Evidence for an excitatory $GABA_A$ response in human motor cortex in idiopathic generalised epilepsy

Benjamin I Silbert[a], Alexandra E Heaton[a], Robin FH Cash[a,b], Ian James[c], John W Dunne[d], Nicholas D Lawn[d], Peter L Silbert[d], Frank L Mastaglia[a], Gary W Thickbroom[a]

[a] Western Australian Neuroscience Research Institute, University of Western Australia, Perth, Western Australia, Australia. [Western Australian Neuroscience Research Institute, 4th Floor, A Block, QEII Medical Centre, Verdun Street, Nedlands, Western Australia 6009, Australia]

[b] Division of Brain, Imaging and Behaviour - Systems Neuroscience, Toronto Western Research Institute, University Health Network, Toronto, Ontario, Canada. [Division of Brain, Imaging and Behaviour - Systems Neuroscience, Toronto Western Research Institute, 339 Bathurst Street, MP14-324, Toronto, Ontario M5T 2S8, Canada]

[c] Centre for Clinical Immunology and Biomedical Statistics, Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Western Australia, Australia. [Centre for Clinical Immunology and Biomedical Statistics, Institute for Immunology and Infectious Diseases, Building 390, Murdoch University, Discovery Way, Murdoch, Western Australia 6150, Australia]

[d] Department of Neurology, Royal Perth Hospital, Perth, Western Australia, Australia. [Department of Neurology, Level 8, A Block, Royal Perth Hospital, GPO Box X2213, Perth, Western Australia 6001, Australia]

Address for Correspondence:

GW Thickbroom

M518, University of Western Australia

Nedlands, Western Australia 6009

Australia

Email: gary.thickbroom@gmail.com Phone: +61 8 9346 4479

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Highlights:

1. TMS was used to explore GABA-mediated inhibition in IGE
2. Post-synaptic $GABA_B$ inhibition (LICI) was normal, but pre-synaptic LICF reduced
3. $GABA_A$ inhibition (SICI) was reduced in untreated but not treated IGE
4. When tested during $GABA_B$ inhibition, the SICI protocol was excitatory
5. During ongoing $GABA_B$ inhibition, $GABA_A$ activation may be excitatory

Abstract

Purpose: Impaired GABAergic inhibition has been implicated in the pathophysiology of epilepsy. The possibility of a paradoxical excitatory effect of GABA in epilepsy has been suggested, but has not been investigated *in vivo*. We investigated pre- and post-synaptic GABAergic mechanisms in patients with idiopathic generalised epilepsy (IGE).

Methods: In 10 patients and 12 control subjects we explored short- and long-interval intracortical inhibition (SICI, LICI; post-synaptic $GABA_A$ and $GABA_B$-mediated respectively) and long-interval intracortical facilitation (LICF; pre-synaptic disinhibition) using transcranial magnetic stimulation.

Results: While post-synaptic $GABA_B$-mediated inhibition was unchanged in IGE (p=0.09), LICF was reduced compared to controls (controls: 141±17% of baseline; untreated patients: 107±12%, p=0.2; treated patients: 79±10%, p=0.003). $GABA_A$-mediated inhibition was reduced in untreated patients (response amplitude 56±4% of baseline vs. 26±6% in controls, p=0.004) and normalised with treatment (37±12%, p=0.5 vs. controls). When measured during LICI, $GABA_A$-mediated inhibition became excitatory in untreated IGE (response amplitude 120±10% of baseline, p=0.017), but not in treated patients.

Conclusions: Pre- and post-synaptic GABA-mediated inhibitory mechanisms are altered in IGE. The findings lend *in vivo* support to evidence from experimental models and *in vitro* studies of human epileptic brain tissue that GABA may have a paradoxical excitatory role in ictogenesis.

Keywords: transcranial magnetic stimulation, epilepsy, cortical excitability, GABA, disinhibition

Abbreviations:

AED: anti-epileptic drug

AED_{NO}: IGE not on AEDs (subject group)

AED_{ON}: IGE on AEDs (subject group)

CS: conditioning stimulus

EEG: electroencephalography

EMG: electromyography

FDI: first dorsal interosseous (muscle)

GABAR: GABA receptor

$GABA_AR$: $GABA_A$ receptor

$GABA_BR$: $GABA_B$ receptor

I_{1mV}: intensity needed to evoke a MEP of 1mV amplitude

IGE: idiopathic generalised epilepsy

ISI: inter-stimulus interval

IPSP: inhibitory post-synaptic potential

JME: juvenile myoclonic epilepsy

KCC2: K^+/Cl^- cotransporter 2

LCD: late cortical disinhibition

LICF: long-interval intracortical facilitation

LICI: long-interval intracortical inhibition

MEP: motor evoked potential

NKCC1: $Na^+/K^+/2Cl^-$ cotransporter 1

PS: priming stimulus

RMT: resting motor threshold

SICI: short-interval intracortical inhibition

TLE: temporal lobe epilepsy

TMS: transcranial magnetic stimulation

TS: test stimulus

TS*: adjusted test stimulus

Introduction

Epilepsy is characterised by neuronal hyperexcitability and hypersynchronicity which manifests as recurrent seizures [1-2]. The pathophysiological processes underlying epilepsy in its various forms remain incompletely understood (for reviews, see Engelborghs et al. [1] and McCormick and Contreras [2]) but an imbalance between excitatory and inhibitory neuronal inputs is usually implicated. Critical to understanding the pathophysiology of epileptogenesis and ictogenesis are abnormalities in synaptic transmission, and in particular in the activity of GABAergic synaptic networks [2-5]. An improved understanding of *in vivo* synaptic function in epilepsy may inform diagnosis and clinical management.

Within the central nervous system, GABAergic activity can regulate neuronal firing, synchronicity and network oscillations [6]. At a cellular level, activation of post-synaptic GABA receptors results in rapid transient ($GABA_AR$-mediated) and longer-lasting ($GABA_BR$-mediated) inhibitory post-synaptic potentials that can modulate or gate firing of the post-synaptic neuron [7-9]. Pre-synaptic $GABA_BRs$ remain active for longer than post-synaptic $GABA_BRs$, and regulate (reduce) further GABA release by inhibiting Ca^{2+} influx, resulting in disinhibition [8, 10-11].

In human motor cortex, the activation of GABARs can be measured with TMS using paired-pulse stimulation. SICI occurs when a sub-threshold conditioning pulse is delivered ~2-6ms before a supra-threshold test pulse; the conditioning pulse elicits a $GABA_AR$-mediated IPSP and thereby reduces the amplitude of the MEP to the test pulse [12-13] . The ratio of the amplitude of the conditioned MEP to the MEP for the test pulse alone can be used as an index of $GABA_AR$ activation. A supra-threshold conditioning pulse results in activation of post-synaptic $GABA_BRs$, reducing test MEP amplitudes for conditioned-test ISIs of up to ~150ms

(LICI) [14-16]. Pre-synaptic $GABA_BRs$ activated by the conditioning pulse limit further GABA release, and as they remain active for longer than post-synaptic $GABA_BRs$ a period arises beyond LICI when disinhibition dominates (LCD), and during which MEP amplitude is increased (LICF) and SICI reduced [17-19].

As pre- and post-synaptic GABARs can exert widespread influence over neuronal firing, their dysfunction has been implicated in the processes of epileptogenesis and ictogenesis [2, 4-5, 20]. Previous TMS studies in patients with untreated IGE have reported reduced levels of SICI compared to non-epileptic control subjects [21-25], suggesting a state of cortical hyperexcitability with altered (decreased) cortical $GABA_A$ activity. Normal levels of SICI have been consistently reported in patients with IGE who are well controlled on AED therapy [21-25], apart from those with JME in whom SICI may [23, 26] or may not [27-28] return to normal levels. The effect of IGE on LICI is not certain. Although not yet explored in human IGE *in vivo*, impaired $GABA_BR$-mediated auto-inhibition has been demonstrated in *in vitro* studies of human brain tissue obtained from patients undergoing surgery for pharmaco-resistant temporal lobe epilepsy [10, 29]. While GABA mediates cortical inhibition and disinhibition in adults, it is known to have excitatory effects early in development [30]. An excitatory role for GABA in epilepsy has also been suggested on the basis of findings in animal models and surgically resected human brain tissue [31-33].

In the present study we used TMS to compare the strengths of short- and long-interval intracortical inhibition and disinhibition in the motor cortex in patients with treated and untreated IGE and in controls.

Methods

Subjects

Ten patients diagnosed with IGE (16-37 years of age, mean 23 years; 4 male, all right hand dominant) were recruited from the Royal Perth Hospital First Seizure Clinic and from a private epilepsy clinic. The diagnosis of IGE was made by an epileptologist (JWD, NDL or PLS) on the basis of clinical assessment and EEG. Patients were divided into two groups according to whether or not they were currently being treated with an AED (not on treatment: AED_{NO}, on treatment: AED_{ON}). Further patient details are presented in Table 1. Patients taking multiple AEDs were not recruited. Four patients underwent repeat studies (at least two weeks apart) after commencing (patients #1, #2 and #4) or ceasing (patients #5) AED treatment as part of their prescribed epilepsy management. In total, seven sets of measurements (three unique, four crossover) were obtained from patients off treatment, and seven from patients on AED treatment (three unique, four crossover). Apart from patient #6, all patients were seizure-free for at least 3 months following testing. Twelve healthy individuals (19-32 years of age, mean 23 years; nine male, all right hand dominant) without a history of epilepsy in first-degree relatives were recruited as a control group. Approval for the clinical arm of the study was obtained from the Royal Perth Hospital Human Ethics Committee, and University of Western Australia Human Research Ethics Committee granted approval for control measurements. All participants provided written informed consent according to the Declaration of Helsinki.

Testing was performed at 9 AM, after a minimum of seven hours uninterrupted sleep the night before. Participants abstained from alcohol in the 24 hours prior to testing and from stimulant drinks (e.g. coffee, ‘energy drinks’) on the day of testing. The IGE group were tested at least one week after their last generalised tonic-clonic seizure, and at least 24 hours

after any other clinical seizure type. No participants were taking medications known to alter seizure threshold (other than a single AED).

TMS

MEPs were recorded from the right FDI by surface EMG (sample rate 10kHz, amplification x500, filtering 0.02–20kHz). TMS was delivered through a 7cm figure-of-eight coil connected to three magnetic stimulators (Magstim 200^2; Magstim Co., UK) linked through a custom-built device. The coil was held tangential to the head and positioned in the parasagittal plane at the optimal site for activation of the right FDI (determined from initial exploration over a 1cm grid). Stimuli were delivered at 0.2Hz with the FDI relaxed, and peak-peak MEP amplitude was measured. RMT was determined according to the Rossini-Rothwell criterion [34]. As recommended by safety guidelines, in all IGE subjects surface EMG of the right deltoid muscle was monitored so as to detect intracortical spread of excitation [35], and a neurologist (PLS) was present for all testing. No adverse events occurred during testing.

LICI/LICF

LICI and LICF curves were generated using paired-pulse TMS. The single-pulse TMS intensity needed to evoke a MEP of 1mV amplitude was first determined (I_{1mV}), and both stimuli in the paired-pulse were set to this intensity. The first pulse in a pair was designated the priming stimulus and the second pulse the test stimulus (Figure 1). Paired-pulses were delivered at 14 ISIs spaced so as to encompass the periods of LICI and LICF (100, 150, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 350ms). ISIs were pseudo-randomised and divided into four blocks, with eight stimuli for each ISI. At each ISI the mean TS-MEP amplitude was calculated as a percentage of the mean PS-MEP. The ISIs corresponding to

each subject's greatest LICI and LICF were used in the triple-pulse SICI measurements ($SICI_{LICI}$ and $SICI_{LICF}$). In participants with IGE who did not show LICF, the ISI with greatest PS-TS amplitude was used to evaluate $SICI_{LICF}$.

SICI

Triple-pulse TMS was used to measure SICI during LICI and LICF (Figure 1). A supra-threshold PS (at I_{1mV}) was followed by a paired-pulse stimulus designed to elicit SICI, consisting of a sub-threshold conditioning stimulus followed 2ms later by a supra-threshold test stimulus (TS*) that was intensity-adjusted so as to give a MEP of 1mV in the presence of LICI or LICF. The CS was delivered at three intensities: 0.7, 0.8 and 0.9 RMT. An ISI of 2ms was chosen to avoid contamination of SICI by SICF [36]. Unprimed SICI was measured in the absence of a PS, with TS at I_{1mV}, and calculated from the ratio (expressed as a percentage) of mean conditioned MEP amplitude (10 stimuli) to mean unconditioned test MEP amplitude (10 stimuli). $SICI_{LICI}$ and $SICI_{LICF}$ were measured in the presence of a PS, and calculated from mean conditioned TS* MEP amplitude to mean unconditioned TS* amplitude (10 stimuli). Stimuli were delivered in blocks of 40 for each of unprimed SICI, $SICI_{LICI}$ and $SICI_{LICF}$, comprised of 10 conditioned stimuli at each level of RMT and 10 unconditioned stimuli, interspersed pseudo-randomly.

Data analysis

Data was compared between Controls, AED_{NO} and AED_{ON}. All data are expressed as mean ± standard deviation.

LICI/LICF: Mixed model analysis with random individual effects was used to compare responses between groups, and to compare each group's responses to baseline. Data was log

transformed where required to better approximate normality for inference purposes. Adjustment for possible differences in baseline values did not alter results.

SICI: Mixed model repeated measures analysis was performed with factors GROUP and RMT for each CONDITION (unprimed SICI, $SICI_{LICI}$, $SICI_{LICF}$). Responses were compared between groups, and within each group responses were compared to baseline for each condition. Adjustment for possible differences in baseline values did not alter results.

Results

LICI and LICF

RMT was similar between groups (p=0.496). There was no significant difference between baseline MEP amplitudes for Controls (1.60±0.30mV), AED_{NO} (1.19±0.15mV) and AED_{ON} (1.35±0.09mV; p=0.46).

Figure 2A presents the LICI/LICF curves for each group. LICI was of similar magnitude in all groups at ISI 100-150ms (p=0.09), with a significant overall reduction in MEP amplitude compared to baseline (mean 46±8% of baseline, p<0.001). At longer ISIs (200-250ms) the Control group demonstrated LICF as expected, with MEP amplitude significantly increased compared to baseline (mean 141±17% of baseline, p=0.015) and peaking at 210ms (151±21% of baseline). At these longer ISIs there was no statistically significant difference in amplitude compared to baseline for AED_{NO} (107±12%, p=0.56), while for AED_{ON} the amplitude was reduced, but with marginal significance (79±10% of baseline, p=0.045). There were significant differences at ISI 200-250ms when groups were compared: amplitude in AED_{ON} was lower than both AED_{NO} (p<0.001) and Controls (p=0.003), while those for AED_{NO} and Controls were not significantly different to each other (p=0.2). Stratifying AED_{ON} according to specific AEDs (Figure 2B) suggests that the difference observed between AED_{ON} and

AED_{NO} may be due to lamotrigine. LICI and LICF values for patients tested both on and off AED treatment are listed in Table 2.

SICI

There was no significant difference in TS*-MEP amplitude between groups (mean 1.20±0.19mV; p=0.387), and no difference between MEPs for PS and TS* (p=0.245). The ISI for $SICI_{LICI}$ was 100ms in all participants, whereas the ISI for $SICI_{LICF}$ varied between 180-250ms (median 210ms, interquartile range 203-230ms). Figure 3 shows SICI as a function of CS intensity (0.7-0.9RMT) for each group (Control, AED_{NO}, AED_{ON}) and each stimulus combination (unprimed SICI, $SICI_{LICI}$, $SICI_{LICF}$). The mean values for unprimed SICI, $SICI_{LICI}$, $SICI_{LICF}$ in patients tested both on and off AED treatment are listed in Table 2.

The overall mean unprimed SICI amplitude in the Control group was 29±4% of baseline. Compared to this, SICI was significantly reduced during LICI (68±11% of baseline, p<0.001), and remained reduced during LICF (53±7% of baseline, p<0.001). The difference between $SICI_{LICI}$ and $SICI_{LICF}$ was also significant (p=0.02).

For the AED_{ON} group, $SICI_{LICI}$ (70±10% of baseline) and $SICI_{LICF}$ (55±14% of baseline) were significantly reduced compared to unprimed SICI (37±12% of baseline; p<0.001 and p=0.03, respectively). Unprimed SICI and $SICI_{LICI}$ in AED_{ON} were no different to the Control group (p=0.500 and p=0.360, respectively).

For the AED_{NO} group, unprimed SICI was reduced compared to AED_{ON} and Controls at CS=0.8RMT (56±4% of baseline, p=0.001 and p=0.004, respectively) and 0.9RMT (52±8% of baseline, p=0.053 and p=0.01, respectively). $SICI_{LICI}$ was 120±10% of baseline, indicating

that CS increased TS* amplitude (p=0.017). This was significantly different to the Control (p=0.007) and AED_{ON} (p=0.009) groups, in which $SICI_{LICI}$ was less than 100%. During LICF, SICI in the AED_{NO} group was reduced compared to unprimed SICI (72±5% of baseline, p=0.015). Figure 4 shows SICI MEP waveforms from a representative participant, demonstrating the expected reduction in conditioned MEP amplitude without PS, but that with PS the conditioned MEP is increased in amplitude relative to the unconditioned MEP.

There was no difference in $SICI_{LICF}$ between any groups (p=0.980).

Discussion

The present study demonstrates significant differences in cortical inhibition between individuals with IGE and healthy control subjects. Although LICI was unchanged in IGE, there was no evidence for the post-LICI period of facilitation (LICF) that is present in healthy individuals. The strength of SICI was also reduced, and when measured during LICI, the SICI protocol elicited a paradoxical excitatory rather than an inhibitory response in untreated epileptics. Treatment with AEDs was associated with restoration of normal levels of SICI. These findings point to altered function of GABAergic inhibition in epilepsy, and suggest that under some circumstances GABA may have an excitatory rather than an inhibitory action in the cortex in IGE.

The conventional GABA hypothesis of epilepsy suggests that “a reduction of GABAergic inhibition results in epilepsy while an enhancement of GABAergic inhibition results in an antiepileptic effect” [37]. In recent years this hypothesis has been challenged by reports of $GABA_A$Rs with excitatory effects, likely due to alterations in chloride homeostasis (for reviews, see [4, 31-33, 38-40]). Activation of the $GABA_A$R opens Cl^- channels allowing a

flow of ions down their electrochemical gradient. The direction of this gradient is set by the neuronal membrane cotransporters NKCC1 (influx of ions) and KCC2 (efflux of ions) [41]. *In utero*, relative over-expression of NKCC1 results in high intracellular Cl^- concentration, and thus opening of the $GABA_AR$ Cl^- channel results in a depolarising (outward) ion flow [42-46]. In normal early human infant life the relative expression of NKCC1 and KCC2 reverses, with over-expression of KCC2, thereby lowering intracellular Cl^- concentration such that $GABA_AR$ activation induces hyperpolarisation [47-48]. *In vitro* studies of adult human brain tissue resected for treatment of pharmaco-resistant TLE demonstrate pathological over-expression of NKCC1 and under-expression of KCC2, in which case activation of $GABA_ARs$ may lead to Cl^- efflux and neuronal depolarisation [49-53]. These same findings have also been reported in epileptogenic peritumoural adult human brain tissue [54], and in combination with data from animal models suggest a potentially epileptogenic/ictogenic role for $GABA_ARs$ in epilepsy [4, 41]. Recent studies have also identified mutations affecting GABAergic signalling in some individuals with familial IGE, including loss-of-function mutations in Cl^- channels resulting in abnormally elevated intracellular Cl^- concentration [55-57].

One possible explanation as to why $SICI_{LICI}$ facilitated MEPs is that LICI may contribute to a reversal of the chloride gradient. During LICI, the $GABA_BR$ IPSP (which is mediated by K^+ outflow [7, 58-59]) may reduce KCC2 activity (which depends on the K^+ gradient to transport Cl^- out of the cell) and result in a temporarily higher intracellular Cl^- concentration [41, 60-63]. A sufficiently high concentration could lead to Cl^- efflux when $GABA_ARs$ are activated, and therefore a depolarising response to SICI. Collapse of the Cl^- gradient with subsequent depolarising responses has been demonstrated in animal models of focal and generalised epilepsy [64-66] and in adult (focal) epileptic human brain tissue [10, 29].

Changes in K^+/Cl^- dynamics are thought to contribute to ictal and inter-ictal activity in epilepsy [4, 40, 67], and may underlie the process of ictogenesis by converting a negative (inhibitory) feedback loop (pyramidal cell activation of inhibitory interneurons) into a positive (excitatory) loop [41, 68-69].

In keeping with our previous findings [19], in healthy individuals LICI at ISIs of 100-150ms was followed by a period of intracortical facilitation (LICF) between 200-250ms, and disinhibition (detectable as a reduction in SICI) was present throughout both of these periods. This pattern was not observed in IGE, where LICF was absent in both treated and untreated groups of patients, although disinhibition (as determined by $SICI_{LICF}$) was present albeit weaker for AED_{NO}. A reduction in the strength (and possibly duration) of pre-synaptic $GABA_BR$ activity has been demonstrated in surgically resected brain tissue from adults with focal epilepsy [29], and this could explain the absence of LICF with weaker $SICI_{LICF}$ in our patients.

Consistent with previous studies in IGE [21-25] and one study of patients with generalised epilepsy secondary to $GABA_AR$ subunit mutation (conferring partial loss of function) [70], we found a reduction in the strength of unprimed SICI in untreated epilepsy patients compared to healthy controls, and interpret this as evidence of impaired $GABA_AR$ function. While this was restored by AED treatment, the number of AEDs in relation to sample size does not enable this restoration of $GABA_AR$ function to be interpreted pharmacologically. However, in contrast to Badawy et al. [21-24, 71-72] we did not observe LICF in IGE. Badaway et al. found no evidence of LICI in their healthy controls, whereas we found LICI in both controls and IGE, suggesting that there may be an interrelationship between the presence of LICI and LICF [19]. As LICI is a well-established phenomenon in healthy individuals, its

the absence in the Badaway et al. control groups complicates comparison with the present findings.

The abnormalities in GABA-mediated measurements in the present study warrant further investigation and may have clinical applications for the diagnosis and treatment of IGE. The diagnosis of IGE is primarily clinical based on history and examination, with EEG performed to support the diagnosis and aid in syndromal subclassification and prognostication [73]. However, up to ~50% of individuals who present with a seizure will have a normal inter-ictal EEG (which does not exclude the diagnosis of epilepsy) and in others the EEG may be abnormal yet non-diagnostic [74-75]. In these individuals, if the history is not clearly diagnostic of IGE then measurement of LICF and $SICI_{LICI}$ may be a useful diagnostic adjunct.

In conclusion, we investigated the relationship between pre-synaptic disinhibition and post-synaptic inhibition in the motor cortex of treated and untreated IGE, and find that, during the period of post-synaptic inhibition, activation of $GABA_A$Rs may have excitatory effects. The findings lend *in vivo* support to a growing body of evidence from experimental models and *in vitro* studies of human epileptic brain tissue that GABA may have an excitatory role in epilepsy. Coupled with alterations in disinhibition, the present findings point to a complex modulation of pre- and post-synaptic GABAergic mechanisms in epilepsy.

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Figure Legends:

Figure 1: Protocol diagram. TOP: LICI/LICF curves were generated from paired-pulse TMS, made up of a supra-threshold PS followed by TS (1mV MEP) delivered 100-350ms later. This curve was used to identify the timing of strongest LICI (∨) and LICF(∧) for each individual. BOTTOM: Paired- and triple-pulse TMS was used to measure LICI, LICF, and SICI during both LICI and LICF. LICI/LICF was calculated at each ISI from the amplitude ratio of primed-to-unprimed TS. Unprimed SICI was calculated from the amplitude ratio of unconditioned-to-conditioned TS at ISI 2ms. At the two post-PS intervals corresponding to LICI and LICF, TS intensity was adjusted to restore MEP amplitude to 1mV (TS*) in the presence of PS and SICI was then measured by delivering a CS prior to TS*.

Figure 2: LICI/LICF curves: Mean paired-pulse (PS-TS) MEP amplitudes at each ISI, expressed as a percentage of single-pulse baseline. Error bars represent standard error. (A) All subject groups; (B) Subjects in AED_{ON} only, according to specific AEDs.

Figure 3: SICI curves: Mean conditioned MEP amplitudes expressed as a percentage of unconditioned amplitudes. Values <100% indicate inhibition (0% corresponds to complete suppression of MEPs), and values >100% indicate a facilitatory response. Error bars represent standard error.

Figure 4: MEP waveforms (overlay of 3) from a representative IGE patient (Subject 1, AED_{NO}). Without the priming stimulus (top row), conditioned MEP amplitude (CS-TS) is reduced compared to unconditioned (TS) amplitude. Measurements made after a PS (during LICI) show the conditioned MEP amplitude to be greater than for the unconditioned MEP.

Tables:

Table 1: Demographics of patients with idiopathic generalised epilepsy

Patient	Gender	Age	AED	Months on current AED[#]	Epilepsy Syndrome	Inter-ictal EEG	Months since last seizure
AED_{NO}							
1*	F	16	-		IGETCS	GSW	3
2*	M	22	-		JME	GSW	0.3
3	F	19	-		IGETCS	GSW	9
4*	M	35	-		JAE	GSW	1
5*	M	22	-		IGETCS	GSW	27
6	F	26	-		JME	GSW, PPR	0.25
7	F	37	-		IGETCS	GSW	14
AED_{ON}							
1*	F	17	LTG	3	IGETCS	GSW	7
2*	M	22	VPA	0.5	JME	GSW	1
4*	M	36	VPA	8	JAE	GSW	6
5*	M	21	LTG	17	IGETCS	GSW	24
8	M	18	VPA	10	IGETCS	GSW	5
9	F	18	LTG	8	JAE	GSW	36
10	F	18	LTG	10	JAE	GSW	6

GSW: sporadic generalised spike and wave pattern, *IGETCS:* idiopathic generalised epilepsy with tonic-clonic seizures, *JAE:* juvenile absence epilepsy, *LTG:* lamotrigine, *PPR:* photoparoxysmal response, *VPA:* sodium valproate.
* indicates subject tested both on and off AEDs
[#] 'current AED' refers to both medication and dosage

Table 2: LICI, LICF and SICI in subjects tested both on and off AED treatment

	LICI		LICF		Unprimed SICI		$SICI_{LICI}$		$SICI_{LICF}$	
Patient	Off	On	Off	On	Off	On	Off	On	Off	On
1	53%	90%	148%	116%	35%	7%	-15%	12%	25%	13%
2	51%	63%	157%	118%	70%	88%	30%	18%	36%	65%
4	3%	12%	117%	75%	46%	41%	-28%	4%	35%	17%
5	1%	4%	65%	63%	44%	73%	-60%	#	43%	-4%

LICI values correspond to ISI 100ms, and LICF values correspond to each subject's peak ISIs (as described under *Methods*). All SICI values are averaged across CS 0.7-0.9RMT.
[#] unable to measure $SICI_{LICI}$ as maximum stimulator output reached

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Tables:

Table 1: Demographics of patients with idiopathic generalised epilepsy

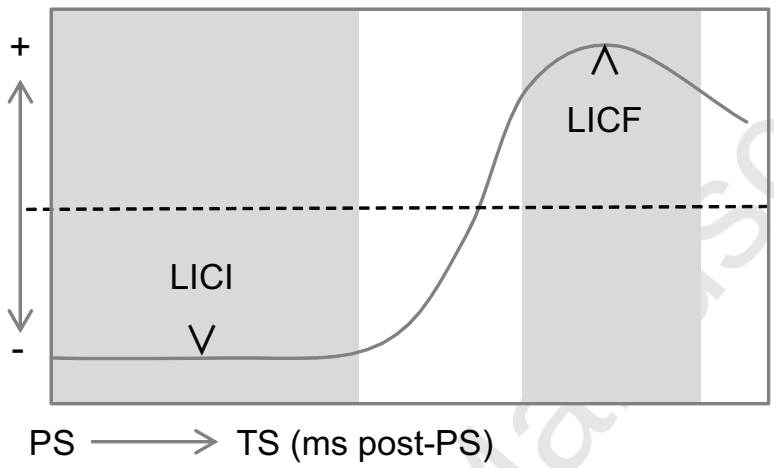
Patient	Gender	Age	AED	Months on current AED[#]	Epilepsy Syndrome	Inter-ictal EEG	Months since last seizure
AED_{NO}							
1*	F	16	-		IGETCS	GSW	3
2*	M	22	-		JME	GSW	0.3
3	F	19	-		IGETCS	GSW	9
4*	M	35	-		JAE	GSW	1
5*	M	22	-		IGETCS	GSW	27
6	F	26	-		JME	GSW, PPR	0.25
7	F	37	-		IGETCS	GSW	14
AED_{ON}							
1*	F	17	LTG	3	IGETCS	GSW	7
2*	M	22	VPA	0.5	JME	GSW	1
4*	M	36	VPA	8	JAE	GSW	6
5*	M	21	LTG	17	IGETCS	GSW	24
8	M	18	VPA	10	IGETCS	GSW	5
9	F	18	LTG	8	JAE	GSW	36
10	F	18	LTG	10	JAE	GSW	6

GSW: sporadic generalised spike and wave pattern, *IGETCS:* idiopathic generalised epilepsy with tonic-clonic seizures, *JAE:* juvenile absence epilepsy, *LTG:* lamotrigine, *PPR:* photoparoxysmal response, *VPA:* sodium valproate.
* indicates subject tested both on and off AEDs
[#] 'current AED' refers to both medication and dosage

Table 2: LICI, LICF and SICI in subjects tested both on and off AED treatment

	LICI		LICF		Unprimed SICI		$SICI_{LICI}$		$SICI_{LICF}$	
Patient	Off	On	Off	On	Off	On	Off	On	Off	On
1	53%	90%	148%	116%	35%	7%	-15%	12%	25%	13%
2	51%	63%	157%	118%	70%	88%	30%	18%	36%	65%
4	3%	12%	117%	75%	46%	41%	-28%	4%	35%	17%
5	1%	4%	65%	63%	44%	73%	-60%	#	43%	-4%

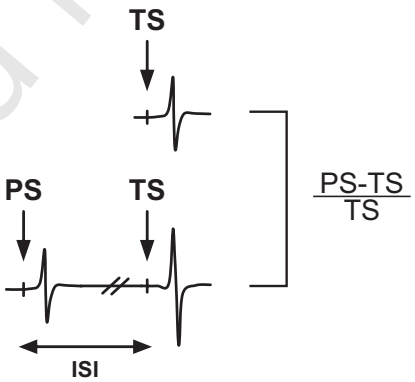
LICI values correspond to ISI 100ms, and LICF values correspond to each subject's peak ISIs (as described under *Methods*). All SICI values are averaged across CS 0.7-0.9RMT.
[#] unable to measure $SICI_{LICI}$ as maximum stimulator output reached



LICI / LICF

Unprimed TS (control)

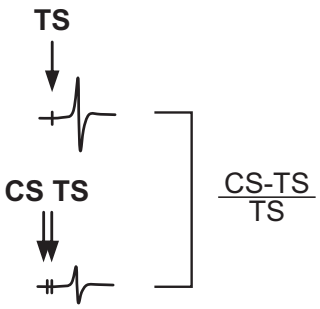
Primed TS



SICI - Unprimed

Unconditioned TS (control)

Conditioned TS



$SICI_{LICI}$ / $SICI_{LICF}$

Primed TS* (control)

Primed conditioned TS*