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Long-interval intracortical inhibition as biomarker for epilepsy: a transcranial magnetic stimulation study

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Abstract

Cortical excitability, as measured by transcranial magnetic stimulation combined with electromyography is a potential biomarker for the diagnosis and follow-up of epilepsy. We report on long-interval intracortical inhibition data measured in four different centres in healthy controls (N = 95), subjects with refractory genetic generalised epilepsy (N = 40) and with refractory focal epilepsy (N = 69). Long-interval intracortical inhibition was measured by applying two supra-threshold stimuli with an interstimulus interval of 50, 100, 150, 200 and 250 ms and calculating the ratio between the response to the second (test stimulus) and to the first (conditioning stimulus). In all subjects, the median response ratio showed inhibition at all interstimulus intervals. Using a mixed linear-effects model, we compared the long-interval intracortical inhibition response ratios between the different subject types. We conducted two analyses; one including data from the four centres and one excluding data from centre 2, as the methods in this centre differed from the others. In the first analysis, we found no differences in long-interval intracortical inhibition between the different subject types. In all subjects, the response ratios at interstimulus intervals 100 and 150 ms showed significantly more inhibition than the response ratios at 50, 200 and 250 ms. Our second analysis showed a significant interaction between interstimulus interval and subject type ($p = 0.0003$). Post-hoc testing showed significant differences between controls and refractory focal epilepsy at interstimulus intervals of 100 ms ($p=0.02$) and 200 ms ($p=0.04$). There were no significant differences between controls and refractory generalised epilepsy groups or between the refractory generalised and focal epilepsy groups. Our results do not support the body of previous work that suggests that long-interval intracortical inhibition is significantly reduced in refractory focal and genetic generalised epilepsy. Results from the second analysis are even in sharper contrast with previous work, showing inhibition in refractory focal epilepsy at 200 ms instead of facilitation previously reported. Methodological differences, especially shorter intervals between the pulse pairs, may have contributed to our inability to reproduce previous findings. Based on our results we suggest that long-interval intracortical inhibition as measured by transcranial magnetic stimulation and electromyography is unlikely to have clinical use as a biomarker of epilepsy.

Keywords: TMS, cortical excitability, paired pulse, LICI, refractory

Abbreviated summary:

Long-interval intracortical inhibition, measured with paired-pulse transcranial magnetic stimulation, may be a promising candidate-biomarker to monitor disease activity in epilepsy. In a large retrospective cohort of people with refractory epilepsy and healthy controls, Bauer *et al* show that long-interval intracortical inhibition is unlikely to be useful as a clinical biomarker.

Abbreviations used in the text:

ADM	abductor digiti minimi
AEDs	anti-epileptic drugs
APB	abductor pollicis brevis
CR	conditioning response
FDI	first dorsal interosseous
ISI	interstimulus interval
LICI	long-interval intracortical inhibition
MEP	motor-evoked potential
(r)MT	(resting) motor threshold
SD	standard deviation
TMS	transcranial magnetic stimulation
TR	test response

Introduction

Epilepsy is a paroxysmal neurological condition characterised by an enduring predisposition to generate epileptic seizures (Fisher *et al.*, 2014). The diagnosis is based on the clinical history, often supported by interictal or ictal epileptic discharges on the EEG (Rosenow *et al.*, 2015). These pathological changes in the EEG are paroxysmal and do not always occur during a short EEG recording (Smith, 2005). The diagnostic sensitivity of routine EEG for epilepsy is estimated at 17% in adults and at 58% in children (Bouma *et al.*, 2016). Sensitivity can be moderately increased by

increasing recording time, or by using activation procedures such as sleep deprivation, hyperventilation or photic stimulation (Rosenow *et al.*, 2015; Smith, 2005). In about 70% of those diagnosed with epilepsy, seizures can be suppressed with AEDs (Brodie *et al.*, 2012), but finding the optimal AED type and dose for an individual patient can be a difficult and time-consuming process. The EEG does not provide a direct measure of seizure proneness and its use in the follow-up of epilepsy is therefore limited, spurring the search for a reliable biomarker of epilepsy activity to improve its management (Engel, 2008; Smith, 2005).

Increased cortical excitability resulting from an imbalance between excitatory and inhibitory activity is thought to play an important role in the pathophysiology of epilepsy (Schwartzkroin, 1994). Cortical excitability can be measured non-invasively using single or paired pulse TMS (Reutens and Berkovic, 1992). Single pulse protocols are used to assess the MT, MEP amplitude and cortical silent period. With paired pulse protocols, short-interval intracortical inhibition, intracortical facilitation and LICI can be measured (Kobayashi and Pascual-Leone, 2003). Several studies showed increased cortical excitability in groups of drug-naïve subjects with generalised or focal epilepsy compared to healthy controls (see for review de Goede *et al.*, 2016). Four studies reported a decrease in excitability after successful treatment with AEDs but not after ineffective treatment (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d).

Of the different variables measured with TMS, those measured with paired pulse protocols appear to hold the greatest potential as a biomarkers for epilepsy for several reasons: firstly, they provide information about *cortical* excitability rather than integrated cortico-spinal excitability as is the case for single pulse measures (Ziemann *et al.*, 1996). Secondly, they can be expressed as dimensionless ratios, enabling comparison across different institutions. Thirdly, paired pulse protocols appear to yield more reliable findings than single pulse protocols (de Goede *et al.*, 2016). Several studies consistently found facilitation at the short (2 and 5 ms) and long (250 and 300 ms) ISIs, instead of inhibition as in healthy controls, providing evidence for cortical hyperexcitability in drug-naïve epilepsy (see for review de Goede *et al.*, 2016). Lastly, this difference between facilitation in drug-naïve epilepsy and inhibition in controls was larger at long than at short ISIs (Badawy *et al.*, 2007). Of

the different TMS variables, LICI may thus be the most suitable as an epilepsy biomarker. LICI is measured by applying two supra-threshold stimuli with an ISI of 50-400 ms and calculating the ratio between the response to the second (test stimulus) and to the first (conditioning stimulus) (a variant consists of the ratio between the response to the test stimulus and to an unconditioned stimulus). A ratio with values < 1 indicates inhibition, while values > 1 indicate facilitation (Valls-Solé *et al.*, 1992). LICI is thought to be linked to GABA-B receptor mediated inhibition (McDonnell *et al.*, 2006; Werhahn *et al.*, 1999).

In epilepsy, LICI was mainly studied by one group of investigators who, in several studies, showed facilitation instead of inhibition at ISIs of 50, 150, 250 and 300 ms in groups of drug-naïve people with different types of genetic generalised epilepsy (Badawy *et al.*, 2007, 2012, 2014; Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013c, 2013d; Badawy and Jackson, 2012; Brodtmann *et al.*, 1999). In drug-naïve focal epilepsy, cortical excitability was consistently increased in the hemisphere ipsilateral to the epileptic focus, but not in the contralateral hemisphere, at ISIs 250 and 300 ms (Badawy *et al.*, 2007, 2012, 2014, 2015; Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013b, 2013c; Badawy and Jackson, 2012). In successfully treated epilepsy, hyperexcitability normalised over time in seizure-free groups, becoming more similar to controls, but it remained increased in refractory groups (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d). Two recent studies from other groups reported contrasting findings, however; the first found significantly lower cortical excitability in subjects with genetic generalised epilepsy on AEDs compared to a healthy control group and a drug-naïve generalised epilepsy group at ISIs between 200 and 250 ms. No significant differences were found between the drug-naïve epilepsy and control groups (Silbert *et al.*, 2015). The second study found inhibition at an ISI of 50 ms in poorly controlled epilepsy, but not in moderately controlled epilepsy or healthy controls. At an ISI of 200 ms, the groups with poorly and moderately controlled epilepsy both showed inhibition (more prominent in the poorly controlled group), whereas healthy controls did not. These results, however, were not significant after correction for multiple comparisons (Pawley *et al.*, 2017).

To establish the true potential of LICI as a clinical biomarker of epilepsy, the promising findings need to be replicated and extended to larger groups. An ideal biomarker needs to provide consistent results across different centres. For it to be useful on an individual level, the inter-individual variability should be low. A difference in excitability between refractory epilepsy and healthy controls would support the use of LICI to rapidly evaluate the effect of treatment with AEDs. We report on LICI data from healthy controls and people with refractory genetic generalised and focal epilepsy from four different centres in two different countries. Our results do not support the utility of LICI as a biomarker for epilepsy.

Methods

Data were collected independently in four different tertiary referral centres, two each in the Netherlands and the UK, and retrospectively pooled. The centres were: 1) Medisch Spectrum Twente (Netherlands); 2) Stichting Epilepsie Instellingen Nederland – SEIN (Netherlands); 3) King's College London (UK) and 4) University College London (UK).

The studies were performed in accordance with guidelines for TMS use in clinical practice and research (Rossi *et al.*, 2009). All study protocols were approved by the local ethics committees of each of the participating centres.

Participants

Informed written consent was provided by all participants. For those younger than 18 years, assent was also obtained from both parents. People with contra-indications to TMS other than epilepsy and pregnant women were excluded.

Centre 1

Healthy adults (aged 18 years or over) were recruited locally through advertisement at the University of Twente and the Medisch Spectrum Twente. People with a history of epilepsy, brain lesions or spinal cord surgery were excluded. Hand dominance was assessed with the Dutch Handedness Questionnaire (van Strien, 1992; Van Strien, 2003).

Centre 2

Healthy participants (aged 12 years or over) were recruited locally through digital and paper adverts. People with a neurological or psychiatric condition, including migraine or epilepsy, diabetes mellitus and people taking medication that could affect cortical excitability (such as psychoactive drugs and β -blockers) were excluded. Hand dominance was assessed with the Dutch version of the Edinburgh handedness questionnaire (Oldfield, 1971).

Centre 3

Adults with a clinical diagnosis of epilepsy were recruited via specialised neurology and epilepsy clinics at King's College Hospital, St Thomas' Hospital, St George's Hospital, London, Kent and Canterbury Hospital and Queen Elizabeth Hospital, Woolwich. For the control group, healthy adults without a personal or family history of neurological or psychiatric conditions were recruited through a local research volunteer's database and friends of participants. Those with epilepsy who had a neuropsychiatric condition other than epilepsy, non-epileptogenic seizures, an estimated IQ < 70 or who did not cooperate with the TMS procedures were excluded. In part of the cohort, hand dominance was assessed with the Edinburgh handedness questionnaire (Oldfield, 1971).

Centre 4

Adults with epilepsy were recruited through specialised epilepsy clinics and an inpatient unit at the National Hospital for Neurology and Neurosurgery (Queen Square and Chalfont sites). Participants with a clinical diagnosis of refractory genetic generalised or focal epilepsy were included. Hand dominance was assessed with the Edinburgh handedness questionnaire (Oldfield, 1971).

Data acquisition

In all centres, participants were seated in a comfortable chair with their hands in a relaxed position and their eyes open. The experimental set-up and stimulation protocol of each centre is summarised in table 1.

Centre 1

TMS was performed with a Magstim Rapid² Stimulator (maximum stimulator output 1.5 T), and a figure-of-eight aircooled 70 mm coil (The Magstim Company Limited, Whitland, UK). Biphasic TMS pulses were given to both motor hot spots of the ADM

muscle. Muscle activity was recorded using two surface Ag/AgCl electrodes placed in a belly-tendon montage. MEPs were recorded from the contralateral ADM muscle with a 72-channel Refa system (TMSi, Oldenzaal, the Netherlands). Data were recorded with a sampling frequency of 5 kHz and stored for offline analysis. Measurements were conducted between 09.00 AM and 5.00 PM.

Centre 2

TMS was performed with a MagPro X100 magnetic stimulator (maximum stimulator output 3.9 T), and a 12 cm diameter parabolic circular MMC-140 coil (Magventure, Farum, Denmark). Biphasic TMS pulses were given on the vertex (Cz). MEPs were recorded bilaterally from the APB muscles with a Nicolet Viking EDX EMG system (Natus, Madison, WI, USA). Data were recorded with a sampling frequency of 4 kHz and stored for offline analysis. Measurements were conducted between 09.00 AM and 4.00 PM.

Centre 3

TMS was performed using two Magstim 200² stimulators connected via a BiStim module, and a figure-of-eight 90 mm coil (The Magstim Company Limited, Whitland, UK). Monophasic TMS pulses were given to both motor hot spots of the FDI muscle. MEPs were recorded from the contralateral FDI muscle with a CED1902 EMG amplifier and CED 1401 Signal 3.13 software (Cambridge Electronic Design, Cambridge, UK) using a sampling rate of 15 kHz, a bandwidth of 10-5,000 Hz, a gain of 1,000 (ranging from -5 to 5 volts), and traces recorded on Signal 3.13 software (CED 1401) and stored for offline analysis. Measurements were conducted between 09.00 AM and 5.00 PM.

Centre 4

Hardware and software for TMS-EMG data collection were the same as in centre 3. A figure-of-eight 70 mm D70 alpha coil was used (The Magstim Company Limited, UK). Monophasic TMS pulses were given to the dominant motor hot spot of the APB muscle. MEPs were recorded with a sampling frequency of 2 kHz and stored for offline analysis. Recordings were obtained between 09.00 AM and 5.00 PM.

Estimation of the resting motor threshold

For the three centres using a figure-of-eight coil, the rMT was determined by applying single pulses to the ADM (centre 1), FDI (centre 3) or APB (centre 4) motor hot spots. The hot spot was defined as the location where the largest MEPs were induced when the TMS coil was placed tangentially with the handle pointed backwards and laterally at an angle of 45° from the midline. Stimulation commenced at 30% of maximum stimulator output and increased in 5% increments until a MEP was seen. 1% changes in intensity were then used to find the threshold, defined as the minimum stimulus intensity which produced a MEP with a peak-to-peak amplitude > 50 μV (> 100 μV in centre 4) in 50% or more of ten trials in the fully relaxed target muscle (Rossini *et al.*, 1994). Relaxation of the target muscle was monitored by continuous visual observation of the EMG.

The approach was different in centre 2, where the rMT was approximated using a single pulse stimulus-response curve, with the coil on the vertex. Stimulation started at 20% of stimulator output with 5% stepwise increments until there was a consistent twitch in the hand contralateral to the stimulated hemisphere in 50% or more of eight trials (approximated rMT). Then, a semi-automated, in-house designed scanning protocol (created in Matlab® (version 7.5.0 R2007b The MathWorks Inc., Natick, MA, USA)) was used to automatically deliver stimuli with a fixed intertrial interval of 2 s, and eight stimuli at each intensity. Scanning started at a stimulator output value of 10-12% below the approximated rMT and increased in 2% steps until a reproducible MEP (> 200 μV) was seen after every stimulus (corresponding to 110-120% rMT). The rMT was defined as the lowest stimulus intensity eliciting a visible twitch in any hand muscle in 50% or more of eight stimuli (Varnava *et al.*, 2011).

Assessment of Long-interval Intracortical Inhibition

Centre 1

Paired pulse stimulation was applied with the coil over the ADM motor hot spot. Both pulses were given at 120% of the rMT with ISIs between 50 to 300 ms, with 50 ms increments (6 intervals). Stimulation was repeated fifty times for each ISI. The stimulus pairs were given randomly with approximately 4 s (range 3.5-4.5 s) between stimulus pairs (intertrial interval). To calculate the LICI for each ISI, the ratio was taken between the mean peak-to-peak amplitude of the responses to the second (test)

stimuli (TR), and the mean peak-to-peak amplitude of the responses to the first (conditioning) stimuli (CR): $\text{mean(TR)}/\text{mean(CR)}$.

Centre 2

Paired pulse stimulation was applied with the coil over the vertex. Both pulses were given at 110% of the rMT, with increasing ISIs between 50 and 400 ms, with 25 ms increments (15 intervals). Stimulation was repeated six times for each ISI. The stimulus pairs were given in a fixed increasing order with an intertrial interval of 1 s. An unconditioned stimulus was given six times immediately before the start of the paired pulse stimulation protocol. To calculate the LICI for each ISI, the mean peak-to-peak amplitude in response to the conditioned, second stimuli (TR) was divided by the mean peak-to-peak amplitude in response to the unconditioned stimuli: $\text{mean(TR)}/\text{mean(unconditioned MEP)}$.

Centre 3

Paired pulse stimulation was applied with the coil over the FDI motor hot spot. Both paired pulses were given at 120% of the rMT. Four ISIs were tested: 50, 150, 200 and 250 ms. Stimulation was repeated ten times for each ISI. The stimulus pairs were given in random order with an intertrial interval of 4 s. To calculate the LICI for each ISI, the ratios were taken between the peak-to-peak amplitudes of the responses to the second test stimuli (TR), and the peak-to-peak amplitudes of the responses to the first conditioning stimuli (CR). Then the mean over all ratios was taken: mean(TR/CR) .

Centre 4

Paired pulse stimulation was applied with the coil over the APB motor hot spot. Both pulses were given at 110% of the rMT. Five ISIs were tested: 50, 100, 150, 200 and 250 ms. Stimulation was repeated ten times for each ISI. The stimulus pairs were given in a random order with a 5 s intertrial interval. LICI was calculated in the same way as in centre 2.

Data analysis

Data analysis was done in R® (R Core Team, 2015). Each centre provided the following individual data for analysis: age, gender, hand dominance (if available), epilepsy diagnosis, including whether epilepsy was refractory to treatment with AEDs (defined as at least one seizure in the year preceding the TMS measurement), number

of different AEDs, side of seizure focus (in case of focal epilepsy), and the mean LICI for each ISI. Only LICI values of healthy controls and people with refractory epilepsy (generalised or focal) were included in the analysis. Based on previous reports, cortical excitability as measured by LICI remains elevated in those who are refractory to pharmacological treatment, whereas it normalises (returns to the levels seen in controls) in those who become seizure free. The refractory epilepsy group is, therefore, the most interesting to assess in this context. For controls and genetic generalised epilepsy, we only analysed the LICI when stimulating the dominant hemisphere (left hemisphere for right-hand dominance). When hand dominance was unknown and in ambidextrous participants, we analysed the LICI when stimulating the left hemisphere. For focal epilepsy, we included the LICI when stimulating the hemisphere ipsilateral to the epileptic focus in the analysis, or the dominant/left hemisphere when epileptic foci were bilateral. We included the LICI measured at ISIs of 50, 100, 150, 200, and 250 ms.

Statistical analysis

As the material and stimulation protocols differed between the centres, we expected not only large inter-individual variability of LICI values, but also a large variability between the centres. This inter-centre variability limits the comparison of data between the centres and means that data cannot simply be pooled for analysis. Linear mixed-effects models (lme4 package, Linear Mixed Effects version 4, (Bates *et al.*, 2015) are the best way to deal with such datasets, as they allow for correction of systematic variability. We accounted for the heterogeneity of LICI values across subjects and centres by defining them as effects with a random intercept, thus instructing the model to correct for any systematic differences between the subjects (inter-individual variability) and centres (inter-centre variability). We then analysed the influence of two possible fixed effects on LICI: 1) the subject type (three levels: controls, refractory genetic generalised epilepsy and refractory focal epilepsy) and 2) the ISIs (five levels: 50, 100, 150, 200 and 250 ms). LICI response ratios were log transformed to better approximate normality (see figure A in the supplementary material). To optimise our model, we checked the normality of the model residual.

We ran a type-II analysis of variance. Wald chi-square tests were used for fixed effects in linear mixed-effects models. For post-hoc tests we used the Lsmean

package (Lsmean version 2.20-23, (Searle *et al.*, 1980)) where p -values were considered as significant at $p < 0.05$ and adjusted for the number of comparisons performed (Tukey method).

Results

Participants

Centre 1

Twenty-five healthy subjects were included. Four were excluded from the analyses: in two stimulation was not possible at an intensity of 120% the rMT, in one the session was terminated prematurely as the subject felt unwell, and in one LICI data were not available from the dominant hemisphere. Twenty-one healthy individuals were included in the analysis (6 males, mean age 28.6 years, range 20-49 years, three left handed), see figure 1.

Centre 2

Thirty-eight controls were included; one was excluded due to non-specific EEG abnormalities. Data of 37 controls were included in the analysis (11 males, mean age 38.1 years, range 15-62 years, four left handed, one ambidextrous), see figure 1.

Centre 3

Thirty-seven controls and 110 subjects with epilepsy were included (54 with genetic generalised epilepsy, 55 with focal epilepsy and one with an unclear diagnosis). All controls were included in the analysis (19 males, mean age 30.2 years, range 18-52 years, four left handed, one ambidextrous). Of the 54 subjects with generalised epilepsy, 31 were excluded from the analysis: in eleven LICI data were not collected (for reasons including too high motor threshold or discomfort during stimulation), in four LICI data was not available from the dominant hemisphere, nine were not taking AEDs at the time of the experiment, and seven were not considered refractory. Thus, 23 subjects with refractory genetic generalised epilepsy were included in the analysis (10 males, mean age 30.1 years, range 18-54 years, hand dominance known in twelve, of these two were left-handed, AEDs: median 1, range 1-4). In 24 of the 55 with focal epilepsy, LICI data were not collected. In three, LICI data were only available for the hemisphere contralateral to the epileptic focus. Thus, 28 subjects with refractory focal epilepsy were included in the analysis (12 males, mean age 39.4 years, range 21-66

years, AEDs: median 1, range 1-3), see figure 1. The hand dominance of this group was unknown.

Centre 4

Nineteen participants with genetic generalised epilepsy were included. One had to be excluded from the analysis as no AEDs was used and one as LICI data from the dominant hemisphere was not available. Seventeen subjects with genetic generalised epilepsy were included in the analysis (8 males, mean age 34.4 years, range 20-51 years, one left handed, two ambidextrous, AEDs: median 3, range 1-5). Fifty-nine with focal epilepsy were included but fifteen were excluded as LICI data from the hemisphere ipsilateral to the epileptic focus were not available. In one participant with a bilateral focus, LICI data from the dominant hemisphere were unavailable. Two participants were not taking AEDs, leaving 41 with refractory focal epilepsy for analysis (18 males, mean age 39.7 years, range 18-61 years, four left handed, AEDs: median 2, range 1-6), see figure 1. A further five participants were included, but as the epilepsy diagnosis was unclear they were excluded from the current analysis.

In total, we included data from 204 subjects in the analysis, 40 with refractory generalised epilepsy, 69 with refractory focal epilepsy and 95 healthy controls (see figure 1). Part of the data from this cohort was previously reported in other studies (centre 1: (de Goede and van Putten, 2017), centre 3: (Chowdhury *et al.*, 2015; Pawley *et al.*, 2017) and centre 4 (including patients with Dravet Syndrome): (Stern *et al.*, 2016, 2017).

LICI recovery curves

For all subject types, the response ratios for each ISI are shown in figure 2 and table 2. As expected, we found inhibition (median LICI value < 1) in healthy controls at all analysed ISIs (50, 100, 150, 200 and 250 ms) (figure 2 and figure B in the supplementary material). Unexpectedly, the median LICI was also < 1 in refractory genetic generalised epilepsy (all ISIs except 50 ms) and refractory focal epilepsy (all ISIs). The mean LICI also showed inhibition in the controls, except in centre 2 (all ISIs), in centre 1 at ISI 50 ms and in centre 3 at ISI 250 ms (table 2). In the refractory epilepsy groups the mean LICI was also < 1 at most ISIs, except for genetic generalised epilepsy at ISI 50 ms and focal epilepsy in centre 3 at ISI 250 ms (table 2). The linear mixed-effects model showed that there was no significant interaction

between subject type and LICI at any of the ISIs. We found a main effect of interaction between ISI and LICI ($p < 0.001$). Post-hoc tests revealed that LICI at ISIs 100 and 150 ms showed significantly more inhibition compared to LICI at ISIs 50, 200 and 250 ms for all three subject types, see figure 3. The methods of centre 2 differ the most from those used in the other centres, as it was the only site to use a round coil and stimulation on the vertex. It also used the shortest intertrial interval and the lowest number of repetitions per interstimulus interval. The statistical model takes this methodological heterogeneity into account but as centre 2 also seems to have less consistent results and more outliers than centre 1 and 3 and other studies in the field (Cash *et al.*, 2010; Caux-Dedeystère *et al.*, 2015; Silbert *et al.*, 2015; Valls-Solé *et al.*, 1992; Wassermann *et al.*, 1996) we re-ran the statistical analysis without centre 2 data. In contrast with the first analysis, this showed a significant interaction between ISI and subject groups ($p=0.0003$). Post-hoc testing revealed a small yet significant difference between controls and refractory focal epilepsy at ISIs of 100 ms ($p=0.02$) and 200 ms ($p=0.04$). There were no significant differences between the control and refractory generalised epilepsy groups or between the refractory generalised and focal epilepsy groups, see figure 4. At an ISI 100 ms, the LICI of both controls and refractory focal epilepsy was <1 , indicating inhibition. At an ISI 200 ms, there was neither inhibition nor facilitation in the controls (LICI ~ 1) but inhibition in the focal epilepsy group (LICI <1).

Discussion

Our analysis of long-interval paired pulse TMS data collected in four different centres do not support previous promising findings (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d). Our first analysis, including data from all centres, showed no significant differences in LICI between those with refractory genetic generalised epilepsy, subjects with refractory focal epilepsy and healthy controls. We observed a statistically significant difference between the ISIs of 50, 200 and 250 ms on one hand, and ISIs 100 and 150 ms on the other hand for all subjects. Inhibition was measured at all five ISIs in all subject types but it was significantly stronger at ISIs 100 and 150 ms. The results from our second analysis without centre 2 data, clearly contrast with previous findings (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et*

al., 2013a, 2013d): firstly, we find inhibition in the refractory epilepsy groups instead of *facilitation* ($LICI > 1$) reported previously. Secondly, the differences in our sample are only found between refractory focal epilepsy and controls and not in generalised refractory epilepsy. Thirdly, the differences are found at other ISIs (100 and 200 ms instead of 50, 150 and 250 ms) than previously reported (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d). Lastly, the differences between controls and refractory focal epilepsy are much smaller in our sample than in previous reports (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d).

One of the main methodological differences between our study protocols and that of previous studies is the intertrial interval, which ranged between 1 and 5 s in our studies but was 15 s in previous studies (Badawy and Jackson, 2012; Badawy *et al.*, 2007, 2012, 2014, 2015; Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013b, 2013c, 2013d; Brodtmann *et al.*, 1999). Another study of LICI in epilepsy, also using an intertrial interval of 5 s did not show a difference between the healthy control and drug-naïve epilepsy groups (Silbert *et al.*, 2015). Several studies have shown that the MEP amplitude is influenced by the intertrial interval but there are no studies assessing the influence of the intertrial interval on LICI. The optimal intertrial interval to obtain reproducible single pulse MEPs is probably between 10 and 20 s (Julkunen *et al.*, 2012; Möller *et al.*, 2009; Pellicciari *et al.*, 2016; Vaseghi *et al.*, 2015), although other studies show that stimulus-response curves can be obtained reliably using shorter intertrial intervals (Mathias *et al.*, 2014; Pearce *et al.*, 2013). Other LICI studies, mostly in relatively small cohorts, used random intertrial intervals between 4 and 15 s, but showed variability similar to our cohort (Kujirai *et al.*, 1993; Sanger *et al.*, 2001; Vallence *et al.*, 2017; Valls-Solé *et al.*, 1992; Wassermann *et al.*, 1996). Interestingly, centre 2, with the shortest intertrial interval (1 s) shows *mean* $LICI > 1$ at all ISIs, but *median* $LICI < 1$ at all ISIs, which may be due to several extreme outliers and speculatively a cumulative effect of the paired pulses. The relatively short intertrial intervals in all centres may thus have contributed to our inability to reproduce previous findings obtained with an interstimulus interval of 15 s (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d). A recent study, however, that also used an intertrial interval of 15 s showed facilitation at ISIs of 50, 80, 110 and 140 ms in a group of 20 healthy volunteers

(Bolden *et al.*, 2017). Future studies are warranted to quantify the influence of intertrial interval on paired pulse TMS protocols.

Another explanation for the different results could be the participant cohorts. We only included data obtained from the dominant hemisphere in controls and generalised epilepsy and the ipsilateral hemisphere in focal epilepsy (i.e. the side of the seizure focus). In centre 4 it was standard practise to stimulate the dominant hemisphere only, leading to the exclusion of those in whom the dominant hemisphere was not the ipsilateral hemisphere. Including the results of both hemispheres in our multi-centre analysis would have lead to missing data for cases in which only one hemisphere was measured. Alternatively we could have estimated a mean of both hemispheres measured, which could have introduced a bias. Our choice lead to several exclusions, especially from centre 4, but we feel that this was the best way to deal with this issue. Even if some bias were introduced this way, we would expect the large differences between controls and subjects reported in other studies to have been visible in our large sample. We included a large number of people with all types of refractory generalised and focal epilepsy as well as healthy controls, while previous studies often report on relatively small samples (~20 participants) of people with specific epileptic syndromes (Badawy *et al.*, 2012, 2014; Badawy, Macdonell, Jackson, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013b, 2013c, 2013d) or AEDs (Silbert *et al.*, 2015). Our retrospective study design did not allow us to go into such detail, limiting the comparison with these studies. It should be noted that the participant cohorts reported in previous studies appear to overlap, potentially leading to multiple publication bias and overestimation of the consistency of these findings (Badawy *et al.*, 2017; Bauer *et al.*, 2017; Brigo *et al.*, 2012).

Comparing TMS data across centres is challenging as variables such as the rMT depend directly on the equipment used. LICI, however, is expressed as a response ratio, and thus is dimensionless. It is, therefore, theoretically better suited for comparison between centres than the rMT. We report on data collected from multiple centres and although it provides a large body of data, it is limited by its retrospective set-up and the different equipment and stimulation protocols used. Clear methodological guidelines for LICI stimulation are currently lacking, as there is insufficient data to define the most robust LICI protocol in terms of stimulator,

stimulation intensity, coil type, stimulation site, target muscle, number of repetitions and intertrial interval. Some of the methodological differences are less likely to hamper the direct comparison between centres. For example, in three of the centres a figure-of-eight coil was used and stimulation was applied to the motor hotspot, while in one of the centres (centre 2) a round coil was used and stimulation was applied on the vertex. The coil design (circular or figure-of-eight) affects the size and depth of the cortical region that is stimulated by the TMS pulse, and may result in different LICI responses. While several studies compared different coils for shorter interstimulus intervals (Badawy *et al.*, 2011; Cantello *et al.*, 2000; Fleming *et al.*, 2012; Shimizu *et al.*, 1999), only one study directly compared these different coils for LICI in a small sample ($N = 8$) (Valzania *et al.*, 1994). The LICI responses obtained at stimulation intensities of 110 and 120% rMT with either a circular or a figure-of-eight coil were similar in this study.

Other methodological differences are more likely to hamper direct comparison between centres. Centre 2 and 4 applied two pulses with an intensity of 110% rMT while the other centres applied two pulses at 120% of the rMT. This was previously shown to yield small differences in the LICI response (Valls-Solé *et al.*, 1992), and may have contributed to the weaker mean LICI (> 1) seen in centre 2. Despite the difference in stimulation intensity used in centre 3 and 4 the mean LICI is < 1 in both refractory epilepsy groups at ISIs 100, 150 and 200 ms (table 2), contrasting with previous studies showing facilitation at these ISIs in refractory epilepsy (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d). In our refractory epilepsy groups, facilitation is only seen at 50 ms in genetic generalised epilepsy (centre 3 and 4) and at 250 ms in focal epilepsy (centre 3, table 2). Two centres (1 and 2) used biphasic pulses for stimulation. Biphasic and monophasic pulses preferentially excite partly different sets of cortical axons when using the same coil orientation (Groppa *et al.*, 2012), potentially contributing to different response patterns. The number of repetitions and stimulation sequence (random or fixed) also differed, being relatively low in all centres, except in centre 1, where each ISI was repeated 50 times. Recent studies suggest the use of 20-30 repetitions for single pulse and short-interval paired-pulse protocols (Chang *et al.*, 2016; Goldsworthy *et al.*, 2016), although 10 repetitions are commonly used in studies of epilepsy (Badawy *et al.*, 2012, 2014; Badawy, Macdonell, Jackson, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013b, 2013c, 2013d). All these methodological

differences result in a net inter-centre variability, which limits the direct comparison of LICI outcomes from different centres. We accounted for inter-centre variability by using a linear mixed-effects model, and although this is a methodological robust solution, it cannot replace a prospective multi-centre study in which all centres use exactly the same materials and methods. Despite the different methods, however, the results of centre 1, 3 and 4 (and 2) are consistent, making the difference with the results of previous studies all the more striking. The individual studies that constituted our sample were set up as prospective TMS trials and part of the data was previously reported (centre 1: (de Goede and van Putten, 2017), centre 3: (Pawley *et al.*, 2017) and centre 4, including patients with Dravet Syndrome: (Stern *et al.*, 2017). In none of these studies were previous promising results replicated (Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013d), spurring the current retrospective multi-centre analysis. Only one centre (3) in our study included all three participant types. Despite the same protocol and equipment being used, there was no significant difference between LICI measured in refractory generalised epilepsy, refractory focal epilepsy and control groups in this centre (see also (Pawley *et al.*, 2017)).

The LICI response ratios were not normally distributed in our sample. This is in line with one previous study of LICI (Silbert *et al.*, 2015). Another study, reporting on a large cohort of healthy subjects, showed that rMT and short-interval intracortical inhibition do not follow a normal distribution (Wassermann, 2002). We suggest that statistical analyses of TMS variables should be done on log-transformed data. Furthermore, responses at different ISIs should be treated as repeated measurements, warranting corrections for multiple comparisons when several ISIs are measured in the same participants (Pawley *et al.*, 2017).

Some authors consistently report low inter-individual variability of LICI (Badawy *et al.*, 2012, 2014, 2015; Badawy, Jackson, *et al.*, 2013; Badawy, Macdonell, Berkovic, *et al.*, 2010; Badawy, Vogrin, *et al.*, 2013a, 2013b, 2013d; Badawy and Jackson, 2012); however, others reported much higher variability of TMS responses (Du *et al.*, 2014; Lang *et al.*, 2011; Nakamura *et al.*, 1997; Sanger *et al.*, 2001; Valls-Solé *et al.*, 1992; Wassermann, 2002; Wassermann *et al.*, 1996). In our cohort, the response ratio to long-interval paired pulse stimulation varied between individuals from strong

inhibition to facilitation supporting some previous studies (Bolden *et al.*, 2017; Valls-Solé *et al.*, 1992). LICI variability was shown to be linked to the time of day and sleep status (Lang *et al.*, 2011), neuropsychological profile (Bolden *et al.*, 2017) and to age and hemispheric dominance (Vallence *et al.*, 2017). While some TMS variables were shown to vary according to the menstrual cycle (Hattemer *et al.*, 2007; Inghilleri *et al.*, 2004; Smith *et al.*, 2002), no data are available for LICI. These and probably other unknown factors contribute to the large interindividual variability of LICI and need to be adequately accounted for. One previous study accounted for the interindividual variability by using a mixed model analysis, similar to our approach (Silbert *et al.*, 2015).

An ideal biomarker for epilepsy diagnosis and management should show a difference in cortical excitability between drug-naïve people with epilepsy and healthy controls. To be of clinical use for the management of epilepsy, it needs to show normalisation of cortical excitability soon after treatment initiation in those who become seizure-free, but not in those with refractory seizures. In the latter group, cortical excitability should remain different from controls despite treatment indicating active epilepsy with (refractory) seizures. Our analysis (both with and without centre 2), however, shows the opposite effect. A good biomarker should also have low inter-individual variability, so that it can also be used to assess disease activity on an individual level. In our analysis, we only included people with refractory seizures and controls. Based on our findings showing both no significant differences between subjects with refractory epilepsy and controls, and a variable response to long-interval paired pulse stimulation, we argue that the use of LICI measured with TMS-EMG as a biomarker for epilepsy is limited and that a prospective trial is urgently needed to confirm this finding. Recent studies show that combining TMS with EEG may provide a more direct method to assess cortical excitability and underlying processes (Ilmoniemi *et al.*, 1997; Ilmoniemi and Kicić, 2010). There is only one report of paired pulse TMS-EEG in epilepsy (Kimiskidis *et al.*, 2017). Using feature selection methods combined with a Bayesian classifier, this study found a cross-validated diagnostic accuracy of 0.92 for differentiating genetic generalised epilepsy from healthy controls and 0.80 for differentiating responders from non-responders, suggesting that paired pulse TMS-EEG may be useful for diagnosis and the assessment of disease severity (Kimiskidis *et al.*, 2017). Further research is needed to extend and confirm these

findings. TMS-EEG may also help to reveal the mechanisms underlying the difference in LICI at ISIs of 100 and 150 ms and LICI at ISIs 50, 200 and 250 ms that we found in all groups of our cohort.

Conclusion

In this retrospective study, we could not replicate the difference in LICI measured with TMS-EMG between people with refractory genetic generalised and focal epilepsy and healthy controls consistently reported in previous studies. Methodological differences, especially shorter intertrial intervals, may have contributed to our inability to replicate previous findings. Further studies are needed to assess the influence of the length of the intertrial interval on LICI and to establish guidelines for LICI stimulation protocols. Based on our findings, LICI measured with TMS-EMG is unlikely to be useful as a biomarker in the clinical management of epilepsy. Future prospective multi-centre trials are needed to confirm this finding.

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Conflicts of interest

SMS has been consulted by, or received fees for lectures, or institutional support, from GSK, Vitaflo, Nutricia, Eisai and UCB Pharma. MJAMvP is co-founder of Clinical Science Systems, the Netherlands. JWS has been consulted by and received fees for lectures from GSK, Eisai and UCB Pharma. All other authors declare no conflicts of interest.

References:

- Badawy R, Curatolo J, Newton M, Berkovic S, Macdonell R. Changes in cortical excitability differentiate generalized and focal epilepsy. *Ann Neurol* 2007; 61: 324–331.
- Badawy R, Jackson G. Cortical excitability in migraine and epilepsy: a common feature? *J Clin Neurophysiol* 2012; 29: 244–249.
- Badawy R, Jackson G, Berkovic S, Macdonell R. Inter-session repeatability of cortical excitability measurements in patients with epilepsy. *Epilepsy Res* 2012; 98: 182–186.
- Badawy R, Jackson G, Berkovic S, Macdonell R. Cortical excitability and refractory epilepsy: a three-year longitudinal transcranial magnetic stimulation study. *Int J Neural Syst* 2013; 23: 1250030.
- Badawy R, Macdonell R, Berkovic S, Newton M, Jackson G. Predicting seizure control: cortical excitability and antiepileptic medication. *Ann Neurol* 2010; 67: 64–73.
- Badawy R, Macdonell R, Berkovic SF, Vogrin SJ, Jackson GD, Cook MJ. Reply: Transcranial magnetic stimulation as a biomarker for epilepsy. *Brain* 2017; doi:10.109: 1–4.
- Badawy R, Macdonell R, Jackson G, Berkovic S. Can changes in cortical excitability distinguish progressive from juvenile myoclonic epilepsy? *Epilepsia* 2010; 51: 2084–2088.
- Badawy R, Tarletti R, Mula M, Varrasi C, Cantello R. The routine circular coil is reliable in paired-TMS studies. *Clin Neurophysiol* 2011; 122: 784–788.
- Badawy R, Vogrin S, Lai A, Cook M. The cortical excitability profile of temporal lobe epilepsy. *Epilepsia* 2013a; 54: 1942–1949.
- Badawy R, Vogrin S, Lai A, Cook M. Capturing the epileptic trait: cortical excitability measures in patients and their unaffected siblings. *Brain* 2013b; 136: 1177–1191.
- Badawy R, Vogrin S, Lai A, Cook M. Cortical excitability changes correlate with fluctuations in glucose levels in patients with epilepsy. *Epilepsy Behav* 2013c; 27: 455–460.
- Badawy R, Vogrin S, Lai A, Cook M. Patterns of cortical hyperexcitability in adolescent/adult-onset generalized epilepsies. *Epilepsia* 2013d; 54: 871–878.
- Badawy R, Vogrin S, Lai A, Cook M. On the midway to epilepsy: is cortical excitability normal in patients with isolated seizures? *Int J Neural Syst* 2014; 24: 1430002.
- Badawy R, Vogrin S, Lai A, Cook M. Does the region of epileptogenicity influence the pattern of change in cortical excitability? *Clin Neurophysiol* 2015; 126: 249–256.

- Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat. Softw* 2015; 67
- Bauer PR, Goede AA De, Braack EM, Putten MJAM Van, Gill RD, Sander JW. Transcranial magnetic stimulation as a biomarker for epilepsy. *Brain* 2017; 10–12.
- Bolden LB, Griffis JC, Pati S, Szaflarski JP. Cortical excitability and neuropsychological functioning in healthy adults. *Neuropsychologia* 2017; 102: 190–196.
- Bouma HK, Labos C, Gore GC, Wolfson C, Keezer MR. The diagnostic accuracy of routine electroencephalography after a first unprovoked seizure. *Eur J Neurol* 2016; 23: 455–463.
- Brigo F, Storti M, Benedetti MD, Rossini F, Nardone R, Tezzon F, et al. Resting motor threshold in idiopathic generalized epilepsies: A systematic review with meta-analysis. *Epilepsy Res* 2012; 101: 3–13.
- Brodie MJ, Barry SJ, Bamagous GA, Norrie JD, Kwan P. Patterns of treatment response in newly diagnosed epilepsy. *Neurology* 2012; 78: 1548–1554.
- Brodthmann A, Macdonell RA, Gilligan AK, Curatolo J, Berkovic SF. Cortical excitability and recovery curve analysis in generalized epilepsy. *Neurology* 1999; 53: 1347–1349.
- Cantello R, Civardi C, Cavalli a, Varrasi C, Tarletti R, Monaco F, et al. Cortical excitability in cryptogenic localization-related epilepsy: interictal transcranial magnetic stimulation studies. *Epilepsia* 2000; 41: 694–704.
- Cash RFH, Ziemann U, Murray K, Thickbroom GW. Late cortical disinhibition in human motor cortex : a triple-pulse transcranial magnetic stimulation study. *J Neurophysiol* 2010; 103: 511–518.
- Caux-Dedeystère A, Derambure P, Devanne H. Late cortical disinhibition in relaxed versus active hand muscles. *Neuroscience* 2015; 298: 52–62.
- Chang WH, Fried PJ, Saxena S, Jannati A, Gomes-Osman J, Kim Y-H, et al. Optimal number of pulses as outcome measures of neuronavigated transcranial magnetic stimulation. *Clin Neurophysiol* 2016; 127: 2892–2897.
- Chowdhury FA, Pawley AD, Ceronie B, Nashef L, Elwes RDC, Richardson MP. Motor evoked potential polyphasia: a novel endophenotype of idiopathic generalized epilepsy. *Neurology* 2015; 84: 1301–1307.
- Du X, Summerfelt A, Chiappelli J, Holcomb HH, Hong LE. Individualized brain inhibition and excitation profile in response to paired-pulse TMS. *J Mot. Behav* 2014; 46: 39–48.
- Engel JJ. Progress in epilepsy: reducing the treatment gap and the promise of biomarkers. *Curr Opin Neurol* 2008; 21: 150–154.
- Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE Official Report: A practical clinical definition of epilepsy. *Epilepsia* 2014; 55: 475–482.
- Fleming MK, Sorinola IO, Newham DJ, Roberts-Lewis, S.F., Bergmann JHM. The effect of coil type and navigation on the reliability of transcranial magnetic stimulation. *IEEE Trans Neural Syst Rehabil Eng* 2012; 20: 617–625.
- de Goede AA, ter Braack EM, van Putten MJAM. Single and paired pulse transcranial magnetic stimulation in drug naïve epilepsy. *Clin Neurophysiol* 2016; 127: 3140–3155.
- de Goede AA, van Putten MJAM. Repeatability of long intracortical inhibition in healthy subjects. *Clin*

- Neurophysiol Pract. 2017; 2: 26–34.
- Goldsworthy MR, Hordacre B, Ridding MC. Minimum number of trials required for within- and between-session reliability of TMS measures of corticospinal excitability. *Neuroscience* 2016; 320: 205–209.
- Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol* 2012; 123: 858–882.
- Hattemer K, Knake S, Reis J, Rochon J, Oertel WH, Rosenow F, et al. Excitability of the motor cortex during ovulatory and anovulatory cycles: a transcranial magnetic stimulation study. *Clin Endocrinol* 2007; 66: 387–393.
- Ilmoniemi RJ, Kicić D. Methodology for combined TMS and EEG. *Brain Topogr* 2010; 22: 233–248.
- Ilmoniemi RJ, Virtanen J, Ruohonen J, Karhu J, Aronen HJ, Näätänen R, et al. Neuronal responses to magnetic stimulation reveal cortical reactivity and connectivity. *Neuroreport* 1997; 8: 3537–3540.
- Inghilleri M, Conte A, Currà A, Frasca V, Lorenzano C, Berardelli A. Ovarian hormones and cortical excitability. An rTMS study in humans. *Clin Neurophysiol* 2004; 115: 1063–1068.
- Julkunen P, Säisänen L, Hukkanen T, Danner N, Könönen M. Does second-scale intertrial interval affect motor evoked potentials induced by single-pulse transcranial magnetic stimulation? *Brain Stimul* 2012; 5: 526–532.
- Kimiskidis VK, Tsimpiris A, Ryvlin P, Kalviainen R, Koutroumanidis M, Valentin A, et al. TMS combined with EEG in genetic generalized epilepsy: A phase II diagnostic accuracy study. *Clin Neurophysiol* 2017; 128: 367–381.
- Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. *Lancet Neurol* 2003; 2: 145–156.
- Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. *J Physiol* 1993; 471: 501–519.
- Lang N, Rothkegel H, Reiber H, Hasan A, Sueske E, Tergau F, et al. Circadian modulation of GABA-mediated cortical inhibition. *Cereb Cortex* 2011; 21: 2299–2306.
- Mathias JP, Barsi GI, Van De Ruit M, Grey MJ. Rapid acquisition of the transcranial magnetic stimulation stimulus response curve. *Brain Stimul* 2014; 7: 59–65.
- McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res* 2006; 173: 86–93.
- Möller C, Arai N, Lücke J, Ziemann U. Hysteresis effects on the input-output curve of motor evoked potentials. *Clin Neurophysiol* 2009; 120: 1003–1008.
- Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. *J Physiol* 1997; 498: 817–823.
- Oldfield R. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 1971; 9: 97–113.
- Pawley AD, Chowdhury FA, Tangwiriyasakul C, Ceronie B, Elwes RDC, Nashef L, et al. Cortical excitability correlates with seizure control and epilepsy duration in chronic epilepsy. *Ann Clin Trans Neurol* 2017; 4: 87–97.

- Pearce AJ, Clark R a, Kidgell DJ. A comparison of two methods in acquiring stimulus-response curves with transcranial magnetic stimulation. *Brain Stimul* 2013; 6: 306–309.
- Pellicciari MC, Miniussi C, Ferrari C, Koch G, Bortoletto M. Ongoing cumulative effects of single TMS pulses on corticospinal excitability: An intra- and inter-block investigation. *Clin Neurophysiol* 2016; 127: 621–628.
- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015
- Reutens DC, Berkovic SF. Epilepsy demonstrated with transcranial magnetic stimulation. *Lancet* 1992; 339: 362–363.
- Rosenow F, Martin Klein K, Hamer HM. Non-invasive EEG evaluation in epilepsy diagnosis. *Expert Rev Neurother* 2015; 15: 425–444.
- Rossi S, Hallett M, Rossini P, Pascual-Leone A, Group and TS of T consensus. Safety, ethical considerations, and application guideline for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* 2009; 120: 2008–2039.
- Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol* 1994; 91: 79–92.
- Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. *J Physiol* 2001; 530: 307–317.
- Schwartzkroin PA. Cellular electrophysiology of human epilepsy. *Epilepsy Res* 1994; 17: 185–192.
- Searle S, Speed F, Milliken G. Population marginal means in the linear model: an alternative to least squares means. *Am. Stat.* 1980; 34: 216–221.
- Shimizu T, Filippi MM, Palmieri MG, Oliveri M, Vernieri F, Pasqualetti P, et al. Modulation of intracortical excitability for different muscles in the upper extremity: paired magnetic stimulation study with focal versus non-focal coils. *Clin Neurophysiol* 1999; 110: 575–581.
- Silbert BI, Heaton AE, Cash RFH, James I, Dunne JW, Lawn ND, et al. Evidence for an excitatory GABAA response in human motor cortex in idiopathic generalised epilepsy. *Seizure* 2015; 26: 36–42.
- Smith MJ, Adams LF, Schmidt PJ, Rubinow DR, Wassermann EM. Effects of ovarian hormones on human cortical excitability. *Ann Neurol* 2002; 51: 599–603.
- Smith S. EEG in the diagnosis, classification, and management of patients with epilepsy. *J Neurol Neurosurg Psychiatry* 2005; 76: ii2-ii7.
- Stern WM, Desikan M, Hoad D, Jaffer F, Strigaro G, Sander JW, et al. Spontaneously fluctuating motor cortex excitability in alternating hemiplegia of childhood: A transcranial magnetic stimulation study. *PLoS One* 2016; 11: 1–18.
- Stern WM, Sander JW, Rothwell JC, Sisodiya SM. Impaired intracortical inhibition demonstrated in vivo in people with Dravet syndrome. *Neurology* 2017; 88: 1–7.
- van Strien J. Classificatie van links-en rechtshandige proefpersonen [Classification of left- and right-handed research participants]. *Ned Tijdschr v Psychol* 1992; 47: 88–92.
- Van Strien J. The Dutch handedness questionnaire. 2003: URL: hdl.handle.net/1765/956 (accessed

10.10.16).

Vallence A-M, Smalley E, Drummond P, Hammond G. Long interval intracortical inhibition is asymmetric in young but not older adults. *J Neurophysiol* 2017; in press

Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. *EEG Clin Neurophysiol* 1992; 85: 355–364.

Valzania F, Quatrone R, Strafella AP, Bombardi R, Santangelo M, Tassinari CA, et al. Pattern of motor evoked response to repetitive transcranial magnetic stimulation. *EEG Clin Neurophysiol* 1994; 93: 312–317.

Varnava A, Stokes MG, Chambers CD. Reliability of the ‘observation of movement’ method for determining motor threshold using transcranial magnetic stimulation. *J Neurosci Methods* 2011; 201: 327–332.

Vaseghi B, Zoghi M, Jaberzadeh S. Inter-pulse interval affects the size of single-pulse TMS-induced motor evoked potentials: A reliability study. *Basic Clin Neurosci.* 2015; 6: 44–51.

Wassermann EM. Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin Neurophysiol* 2002; 113: 1165–1171.

Wassermann EM, Samii A, Mercuri B, Ikoma K, Oddo D, Grill SE, et al. Responses to paired transcranial magnetic stimuli in resting, active, and recently activated muscles. *Exp Brain Res* 1996; 109: 158–163.

Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol* 1999; 517: 591–597.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann Neurol* 1996; 40: 367–378.

Table 1: TMS set-up and stimulation protocol per centre

	Centre 1	Centre 2	Centre 3	Centre 4
Stimulator type	Magstim Rapid ²	MagPro X100	Magstim BiStim ²	Magstim BiStim ²
Maximum stimulator output (T)	1.5	3.9	1.5	1.5
Magnetic pulse waveform	biphasic	biphasic	monophasic	monophasic
Coil type	figure-of-eight	round	figure-of-eight	figure-of-eight
Stimulation location	motor hot spot	vertex (Cz)	motor hot spot	motor hot spot
Target muscle	ADM	APB	FDI	APB
Intertrial interval	~4 s	1 s	4 s	5 s
Repetitions per ISI	50	6	10	10
Order of ISI's	random	increasing	random	random
Conditioning pulse intensity (%rMT)	120	110	120	110
Test pulse intensity (%rMT)	120	110	120	110
LICI response ratio	Mean(TR)/mean(CR)	Mean(TR)/mean(unconditionedMEP)	Mean(TR/CR)	Mean(TR)/mean(unconditionedMEP)
Time of day measurement	9AM-5PM	9AM-4PM	9AM-5PM	9AM-5PM

ADM = abductor digiti minimi, APB = abductor pollicis brevis, FDI = first dorsal interosseous, ISI = interstimulus interval, rMT = resting motor threshold, LICI = long-interval intracortical inhibition, TR = test response, CR = conditioning response, MEP = motor-evoked potential

Table 2: LICI results

Subject type	Centre	ISI 50 ms	ISI 100 ms	ISI 150 ms	ISI 200 ms	ISI 250 ms
Controls	1	1.32 ± 1.69	0.28 ± 0.30	0.39 ± 0.36	0.81 ± 0.55	0.82 ± 0.40
	2	3.76 ± 8.74	1.14 ± 1.80	1.15 ± 2.43	1.32 ± 1.91	1.37 ± 1.50
	3	0.88 ± 0.84	-	0.58 ± 0.42	1.00 ± 0.52	1.06 ± 0.63
Generalised epilepsy	3	1.39 ± 1.84	-	0.45 ± 0.34	0.82 ± 0.47	0.91 ± 0.42
	4	1.37 ± 1.51	0.27 ± 0.23	0.40 ± 0.35	0.63 ± 0.34	0.78 ± 0.49
Focal epilepsy	3	0.73 ± 0.60	-	0.70 ± 0.95	0.88 ± 0.85	1.27 ± 1.36
	4	0.78 ± 0.89	0.33 ± 0.25	0.34 ± 0.24	0.46 ± 0.28	0.64 ± 0.66

LICI (mean ± SD) at each ISI per centre and subject type. For controls and refractory genetic generalised epilepsy LICI measured when stimulating the dominant hemisphere is shown, for refractory focal epilepsy when stimulating the ipsilateral hemisphere.

Figure legends:

Figure 1: Included and excluded subjects per centre. Exclusion criteria:

¹No LICI data, ²no LICI data for the dominant hemisphere, ³aspecific abnormalities on the EEG, ⁴no treatment with AEDs, ⁵not refractory, ⁶no LICI data for the hemisphere ipsilateral to the epileptic focus, ⁷type of epilepsy unknown.

Figure 2: LICI at each ISI per centre and subject type. The boxplots show the median \pm 25th percentiles; the whiskers show 1.5 times the interquartile range; the dots show outliers outside the whiskers. All outliers were included in the analysis but several are outside the boundaries of the y-axis of the figure: healthy controls – centre 1: LICI = 7.00 (ISI 50 ms); centre 2: LICI = 5.37, 5.42, 18.50, 24.20, 45.24 (ISI 50 ms), 5.56, 6.02, 7.37 (ISI 100 ms), 10.23, 10.31 (ISI 150 ms), 6.15, 9.68 (ISI 200 ms), 6.11, 6.61 (ISI 250 ms). Generalised epilepsy – centre 3: LICI = 8.86 (ISI 50 ms). Focal epilepsy – centre 3: LICI = 6.73 (ISI 250 ms).

Figure 3: LICI at all ISIs per subject type pooled across centres. The boxplots show the median \pm 25th percentiles; the whiskers show 1.5 times the interquartile range. LICI at ISIs 100 and 150 ms (diagonal pattern) shows significantly more inhibition than LICI at ISIs 50, 200 and 250 ms for all subject types.

Figure 4: LICI at all ISIs per subject type pooled across centres 1, 2, 3 and 4. The boxplots show the median \pm 25th percentiles; the whiskers show 1.5 times the interquartile range. * significant difference between controls (open boxes, without centre 2) and refractory focal epilepsy at ISIs of 100 ms ($p=0.02$) and 200 ms ($p=0.04$). There were no significant differences between the control (open boxes, without centre 2) and refractory generalised epilepsy group or between the refractory generalised and focal epilepsy groups. There were no differences between the groups when data from all centres were included in the analysis (filled boxes).

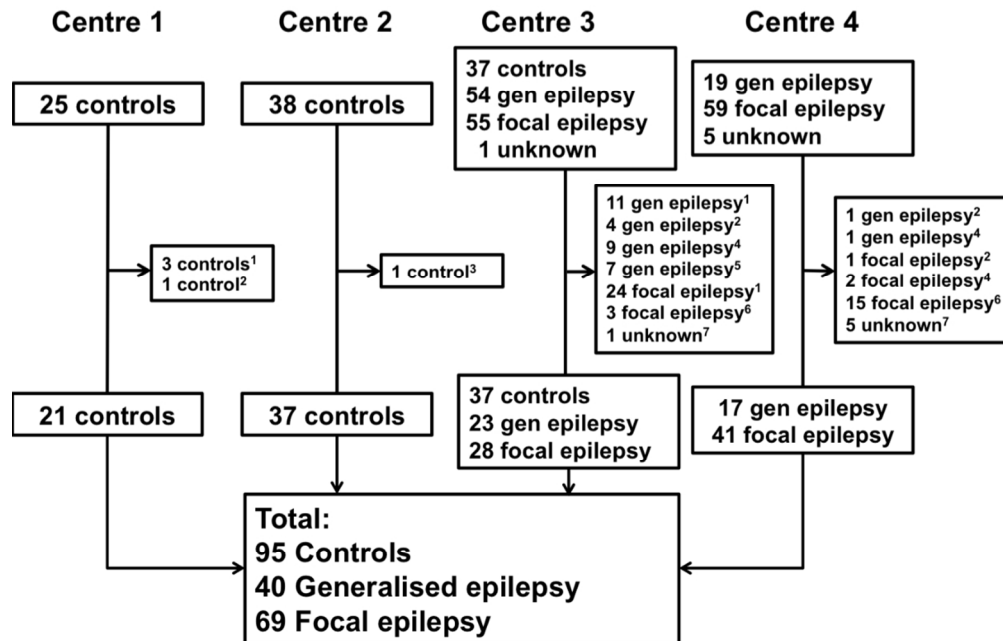


Figure 1: Included and excluded subjects per centre. Exclusion criteria: 1No LICI data, 2no LICI data for the dominant hemisphere, 3specific abnormalities on the EEG, 4no treatment with anti-epileptic drugs, 5not refractory, 6no LICI data for the hemisphere ipsilateral to the epileptic focus, 7type of epilepsy unknown.

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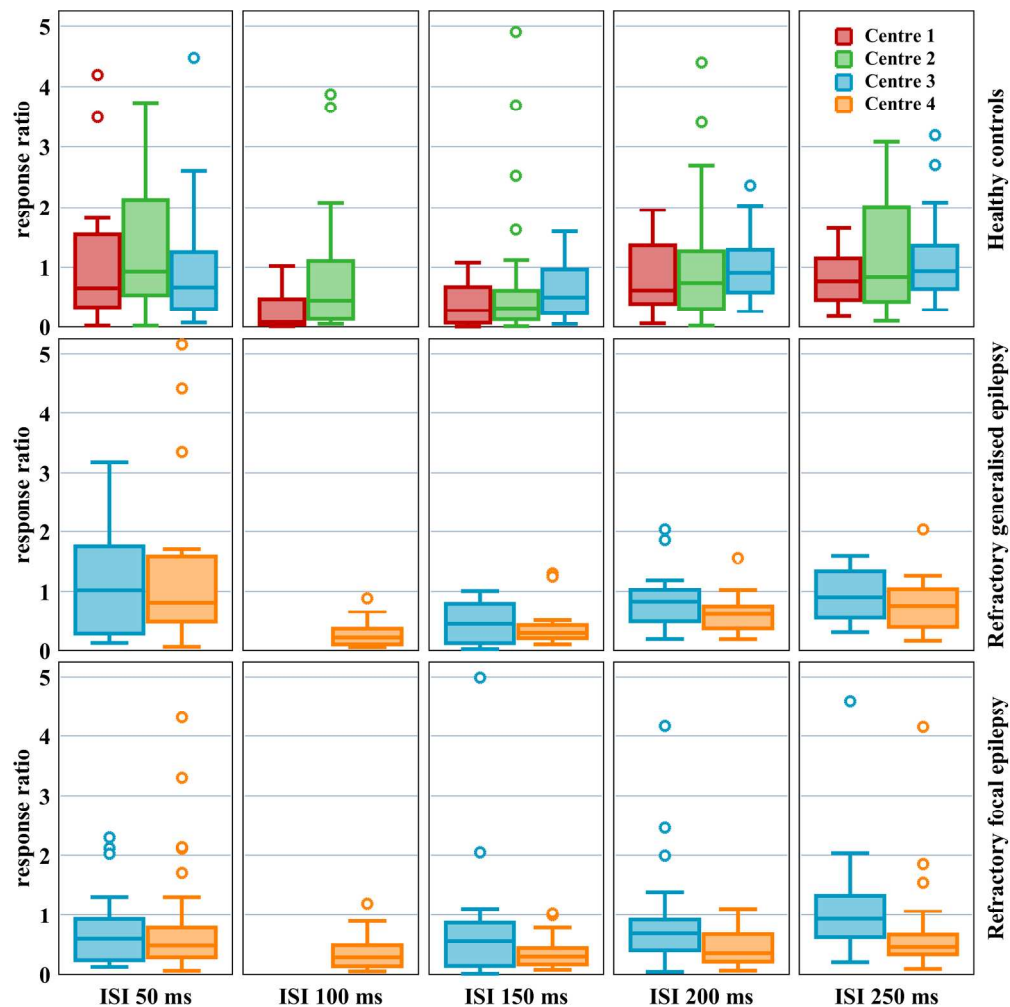


Figure 2: LICI at each ISI per centre and subject type. The boxplots show the median \pm 25th percentiles; the whiskers show 1.5 times the interquartile range; the dots show outliers outside the whiskers. All outliers were included in the analysis but several are outside the boundaries of the y-axis of the figure: healthy controls - centre 1: LICI = 7.00 (ISI 50 ms); centre 2: LICI = 5.37, 5.42, 18.50, 24.20, 45.24 (ISI 50 ms), 5.56, 6.02, 7.37 (ISI 100 ms), 10.23, 10.31 (ISI 150 ms), 6.15, 9.68 (ISI 200 ms), 6.11, 6.61 (ISI 250 ms). Generalised epilepsy - centre 3: LICI = 8.86 (ISI 50 ms). Focal epilepsy - centre 3: LICI = 6.73 (ISI 250 ms).

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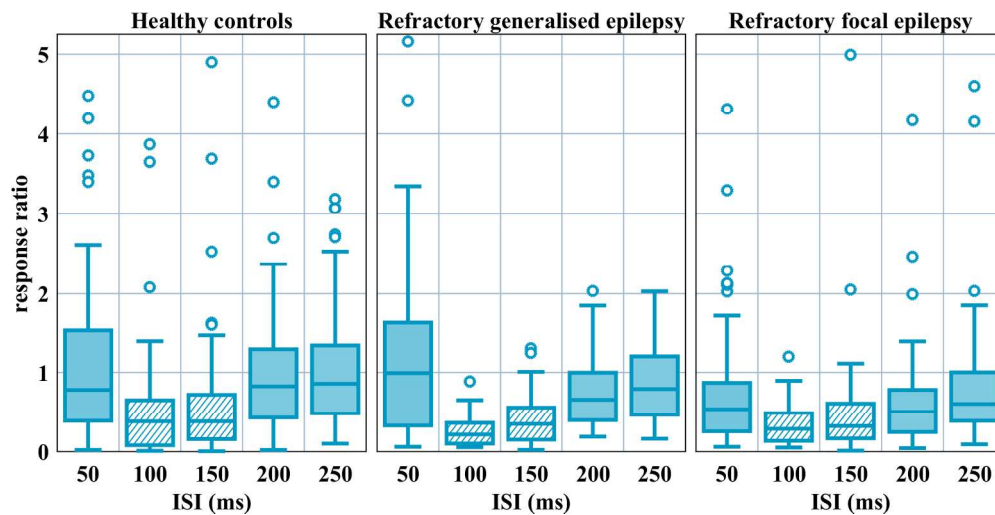


Figure 3: LICI at all ISIs per subject type pooled across centres. The boxplots show the median \pm 25th percentiles; the whiskers show 1.5 times the interquartile range. LICI at ISIs 100 and 150 ms (diagonal pattern) shows significantly more inhibition than LICI at ISIs 50, 200 and 250 ms for all subject types.

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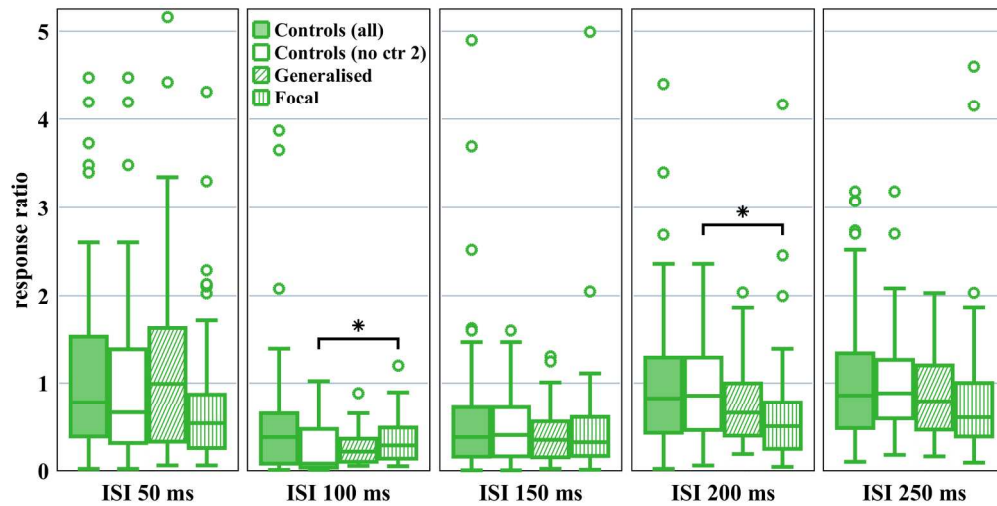


Figure 4: LICI at all ISIs per subject type pooled across centres 1, 2, 3 and 4. The boxplots show the median \pm 25th percentiles; the whiskers show 1.5 times the interquartile range. * significant difference between controls (open boxes, without centre 2) and refractory focal epilepsy at ISIs of 100 ms ($p=0.02$) and 200 ms ($p=0.04$). There were no significant differences between the control (open boxes, without centre 2) and refractory generalised epilepsy group or between the refractory generalised and focal epilepsy groups. There were no differences between the groups when data from all centres were included in the analysis (filled boxes).

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Review

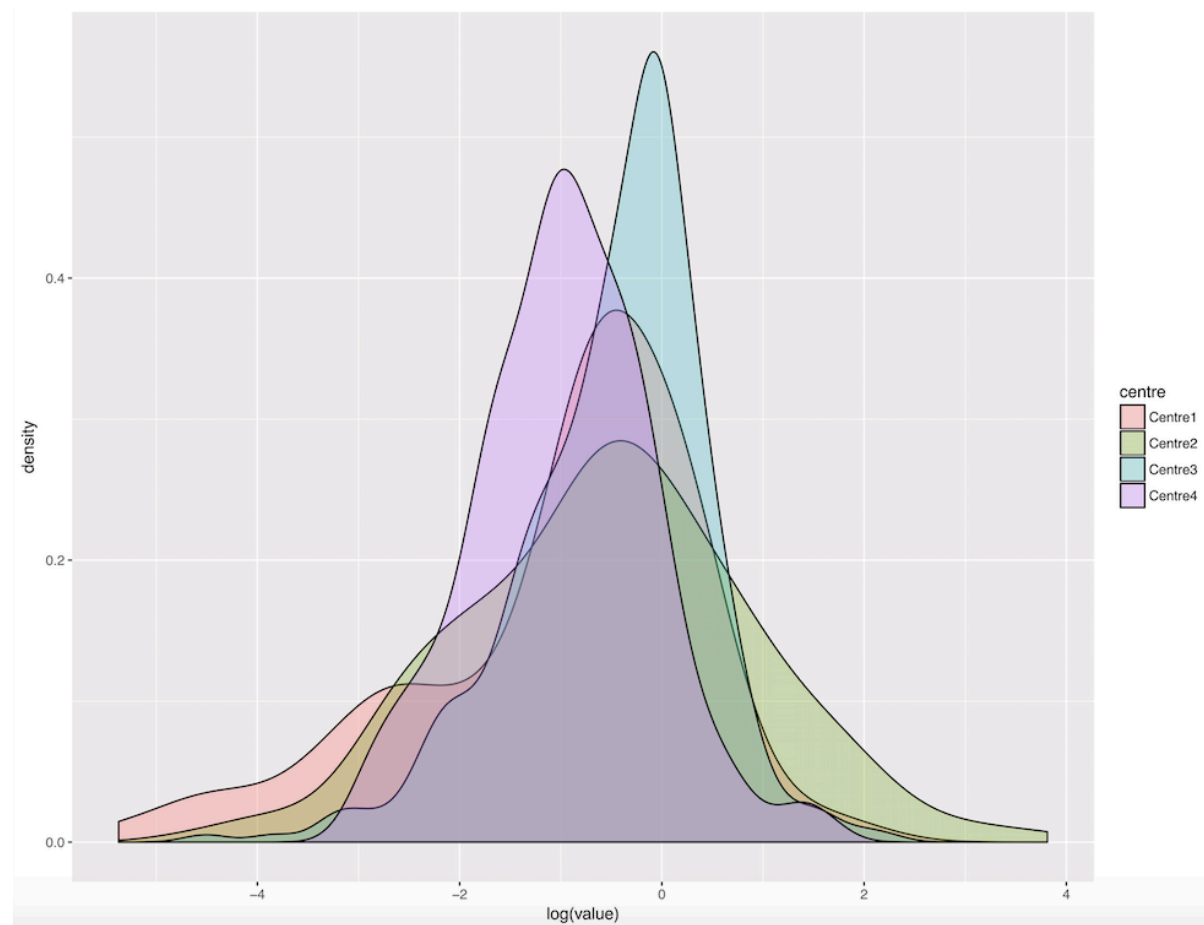
Supplementary material:

Figure A: Distribution of log-transformed LICI data in all centres. Data of controls and patients are pooled together and shown for Centre 1, 2, 3 and 4. The different results with and without the data from centre 2 may be explained by the difference in distribution. The distribution of the data from centre 2 is wider than that of the other centres, indicating a larger variability, which may have been caused by the stimulation protocol used (global stimulation, short intertrial interval, biphasic stimuli).

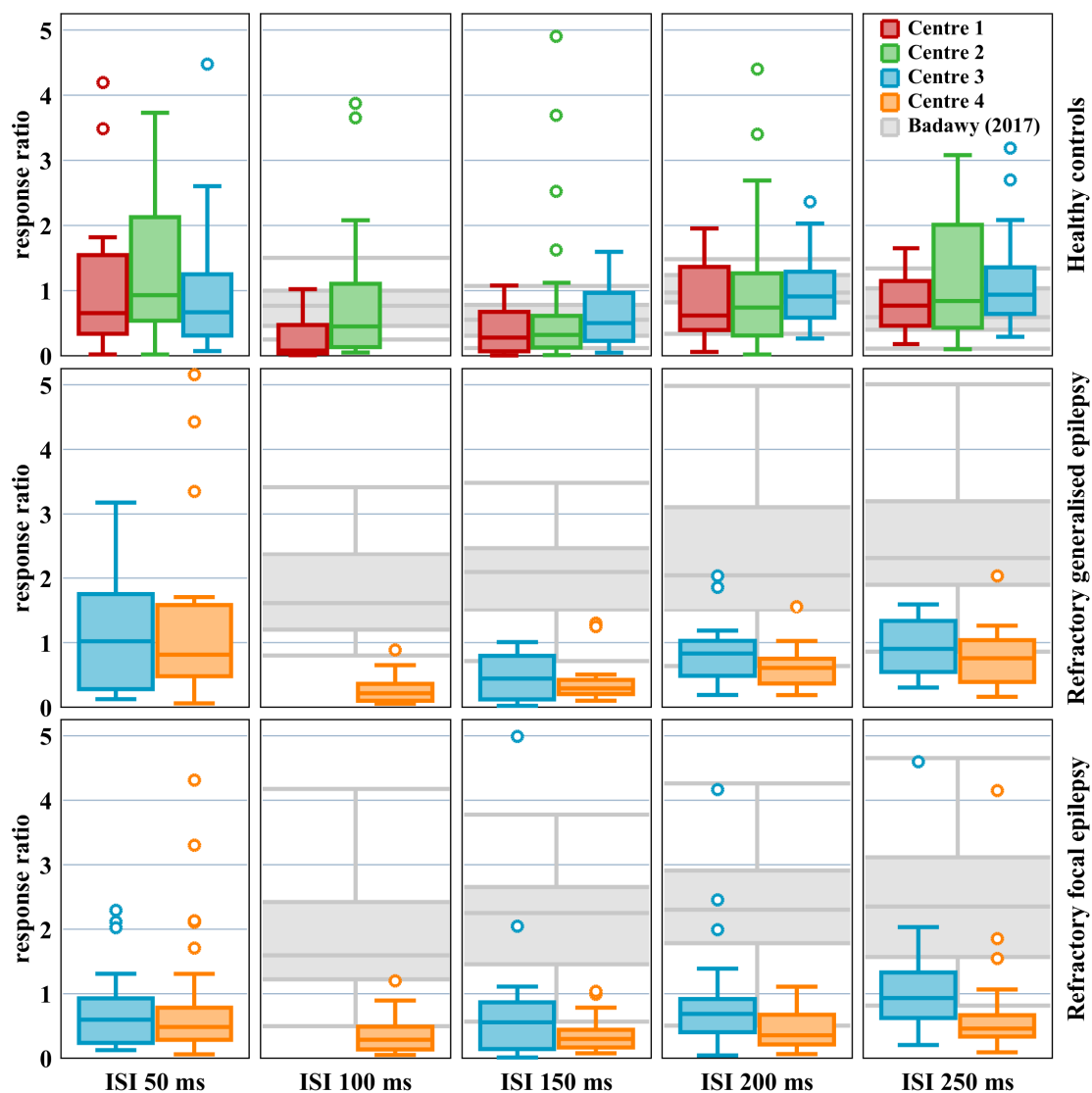


Figure B: LICI at each ISI per centre and subject type. (see also figure 2 in the main text.) The grey boxplots represent the long ISI response ratios from Badawy et al., obtained from the online supplementary material (see Badawy et al., 2017): healthy controls C-1 to C-20; refractory generalised epilepsy JM-RF-1 to JM-RF-16, JA-RF-1 to JA-RF-15 and GTC-RF-1 to GTC-RF-18; refractory focal epilepsy TL-RF-1 to TL-RF-20, ETL-RF-1 to ETL-RF-18 and TL-TL-RF-1 to TL-TL-RF-7. Boxplots show the median \pm 25th percentiles, whiskers show 1.5 times the interquartile range; the dots show outliers outside the whiskers. All outliers were included in the analysis but several are outside the boundaries of the y-axis of the figure: healthy controls – centre 1: LICI = 7.00 (ISI 50 ms); centre 2: LICI = 5.37, 5.42, 18.50, 24.20, 45.24 (ISI 50 ms), 5.56, 6.02, 7.37 (ISI 100 ms), 10.23, 10.31 (ISI 150 ms), 6.15, 9.68 (ISI 200 ms), 6.11, 6.61 (ISI 250 ms). Generalised epilepsy – centre 3: LICI = 8.86 (ISI 50 ms). Focal epilepsy – centre 3: LICI = 6.73 (ISI 250 ms).