The exciting migraine brain: towards neurophysiological prediction of migraine attacks

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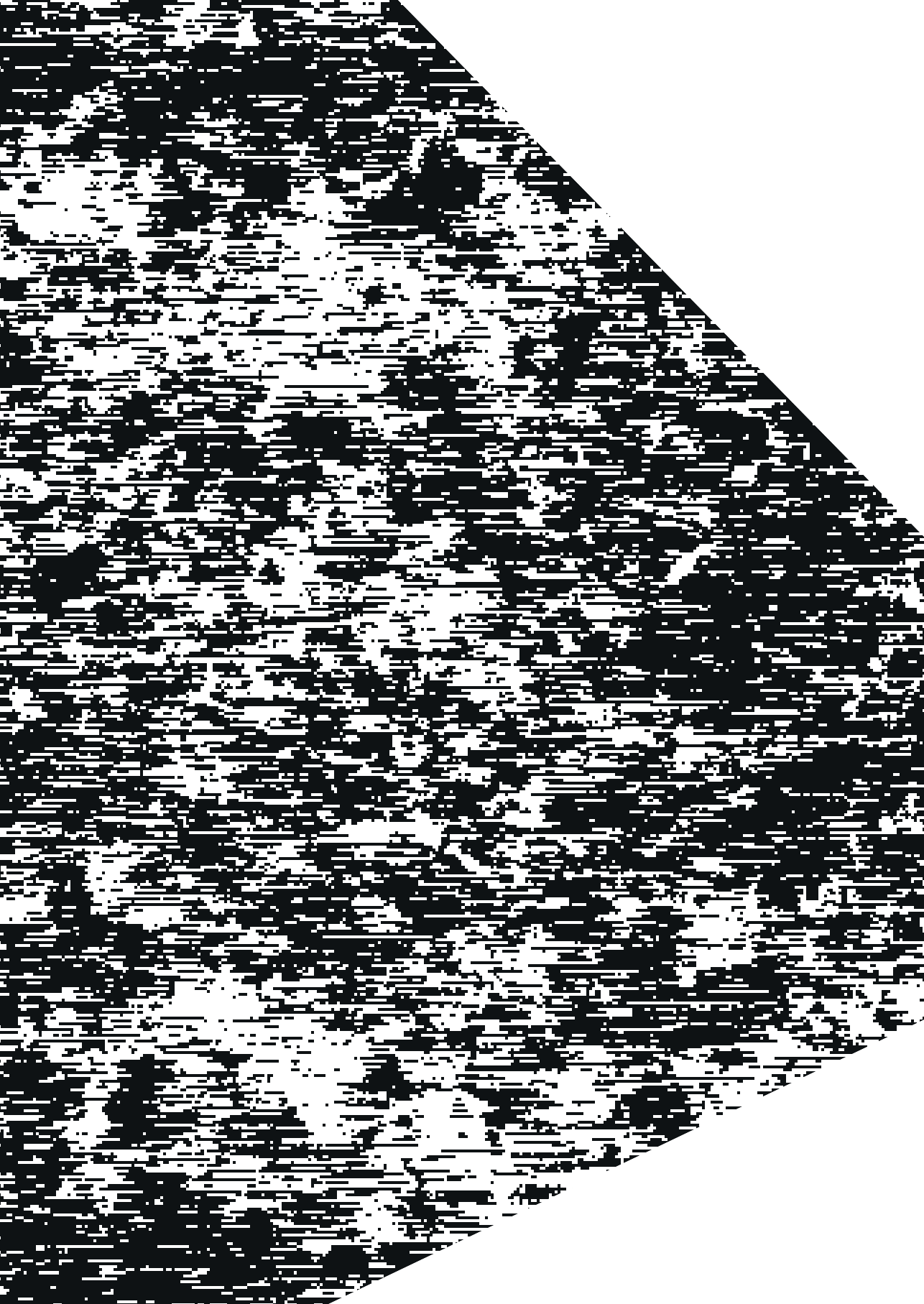

Chapter 6

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

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Abstract

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. 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.

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.

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.

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.

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] 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 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 epilepsy compared to 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 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 JME compared to controls, and increased amplitude of late peaks when participants with JME were sleep deprived, demonstrating cortical hyperexcitability.[21] 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 align between trials shortly after the TMS pulse. A recent study suggests that phase clustering 20–60 ms post-stimulus 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 magneto-encephalography 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 on-going EEG was linked to increased neuronal excitability in epilepsy.[31] Phase synchrony in response to photic stimulation was also elevated in migraine with and without aura compared to 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 to controls or people with migraine with aura.

Methods

Participants

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]

Juvenile Myoclonic Epilepsy

Participants, diagnosed with Juvenile Myoclonic Epilepsy or possible Juvenile Myoclonic Epilepsy 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 and over, with a history of myoclonic seizures and/or at least one generalized tonic-clonic seizure, who were either not taking anti-epileptic drugs (active epilepsy off-drugs) or considering tapering anti-epileptic drugs (in remission) in conjunction with the attending neurologist could be included. Subjects with co-morbid 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]

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'.

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.

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.

Material

Transcranial Magnetic Stimulation

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 a.m. or 02.00 p.m. and spread evenly between a.m. and p.m. No significant differences in TMS measures were reported between these times of the day,[41] except a larger TMS-evoked potential 100ms after the stimulus.[42] Soft earplugs were used to reduce the coil click.

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.

Electroencephalography

EEG 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.

Stimulation protocols

Photic stimulation

After a 10-minute baseline EEG recording, photic stimulation (Sigma, Is FSA 10-2D-I, SIGMA Medizin-Technil 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.

Single-pulse TMS stimulus response curve

The resting motor threshold, 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 semi-automated, in-house designed scanning protocol (created in Matlab® (version 7.5.0 R2007b The MathWorks Inc., Natick, MA, USA)) was used to determine the resting motor threshold 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 2s. This frequency was not shown to alter motor evoked potentials.[44,45] The scanning procedure was performed using anticlockwise (right hemisphere) and clockwise (left hemisphere) stimulation as part of the artifact reduction strategy 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 re-measured after 10–12 months at the same time of 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, 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 minimising the number of pulses.[46] In the epilepsy on medication group 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.

Data analysis

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

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.

Phase clustering and Neuronal Network Excitability Index

EEG 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 s / 100 ms = 10 Hz) starting 15 ms after TMS- or sham stimulation (see also below regarding TMS artefact reduction) and without delay (0 ms) for photic stimulation. After linear de-trending, 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 analysed 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 equation (1).

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

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

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

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

$$NNEI_c = \langle 1 - PCI_c^1 \rangle_c \tag{3}$$

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.

Time-Frequency Analysis

For TMS time-frequency analyses of the PCI 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 (0–6ms 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 – 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 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 equation (1):

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

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 equation 1A, and the results were averaged over all channels.

TMS and muscle artifact reduction

We included several strategies to reduce stimulation and muscle artifacts related to magnetic stimulation. Firstly, equation (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 artefacts are expected within the first 15 ms after the stimulus. To ensure that our results are not due to muscle artefact 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 anticlockwise stimulation responses offline in a pairwise fashion to compensate the linear component, containing the artefact, in the response to each polarity (eq. (4))[48]:

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

$F_{c,i}^{+}$ and $F_{c,i}^{-}$ are the response amplitudes to the clockwise and counterclockwise current stimulations from 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 equations (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.

Statistical analyses

We took the small sample size of the epilepsy (on and off medication) and migraine groups 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 resting motor threshold 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 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 stimulation 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 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 stimulation in the control groups.

Results

Participants

We included 38 controls (25 females, mean age 38.1 years, range 15–62 years) between May 2014 and October 2014. Five were left handed. 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 due to

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 analysed the results with and without two of these family members. Given the small differences between the 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.

Table 1. Clinical features of participants with juvenile myoclonic epilepsy.

Nr	M/F	age	age onset	Handed-ness	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 6-40Hz) accompanied by myoclonic jerks	0.24	0.49	0.29	0.79
E4	F	34	16	7	Y	8 yrs	myoclonic jerks + TCs	normal background activity with spontaneous 3-4Hz (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 3Hz (poly)spike-and-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: [1]depakine chrono 2000mg 1/day, [2]depakine 750mg 2/day, [3]depakine 500mg 2/day.

Table 2. Characteristics of participants with migraine with aura.

Nr	M/F	age at inclusion	age at onset	Handed-ness	attacks per month	% of attacks with aura	TMS rPCI	TMS NNEI
M1	F*	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*	62	17	-8	0.5	100	0.15	0.52
M9	F	51	18	9	1	100	0.14	0.44
M10	F*	31	11	7	1.5	35	0.18	0.46

Handedness: according to the Edinburgh handedness questionnaire (scores <-5 indicate left-hand dominance). *first-degree family members.

Resting motor threshold

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

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.

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 Figure 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 neighbouring electrodes) where the evoked response should be most prominent showed a difference between the first measurement of the controls and epilepsy off medication group (p = 0.016, see Figure 1A for the cluster). The visual-evoked potential shown in Figure 2A did not differ between the control and 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), Figures 1B and 2B). The TMS spectrum differed between epilepsy off medication and the first measurement of the controls (Figure 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 (Figure 3B, p = 0.004). There were no differences in the other group comparisons. The analysis of Figure 3A suggests that the feature which best distinguishes TMS-evoked responses in epilepsy from controls is the rPCI defined in equation (2), as the high-frequency phase information is taken into account. For photic-evoked responses, in contrast, Figure 3B suggests that the rPCI and the NNEI (equation (3)) may be suitable markers as they reflect phase clustering in the lower frequencies. As shown in equation (2), the rPCI can increase either due to an *in*crease of PCI or to a *de*crease of PCI. The NNEI is useful to discriminate between these two alternatives. This is further tested in the next section.

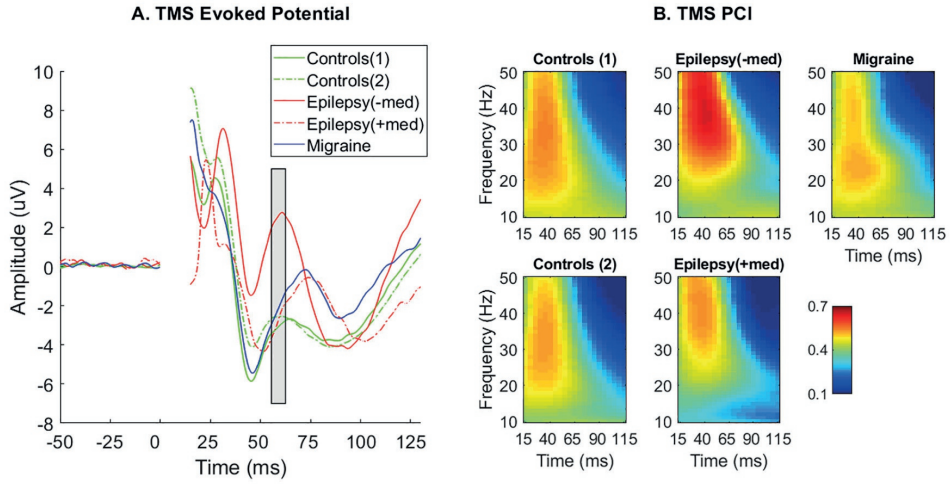

Figure 1. (A) TMS-evoked potential over the central electrode cluster for control, group with epilepsy, and group with migraine. Evoked responses averaged over a central electrode cluster, consisting of electrode Cz (the TMS target) and the neighboring electrodes surrounding electrode Cz. The gray area 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.

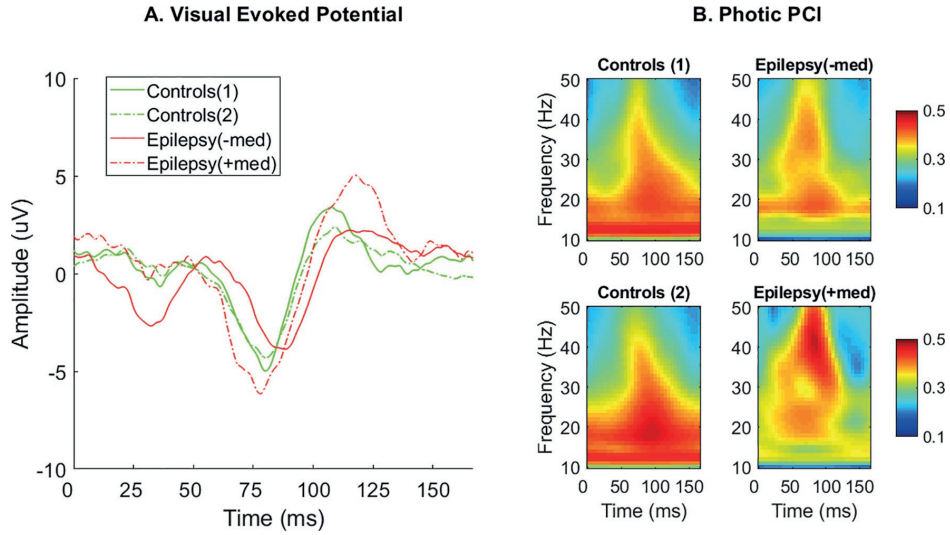

Figure 2. (A) Visual-evoked potential averaged over the occipital electrode cluster. Evoked photic response for the occipital electrode cluster consisting of Oz and the neighboring

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.

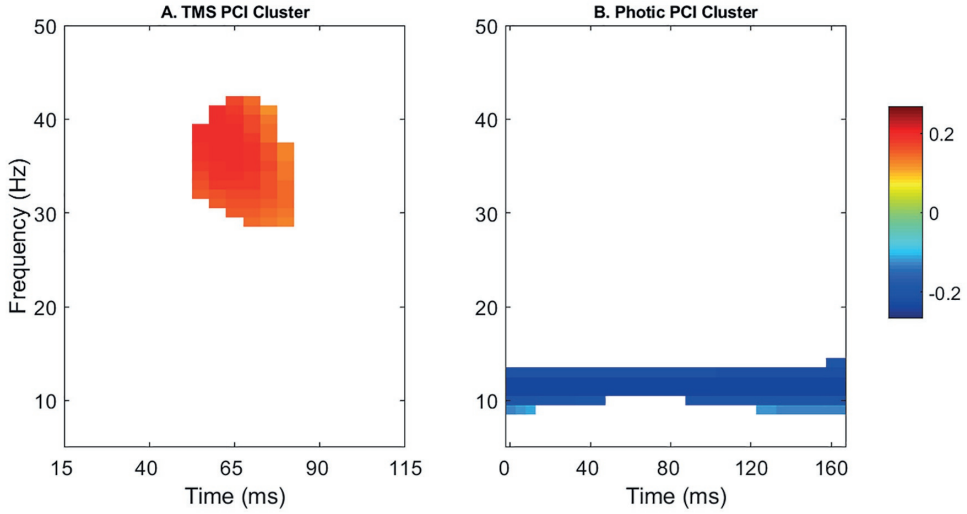

Figure 3. Monte Carlo permutation testing revealed significant differences in TMS (A) and photic stimulation (B) for the epilepsy(−med) versus controls(1) group comparison. Monte Carlo permutation testing with 2500 permutations, a cluster-alpha of 0.01 and significance of 0.025 revealed a significant difference between epilepsy without medication and controls(1). (A) TMS PCI cluster. The cluster is located from50ms to 80ms 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.

rPCI and NNEI for TMS and photic stimulation

To quantify the difference in PCI between the different groups, we used the rPCI (equation (2)) and the NNEI (equation (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 epilepsy off medication compared with controls. The rPCI and NNEI elicited by sham stimulation did not differ between controls and the epilepsy groups. The rPCI and NNEI in the group with migraine did not significantly differ from controls (Figure 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 day the measurement took place (a.m. or p.m.). 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.

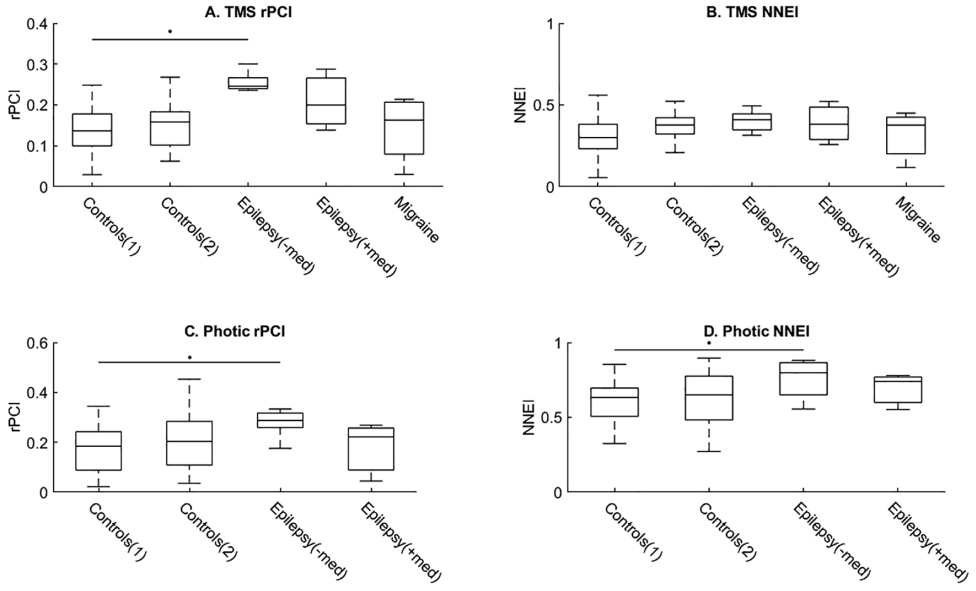

Figure 4. Excitability biomarker boxplots for all groups. Median TMS polarity-compensated relative phase clustering index (rPCI) and neural network excitability index (NNEI) for all groups and stimulation modalities. The boxes show the 25–75th percentiles, the line in the box is the sample median. The polarity-compensated transcranial magnetic stimulation (0.5Hz) 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.

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

		controls(1)	Controls(2)	Epilepsy(-med)	Epilepsy(+med)	Migraine
	N	36	30	5	3	10
TMS	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)
	N	35	29	5	3	-
Photic	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)	-
	N	35	29	4	3	10
Sham	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 migraine group. * indicates significant difference with the respective control population.

An example of the rPCI and NNEI following changes in the dose of levetiracetam in one participant with epilepsy is shown in Figure 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).

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 to controls but not in those with migraine with aura. The rPCI elicited by photic stimulation was also increased in 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

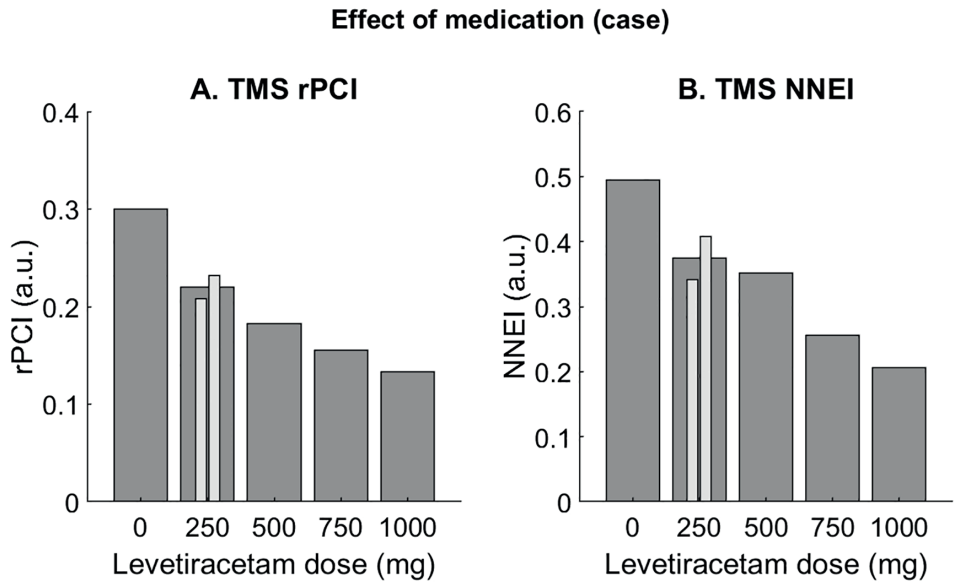

Figure 5. Effect of medication (levetiracetam) on rPCI and NNEI for one participant with juvenile myoclonic epilepsy. For case E3 of Table 1, the evolution of the polarity-compensated rPCI and NNEI against the levetiracetam dose is depicted. This is the only participant in whom several measurements were 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.

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 equation (3), a low PCI value at the base frequency corresponds to a high NNEI, i.e., a high neural network excitability. We confirmed this as 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 to controls. The net result was a higher relative PCI in the epilepsy off medication group 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 desynchronisation 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 synchronisation in healthy subjects.[54] Interestingly, diazepam is used to terminate seizures. The decreased phase clustering in the alpha range after photic stimulation in epilepsy off drugs may thus indicate decreased GABA-ergic inhibition,[55,56] and may facilitate phase clustering in the gamma range. In migraine, phase synchronisation 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 synchronisation and entrainment of neural populations due to recurrent connectivity.[28] Recurrent connectivity and reduced GABA-ergic 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.

In all groups, the highest phase clustering index following magnetic and photic stimulation was found in the gamma range (30–40 Hz), consistent with previous findings.[20] Artifacts elicited by TMS stimulation (muscle and stimulation artifacts) can also occur in the gamma frequency range. TMS-induced muscle artifacts usually peak around 7 ms and return to baseline around 15 ms.[61] We therefore analyzed the rPCI in epochs which 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 (equation (2)) corrects large stimulus-locked artifacts. The NNEI is, however, still affected by these artifacts. Secondly, we compensated the magnetic charge of the stimulation (equation (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.

Similarly 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 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 *synchronisation between* EEG channels (see for review[62]). A recent TMS-EEG study showed that TMS-induced activity persists up to 800 ms post-stimulus.[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 (>20Hz) 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 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 a.m. or 2 p.m. No significant differences in TMS measures were reported between these times of day,[41] except a larger TMS-

evoked potential 100ms after the stimulus.[42] We did not find a difference in rPCI between the people measured at 9 a.m. and those measured at 2 p.m. 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.

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References

1. Jefferys JGR. Advances in understanding basic mechanisms of epilepsy and seizures. *Seizure*. 2010;19(10):638–646.
2. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. *Annu. Rev. Physiol.* 2013;75(1):365–391.
3. Tolner EA, Houben T, Terwindt GM, De Vries B, Ferrari MD, Van Den Maagdenberg AMJM. From migraine genes to mechanisms. *Pain*. 2015;156(4):S64–S74.
4. Bauer PR, Carpay J a, Terwindt GM, Sander JW, Thijs RJ, Haan J, Visser GH. Headache and epilepsy. *Curr. Pain Headache Rep.* 2013;17(8):351.
5. Mantegazza M, Cestèle S. Pathophysiological mechanisms of migraine and epilepsy: Similarities and differences. *Neurosci. Lett.* 2018;667:92–102.
6. Haut SR, Bigal ME, Lipton RB. Chronic disorders with episodic manifestations: Focus on epilepsy and migraine. *Lancet Neurol.* 2006;5(2):148–157.
7. Rogawski MA. Common Pathophysiologic Mechanisms in Migraine and Epilepsy. *Arch Neurol.* 2008;65(6):709–714.
8. Vecchia D, Pietrobon D. Migraine: a disorder of brain excitatory–inhibitory balance? *Trends Neurosci.* 2012;35(8):507–520.
9. de Goede AA, ter Braack EM, van Putten MJAM. Single and paired pulse transcranial magnetic stimulation in drug naïve epilepsy. *Clin. Neurophysiol.* 2016;127(9):3140–3155.
10. Brigo F, Storti M, Benedetti MD, Rossini F, Nardone R, Tezzon F, Fiaschi A, Bongiovanni LG, Manganotti P. Resting motor threshold in idiopathic generalized epilepsies: A systematic review with meta-analysis. *Epilepsy Res.* 2012;101(1–2):3–13.
11. Kelley SA, Hartman AL, Kossoff EH. Comorbidity of migraine in children presenting with epilepsy to a tertiary care center. *Neurology*. 2012;79(5):468–473.
12. Schankin CJ, Rémi J, Klaus I, Sostak P, Reinisch VM, Noachtar S, Straube A. Headache in juvenile myoclonic epilepsy. *J. Headache Pain*. 2011;12(2):227–233.
13. Brigo F, Storti M, Nardone R, Fiaschi A, Bongiovanni LG, Tezzon F, Manganotti P. Transcranial magnetic stimulation of visual cortex in migraine patients: a systematic review with meta-analysis. *J. Headache Pain*. 2012;13(5):339–49.
14. Cortese F, Coppola G, Di Lenola D, Serrao M, Di Lorenzo C, Parisi V, Pierelli F. Excitability of the motor cortex in patients with migraine changes with the time elapsed from the last attack. *J. Headache Pain*. 2017;18(1):16–21.
15. Aurora S, Al-Sayeed F, Welch K. The Cortical Silent Period is Shortened in Migraine with

Aura. *Cephalalgia*. 1999;19(8):708–712.
16. Werhahn KJ, Wiseman K, Herzog J, Förderreuther S, Dichgans M, Straube A. Motor cortex excitability in patients with migraine with aura and hemiplegic migraine. *Cephalalgia*. 2000;20(1):45–50.
17. Siniatchkin M, Reich A-L, Shepherd AJ, van Baalen A, Siebner HR, Stephani U. Peri-ictal changes of cortical excitability in children suffering from migraine without aura. *Pain*. 2009;147(1–3):132–140.
18. Neverdahl JP, Omland PM, Uglem M, Engstrøm M, Sand T. Reduced motor cortical inhibition in migraine: A blinded transcranial magnetic stimulation study. *Clin. Neurophysiol.* 2017;128(12):2411–2418.
19. Miniussi C, Thut G. Combining TMS and EEG offers new prospects in cognitive neuroscience. *Brain Topogr.* 2010;22(4):249–256.
20. Saari J, Kallioniemi E, Tarvainen M, Julkunen P. Oscillatory TMS-EEG-Responses as a Measure of the Cortical Excitability Threshold. *IEEE Trans. Neural Syst. Rehabil. Eng.* 2018;26(2):383–391.
21. Del Felice A, Fiaschi A, Bongiovanni GL, Savazzi S, Manganotti P. The sleep-deprived brain in normals and patients with juvenile myoclonic epilepsy: A perturbational approach to measuring cortical reactivity. *Epilepsy Res.* 2011;96(1–2):123–131.
22. Julkunen P, Säisänen L, Könönen M, Vanninen R, Kälviäinen R, Mervaala E. TMS-EEG reveals impaired intracortical interactions and coherence in Unverricht-Lundborg type progressive myoclonus epilepsy (EPM1). *Epilepsy Res.* 2013;106(1–2):103–112.
23. Valentin A, Arunachalam R, Mesquita-Rodrigues A, Garcia Seoane JJ, Richardson MP, Mills KR, Alarcon G. Late EEG responses triggered by transcranial magnetic stimulation (TMS) in the evaluation of focal epilepsy. *Epilepsia*. 2008;49(3):470–480.
24. Kimiskidis VK, Kugiumtzis D, Papagiannopoulos S, Vlaikidis N. Transcranial magnetic stimulation (TMS) modulates epileptiform discharges in patients with frontal lobe epilepsy: A preliminary EEG-TMS study. *Int. J. Neural Syst.* 2013;23(1).
25. Shafi MM, Vernet M, Klooster D, Chu CJ, Boric K, Barnard ME, Romatoski K, Westover MB, Christodoulou J a, Gabrieli JDE, Whitfield-Gabrieli S, Pascual-Leone A, Chang BS. Physiological consequences of abnormal connectivity in a developmental epilepsy. *Ann. Neurol.* 2015;77(3):487–503.
26. Kimiskidis VK, Tsimpiris A, Ryvlin P, Kalviainen R, Koutroumanidis M, Valentin A, Laskaris N, Kugiumtzis D. TMS combined with EEG in genetic generalized epilepsy: A phase II diagnostic accuracy study. *Clin. Neurophysiol.* 2017;128(2):367–381.
27. ter Braack EM, Koopman MSc A-WE, van Putten MJAM. Early TMS evoked potentials in epilepsy: a pilot study. *Clin. Neurophysiol.* 2016;127(9):3025–3032.
28. Parra J, Kalitzin SN, Iriarte J, Blanes W, Velis DN, Lopes da Silva FH. Gamma-band phase clustering and photosensitivity: is there an underlying mechanism common to photosensitive epilepsy and visual perception? *Brain*. 2003;126(5):1164–1172.
29. Kalitzin S, Velis D, Suffczynski P, Parra J, Lopes Da Silva F. Electrical brain-stimulation paradigm for estimating the seizure onset site and the time to ictal transition in temporal lobe epilepsy. *Clin. Neurophysiol.* 2005;116(3):718–728.
30. Wendling F, Gerber U, Cosandier-Rimele D, Nica A, De Montigny J, Raineteau O, Kalitzin

S, Lopes da Silva F, Benquet P. Brain (Hyper)Excitability Revealed by Optimal Electrical Stimulation of GABAergic Interneurons. *Brain Stimul.* 2016;9(6):919–932.
31. Meisel C, Schulze-Bonhage A, Freestone D, Cook MJ, Achermann P, Plenz D. Intrinsic excitability measures track antiepileptic drug action and uncover increasing/decreasing excitability over the wake/sleep cycle. *Proc. Natl. Acad. Sci.* 2015;112(47):14694–14699.
32. Yum MK, Moon JH, Kang JK, Kwon OY, Park KJ, Shon YM, Lee IK, Jung KY. Timely event-related synchronization fading and phase de-locking and their defects in migraine. *Clin. Neurophysiol.* 2014;125(7):1400–1406.
33. Angelini L, Tommaso M De, Guido M, Hu K, Ivanov PC, Marinazzo D, Nardulli G, Nitti L, Pellicoro M, Pierro C, Stramaglia S. Steady-State Visual Evoked Potentials and Phase Synchronization in Migraine Patients. *Phys. Rev. Lett.* 2004;93(3):038103.
34. Gantenbein AR, Sandor PS, Fritschy J, Turner R, Goadsby PJ, Kaube H. Sensory information processing may be neuroenergetically more demanding in migraine patients. *Neuroreport*. 2013;24(4):202–5.
35. De Tommaso M, Marinazzo D, Guido M, Libro G, Stramaglia S, Nitti L, Lattanzi G, Angelini L, Pellicoro M. Visually evoked phase synchronization changes of alpha rhythm in migraine: Correlations with clinical features. *Int. J. Psychophysiol.* 2005;57(3):203–210.
36. de Tommaso M, Stramaglia S, Marinazzo D, Trotta G, Pellicoro M. Functional and effective connectivity in EEG alpha and beta bands during intermittent flash stimulation in migraine with and without aura. *Cephalalgia*. 2013;33(11):938–47.
37. De Tommaso M, Ambrosini A, Brighina F, Coppola G, Perrotta A, Pierelli F, Sandrini G, Valeriani M, Marinazzo D, Stramaglia S, Schoenen J. Altered processing of sensory stimuli in patients with migraine. *Nat. Rev. Neurol.* 2014;10(3):144–155.
38. Oldfield R. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*. 1971;9:97–113.
39. Kasteleijn- Nolst Trenité DGA, Schmitz B, Janz D, Delgado-Escueta A V., Thomas P, Hirsch E, Lerche H, Camfield C, Baykan B, Feucht M, Martínez-Juárez IE, Duron RM, Medina MT, Rubboli G, Jerney J, et al. Consensus on diagnosis and management of JME: From founder's observations to current trends. *Epilepsy Behav.* 2013;28(1):S87–S90.
40. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia*. 2013;33(9):629–808.
41. Koski L, Schrader LM, Wu AD, Stern JM. Normative data on changes in transcranial magnetic stimulation measures over a ten hour period. *Clin. Neurophysiol.* 2005;116:2099–2109.
42. ter Braack EM, de Goede AA, van Putten MJAM. Resting Motor Threshold, MEP and TEP Variability During Daytime. *Brain Topogr.* 2019;32(1):17–27.
43. Groppa S, Oliviero a, Eisen a, Quartarone a, Cohen LG, Mall V, Kaelin-Lang a, Mima T, Rossi S, Thickbroom GW, Rossini PM, Ziemann U, Valls-Solé J, Siebner HR. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin. Neurophysiol.* 2012;123(5):858–82.
44. Pascual-Leone A, Valls-Solé J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain*. 1994;117(4):847–858.

45. Gorsler A, Bäumer T, Weiller C, Münchau A, Liepert J. Interhemispheric effects of high and low frequency rTMS in healthy humans. *Clin. Neurophysiol.* 2003;114(10):1800–1807.
46. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Avanzini G, Bestmann S, Berardelli A, Brewer C, Canli T, Cantello R, Chen R, Classen J, Demitrack M, Di Lazzaro V, Epstein CM, et al. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 2009;120(12):2008–2039.
47. Kalitzin S, Parra J, Velis DN, Lopes da Silva FH. Enhancement of phase clustering in the EEG/MEG gamma frequency band anticipates transitions to paroxysmal epileptiform activity in epileptic patients with known visual sensitivity. *IEEE Trans. Biomed. Eng.* 2002;49(11):1279–86.
48. Kalitzin S, Zijlmans M, Petkov G, Velis D, Claus S, Visser G, Koppert M, Lopes Da Silva F. Quantification of spontaneous and evoked HFO's in SEEG recording and prospective for pre-surgical diagnostics. Case study. *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS*. 2012:1024–1027.
49. Groppe DM, Urbach TP, Kutas M. Mass univariate analysis of event-related brain potentials/fields I: A critical tutorial review. *Psychophysiology*. 2011;48(12):1711–1725.
50. Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. *J. Neurosci. Methods*. 2007;164(1):177–190.
51. Toro C, Deuschl G, Thatcher R, Sato S, Kufta C, Hallett M. Event-related desynchronization and movement-related cortical potentials on the ECoG and EEG. *Electroencephalogr. Clin. Neurophysiol. Evoked Potentials*. 1994;93(5):380–389.
52. Lopes da Silva F. Neural mechanisms underlying brain waves: from neural membranes to networks. *Electroencephalogr. Clin. Neurophysiol.* 1991;79(2):81–93.
53. Thut G, Théoret H, Pfennig A, Ives J, Kampmann F, Northoff G, Pascual-Leone A. Differential effects of low-frequency rTMS at the occipital pole on visual-induced alpha desynchronization and visual-evoked potentials. *Neuroimage*. 2003;18(2):334–347.
54. Premoli I, Bergmann TO, Fecchio M, Rosanova M, Biondi A, Belardinelli P, Ziemann U. The impact of GABAergic drugs on TMS-induced brain oscillations in human motor cortex. *Neuroimage*. 2017;163:1–12.
55. Wendling F, Bartolomei F, Bellanger JJ, Chauvel P. Epileptic fast activity can be explained by a model of impaired GABAergic dendritic inhibition. *Eur. J. Neurosci.* 2002;15(9):1499–1508.
56. Avoli M, de Curtis M. GABAergic synchronization in the limbic system and its role in the generation of epileptiform activity. *Prog. Neurobiol.* 2011;95(2):104–132.
57. Kolev V, Yordanova J, Basar-Eroglu C, Basar E. Age effects on visual EEG responses reveal distinct frontal alpha networks. *Clin. Neurophysiol.* 2002;113(6):901–910.
58. Reutens DC, Berkovic SF, Macdonell RAL, Bladin PF. Magnetic stimulation of the brain in generalized epilepsy: Reversal of cortical hyperexcitability by anticonvulsants. *Ann. Neurol.* 1993;34(3):351–355.
59. Akgun Y, Soysal A, Atakli D, Yuksel B, Dayan C, Arpaci B. Cortical excitability in juvenile myoclonic epileptic patients and their asymptomatic siblings: A transcranial magnetic stimulation study. *Seizure*. 2009;18(6):387–391.

60. Manganotti P, Bongiovanni LG, Fuggetta G, Zanette G, Fiaschi A. Effects of sleep deprivation on cortical excitability in patients affected by juvenile myoclonic epilepsy: A combined transcranial magnetic stimulation and EEG study. *J. Neurol. Neurosurg. Psychiatry*. 2006;77(1):56–60.
61. Rogasch NC, Thomson RH, Farzan F, Fitzgibbon BM, Bailey NW, Hernandez-Pavon JC, Daskalakis ZJ, Fitzgerald PB. Removing artefacts from TMS-EEG recordings using independent component analysis: Importance for assessing prefrontal and motor cortex network properties. *Neuroimage*. 2014;101:425–439.
62. Varela F, Lachaux JP, Rodriguez E, Martinerie J. The brainweb: Phase synchronization and large-scale integration. *Nat. Rev. Neurosci.* 2001;2(4):229–239.
63. Gordon PC, Desideri D, Belardinelli P, Zrenner C, Ziemann U. Comparison of cortical EEG responses to realistic sham versus real TMS of human motor cortex. *Brain Stimul.* 2018;11(6):1322–1330.
64. Legendre P, Legendre L. Statistical testing by permutation. *Numer. Ecol.* 1998;2:17–26.
65. 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(4):526–532.
66. 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(1):621–628.
67. Van Der Werf YD, Paus T. The neural response to transcranial magnetic stimulation of the human motor cortex. I. Intracortical and cortico-cortical contributions. *Exp. Brain Res.* 2006;175(2):231–245.
68. Mathias JP, Barsi GI, Van De Ruit M, Grey MJ. Rapid acquisition of the transcranial magnetic stimulation stimulus response curve. *Brain Stimul.* 2014;7(1):59–65.
69. Pearce AJ, Clark RA, Kidgell DJ. A comparison of two methods in acquiring stimulus-response curves with transcranial magnetic stimulation. *Brain Stimul.* 2013;6(3):306–309.
70. Komssi S, Kähkönen S, Ilmoniemi RJ. The Effect of Stimulus Intensity on Brain Responses Evoked by Transcranial Magnetic Stimulation. *Hum. Brain Mapp.* 2004;21(3):154–164.
71. Chellappa SL, Gaggioni G, Ly JQM, Papachilleos S, Borsu C, Brzozowski A, Rosanova M, Sarasso S, Luxen A, Middleton B, Archer SN, Dijk DJ, Massimini M, Maquet P, Phillips C, et al. Circadian dynamics in measures of cortical excitation and inhibition balance. *Sci. Rep.* 2016;6(1):1–13.
72. Cosentino G, Fierro B, Vigneri S, Talamanca S, Paladino P, Baschi R, Indovino S, Maccora S, Valentino F, Fileccia E, Giglia G, Brighina F. Cyclical changes of cortical excitability and metaplasticity in migraine: evidence from a repetitive transcranial magnetic stimulation study. *Pain*. 2014;155(6):1070–8.
73. Delvaux V, Alagona G, Gérard P, De Pasqua V, Delwaide PJ, Maertens De Noordhout A. Reduced excitability of the motor cortex in untreated patients with de novo idiopathic "grand mal" seizures. *J. Neurol. Neurosurg. Psychiatry*. 2001;71(6):772–776.
74. Badawy R, Macdonell R, Jackson G, Berkovic S. The peri-ictal state: cortical excitability changes within 24 h of a seizure. *Brain*. 2009;132(4):1013–1021.

Supplementary material

Interpretation of the Phase Clustering Index (PCI) in terms of system dynamics

Definitions (1), (2) and (3) of the main text, give a formal signal-analytical algorithm but do not reveal the properties of the dynamic system that may generate those features of phase clustering. Here we present a simple, analytical model of the response of a neuronal system to an external perturbation:

$$F_{ci}^{(\pm)f} = A_c^{(\pm)} V^f + R_c^{(\pm)f} + B_{ci}^f \tag{S1}$$

In the above equation F are the Fourier response amplitudes as introduced previously; V is volume conductance term including all linear artifacts related to the stimulus; R is the polarity dependent physiological response and B is the background activity, not locked in time to the stimulus. It follows that the stimulation amplitude $A_{ci}^{(+)} = -A_c^{(-)}$ if the stimulation current is matched exactly for both polarities.

Inserting the response model (S1) into the combined, polarity-compensated amplitudes in equation (4) of the main text, the first term from (S1) cancels.

Note that the norm in the denominator in equation (S1) can also be written as follows:

$$PCI_c^f = PCI_c^f = \frac{\left\langle \sqrt{F_{c,i}^{f\,2}} \right\rangle_i}{\sqrt{\left\langle F_{c,i}^{f\,2} \right\rangle_i}} \tag{S2}$$

This form is different from earlier publications [28,47]. While the results calculated in both ways are similar, this norm allows for a better pathophysiological interpretation of the underlying mechanism.

Substituting the result into the PCI definition equation (S2) we can express this definition in terms of the background EEG activity B and the physiological response to the stimulation R. Assuming that B and R are not correlated, we obtain the following expression for PCI_c^f:

$$PCI_c^f = \frac{RBR_c^f}{\sqrt{1+\left|RBR_c^f\right|^2}}; RBR_c^f \equiv R_c^f \tag{S3}$$

In the above equation, *RBR* is the ratio between the evoked physiological response and the magnitude of on-going background activity (the factor 2 under the root in the denominator reflects the summation of the two polarities). We can therefore interpret this quantity as a measure of the sensitivity of the system to external perturbations. The PCI is then just the *RBR* but with its magnitude functionally mapped to the [0,1] interval.

The above response model (S1) and the assumptions related to it, are, although realistic, purely "ad hoc" at this stage. A more detailed response model of the neuronal dynamics underlying the PCI will be reported elsewhere.