Phase clustering in transcranial magnetic stimulation-evoked EEG responses in juvenile myoclonic epilepsy and migraine.

Running head: *Phase clustering in epilepsy and migraine.*

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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: To investigate EEG phase clustering in response to transcranial magnetic stimulation (TMS), compare it to photic stimulation in controls and explore its potential as a biomarker of juvenile myoclonic epilepsy or migraine with aura.

Methods: People with juvenile myoclonic epilepsy, 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 juvenile myoclonic epilepsy (five off medication, three on), 10 with migraine with aura and 37 controls. TMS and photic phase clustering spectra showed significant differences between 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 anti-epileptic 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 of brain network activity in epilepsy. Our results suggest that the higher propensity to phase clustering is not shared between epilepsy and migraine.

1. Introduction[1]

Epilepsy and migraine are paroxysmal conditions characterised by a temporary disruption of normal neurological function. Recurrent epileptic seizures are linked to hypersynchronous neuronal activity [1]. Migraine attacks are characterised 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 generalised epilepsy (ref de Goede + Brigo), which is characterised by myoclonus and generalised tonic-clonic seizures shortly after awakening. In children, JME is more often associated with migraine than are other types of epilepsy, such as absence epilepsy (Kelley *et al.*,

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[1] Abbreviations used in this article: TMS=Transcranial Magnetic Stimulation, EEG=electroencephalography, NNEI=Neural Network Excitability Index, PCI=phase clustering index, rPCI=relative phase clustering index

2012). People with JME are more than four times as likely to have migraine than people without JME (Schankin *et al.*, 2011).

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 Brigo *et al.*, 2012 for a review). Several studies show no difference in resting motor threshold between people with migraine and controls [11–15]. Combining TMS with EEG offers new options to assess cortical excitability, bypassing sensory and motor areas [16,17]. Previous TMS-EEG studies in epilepsy investigating TMS-evoked potential and the epileptiform EEG discharges triggered by TMS have identified aberrant excitability and connectivity [18–24] 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 (Del Felice 2011). 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, [17]. On a single electrode, the phase of TMS evoked responses align between trials shortly after the TMS pulse. Empirical evidence from computational models and *in vivo* studies suggests that a more excitable brain results in more synchronised phases in the higher frequency bands and reduced synchronisation in the lower frequency bands (8-14 Hz) (Saari et al., 2018). Measures of inter-trial phase clustering could thus be a measure of cortical excitability. One such measure, the relative Phase Clustering Index (rPCI), was successfully used to quantify the neural response to periodic photic stimulation in magneto-encephalography and to identify dynamic states leading to photoparoxysmal

responses in epilepsy (Parra et al 2003). In temporal lobe epilepsy, it was shown that high values of rPCI were correlated with the probability of occurrence of epileptic seizures (Kalitzin et al 2005). 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 (Wendling et al (2016)).

Icreased phase synchronisation in the gamma frequency range in the on-going EEG was linked to increased neuronal excitability in epilepsy [28]. 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 [29–32]. One study showed beta frequency desynchronisation in migraine with aura [33], potentially linked to hyperresponsivity of the sensory cortices [34].

We assessed whether phase clustering in the TMS-EEG response differs in people with JME compared to 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 [35].

2.1.2 Juvenile Myoclonic Epilepsy

Participants, diagnosed with 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 generalised 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 compulsory for the diagnosis of JME (Kasteleijn et al., 2013).

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 [36]. 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: contra-indications to TMS, pregnancy, any neurological condition other than epilepsy or migraine, any psychiatric condition, the use of medication affecting cortical excitability other than anti-epileptic 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 hours after a convulsive seizure and more than 72 hours after a migraine attack; sessions followed by a convulsive seizure within 24 hours and a migraine attack within 72 hours, identified at follow-up, were also excluded. Participants were asked not to smoke, take drugs or drink alcohol or coffee 12 hours 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 Transcranial Magnetic Stimulation

Magnetic stimulation was performed with a MagPro X100 stimulator (Magventure, Denmark), a 14cm diameter parabolic circular coil (type MMC-140), and a sham coil (type MCF-P-B65). Measurements were conducted at 09.00 AM or 02.00PM and spread evenly between AM and PM. No significant differences in TMS measures were reported between these times of day [37], except a larger TMS-evoked potential 100ms after the stimulus [38]. 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 Electroencephalography

EEG was recorded during the TMS sessions with a 64-channel TMS-compatible EEG system (Waveguard[TM] cap and ASAlab[TM] 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-Technil GmbH, Gelenau, Germany) was performed according to a standard clinical protocol: stimulation started at 2 Hz; followed by 10s runs of increasing frequency at 6, 12, 20, 30, 40, 50 and 60 Hz with eyes closed and open ($\pm$ 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 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 [39], 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 [40,41] The scanning procedure was performed using anticlockwise (right hemisphere) and clockwise (left hemisphere) stimulation as part of the artefact 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 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 [42]. In the 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

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

2.4.1 Removal of artefactual channels

For each subject, artefactual channels were automatically detected: for each channel, the norm covariance matrix was computed for the window -0.1 to 0s 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

EEG phase clustering analysis was previously described [25,26]. 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 100ms (corresponding to a base frequency of 1s / 100ms = 10Hz) starting 15ms after TMS- or sham stimulation (see also below regarding TMS artefact reduction) and without

delay (0ms) 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 6Hz 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).

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

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 Phase Clustering Index (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 =10Hz), 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 previous work [27] is determined by the PCI at the base frequency:

$$NNEI_c = \langle 1 - PCI_c^1 \rangle_c \qquad\qquad (3)$$

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

2.4.3 Time-Frequency Analysis

For TMS time-frequency analyses we used epochs of 1s (4000 samples), starting 0.5s before the magnetic stimulus to avoid convolution edge effects in the window of interest from 15ms to 115ms. The part of the signal containing TMS ringing artefacts (0–6ms after the stimulus) were cut. Cubic interpolation was used from 0-15ms around the stimulus to reduce muscle artefact contamination. The trials were baseline-corrected using a baseline window from -50ms - 0ms relative to the TMS stimulus. The time-frequency Wavelet components for frequencies between 8 and 50Hz were computed using Morlet wavelets with a width 5 for the window of 15ms to 115ms in steps of 5ms in order to gain sufficient temporal resolution for the low frequency content with adequate frequency resolution in the higher frequencies. Due to our window selection

of [-0.5:0.5 sec], we can compute the TF with the chosen cycle width for the window [15ms:115ms] without any border distortions.

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

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

where t is time. For the photic stimulation time-frequency analysis of the PCI, the interval of interest was an epoch of 167ms, with a mirror buffer of 500ms on each side to avoid convolution edge effects in the time-frequency analysis. De-trending 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 5ms. The PCI was again computed using equation 1A, and the results were averaged over all channels.

2.4.4 TMS and muscle artefact reduction

We included several strategies to reduce stimulation and muscle artefacts related to magnetic stimulation. First, equation (2) allows to cancel out broad-band artefacts, 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 15ms after the magnetic stimulation. The largest TMS and muscle artefacts are expected within the first 15ms after the stimulus. To ensure that our results are not due to muscle artefact contamination, the analysis was repeated for epochs starting at 20ms, 25ms, and 30ms relative to the TMS stimulus, with similar results. Only data from the final analysis with a window length of 100ms starting 15ms 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 off-line in a pair-wise fashion to compensate the linear component, containing the artefact, in the response to each polarity (eq. 4)[43]:

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

$F_{c,i}^{+}$ and $F_{c,i}^{-}$ are the response amplitudes to the clockwise and anticlockwise 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 artefacts (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 epilepsy and control group. In the migraine group, we compare TMS stimulation with sham stimulation.

2.4.5 Statistical analyses

We took the small sample size of the epilepsy (on and off medication) and migraine groups into account by using non-parametric, Monte Carlo-based statistics, which were shown to be robust in such small sample sizes [44]. For all statistical analyses, the off medication epilepsy group was compared with the first measurement of the

controls (8 stimuli per intensity), while the epilepsy on medication and the migraine group 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 [45] 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 64 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, anticlockwise, sham, polarity-compensated and photic stimulation 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.

3. Results

3.1 Participants

We included 38 controls (25 women, mean age 38.1yrs, 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 due to non-specific EEG abnormalities. From another control, we excluded the first measurement as it contained a large artefact 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 juvenile myoclonic epilepsy 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 anti-epileptic drugs at inclusion (E1-E5). Two were photosensitive (E3 and E4). Three were treated with anti-epileptic 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 due to 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 here report 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	Handedness	PS	last seizure	clinical features	EEG features	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 myoclinic 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 generalised 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

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

3.2 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.

3.3 Time and frequency characteristics of the Phase Clustering Index (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. To analyse the robustness of the biomarkers with respect to the montage, post-hoc analysis of the central electrode cluster was performed (Cz and neighbouring electrodes), which showed a difference between the first measurement of the controls and epilepsy off medication group (p= 0.016, see figure 1A for the cluster). Theoretically, electrode Cz should be least affected by artefacts elicited by the TMS stimulation with a round coil above the vertex, as the magnetic field has a 90 degree angle with the electrode, thus not affecting the electrode. This is why we chose Cz as the common reference electrode. The results of the Cz common reference montage were very similar to the common average reference montage results, indicating that the method was not dependent on the chosen montage. The visual evoked potential shown in fig. 2A did not differ between the control and epilepsy groups. Photic stimulation was not done in the migraine group.

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 epilepsy off medication group in the gamma frequency band (30-40 Hz) around 50 to 80ms. The PCI spectrum, in contrast, showed decreased PCI in the epilepsy off medication group 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 Eq. 2, as it the high frequency phase information takes into account. For photic evoked responses in contrast, Figure 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 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.

3.5 Relative Phase Clustering and Neural Network Excitability Indices for TMS and photic stimulation

To quantify the difference in PCI between the different groups, we used the *relative* phase clustering index (eq.2) and the neural network excitability index (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 epilepsy off medication group than in controls (p=0.023), while the NNEI showed a weak trend for being higher (p=0.147). The epilepsy off medication group 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 epilepsy off medication compared to controls. The rPCI and NNEI elicited by sham stimulation did not differ between

controls and the epilepsy groups. The rPCI and NNEI in the migraine group did not significantly differ from controls.

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

Table 4: median relative Phase Clustering Index and 5-95 percentile for all groups.

		controls(1)	Controls(2)	Epilepsy(-med)	Epilepsy(+med)	Migraine
TMS	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. PC=Polarity-compensated (age adjusted in the epilepsy groups only). Photic stimulation at 6Hz 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).

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 juvenile myoclonic epilepsy on and off medication compared to controls but not in migraine with aura. The rPCI elicited by photic stimulation was also increased in juvenile myoclonic epilepsy off medication compared to controls. In line with a recent study, we show that phase clustering of evoked responses may be a candidate biomarker to monitor cortical excitability [17], 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 which reported an elevated rPCI in photosensitive absence epilepsy; it increased gradually in the period preceding the occurrence of a paroxysmal response [25].

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 [27]. 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 [27]. 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 as after photic stimulation, we found lower phase clustering in lower frequency ranges (alpha and beta bands) and a higher NNEI in the off-medication epilepsy group compared to controls. Conversely, after TMS, we found increased phase clustering in

gamma range frequencies in the epilepsy without medication group 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 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 [46–48]. It was recently shown that diazepam, a GABA-A receptor agonist, increased TMS-induced alpha band synchronisation in healthy subjects [49]. 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 [50,51], and may facilitate phase clustering in the gamma range. In migraine, phase synchronisation in the alpha band following visual stimulation was increased [32]. 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 [52]. Our finding of high NNEI and reduced photic stimulation phase clustering in the alpha band in the epilepsy group 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 [25]. 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 [10,18,53–56]. 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 (30-40Hz), consistent with previous findings [17]. Artefacts elicited by TMS stimulation (muscle and stimulation artefacts) can also occur in the gamma frequency range. TMS-induced muscle artefacts usually peak around 7ms and return to baseline around 15ms [57]. We therefore analysed the rPCI in epochs which theoretically start after or at the tail-end of the muscle artefact and repeated the analysis for windows starting at 20, 25 and 30ms without changing the results. We introduced several novel strategies to reduce artefacts. Firstly, the rPCI analysis (eq 2) corrects large stimulus-locked artefacts. NNEI is, however, still affected by these artefacts. 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 epilepsy compared to controls represent a neuronal process rather than a measurement artefact.

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 straight-forward.

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 localisation, but in a design with image-guided focal magnetic stimulation in focal epilepsy, the rPCI may potentially help localise cortical areas with aberrant inhibition. Image-guided focal magnetic stimulation was previously successful in localising cortical areas connected to subcortical heterotopic grey matter in periventricular nodular heterotopia using the TMS-evoked potential [22].

The phase clustering measures reported here, are obtained from the TMS-triggered responses per channel over stimulation trials. We did not address phase clustering *between* EEG channels, which is often associated with the concepts of post-stimulus *spatial* synchronisation or desynchronisation (Gordon et al., 2018). The inter-trial phase clustering decays shortly after the TMS stimulus, with clustering at higher frequencies decaying faster than at low frequencies. We have studied the response up to 750 ms after the stimulus. In our data, there is 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).

Limitations of our study include the small sample size in the epilepsy and migraine groups and the need to optimise the stimulation protocol for the analysis of phase clustering. Repetitive magnetic stimulation can alter cortical excitability, and 5Hz, but not 0.5Hz stimulation, significantly increased the Motor Evoked Potential [41]. A

subsequent study did show a small inhibitory effect of 0.5Hz stimulation, especially during the first 20 stimuli [58]. Others showed that the MEP amplitude increased after 200 TMS pulses given every 4s [59]. Only one study investigated the effect of 15-minute trains of 0.6Hz stimulation on the EEG and found a significant increase of the N45 amplitude [60]. Our choice for a ramped stimulus-response curve with an interstimulus interval of 0.5Hz was based on the fact that stimulus-response curves were shown to be invariant to interstimulus intervals from 1.4 to 4s [61], and that there was no difference between stimulus-response curves acquired with a ramped (increasing) or random stimulation intensity order [62]. Several studies have shown the effect of stimulation intensity on the EEG response, such that a cortical excitability threshold could be measured [17,63]. 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 [64]. Our measurements were conducted at 9AM or 2PM. No significant differences in TMS measures were reported between these times of day [37], except a larger TMS-evoked potential 100ms after the stimulus [38]. We did not find a difference in rPCI between the people measured at 9AM and those measured at 2PM. Cortical excitability was also shown to change between, before and after epileptic seizures [65–67] and migraine attacks [11]. 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 [25]. 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 anti-epileptic therapy from non-responders.

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

Potential conflicts of interests

Supplementary information 1
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 artefacts 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 A_α if the
stimulation current is matched exactly for both polarities.
Inserting the response model (S1) into the combined, polarity-compensated amplitudes
in   eq (4) of the main text, the first term from (S1) cancels.

Note that the norm in the denominator in Eq (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 [25,26]. 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 Eq (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 :

$$PCI_c^f = \frac{RBR_c^f}{\sqrt{1+\left|RBR_c^f\right|^2}} ; RBR_c^f \equiv R_c \qquad (S2)$$

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.

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