onset groups only: exposure to anti-epileptic drugs prior to the TMS study; and (vi) previous cortical resections or craniotomies.

**Table 2 Findings on MRI in each of the focal epilepsy groups**

| Epilepsy syndrome | Subgroup | Findings |
|---|---|---|
| Temporal lobe epilepsy | Drug naïve new onset | 1 patient with hypertrophied amygdala<br>5 patients lesion negative |
| | Refractory seizures | 4 patients with hippocampal sclerosis<br>2 patients with cortical dysplasia<br>1 patient with a dysembryoplastic neuroepithelial tumour<br>7 patients lesion negative |
| | Seizure free | 2 patients with hippocampal sclerosis<br>1 patient with temporal cyst<br>11 patients lesion negative |
| Extra-temporal lobe epilepsy | Drug naïve new onset | 1 patient with frontal cyst<br>4 patients lesion negative |
| | Refractory seizures | 1 patient with parieto-occipital gliosis<br>1 patient with parietal cortical dysplasia<br>2 patients with frontal cortical dysplasia<br>1 patient with frontal gliosis<br>1 patient with occipital gliosis<br>1 patient with occipital cyst<br>8 patients lesion negative |
| | Seizure free | 1 patients with frontal cyst<br>1 patient with occipital cyst<br>9 patients lesion negative |

### Sibling exclusion criteria

Siblings of patients were excluded from the study for the following: (i) a history of seizures, migraine or any other neurological condition; (ii) suffering from any medical condition at the time of the study; (iii) history of head trauma, or craniotomies; (iv) previous exposure to anti-epileptic drugs; and (v) currently taking medication of any kind.

## Electroencephalography recording

All patients and each of their siblings underwent a routine clinical EEG recording using the standard 10–20 electrode system on the same day of TMS before the testing session. Digital EEG Recordings were performed on an E-series EEG amplifier using Profusion EEG4 software (Compumedics Ltd.). The EEG recording session lasted at least 25 min, including hyperventilation and photic stimulation.

## Transcranial magnetic stimulation

Both hemispheres were studied in each participant (patients and control subjects). During TMS, the participants sat in a comfortable, reclining chair. Surface electromyographic (EMG) recording was made from the abductor pollicis brevis muscle. Stimuli were delivered to the contralateral cerebral hemisphere by applying the appropriate direction of coil current flow (anticlockwise for left cortical stimulation and clockwise for right cortical stimulation), using a flat circular 9-cm diameter magnetic coil (14 cm external diameter) with the centre of the coil positioned over the vertex and held in a plane tangential to it using a pair of Magstim 200 magnetic stimulators. Paired stimulation at various interstimulus intervals was performed using a Bistim module to connect two stimulators to the coil.

The motor evoked potentials were recorded and digitized online through a CED 1401 interface (Cambridge Electronic Design Ltd) and stored on computer for offline analysis. Signal software (Cambridge Electronic Design Ltd) was used for automated acquisition and marking of the recorded motor evoked potentials. Filters for the acquisition were set to low frequency of 10 Hz and high frequency of 5 kHz. Sweep speed for threshold determination and paired pulse TMS at short interstimulus intervals was 100 ms and the sensitivity was set to 100 μV/division. For longer interstimulus intervals the sweep was adjusted to 500 ms and sensitivity to 2 mV/division. The motor evoked potential amplitude was measured from peak to peak.

The experimental session lasted for 60–90 min and the following parameters were recorded.

### Motor threshold

Motor threshold was determined in all tested hemispheres while the participant was at rest, verified by continuous visual and auditory EMG feedback. Stimulation commenced at 30% of maximum output and increased in 5% increments until the motor evoked potential was established. One per cent changes in intensity were then used to measure the threshold value. Motor threshold was defined as the lowest level of stimulus intensity which produced a motor evoked potential in the target muscle of peak-to-peak amplitude $>100\,\mu V$ on $\geqslant 50\%$ of 10 trials (Rossini *et al*., 1994).

### Intracortical inhibition and facilitation

Cortical recovery curves were derived using paired pulse TMS. For the short interstimulus intervals of 2, 5, 10 and 15 ms, the first stimulus was given at 80% of motor threshold and the second stimulus 20% above motor threshold. Ten stimuli at 20% above motor threshold without a preconditioning stimulus were also given. For longer interstimulus intervals, the stimulation intensity was 20% above motor threshold using paired stimuli in 50 ms increments at interstimulus intervals of 100–300 ms. A minimum interval of 15 s was kept between the delivery of each pair of stimuli. Stimuli were given at randomly selected interstimulus intervals until a total of 10 at each interstimulus interval was achieved.

Recovery curves at short interstimulus intervals (2–15 ms) were constructed for each hemisphere using the ratio of the mean peak to peak amplitude of the response [termed test response (TR)] at each interstimulus interval following the conditioning stimulus given below motor threshold expressed as the percentage of the mean motor evoked potential (MEP) when the test stimulus was given alone without a preconditioning stimulus (TR/MEP%).

Recovery curves at longer interstimulus intervals (100–300 ms) were constructed for each hemisphere using the ratio of the mean peak-to-peak amplitudes of the response to the second stimulus termed the test response (TR) and the response to the first stimulus termed the conditioning response (CR) at each interstimulus interval measured as a percentage (TR/CR%).

To avoid any effect of diurnal variation in cortical excitability all studies were performed between 10 am and 3 pm. Care was taken to avoid clustering of any of the participants in a group to a particular time, and the studies were spread evenly over this time interval in all groups. Similarly, to avoid any hormonal effects related to variations across the menstrual cycle, care was taken to avoid clustering of the female participants in each group to a particular phase of the cycle and they were spread evenly across the two phases (luteal and follicular) in each group. It was requested of all participants to maintain regular sleep patterns with 7 to 9 h of sleep the night before the test. In patients, the results were only analysed after a minimum of 2 days of seizure freedom on either side of the study was confirmed. This was based on seizure diaries. No patients were excluded as a result of seizures. In addition, no patient suffered a seizure during the TMS study.

Each participant was given a unique alpha numeric code. This was the only identifying feature on the TMS data acquired. The analysis was performed after all participants had been tested. This ensured that the investigator analysing the TMS results was blinded to clinical information during the analysis.

## Statistical analysis

The results from patients with generalized epilepsy, non-epilepsy control subjects and all their respective siblings were analysed according to hemisphere dominance. This was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). In patients with focal epilepsy the results were analysed according to the ipsilateral (hemisphere with presumed seizure focus) and contralateral hemisphere. This was based on electroclinical and imaging findings.

Intergroup comparisons between clinical features (age, age at onset of seizures, gender and seizure frequency) and anti-epileptic drug type with change in cortical excitability was performed using paired *t*-test and the chi-square test. This was done for each subgroup within each syndrome and the homologous subgroup in the other syndromes within its group (e.g. refractory juvenile myoclonic epilepsy with refractory juvenile absence epilepsy and refractory generalized epilepsy with tonic-clonic seizures etc.).

For cortical excitability measures (motor threshold and interstimulus intervals) a two-way ANOVA was used. Each ANOVA had a between-participants factor 'group' (patients and siblings of each group with juvenile myoclonic epilepsy, juvenile absence epilepsy, generalized epilepsy with tonic-clonic seizures, non-epilepsy control subjects and patients and siblings of each group with temporal lobe epilepsy, extra-temporal lobe epilepsy, non-epilepsy) and a within-participant factor 'hemisphere' (interhemispheric comparison).

For all analyses, $P < 0.05$ was chosen as the significance level. Fisher's Protected Least Significant Difference *post hoc* tests were performed as appropriate. The analysis was performed on SPSS, 15.0 for Windows®.

The effect size was calculated for the significant results (motor threshold and each interstimulus interval) using the formula:

Effect size = mean of siblings – mean of non-epilepsy controls / standard deviation of non-epilepsy controls

The same formula was used comparing patients in each group to their respective siblings, where siblings were considered the control group.

Effect size 0.2 was considered small, 0.5 medium and $\geqslant$ 0.8 large (Cohen, 1969).

# Results

## Electroclinical findings

There were no intergroup differences in age, gender, age of seizure onset, seizure frequency or different combinations of the anti-epileptic drugs used in any of the patient sub-groups. There were also no intergroup differences in age and gender between patients and their respective siblings or between any of the sibling groups (Table 1).

Table 3 summarizes the EEG findings recorded from each patient group and their respective siblings on the day TMS was performed.

## Cortical excitability measures

The results from both hemispheres were analysed in all patients. The only group that showed interhemispheric differences was the drug naïve new onset focal epilepsy group. In all the others, there were no interhemispheric differences. Consequently the results of both hemispheres will only be shown for the focal epilepsy groups. For the remaining groups (generalized epilepsy and non-epilepsy control subjects and their respective siblings) only the results of the dominant hemisphere are shown.

The results in patients with different epilepsy syndromes are not the subject of this paper and will be presented in detail and discussed in a separate report. Here they are shown for reference only and are summarized in Table 4.

### Cortical excitability amongst siblings in healthy non-epilepsy control subjects

The control group showed the expected results in both the short and long interstimulus interval recovery curves, with inhibition of the test response at interstimulus intervals 2 and 5 ms and facilitation at interstimulus intervals 10 and 15 ms, and test responses approaching unity at all the tested long interstimulus intervals. There was no difference between control subjects and their siblings in motor threshold (Table 4) or at any interstimulus interval (Fig. 1).

### Cortical excitability in siblings of patients with different 'genetic' generalized epilepsy syndromes

#### Motor threshold

The only difference in motor threshold was observed between patients in the drug naïve new onset juvenile myoclonic epilepsy

**Table 3 Percentage of participants with EEG abnormalities recorded on the day of the TMS test in each group**

| Group | Subgroup | Per cent with definite epileptiform discharges | Per cent with non-specific abnormalities |
|---|---|---|---|
| **Juvenile myoclonic epilepsy** | Drug naïve, new onset | 71 (12) | 29 (18) |
| | Refractory seizures | 83 (13) | 17 (20) |
| | Seizure free | 42 (11) | 44 (18) |
| **Juvenile absence epilepsy** | Drug naïve, new onset | 75 (11) | 25 (18) |
| | Refractory seizures | 75 (11) | 25 (20) |
| | Seizure free | 41 (9) | 42 (15) |
| **Generalized epilepsy with tonic-clonic seizures** | Drug naïve, new onset | 57 (8) | 43 (14) |
| | Refractory seizures | 71 (10) | 29 (10) |
| | Seizure free | 38 (8) | 30 (9) |
| **Temporal lobe epilepsy** | Drug naïve, new onset | 23 (0) | 40 (14) |
| | Refractory seizures | 36 (7) | 42 (12) |
| | Seizure free | 15 (0) | 21 (15) |
| **Extra-temporal lobe epilepsy** | Drug naïve, new onset | 23 (0) | 30 (11) |
| | Refractory seizures | 33 (7) | 36 (14) |
| | Seizure free | 17 (0) | 27 (11) |

Values refer to patients and numbers in brackets refer to siblings. Definite epileptiform discharges included spike or poly-spike-waves, sharp-slow waves and temporal intermittent rhythmic delta activity. Non-specific abnormalities included slowing (diffuse or lateralized) and/or sharp waves.

group and their siblings (Table 5). Motor threshold was lower (denoting increased excitability) in patients compared with their siblings. There were no other differences in motor threshold between the patients and their siblings in the other two juvenile myoclonic epilepsy groups or in any of the groups with juvenile absence epilepsy or generalized epilepsy with tonic-clonic seizures.

There were no intergroup differences in motor threshold between the siblings of any of the generalized epilepsy groups or on comparison with non-epilepsy control subjects (Table 5).

### Intracortical inhibition and facilitation

*Comparison with non-epilepsy control subjects*

An increase in cortical excitability was observed on comparing patients' siblings and non-epilepsy control subjects at the short interstimulus interval 2 ms ($P < 0.05$, effect sizes 0.2–0.4) and long interstimulus intervals 150, 250 and 300 ms ($P < 0.05$, effect sizes 0.3–0.7), being most prominent in siblings of patients with juvenile myoclonic epilepsy, more so those with refractory seizures (Fig. 2).

### Comparison with patients with generalized epilepsy

In the drug naïve-new onset groups, cortical excitability was higher in patients compared with their siblings at the 150, 250 and 300 ms interstimulus intervals ($P < 0.01$, effect sizes ranging 0.5–0.7; maximum in juvenile myoclonic epilepsy). There were no other differences at any of the short or remaining long interstimulus intervals (Fig. 2).

In patients with refractory seizures, cortical excitability was higher at all the long interstimulus intervals in patients compared with their siblings ($P < 0.01$, effect sizes ranging 0.4–0.9; maximum in juvenile myoclonic epilepsy; Fig. 2). There were no differences at any of the short interstimulus intervals.

There were no differences at any interstimulus interval between seizure free patients with juvenile myoclonic epilepsy, juvenile absence epilepsy or generalized epilepsy with tonic-clonic seizures and their respective siblings (Fig. 2).

There were no intergroup differences at any interstimulus interval between the siblings of any of the generalized epilepsy groups.

## Cortical excitability in siblings of patients with different focal epilepsy syndromes

### Motor threshold

There were no differences between any of the patient groups with either temporal lobe epilepsy or extra-temporal lobe epilepsy and their respective siblings.

There was no intergroup difference in motor threshold between the siblings of any of the focal epilepsy groups or on comparison with non-epilepsy control subjects (Table 5).

### Intracortical inhibition and facilitation

There were no interhemispheric differences in the siblings of either group (temporal lobe epilepsy or extra-temporal lobe epilepsy).

### Comparison with non-epilepsy control subjects

An increase in cortical excitability was observed on comparing patient's siblings and non-epilepsy control subjects at the long interstimulus intervals 250 and 300 ms ($P < 0.05$, effect sizes 0.3–0.6), being most prominent in siblings of patients with refractory seizures in both groups (Fig. 3).

### Comparison with patients with focal epilepsy

In the drug naïve-new onset groups, cortical excitability was higher in the ipsilateral hemispheres of patients (both temporal lobe epilepsy and extra-temporal lobe epilepsy) compared with both hemispheres of their siblings at the 250 and 300 ms interstimulus intervals ($P < 0.05$, effect sizes ranging 0.3–0.6). There were no other differences at any of the short or remaining

**Table 4** Main TMS findings in all the patient groups and their respective siblings

| Group | Subgroup | Patients | | Siblings | |
|---|---|---|---|---|---|
| | | Motor threshold | Paired-pulse TMS | Motor threshold | Paired-pulse TMS |
| **JME** | Drug naïve, new onset | Decreased | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with JAE and GE-TCS and controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at the 150, 250 and 300 ms ISI compared with patients.<br>No differences in short ISIs compared with patients. |
| | Refractory seizures | – | Increased excitability at the 2 and 5 ms and all long ISIs compared with JAE and GE-TCS and controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at all long ISIs compared with patients.<br>No differences in short ISIs compared with patients. |
| | Seizure free | – | Increased excitability at the 150, 250 and 300 ms ISIs compared with controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>No differences in short or long ISIs compared with patients. |
| **JAE** | Drug naïve, new onset | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at the 150, 250 and 300 ms ISI compared with patients.<br>No differences in short ISIs compared with patients. |
| | Refractory seizures | – | Increased excitability at the 2 and 5 ms and all long ISIs compared with controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at all long ISIs compared with patients.<br>No differences in short ISIs compared with patients. |
| | Seizure free | – | Increased excitability at the 250 and 300 ms ISIs compared with controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>No differences in short or long ISIs compared with patients. |
| **GE-TCS** | Drug naïve, new onset | | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls. | | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at the 150, 250 and 300 ms ISI compared with patients.<br>No differences in short ISIs compared with patients. |

(continued)

**Table 4** Continued

| Group | Subgroup | Patients | | Siblings | |
|---|---|---|---|---|---|
| | | Motor threshold | Paired-pulse TMS | Motor threshold | Paired-pulse TMS |
| | Refractory seizures | – | Increased excitability at the 2 and 5 ms and all long ISIs compared with controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at all long ISIs compared with patients.<br>No differences in short ISIs compared with patients. |
| | Seizure free | – | Increased excitability at the 250 and 300 ms ISIs compared with controls. | – | Increased excitability at the 2 and 5 ms and 150, 250 and 300 ms ISIs compared with controls.<br>No differences in short or long ISIs compared with patients. |
| **TLE** | Drug naïve, new onset | Increased in ipsilateral hemisphere | Increased excitability at the 2 and 5 ms as well as 250 and 300 ms ISIs in ipsilateral hemisphere compared with contralateral hemisphere and controls. | | Increased excitability at the 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at the 250 and 300 ms ISI compared with ipsilateral hemisphere in patients. No differences in short ISIs compared with either hemisphere in patients. |
| | Refractory seizures | Increased in both hemispheres | Increased excitability at the 2 and 5 ms and all long ISIs in both hemispheres compared with controls. | – | Increased excitability at the 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at all long ISIs compared with both hemispheres in patients.<br>No differences in short ISIs compared with patients. |
| | Seizure free | Increased in both hemispheres | Increased excitability at the 250 ms ISI in the ipsilateral hemisphere compared with controls.No differences compared with controls at short ISIs in either hemisphere. | – | Increased excitability at the 250 and 300 ms ISIs compared with controls.<br>No differences in short or long ISIs compared with either hemisphere in patients. |
| **Extra-TLE** | Drug naïve, new onset | – | Increased excitability at the 250 and 300 ms ISIs in ipsilateral hemisphere compared with contralateral hemisphere.<br>Increased excitability at the 2 and 5 ms as well as 250 and 300 ms ISIs in ipsilateral hemisphere compared with controls. | – | Increased excitability at the 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at the 250 and 300 ms ISI compared with ipsilateral hemisphere in patients.<br>No differences in short ISIs compared with either hemisphere in patients. |
| | Refractory seizures | Increased in both hemispheres | Increased excitability at the 2 and 5 ms and all long ISIs in both hemispheres compared with controls. | – | Increased excitability at the 250 and 300 ms ISIs compared with controls.<br>Decreased excitability at all long ISIs compared with both hemispheres in patients.<br>No differences in short ISIs compared with patients. |
| | Seizure free | Increased in both hemispheres | Increased excitability at the 250 ms ISI in the ipsilateral hemisphere compared with controls.<br>No differences compared with controls at short ISIs in either hemisphere. | – | Increased excitability at the 250 and 300 ms ISIs compared with controls.<br>No differences in short or long ISIs compared with either hemisphere in patients. |

GE-TCS = generalized epilepsy with tonic-clonic seizures; ISI = interstimulus interval; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; TLE = temporal lobe epilepsy.

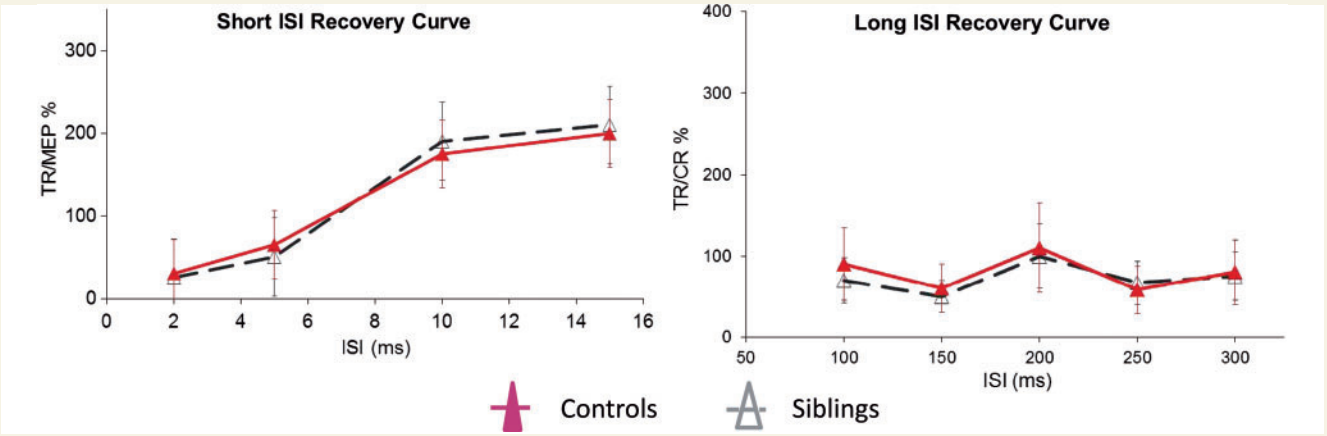


**Figure 1** Short and long interstimulus interval (ISI) recovery curves with error bars for the dominant hemisphere in non-epilepsy control subjects and their siblings. Ratios <100% indicate inhibition and ratios >100% indicate facilitation. CR = conditioning response; MEP = motor evoked potential; TR = test response.

**Table 5 Motor threshold (mean ± SD) for each participant group (patients and control subjects) and their siblings**

| Group | Subgroup | | Participants /patients motor threshold (stimulus intensity %) | Siblings motor threshold (stimulus intensity %) |
|---|---|---|---|---|
| **Non-epilepsy controls** | | | 55.4 ± 5.7 | 54.9 ± 4.8 |
| **Juvenile myoclonic epilepsy** | Drug naïve, new onset | | 49.3 ± 7.1 | 54.2 ± 5.5 |
| | Refractory seizures | | 53.8 ± 5.2 | 55.0 ± 5.2 |
| | Seizure free | | 56.4 ± 7.1 | 54.6 ± 6.6 |
| **Juvenile absence epilepsy** | Drug naïve, new onset | | 54.7 ± 5.3 | 55.2 ± 6.2 |
| | Refractory seizures | | 55.3 ± 5.5 | 56.2 ± 4.7 |
| | Seizure free | | 56.9 ± 4.8 | 55.6 ± 6.4 |
| **Generalized epilepsy with tonic-clonic seizures** | Drug naïve, new onset | | 53.9 ± 7.9 | 53.7 ± 4.6 |
| | Refractory seizures | | 55.7 ± 4.6 | 53.9 ± 7.3 |
| | Seizure free | | 57.1 ± 3.7 | 55.9 ± 5.6 |
| **Temporal lobe epilepsy** | Drug naïve, new onset | Hemisphere A | 59.4 ± 5.8 | 53.4 ± 6.3 |
| | | Hemisphere B | 54.2 ± 5.7 | 55.1 ± 3.9 |
| | Refractory seizures | Hemisphere A | 60.6 ± 5.2 | 52.7 ± 6.6 |
| | | Hemisphere B | 60.7 ± 4.8 | 55.8 ± 7.0 |
| | Seizure free | Hemisphere A | 62.6 ± 5.1 | 54.9 ± 5.6 |
| | | Hemisphere B | 60.9 ± 5.7 | 55.7 ± 6.1 |
| | | | 61.1 ± 4.9 | 55.2 ± 5.6 |
| **Extra-temporal lobe epilepsy** | Drug naïve, new onset | Hemisphere A | 57.3 ± 6.1 | 54.8 ± 6.2 |
| | | Hemisphere B | 55.5 ± 5.6 | 55.1 ± 7.3 |
| | Refractory seizures | Hemisphere A | 61.1 ± 4.8 | 52.7 ± 5.5 |
| | | Hemisphere B | 60.5 ± 6.3 | 53.5 ± 6.4 |
| | Seizure free | Hemisphere A | 63.0 ± 5.9 | 52.9 ± 7.6 |
| | | Hemisphere B | 61.7 ± 5.9 | 54.6 ± 4.9 |

Hemisphere A = the ipsilateral hemisphere (hemisphere with seizure focus) in patients and the dominant hemisphere in siblings; Hemisphere B = the contralateral hemisphere in patients and the non-dominant hemisphere in siblings.

long interstimulus intervals (Fig. 3). There were no differences between the contralateral hemispheres and either hemisphere in their siblings.

In patients with refractory seizures, cortical excitability was higher in both hemispheres at all the long interstimulus intervals in patients with temporal lobe epilepsy and extra-temporal lobe epilepsy compared with their siblings ($P < 0.01$, effect sizes ranging 0.4–0.7; Fig. 3). There were no differences at any of the short interstimulus intervals.

There were no differences at any interstimulus interval between seizure free patients with temporal lobe epilepsy or extra-temporal lobe epilepsy and their respective siblings (Fig. 3).

There was no inter-group difference at any interstimulus interval between the siblings of any of the focal epilepsy groups.

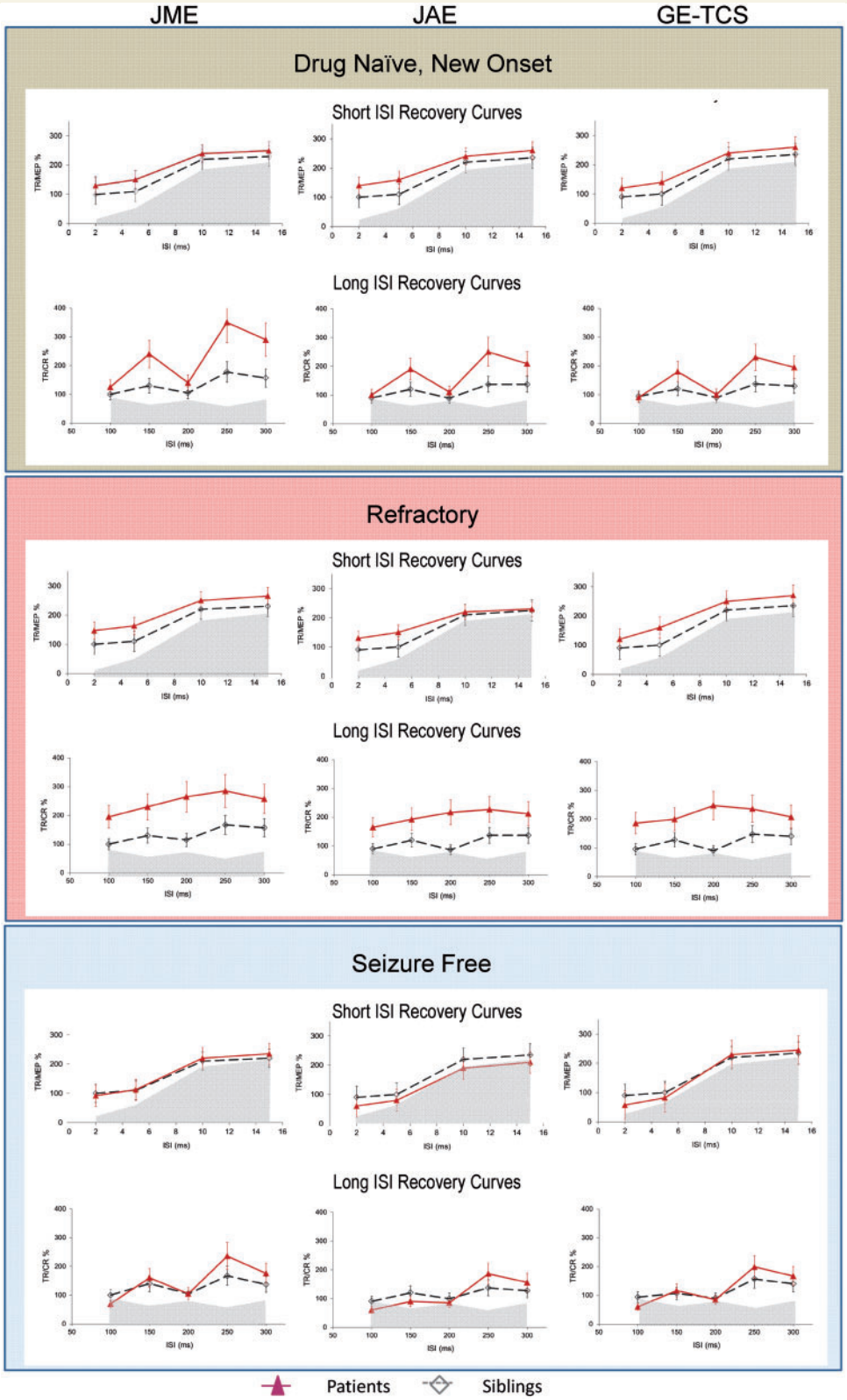


**Figure 2** Short and long interstimulus interval (ISI) recovery curves with error bars for the dominant hemisphere in patients with generalized epilepsy and their siblings. Ratios <100% indicate inhibition and ratios >100% indicate facilitation. The upper boundary of the grey shaded area represents non-epilepsy controls. GE-TCS = generalized epilepsy with tonic-clonic seizures; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; TLE = temporal lobe epilepsy.

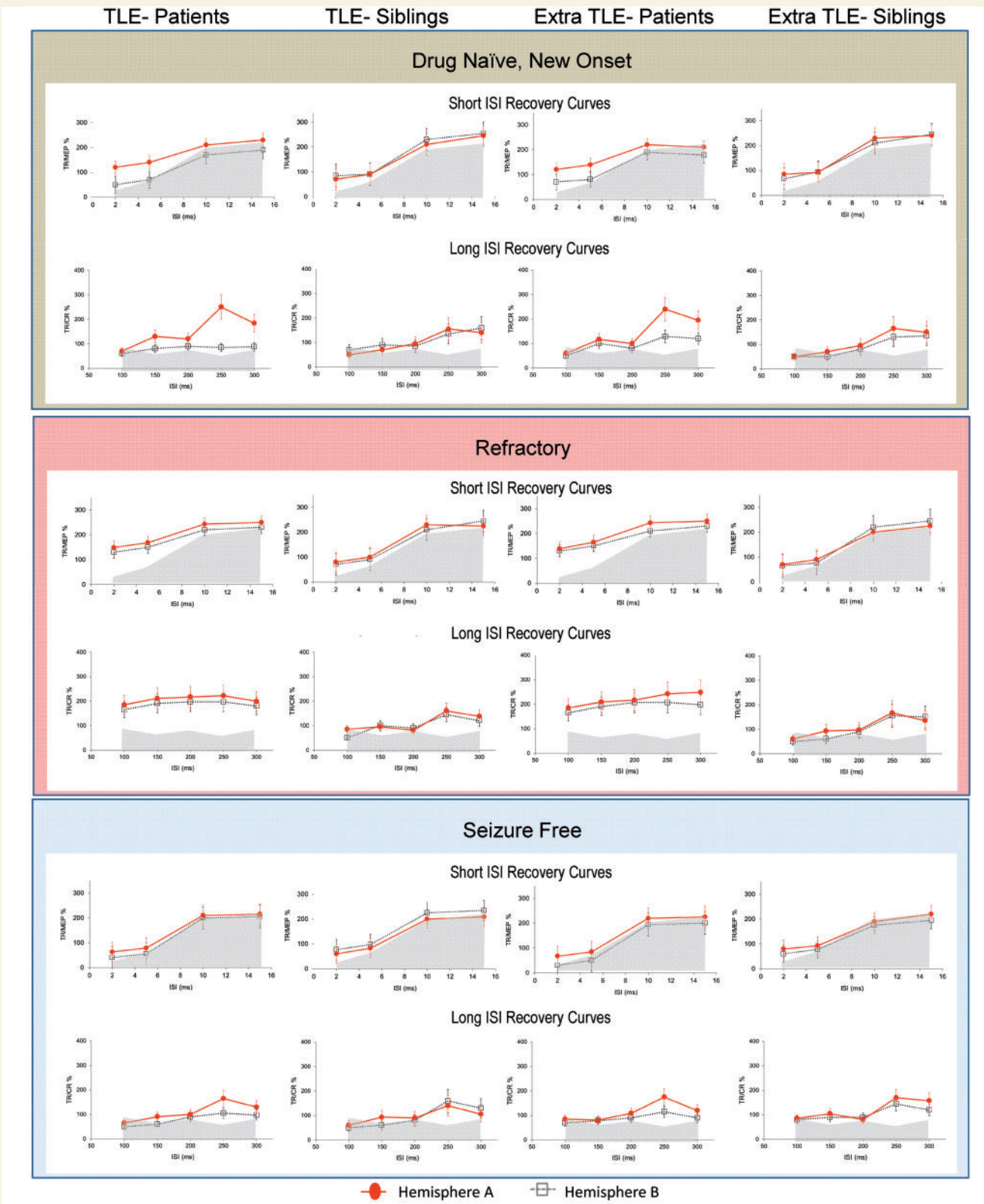


**Figure 3** Short and long ISI recovery curves with error bars for both hemispheres in patients with focal epilepsy and their siblings. Ratios <100% indicate inhibition and ratios >100% indicate facilitation. Hemisphere A = the ipsilateral hemisphere (hemisphere with seizure focus) in patients and the dominant hemisphere in siblings. Hemisphere B = the contralateral hemisphere in patients and the non-dominant hemisphere in siblings. The upper boundary of the grey shaded area represents non-epilepsy controls. GE-TCS = generalized epilepsy with tonic-clonic seizures; JAE = juvenile absence epilepsy; JME = juvenile myoclonic epilepsy; TLE = temporal lobe epilepsy.

# Discussion

In the present study we report that asymptomatic/unaffected siblings of patients with epilepsy have a similar cortical excitability profile to their affected siblings. We found evidence of cortical hyper-excitability in the asymptomatic siblings of patients with various types of generalized and focal epilepsy, being more prominent in siblings of patients with generalized epilepsy syndromes. This suggests an underlying increased susceptibility for a lowered seizure threshold in families with epilepsy.

## Electroencephalography abnormalities

While the current investigation concentrated on using TMS to study cortical excitability, we also conducted a routine EEG on patients on the day of study and the results of those studies also revealed aspects of increased excitability. We found epileptiform discharges in the EEG of 83% of patients with generalized epilepsy and 30% of patients with focal epilepsy. This is similar to previous reports (Goodin and Aminoff, 1984; Gregory *et al*., 1993; Glick, 2002). Also as expected, the percentage was highest in juvenile myoclonic epilepsy, and in all groups was more frequently seen in patients with refractory seizures (Sundaram *et al*., 1990).

Furthermore, in asymptomatic siblings definite epileptiform discharges were recorded in up to 13% and non-specific abnormalities were found in a further 20%. Again this was more common in the siblings of patients with juvenile myoclonic epilepsy and notably in most groups was more common if the patients were refractory. These findings of increased prevalence of interictal epileptiform discharges in siblings of patients with epilepsy is similar to that reported elsewhere (Doose *et al*., 1977; Atakli *et al*., 1999; Akgun *et al*., 2009). Abnormalities were reported in many more siblings of patients with generalized (50%) and focal epilepsy (33%) in an earlier study (Rodin and Gonzalez, 1966). This latter study, however, did not categorize the abnormalities into definitely epileptiform and non-specific categories, and this could explain the higher incidence. It is known that ~10% of normal people may have non-specific EEG abnormalities and ~1% may have epileptiform paroxysmal activity without seizures (Gregory *et al*., 1993). Thus our results indicate a much higher prevalence of epileptiform discharges in families with epilepsy compared with the general population and suggest an underlying increased excitability in those cohorts. The substrate underlying this increase in cortical excitability was investigated using TMS to quantify motor threshold as well as intracortical inhibition and facilitation (using short and long recovery curves).

## Patterns of disturbances in cortical excitability

In the current study, cortical excitability was higher in patients with juvenile myoclonic epilepsy compared with the other two generalized epilepsy syndromes. This effect was not replicated in the siblings of these cohorts who all showed a similar increase in cortical excitability regardless of syndrome. In all groups with generalized epilepsy the siblings showed increased cortical excitability at the short interstimulus intervals of 2 and 5 ms and the long interstimulus intervals 150, 250 and 300 ms compared with control subjects. Cortical excitability was lower at the same long interstimulus intervals in siblings compared with patients, with no differences at short interstimulus intervals observed. These findings indicate that there is indeed a degree of cortical hyper-excitability or defective inhibition in siblings of patients with generalized epilepsy regardless of type of syndrome. Similarly, despite the differences at least in the early drug naïve state between patients with temporal lobe epilepsy and extra-temporal lobe epilepsy, cortical excitability is increased in the siblings of these two cohorts in a similar manner. It was only observed in the long interstimulus intervals of 250 and 300 ms compared with control subjects. But when compared with patients, there were also no differences at any of the short interstimulus intervals suggesting a mild degree of hyperexcitability at the short interstimulus intervals, although it is not as prominent as that observed in generalized epilepsies. In addition, as seen in the figures, in control participants, the long recovery curve showed a small peak at 200 ms, with a dip at 250 ms and a gradual increase toward the end of the curve. This pattern was absent in the siblings of patients (generalized and focal) where a pattern similar to that observed in drug naïve patients (uninfluenced by the effects of anti-epileptic drugs or chronic seizures) was seen. In both those cohorts, the measures at 200 ms remained fairly constant (at ~100% regardless of group), and cortical excitability at the 250–300 ms interstimulus interval increased significantly.

Increased excitability at interstimulus intervals of 2–5 ms most likely represents defective $GABA_A$ mediated mechanisms (Boroojerdi, 2002) whereas the increase at the longer interstimulus intervals is most likely mediated by $GABA_B$ circuits (Mott and Lewis, 1994; McDonnell *et al*., 2007; Florian *et al*., 2008). The findings thus indicate defective function within inhibitory circuits that is likely mediated through abnormal genetic mechanisms. These abnormalities are not only present in generalized epilepsies with a presumed genetic basis, but also in patients with focal epilepsy resulting from a clear structural abnormality known to provoke seizures. This confirms the postulated overlap in the neurodevelopmental genes responsible for both brain structure and the expression of the disease in patients with lesional and post-traumatic epilepsies (Scher *et al*., 2011; Kabat and Krol, 2012).

The findings suggest that there are both shared and distinct genetic components across generalized and focal epilepsy syndromes. Furthermore, given that the vast majority of relatives of patients with epilepsy are clinically healthy (all the siblings investigated in our study were asymptomatic) it would appear that the inheritable genetic trait alone is insufficient to determine the phenotypic presentation, other factors be they environmental or acquired play a role as well.

Studies have shown that although the risk of seizures is higher in first-degree relatives of patients with idiopathic epilepsies than in those of patients with symptomatic epilepsies, the risk is still higher in the latter than in the relatives of control subjects (Ottman *et al*., 1996). In animal models, the expression of

$GABA_A$ receptor subunits have been shown to occur before the development of seizures, suggesting that these changes are causative and that alterations in receptor density occur when seizures are established (Brooks-Kayal *et al*., 1998). Moreover, age dependence of such changes has been observed (Zhang *et al*., 2004). Clinical studies of families that have 'idiopathic epilepsies', including some with genes of large effect, can show diverse phenotypes. This demonstrates that factors other than the 'epilepsy genes'—be they acquired factors or modifier genes—in part determine the phenotype (Scheffer and Berkovic, 1997). The role of minor acquired factors (e.g. breech birth and minor head injuries), acting on a presumed genetic predisposition, is the most contentious (Deymeer and Leviton, 1985). Thus, non-genetic factors might well contribute to epilepsies that have a predominant genetic aetiology; however, the nature and attributable risk of those factors has not yet been elucidated (Berkovic *et al*., 2006).

Another interesting finding in our focal epilepsy cohorts was that cortical excitability increased in both hemispheres in siblings whereas in drug naïve patients with new onset epilepsy, cortical excitability measures in the unaffected hemisphere did not differ compared with control subjects. Cortical excitability measures in that hemisphere were only abnormal in patients with refractory seizures. The lack of lateralization in siblings suggest that in these patients cortical excitability changes are not reflective of focal structural abnormalities but merely as the partial expression of a more diffuse disturbance of excitability within neuronal networks. It would appear that at least early in the course of the disease and prior to the detrimental effect of recurrent seizures, cortical excitability decreases in the unaffected hemisphere possibly as a protective mechanism to prevent seizure spread. This effect is lost with time as a result of recurrent seizures or even (less likely) may be the cause of more seizures. The relationship of neuronal plasticity to epilepsy is complex, with evidence that seizures themselves can alter ion channel gene expression and subunit stoichiometry (Shah *et al*., 2004; Brewster *et al*., 2005), and that resistance to anti-epileptic drugs might be associated with alteration in the function of various channels (Remy and Beck, 2006). Further support for this comes from our recent findings of progressive changes in cortical excitability associated with refractory seizures (Badawy *et al*., 2013). This may be why patients with a seemingly identical diagnosis and drug choice are refractory to medication while others become seizure free.

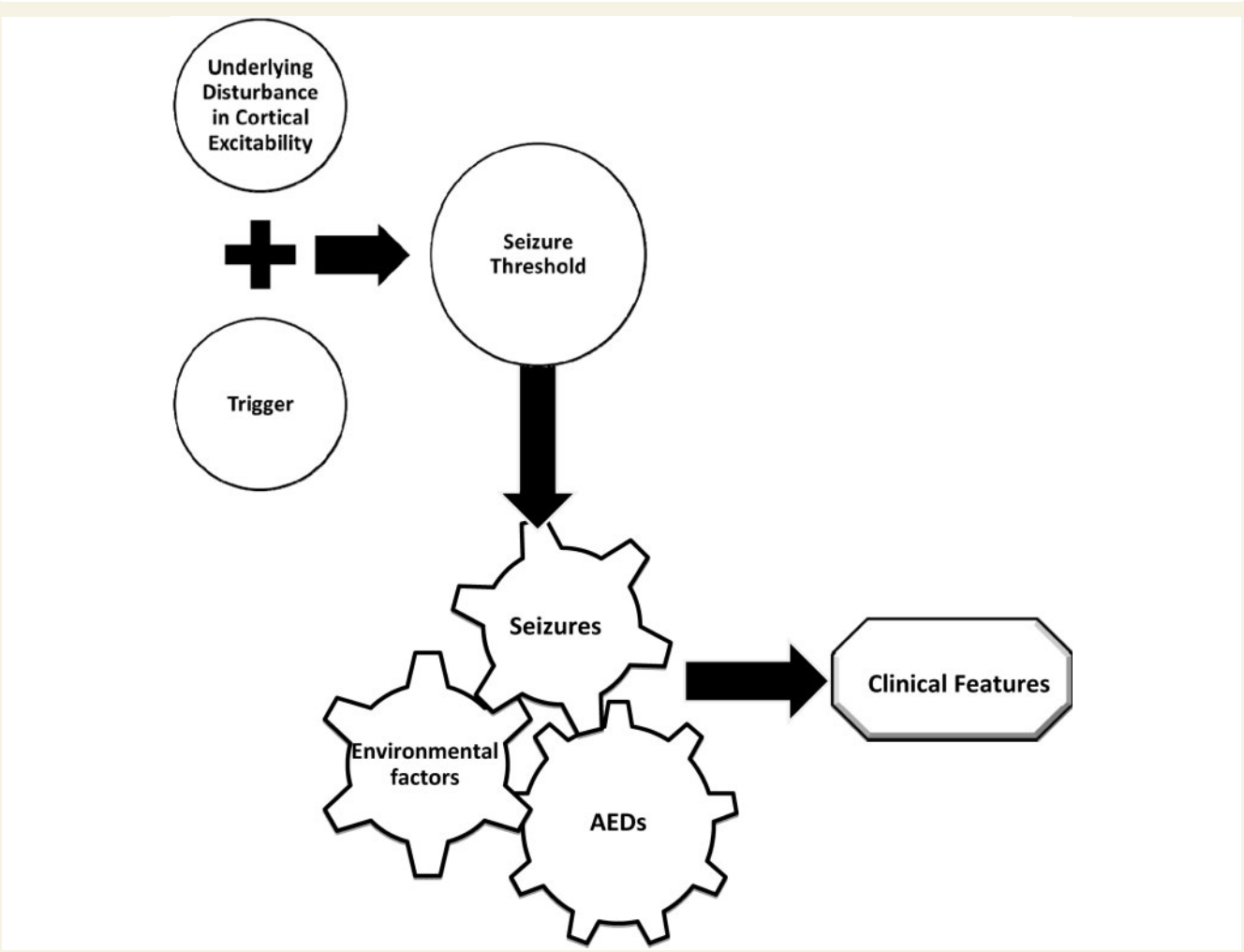


**Figure 4** Schematic illustrating the proposed interaction underlying the epileptic phenotypic presentation. AEDs = anti-epileptic drugs.

## Concluding remarks

An underlying genetic susceptibility for patients to develop epilepsy has been noted from a number of epidemiological studies, and is supported by direct measurement of cortical excitability using TMS. The evidence suggests that while epilepsy is a multifactorial condition, it appears to be unified by channel dysfunction and in turn implicates genes at some basic level, even for the lesional cases. As summarized in Fig. 4, a seizure is often triggered by an initial precipitating event such as an acquired insult or other environmental factors known to be associated with increased likelihood of seizure expression. This underlying genetic susceptibility for seizures subsequent to the initial event is prone to increase, and hence be represented as an epilepsy syndrome. The phenotypic presentation, however, including the frequency of seizures, is an intrinsically complex interaction of various factors including genetic dysfunction, actions of anticonvulsants and their efficacy, along with other concomitant environmental factors influencing seizure susceptibility. The opportunities presented by TMS in directly evoking features of these dynamic susceptibility processes may further elucidate how transitions in clinical presentation are characterized and lead to increased efficacy when tailoring management for these often complex syndromes.

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