RESEARCH ARTICLE

Tracking cortical excitability dynamics with transcranial magnetic stimulation in focal epilepsy

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Abstract

Introduction: The lack of reliable biomarkers constrain epilepsy management. We assessed the potential of repeated transcranial magnetic stimulation with electromyography (TMS-EMG) to track dynamical changes in cortical excitability on a within-subject basis. **Methods**: We recruited people with refractory focal epilepsy who underwent video-EEG monitoring and drug tapering as part of the presurgical evaluation. We performed daily TMS-EMG measurements with additional postictal assessments 1–6 h following seizures to assess resting motor threshold (rMT), and motor evoked potentials (MEPs) with single- and paired-pulse protocols. Anti-seizure medication (ASM) regimens were recorded for the day before each measurement and expressed in proportion to the dosage before tapering. Additional measurements were performed in healthy controls to evaluate day-to-day rMT variability. **Results**: We performed 77 (58 baseline, 19 postictal) measurements in 16 people with focal epilepsy and 35 in seven healthy controls. Controls showed minimal day-to-day rMT variation. Withdrawal of ASMs was associated with a lower rMT without affecting MEPs of single- and paired-pulse TMS-EMG paradigms. Postictal measurements following focal to bilateral tonic–clonic seizures demonstrated unaltered rMT and increased short interval intracortical inhibition, while measurements following focal seizures with impaired awareness showed decreased rMT's and reduced short and long interval intracortical inhibition. **Conclusion**: Serial within-subject rMT measurements yielded reproducible, stable results in healthy controls. ASM tapering and seizures had distinct effects on TMS-EMG excitability indices in people with epilepsy. Drug tapering decreased rMT, indicating increased overall corticospinal excitability, whereas seizures affected intracortical inhibition with contrasting effects between seizure types.

Introduction

Epilepsy is characterized by neuronal hyperexcitability and hypersynchrony involving a disturbed balance between cortical excitatory and inhibitory inputs.[1–3] Seizures may be difficult to control and impact the quality of life.[4] Biomarkers that measure disease severity and help to evaluate pharmacotherapy are needed.

Transcranial magnetic stimulation with electromyography (TMS-EMG) has been utilized for the noninvasive assessment of cortical excitability.[5] It yield various readouts, including the resting motor threshold (rMT) reflecting membrane excitability of neurons within the corticospinal tract, and measures reflecting the activity of excitatory and inhibitory intracortical circuits.[6–8] The rMT is determined with single-pulse TMS (spTMS) while paired-pulse TMS (ppTMS) paradigms are used to determine short interval cortical inhibition (SICI), a marker for $GABA_A$-receptor-mediated inhibition,[9] and the long interval cortical inhibition (LICI) a measure of $GABA_B$-

receptor-mediated inhibition.[10] Clinical studies demonstrated that various anti-seizure medications (ASM) influence rMT.[7,11–16] SICI and LICI have been used to investigate the GABA-ergic properties of pharmacological compounds[17–19] and investigate aberrant inhibition in epilepsy.[2,20,21] For instance, a TMS-EMG study in people with Dravet syndrome reported facilitation, rather than suppression, of the response with short interval ppTMS, indicating reduced recruitment of inhibitory neurons by the conditioning pulse.[21] Combining spTMS and ppTMS may help assess the different aspects of cortical motor excitability.

The use of TMS to differentiate between people with epilepsy and healthy controls proved inadequate because of high inter-subject variability.[22,23] Serial within-subject TMS measurements, however, may potentially trace the cortical excitation-inhibition balance within individuals over time. Accordingly, a longitudinal study in 20 healthy controls demonstrated that the use of carbamazepine and lamotrigine exerts a dose-dependent effect on the rMT.[14] Likewise, the initiation of a ketogenic diet in eight people with epilepsy was associated with increased attenuation following short-latency ppTMS, indicating increased GABA-mediated inhibition.[24] Serial TMS may thus be attractive to monitor treatment response. A previous study demonstrated that seizures impacted ppTMS readouts with more attenuated conditioned responses (CRs), indicating increased recruitment of inhibitory neurons by the conditioning stimulus after seizures.[20] TMS could therefore help to assess cortical excitability in the postictal state, especially for seizures followed by postictal generalized EEG suppression (PGES), an EEG marker related to excessive inhibition.[25–27] This approach could further our understanding of seizure termination mechanisms in focal impaired awareness (FIA) and focal to bilateral tonic–clonic (fbTC) seizures.

We aimed to explore the potential of TMS-EMG measures to assess the impact of ASM tapering and seizures on cortical excitability measures. We performed daily, and postictal assessments in people admitted for seizure recordings as part of a presurgical evaluation at the epilepsy monitoring unit (EMU).[28] We hypothesized that ASM tapering would result in increased TMS-EMG measures of excitatory control, while the occurrence of a seizure would increase TMS-EMG measures reflecting inhibitory control.

Methods

Participants

Adults admitted to the EMU for presurgical evaluation were consecutively included between May 2017 and July 2019 if they had (1) a history of fbTC seizures and (2) $\geq$1 fbTC seizures in the year before admission. Healthy controls were recruited among employees of the institution. Cases and controls were excluded in case of contraindications to TMS other than epilepsy, including pregnancy, inability to follow the experimental protocol, and in case of any medication changes other than the ASM scheduled during the trial period. The study was approved by the ethics committee of Leiden University Medical Center. All participants provided written informed consent before entry.

Experimental design

Daily records were kept of seizures (based on video-EEG) and drug regimens. Clinical observation included continuous video-EEG and ECG, recordings. On the day of admission, a baseline TMS-EMG measurement was performed at approximately 1:30 PM. Subsequent TMS-EMG measurements were performed daily around 8:00 AM. Postictal measurements were performed 1–6 h after the end of any fbTC or FIA seizure. Each individual underwent a maximum of three postictal measurements of their most common seizure type. In the case of two distinct seizure types, we limited the postictal measurements to a single assessment if we had already obtained three postictal measurements for another seizure type. Each control underwent five consecutive daily rMT assessments performed at approximately the same time.

Measurement setup and protocol

Magnetic stimulation was performed using a Magpro X100 Magnetic stimulator (Magventure, Farum, Denmark) using a large 140-mm diameter circular coil (MMC-140) centered above the vertex (Cz-EEG electrode position).[29] The circular coil allows for a diffuse stimulation of the cortex, minimizes the impact of small changes in coil position, and reduces the length of a measurement session, as motor hotspot determination is not needed.[29] The muscle response was recorded using disposable self-adhesive pre-gelled (16 $\times$ 20) mm rectangular Ag/AgCl surface electrodes. The EMG signal was acquired with a 16-kHz sampling frequency using the Nicolet Viking EMG system (Carefusion, San Diego, CA, USA), connected to a computer running MATLAB (The Math Works, Inc. MATLAB, version 2018a, Natick, MA, USA).

Participants were seated in a comfortable chair. Muscle activity was recorded bilaterally using a belly-tendon montage of the thenar muscles. They were asked to relax and were provided with foam ear-inserts. Participants were asked to keep their eyes open during the TMS-evaluation, including during postictal measurements. If the person closed their eyes they were reinstructed to

keep their eyes open. Each measurement started by assessing left and right rMT, determined as the minimal mean stimulator output (MSO) required to evoke motor responses above 50 μV in five out of 10 trials. Next, for each current direction, the following stimulations were given: spTMS (50 trials, 110% rMT, 5-sec intertrial interval), short-latency ppTMS to assess SICI (30 trials, 80% rMT conditioning stimulus, 110% rMT test stimulus, 5 msec inter-stimulus interval, 5 sec in between trials), and long latency ppTMS to assess LICI (30 trials, 110% rMT conditioning, 110% RMT test stimulus, 100 msec interstimulus interval, 5 sec in between trials). Each measurement session lasted approximately 30 min.

Data processing and MEP analysis

EMG signals were extracted starting 20 msec before and ending 50 msec after TMS pulses. Trials with significant pre-activation (>20 μV amplitude) of the abductor polices brevis muscle in the 20 msec window before stimulation were discarded from the analysis. For each trial, the peak-to-peak amplitude of the motor evoked potentials (MEPs) was determined in the window starting 15 msec after and ending 50 msec after the stimulus trigger. For ppTMS the conditioned peak-to-peak MEP amplitude was divided by the unconditioned peak-to-peak amplitude. Values below one thus indicate suppression of the response, while values above one indicate facilitation.

Medication effects

To investigate the effect of ASM dosage on TMS indices we normalized the summed dosage for each ASM type 24 h prior to measurement and divided this value by the 24 h summed medication taken at home. Next, to calculate a combined normalized ASM load, we summed the normalized values per ASM type and divided this by the total number of ASMs. Consider S as the set containing all the ASM types an individual with epilepsy takes, then we can estimate the overall ASM load L at measurement m as follows:

$$L(m) = \frac{1}{N} \sum_{x \in S} \frac{x_{24}(m)}{x_h}$$

Where for every type of ASM $x \in S$, x_{24} is the summed dosage of ASM x in the 24 h prior to measurement m, x_h is the summed daily at home dosage of ASM x, and N is the total number of elements in set S.

Statistical analysis

We used regression analysis to determine correlations between TMS-EMG indices and ASM dosage and investigate the impact of single seizures on the TMS-EMG indices. For rMT a linear mixed effects model was used with fixed effects for the intercept, ASM load, handedness, lateralization of the epileptic focus (according to the ictal EEG onset and/or clinical semiology or interictal epileptiform EEG activity), seizure occurrence before measurement and type (none, FIA, fbTC), and random intercept by-subject (to account for high between-subject variation in baseline rMT). For MEP, SICI, and LICI we used a linear mixed-effect model with ASM load and seizure type entered as fixed effects, and a random effect model for intercept by-subject. The best linear unbiased predictor estimates and corresponding 95% confidence interval (CI) for each predictor are presented.

We calculated the intraclass correlation coefficient (ICC) to estimate the agreement between repeated sessions within healthy controls. ICC varies between 0 and 1, where 1 represents perfect repeatability.

Results

Population characteristics

Characteristics of participants are summarized in Table 1 and an overview of the ASMs for each individual with epilepsy is given in Table 2. In total, 77 measurements (58 baseline and 19 postictal) were performed in 16 people with epilepsy (mean age 32 years, range: 19–51 years; 9 male, 7 female), and 35 in seven controls (mean age 34 years, range 19–57 years; 3 male, 4 female). Two individuals with epilepsy terminated the study prematurely; one due to a self-reported high emotional burden of the TMS measurement in combination with the presurgical evaluation, the second due to fear of seizure induction by TMS. The remaining 14 tolerated the TMS-EMG procedures well. One was rejected from analysis due to insufficient TMS-EMG data as evaluation was terminated after 2 days.

A total of 34 seizures (range 1–9) were recorded in nine people, including nine fbTC seizures in four people. In four, no seizures occurred. Postictal generalized EEG supression was observed in the EEG for four out nine fbTC seizures (mean postictal generalized EEG supression duration 40 sec, range: 14–59 sec). The remaining 25 seizures in seven people were FIA seizures.

Postictal TMS-EMG measurements were performed for six out of nine fbTC seizures and 13 out of 25 FIA seizures. All participants were awake, able, and willing to undergo the postictal evaluations and had their eyes open during the measurement. Postictal measurements were not performed following the remaining 15 seizures due to either the occurrence of seizure clusters ($n = 11$), presence of at least three previous postictal recordings following the same seizure type ($n = 3$), or general fatigue/

Table 1. Population demographics.

					Epilepsy lateralization				Seizures		
Case	F/M	Age	Epi dur (years)	Handedness	Interictal EEG	Ictal onset EEG	Semiology	MRI findings	FIA	fbTC	Meas
301	M	37	20	Left	Left	Left	Left	MTS left	8	1	2
302	M	51	45	Right	Left	Left	Left	MTS left	4	–	1
303	F	19	16	Left	–	–	Left	–	–	–	–
304	F	45	13	Right	Bilat.	–	Right	Bilat. white-matter abnormalities	–	–	–
305	M	29	6	Left	–	–	–	MTS left	–	–	–
306	M	20	13	Right	Bilat. (R > L)	Right	Right	–	–	2	1
308	M	34	13	Right	–	Left	–	Left sided DVA with cavernoma temporal lobe	3	–	3
309	F	30	18	Right	–	–	Right	–	–	–	–
310	M	41	27	Right	Left	Left	–	MAP abnormality left frontal lobe	3	–	3
311	M	23	8	Right	Right	Right	Right	–	1	3	3
312	M	24	5	Right	Bilat. (R > L)	Right	Right	–	–	3	2
313	F	34	10	Right	Left	–		MTS left	1	–	1
316	M	38	11	Right	Bilat. (L > R)	Left	Left	MTS left	5	–	2

F, female; M, male; Epi dur, years living with epilepsy; Bilat, bilateral; R, right; L, left; MTS, mesiotemporal sclerosis; DVA, developmental venous anomaly; MAP, morphometric analysis program; FIA, focal seizures with impaired awareness; fbTC, focal to bilateral tonic–clonic seizures.

Table 2. Anti-seizure medication per individual with epilepsy.

	Medication type (mg)								
Case	CBZ	CLB	LCM	LTG	LEV	OCB	TPM	VPA	Total ASMs per case
301	1600				3000				2
302	1600	10						2250	3
303	1200								1
304					1500	900			2
305			150						1
306			350			1500			2
308			200					1250	2
309		20		450					2
310			300		1000		150	2000	4
311		15			2000	1299			3
312	1000				2500			2250	3
313				300					1
316	1400	10							2
Total number of cases on ASM	5	4	4	2	5	3	1	4	

ASM, anti-seizure medication; *N*, number of ASMs; CBZ, carbamazepine; LEV, levetiracetam; VPA, valproatic acid; CLB, clobozapam; OCB, oxcarbamazepine; LCM, lacosamide; LTG, lamotrigine; TPM, topirimate.

exhaustion ($n = 1$). Examples of serial TMS-EMG measurements in acasewith fbTC seizures and a case with FIA seizures are shown in Figures 1, 2, respectively.

TMS-EMG parameter changes

A schematic overview of the results is shown in Table 3. The difference in the spTMS and ppTMS-EMG parameters in the postictal evaluations relative to the previous baseline measurement is shown per seizure type in Figure 3.

rMT changes

Intersession reproducibility of the rMT across the different testing days in the healthy controls was high (ICC:

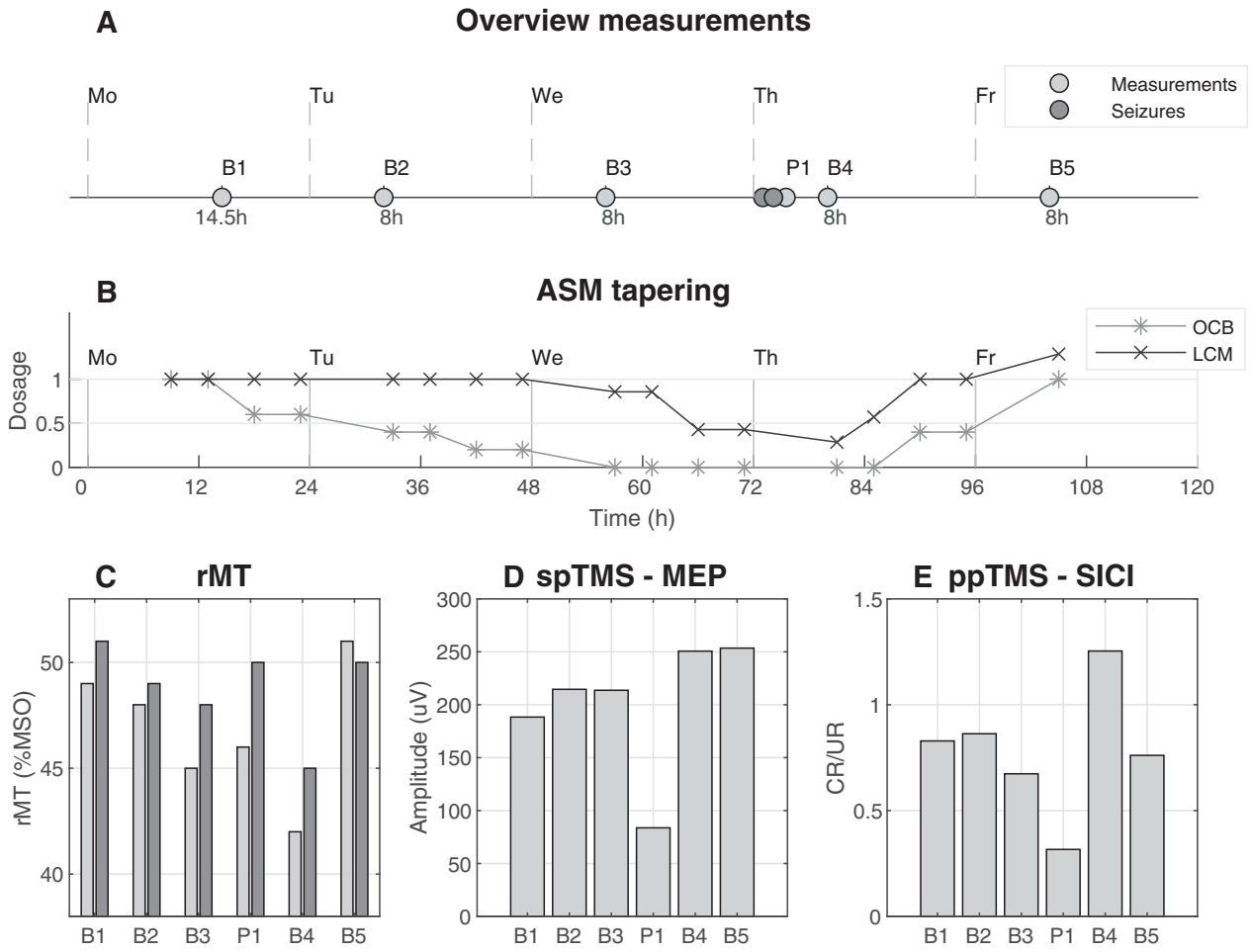

Figure 1. Case 306 with multiple focal to bilateral tonic–clonic seizures with right hemispheric onset. Panel (A) provides an overview of the timing of the TMS-EMG measurements (open blue circles indicated as B1–B5 for baseline evaluations and P1 for the postictal evaluation) and detected fbTC seizures (red circles). Panel (B) displays the ASM regimen changes during tapering, as expressed by the normalized dosage (i.e., the summed dosage over the 24 h prior to each measurement timepoint, divided by the standard at-home dosage summed over 24 h); changes of individual ASMs are depicted with separate lines. Panels (C–E) show the cortical excitability indices for all measurements that showed significant ASM- and seizure-related changes in the postictal phase. The individual left-hand (light gray) and right-hand (dark gray) rMT values are shown in panel (C). Note that the rMT shows a gradual reduction with a reduction in medication dosage. The postictal P1 measurements demonstrated an increased rMT when compared to the surrounding baseline measurements. MEP amplitude for single-pulse TMS, measured at 110% rMT (panel D) was significantly reduced for the postictal measurement when compared to surrounding baseline measurements. The measure of short interval ppTMS (SICI; CR/UR) showed a diminished postictal ratio (panel E), suggesting an increase in $GABA_A$-mediated inhibition in the postictal phase. Measures of long interval ppTMS (LICI) were not significant for fbTC seizures and are not shown. ASM, anti-seizure medication; OCB, oxcarbazepine; LCM, lacosamide; rMT, resting motor threshold; MSO, mean stimulator output; MEP, motor evoked potential; spTMS, single-pulse TMS; ppTMS, paired pulse TMS; SICI, short interval cortical inhibition; CR, conditioned response; UR, unconditioned response; fbTC, focal to bilateral tonic–clonic; TMS-EMG, transcranial magnetic stimulation with electromyography.

0.996). The changes in rMT as a function of normalized ASM load, including the model's significant curve fits and their corresponding confidence intervals, are shown for the individual subjects in Figure 4. Decreasing ASM load in people with epilepsy was associated with lower rMT values (5.3% MSO, 95%CI: 3.1–7.4% MSO). The occurrence of fbTC seizures did not have a significant effect on rMT (estimate: −0.2% MSO, 95%CI: −2.4% to 2.0% MSO). Conversely, following FIA seizures a decrease in rMT was found (estimate: −2.2% MSO, 95% CI: −3.7% to −0.6% MSO). Both handedness and lateralization of the seizure onset zone had an effect on the rMT with lower values for the dominant hemisphere (estimate: 1.2% MSO, 95% CI: 0.2–2.3% MSO) and the hemisphere ipsilateral to the seizure onset zone (estimate: 1.6% MSO, 95% CI: 0.6–2.7% MSO).

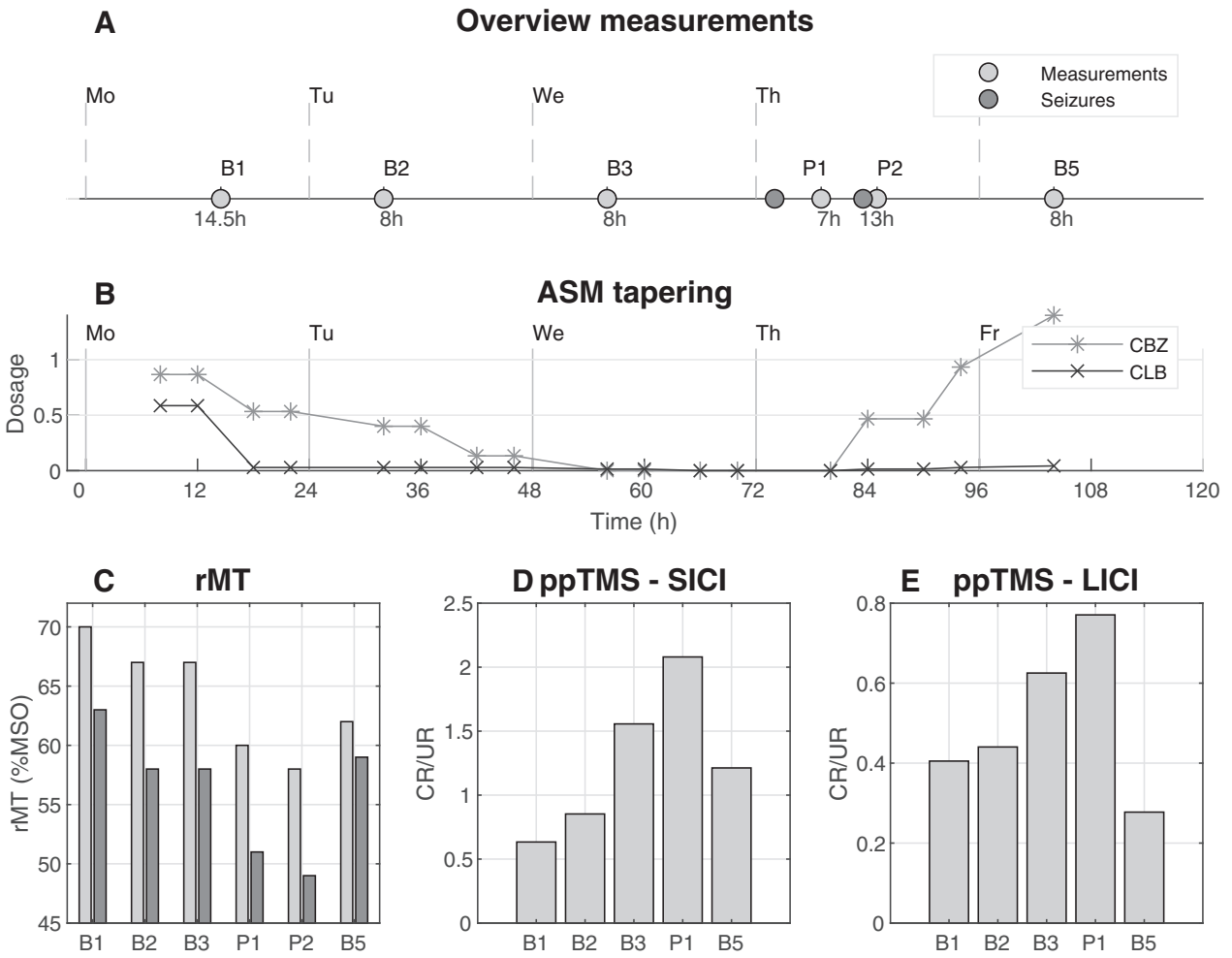

Figure 2. Case 316 with multiple focal impaired awareness seizures with left hemispheric onset. Panel (A) provides an overview of the serial TMS-EMG measurements (open blue circles indicated as B1–B5 for baseline evaluations and P1-P2 for the postictal evaluations) and detected FIA seizures (red circles). Note that P1 coincided with the planned baseline measurement B4 for this case and thus replaced B4. Panel (B) displays the ASM regimen changes during tapering, as expressed by the normalized dosage (i.e., the summed dosage over the 24 h prior to each measurement timepoint, divided by the standard at-home dosage summed over 24 h); changes of individual ASMs are depicted with separate lines. Panels (C–E) show the cortical excitability indices that showed significant ASM- and seizure-related changes in the postictal phase. The individual left-hand (light gray) and right-hand (dark gray) rMT values are shown in panel (C). Note that the rMT was further reduced in the postictal evaluations relative to the baseline measurements, while SICI and LICI (panels D–E) both showed increased conditioned to unconditioned response ratios, suggesting reduced $GABA_A$-mediated inhibition in the postictal phase. Note that after the second seizure, no ppTMS paradigms were performed. The spTMS MEP responses at 110% rMT were not significant for FIA seizures and are not shown. ASM, anti-seizure medication; CBZ, carbamazepine; CLB, clobazam; rMT, resting motor threshold; MSO, mean stimulator output; ppTMS, paired pulse TMS; SICI, short interval cortical inhibition; LICI, long interval cortical inhibition; CR, conditioned response; UR, unconditioned response; FIA, focal impaired awareness; TMS-EMG, transcranial magnetic stimulation with electromyography.

Single and paired pulse MEP changes

Single pulse MEP amplitudes did not correlate with the normalized ASM load *L* (estimate: −3.4 μV, 95% CI: −74.1 to 67.1 μV). Postictal measurements showed a reduction in MEP amplitude measured at 110% rMT after fbTC seizures (estimate: −106.8 μV, 95% CI: −181.6 to −32.1 μV), but not following FIA seizures (estimate: −9.3 μV, 95% CI: −64.5 to 45.8 μV).

The change in ppTMS SICI and LICI as a function of normalized ASM load, including the model's significant curve fits and corresponding confidence intervals, are shown in Figures S1, S2, respectively.

Short interval ppTMS evoked responses did not correlate with the normalized ASM load *L* (estimate: −0.3, 95% CI: −0.7 to 0.1). Postictal short interval measurements performed after fbTC seizures showed a decrease in the SICI CR/unconditioned response (UR)-ratio

Table 3. Overview of the TMS-EMG outcome measures for ASM tapering and postictal measurements after FIA and fbTC seizures.

Outcome measure	ASM ↓	FIA postictal	fbTC postictal
rMT (%MSO)	↓	↓	=
MEP (mV)	=	=	↓
SICI (CR/UR-ratio)	=	↑	↓
LICI (CR/UR-ratio)	=	↑	=

For FIA seizures SICI and LICI show increased CR/UR ratio's, indicating reduced attenuation of the conditioned response, while fbTC seizures show reduced CR/UR ratio's indicating increased attenuation of the conditioned response. ASM, anti-seizure medication; FIA, focal impaired awareness; fbTC, focal to bilateral tonic–clonic; rMT, resting motor threshold; MEP, motor evoked potential; CR, conditioned response; UR, unconditioned response; TMS-EMG, transcranial magnetic stimulation with electromyography; SICI, short interval cortical inhibition; LICI, long interval cortical inhibition.

(estimate: −0.5, 95% CI: −1.0 to −0.1), whereas a significant increase in the SICI measure was observed following FIA seizures (estimate: 0.8, 95% CI: 0.4–1.1).

Long interval paired-pulse evoked responses did not correlate with the normalized ASM load *L* (estimate: 0.2, 95% CI: −0.3 to 0.6), or fbTC seizures (estimate: −0.2, 95% CI: −0.9 to 0.6). Similar as for the SICI measure, after FIA seizures the LICI CR/UR-ratio was increased (estimate: 0.8, 95% CI: 0.3–1.4).

Discussion

We demonstrated that ASM tapering and seizures impact motor cortex excitability with distinct effects on TMS-EMG-based excitability measures. Drug tapering resulted in decreased rMT, suggestive of increased corticospinal excitability. Seizures affected intracortical inhibition with contrasting effects between fbTC and FIA seizure types. Postictal TMS evaluations following fbTC seizures were associated with increased cortical inhibition (presumptively mediated by altered $GABA_A$-mediated mechanisms). Conversely, FIA seizures were associated with reduced cortical inhibition and elevated corticospinal excitability.

Limitations

The EMU offered an ideal environment to study peri-ictal and ASM dose–response effects on cortical excitability, but the setting also limited our analysis in several ways. The heterogeneity of drug regimens and tapering schemes did not allow us to assess the effects of each drug individually. Instead, we used normalized medication levels to estimate the overall ASM load. We could not account for the pharmacokinetic contrasts between ASMs, but we found a clear correlation between various ASM regimes drug load. Previous TMS-EEG studies suggested specific fingerprints per ASM type.[7,8] Further studies are needed to explore the individual ASM effects on the rMT.

The sample of postictal measurements after fbTC seizures (six measurements in three people) was low, increasing the probability of a type-II error. Nevertheless, we found effects of ASM tapering and seizure occurrence and type with small confidence intervals suggesting that these effects were robust.

We also assessed TMS-EEG, but we did not include these measurements in the final analysis as the EEG contained too many artifacts for low-density EEG recordings with the limited number of trials used in this study. TMS-EEG measures could provide a valuable addition,[30] but would in retrospect, require more extensive EEG coverage and extended measurement sessions with more trials per protocol to allow for better post-processing of the recordings.

The spTMS and ppTMS protocols were performed at the lowest rMT of both hemispheres to compare clockwise versus counter-clockwise stimulation directly. This resulted in subthreshold stimulation intensities for the hemisphere with higher rMT. TMS-EMG measures thus were only compared for the hemisphere with the lowest within-subject rMT. We did not repeat TMS protocols at different stimulation intensities to limit the study burden.

Changes in ASMs

ASM tapering strongly correlated with lower rMT thresholds, suggesting increased corticospinal excitability. Previous pharmacological studies showed dose–response effects with an increased rMT (i.e., indicating reduced excitability) following a single ASM dose.[7,11–16] One study performed multiple TMS measurements over 8 weeks to evaluate the effect of carbamazepine and lamotrigine on rMT in healthy volunteers.[14] While the increase in ASM blood levels following ASM initiation correlated with higher rMT values, a weaker correlation was found between ASM blood levels and rMT in the TMS trials 1–3 days following acute withdrawal. This indicates that recovery of the rMT to baseline values is slower than the recovery of the ASM blood levels. Following ASM withdrawal, we found a reduction in rMT thresholds, indicating enhanced corticospinal excitability and an increase in rMT when medication returned back to at-home levels. While our experiment was not designed to compare the up-titration and tapering period directly, no significant differences were observed in post hoc analysis. We speculate that pharmacokinetic and pharmacodynamic factors may differ between people on chronic drug regimens and those starting with medication. ASM tapering did not impact the read-outs of the ppTMS paradigms. This is in

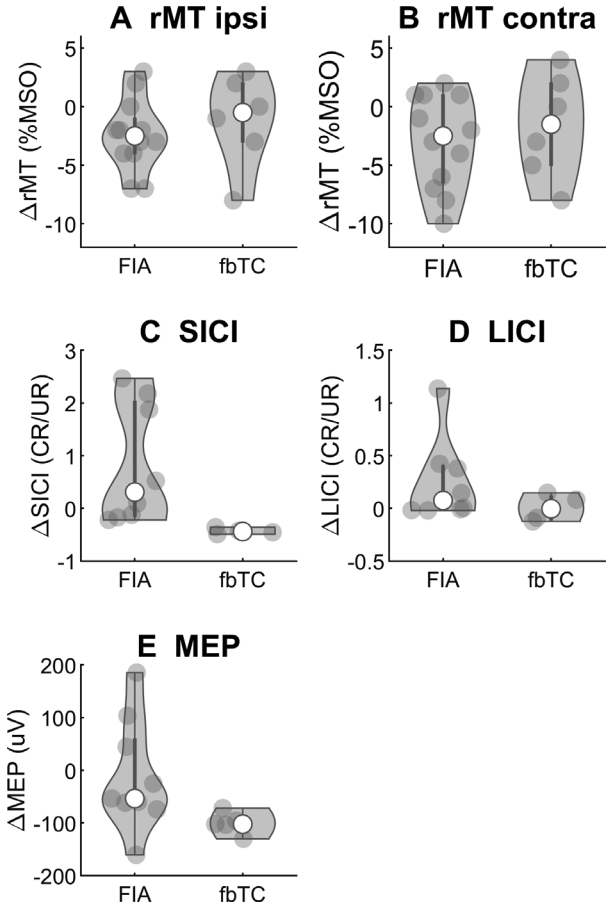

Figure 3. Violin plots of the postictal change in TMS-EMG parameters per seizure type. Each panel depicts the change (Δ) in the postictal TMS-EMG parameters compared to the baseline evaluation. The gray dots represent the individual measurements, the white circle represents the median value, the dark gray bars represent the interquartile range, and the gray area represents the smoothed probability density. Panels (A and B) show the postictal change in resting motor threshold for the hemisphere ipsilateral and contralateral to the seizure onset zone; panels (C and D) display the postictal paired pulse TMS-EMG changes related to short and LICI; panel (E) shows the postictal change in the MEP measured at 110% resting motor threshold. FIA, focal seizures with impaired awareness; fbTC, focal to bilateral tonic–clonic seizures; rMT, resting motor threshold; MSO, mean stimulator output; SICI, short interval cortical inhibition; LICI, long interval cortical inhibition; CR, conditioned response; UR, unconditioned response; TMS-EMG, transcranial magnetic stimulation with electromyography.

agreement with previous single-dose studies of several ASMs, where no direct effect on the ppTMS read-outs was found.[2,7,8,31]

Postictal measurements

Postictal measurements following fbTC seizures showed marked SICI enhancement with increased response attenuation. SICI increase after fbTC seizures is congruent with a previous study where a similar enhancement of SICI was found up to 24 h after seizure onset.[28] Postictal MEPs measured at 110% rMT were significantly reduced in amplitude compared to baseline measurements, suggesting a reduction in the input–output recruitment slope of the motor system after fbTC seizures. A single-dose study of lorazepam, a $GABA_A$-receptor agonist, demonstrated depressed input–output curves following administration and decreased MEP amplitudes, especially in the high-intensity part of the input–output curve.[32] Therefore, we speculate that our finding of SICI enhancement and MEP amplitude decrease following fbTC seizures reflects increased $GABA_A$-mediated inhibition. We found no significant effect on rMT or LICI, which has been demonstrated to involve mainly $GABA_B$ rather than $GABA_A$-mediated inhibition.[9] This suggests that enhanced postictal inhibition after fbTC seizures is primarily mediated by $GABA_A$-receptors.

Postictal TMS-EMG measures following FIA seizures, in contrast, showed signs of increased excitability due to reduced inhibition. rMT following FIA seizures was lower, and SICI and LICI read-outs showed signs of reduced inhibition causing increased excitability, reflected in facilitation of the MEP CR relative to the UR. We speculate that increased excitability after FIA seizures may reflect an ictal focus to be more excitable (less inhibited) following a first seizure, thus lowering the threshold for a seizure cluster. Seizure clusters are common in refractory epilepsy and imply impaired seizure termination or increased cortical excitability.[33] Both are potential consequences of secondary alterations from an initial seizure that promotes a second seizure or excess seizure-promoting factors.[34] Our finding of increased excitability following FIA seizures contrasts with a previous study, where postictal SICI and LICI both were enhanced, that is, more attenuated CRs, for almost all interstimulus intervals in focal and generalized epilepsy.[28] They all had newly diagnosed epilepsy, thus contrasting with our population of refractory focal epilepsy. We speculate that in people with refractory epilepsy, there may be aberrant inhibition in the postictal state, resulting in an increased tendency for seizure clusters and secondary fbTC seizures. However, another important difference is the contrasts in the timing of the TMS measurements. We performed measurements on average 2.25 h (range: 1–7 h) after seizures, while the referred study performed measurements on average 17 h after seizure occurrence. We postulate that measurements performed with significant time lag between the seizure and the TMS evaluation will miss the proictal state changes observed in our study.

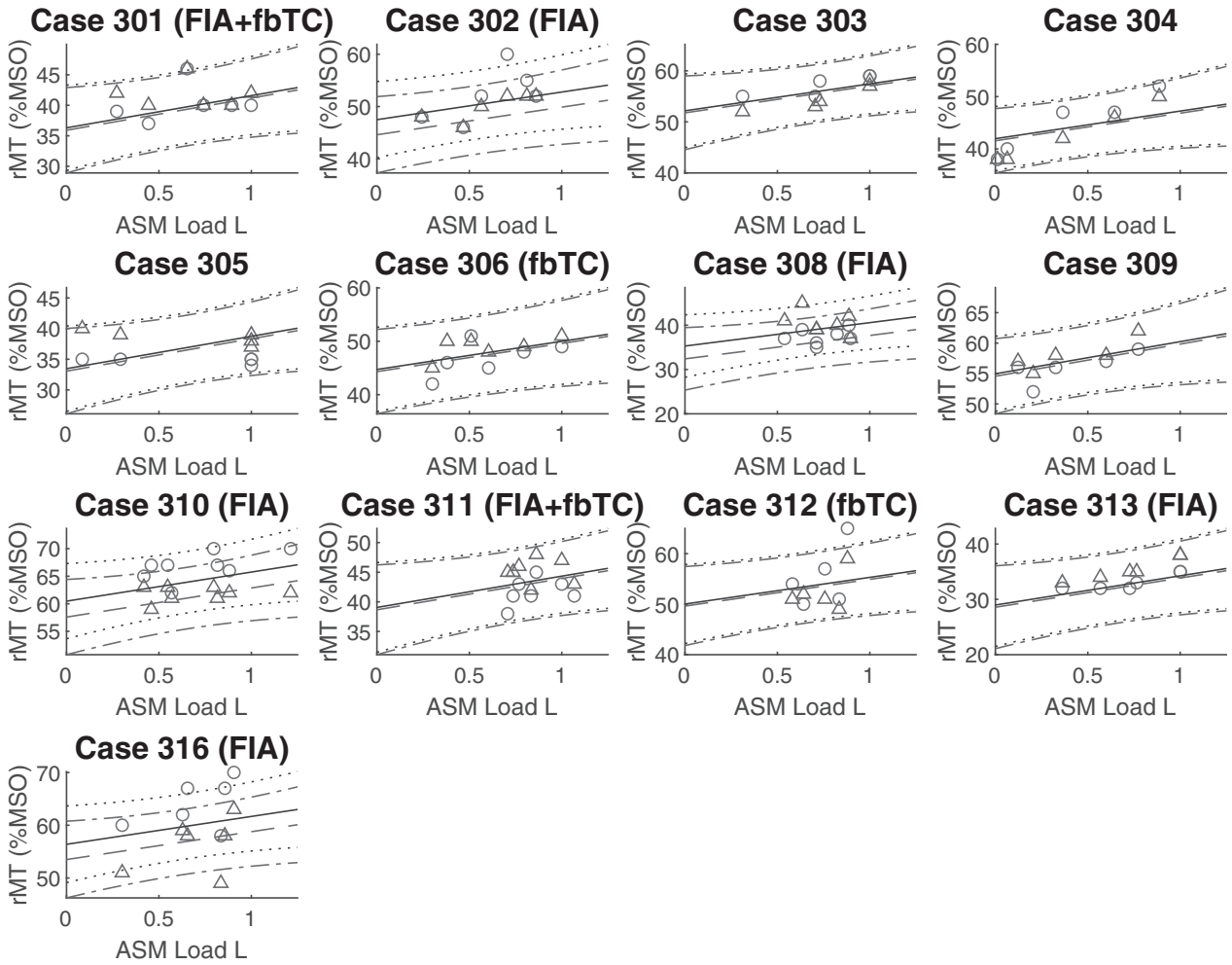

Figure 4. Resting motor threshold as function of normalized anti-seizure medication dose for all individuals with epilepsy. For each case, the seizures types that occurred during their admittance to the epilepsy monitoring unit are shown within the parenthesis. Four cases had no seizures during the study period. The circles depict the resting motor threshold measurements ipsilateral to the hemisphere of the seizure onset zone, while the triangles display the contralateral hemisphere measurements. The solid lines show the significant curve fits for the ipsilateral measurements with the corresponding confidence interval shown by the dotted line. Similarly, the dashed lines shows the significant curve fit for the contralateral measurements with the corresponding confidence interval shown by the dash-dotted lines. ASM, anti-seizure medication; rMT, resting motor threshold; MSO, mean stimulator output.

Interhemispheric differences

Handedness is the most outward example of motor laterality. When accounting for various other factors, we found that handedness was correlated with slightly lower thresholds in the hemisphere corresponding to the dominant hand. Similarly, lateralization of the seizure onset zone was associated with lower rMT in the ipsilateral hemisphere relative to the contralateral hemisphere. This may reflect increased excitability of the hemisphere ipsilateral to the seizure focus (due to reduced inhibition or increased excitation) or decreased excitability of the contralateral hemisphere. Considering that epilepsy is generally regarded as a condition with an aberrant inhibition-excitation balance, we find the prior explanation more likely. Previous rMT studies on the lateralization of handedness[35–38] and seizure onset zone[20,39,40] yielded mixed results. Our study differs from the above report in three significant aspects: coil type, serial measurements, and the EMU setting. We employed round-coil TMS in contrast to figure-of-eight coils commonly used in TMS-EMG studies. We speculate that more broad activation of the cortex by round coil TMS results in more widespread activation patterns of inhibitory and excitatory networks resulting in different downstream effects than expected with a figure-of-eight coil with

effects on TMS and MEP features. We used serial measurements within individuals to demonstrate the group-level fixed effects. Single TMS measurements not taking into account on the physiological fluctuations in cortical excitability may lack sufficient power to establish the observed effect. Lastly, we performed measurements in a setting where the balance between excitation and inhibition fluctuated due to ASM load changes and a relative high seizure burden. Our findings suggest that these fluctuations affect the interhemispheric rMT differences over time. The interhemispheric rMT differences and the relation with lateralization of handedness and seizure onset zone is, however, anything but straightforward and more research is needed to further explore the observed effects.

Safety of TMS in people with epilepsy

Seizure induction is the most severe complication of TMS.[41] In our study, where participants were inpatients for seizure recordings, induced seizures were not considered adverse events provided that the provoked seizure in an individual had similar semiology to unprovoked seizures. Two seizures occurred during a TMS evaluation; in one case, seizure onset occurred during a spTMS session. In the second, it was within 1 min after rMT determination. Seizure semiology for TMS-related seizures was similar to their unprovoked seizures. The provoked seizures occurred within a seizure cluster of multiple FIA seizures for both cases. It, therefore, remains questionable whether these two clusters were started by the TMS session or were coincidental.

Concluding statements and future perspectives

We demonstrated that serial TMS-EMG evaluations, using various spTMS and ppTMS EMG parameters, can be used to monitor changes in motor cortex excitability in the context of epilepsy. Longitudinal measurements can be applied to unveil effects related to changes in ASM regiment changes and effects related to the occurrence of seizures that can be distinct per seizure type. The observation of increased excitability after FIA seizures, that could be due to a period of reduced inhibition, may play a role in the occurrence of seizure clusters, thus reflecting a proictal state. Conversely, the finding of increased inhibition after fbTC seizures suggests a shift of the excitation/inhibition-axis toward a condition of increased inhibition or reduced excitation. We postulate that the PGES seen after some fbTCs may be a phenomenon related to such a shift. However, more research is needed to better understand the mechanism behind seizure clusters and PGES. Studies using within-subject designs may help to elucidate the role of aberrant inhibition or excitation levels in the peri-ictal state and relate these to clinical outcome. Yet, another underexplored prospect of serial TMS evaluations is to predict the individual treatment response.

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Author Contributions

R. M. H., S. S., P. R. B., G. H. V., and R. D. T. contributed to the conception of the study; all authors contributed to the design of the study; R. M. H. and S. S. contributed to the acquisition of the data; R. M. H. prepared the figures; R. M. H., P. R. B., E. A. T., G. H. V., and R. D. T. contributed to the analysis of the data; all authors contributed to editing.

Conflict of Interest

R. M. H. and R. D. T. receive support from the Christelijke Vereniging voor de Verpleging van Lijders aan Epilepsie. R. D. T. reports lecture and consultancy fees from Medtronic, UCB, Theravarance, Zogenix, Novartis and Arvelle and grants from EpilepsieNL, Medtronic, Michael J Fox Foundation, NewLife Wearables and The Netherlands Organisation for Health Research and Development (843002707). P. R. B. receives lecture fees from NovoCure and Aurikamed. G. H. V. reports grants from EpilepsieNL. All other authors have no disclosures to make.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Short interval cortical inhibition as function of normalized anti-seizure medication dose for all epilepsy subjects.
Figure S2. Long interval cortical inhibition as function of normalized anti-seizure medication dose for all epilepsy subjects.

