



Phase clustering in transcranial magnetic stimulation-evoked EEG responses in genetic generalized epilepsy and migraine

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ARTICLE INFO

Article history:

Received 26 September 2018

Revised 21 January 2019

Accepted 22 January 2019

Available online 13 March 2019

Keywords:

TMS–EEG

Excitability

Juvenile myoclonic epilepsy

Phase synchronization

Biomarker

ABSTRACT

Background: Epilepsy and migraine are paroxysmal neurological conditions associated with disturbances of cortical excitability. No useful biomarkers to monitor disease activity in these conditions are available. Phase clustering was previously described in electroencephalographic (EEG) responses to photic stimulation and may be a potential epilepsy biomarker.

Objective: The objective of this study was to investigate EEG phase clustering in response to transcranial magnetic stimulation (TMS), compare it with photic stimulation in controls, and explore its potential as a biomarker of genetic generalized epilepsy or migraine with aura.

Methods: People with (possible) juvenile myoclonic epilepsy (JME), migraine with aura, and healthy controls underwent single-pulse TMS with concomitant EEG recording during the interictal period. We compared phase clustering after TMS with photic stimulation across the groups using permutation-based testing.

Results: We included eight people with (possible) JME (five off medication, three on), 10 with migraine with aura, and 37 controls. The TMS and photic phase clustering spectra showed significant differences between those with epilepsy without medication and controls. Two phase clustering-based indices successfully captured these differences between groups. One participant was tested multiple times. In this case, the phase clustering-based indices were inversely correlated with the dose of antiepileptic medication. Phase clustering did not differ between people with migraine and controls.

Conclusion: We present methods to quantify phase clustering using TMS–EEG and show its potential value as a measure of brain network activity in genetic generalized epilepsy. Our results suggest that the higher propensity to phase clustering is not shared between genetic generalized epilepsy and migraine.

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1. Introduction

Epilepsy and migraine are paroxysmal conditions characterized by a temporary disruption of normal neurological function. Recurrent epileptic seizures are linked to hypersynchronous neuronal activity [1]. Migraine attacks are characterized by headache and sensory hypersensitivity without excessive synchronous neuronal activity [2,3].

Abbreviations: TMS, transcranial magnetic stimulation; EEG, electroencephalography; NNEI, neural network excitability index; PCI, phase clustering index; rPCI, relative phase clustering index.

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Epilepsy and migraine were suggested to share pathophysiological mechanisms based on epidemiological and genetic evidence [4,5]. The diagnosis of both conditions is made on clinical grounds and is, for epilepsy, often supported by electroencephalographical (EEG) findings. There are no reliable markers to assess the likelihood of a paroxysmal event occurring. In migraine and epilepsy, it is thought that altered neuronal excitation–inhibition dynamics, resulting in cerebral hyperexcitability, underlie attack susceptibility [5–8]. Cortical excitability, measured using transcranial magnetic stimulation (TMS), was shown to be elevated in those with epilepsy compared with controls on group level [9]. This was also the case in several studies of juvenile myoclonic epilepsy (JME), one of the most common forms of genetic generalized epilepsy [9,10], which is characterized by myoclonus and generalized tonic–clonic seizures shortly after awakening. In children,

JME is more often associated with migraine than other types of epilepsy, such as absence epilepsy [11]. People with JME are more than four times as likely to have migraine than people without JME [12].

Findings of TMS studies in people with migraine are more complex, with several studies showing increased excitability of the visual cortex, reflected by a lower phosphene threshold, especially in migraine with aura (see for review [13]). Several studies show no difference in resting motor threshold (rMT) between people with migraine and controls [14–18]. Combining TMS with EEG offers new options to assess cortical excitability, bypassing sensory and motor areas [19,20]. Previous TMS–EEG studies in epilepsy investigating TMS-evoked potential and the epileptiform EEG discharges triggered by TMS have identified aberrant excitability and connectivity [21–27]. The only TMS–EEG study in JME to date found increased amplitude potentials in those with JME compared with controls and increased amplitude of late peaks when participants with JME were sleep-deprived, demonstrating cortical hyperexcitability [21]. Such TMS–EEG studies were thus far not conducted in people with migraine.

One novel way of assessing cortical excitability using TMS–EEG is by determining the uniformity of phase angles across trials in EEG responses, [20]. On a single electrode, the phase of TMS-evoked responses aligns between trials shortly after the TMS pulse. A recent study suggests that phase clustering 20–60 ms poststimulus in the 8–70-Hz frequency band may be a good candidate for measuring cortical excitability [20]. One measure of phase clustering, the relative phase clustering index (rPCI), was successfully used in magnetoencephalography to quantify the neural response to periodic photic stimulation and to identify dynamic states leading to photoparoxysmal responses in epilepsy [28]. In temporal lobe epilepsy, it was shown that high values of rPCI were correlated with the probability of occurrence of epileptic seizures [29]. Recently, it was demonstrated that an index derived from the PCI, computed from local field potentials recorded *in vitro* or *in vivo* using intracranial recordings during very weak periodic pulse stimulation, can be used to quantify the state of excitability of neuronal networks in epileptogenic brain tissue [30].

Increased phase synchronization in the gamma frequency range in the ongoing EEG was linked to increased neuronal excitability in epilepsy [31]. Phase synchrony in response to photic stimulation was also elevated in those with migraine with and without aura compared with controls, especially in the alpha frequency range [32–35]. One study showed beta frequency desynchronization in migraine with aura [36], potentially linked to hyperresponsivity of the sensory cortices [37].

We assessed whether phase clustering in the TMS–EEG response differs in people with JME compared with controls or people with migraine with aura.

2. Methods

2.1. Participants

2.1.1. Controls

Healthy volunteers aged 12 years or over were recruited locally through digital and paper adverts. Those with a history of epilepsy or migraine were excluded. Hand dominance was assessed with a clinically validated questionnaire [38].

2.1.2. JME

Participants, diagnosed with JME or possible JME by their treating neurologist, were recruited from outpatient clinics. The diagnosis was based on the clinical history and a clinical interictal EEG recording performed at least one week prior to the TMS–EEG session. Participants aged 12 years and over, with a history of myoclonic seizures and/or at least one generalized tonic-clonic seizure, who were either not taking antiepileptic drugs (active epilepsy off drugs) or considering tapering antiepileptic drugs (in remission) in conjunction with the attending

neurologist, could be included. Subjects with comorbid migraine were excluded. In the Netherlands, where this study was conducted, the presence of myoclonus is not considered compulsory for the diagnosis of JME [39].

2.1.3. Migraine with visual aura

Participants with migraine with visual aura were recruited locally through digital and paper adverts at a clinic. The diagnosis was based on the International Classification of Headache Disorders criteria [40]. People aged 18 years and over with migraine headache preceded by visual aura in at least 30% of the attacks were included. Subjects needed to have at least one migraine attack per year, at least one in the preceding year, and less than eight attacks or 15 headache days per month. We excluded people using prophylactic medication and those with a history of epilepsy, and those without aura and with “aura sans migraine”.

2.1.4. Exclusion criteria for all groups

These were the exclusion criteria: contraindications to TMS, pregnancy, any neurological condition other than epilepsy or migraine, any psychiatric condition, the use of medication affecting cortical excitability other than antiepileptic drugs (such as psychoactive drugs and beta blockers), and diabetes mellitus, as this can affect peripheral nerves which were investigated for a separate study (not reported here). Experimental sessions were performed more than 24 h after a convulsive seizure and more than 72 h after a migraine attack; sessions followed by a convulsive seizure within 24 h and a migraine attack within 72 h, identified at follow-up, were also excluded. Participants were asked not to smoke, take drugs, or drink alcohol or coffee 12 h preceding the measurement and to maintain a normal sleep pattern the night prior to the measurement.

2.1.5. Informed consent & ethical approval

The study was approved by the Ethics Committee of Erasmus University Medical Centre, Rotterdam. All participants gave written informed consent. Assent was also obtained from parents of participants younger than 18.

2.2. Material

2.2.1. TMS

Magnetic stimulation was performed with a MagPro X100 stimulator (Magventure, Denmark), a 14-cm diameter parabolic circular coil (type MMC-140), and a sham coil (type MCF-P-B65). Measurements were conducted at 09.00 AM or 02.00 PM and spread evenly between AM and PM. No significant differences in TMS measures were reported between these times of the day [41], except a larger TMS-evoked potential 100 ms after the stimulus [42]. Soft earplugs were used to reduce the coil click.

2.2.2. Electromyography

Motor-evoked potentials were recorded bilaterally from the abductor pollicis brevis muscles with a Nicolet Viking EDX electromyograph (Natus, Madison, WI, USA). The coil size and design activated these muscles in >90% of participants. Muscle activity was monitored using real-time visual feedback. Data were recorded with a sampling frequency of 4 kHz and stored for offline analysis.

2.2.3. EEG

Electroencephalography was recorded during the TMS sessions with a 64-channel TMS-compatible EEG system (Waveguard™ cap and ASALab™ software, ANT-neuro, Enschede, The Netherlands), a sampling frequency of 4 kHz, and a ground electrode located on the AFz electrode position. Participants were seated in a comfortable chair with their eyes open and arms in supine position.

2.3. Stimulation protocols

2.3.1. Photic stimulation

After a 10-minute baseline EEG recording, photic stimulation (Sigma, Is FSA 10-2D-I, SIGMA Medizin-Technik GmbH, Gelenau, Germany) was performed according to a standard clinical protocol: stimulation started at 2 Hz; followed by 10-s runs of increasing frequency at 6, 12, 20, 30, 40, 50, and 60 Hz with eyes closed and open (± 5 s each). If an epileptiform discharge was elicited, stimulation was stopped and resumed at 60 Hz. Stimulation was thereafter performed at decreasing frequencies until another discharge occurred, to determine the range of frequencies to which an individual was sensitive. Photic stimulation was performed in controls and people with epilepsy but not in people with migraine, as several people in our sample indicated that this could trigger a migraine attack. The aim of this study was to assess TMS–EEG parameters of cortical excitability outside migraine attacks, and thus, we avoided to trigger attacks. We used the photic stimulation in controls and people with epilepsy to validate the results obtained with TMS–EEG.

2.3.2. Single-pulse TMS stimulus response curve

The rMT, defined as the lowest stimulation intensity that evokes a peak-to-peak electromyographic amplitude larger than 50 μV in 50% of the trials [43], was measured with the coil on the vertex (electrode position Cz) and a scanning procedure described hereafter. For a first approximation of the motor threshold, stimulation was started at 20% stimulator output and increased with 5% steps until a consistent twitch in the hand contralateral to the stimulated hemisphere was seen in 50% of the trials. Then, a semiautomated, in-house designed scanning protocol (created in Matlab® (version 7.5.0 R2007b The MathWorks Inc., Natick, MA, USA)) was used to determine the rMT as follows: scanning started at a stimulator output value of 10–12% below the visually determined motor threshold and increased in 2% steps until a reproducible motor-evoked potential (>200 μV) was seen after every stimulus (± 110 –120% rMT). Stimuli were given with interstimulus intervals of 2 s. This frequency was not shown to alter motor-evoked potentials [44,45]. The scanning procedure was performed using counterclockwise (right hemisphere) and clockwise (left hemisphere) stimulation as part of the artifact reduction strategy (see Section 2.4.4) and repeated with the sham coil. To be useful in clinical settings, the stimulation protocol was designed to be a short protocol yielding maximum information at once.

To assess long-term reproducibility of the TMS–EEG parameters, controls were remeasured after 10–12 months at the same time of the day. We also explored whether the measure of EEG phase clustering (see below) is affected by the number of stimuli per intensity. The control group was measured twice with different numbers of stimuli per intensity: during the first measurement, we used eight stimuli per stimulus, and in the second measurement, we used 20 stimuli per stimulus intensity. People with epilepsy were measured following each medication change. To reduce the theoretical risk of eliciting a seizure in participants with epilepsy off medication, we used eight stimuli per stimulus intensity minimizing the number of pulses [46]. In the group with epilepsy on medication, we used 20 stimuli per stimulus intensity, as the theoretical risk of a seizure is lower in these groups. People with migraine were measured only once using 20 stimuli per stimulus intensity.

2.4. Data analysis

Offline analyses were performed in Matlab® (8.5.0 R2015a). The phase clustering analysis described below was applied on data acquired with the two TMS polarities, sham stimulation, and photic stimulation.

2.4.1. Removal of artifactual channels

For each subject, artifactual channels were automatically detected: for each channel, the norm covariance matrix was computed for the window -0.1 to 0 s relative to the TMS stimulus. Then, the Z-score was computed from the norm covariance of each channel relative to the other channels. Channels with a Z-score > 3 were excluded from the reference montage and subsequent analyses. On average, 4 channels were removed for each subject (range: 2–7 channels). The M1, M2, T7, and T8 electrodes were most often detected as ‘outlier’ channels.

2.4.2. Phase clustering and neuronal network excitability indices

Electroencephalography phase clustering analysis was previously described [28,47]. The phase clustering index (PCI) describes the phase consistency of the complex Fourier components across the stimulation trials, with zero representing completely scattered phases and one maximal phase grouping. To obtain the PCI, we used epochs of 100 ms (corresponding to a base frequency of $1 \text{ s} / 100 \text{ ms} = 10 \text{ Hz}$) starting 15 ms after TMS or sham stimulation (see also below regarding TMS artifact reduction) and without delay (0 ms) for photic stimulation. After linear detrending, the complex Fourier components of the signal were computed using the fast Fourier transform after application of a Hamming taper, yielding complete frequency and phase representation of the responses. The length of the window defines the base frequency of the representation with the harmonic component representing an integer multiple of the base frequency. For photic stimulation, only responses to 6 Hz stimulation when subjects had their eyes closed were analyzed to ensure enough stimulation trials (30 trials for each subject).

The PCI was computed for each complex number F obtained from the Fourier transform using Eq. (1).

$$PCI_c^f = \frac{\left\langle \left| F_{c,i}^f \right| \right\rangle_i}{\left\langle \left| F_{c,i}^f \right| \right\rangle_i} \quad (1)$$

where f is frequency band, i is stimulus number (from N_i in total), c is the EEG channel, the symbol $|z|$ represents the magnitude (the absolute value) of a complex number z , and $\langle \cdot \rangle_i$ indicates averaging over all stimuli. For more information regarding the pathophysiological interpretation of the PCI in terms of system dynamics, see Supplementary information S1.

The rPCI, i.e., the maximal PCI at a given frequency relative to the PCI at the base frequency ($PCI^1 = 10 \text{ Hz}$), was then computed by:

$$rPCI_c = \left\langle \max_f \left(PCI_c^f - PCI_c^1 \right) \right\rangle_c \quad (2)$$

The neural network excitability index (NNEI) introduced in the previous work [30] is determined by the PCI at the base frequency:

$$NNEI_c = \left\langle 1 - PCI_c^1 \right\rangle_c \quad (3)$$

While both measures were initially computed using the whole epoch in-between successive stimuli, TMS has restrictions because of the ringing and muscle artifacts present in the window shortly after the stimulus (see below), so we calculated the PCI for a fixed window length of 100 ms starting 15 ms after a TMS stimulus. In theory, the window length can influence the general spectral resolution of the PCI. In our sample, windows of 50 ms to 500 ms (base frequencies from 20 Hz to 2 Hz) showed a similar PCI spectrum with comparable rPCI values.

2.4.3. Time–frequency analysis

For TMS time–frequency analyses, we used epochs of 1 s (4000 samples), starting 0.5 s before the magnetic stimulus to avoid convolution edge effects in the window of interest from 15 ms to 115 ms. The

part of the signal containing TMS ringing artifacts (0–6 ms after the stimulus) was cut. Cubic interpolation was used from 0 to 15 ms around the stimulus to reduce muscle artifact contamination. The trials were baseline-corrected using a baseline window from –50 ms to 0 ms relative to the TMS stimulus. The time–frequency wavelet components for frequencies between 8 and 50 Hz were computed using Morlet wavelets with a width of 5 for the window of 15 ms to 115 ms in steps of 5 ms in order to gain sufficient temporal resolution for the low frequency content with adequate frequency resolution in the higher frequencies. Because of our window selection of [–0.5:0.5 s], we can compute the Time-frequency (TF) with the chosen cycle width for the window [15 ms:115 ms] without any border distortions.

Next, the time-phase clustering response was computed using a modified version of Eq. (1):

$$PCI_{t,c}^f = \frac{\left| \left\langle F_{t,c,i}^f \right\rangle_i \right|}{\left| \left\langle F_{t,c,i}^f \right\rangle_i \right|} \quad (1A)$$

where t is time. For the photic stimulation time–frequency analysis of the PCI, the interval of interest was an epoch of 167 ms, with a mirror buffer of 500 ms on each side to avoid convolution edge effects in the time–frequency analysis. Detrending was applied before computing the time–frequency Fourier components for frequencies between 5 and 50 Hz using Morlet wavelets with a width of 5 cycles for the whole window of interest in steps of 5 ms. The PCI was again computed using Eq. (1A), and the results were averaged over all channels.

2.4.4. TMS and muscle artifact reduction

We included several strategies to reduce stimulation and muscle artifacts related to magnetic stimulation. Firstly, Eq. (2) allows to cancel out broadband artifacts, such as sharp spikes induced by, and time-locked to, the magnetic stimulus as they will result in a high PCI for all frequencies. Secondly, we performed the phase clustering analysis using a window that started 15 ms after the magnetic stimulation. The largest TMS and muscle artifacts are expected within the first 15 ms after the stimulus. To ensure that our results are not due to muscle artifact contamination, the analysis was repeated for epochs starting at 20 ms, 25 ms, and 30 ms relative to the TMS stimulus, with similar results. Only data from the final analysis with a window length of 100 ms starting 15 ms after the TMS stimulus were included. Thirdly, to reduce linear volume conduction effects caused by the magnetic stimulus, we added the clockwise and counterclockwise stimulation responses offline in a pairwise fashion to compensate the linear component, containing the artifact, in the response to each polarity (Eq. (4)) [48]:

$$F_{c,i}^{(c)f} \equiv F_{c,i}^+ + F_{c,i}^- \quad (4)$$

$F_{c,i}^+$ and $F_{c,i}^-$ are the response amplitudes to the clockwise and counterclockwise current stimulations from a series of equal number of stimuli. We will refer to this as *polarity compensation* and to $F_{c,i}^{(c)f}$ as *polarity-compensated amplitudes*, which were used in Eqs. (1) and (2). All analyses were done on polarity-compensated signal as theoretically, it is less affected by artifacts (see Eq. (4)). Unless stated otherwise, “rPCI” refers to polarity-compensated rPCI. Sham stimulation was done in the three groups to evaluate the effect of the audible coil, as the earplugs did not mask the click completely.

In controls, we compare the compensated stimulation with the individual stimulation polarities, and in addition, we compare TMS to sham stimulation and photic stimulation in the group with epilepsy and the control group. In the group with migraine, we compare TMS with sham stimulation.

2.4.5. Statistical analyses

We took the small sample size of the group with epilepsy (on and off medication) and the group with migraine into account by using nonparametric, Monte Carlo-based statistics, which were shown

to be robust in such small sample sizes [49]. For all statistical analyses, the group with epilepsy off medication was compared with the first measurement of the controls (8 stimuli per intensity) while the group with epilepsy on medication and the group with migraine were compared with the second measurement of the controls (20 stimuli per intensity).

The rMT was compared across groups using an independent sample permutation test using 10,000 permutations and a significance level of 0.05.

The TMS-evoked potentials and time–frequency PCI spectra were compared across groups using the cluster-based Monte Carlo permutation testing [50] using 2500 permutations, a cluster-alpha of 0.01, and a significance level of 0.025.

To assess possible biomarkers of epileptogenicity, we quantified the rPCI (Eq. (2)) and NNEI (Eq. (3)) averaged over all EEG channels after magnetic, sham, and photic stimulations in controls, people with epilepsy on and off medication, and participants with migraine. These rPCI and NNEI values averaged over all channels were compared across groups using an independent sample permutation test using 10,000 permutations with a significance level of 0.05.

To assess the robustness of TMS-evoked rPCI, we compared the rPCI obtained after clockwise, counterclockwise, sham, polarity-compensated, and photic stimulations in the control group using the independent sample permutation test. Still in the control group, for polarity-compensated stimulation and sham stimulation, we compared the rPCI after 8 pulses per intensity (the first measurement) and after 20 stimuli per intensity (the second measurement) using the paired sample permutation test. For polarity-compensated stimulation, sham stimulation, and photic stimulation, we also compared the rPCIs measured during the morning with those measured in the afternoon, and the rPCIs measured in men and women using the independent sample permutation test. We used a permutation test based on Spearman's rho correlation coefficient to estimate the effect of age on the polarity-compensated rPCI, and rPCI as estimated by sham and photic stimulations in the control groups.

3. Results

3.1. Participants

We included 38 controls (25 women, mean age: 38.1 years, range: 15–62 years) between May 2014 and October 2014. Five were left-handed. Of those 38 controls, thirty were measured a second time after an average of 350 days (range: 296–378 days). One participant was excluded from the analyses because of nonspecific EEG abnormalities. From another control, we excluded the first measurement as it contained a large artifact due to incorrect settings of the magnetic stimulator. Thus, the analysis of the first measurement was based on 36 controls, and the analysis of the second measurement on 29 controls.

Eight participants with JME were included (4 women, mean age: 31.5 years, range: 14–59) between May 2014 and October 2015. All were right-handed (Table 1). Five were not taking antiepileptic drugs at inclusion (E1–E5). Two were photosensitive (E3 and E4). Three were treated with antiepileptic drugs for two years or more and were contemplating drug withdrawal (EM1, EM2, EM3). To ensure adherence, drug levels were monitored. None of the participants had a seizure during the time that they were included in the study (7–12 months).

Twelve people with migraine were recruited (10 women, mean age: 38 years; range: 21–62, 4 left-handed, Table 2). One female was excluded because of beta blocker use; one male was excluded, as he did not have an attack in the preceding year. The attack frequency for the remaining ten participants was between 0.3 and 2 per month. Apart from one participant who habitually drank seven cups of coffee per day, daily coffee consumption in this group was limited. Three female participants were first-degree relatives. We analyzed the results with and without two of these family members. Given the small differences between the

Table 1
Clinical features of participants with juvenile myoclonic epilepsy.

Nr	M/F	Age	Age at onset	Handedness	PS	Last seizure	Clinical features	EEG features at diagnosis	TMS rPCI	TMS NNEI	Photic rPCI	Photic NNEI
E1	F	14	14	9	N	28 days	TC, 1 febrile seizure	Normal background activity, spikes and spike-and-wave complexes with anterior maximum	0.22	0.40	0.30	0.84
E2	M	29	22	8	N	158 days	Nocturnal TCs triggered by alcohol	Normal background activity, (poly) spike-and-wave complexes with anterior maximum, increased abnormalities under hyperventilation	0.23	0.44	0.29	0.87
E3	M	20	20	9	Y	6 days	Nocturnal TCs triggered by alcohol, myoclonic jerks upon photic stimulation	Normal background activity without spontaneous epileptic abnormalities. Very clear photosensitivity (Waltz 3 between 6 and 40 Hz) accompanied by myoclonic jerks	0.24	0.49	0.29	0.79
E4	F	34	16	7	Y	8 years	Myoclonic jerks + TCs	Normal background activity with spontaneous 3–4 Hz (poly) spike-and-wave complexes with alternating maximum, sometimes accompanied by myoclonic jerks	0.20	0.45	0.19	0.62
E5	M	17	15	9	N	3 months	Myoclonic jerks + TCs	Normal background activity with 3 Hz (poly) spike-and-wave complexes with frontal maximum	0.22	0.33	0.28	0.68
EM1 ¹	F	59	16	9	N	24 months	Myoclonic jerks + TCs + absences	Normal background activity without epileptiform discharges	0.14	0.29	0.14	0.72
EM2 ²	M	24	15	8	N	42 months	Myoclonic jerks + TCs + absences	Normal background activity with subtle generalized epileptiform discharges	0.29	0.58	0.26	0.77
EM3 ³	F	55	8	8	N	18 years	Myoclonic jerks + TCs	Not available	0.19	0.41	0.02	0.54

M = male, F = female, PS = photic sensitivity, N = no, Y = yes, Handedness = according to the Edinburgh handedness questionnaire, TC = tonic-clonic seizures. Medication at time of measurement: ¹depakine chrono 2000 mg 1/day, ²depakine 750 mg 2/day, ³depakine 500 mg 2/day.

two analyses, we report here the results including the three family members.

All participants tolerated the experimental sessions. None had a seizure or migraine attack following stimulation.

3.2. rMT

The median rMT data and number of stimuli during each TMS procedure and photic stimulation are shown in Table 3. There was no significant difference in rMT between the groups.

3.3. Time and frequency characteristics of the PCI of magnetic and photic stimulation

We first explored the polarity-compensated TMS-evoked potential for each group (see Fig. 1A). Permutation testing revealed no significant clusters in the group comparisons of the averaged time–amplitude results. Post hoc analysis of the stimulated area (central electrode cluster consisting of electrode Cz and neighboring electrodes) where the evoked

Table 2
Characteristics of participants with migraine with aura.

Nr	M/F	Age at inclusion	Age at onset	Handedness	Attacks per month	% of attacks with aura	TMS rPCI	TMS NNEI
M1	F ^a	29	11	−5	1	40	0.04	0.20
M2	M	50	15	−7	1	100	0.14	0.37
M3	F	27	15	9	0.3	90	0.01	0.18
M4	F	21	19	9	0.3	100	0.22	0.38
M5	F	45	13	8	1	100	0.12	0.45
M6	F	35	22	8	0.5	30	0.02	0.11
M7	F	40	25	9	2	100	0.13	0.44
M8	F ^a	62	17	−8	0.5	100	0.15	0.52
M9	F	51	18	9	1	100	0.14	0.44
M10	F ^a	31	11	7	1.5	35	0.18	0.46

Handedness = according to the Edinburgh handedness questionnaire (scores < −5 indicate left-hand dominance).

^a First-degree family members.

response should be most prominent showed a difference between the first measurement of the controls and the group with epilepsy off medication ($p = 0.016$, see Fig. 1A for the cluster). The visual-evoked potential shown in Fig. 2A did not differ between the control and the groups with epilepsy. Photic stimulation was not done in the group with migraine.

Next, we explored the time–frequency characteristics of the TMS and photic stimulation PCI spectra (Eq. (1A), Figs. 1B and 2B). The TMS spectrum differed between the group with epilepsy off medication and the first measurement of the controls (Fig. 3A, $p = 0.024$). This cluster showed increased PCI in the group with epilepsy off medication in the gamma frequency band (30–40 Hz) around 50 to 80 ms. The PCI spectrum, in contrast, showed decreased PCI in the group with epilepsy off medication in the 10–14-Hz frequency band over the whole epoch (Fig. 3B, $p = 0.004$). There were no differences in the other group comparisons. The analysis of Fig. 3A suggests that the feature that best distinguishes TMS-evoked responses in epilepsy from controls is the rPCI defined in Eq. (2), as the high-frequency phase information is taken into account. For photic-evoked responses, in contrast, Fig. 3B suggests that the rPCI and the NNEI (Eq. (3)) may be suitable markers as they reflect phase clustering in the lower frequencies. As shown in Eq. (2), the rPCI can increase either due to an increase of PCI or to a decrease of PCI. The NNEI is useful to discriminate between these two alternatives. This is further tested in the next section.

3.4. rPCI and NNEI for TMS and photic stimulation

To quantify the difference in PCI between the different groups, we used the rPCI (Eq. (2)) and the NNEI (Eq. (3)). The median rPCI and NNEI elicited by the different stimulation modalities (polarity-compensated, sham, photic) in the different groups and the corresponding 5–95 percentiles are shown in Table 4.

The polarity-compensated rPCI was significantly higher in the group with epilepsy off medication than in controls ($p = 0.023$) while the NNEI showed a weak trend for being higher ($p = 0.147$). The group with epilepsy off medication also had significantly higher rPCI values than controls ($p = 0.021$). Photic stimulation showed higher rPCI ($p = 0.009$) and NNEI ($p = 0.025$) values in the group with

Table 3
Median (range) number of TMS and photic stimuli and resting motor threshold (rMT) values.

	# TMS stimuli	# photic stimuli	rMT right hemisphere	rMT left hemisphere
Controls 1	112 (96–208)	30	42% (31–68%)	40% (31–59%)
Controls 2	400 (280–480)	30	39% (29–57%)	43% (25–59%)
Epilepsy no med	176 (112–290)	30	51% (41–53%)	46% (39–53%)
Epilepsy + med	280 (160–320)	30	61.5% (45–78%)	47% (43–74%)
Migraine	340 (280–440)		43% (33–57%)	45% (31–47%)

There was no significant difference in rMT between the groups.

epilepsy off medication compared with controls. The rPCI and NNEI elicited by sham stimulation did not differ between controls and the groups with epilepsy. The rPCI and NNEI in the group with migraine did not significantly differ from controls (Fig. 4).

In controls, the polarity-compensated rPCI, photic rPCI, and sham rPCI did not differ between the first and second measurement, between men and women, nor between the times of the day the measurement took place (AM or PM). Age correlated with photic rPCI ($r = 0.399$, $p = 0.012$) and photic NNEI ($r = 0.411$, $p = 0.010$) in the control group but not with TMS rPCI and NNEI.

An example of the rPCI and NNEI following changes in the dose of levetiracetam in one participant with epilepsy is shown in Fig. 5. The decrease of the rPCI and NNEI is inversely proportional to the dose. A similar trend was seen for the photic rPCI but not for the photic NNEI (figure not shown).

4. Discussion

We confirmed the feasibility of assessing EEG phase clustering using a TMS single-pulse paradigm and validate the results with photic stimulation. We found that rPCI elicited by TMS was increased in those with JME on and off medication compared with controls but not in those with migraine with aura. The rPCI elicited by photic stimulation was also increased in those with JME off medication compared with controls. In line with a recent study, we show that phase clustering of evoked responses may be a candidate biomarker to monitor cortical excitability [20], and we show its potential for diagnostic value in epilepsy. An interesting additional finding, although preliminary, is that in one participant, the decrease of the rPCI and NNEI was linked to increased doses of levetiracetam. Replication of this finding is needed to evaluate

the value of rPCI as cortical excitability marker. These findings are in line with a previous study using magnetoencephalography and photic stimulation that reported an elevated rPCI in photosensitive absence epilepsy; it increased gradually in the period preceding the occurrence of a paroxysmal response [28].

The rPCI is a relative measure. Reduced phase clustering at lower frequencies and increased phase clustering at higher frequencies can theoretically result in high rPCI values. We previously introduced the NNEI to quantify excitability determined at the neuronal level [30]. The NNEI specifically reflects the low frequency spectral components. We previously showed that NNEI is small at low excitability levels but is high at high excitability levels [30]. Thus, given Eq. (3), a low PCI value at the base frequency corresponds to a high NNEI, i.e., a high neural network excitability. We confirmed this after photic stimulation. We found lower phase clustering in lower frequency ranges (alpha and beta bands) and a higher NNEI in the group with epilepsy off medication compared with controls. Conversely, after TMS, we found increased phase clustering in gamma range frequencies in the group with epilepsy without medication compared with controls. The net result was a higher relative PCI in the group with epilepsy off medication for both stimulation modalities. This suggests that different mechanisms are at play following TMS and photic stimulation. In our sample, the NNEI only differentiates the group with epilepsy from controls after photic stimulation. Alpha desynchronization was previously shown to be linked to an increase in oscillations at higher frequencies while an increase of activity in the alpha band is as a sign of cortical hypoexcitability [51–53]. It was recently shown that diazepam, a gamma aminobutyric acid - A (GABA-A) receptor agonist, increased TMS-induced alpha band synchronization in healthy subjects [54]. Interestingly, diazepam is used to terminate seizures. The

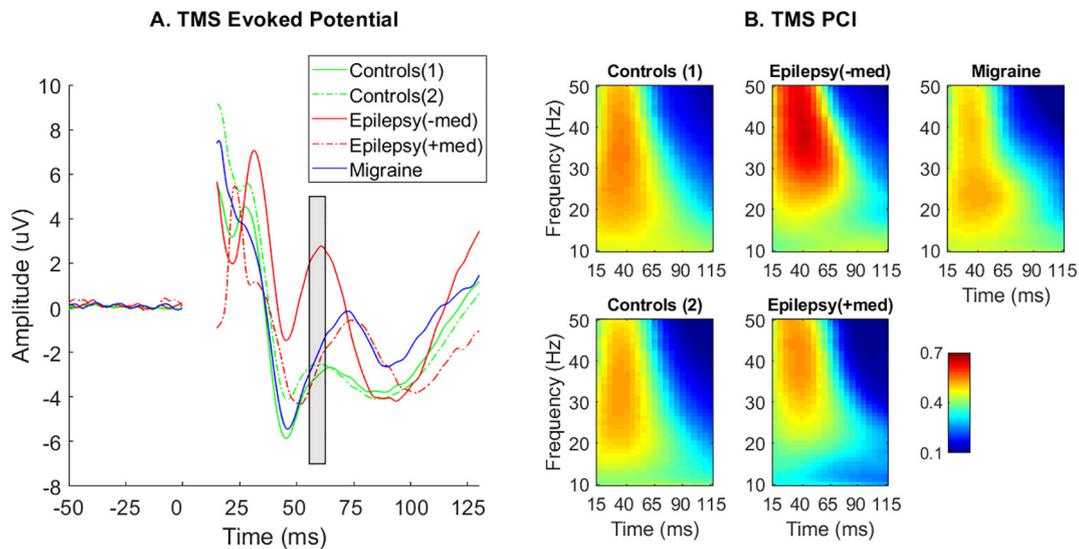


Fig. 1. A. TMS-evoked potential over the central electrode cluster for control, group with epilepsy, and group with migraine. Evoked responses averaged over a central electrode cluster, consisting of electrode Cz (the TMS target) and the neighboring electrodes surrounding electrode Cz. The gray area highlights the significantly different time samples between epilepsy (– med) and controls(1) ($p = 0.016$). B. Time–frequency representation of polarity-compensated PCI averaged over all channels. For Controls 1st, Controls 2nd, Migraine, Epilepsy without medication, and Epilepsy with medication. TMS frequency was 0.5 Hz. Wavelet analysis was performed using Morlet wavelets with 5 cycles.

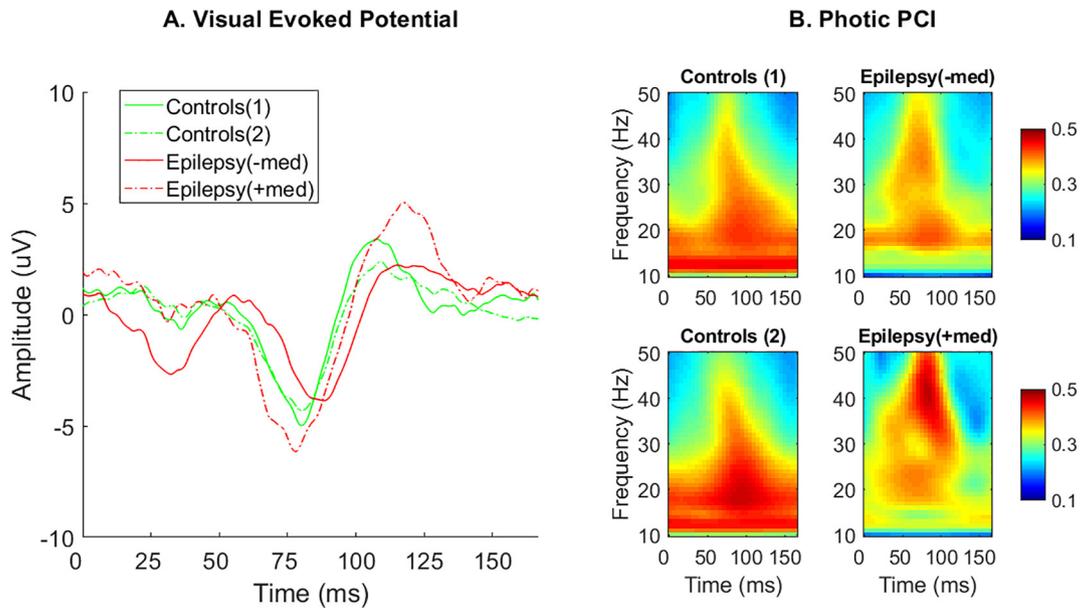


Fig. 2. A. Visual-evoked potential averaged over the occipital electrode cluster. Evoked photic response for the occipital electrode cluster consisting of Oz and the neighboring electrodes. B. Time–frequency profile of 6 Hz photic PCI from controls and groups with epilepsy, averaged over all channels. For Controls 1st, Controls 2nd, Epilepsy without medication, and Epilepsy with medication. The group with migraine was not visually stimulated. Wavelet analysis was performed using Morlet wavelets with 5 cycles.

decreased phase clustering in the alpha range after photic stimulation in epilepsy off drugs may thus indicate decreased GABAergic inhibition [55,56] and may facilitate phase clustering in the gamma range. In migraine, phase synchronization in the alpha band following visual stimulation was increased [35]. As we did not visually stimulate participants with migraine, we cannot confirm this finding. In controls, age positively correlated with NNEI and rPCI, in line with previous observations of decreasing alpha band phase locking with increasing age, especially in occipital regions [57]. Our finding of high NNEI and reduced photic stimulation phase clustering in the alpha band in the group with epilepsy may be age related. High NNEI, reflecting low phase clustering in the alpha band (corresponding to a low value of PCI^1), suggests a

state of high excitability which may contribute to this form of epilepsy affecting mainly young adults between 12 and 20 years old.

The increased phase clustering in the gamma range in epilepsy off medication after TMS and photic stimulation may indicate increased propensity to synchronization and entrainment of neural populations due to recurrent connectivity [28]. Recurrent connectivity and reduced GABAergic inhibition may set migraine and epilepsy apart, as the rPCI and PCI frequency spectrum of migraine did not differ from controls. Migraine and epilepsy showed increased cortical excitability in previous studies [13,21,58–60]. Further studies are needed to understand the mechanisms underlying the reported cortical hyperexcitability in migraine.

Epilepsy Controls Comparison

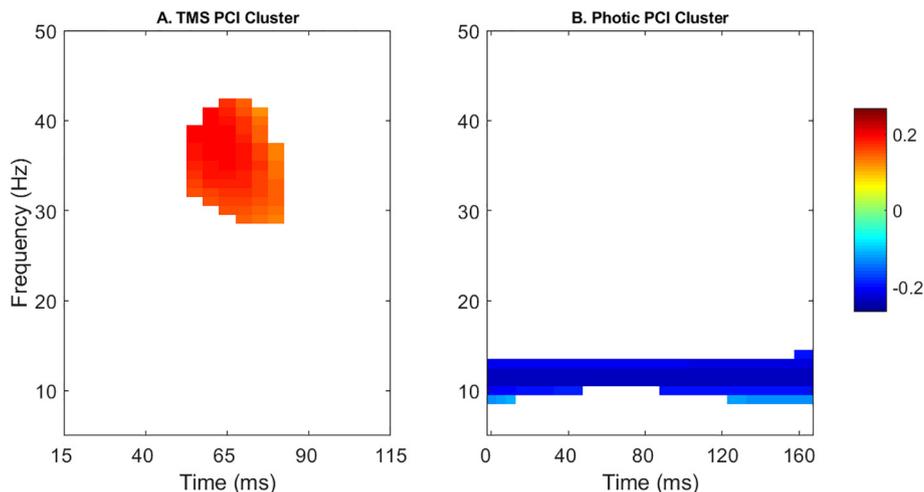


Fig. 3. Monte Carlo permutation testing revealed significant differences in TMS (A) and photic stimulation (B) for the epilepsy (–med) versus controls(1) group comparison. Monte Carlo permutation testing with 2500 permutations, a cluster-alpha of 0.01 and significance of 0.025 revealed a significant difference between epilepsy without medication and controls(1). A. TMS PCI cluster. The cluster is located from 50 ms to 80 ms in the gamma frequency range, with increased PCI in the group with epilepsy when compared with the control group. B. Photic PCI cluster. The photic PCI cluster is located over the whole time window in the 10–14 Hz frequency band, with decreased PCI in the group with epilepsy when compared with the control group.

Table 4
Median relative phase clustering index and 5–95 percentile for all groups.

		Controls(1)	Controls(2)	Epilepsy(– med)	Epilepsy(+ med)	Migraine
TMS PC	N	36	30	5	3	10
	rPCI	0.11 (0.03–0.23)	0.11 (0.05–0.22)	0.22 (0.18–0.24)*	0.19 (0.14–0.29)*	0.13 (0.01–0.22)
	NNEI	0.33 (0.13–0.58)	0.40 (0.19–0.56)	0.44 (0.34–0.49)	0.41 (0.29–0.58)	0.41 (0.11–0.52)
Photic	N	35	29	5	3	–
	rPCI	0.14 (0.040–0.32)	0.17 (0.04–0.35)	0.29 (0.19–0.30)*	0.14 (0.02–0.26)	–
	NNEI	0.63 (0.40–0.80)	0.62 (0.32–0.87)	0.79 (0.62–0.87)*	0.72 (0.54–0.77)	–
Sham	N	35	29	4	3	10
	rPCI	0.09 (0.03–0.18)	0.05 (0.02–0.12)	0.11 (0.03–0.13)	0.06 (0.03–0.11)	0.05 (0.02–0.08)
	NNEI	0.76 (0.53–0.85)	0.82 (0.69–0.89)	0.80 (0.51–0.87)	0.86 (0.51–0.92)	0.81 (0.72–0.89)

N = number of participants in whom data were collected. TMS PC = Polarity-compensated (age adjusted in the groups with epilepsy only). Photic stimulation at 6 Hz was not performed in the group with migraine.

* Indicates significant difference with the respective control population.

In all groups, the highest PCI following magnetic and photic stimulations was found in the gamma range (30–40 Hz), consistent with previous findings [20]. Artifacts elicited by TMS (muscle and stimulation artifacts) can also occur in the gamma frequency range. Transcranial magnetic stimulation-induced muscle artifacts usually peak around 7 ms and return to baseline around 15 ms [61]. We therefore analyzed the rPCI in epochs that theoretically start after or at the tail end of the muscle artifact and repeated the analysis for windows starting at 20, 25, and 30 ms without changing the results. We introduced several novel strategies to reduce artifacts. Firstly, the rPCI analysis (Eq. (2)) corrects large stimulus-locked artifacts. The NNEI is, however, still affected by these artifacts. Secondly, we compensated the magnetic charge of the stimulation (Eq. (3)), cancelling volume conductance and polarity-dependent TMS decay artifacts. Lastly, the rPCI obtained with TMS is consistent with the rPCI obtained with photic stimulation.

Both stimulation modalities, however, differ in terms of PCI. We therefore conclude that the rPCI and its elevation in the group with epilepsy compared to controls represent a neuronal process rather than a measurement artifact.

Our comparison of the rPCI elicited by magnetic and photic stimulation modalities shows that magnetic stimulation elicits a larger rPCI difference between people with epilepsy and controls and may have greater potential for clinical application. The rPCI analysis yields one mean value per individual, making statistical analysis relatively straightforward.

Similar to TMS-evoked potential analysis, rPCI analysis can also be done on each EEG channel. Our experimental set-up with a circular coil was not directed towards localization, but in a design with image-guided focal magnetic stimulation in focal epilepsy, the rPCI may potentially help localize cortical areas with aberrant

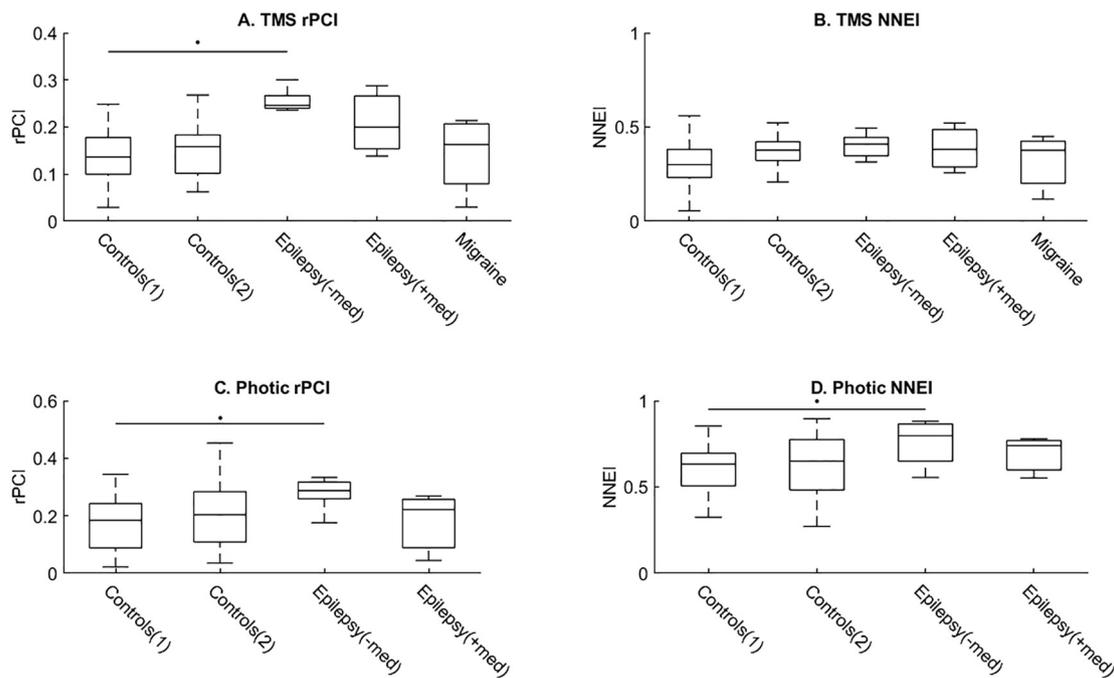


Fig. 4. Excitability biomarker boxplots for all groups. Median TMS polarity-compensated relative phase clustering index (rPCI) and neural network excitability index (NNEI) for all groups and stimulation modalities. The boxes show the 25–75th percentiles, the line in the box is the sample median. The polarity-compensated transcranial magnetic stimulation (0.5 Hz) results are shown in panels A. and B. 6 Hz photic stimulation results are shown in panels C. and D. Photic stimulation was not done in the group with migraine. * indicates significant difference between the indicated groups.

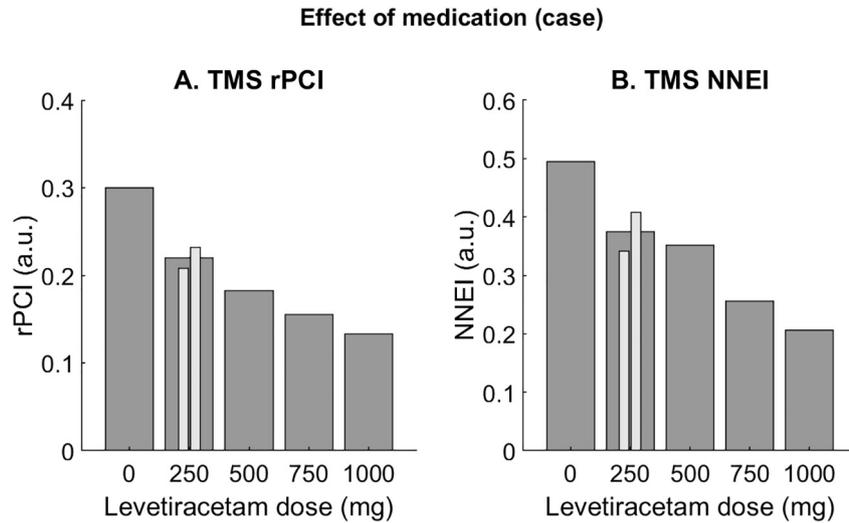


Fig. 5. Effect of medication (levetiracetam) on rPCI and NNEI for one participant with juvenile myoclonic epilepsy. For case E3 of Table 1, the evolution of the polarity-compensated rPCI & NNEI against the levetiracetam dose is depicted. This is the only participant in whom several measurements were done with different medication doses. The polarity-compensated rPCI and NNEI are shown on the y-axis and each dose of levetiracetam on the x-axis. The plots are not shown in chronological order, as the participant started with 1000 mg levetiracetam. The dose was gradually lowered to 250 mg because of side effects. Two measurements were done while the participant was taking 250 mg levetiracetam; the average is shown in gray. The participant remained seizure-free for the duration of the study. During the last measurement (250 mg), no photoparoxysmal reaction was seen whereas this had been present during the other measurements.

inhibition. Image-guided focal magnetic stimulation was previously successful in localizing cortical areas connected to subcortical heterotopic gray matter in periventricular nodular heterotopia using the TMS-evoked potential [25].

The phase clustering measures reported here are obtained from the TMS-triggered responses per channel over stimulation trials. We did not address phase synchronization between EEG channels (see for review [62]). A recent TMS-EEG study showed that TMS-induced activity persists up to 800 ms poststimulus [63]. We have studied the TMS intertrial phase clustering response up to 750 ms after the stimulus. In our data, phase clustering decays shortly after the TMS stimulus, with clustering at higher frequencies decaying faster than at low frequencies. There was no apparent clustering of phases of the higher frequencies (>20 Hz) after ~120 ms while there is no clustering of lower frequencies (<20 Hz) after 400 ms. More than 400 ms after the TMS stimulus, phase clustering was only present in the low frequency bands (<8 Hz).

The limitations of our study include the small sample size in the groups with epilepsy and with migraine, which we dealt with by using permutation-based statistics that are robust even when small and groups of varying sample size are considered [64], and the need to optimize the stimulation protocol for the analysis of phase clustering. Repetitive magnetic stimulation can alter cortical excitability, and 5 Hz, but not 0.5 Hz stimulation, significantly increased the motor-evoked potential [45]. A subsequent study did show a small inhibitory effect of 0.5 Hz stimulation, especially during the first 20 stimuli [65]. Others showed that the motor evoked potential (MEP) amplitude increased after 200 TMS pulses given every 4 s [66]. Only one study investigated the effect of 15-minute trains of 0.6 Hz stimulation on the EEG and found a significant increase of the N45 amplitude [67]. Our choice for a ramped stimulus–response curve with an interstimulus interval of 0.5 Hz was based on the fact that stimulus–response curves were shown to be invariant to interstimulus intervals from 1.4 to 4 s [68], and that there was no difference between stimulus–response curves acquired with a ramped (increasing) or random stimulation intensity order [69]. Several studies have shown the effect of stimulation intensity on the EEG response, such that a cortical excitability threshold could be measured [20,70]. As a first approach, we chose to pool different stimulus intensities to calculate the rPCI, further research will include the identification of stimulus intensity effects on this parameter. Cortical excitability is dynamic and changes throughout the day [71].

Our measurements were conducted at 9 AM or 2 PM. No significant differences in TMS measures were reported between these times of the day [41], except a larger TMS-evoked potential 100 ms after the stimulus [42]. We did not find a difference in rPCI between the people measured at 9 AM and those measured at 2 PM. Cortical excitability was also shown to change between, before, and after epileptic seizures [72–74] and migraine attacks [14]. We took care to conduct our measurements in the interictal period. Previously, the rPCI was shown to increase when photic stimulation was followed by an epileptic discharge [28]. To improve the understanding of the clinical significance of the rPCI and NNEI as biomarkers for a brain state with increased cortical excitability and seizure propensity, further studies will need to assess its change just before, after, and between seizures. Another important clinical question is whether the rPCI could help differentiate responders to antiepileptic therapy from nonresponders.

We showed that EEG phase clustering elicited by TMS and photic stimulation is a potential marker of epileptogenicity in people with JME. The systematic application of rPCI may contribute to a better understanding of pathophysiological mechanisms in epilepsy and may have a direct clinical application.

Acknowledgments

The authors thank the participants for their time, Paul Kallansee and Jan de Vries for their help in EEG data collection, Anneliek Schooneman, Trusjen Punte, and Donja de Graaf for logistical support, Holger Westerhuis, Frank van Engelen, and Erik Kuitert for technical support, and Dr. A. Vlaar, Dr. F.S.S. Leijten, Mrs. Bloemen-Carlier, Dr. P. Augustijn, Dr. S. Van Beek, Dr. G.-J. de Haan for referring participants with JME, and Dr. A. Del Felice and anonymous reviewers for critically reviewing a final version of the manuscript.

This work was partly undertaken at NIHR University College London Hospitals Comprehensive Bio-Medical Research Centre, which received a proportion of funding from the Department of Health's Research Centres funding scheme.

PRB is supported by the Christelijke Vereniging voor de Verpleging van Lijders aan Epilepsie (Nederland). MDF receives research support from the European Union Seventh Framework Programme ("EUROHEADPAIN", grant agreement no. 602633) and the Dutch national science prize, Spinoza Award. EAT receives research support from the European Union's

Seventh Framework programme (“EUROHEADPAIN”, grant agreement no. 602633 and “BRAINPATH”, grant agreement no. 61236).

JWS receives research support from the Dr. Marvin Weil Epilepsy Research Fund and UK Epilepsy Society.

Potential conflicts of interests

JWS has been consulted by and received fees for lectures from Eisai, Bial, Janssen, and UCB Pharma.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yebeh.2019.01.029>.

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