The exciting migraine brain: towards neurophysiological prediction of migraine attacks

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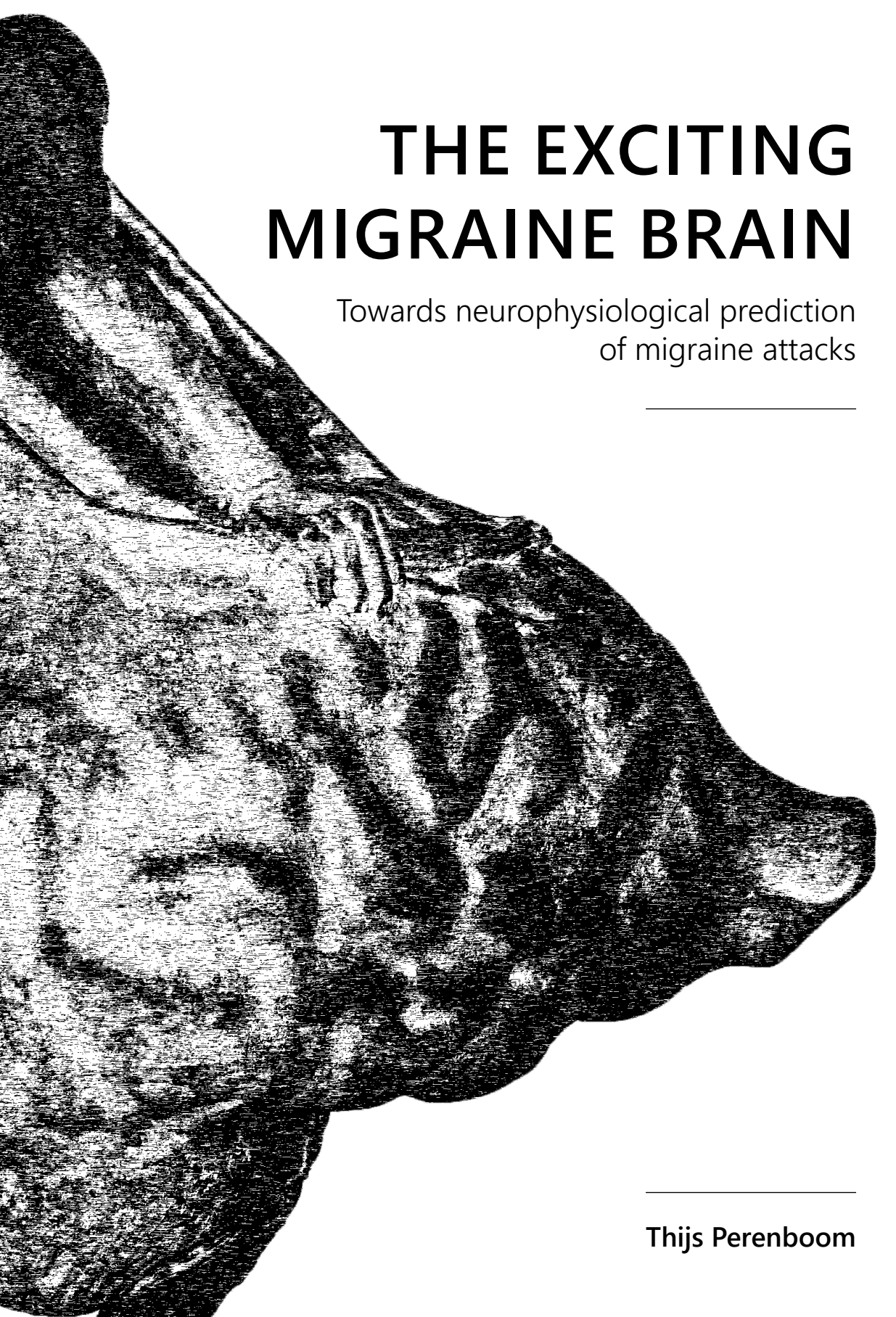

THE EXCITING MIGRAINE BRAIN

Towards neurophysiological prediction of migraine attacks

Thijs Perenboom

The exciting migraine brain

Towards neurophysiological prediction of migraine attacks

Matthijs Johannes Lambertus Perenboom

Colophon

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The exciting migraine brain

Towards neurophysiological prediction of migraine attacks

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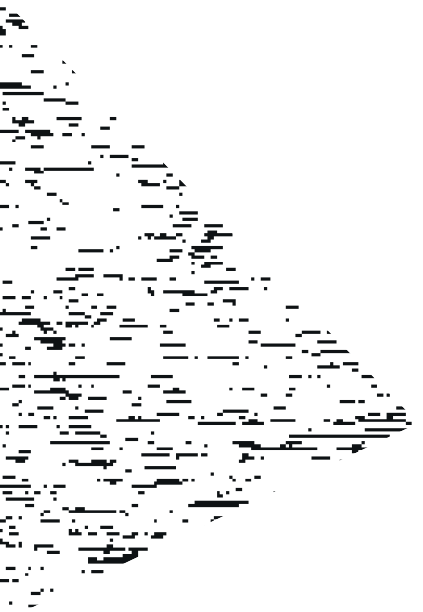
General introduction



Migraine

Migraine is a common, disabling brain disorder, characterized by recurrent attacks of headache associated with nausea and/or vomiting and hypersensitivity to sensory inputs like light (photophobia) and sound (photophobia).[1] Migraine attacks by definition last between 4 and 72 hours, and present with severe, often unilateral, pulsating headache.[1] Migraine is divided in two main subtypes: migraine without aura and migraine with aura; in about one-third of migraine patients, the headache is for the majority of attacks preceded by transient neurological symptoms (the migraine aura), mostly consisting of visual disturbances but sometimes also concerning motor or speech impairments.[2] The prevalence of migraine is different between men and women; in western countries, at least 12% of the general population suffers from recurrent migraine attacks, of which more than two-thirds are women mainly in the age of 35-50 years.[3] The worldwide impact of migraine on the quality of life is substantial,[4] making migraine the second leading cause of years lived with disability.[5] The median attack frequency is 1-2 per month and the median attack duration is one day; at least 10% of the patients have weekly attacks of 2-3 days each. Since the last revision of the International Classification of Headache Disorders,[1] migraine is divided into two subgroups based on the number of monthly migraine days and monthly headache days: patients with *episodic migraine* have fewer than 15 days of headache per month, patients with *chronic migraine* experience at least 15 headache days per month of which at least 8 migraine days.[6] Treatment of migraine remains challenging, with both acute and prophylactic treatment seldom resulting in complete remission of symptoms and often causing bothersome physical and cognitive side effects.[7] Ineffective treatment of episodic migraine using acute medication could even lead to chronification of migraine.[8]

Patients with migraine have an increased risk to develop comorbid disorders like depression and epilepsy, and vice versa.[9,10] Not only in adults,[11] but also in children with juvenile myoclonic epilepsy, migraine was more prevalent than in the general population.[12] Some antiepileptic drugs are also effective in the treatment of migraine,[13] in particular those that act by reducing neuronal excitability, suggesting shared mechanisms involving network hyperexcitability underlying attack initiation in migraine and epilepsy.[13,14] Also, mutations that underly a rare monogenic form

of migraine can cause epilepsy,[15] which can be modelled in animals and has indicated hyperexcitability as key mechanism.[16] Lastly, shared features of cortical excitability that were identified in people with migraine or epilepsy[17] further point to shared disease and treatment mechanisms involving disturbances in neuronal network excitability.

The combination of the unpredictable recurrence of migraine attacks, the migraine-related complaints in between and during attacks, side effects of migraine drugs and migraine comorbidities contribute to a substantial burden to people with migraine. While various clinical studies investigated whether in migraine functional processing in brain networks including the cortex is altered, no consistent results were obtained. With respect to structural abnormalities, only minor changes were found, in particular for migraine with aura.[18] Both clinical and experimental studies of migraine are hampered by the fact that clinical symptoms in migraine are largely subjective, which underscores the need of a reliable biological marker of migraine susceptibility, and clinical animal models of migraine with recurring attacks.

Phases of the migraine attack

Migraine is a cyclical disease, where the headache occurs in episodes. Traditionally, four different phases are distinguished in a migraine attack.[19]

The premonitory, or prodromal, phase is characterized by a variety of symptoms; the most frequently reported premonitory symptoms are fatigue, weariness, phonophobia, yawning, stiff neck, gastrointestinal symptoms, mood and cognitive changes, temperature change, smell and taste distortion, and food craving.[20,21] The duration of the prodromal phase varies amongst patients and ranges from a few hours until one to two days.[22]

The aura phase occurs in about one in three migraine patients, and is characterized by transient neurological aura symptoms and ranges from 5 until 60 minutes.[19] The aura phase usually precedes the headache phase, but could also overlap with the start of the headache phase.[23] The visual aura is the most prevalent type of aura, with symptoms varying from simple flashes, lights to fortification scotoma ('zig-zag

patterns') or complex hallucinations. Other aura symptoms such as sensory, motor or speech disturbances rarely occur without coexisting visual disturbances.[2]

The headache phase is characterized by a moderate to severe, often unilateral throbbing headache, accompanied by nausea, vomiting and photo- and phonophobia, that typically lasts between 4 and 72 hours when no rescue medication is used. The headache can be aggravated by mild physical activity, and many patients require bedrest during an attack.[24]

The post-headache phase (also called postdromal or recovery phase) is typically characterized by decreased cognitive functioning, mood changes, drowsiness and tiredness. The postdromal phase is present in most migraine patients and can last from several days to one week.[25]

In addition to the symptoms related to the attack, migraine patients suffer from various types of complaints in periods between attacks, such as enhanced sensitivity to light.[26] Thus, the clinical manifestations of migraine are not limited to the headache episodes, which makes migraine a disease with a fluctuating representation of symptoms of which headache is only one.[27] The start of the 'migraine attack' remains elusive, and has been suggested to represent a tipping point in brain dynamics.[28] Any technique and readout parameter that can reliably indicate and/or explain the mechanisms underlying the onset of a migraine attack, opens up a new possibility for studies into migraine attack prediction and prevention. In this thesis, we focus on the development of a 'toolbox' of methods and paradigms aimed at identifying functional markers that can help predict an impending migraine attack.

Migraine pathophysiology

Although the pathophysiological mechanisms for the different phases of a migraine attack are extensively studied in experimental models, little is known about how and why attacks actually begin in a patient.[19] Several mechanisms seem to contribute to migraine attack susceptibility, each with a distinct time scale (Figure 1). First, genetic predisposition, that may cause dysfunction of ion channels or transporters and subsequent neuronal hyperexcitability, can underlie lifelong disease

susceptibility.[29] Second, fluctuating factors like stress and relaxation, circadian and hormonal rhythms that all influence brain activity may temporarily increase the susceptibility to develop an attack.[30,31] Finally, patient-specific attack triggers – such as certain types of food, or extensive exercise – could be an additional mechanism leading to the migraine attack onset.[22,32]

The genetic component of migraine has evidence in the hereditary predisposition demonstrated in family and twin studies, and population research.[33] Large genome-wide association studies indicated the involvement of multiple gene variants in the susceptibility to migraine, which (besides several variants linked to vascular function) include variants in genes associated with ion channel activity.[33,34] Genetics studies on the rare migraine form of (familial) hemiplegic migraine (FHM) identified three causal genes: *CACNA1A* (FHM1), *ATP1A2* (FHM2), and *SCN1A* (FHM3), which all encode proteins that affect ion activity in the brain.[9] At the cellular level, as shown by mouse studies for the cortex, a consequence of each of the three FHM mutations is that the release and concentration of the excitatory neurotransmitter glutamate in the synaptic cleft is enhanced, leading to increased neuronal excitability.[9,16]

The migraine headache is preceded by the premonitory phase, which is hypothesized to start in the hypothalamus.[19,27] Already before the presence of headache, neuroimaging demonstrated specific activation of the hypothalamus.[36] Common premonitory symptoms such as tiredness, yawning and concentration problems, and often reported migraine attack triggers like lack of sleep, stress and food deprivation are likely under control of the hypothalamus.[19] Also, several of the fluctuating factors involved in attack susceptibility, like circadian and hormonal rhythms, point toward hypothalamic involvement in the onset of a migraine attack.[27]

Visual aura symptoms are most likely caused by the phenomenon of cortical spreading depolarization (CSD).[9] Based on experimental studies in rodents, CSD is a propagating depolarizing wave of electrophysiological neuronal and glial hyperactivity, followed by depression of the formerly hyperactive neurons. When induced in the occipital (visual) cortex, CSD spreads frontally across the cortex. The speed of the depolarizing wave front is about 2–4 mm per minute, while the

subsequent neuronal depression might last several minutes to an hour.[37,38] The variety in the presentation of aura symptoms correlates with different cortical activation patterns, as demonstrated by neuroimaging.[39] Direct measurement of CSD during the aura phase is notoriously difficult. First, because of the unpredictability of attacks, and second, because the slow DC-features characteristic of a CSD are difficult to reliably identify from scalp EEG.[40,41] Using blood oxygen level-dependent (BOLD) neuroimaging as an indirect brain activity measure during the visual aura phase of migraine patients, brain activation patterns reflecting the predicted spreading and depolarizing nature of CSD were observed.[42] Using magneto-encephalography, a comparison of neurophysiological activity features during an induced (by visual pattern stimulation) and spontaneous visual aura with brain activity during rest showed spreading potential shifts indicative of CSD in the occipital cortex.[43]

Visual aura symptoms are most likely caused by the phenomenon of cortical spreading depolarization (CSD).[9] Based on experimental studies in rodents, CSD is a propagating depolarizing wave of electrophysiological neuronal and glial hyperactivity, followed by depression of the formerly hyperactive neurons. When induced in the occipital (visual) cortex, CSD spreads frontally across the cortex. The speed of the depolarizing wave front is about 2–4 mm per minute, while the subsequent neuronal depression might last several minutes to an hour.[37,38] The variety in the presentation of aura symptoms correlates with different cortical activation patterns, as demonstrated by neuroimaging.[39] Direct measurement of CSD during the aura phase is notoriously difficult. First, because of the unpredictability of attacks, and second, because the slow DC-features characteristic of a CSD are difficult to reliably identify from scalp EEG.[40,41] Using blood oxygen level-dependent (BOLD) neuroimaging as an indirect brain activity measure during the visual aura phase of migraine patients, brain activation patterns reflecting the predicted spreading and depolarizing nature of CSD were observed.[42] Using magneto-encephalography, a comparison of neurophysiological activity features during an induced (by visual pattern stimulation) and spontaneous visual aura with brain activity during rest showed spreading potential shifts indicative of CSD in the occipital cortex.[43]

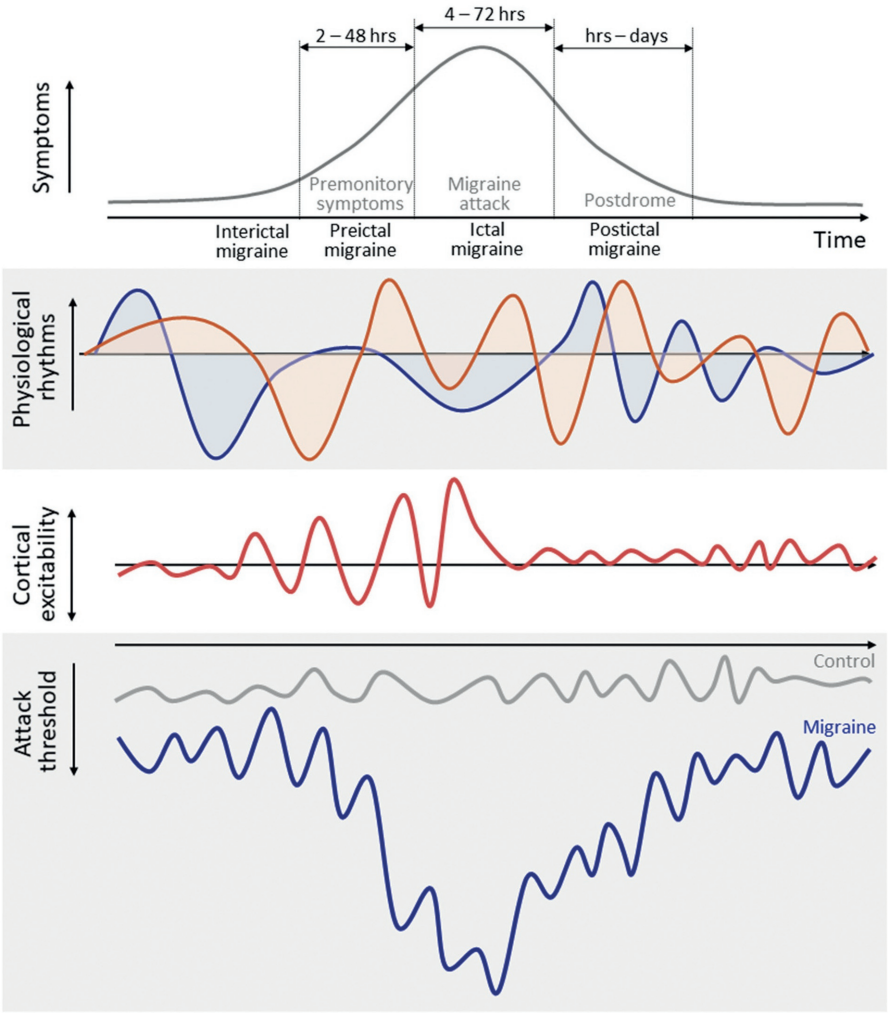


Figure 1. Migraine is a paroxysmal disorder, characterized by unpredictable recurrent attacks of headache and associated symptoms. The susceptibility to develop an attack is likely affected by the periodicity of several physiological rhythms (e.g., circadian, hormonal) and external factors (e.g., food intake, stressors) as well as a lower overall attack threshold due to genetic predisposition. Cortical excitability varies over the migraine cycle, and is likely affected by the underlying physiological rhythms as well as the acute impact of external factors on brain activity. The start of an attack might thereby be caused by the combination of patient-specific 'migraine triggers' in combination with a lowered threshold. (Figure based on Stankewitz & May (2009)[30] and Peng & May (2019)[35])

The migraine headache is thought to result from activation of the trigeminovascular system, which involves dural nociceptive trigeminal afferents from trigeminal ganglion sensory neurons, and brainstem centers and thalamocortical areas involved in processing head pain.[19] The neurological symptoms that accompany a migraine headache, like photophobia, phonophobia and allodynia, appear to be the result of sensitization of neurons in and around the thalamus.[19,44] Which factors underlie the activation of the trigeminovascular system during a spontaneous migraine attack remains an enigma. Based on experimental studies, several factors can activate the trigeminovascular system at the dural level. These factors include the build-up of diffusible substances such as extracellular K^+, release of vasoactive mediators such as calcitonin gene-related peptide (CGRP)[9] as well as inflammatory mechanisms.[19,45] In animal experiments, CSD was demonstrated to be capable of activating the trigeminovascular system,[46] involving a neuroinflammatory response leading to activation of trigeminal afferents.[47] In addition or alternatively, CSD may also directly contribute to headache initiation via cortico-trigeminal projections.[19] In migraine without aura, where the presence of CSD is not indicated as in the aura phase, a 'silent aura' in subcortical brain regions might trigger the headache phase.[44] Also in the absence of a spreading depolarization, however, it is possible that overall hyperexcitability in neurons within the trigeminovascular system might lower the threshold for activating head pain pathways resulting in an attack.

Visual sensitivity in migraine

Multiple symptoms related to the migraine attack are linked to the visual system. For instance, visual stimuli such as flashing lights or striped patterns are commonly reported by patients to be triggers of a migraine attack.[48,49] Interictally, migraine patients with and without aura reported more optical illusions when looking at striped patterns, the so-called 'pattern glare', in comparison to healthy controls,[50] and also moving imagery was processed differently by migraineurs.[51] During and in between attacks, more than half of migraine patients experience enhanced sensitivity to light, i.e. 'photophobia'.[26] Photophobia is one of the diagnostic criteria of migraine, not only in migraine with aura but also in migraine without aura.[1] Lastly, the migraine aura, when present, is almost always at first visual.[52]

Hyperexcitability of the visual cortex is suggested to underlie the visual sensitivity,[53] and may also prime the initiation of CSD.[18]

In between attacks, the visual cortex of migraine patients showed a more pronounced response, as measured by position emission tomography (PET), to stimulation by light compared to controls, that was even further enhanced by additional painful heat stimulation.[54] Such enhanced responsivity may be even more specific for migraine with aura patients, indicated by an increased BOLD activity in response to visual stimulation.[55] With similar study methods, it was shown that in migraine with aura, visual cortex activation also correlated positively with visual discomfort and photophobia.[56] In the pre-ictal phase, patients experiencing photophobia showed a larger enhancement of visual cortex activity (measured by PET) compared to patients with no photophobia symptoms.[57] During the attack, low intensity light stimulation which did not activate the cortex interictally induced measurable increases in BOLD activation of the visual cortex, and, to a lesser extent, also after headache relief.[58]

Several pathophysiological processes might underlie photophobia and other forms of visual sensitivity.[44] Visually-impaired migraine patients, who were capable of detecting light but had severe retinal rod-cone degeneration, still reported exacerbation of the migraine headache by light.[59] This led to the discovery, in rats, of dura-sensitive thalamic neurons that are indirectly responsive to light via input received from photosensitive retinal ganglions cells containing the photoreceptor melanopsin. These dura-sensitive and light-responsive thalamic neurons project further towards somatosensory and visual cortices, thereby representing an integration of light and painful stimuli (Figure 2).[59] In migraine patients, specific colors of light were found to either enhance (blue, red) or decrease (green) the headache pain, and visual flash stimulation with green light resulted in significantly smaller responses than stimulation with red and blue light flashes.[60] In rats, the light-responsive thalamic neurons were driven to a lesser extent by green compared to white, blue and red light, pointing towards an important role of the trigeminovascular system in visual sensitivity.[60] In addition, reversal of photophobia has been reported in several studies on efficacy of migraine drugs. Drugs that are able to abort migraine headache usually reverse photophobia, which suggests a shared mechanism involving activation of the trigeminovascular system.[61]

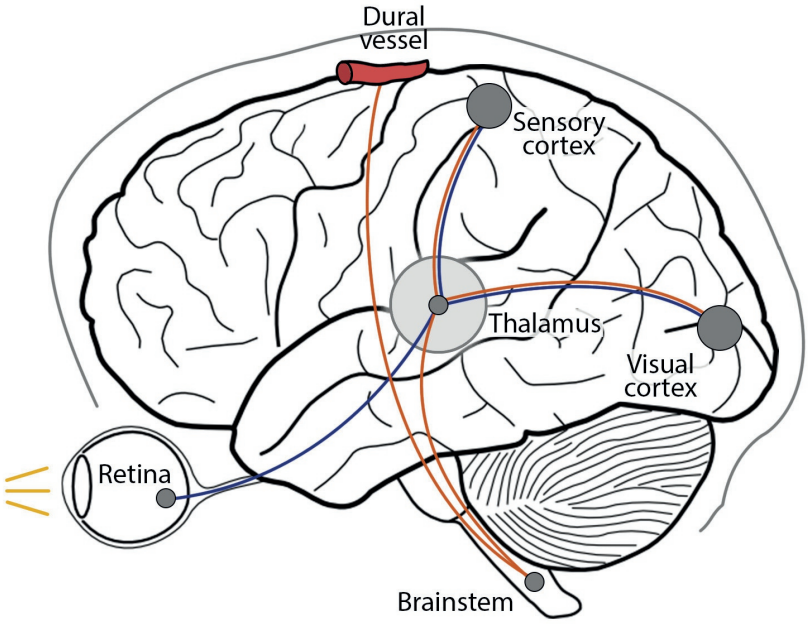


Figure 2. Neuronal pathways processing responses of the retina to light converge onto pathways processing pain signals from the dura towards the thalamus. This convergence proposedly worsens the migraine headache by projection of these thalamic neurons to somatosensory cortices involved in pain perception. In a similar way, enhanced sensitivity to light during a migraine attack might result from the same convergence, as thalamic neurons also project to visual cortices involved in the perception of light. (Figure based on work by Noseda et al. (2011)[61])

Cortical excitability in migraine

An important hypothesis concerning mechanisms underlying migraine is the theory that the excitability of the cortex is specifically enhanced,[30,53] which may be most pronounced for the visual cortex given the often visual nature of the aura.[42] As for the rest of the brain, cortical excitability is affected by factors that influence neuronal function such as the balance in neuronal ion concentrations and the functioning of neuronal and astrocytic ion channels or transporters.[62] Intrinsic enhancement of neuronal excitability in migraine is likely to have a genetic basis, which is evident for the FHM mutations that are linked to dysfunctional ion channels which cause a

disturbed balance between glutamatergic and GABAergic transmission.[18,63] When measured using magnetic resonance spectroscopy, indeed enhanced glutamate levels,[64,65] and elevated levels of GABA have been measured in the brain of migraine patients.[66]

Several approaches have been developed to study in which way abnormal excitability of the cortex may play a role in migraine and the initiation of attacks, using neurophysiological, neuroimaging, metabolomic and animal model approaches.[53]

The FHM1 mutant mouse model to study cortical hyperexcitability in migraine

Animal models are valuable tools to explore mechanisms of cortical dysfunction in the context of migraine. Translational approaches include the introduction of human pathogenetic mutations, such as the mutations in the three FHM genes *CACNA1A*, *SCN1A* and *ATP1A2*, and investigating the neuronal, network and behavioral consequences in transgenic mouse models.[16,29] FHM1 mice displaying the gain-of-function missense mutation R192Q in the *Cacna1a* gene encoding a subunit of neuronal voltage-gated $Ca_V2.1$ Ca^{2+} channels show enhanced susceptibility to experimentally induced CSD.[67,68] This CSD susceptibility could be explained by an enhanced glutamatergic transmission resulting from the effect of the R192Q mutation on increasing presynaptic calcium-influx in glutamatergic neurons.[69] At the morphological level, FHM1 mutant mice display altered axonal and dendritic morphology in the sensorimotor cortex, with larger axonal boutons and a higher percentage of highly excitable mushroom-type dendritic spines, which are densely populated with excitatory NMDA receptors compared to wild-type, suggesting stronger and more excitable synapses.[70] With respect to modelling effects of migraine triggers, the CSD susceptibility in FHM1 R192Q mutant mice (but not in wild-type controls) was specifically enhanced by an acute administration of the stress hormone corticosterone,[71] possibly resembling patient reports of stress as attack trigger.[72] Also, in line with clinical photophobia symptoms in patients,[73] FHM1 mutant mice showed signs of light aversion that could reflect an increase in visual system responsivity.[74]

Neurophysiological techniques to investigate cortical excitability

Electroencephalography (EEG) is the measurement of electrical activity generated by neurons firing action potentials. With electrodes on the scalp, several cortical rhythms are distinguished, which are related to sleep stages and levels of arousal; these rhythms are named delta (< 4 Hz), theta (4–7 Hz), alpha (8–13 Hz), beta (14–30 Hz) and gamma (> 30 Hz).[75] The activity levels in those frequency bands, as well as the relationship between activities in different frequency bands (cross-frequency coupling) or between different regions of the cortex (connectivity analysis), are studied as possible indicators of neurological disease presence or severity.[76–78] EEG has an excellent temporal resolution (milliseconds) while the spatial resolution is, due to filtering effects of the skull, low compared to neuroimaging methods like magnetic resonance imaging or position emission topography. However, when compared to those techniques, EEG is much more easy and quicker to apply in both clinical and research setting.[79] With automated parameter extraction like peak amplitude or band power, a blinded EEG analysis of different groups is possible, circumventing the large inter-observer variability present in traditional EEG analysis with visual determination of those parameters.[80]

External perturbations of the brain using different modalities (e.g. visual, auditory or somatosensory stimuli) generate synchronized electrical activity that can be recorded as evoked potentials using scalp EEG. Altered neurophysiological activity in response to perturbations could possibly be used as biomarker of migraine type (with or without aura) or as predictor of an impending migraine attack.[28,81] Due to its ease-of-use and excellent temporal resolution, EEG is a useful tool to study the effect of such perturbations in migraine patients.

Quantitative EEG changes in migraine patients

The study of EEG rhythms in the absence of any external perturbation ('resting-state') is named quantitative EEG. Early studies (reviewed by Sand[82]) remained inconclusive regarding the effect of migraine and migraine phase on e.g. peak frequency, band power and hemispheric symmetry.[82,83] More recently, reduced alpha power in the occipital cortex for migraine without aura was reported.[84] Another resting-state study demonstrated, in the interictal phase for patients with migraine without aura, reduced EEG power and reduced coherence in the delta,

alpha, beta and gamma bands, except for an increased delta, alpha and beta connectivity in the fronto-occipital network.[85] In a group of patients with migraine, an increased theta power in all cortical regions, and increased delta power in the fronto-central region was demonstrated; this effect was more pronounced in patients without compared to with aura.[86] Overall, results appear to vary depending on the parameter that is derived from the EEG recordings, which indicates that quantitative EEG parameters are inadequate as reliable readout of cortical excitability.

Visual evoked potential changes in migraine patients

The functioning of the visual system including the cortex can be studied non-invasively by recording the electrophysiological response to visual stimulation to the eye, the so-called visual evoked potential (VEP), using scalp EEG. The VEP represents the summation of electrical potentials recorded over the scalp, mirroring the neurophysiological activation along the visual pathway from retina up to the visual cortex.[79] Visual stimulation can be presented to people using flashes of light or patterned stimuli such as a shifting black-and-white checkerboard-like pattern. Besides application to human subjects, the VEP response can be used to study visual system functioning in animal models, for instance in freely-behaving mice, allowing invasive EEG recordings to study the evoked responses also locally within the cortex.[87]

The averaged EEG response to multiple transient stimuli (the 'single VEP') consists of multiple positive and negative peaks in the EEG trace, of which the amplitude and latency have been studied as potential markers of altered cortical excitability in migraine.[88,89] Lack of habituation to repeated visual stimulation has long been considered a hallmark of altered cortical excitability in migraine.[90] Whereas healthy controls showed a decreasing VEP response after about 600 repeated stimuli, the VEP response of migraine patients remained stable.[79,91] However, lack of repeatability of those results in studies with a blinded study design challenged the concept of 'lack of habituation' as migraine biomarker.[80,92]

A repetitive visual stimulus presented at a frequency above ~3.5 Hz generates a stationary ('steady-state' or 'photic drive') neurophysiological response consisting of the stimulation frequency and multiples of the stimulation frequency (harmonics) in the EEG signal. Enhanced photic drive for several stimulation frequencies (but

mainly between 10 and 30 Hz) was reported interictally for migraine, at the stimulated frequency[93–95] or at harmonic frequencies.[96,97] Combining multiple frequencies in one stimulation paradigm, by presenting light flashes at increasing frequency (the so-called 'chirp' stimulation) showed an interictal increased EEG response for 18–26 Hz stimulation, but not above or below those frequencies.[98] With similar stimulation frequencies, the synchronization and connectivity of EEG responses showed different responses in migraine with versus without aura, with specifically increased cortical activation in patients with aura.[99]

Alterations in responsivity to visual stimulation are considered to be related to alterations in cortical excitability, but it remains debated whether changes are caused by hypo- or hyperexcitability.[100,101] EEG activity reflects the summation of a population of inhibitory and excitatory neurons, often obscuring the underlying pathophysiological processes.[101] In addition, by using VEPs not only cortical but also subcortical responses, and their interactions, are measured. More direct measures of cortical excitability are therefore required to provide direct insight in changes in cortical excitability in migraine.

Transcranial magnetic stimulation as a tool to study cortical excitability

Another way to study excitability of the (visual) cortex is by directly exciting cortical neurons with a magnetic pulse over the scalp, a method known as transcranial magnetic stimulation (TMS), while measuring the response in muscular activity using electromyography (EMG) or cortical activity using EEG.[102] The magnetic pulse activates neurons to a depth of up to 2 cm below the skull, so the technique is limited to influencing activity of superficial layers of the cortex. Focal stimulation is achieved using a figure-of-eight coil, while the use of a circular coil activates the cortex more diffusely.[103]

When applied over the motor cortex, triggered motor cortical neurons subsequently activate spinal motor neurons resulting in a motor evoked response (MEP) that is measurable by EMG.[104] While the peaks and latencies of the MEP are highly variable within and between participants, the lowest stimulation intensity at which an MEP is induced – the resting motor threshold (RMT) – is often used as a reflection of motor cortex excitability.[17] Interictally, patients with migraine with and without aura, as well as patients with familial hemiplegic migraine, showed similar RMT

values compared to controls.[90,105,106] However, when using stepwise increasing stimulation intensities (a so-called input-output curve), enhanced MEP responses were seen in patients with migraine with and without aura, specifically for higher stimulation intensities, indicative of motor cortex hyperexcitability.[107,108]

Applying TMS over the visual cortex results in the induction of magnetophosphenes, which are visually perceptible flashes and patterns of light and color. The minimum stimulation intensity necessary to induces phosphenes is inversely related to the level of visual cortex excitability,[109] and could as such be used as subjective marker of hyperexcitability.[110] Combining several studies in a meta-analysis, it appears the phosphene threshold was reduced in migraine with and without aura with circular coil stimulation, whereas focal stimulation only demonstrated an increased phosphene prevalence in migraine with aura.[111]

As both cortical and subcortical pathways contribute to the MEP, the RMT is not a direct measure of motor cortical excitability,[112] and TMS-evoked magnetophosphenes represent a subjective readout. To measure cortical excitability objectively or in behaviorally silent areas (i.e., without measurable motor or visual response), TMS can be combined with EEG.[113] The TMS-evoked potential (TEP) consists of several positive and negative peaks which are reproducible within participants.[114,115] In healthy subjects, distinct peaks have been assessed to specifically reflect network activity influenced by excitatory or inhibitory networks based on observed effects of neuroactive drugs.[116,117] In conditions with implied altered cortical excitability like epilepsy[118,119] and schizophrenia,[120] TEP responses were demonstrated as possible biomarker of changes in cortical excitability. In migraine, the combination of TMS with concurrent EEG has not yet been described.

Changes in cortical network excitability over the migraine cycle

Surprisingly, there have been relatively few studies on the development of parameters reflecting the onset of migraine attacks. Any technique that can reliably predict or explain the mechanisms behind attack onset in migraine opens up new targets for studies into migraine attack prevention. To be able to study fluctuations

in cortical excitability over the migraine cycle, ideally the same patients are each monitored at multiple timepoints. To circumvent the difficulty of predicting the migraine attack, some studies have focused on multiple randomly timed measurements in the same patients, which afterwards could be timed to the nearest attack.[121,122] Another method is to study female patients with periodic menstrual migraine, in whom attacks often coincide with the menstrual phase.[123,124]

One of the earliest attempts to study neurophysiological changes before a migraine attack used a slow cortical potential change, the contingent negative variation (CNV) that is evident in EEG recordings following a 'warning' sound and subsequent 'test' sound followed by a motor response (button press). During the 24-48 hours prior to the onset of a migraine attack, an increase in the characteristic negative EEG amplitudes of the repeated CNV had a predictive value for attack onset.[125] Another study confirmed this finding, and uncovered that the differences in CNV amplitude and habituation over repeated stimuli was at its maximum compared to non-migraineurs in a window of 24 hours prior to the attack.[126] Using TMS-EMG, in the 48 hours before an attack, repeated motor responses to TMS were found to be facilitated suggestive of enhanced network excitability and predisposition to attack triggers, whereas during and in the 48 hours after the attack the responses showed a pattern of suppression suggestive of enhanced network inhibition.[127] In line with these findings, the photic drive to visual stimulation at 12 Hz increased in migraine patients in the same 48-hour time window before an attack.[128] Using resting-state EEG, increased hemispheric asymmetry (alpha and theta bands) and decreased (alpha and theta) or increased (delta band) EEG power demonstrated that 36 hours, but not 72 hours, before an attack migraine patients showed altered brain activity compared to controls; those differences are proposed to be indicative of fluctuating cortical activity over the migraine cycle, possibly caused by thalamocortical dysfunction.[129]

In patients with periodic menstrual migraine, an increased EEG power in delta and theta frequency bands, increased alpha asymmetry and enhanced early CNV amplitude were observed in the 1-4 days before an attack, when compared to measurements in the same patients after the attack.[122] In a neuroimaging study of migraine patients (with and without aura), an increased BOLD activity in the spinal trigeminal nuclei during nociceptive stimulation is in line with a rise in excitability

towards the next migraine attack, also in subcortical regions.[130] Expanding on this work, when a patient with migraine without aura was examined over 30 consecutive days, clear cyclical patterns of increased BOLD activity in the visual cortex following painful stimulation was visible before and immediately after the three captured migraine attacks.[36] Again, the response to nociceptive stimuli was specifically enhanced 24 hours prior to the headache onset, and normalized after the attack. These cortical and subcortical changes in brain activity were proposed to reflect an increased susceptibility of the migraineous brain to precipitating factors and the neurophysiological readiness to generate an attack.[36,122,130]

Subjective complaints in the phase preceding migraine attacks have also been studied to help predict attacks. A lot of clinical studies have assessed these premonitory symptoms,[20,21] and some studies tried to predict a migraine attack based on detection of premonitory symptoms.[131] Nevertheless, none succeeded at finding a premonitory parameter that was reliable and precise enough to be used as a predictive indicator for the onset of migraine attacks. Therefore, further research is needed to identify easy-to-use and reliable predictive readouts for migraine attacks.

Scope and outline of this thesis

The aim of this thesis was to investigate the initiation phase of migraine attacks based on neurophysiological outcome parameters. The main focus of the work was on the development of methodologies to measure cortical excitability dynamics over the migraine cycle. The research described in this thesis is divided into two parts. **Part I** describes the development of measurements of visual cortex excitability in migraine patients and a transgenic migraine mouse model. We developed a questionnaire to assess visual allodynia in patients, and combined preclinical and clinical studies to develop several visual stimulation paradigms in combination with EEG measurements. **Part II** describes applications of transcranial magnetic stimulation (TMS) with concurrent EEG recordings in people with migraine or epilepsy. TMS-EEG measures the direct neuronal response to stimulation over the whole cortex, rather than the indirect activation of the visual cortex in response to visual stimulation. We studied the TMS-evoked potential and phase clustering of those potentials as possible disease biomarkers.

Part I: Visual system excitability as migraine attack predictor

Chapter 2 describes the development, validation and application of the 'Leiden Visual Sensitivity Scale' (L-VISS), a questionnaire to assess visual allodynia. This tool has potential use in longitudinal assessments of a patient's sensitivity to light and patterns, as it is quick to apply and not dependent on any recording technology. Besides, it could be used in conjunction with more elaborate (e.g., neurophysiological) recordings to provide a personalized (subjective) assessment of changes in cortical excitability over the migraine cycle.

Chapter 3 describes the translational application of common and newly developed visual stimulation paradigms during EEG recordings in a migraine mouse model. To bridge the gap between measurements of surface EEG in patients and direct neuronal network measurements in animal models, we studied the effect of the FHM1 missense mutation R192Q, that leads to enhanced glutamatergic neurotransmission, on visual cortex responsivity using visual evoked potential recordings.

In **Chapter 4,** we explore the use of the 'chirp' visual stimulation to measure cortical excitability in migraine patients. Using light flashes at increasing stimulation frequency, chirp stimulation allows comparison of responsivity at various driving frequencies and related harmonic frequencies. We applied this stimulation paradigm in groups of migraine patients with and without aura in the interictal and pre-ictal phases, to study the effect of migraine aura and migraine phase on cortical excitability.

Part II: TMS-EEG, a novel method to measure cortical excitability in migraine

Chapter 5 described the application of transcranial magnetic stimulation (TMS) with concurrent EEG to enable comparison of TMS-evoked potentials between migraine with aura and controls, as direct measure of cortical excitability. In **Chapter 6**, differences in TMS-induced EEG 'phase clustering' were investigated in migraine with aura and juvenile myoclonic epilepsy, to explore the potential of this EEG feature as a biomarker of genetic generalized epilepsy or migraine with aura.

Chapter 7 provides a general discussion of this thesis, with considerations for future translational research into migraine attack prediction using neurophysiological methods.

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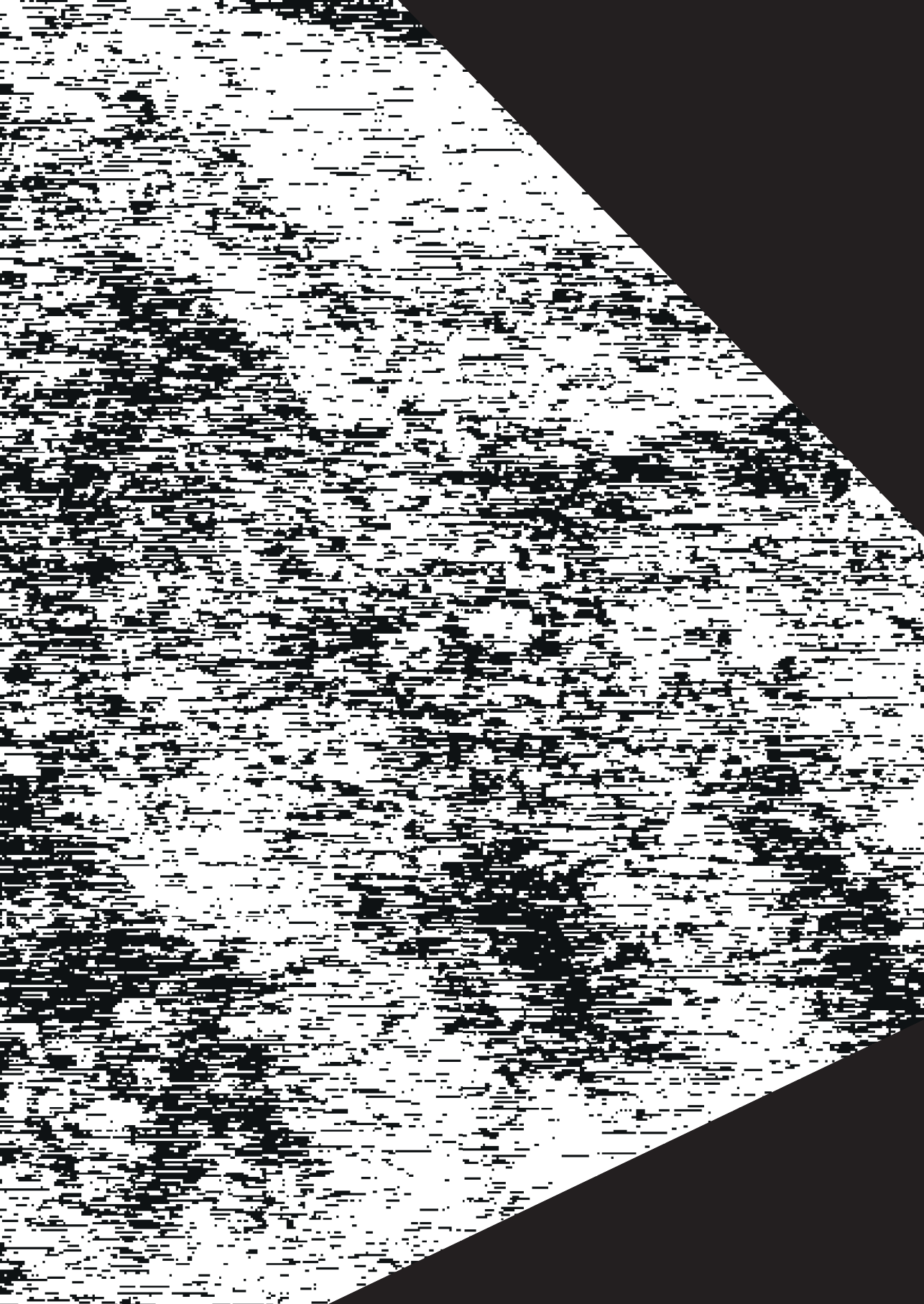
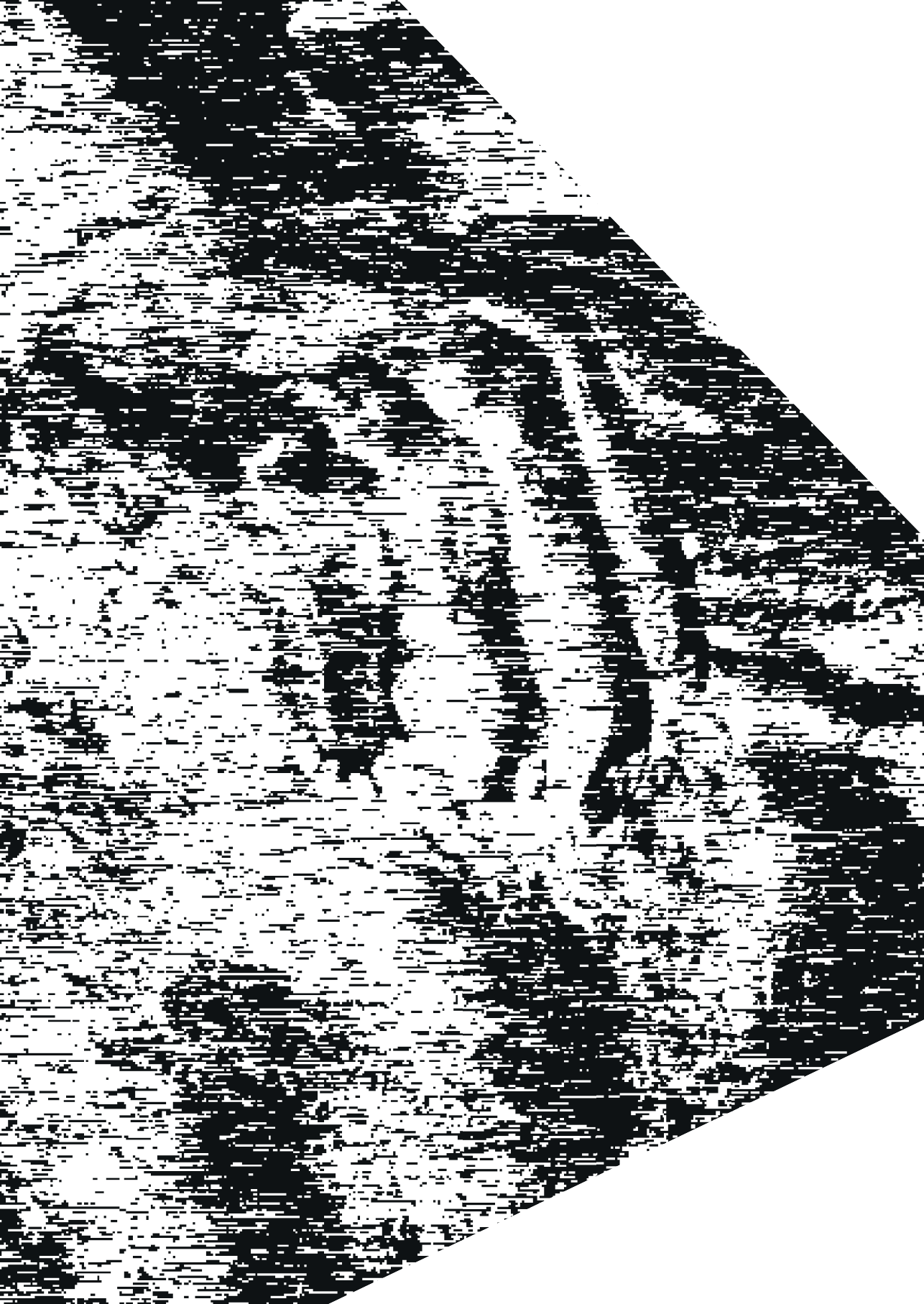


Part I

Visual cortex excitability as migraine attack predictor



Chapter 2

Quantifying visual allodynia across migraine subtypes: the Leiden Visual Sensitivity Scale

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Abstract

Enhanced sensitivity to light (photophobia) and patterns is common in migraine and can be regarded as visual allodynia. We aimed to develop and validate a questionnaire to easily quantify sensitivity to light and patterns in large populations, and to assess and compare visual allodynia across different migraine subtypes and states.

We developed the Leiden Visual Sensitivity Scale (L-VISS), a 9-item scale (score range 0-36 points), based on literature and patient interviews, and examined its construct validity. Furthermore, we assessed ictal and interictal visual sensitivity in episodic migraine with (n = 67) and without (n = 66) aura and chronic migraine with (n = 20) and without (n = 19) aura, and in healthy controls (n = 86). Differences between migraine subtypes and states were tested using a linear mixed model with 3 fixed factors (episodic/chronic, with/without aura, and ictal/interictal).

Test–retest reliability and construct validity of L-VISS were good. Leiden Visual Sensitivity Scale scores correlated in the expected direction with light discomfort (Kendall's T = -0.25) and pattern glare tests (T = 0.35). Known-group comparisons confirmed its construct validity. Within migraine subtypes, L-VISS scores were higher in migraine with aura versus without aura and in chronic versus episodic migraine. The linear mixed model showed all factors affected the outcome ($P < 0.001$).

The L-VISS is an easy-to-use scale to quantify and monitor the burden of bothersome visual sensitivity to light and patterns in large populations. There are remarkable ictal and interictal differences in visual allodynia across migraine subtypes, possibly reflecting dynamic differences in cortical excitability.

Introduction

Migraine is a common, multifactorial brain disorder characterized by recurring disabling attacks of headache and associated features (migraine without aura) and, in one-third of patients, neurological aura features (migraine with aura).[1] Visual symptoms are common; most auras are visual[2] and up to 90% of patients report photophobia during attacks.[3] In-between attacks, 60% of migraineurs experience at least some enhanced sensitivity to light and many notice abnormal sensitivity to visual patterns[4] or visual hallucinations,[5] suggesting permanent patient burden caused by disturbed visual processing.

In concordance with 'tactile allodynia', i.e., the painful response to non-painful stimuli, this increased visual sensitivity has been termed "visual allodynia".[6] Tactile allodynia is a common phenomenon among migraineurs,[7] in particular, those with chronic migraine[8] or migraine with aura.[9] Notably, in patients with chronic migraine,[10] migraine with aura,[11,12] and pre-ictal photophobia,[13] neurophysiological and neuroimaging evidence supporting ictal and interictal hyperexcitability of the visual migraine cortex[12,14,15] is accumulating.[16] Enhanced visual sensitivity might thus reflect visual cortex hyperexcitability,[17] which in turn might predispose to cortical spreading depolarization, the likely mechanism for aura.[16] These studies, however, were all using complex methods and, accordingly, could only investigate limited numbers of patients and migraine subtypes.

Quantifying sensitivity to light[18] and visual patterns[19] using questionnaires might be a promising non-invasive method to compare visual allodynia as a proxy for visual cortex excitability across large groups of patients with different migraine subtypes outside and during attacks. Existing questionnaires, however, measure only light or indirect pattern sensitivity, and are mostly dichotomous.[20–22] Moreover, studies applying these questionnaires were small and were focusing on only a single migraine subtype and state, precluding direct comparison between migraine subtypes and states.

We aimed to compare visual allodynia between large study populations across a spectrum of migraine subtypes both during and outside attacks. Therefore, we developed and validated an easy-to-use, self-report instrument to quantify visual

sensitivity to light and patterns on a near-continuous linear scale (the 'Leiden Visual Sensitivity Scale'(L-VISS)). Subsequently, we applied L-VISS to measure ictal and interictal visual allodynia in four large and clinically well-defined migraine subgroups with episodic or chronic migraine with or without aura.

Methods

The present study consisted of three parts: (i) development of the L-VISS questionnaire; (ii) validation of L-VISS as a reproducible and reliable easy-to-use self-report instrument to assess visual sensitivity; and (iii) assessing and comparing ictal and interictal visual sensitivity by using L-VISS in four migraine subgroups.

Participants

Subjects aged 18 to 65 with sufficient command of Dutch to fully understand the questionnaire were recruited from: (i) the headache clinics of Leiden University Medical Centre and Tergooi Hospital; and (ii) the Leiden University Migraine Neuro-Analysis (LUMINA) database,[23] which includes pre-screened non-headache controls and people with episodic or chronic migraine, willing to participate in studies on migraine.

Exclusion criteria for all participants were: psychiatric or neurological disorder (except migraine for participants with migraine); use of chronic medication (other than oral contraceptives), including migraine prophylactics, in the four weeks preceding the measurements (except for participants with chronic migraine); and history of malignancy. Diagnosis was confirmed before participation by telephone interview for participants with episodic or chronic migraine (i.e., headache on ≥15 days/month of which at least eight fulfil migraine criteria) according to the International Classification of Headache Disorders (ICHD)-III-beta criteria.[1] They were to have at least one attack per month in the six months before the measurement day. Controls and their first-degree relatives could not have migraine nor could they have any other form of headache on more than one day per month. The study was approved by the Medical Ethics Committee of Leiden University Medical Center, and all participants provided written informed consent prior to inclusion.

Development of L-VISS and data collection

Items for the self-report scale to quantify sensitivity for light and patterns were based on the migraine literature[24,25] and structured in-depth interviews with patients with migraine. After several revisions, using the feedback of patients with migraine from think-aloud interview sessions, we selected the items. For all items we used a 5-point Likert-type response scale, to measure the degree rather than presence of visual sensitivity, and 5-point scales yield the best data quality.[26] Per item, these five options were provided: 'not at all' (0 points), 'slightly' (1 point), 'moderately' (2 points), 'severely' (3 points) and 'very severely' (4 points). Outcome of the scale (L-VISS score) is defined as the sum of the responses to all nine questions (range 0-36 points). Participants were instructed to complete the questionnaire (a web-based or identical printed version) based on their experiences during the last month. Controls completed the questionnaire once. Participants with migraine completed the questionnaire twice, once while focusing on the interictal state and once while focusing on the ictal state. Patients with chronic migraine could opt-out for the interictal part of the questionnaire if they felt unable to identify an interictal state.

Measuring pattern glare and light discomfort

As part of the validation process of L-VISS, pattern glare and light sensitivity were measured in subgroups of controls and participants with episodic migraine in-between attacks, i.e., at least three days after the last attack and at least three days before the next attack. Those who got an attack within three days after the measurement day were excluded. Measurements took place on the same day upon which the participants completed L-VISS for the first time: first the pattern glare test and then, after an interval of at least 5 minutes, the light discomfort test. Participants with chronic migraine were not included in these experiments because they were not expected to be free of migraine for six consecutive days. We considered ictal state tests too burdensome for patients.

Pattern Glare Test

This test is used to measure pattern glare in response to printed patterns.[27] Participants are presented with three black-and-white horizontally striped patterns with a different spatial frequency (pattern 1: 0.6 cycles per degree [cpd], pattern 2: 4.0 cpd, pattern 3: 12 cpd). Participants were seated in a lighted room at 70-cm

distance of the pattern and instructed to binocularly focus for 5 seconds on the fixation dot in the middle of the pattern. Three variants of visual distortion were rated: color, motion (bending of lines, shimmer/flicker) and shapes (blurring of lines, fading, and shadowy shapes). After each measurement, patients were asked whether they suffered from afterimages. Test result was the pattern glare score, defined as the number of the reported visual distortions summed over the three patterns (0–9 points; modified from Tibber et al[28]).

Light Discomfort Test

Individual discomfort to light was quantified using a custom-made setup comparable with other studies.[29] All tests were performed in the same room with minimal background lighting. Participants were seated facing a 1,000-W halogen lamp (QLT-1000; Falcon Eyes Ltd, Hong Kong) with their head positioned on a headrest. Heat reducing and light diffusing glass was mounted between the lamp and the headrest. A light intensity sequence was programmed through custom-written software, increasing from 1.6 loglux to 4.4 loglux in 2-second steps of 0.1 loglux with 2-second rest between each step. Light intensity was kept stable by automatic adjustments every 20 ms based on feedback from a luxmeter attached to the headrest above both eyes (SLD-70 BG2A Photodiode; Advanced Photonix, Inc., Ann Arbor, MI). Participants were instructed to indicate when the light intensity became uncomfortable; the test was stopped at that moment. The light discomfort test was repeated three times, with intervals of at least three minutes between each measurement to avoid habituation to the light stimulus. After each measurement, patients were asked whether they suffered from afterimages. Test outcome was the median light discomfort threshold of three subsequent tests.

Data analysis and statistics

Validity of standardized measurements

To validate our setup for the pattern glare and light discomfort tests, we compared our results to previous findings using these tests in migraine.[28,29] Pattern glare scores and light discomfort threshold were compared between participants with episodic migraine and controls using independent-samples *t*-tests. Presence of afterimages

was compared between groups using Fisher exact test (light discomfort threshold test) and Pearson χ^2 test (pattern glare test).

Internal consistency and test-retest reproducibility

Internal consistency was assessed in participants with episodic migraine (interictal state score) and controls, using Cronbach α coefficient ($\alpha \geq 0.70$ considered acceptable), inter-item correlation (recommended 0.15-0.50), and item-total correlation (criterion value ≥ 0.30).[30] Test-retest reproducibility was assessed using one way intra-class correlation coefficients (ICCs) for the sum score of the L-VISS and the individual items: ICC$\geq$ 0.41 acceptable; ICC$\geq$ 0.61 good; ICC$\geq$ 0.81 excellent.[31] For this purpose, both subgroups completed the questionnaire a second time two to three weeks later.

Comparisons between sensory and behavioral testing

Leiden Visual Sensitivity Scale scores were correlated with pattern glare and light discomfort tests, two established measures of pattern and light sensitivity (see above). We hypothesized that L-VISS scores would correlate positively with pattern glare scores (i.e., increased visual discomfort correlates with more visual sensitivity) and negatively with light discomfort threshold (i.e., increased visual sensitivity correlates with lower light discomfort threshold). Correlations were assessed using Kendall's τ as the L-VISS scores in the validation subgroup (controls and participants with episodic migraine) were skewed to the left and our dataset contains ties between scores. Correlations below 0.30 were considered poor, between 0.30 and 0.60 moderate, and above 0.60 good.[32] Using independent-samples *t*-tests, we assessed whether L-VISS scores were higher in those who had afterimages after the pattern glare and light discomfort tests compared to those who did not have afterimages.

Two known-group comparisons, i.e., comparisons with expected outcome based on information from literature, were conducted. Photophobia is reported by 90% of migraineurs during attacks versus 60% outside attacks,[3,33] and by only less than 5% of controls.[33,34] We hypothesized that L-VISS scores (i) within participants with migraine are higher during compared to outside attacks (paired-samples *t*-test); and (ii) in participants with migraine are higher compared to those in controls (independent-samples *t*-test).

Comparison across migraine sub-types

Visual sensitivity was assessed in controls and four migraine subtypes outside and during attacks: (i) episodic migraine without aura; (ii) episodic migraine with aura; (iii) chronic migraine without aura; and (iv) chronic migraine with aura. A linear mixed model was fitted on the L-VISS scores. The repeated-measures factor was set to compare the interictal vs ictal scores. Three fixed factors were included: (i) diagnosis: episodic vs chronic migraine; (ii) aura status: migraine with vs without aura; and (iii) attack status: in-between or during the attack. Sex was included as covariate. The two-way interactions between these factors, and between factors and covariates were also tested.

Baseline subject characteristics and L-VISS scores are reported as mean and SD. Independent *t*-tests, χ^2 tests and Fisher exact test were used for comparison of baseline characteristics when appropriate. For all analyses, *P* values were considered significant when lower than 0.05. All data analyses were performed with IBM SPSS (version 22.0; Armonk, NY).

Results

Study population

We included in total 258 participants: 133 with episodic migraine (n = 66 with and n = 67 without aura), 39 with chronic migraine (n = 20 with and n = 19 without aura; 19 participants (9 with, 10 without aura) had medication overuse according to ICHD-III-beta criteria[1]), and 86 age- and sex-balanced non-headache controls (Table 1). As expected, participants with chronic migraine report more migraine days and attacks per month and higher triptan use compared with those with episodic migraine. Participants with chronic migraine reported sufficient days per month without headache to complete the questionnaire based on interictal days (chronic migraine without aura: 8.0 ± 6.0 headache-free days per month, chronic migraine with aura: 9.3 ± 5.5 days). Chronic migraine groups included more female participants than episodic migraine groups and controls. Triptan users' rate and monthly migraine attack and migraine day frequency were higher in participants with episodic migraine without aura compared to those with aura. Otherwise baseline characteristics of the control and migraine subgroups were similar.

A sample of 146 participants (control: n = 46, episodic migraine without aura: n = 56; episodic migraine with aura: n = 44) completed the pattern glare test and 64 participants (control: n = 20; episodic migraine without aura: n = 23; episodic migraine with aura: n = 21) the light discomfort test. Five of these participants were subsequently excluded from the analysis; four (without aura: n = 3) developed a migraine attack within three days after the test and one (without aura) had started using prophylactic medication in the period between the telephone interview and the measurement day. Two (without aura: n = 1; with aura: n = 1) participants were excluded from the test-retest analysis because they had completed the retest questionnaire after more than three weeks.

Table 1. Baseline characteristics of controls and migraine subgroups

Variable	Headache-free controls (n=86)	Episodic migraine without aura (n=67)	Episodic migraine with aura (n=66)	Chronic migraine without aura (n=19)	Chronic migraine with aura (n=20)	p-value
Female (n (%))	55 (64)	43 (64)	42 (64)	18 (95)	19 (95)	0.005
Age (years)	38.9 ± 12.5	37.0 ± 9.8	35.2 ± 10.8	41.4 ± 10.8	37.8 ± 10.2	0.17
Age at onset migraine	-	13.1 ± 16.3	14.4 ± 8.3	17.1 ± 9.7	15.0 ± 6.6	0.64
Migraine duration (years)	-	23.7 ± 17.2	21.2 ± 11.0	24.4 ± 15.2	22.9 ± 12.2	0.74
Migraine attacks per month	-	2.3 ± 1.4[a]	1.7 ± 1.7[a]	9.4 ± 8.2	10.2 ± 5.1	<0.001
Migraine days per month	-	4.0 ± 3.3[b]	2.3 ± 2.6[b]	13.8 ± 6.8	13.6 ± 4.6	<0.001
Use of triptans (n (%))	-	45 (67)[c]	35 (53)[c]	18 (95)	16 (80)	0.004
Use of prophylaxis (n (%))	-	0	0	4 (21)	1 (5)	0.18

Values are presented as mean with standard deviations, or number with percentage. Extra between-group comparisons: [a] two-tailed *t*-test: $p = 0.03$; [b] two-tailed *t*-test: $p = 0.002$; [c] Chi square test: $p = 0.04$.

Development of Leiden Visual Sensitivity Scale

We identified nine items from the literature[24,25] and semi-structured interviews with 10 patients with migraine based on their daily life experiences with visual allodynia. Think aloud interviews with four controls and four patients with episodic migraine did not reveal any missed aspects of visual allodynia, indicating completeness of the questions. These interviews also confirmed content validity (relevance and comprehensiveness) and acceptability of the questions (see Table 2 for an English translation and Supplementary Table 1 for the original questions in Dutch).

Table 2. English translation of the Leiden Visual Sensitivity Scale

#	Question
1.	To what extent does sunlight bother you when you're not wearing sunglasses?
2.	To what extent are you bothered by artificial lighting?
3.	To what extent are you bothered by flickering lights (e.g., a flickering lamp, during films or at the discotheque)
4.	When you look at a bright light, is your eyesight worse afterwards (e.g., blurred or distorted vision)
5.	To what extent does looking at patterns bother you? (e.g., patterns in clothing, materials, luxaflex)?
6.	When you look at everyday patterns, do you experience afterimages? (seeing an image of the pattern elsewhere, for instance, on a white wall)
7.	When you look at patterns, is your eyesight worse? (e.g., blurred or distorted vision)
8.	When you look at a computer or TV screen, do you see afterimages? (seeing an image of the pattern elsewhere, such as on a white wall)
9.	When you look at a computer or TV screen, is your eyesight worse? (e.g., blurred or distorted vision)

Standardized measurement of pattern glare and light discomfort

Differences between participants with episodic migraine and non-headache controls on pattern glare test and light discomfort tests were in line with earlier reports,[28,29] confirming the utility of these methods for validation purposes. Participants with migraine experienced more induced illusions when looking at patterns (pattern glare score: 4.9 ± 2.0 vs 3.2 ± 2.3; $p < 0.001$; Fig. 1A) and had a lower light discomfort threshold (mean threshold: 2.64 ± 0.5 vs 2.98 ± 0.5 loglux; $p = 0.02$; Fig. 1B).

Of the participants with episodic migraine, 9/39 (23%) reported non-persistent afterimages after the light discomfort threshold test compared to 0/20 of non-headache controls (p = 0.022). For the pattern glare test, the occurrence of afterimages depended on the cycles per degree and was only significant for the 4.0 cpd pattern (Pearson χ^2 test, reported as control vs episodic migraine: 0.6 cpd: 26/46 vs 71/100, p = 0.085; 4.0 cpd: 28/47 vs 83/101, p = 0.003; 12 cpd: 32/47 vs 79/101, p = 0.185).

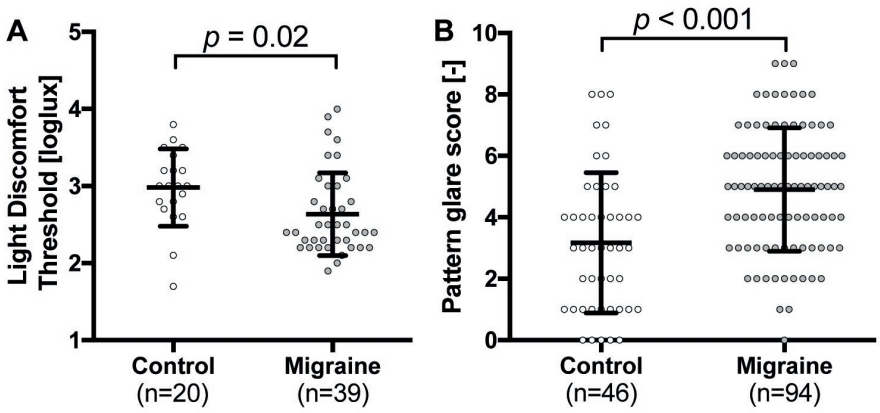

Figure 1. Light discomfort threshold and pattern glare score illustrate visual allodynia in episodic migraine. (A) Light discomfort threshold is decreased in patients with episodicmigraine (n = 39, mean ± SD: 2.64 ± 0.5 loglux) compared with healthy controls (n = 20; 2.98 ± 0.5, p = 0.02). (B) Pattern glare score is enhanced in patients with episodic migraine (n = 94; 3.2 ± 2.3) when compared with controls (n = 46; 4.9 ± 2.0, p < 0.001).

Internal consistency and test-retest reproducibility

Results of the reliability analysis are presented in Table 3 (see Supplementary Table 2, which contains results per group). Internal consistency of the L-VISS was excellent: Cronbach's alpha ranged from 0.73 (controls) to 0.83 (migraine); item-total correlations were above 0.30 except for one question in the control but not migraine group (Q6, correlation 0.23); inter-item correlations ranged from 0.15 to 0.61 over all participants, except for one correlation of 0.08 between Q6 and Q8. Test-retest reliability was good to excellent: ICC of L-VISS scores ranged from 0.78

(controls) to 0.93 (migraine). No floor (2.6% reported lowest score) or ceiling (1.5% reported highest score) effects were present in the responses.

Table 3. Reliability analysis assessed by internal consistency and test-retest reliability

#	Cronbach α (n=219)	Item-total correlations (n=219)	Test-retest correlation (n=57)
Q1	0.85	0.63	0.90
Q2	0.84	0.68	0.93
Q3	0.84	0.75	0.90
Q4	0.85	0.66	0.76
Q5	0.84	0.72	0.91
Q6	0.86	0.54	0.80
Q7	0.85	0.60	0.61
Q8	0.86	0.49	0.83
Q9	0.87	0.37	0.74
Total	0.87	NA	0.93

Data shown for the validation subgroup (control and participants with episodic migraine). Cronbach α is shown for the complete questionnaire, and per question the reliability of the questionnaire without that specific question.

Comparisons between sensory and behavioral testing

Construct validity of light- and pattern-related questions was demonstrated by confirming that the pattern glare score and light discomfort threshold correlated with the L-VISS scores in the expected directions (pattern glare score: Kendall's τ = 0.35, $p < 0.001$; light discomfort threshold: τ = -0.25, $p = 0.01$; Fig. 2A and B). Presence of afterimages was also associated with a higher L-VISS score for the 4.0 cpd pattern (9.1 ± 5.9 vs 5.9 ± 5.1; $p = 0.003$), but not for the 0.6 cpd (9.0 ± 5.8 vs 7.1 ± 5.8; $p = 0.054$) and 12.0 cpd patterns (8.4 ± 5.7 vs 7.8 ± 6.2; $p = 0.58$) or light

discomfort threshold (8.4 ± 6.2 vs 9.1 ± 6.1, $p = 0.75$). Construct validity was also established by confirming the pretest hypotheses that L-VISS scores are higher: (i) in 133 interictal participants with episodic migraine with or without aura (9.9 ± 5.7) than in 86 controls (3.6 ± 2.8; $p < 0.001$); and (ii) within 133 participants with episodic migraine during (19.7 ± 7.2) compared to outside attacks (9.9 ± 5.7; $p < 0.001$) (Fig. 2C).

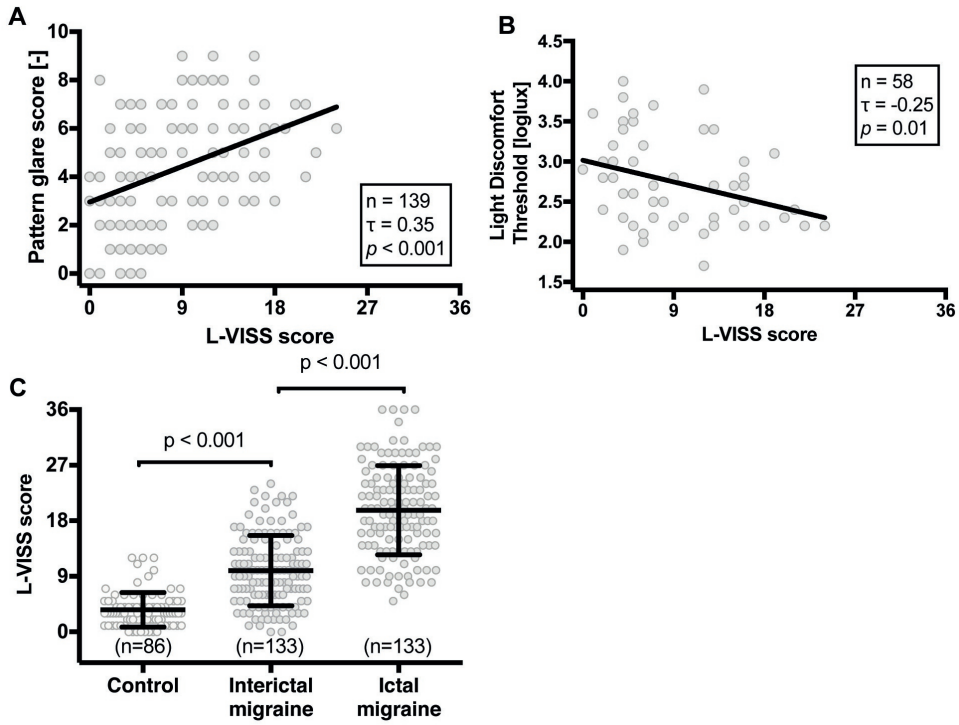

Figure 2. Construct validity was demonstrated by correlations of L-VISS score and standardized measures, and known-group comparisons. (A) Correlation of L-VISS score with pattern glare score (n = 139, of which episodic migraine n = 99; Kendall's T = 0.35, $p < 0.001$). (B) Correlation of L-VISS score with light discomfort threshold (n = 58, of which episodic migraine n = 38; Kendall's T = -0.25, $p < 0.01$). (C) Known-group comparisons show interictal L-VISS scores are higher in patients with episodic migraine (9.9 ± 5.7) compared with controls (3.6 ± 2.8; p < 0.001), and ictal L-VISS scores (19.7 ± 7.2) are increased compared with interictal scores ($p < 0.001$). L-VISS, Leiden Visual Sensitivity Scale.

Comparison across migraine sub-types and states

Mean L-VISS scores were lowest in controls (n = 86; 3.6 ± 2.8; see Fig. 3) and highest in participants with chronic migraine with aura during attacks (n = 20; 25.8 ± 7.9). In between these extremes, L-VISS scores outside attacks were 8.5 ± 5.7 for episodic migraine without aura (n = 67), 11.3 ± 5.4 for episodic migraine with aura (n = 66), 10.9 ± 6.2 for chronic migraine without aura (n = 19), and 17.8 ± 6.9 for chronic migraine with aura (n = 20). During attacks, L-VISS scores were 18.3 ± 7.8 for episodic migraine without aura (n = 67), 21.2 ± 6.3 for episodic migraine with aura (n = 66), and 23.0 ± 8.0 for chronic migraine without aura (n = 19). Diagnosis, aura status and attack status all influenced outcome ($p < 0.001$ for each factor). There were no significant two-way interactions between these factors (all $p > 0.11$). Sex ($p = 0.77$) nor its interactions with three factors did affect outcome (all $p > 0.12$). Thus, L-VISS scores were higher: (i) in chronic vs episodic migraine, both for migraine with and without aura as well as during and outside attacks; (ii) in migraine with aura vs without aura, both in episodic and chronic migraine as well as during and outside attacks; and (iii) during vs outside attacks, both in episodic and chronic migraine as well as in migraine with and without aura.

Migraine attack frequency was weakly correlated with the L-VISS scores outside ($r = 0.263$; $p = 0.001$) and during attacks ($r = 0.241$; $p = 0.002$); aura frequency, however, was not correlated ($r = -0.073$; $p = 0.570$). The use of prophylactic medication in chronic migraine did not affect the L-VISS score in-between ($p = 0.52$) or during attacks ($p = 0.16$).

Discussion

We developed, validated and applied L-VISS, an easy-to-use, nine-item, self-report questionnaire to rapidly and reliably quantify sensitivity to light and patterns on a near-continuous linear scale in large study populations. L-VISS scores were higher in migraineurs: (i) with aura vs without aura; (ii) with chronic vs episodic migraine; (iii) during vs outside attacks; and (iv) vs non-headache controls, for all four migraine subtypes and both during and outside attacks. These findings reveal a fluctuating burden of visual allodynia, in particular, in patients with chronic migraine or migraine with aura, both outside and even more during attacks, and are

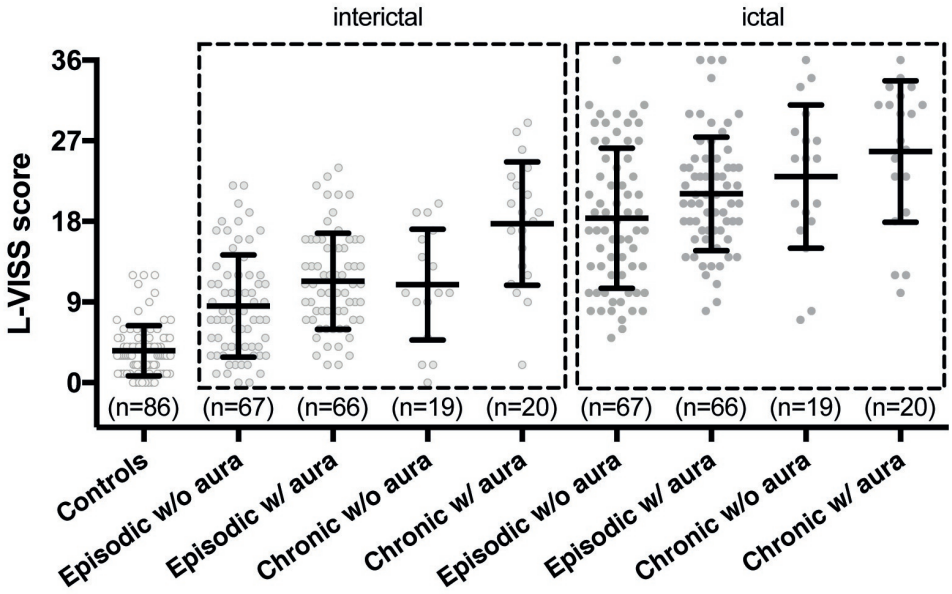

Figure 3. Individual L-VISS scores and mean per subgroup demonstrate effect of aura and chronic migraine on visual sensitivity. Participants with migraine reported interictal (light gray) and ictal (dark gray) scores. Subgroup scores are presented as mean and SD. Healthy controls (mean L-VISS score 3.6), and participants with episodic migraine without (interictal: 8.5/ictal: 18.3) and with aura (11.3/21.2) and participants with chronic migraine without (10.9/23.0) and with aura (17.8/25.8) were compared in-between and during attacks. Diagnosis, aura status, and attack status all affected the outcome (p < 0.001 per factor). L-VISS, Leiden Visual Sensitivity Scale.

well in line with, but do not prove, the hypothesis that the migraine brain is hyperexcitable.[16]

To the best of our knowledge, L-VISS is the first instrument to quantify visual sensitivity to light and patterns on a single, near-continuous, linear scale, enabling direct comparisons across multiple groups. Other instruments all use dichotomous or qualitative scales.[20–22] Items included in the questionnaire were selected based on interviews with migraine patients and their feedback on the relevance and acceptability of these items. Validity was established over a broad range of tests. Internal consistency and test-retest reliability were both good to excellent and there were no floor or ceiling effects. In an experimental setting, L-VISS scores increased with increasing light discomfort and pattern glare as measured with standard

established psychophysical and behavioral tests,[28,29] indeed suggesting that changes in L-VISS scores reflect changes in both phenomena.[32] In known-group comparisons, L-VISS scores were higher in interictal migraineurs compared to controls and, within migraineurs, during compared with outside attacks. Construct validity was also confirmed by the finding that participants with afterimages to pattern glare reported higher L-VISS scores.

Various pathophysiological processes have been proposed to underlie photophobia, and probably other forms of visual sensitivity, including enhanced excitability of the visual cortex.[35] Leiden Visual Sensitivity Scale cannot differentiate between these different mechanisms. However, results from neurophysiological and neuroimaging studies, measuring cerebral excitability more directly,[18] support the view, albeit indirectly, that differences in L-VISS scores might reflect differences in visual cortex excitability. Cortical excitability profiles across migraine subgroups and states in these studies[10–12,15,18] were remarkably similar to the inter-subgroup differences we found for L-VISS scores. Interictal excitability was higher in migraine with aura versus migraine without aura[11,12,18] and in chronic vs episodic migraine.[10] During attacks, visual sensitivity scores were increased even further, probably reflecting the symptom photophobia that might be caused by ictal increase of visual cortical excitability.[11,15] Moreover, self-reported photophobia correlated well with visual cortex excitability as measured with positron emission tomography[13] and blood-oxygen-level dependent activation after visual stimulation.[18,19]

Tactile allodynia and photophobia have both been linked to elevated levels of calcitonin gene-related peptide (CGRP),[36] an important neurotransmitter in migraine pathophysiology.[16] Moreover, the CGRP receptor-antagonist telcagepant has been shown to improve photophobia.[37] Speculatively, increased visual sensitivity in chronic vs episodic migraine might thus reflect chronic central sensitization similar to what has been proposed for tactile allodynia.[38,39] As CGRP plasma levels were higher in people with chronic migraine, in particular, in those with chronic migraine with aura,[40] increased visual sensitivity might potentially reflect increased CGRP activity. Also, in triptan therapy it was shown that treatment is more effective in migraine patients with signs of tactile allodynia when triptans are administered before establishment of allodynic symptoms.[41] The analgesic action of triptans seems to be specifically effective before central sensitization

increases during the migraine attack.[42] The level of visual allodynia as measured using the L-VISS might thus potentially prove a simple predictive test for migraine prophylactic efficacy of CGRP-blocking therapies,[43] and possibly be helpful in selecting candidates for early initiation of triptan treatment.

Recall and selection bias might have influenced our results, but we deem the risk and potential impact limited. Risk of recall bias, e.g., by focusing while responding to L-VISS questions on the most recent days or on days with the most extreme visual hypersensitivity rather than on the whole month, or for chronic migraine by focusing not only on headache-free days but also tension-type headache days, cannot be excluded but is unlikely to explain differences between migraine subgroups. Participants with migraine with visual aura might perhaps have been focused more on visual symptoms. Selection bias, e.g., because subjects with abnormal visual sensitivity were more likely to participate in the present study than those without abnormal visual sensitivity, is also unlikely to have had a major effect. Most (76%) controls and participants with episodic migraine were in fact participating in studies which were unrelated to visual sensitivity and to which completing the L-VISS was added.

Leiden Visual Sensitivity Scale is a well-validated and inexpensive, easy-to-use, self-report instrument to reliably quantify and monitor visual allodynia in large study populations. Visual allodynia contributes to the burden of migraine, not only during but also outside migraine days. Our findings add to the clinical evidence that suggests hyperexcitability of the visual cortex is related to visual symptoms in patients with migraine, particularly in migraine with aura and in chronic migraine, and is increased during migraine attacks.

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Supplementary material

Supp Table 1. Leiden Visual Sensitivity Scale (L-VISS) – Original Dutch version (as used in this study) and English translation.

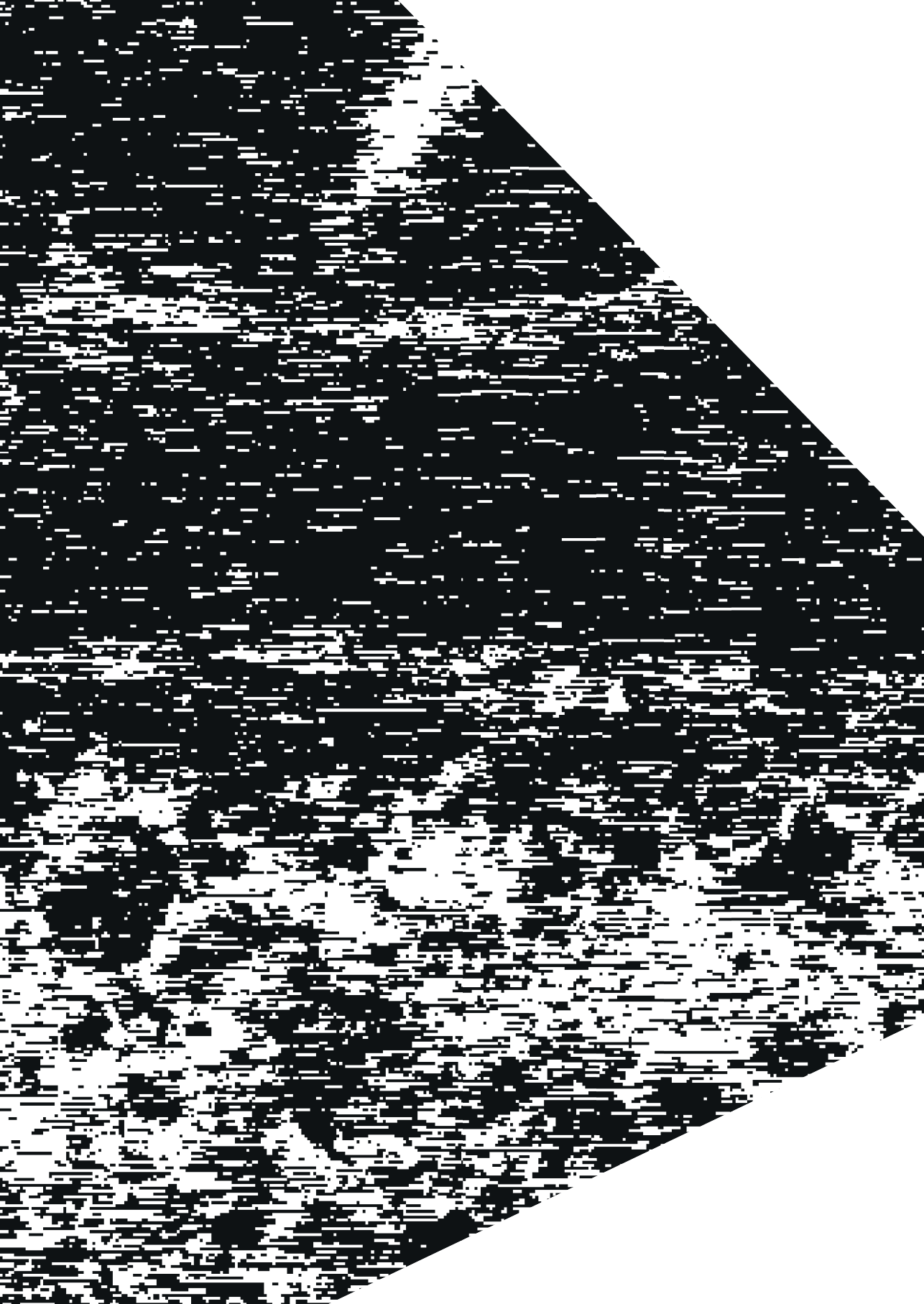
#	Dutch	English
1.	Hoeveel last heeft u van het zonlicht buiten zonder zonnebril?	To what extent does sunlight bother you when you're not wearing sunglasses?
2.	Hoeveel last heeft u van kunstmatige verlichting?	To what extent are you bothered by artificial lighting?
3.	Hoeveel last heeft u van knipperend licht? (Bijvoorbeeld een knipperende lamp of tijdens films of in de discotheek.)	To what extent are you bothered by flickering lights (e.g., a flickering lamp, during films or in a nightclub)
4.	Als u in fel licht kijkt, heeft u daarna last van een verminderd gezichtsvermogen? (Zoals onscherp zien of vervormd zicht.)	When you look at a bright light, is your eyesight worse afterwards (e.g., blurred or distorted vision)
5.	Hoeveel last heeft u van het kijken naar patronen? (Bijvoorbeeld patronen in kleding, stoffen, luxaflex.)	To what extent does looking at patterns bother you? (e.g., patterns in clothing, materials, blinds)?
6.	Als u naar alledaagse patronen kijkt, heeft u dan last van nabeelden? (Dat wil zeggen dat u het beeld van de patronen nog ergens anders ziet, bijvoorbeeld bij het kijken naar een witte muur.)	When you look at everyday patterns, do you experience afterimages? (seeing an image of the pattern elsewhere, for instance, on a white wall)
7.	Als u naar patronen kijkt, heeft u dan last van een verminderd gezichtsvermogen? (Zoals onscherp zien of vervormd zicht.)	When you look at patterns, is your eyesight worse? (e.g., blurred or distorted vision)
8.	Als u naar een beeldscherm kijkt, heeft u dan last van nabeelden? (Dat wil zeggen dat u het beeld van de patronen nog ergens anders ziet, bijvoorbeeld bij het kijken naar een witte muur.)	When you look at a computer or TV screen, do you see afterimages? (seeing an image of the pattern elsewhere, such as on a white wall)
9.	Als u naar een beeldscherm kijkt, heeft u dan last van een verminderd gezichtsvermogen? (Zoals onscherp zien of vervormd zicht.)	When you look at a computer or TV screen, is your eyesight worse? (e.g., blurred or distorted vision)

A 5-point Likert-type response scale was used per question: Not at all (0 points), Slightly (1 point), Moderately (2 points), Severely (3 points) and Very severely (4 points). Patients received written instructions to complete two identical sets of questions, one for the days in the past month without migraine, one for the days in the past month with migraine. Patients could indicate that they did not experience any days without migraine.

Supp Table 2. Reliability analysis assessed by internal consistency and test-retest reliability

	Internal consistency						Reliability		
	Cronbach's alpha			Item-total correlations			Test-retest correlation		
	Total	*Control*	*Episodic migraine*	*Total*	*Contro*	*Episodic migraine*	*Total*	*Control*	*Episodic migraine*
	(n=219)	*(n=86)*	*(n=133)*	*(n=219)*	*(n=86)*	*(n=133)*	*(n=57)*	*(n=19)*	*(n=38)*
Q1	0.85	0.74	0.81	0.63	0.33	0.56	0.90	0.75	0.90
Q2	0.84	0.70	0.81	0.68	0.48	0.60	0.93	0.73	0.92
Q3	0.84	0.69	0.79	0.75	0.53	0.71	0.90	0.82	0.89
Q4	0.85	0.68	0.81	0.66	0.55	0.58	0.76	0.64	0.76
Q5	0.84	0.70	0.80	0.72	0.48	0.66	0.91	0.77	0.90
Q6	0.86	0.74	0.83	0.54	0.23	0.46	0.80	0.73	0.77
Q7	0.85	0.70	0.82	0.60	0.58	0.55	0.61	0.70	0.57
Q8	0.86	0.72	0.83	0.49	0.36	0.43	0.83	0.63	0.85
Q9	0.87	0.71	0.84	0.37	0.44	0.30	0.74	0.78	0.73
Total	0.87	0.73	0.83	NA	NA	NA	0.93	0.78	0.93

Data shown for the validation subgroup (headache-free control and participants with episodic migraine) and separately for headache-free controls and participants with episodic migraine. Cronbach's alpha is shown for the complete questionnaire, and per question the reliability of the questionnaire without that specific question; all are sufficient (Cronbach's alpha > 0.70). Item-total correlations are all above the criterion value (above 0.30), except question 6 in headache-free controls. Test-retest correlation was assessed using intraclass correlation coefficients and were mostly good (above 0.61) to excellent (above 0.81).



Chapter 3

Responsivity to light in familial hemiplegic migraine type 1 mutant mice reveals frequency-dependent enhancement of visual network excitability

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Abstract

Migraine patients often report (inter)ictal hypersensitivity to light, but the underlying mechanisms remain an enigma. Both hypo- and hyperresponsivity of the visual network have been reported, which may reflect either intra-individual dynamics of the network or large inter-individual variation in the measurement of human visual evoked potential data.

We studied visual system responsivity in freely behaving mice using combined epidural electroencephalography and intracortical multi-unit activity to reduce variation in recordings and gain insight into visual cortex dynamics. For better clinical translation, we investigated transgenic mice that carry the human pathogenic R192Q missense mutation in the α_{1A} subunit of voltage-gated $Ca_V2.1$ Ca^{2+} channels leading to enhanced neurotransmission and familial hemiplegic migraine type 1 in patients. Visual evoked potentials were studied in response to visual stimulation paradigms with flashes of light.

Following intensity-dependent visual stimulation, FHM1 mutant mice displayed faster visual evoked potential responses, with lower initial amplitude, followed by less pronounced neuronal suppression compared to wild-type mice. Similar to what was reported for migraine patients, frequency-dependent stimulation in mutant mice revealed enhanced photic drive in the EEG beta-gamma band.

The frequency-dependent increases in visual network responses in mutant mice may reflect the context-dependent enhancement of visual cortex excitability, which could contribute to our understanding of sensory hypersensitivity in migraine.

Introduction

Migraine is a common episodic brain disorder characterized by severe recurrent attacks of headache, associated with phono- and photophobia and other autonomic and neurological symptoms.[1] Many patients report abnormal sensitivity or intolerance to light, not only during but also outside attacks, and show abnormal cortical activation in response to visual stimulation in imaging studies.[2,3] Enhanced visual sensitivity before the onset of headache has been regarded a sign that an attack has started.[4] The light sensitivity may result from cortical 'hyper-responsivity',[5] that is not restricted to the visual cortex as it was also reported for other brain structures implicated in migraine pathophysiology.[6–9]

It remains unresolved whether findings of altered visual responsivity in migraine patients translate to increased or decreased excitability of the visual cortex[5,10] as unaltered,[11] reduced,[12,13] and enhanced[14,15] visual evoked potential (VEP) responses in between attacks have been reported. Apart from transient visual stimulation paradigms, visual processing in migraine has also been studied using steady-state stimulation resulting in 'photic driving' responses. Photic drive (also known as entrainment) is the frequency-following EEG response of the visual cortex to various stimulation frequencies, resulting in a dominant EEG frequency.[16] In migraineurs, an enhanced photic drive response between 10 and 20 Hz was observed that could reflect plasticity changes involving the visual cortex.[7,17,18] A shortened photic driving paradigm ('chirp' stimulation) showed enhanced responses in the beta band (18 to 26 Hz) in between migraine attacks.[19] Using this paradigm, we recently observed a similar enhanced photic drive response that was evident in the harmonics of the beta-gamma band (22–32 Hz) in migraineurs, albeit not in between attacks but toward an impending attack.[20] These observations support the view that enhanced visual network excitability contributes to attack initiation.

Contradictory findings of cortical hyper- or hyporesponsivity in migraine may be explained by the dynamics of the network and, even more likely, can be due to differences in stimulation procedures and readout parameters in clinical studies.[10] Also, large inter-individual variation may be caused by the low signal-to-noise ratio of scalp EEG in humans, which hampers the interpretation of human VEP findings. Performing VEP measurements in animals can circumvent most of the issues, as

VEPs with an improved signal-to-noise ratio can be obtained (i) when intracortical or epidural electrodes are used, and (ii) by controlling the influence of genetic background by using inbred strains.

Visual stimulation by flashes of light has been widely used to elicit VEP responses in anaesthetized,[21,22] head-fixed,[23] and freely behaving mice.[24–26] To investigate changes in visual network responsivity, we here examined flash VEP responses in freely behaving mice. To better capture dynamical changes in visual system responsivity, as reported in migraineurs for stimulation at varying frequencies,[19,20,27,28] both steady state responses and transitions between stimulation frequencies were investigated. We studied both wild-type mice and FHM1 mutant mice that carry the R192Q missense mutation in the α_{1A} subunit of neuronal $Ca_V2.1$ calcium channels,[29] known to cause familial hemiplegic migraine type 1 (FHM1), a subtype of migraine with aura.[8,30] The mutant mice display a gain of $Ca_V2.1$ channel function with enhanced glutamatergic neurotransmission in the cortex,[31,32] and are considered a relevant model for studying mechanisms by which neuronal hyperexcitability contributes to migraine pathophysiology.[8,33] Our mouse model is also relevant given that triggers of attacks, including bright light, reported by FHM1 patients are similar to triggers reported by patients suffering from migraine with aura.[34] In addition, in line with clinical reports of photophobia symptoms in migraineurs during and sometimes also outside attacks,[35,36] FHM1 mutant mice displayed behavioural signs of photophobia[37] that may reflect enhanced visual system responsivity. Hence, insight in altered responses to visual stimulation in the transgenic migraine mice may help understand how visual system alterations are brought about in a migraine context.

Materials and Methods

Animals

Male homozygous FHM1 R192Q knock-in ('FHM1 mutant') and wild-type ('WT') mice of 3–6 months were used. The mutant mice were generated by introducing the human pathogenic FHM1 R192Q missense mutation in the orthologous mouse *Cacna1a* gene using a gene-targeting approach.[29] Mice, backcrossed for 20 generations to C57BL/6J, were maintained on a normal 12:12 light-dark cycle with

water and food available ad libitum. All experiments were approved by the Animal Experiment Ethics Committee of Leiden University Medical Center and were carried out in accordance with ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments. All efforts were made to minimize the suffering of the mice.

EEG recordings and visual stimulation in freely behaving mice

Under isoflurane anaesthesia (1.5%, in oxygen-enriched air), seven electrodes were stereotaxically implanted at the following coordinates (in mm relative to bregma): a pair of platinum (Pt) electrodes 3.5 posterior/2.0 lateral/0.8 ventral from dura (right visual cortex); a pair of Pt electrodes 1.5 anterior/1.5 lateral/0.8 ventral from dura (right motor cortex); a silver (Ag) ball-tip electrode 3.5 posterior/2.0 lateral on the dura (left visual cortex); an Ag ball-tip electrode and Ag-AgCl ball-tip electrode were placed above cerebellum to serve as reference and ground electrodes, respectively (Figure 1A-top). Electrodes were attached to the skull using light-activated bonding primer and dental cement (Kerr optibond / premise flowable, DiaDent Europe, Almere, the Netherlands). Carprofen (5 mg/kg, s.c.) was administered for post-operative pain relief.

After a recovery period of 7 days, animals were placed in a shielded recording cage and connected to the recording hardware through a counterbalanced, low-torque custom-built electrical commutator. Epidural EEG and intracortical local field potential signals were pre-amplified 3X and fed into separate amplifiers for EEG/local field potential and neuronal multi-unit activity (MUA) recordings. EEG/local field potential signals were band-pass filtered (0.05 to 500 Hz) and amplified 1,200X, and digitized (Power 1401, CED, Cambridge, UK) at a rate of 5,000 Hz. In addition, differential signals from paired intracortical Pt electrodes were used for MUA recordings by 36,000X amplification, band-pass filtering (500 to 5,000 Hz) and digitizing at 25,000 Hz. For VEP measurements, the tethered mouse was placed inside a computer-controlled custom-built light-emitting diode (LED) illuminated sphere in which it was able to move freely (van Diepen et al., 2013; Figure 1A-bottom). The wavelength of blue light and irradiance were measured using a calibrated spectrometer (AvaSpec2048; Avantes, Apeldoorn, The Netherlands). The sphere was 30 cm in diameter, with the inside coated with high-reflectance paint. On top of the sphere, around an opening for the swivel,

monochromatic blue (wavelength: 469 nm) 1-ms light flashes were presented at 1-V stimulator output voltage, corresponding to a light intensity of ~2.2 log cd/m^2, unless mentioned otherwise. A baffle prevented mice from looking directly at the LEDs. Water and food were provided inside the sphere during the experiment.

EEG/local field potential data were down-sampled offline to 1,000 Hz. MUA was reduced in complexity by calculating the root mean square amplitude per 25 samples (1 ms), as the root mean square correlates to spiking rate using template matching,[39,40] and next down-sampled to 1,000 Hz. Stimulation sequence, data processing and analysis using custom-written scripts in Matlab (version R2013b; The Mathworks, Natick, MA) varied per paradigm. Resting periods in between different stimulation paradigms were at least 1 minute for both WT and FHM1 mutant groups.

Single-VEP paradigm

To assess whether light flashes evoked responses in the visual cortex, 100 flashes were presented at 1 Hz. Single-VEP responses were high-pass filtered at 1 Hz and low-pass filtered at 35 Hz (4th order Butterworth, zero-phase shift) and averaged between 100 ms prior and 500 ms after stimulation. N1 peak amplitude and latency were detected between 20 and 80 ms after stimulation, P1 peak amplitude and latency between the N1 latency and 120 ms, and N2 peak amplitude and latency between P1 latency and 250 ms. MUA activation was defined as the area-under-curve (AUC) between 20 and 80 ms after stimulation, the subsequent suppression phase was defined as AUC between 90 and 300 ms. EEG or MUA data per animal were excluded from further analyses in case of an absent response to the single-VEP paradigm in the averaged traces.

Input-output paradigm

To assess the intensity dependency of VEP responses, 60 flashes of increasing light intensity between 0.01 V and 0.1 V stimulator output (~0.4 to 1.1 log cd/m^2) were presented at 2 Hz, and five flashes of increasing intensity between 0.2 and 1 V stimulator output (~1.4 to 2.2 log cd/m^2) at 0.5 Hz (Figure 2A-top). The paradigm was repeated 50 times with 20 s rest in-between blocks. Input-output (IO) curves were averaged over 50 repeats and N1 and P1 amplitude were determined as for single-VEP analysis. P1–N1 amplitudes between 0.01 V and 0.1 V light intensity

were fitted with the Naka-Rushton equation,[22] providing V_{max}, the maximum saturated amplitude, k, the semi-saturation intensity, and n, the slope of the fitted line. P1-N1 amplitudes between 0.2 V and 1 V light intensity were fitted using least-squares linear regression, providing slope and intercept (amplitude) parameters.

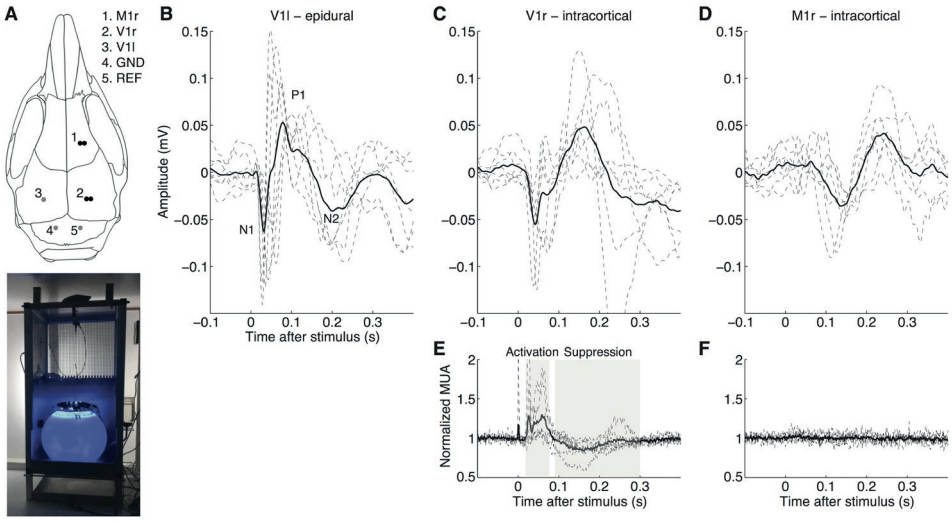


Figure 1. Approach and validation of single-flash visual evoked potential measurements in freely behaving wild-type mice. (A) Top: Electrode locations used for EEG (single epidural electrode in left V1; grey dot), or local field potential/neuronal multi-unit activity (bipolar intracortical electrodes in right V1 and M1; black dots) recordings. Bottom: Home cage with light sphere (cf. van Diepen et al., (2013) for details) for housing a mouse during VEP recordings. (B-F) Individual (dashed lines) and group-averaged (thick line) responses to 100 single flashes (presented at 1 Hz, 1 V) in visual and motor cortex. (B) EEG network responses recorded epidurally over the left visual cortex (n = 8) showing N1, P1, and N2 responses. (C) Local field potential (n = 6) and (E) baseline normalized multi-unit activity (n = 6) recorded in the right visual cortex with activation between 20 and 80 ms, and transient suppression of activity between 90 and 300 ms. (D) local field potential and (F) normalized multi-unit activity in the right motor cortex. Note the absence of time-locked neuronal (multi-unit) activity in relation to light stimulation in the motor cortex. MUA: multi-unit activity; r = right; l = left; GND = ground; REF = reference

Paired-pulse paradigm

To determine the recovery after evoked potentials, double light flashes at 13 inter-stimulus intervals between the conditioning and the test stimulus (ISI; 1000, 750,

500, 400, 350, 300, 250, 225, 200, 175, 150, 100, and 50 ms) were presented at 0.5 Hz and repeated 50 times per ISI (Figure 2C-top). The paradigm was presented at two light intensities corresponding to 0.1 V and 1 V stimulator output. Paired-pulse responses were averaged over the 50 repeats per ISI, and P1–N1 amplitude of the conditioning (Pc–Nc) and test stimuli (Pt–Nt) were determined. Recovery of the response amplitudes to the test stimuli was determined by calculating a paired-pulse response curve. Test stimuli responses were normalized to responses to the conditioning stimulus, where a ratio of 1 indicates return of the test response amplitude to the amplitude of the conditioning response. Possible habituation effects of the long duration of the paradigm on paired-pulse responses were assessed by comparing the first 100 and the last 100 conditioning responses at the 1 V stimulator output.

Habituation VEP paradigm

To assess habituation to repeated light flashes, 600 flashes were presented at 3.1 Hz. Six consecutive blocks of 100 responses were filtered (see "Single-VEP paradigm") and averaged between 50 ms prior and 250 ms after stimulation. N1 and P1 peaks were extracted, and the ratio between the P1-N1 amplitude of the 6th block and the P1-N1 amplitude of the 1st block was calculated (cf. Omland et al., 2013). A ratio below 1 indicates habituation of the 600 pulses, whereas a ratio above 1 indicates potentiation.

Frequency-chirp paradigm

To assess frequency-dependent entrainment, 'chirp' stimulation consisting of four flashes per frequency between 10 and 40 Hz with 1-Hz increments (Figure 5A-top; cf. Gantenbein et al., 2014) was repeated 25 times with 15 s rest in-between blocks. Chirp responses between 2 s prior to 8 s after the start of stimulation were subjected to Morlet wavelet analyses between 5 and 125 Hz, in 1-Hz frequency steps. Wavelet scales increased logarithmically between 3 and 10 cycles (from lowest to highest frequency). The averaged response power over all repetitions was baseline-corrected by calculating the decibel (dB) change in power relative to the mean power between 1.6 and 0.1 s prior to the start of stimulation. For each stimulation frequency, the total response power was calculated as average power between 5 and 125 Hz in the time window between the four flashes in the particular frequency plus 50 ms (cf.

Gantenbein et al., 2014). In the same time windows, we also extracted the response power at driving frequencies (EEG responses between 10 and 40 Hz) and 2nd and 3rd harmonic frequencies (responses between 20 and 80 Hz and between 30 and 120 Hz, respectively) by averaging the time-frequency response power at the frequencies between –0.5 and +0.5 Hz of each particular stimulation frequency (i.e., for driving frequencies), the stimulation frequency times two (for 2nd harmonic frequencies) or times three (3rd harmonic frequencies) (cf. Perenboom et al., 2020). Next, the mean of the total response power and the mean response power at driving frequencies and 2nd and 3rd harmonic frequencies were calculated within three frequency bands: 10–15 Hz (alpha band), 16–30 Hz (beta band), and 31–40 Hz (gamma band).

Frequency-shift paradigm

To investigate transitions between two stimulation frequencies, stimulation consisting of two blocks of flashes at 8 Hz (24 flashes) and 14 Hz (42 flashes) was repeated 50 times without rest (Figure 6A-top). Frequency-shift transition responses were subjected to Morlet wavelet analyses between 3 and 45 Hz in 0.5-Hz frequency steps. Wavelet scales increased logarithmically between 3 and 10 (from lowest to highest frequency). Time-frequency power per response was averaged over all repetitions, and the mean frequency of the response was calculated for each time point. For each stimulation frequency the averaged power in the EEG and MUA signals was determined for a –0.5- to +0.5-Hz window around stimulation frequencies and 2nd and 3rd harmonic frequencies (i.e., 7.5–8.5 Hz, 15.5–16.5 Hz and 23.5–24.5 Hz for 8-Hz stimulation; 13.5–14.5 Hz, 27.5–28.5 Hz and 41.5–42.5 Hz for 14-Hz stimulation). In addition, the average EEG power over time was normalized for each stimulation frequency (8 Hz, resp. 14 Hz) to the EEG power during stimulation at the other frequency (14 Hz, resp. 8 Hz). As an outcome, the multiplicative effect of the photic drive on EEG power for both stimulation frequencies is presented (Figure 6B). The responsivity of the visual cortex to each stimulation frequency was calculated as a difference in normalized power during stimulation at that frequency (average of 1 to 2 s after stimulation frequency onset) compared to stimulation at the other frequency (1 to 2 s after frequency switch).

Statistical analysis

Non-parametric two-sided Wilcoxon rank-sum tests were used to determine whether differences between two or multiple groups were significant. Test-retest reproducibility of IO curve readouts were compared using intraclass correlation coefficients. Intensity-specific effects of IO responses on peak amplitude and latency were tested using two-way Analysis of Variance (ANOVA) using intensity (0.01 to 1.0 V) and group (WT and mutant) as factors. Paired-pulse response recovery was tested using a two-tailed one-sample *t*-test per group using the ratio of conditioning and test stimulus amplitude versus a ratio of 1. Presence of chirp responses (mean dB change from 0) was tested as indicated for paired-pulse recovery. Multiple comparisons were corrected for with false discovery rate (FDR) using the Benjamini-Hochberg procedure. All statistical tests parameters were tested with Graphpad Prism 7 software (GraphPad Software, La Jolla, CA). *P*-values < 0.05 were considered significant.

Results

Visual evoked potentials induced by blue light specifically activate the visual cortex in freely behaving wild-type mice

Visual evoked potentials (VEPs) in response to single-flash blue light pulses, presented at suprathreshold intensity at 1 Hz, were clearly recognizable in the EEG recorded with an epidural electrode over the visual cortex of all (n = 8) freely behaving wild-type (WT) mice (Figure 1B). Averaged N1 amplitude and latency were –0.10 ± 0.03 mV at 45 ± 15 ms; P1 amplitude was 0.09 ± 0.04 mV at latency 80 ± 26 ms (in line with literature[25]), whereas N2 amplitude and latency were –0.05 ± 0.04 mV at 197 ± 40 ms. Combining neuronal MUA and local field potential data from intracortical electrodes in the visual and motor cortex (n = 6 animals; Figure 1C-F) revealed that blue light stimulation specifically activated the visual cortex (Figure 1C, E) and not the motor cortex, as no time-locked multi-unit neuronal activity was observed in the motor cortex (Figure 1F). The time-locked local field potential activity in the motor cortex (Figure 1D) was later than the light-evoked activity in the visual cortex and likely the result of volume conduction. Visual cortex neuronal activity showed an activation phase between 20 and 80 ms (AUC: 14.6 ±

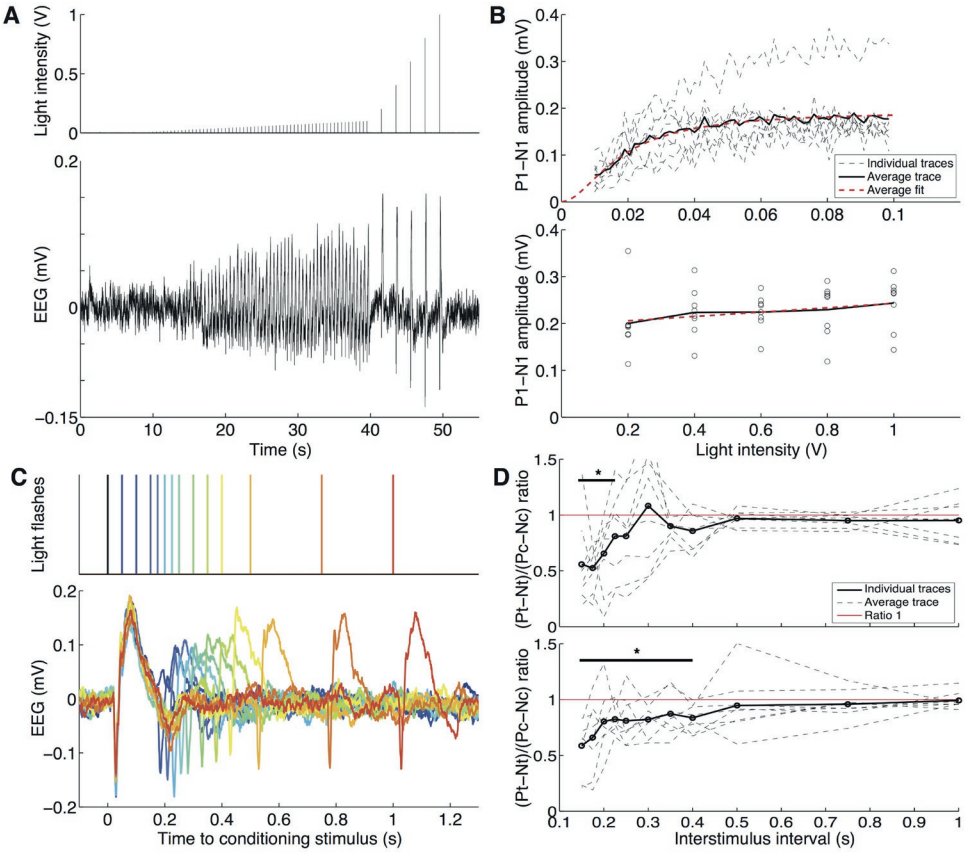
10.1 mVms followed by a suppression below baseline between 90 and 300 ms (AUC: –17.3 ± 5.6 mVms). For subsequent VEP analyses, we used the less invasive epidurally recorded EEG signals from the primary visual cortex to assess overall visual network activity changes in response to light without confounding effects of varying electrode depth. Moreover, recording epidural VEP responses may allow for a more direct comparison with human studies that use scalp EEG. Additional visual cortex measures of intracortically recorded neuronal MUA were used to provide information on cortical neuronal network changes underlying VEP features.

Visual cortex responses in wild-type mice are intensity-dependent and show paired-pulse suppression

The intensity-dependence of VEP responses and time to recover to baseline following stimulation were tested in WT mice by assessing IO and paired-pulse responses, respectively. IO responses were clearly visible in the averaged EEG data in response to light intensities ranging between 0.01 and 1 V (Figure 2A-bottom) and comparable between animals (Figure 2B). Naka-Rushton fitting of the first 60 pulses (Figure 2B-top; between 0.01 and 0.1 V) indicated a V_{max} of 0.19 ± 0.07 mV, a slope n of 2.0 ± 0.3 mV/V, and a semi-saturation intensity k of 0.02 ± 0.008 mV. Linear regression over the 5 pulses with highest intensity (Figure 2B-bottom; between 0.2 and 1 V) showed a V_{max} of 0.20 ± 0.07 mV and slope of 0.05 ± 0.07 mV/V. The amplitude parameters of IO curves were reproducible when comparing two measurements separated by ~3 hr; test-retest reliability was good for amplitude (Naka-Rushton amplitude: ICC of 0.81; linear amplitude: ICC 0.80) but poor to medium for slope (Naka-Rushton slope: ICC 0.53 and linear slope: ICC 0.37), and poor to medium for semi-saturation intensity (Naka-Rushton semi-saturation intensity: ICC 0.53). In subsequent IO curve analyses, therefore, only amplitude parameters were investigated.

Recovery from flash light stimulation at low (0.1 V) and high (1 V) intensity in WT mice was tested using a paired-pulse paradigm with interstimulus intervals between 50 and 1000 ms (Figure 2C-bottom). Test responses at intervals 50 and 100 ms overlapped with the response to the conditioning pulse and were omitted from further analyses. The total duration of the paired-pulse paradigm did not affect the responses, as the ratio of the conditioning responses of the last 100 pulses to the first 100 pulses was neither reduced nor enhanced (P1-N1 amplitude ratio: 0.96 ± 0.34),

indicating that habituation to repeated stimulation did not occur. Therefore, the averaged response per interval was calculated over all stimulation blocks. For a low stimulation intensity of 0.1 V, paired-pulse suppression was observed for intervals of 225 ms and shorter, with the response amplitude to the test pulse recovering to that of the conditioning amplitude (i.e., reaching a ratio of 1) at intervals longer than 225 ms (Figure 2D-top). At the high-intensity stimulation of 1 V, the test response amplitude showed later recovery, i.e. after 500 ms (Figure 2D-bottom). When using a VEP habituation paradigm consisting of 6 blocks of 100 repeated single-VEP stimuli at 3.1 Hz, also no habituation of P1-N1 responses was observed (block 6 to block 1 ratio: 0.83 ± 0.30), in line with the absence of habituation to conditioning stimuli observed in the paired-pulse paradigm.



Familial hemiplegic migraine type 1 mutant mice show aberrant intensity-dependent visual responses to single-pulse stimulation

To assess migraine-relevant network changes in the visual cortex, we next compared responses to the above-described paradigms between FHM1 mutant and WT mice (n = 8 per genotype) (Figure 3A). Averaged single-VEP N1 peak responses to suprathreshold stimulation at 1 Hz in mutant compared to WT mice were reduced in amplitude (WT vs FHM1: –0.10 ± 0.03 mV vs. –0.06 ± 0.08 mV; $p = 0.04$; Figure 3B) and faster (45 ± 15 ms vs. 28 ± 5 ms; $p = 0.01$; Figure 3D), but did not differ for P1 peak amplitude (0.09 ± 0.04 mV vs. 0.08 ± 0.06 mV; $p = 0.88$; Figure 3C) and latency (80 ± 26 ms vs. 75 ± 15 ms; $p = 0.70$; Figure 3E) nor for N2 peak amplitude (–0.05 ± 0.04 mV vs. –0.05 ± 0.04 mV; $p = 0.88$; Figure 3G) and latency (197 ± 40 ms vs. 184 ± 53 ms; $p = 0.49$; Figure 3H). Neuronal activity in the visual cortex of FHM1 mutant and WT mice (example MUA traces in Figure 3F) showed similar initial activation between 20 and 80 ms (AUC: 14.6 ± 10.1 mVms vs. 13.1 ± 11.2 mVms, $p = 0.81$; Figure 3I) but less neuronal suppression between 90 and 300 ms (AUC: –17.3 ± 5.6 mVms vs. –5.0 ± 5.0 mVms; $p = 0.01$; Figure 3J) in mutant mice. These data indicate a faster recovery of visual cortex activity following flash light stimulation in

Figure 2. Visual evoked potential responses in freely behaving wild-type mice show light intensity dependency with a plateau that is stable across animals, and show light-intensity dependent recovery in a paired-pulse paradigm after 225 or 500 ms. (A) Top: Light flash stimulation protocol used for generating input-output curves, consisting of 60 flashes between 0.01 and 0.1 V at 2 Hz and five flashes between 0.2 and 1 V at 0.5 Hz. Bottom: Example EEG trace showing VEP responses to increasing stimulation intensity, illustrating increasing N1-P1 peaks up to 0.1-V stimulation intensity, reaching a plateau between 0.2- and 1-V intensity. (B) Individual (dashed black line) and averaged (thick black line) P1-N1 peak amplitudes for each light intensity. Top: For stimulation between 0.01- and 0.1-V stimulation intensity; bottom: for stimulation between 0.2 and 1 V, with fitted Naka- Rushton (top; dashed red line) and least-squares linear regression lines (bottom). (C) Top: Paired light flashes were presented at 13 intervals after the conditioning stimulus (black), from blue to red: 50, 100, 150, 175, 200, 225, 250, 300, 350, 400, 500, 750, and 1,000 ms. Bottom: Example EEG traces per interval using the same color coding. (D) Top: For 0.1-V stimulation voltage, individual (dashed black) and averaged (thick black line) ratio of P1- N1 peak amplitudes in response to a test (Pt-Nt) and conditioning stimulus (Pc-Nc). Red line indicates a ratio of 1 at which the test stimulus amplitude equals that of the conditioning stimulus. Recovery of responses is present after 225 ms (difference not significant from 1). Bottom: Similar paired-pulse response ratios shown for stimulation at 1 V, showing full recovery of the test response to that of the conditioning response at 500 ms.

mutant mice. Paired-pulse conditioning and test responses, including their ratio and effect of the duration of the paired-pulse paradigm with respect to the observed suppression and recovery, did not differ between mutant and WT mice. In mutant mice, responses to the test pulse showed recovery to the conditioning amplitude for low (0.1 V) and high intensity (1 V) stimulation after about 225 and 500 ms, respectively (Figure 4A, B), similar to WT mice. The repeated conditioning stimulations in the paired-pulse paradigm did not result in habituation of the VEP responses in FHM1 mice (P1-N1 amplitude ratio WT vs. FHM1: 0.96 ± 0.34 vs. 0.98 ± 0.55; $p = 0.96$). Also for the stimulation paradigm of six blocks of 100 pulses at 3.1 Hz, in FHM1 mice no habituation was observed, similar as was observed for WT mice (block 6 to block 1 ratio, WT vs. FHM1: 0.83 ± 0.30 vs. 1.53 ± 1.25; $p = 0.23$).

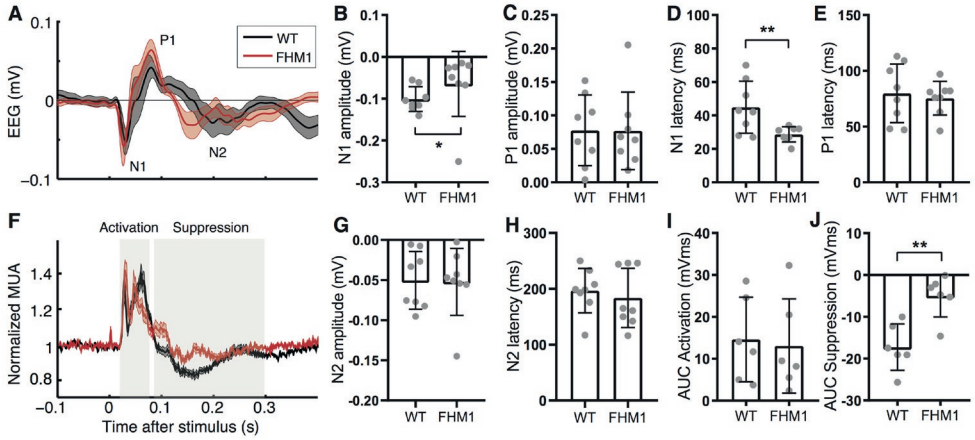


Figure 3. Single-flash VEP responses differ between familial hemiplegic migraine type 1 (FHM1) mutant and wild-type (WT) mice. (A) Average traces (mean with the shaded standard error of the mean) of epidural recordings over the visual cortex in WT (black) and FHM1 (red) mice. (B-E) Individual and mean amplitude and latency for N1 and P1 peaks (n = 8 mice per group). (B) N1 amplitude is smaller in FHM1 (significance indicated: *p = 0.04). (C) P1 amplitude is similar. (D) N1 latency is shorter in FHM1 (**p = 0.01). (E) P1 latency is similar. (F) Average traces of intracortical neuronal MUA recordings over the visual cortex of WT (black) and FHM1 (red) mice. (G, H) Individual and mean amplitude and latency for N2 peak (n = 8 mice per group). (G) N2 amplitude is similar. (H) N2 latency is similar. (I, J) Individual and mean area-under-curve (AUC) for two phases of the neuronal response to visual stimulation (n = 6 mice per group). (I) AUC for initial activation (between 20 and 80 ms) is similar between groups. (J) AUC for suppression (between 90 and 300 ms) is smaller in FHM1 mice (**p = 0.01). Error bars in B-E and G-J show standard deviation.

With respect to IO responses, the maximum P1–N1 amplitude was reduced in FHM1 mutant compared to WT mice for low (0.19 ± 0.07 mV vs. 0.11 ± 0.03 mV; p = 0.0002; Figure 4C), as well as higher stimulation intensities (0.20 ± 0.07 mV vs. 0.14 ± 0.05 mV; p = 0.03; Figure 4C). Also, N1 peaks were of smaller amplitude and had a shorter latency in mutant mice, as was also the case for the P1 peaks (all p < 0.001 for group effects between WT and mutants). Intensity-specificity of the effect was observed for N1 peak amplitude, and P1 peak amplitude and latency (intensity effect: all p < 0.001). Only for N1 amplitude, an interaction effect was present between group and intensity (p = 0.003), with post hoc tests showing differences between WT and mutants for stimulation intensities of 0.02 V and higher (all p < 0.01 with *post hoc* FDR correction). Such interaction effects per intensity level were not observed for N1 latency nor for P1 peak amplitude and latency (interaction group and intensity: all p > 0.88). Mutant mice, compared to WT mice, showed similar local visual cortex neuronal activity levels during the N1 peak during activation (not shown; p = 0.27), albeit with less suppression afterwards (p < 0.001). The intensity-dependence of the MUA data was similar for mutants compared to WT mice (interaction effects: p > 0.99). Our findings reveal that FHM1 mutant mice displayed lower VEP amplitude and a shorter latency, with reduced neuronal suppression, following single-flash stimulation over a range of stimulation intensities.

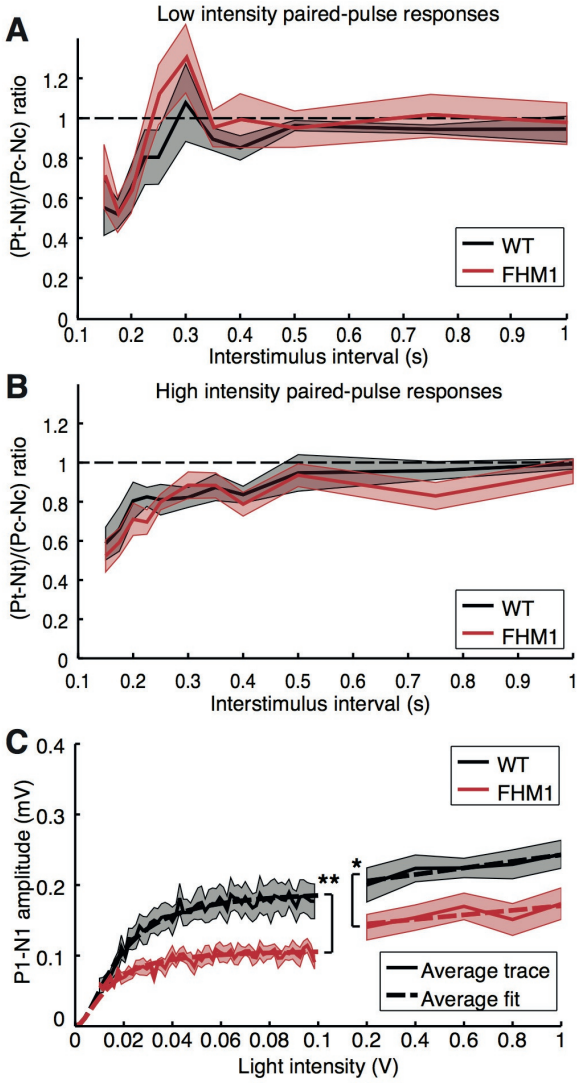


Figure 4. Decreased visual evoked potential input-output (IO) responses, but similar paired-pulse responses, in familial hemiplegic migraine type 1 (FHM1) mutant compared to wildtype (WT) mice. (A) Averaged mutant (red; n = 8) and WT mice (black; n = 8) responses to paired-pulse stimulation at low intensity (0.1 V) reveal recovery in both genotypes after 225 ms. Dashed line indicates recovery to baseline, at a ratio of 1. (B) Paired-pulse responses at high intensity (1 V) show recovery at 500 ms, for both mutant and WT mice. (C) IO curves show lower VEP amplitude in mutant compared to WT mice in response to stimulation between 0.01- and 0.1-V (significance indicated: $^{**}p = 0.005$) and between 0.2- and 0.1-V ($^{*}p = 0.028$) stimulation. Dashed lines indicate averaged Naka-Rushton fit for lower stimulation intensities and averaged least-squares fit for higher intensities. All plots: mean and standard error of the mean (patched).

Familial hemiplegic migraine type 1 mutant mice show enhanced beta-gamma band power during chirp stimulation

To assess changes in frequency dependency of VEP responses in FHM1 mutant mice, photic driving of visual cortex responses was tested using chirp stimulation (Figure 5A). Validation of this paradigm in WT mice revealed that the averaged EEG response showed frequency-following between 10 and 25 Hz (Figure 5A-middle), indicating a photic drive phenomenon. Higher-order responses at multiples of the stimulation frequencies were also present (Figure 5B). The mutant mice showed photic drive in response to chirp stimulation between 10 and 40 Hz (Figure 5A-bottom). Non-baseline-corrected EEG response power did not differ between mutant and WT mice (Figure 5C), indicating that differences in chirp responses are not due to altered EEG spectra. Mutant mice showed an increased overall EEG power during stimulation in the 31–40 Hz gamma band (Figure 5D; $p = 0.028$) but not in the 10-15 Hz alpha ($p = 0.80$) or 16-30 Hz beta ($p = 0.10$) bands, compared to WT mice. Analysis of the separate EEG response power at the driving and harmonic frequencies revealed an increased response power in mutant mice in the gamma band for driving ($p = 0.021$) and 2nd harmonic ($p = 0.038$) frequencies, but not for 3rd harmonic frequencies ($p = 0.16$). In addition, EEG response power was enhanced in the beta frequency range for the driving ($p = 0.028$), but not 2nd harmonic ($p = 0.083$) or 3rd harmonic ($p = 0.57$) frequencies. Local visual cortex neuronal MUA did not display a clear driving or harmonic response above 15 Hz; for the alpha band MUA response no group difference was observed ($p = 0.10$). Enhanced photic drive in the EEG beta and gamma band in response to chirp stimulation in mutant mice is in line with findings in migraine patients,[19] and suggestive of hyperresponsivity of the visual system.

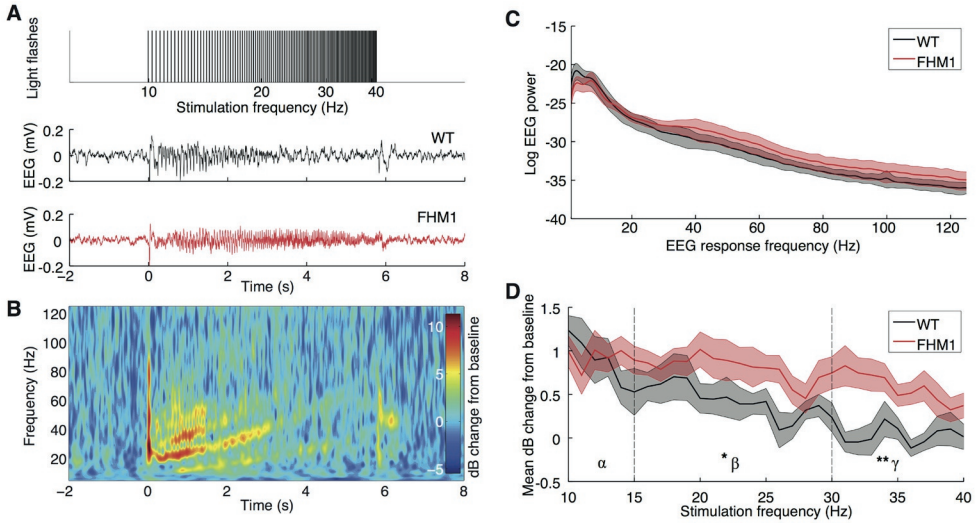


Figure 5. Chirp-stimulation-induced "photic drive" is more pronounced in the EEG beta-gamma bands of the visual cortex in familial hemiplegic migraine type 1 (FHM1) mutant compared to wild-type (WT) mice. (A) Top: Stimulation at increasing frequencies between 10 and 40 Hz in 1-Hz steps, with four light flashes per frequency, is used to generate a chirp stimulation of ~6 s. Example traces of the averaged EEG response to the chirp stimulation paradigm of a WT (middle) and a mutant (bottom) animal. (B) Time-frequency domain representation of averaged and baseline-corrected EEG responses of the WT example trace. Baseline correction was performed over the averaged trials by calculating the log10 decibel (dB) change with respect to EEG activity 160 to 10 ms prior to stimulation onset. Note the presence of higher-order responses, especially the second harmonic (20 to 80 Hz), up to halfway the chirp stimulation at 3 s. (C) Power spectral density of the non-baseline-normalized EEG response power, showing similar EEG spectra for WT and mutant mice. (D) Averaged EEG response power between 5 and 125 Hz for each stimulation frequency is enhanced in mutant mice in the gamma band (30–40 Hz) compared to WT mice but not in the alpha (10–15 Hz) and beta (15–30 Hz) bands (significance indicated: $^{**}p = 0.028$). EEG response power at the driving frequencies in the beta band is enhanced for mutant mice in the beta band ($^{*}p = 0.028$), whereas response power at driving and 2nd harmonic frequencies is increased in the gamma band (driving: $p = 0.021$; 2nd harmonics: $p = 0.038$; see text for details).

Familial hemiplegic migraine type 1 mutant mice show enhanced VEP amplitude during 14-Hz stimulation in a frequency-shift paradigm

We next assessed the visual system dynamics in FHM1 mutant and WT mice using a novel frequency-shift paradigm with alternating frequencies of 8 and 14 Hz (i.e., below and in the alpha band range). The frequency-shifted transition between 8 and

14 Hz in WT mice (Figure 6A-bottom) was parameterized by two amplitude shifts in the normalized EEG-following response (drop in 8-Hz power upon transition from 8 to 14 Hz: 1.50 ± 0.85; drop in 14-Hz power upon transition from 14 to 8 Hz: 0.16 ± 0.31. Mutant mice showed an enhanced response power to 14-Hz stimulation compared to WT mice (drop in 14-Hz power: 0.77 ± 0.44; p = 0.005; Figure 6B-bottom), while the response to 8 Hz was similar between genotypes (drop in 8-Hz power: 0.91 ± 0.55; p = 0.19; Figure 6B-top). Amplitude drops in the normalized visual cortex MUA were not different between genotypes (8 Hz: p = 0.94; 14 Hz: p = 0.13; data not shown).

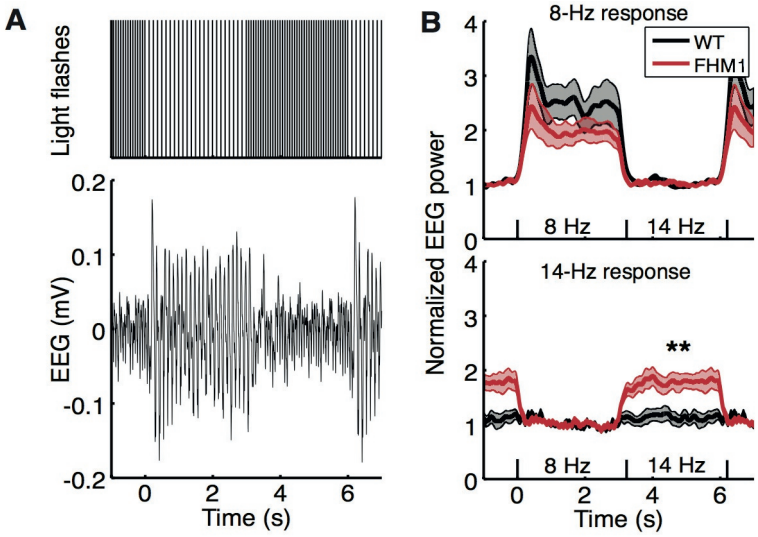


Figure 6. Stronger response to 14-Hz stimulation in the frequency-shifted paradigm in familial hemiplegic migraine type 1 (FHM1) mutant compared to wild-type (WT) mice. (A) Top: Frequency-shifted stimulation paradigm consisting of alternating blocks of 24 flashes at 8 Hz (duration: 3 s) and blocks of 42 flashes at 14 Hz (duration: 3 s). Bottom: Example trace of the averaged EEG response to the frequency-shifted stimulation paradigm. (B) Top: Normalized EEG power at 8 Hz in response to the frequency-shifted paradigm shows a similar response and rest pattern in WT and mutant mice. Bottom: Normalized EEG power at 14 Hz shows increased response to 14 Hz stimulation in mutant mice compared to WT (significance indicated: **p = 0.005).

Discussion

Here we investigated visual system responsivity to existing and novel flash-VEP paradigms in freely behaving mice to gain insight in mechanisms underlying visual sensitivity, particularly in the context of migraine. VEP responses were first assessed in WT mice, which showed time-locked neuronal activation – as indicated by local multi-unit activity (MUA) responses – in the visual cortex and intensity-dependence. Compared to WT animals, FHM1 mutant mice carrying the R192Q missense mutation in the α_{1A} subunit of $Ca_V2.1$ channels displayed: (i) shorter latency of the VEP N1, as well as lower VEP N1 amplitude followed by less pronounced neuronal suppression, in response to single-flash stimulation over a range of light intensities, (ii) enhanced EEG photic drive for the beta (15–30 Hz) and gamma (31–40 Hz) frequency bands in response to visual 'chirp' stimulation, and (iii) enhanced power in the EEG response to 14-Hz stimulation. Together these findings indicate frequency-dependent enhancement of visual system responsivity in FHM1 mutant mice. The findings are in line with observations that functional effects of the FHM1-related gain of $Ca_V2.1$ channel function across cortical (and other) regions may be context-dependent, due to dynamic disturbances in the balance between neuronal excitation and inhibition.[33,41]

By combining EEG and local field potential with intracortical neuronal MUA recordings, we could obtain direct information on visual cortex neuronal activity, thus complementing standard VEP approaches using only EEG or local field potentials. We could thereby distinguish between local (based on MUA responses) and global (based on EEG responses) cortical network interactions in response to the different visual stimulation paradigms. A previous study in anaesthetized mice indicated that VEPs largely reflect visual cortex activity and thus can be used as measure of visual cortex responsivity to light.[22] The time-locked MUA confined to the visual cortex during local field responses in our study demonstrates specificity of the flash stimulations to activate the visual cortex, in accordance with MUA data from anaesthetized mice.[42] While we used blue light flashes, the shape, and characteristics of our single-pulse flash VEPs in WT mice had similar intensity-dependent peak amplitudes and latencies as reported studies in freely behaving mice in which white light flashes were used.[24–26] Blue light flashes have been used earlier

for VEP studies in anaesthetized rats,[43,44] and mice,[22] as well as in freely behaving mice in which effects of light-dark shifts on neuronal activity in the suprachiasmatic nucleus were investigated.[38] Paired-pulse VEP responses showed intensity-dependent suppression, at low-intensity stimulation for intervals up to 225 ms (i.e., above 4.4 Hz) and at high-intensity stimulation up to 500 ms (i.e., 2 Hz). Differences with paired-pulse VEP data from anaesthetized mice, for which suppression occurred for intervals up to 1,000 ms (i.e., 1 Hz)[21] are likely due to slowing down of visual evoked potential components by anaesthesia.[26]

In migraine patients, lack of habituation to visual pattern stimulation is an often (e.g., de Tomasso et al.[7]) but not always (e.g., Omland et al.[45]), reported feature distinguishing patients from controls. A paired-pulse paradigm in migraine patients showed lack of paired-pulse suppression in the 80-130 ms interval range,[13] but longer intervals, as we presented in mice, were not studied. In our mouse experiments, we observed paired-pulse suppression for both the WT and FHM1 mutant groups for intervals between 75 and 150 ms, as well as for longer time intervals. Both lack of habituation and lack of paired-pulse suppression have been attributed to cortical hyperexcitability or 'hyper-responsitivity',[5,13] but to our knowledge a possible mechanistic link between the two experimental observations has not been studied directly. Contradictory findings supporting hyper- or hyporesponsivity across patient studies might be due to various factors: (i) in which phase of the attack the patient is when being investigated, (ii) features of the stimulation paradigm such as the (spatial) frequency, and (iii) differences in readout parameters.[10,45] In our study, the clinically used habituation paradigm (consisting of 600 flashes at 3.1 Hz) did not result in habituation of the P1–N1 component in WT or FHM1 mutant mice; neither did the longer paired-pulse paradigm involving 650 paired flashes at 0.5 Hz. In awake restrained rats, using five blocks of 50 repeated single-pulse light flashes at 1 Hz, for N1 and P1 components no habituation but potentiation was observed, that was influenced by dark- or light-adaptation; habituation was evident though for the later P2 VEP component.[46] In anaesthetized mice, local post-synaptic potentials in the visual cortex showed rapid habituation to 4 Hz light flashes after the first of 10 pulses, that where stable for later pulses.[47] To allow comparison to the clinical studies, we averaged over blocks of 100 pulses without investigating possible short-term habituation changes to single light flashes. To further study habituation to

visual stimuli in freely behaving (mutant) mice, it will be useful to test other paradigms including effects of prior dark- or light adaptation and investigate both short-term and longer term changes.

To better capture dynamic changes in cortical excitability two frequency-dependent visual stimulation paradigms (i.e., chirp and frequency-shift) were used, for the first time in mice. Visual chirp stimulation has been used to discriminate between migraine patients and controls.[19,20] We showed the applicability of this paradigm to freely behaving mice by the presence of EEG-following and higher harmonic responses for stimulations between 10 and 25 Hz. The frequency-shift paradigm around the alpha band (with a shift between 8 and 14 Hz) revealed entrainment of the lower (8 Hz) but not the higher (14 Hz) frequency in WT mice. Lower responsivity to 14-Hz stimulation in WT mice was also evident in the chirp response, which showed a dip around 14-15 Hz. This frequency-dependency might relate to the "critical flicker frequency", i.e., the maximum stimulation frequency at which EEG-following responses can be measured.[48] For mice, this maximum was estimated between 7 and 9 Hz for flash VEPs,[22] and around 12 Hz for pattern VEPs.[49] Visual frequency-following responses up to 15 Hz have been related to thalamo-cortical interactions.[50] The EEG-following response above 14–15 Hz, i.e., up to 25 Hz with even higher harmonics, in our experiments, likely reflects global (including thalamic interactions) rather than local cortical network activity.[16] This is further supported by the observation that local visual cortex neuronal activity did not follow chirp stimulation above ~15 Hz.

The shorter N1 latency observed for single-VEP and input-output paradigms in FHM1 mutant mice suggests hyperresponsivity of the visual system in mutants, likely as the result of genetically enhanced neuronal glutamatergic transmission. Earlier studies showed effects on enhancing excitability of the R192Q mutation in cortical neuronal cultures,[32] sensorimotor cortex brain slices in vitro,[31] and hippocampus in anesthetized mice in vivo.[51] The reduced suppression of neuronal activity following single-VEP N1 responses suggests faster recovery of visual cortex activity following stimulation. Faster recovery of neuronal activity following stimulus-related synaptic depression was also observed in brainstem slices of FHM1 mutant mice, which was hypothesized to be linked with enhanced presynaptic residual calcium levels.[52]

VEP P1-N1 amplitude responses were highly repeatable in both WT and mutant mice, as shown by input-output curve retests, whereas, in humans, VEP features can show profound temporal fluctuations.[53] The reduction of N1 VEP amplitude in mutant mice was accompanied by levels of local neuronal MUA that did not differ from that in WT animals. While VEPs are local field potentials reflecting activity within a brain region spanning at least hundreds of micrometers, the underlying MUA reflects extracellular spike activity of groups of neurons directly surrounding the tip of the electrode, dominated by activity from large (pyramidal) excitatory neurons.[42,54,55] This suggests that the reduced VEP N1 response in mutant mice is likely caused by stronger recruitment of inhibitory neurons, also of more distantly located neurons. Enhanced inhibitory recruitment was previously implicated for the somatosensory cortex in brain slices of FHM1 mutant mice.[32] The apparent conflicting observation of reduced neuronal suppression following N1 suggests that inhibitory networks contributing to this suppression phase are distinct from those impacting the initial N1 response.

For the VEP N2 component, amplitude and latency did not differ between FHM1 and WT mice. In migraine patients, using pattern-VEP stimulation, N2 amplitude was reported to be enhanced,[56] and latency prolonged,[57] which may explain aversive responses of migraineurs to specific patterns and frequencies of light.[56,57] Since early and late N2 components are proposed to reflect distinct visual system responsivity to contour and luminescence,[56–58] respectively, pattern stimulation may reveal whether similar N2 changes exist for FHM1 mice.

Given the complexity of the neuronal networks involved in sensory evoked responses, it is not surprising that effects of mutated $Ca_V2.1$ channels on VEP responses are not identical for the different VEP features and paradigms. For instance, possible brain region-specific effects of the R192Q mutation may also explain the absence of a difference between FHM1 and WT mice for evoked response features following somatosensory stimulation.[59] Hence, network-specific changes in excitability may contribute to variable reports on hypo- versus hyperresponsivity in patient studies with different experimental designs.[10] Regardless, extrapolation of findings from mouse studies to the human situation needs to be done with great caution, not only because of species differences, but also

because findings from hemiplegic migraine may not extend to the common forms of migraine.

We observed a clear effect of the FHM1 mutation on frequency-following responses using visual chirp stimulation, whereby mutant mice were able to follow the chirp frequency stimulation up to 40 Hz, compared to a maximum of 25 Hz in WT mice. The increased EEG response power in the beta-band (15–30 Hz) and lower gamma-band (30–40 Hz) following chirp stimulation is in line with an enhanced beta-gamma band response (18–26 Hz) reported interictally in migraine patients.[19] For patients, enhanced gamma-power reported for pattern VEP responses was proposed to reflect dysfunctional thalamocortical connectivity.[60] $Ca_V2.1$ Ca^{2+} channels were shown to play a key role in thalamocortical gamma-oscillatory activity in mice, as evidenced from in vitro and in vivo experiments in mice lacking $Ca_V2.1$ channels, for which EEG data showed strongly reduced gamma-band power.[61] This suggests that the enhanced beta-gamma power for FHM1 mutant mice in the chirp experiments may reflect enhanced thalamocortical excitability. A role of network interactions outside the visual cortex is reinforced by the absence of local neuronal activity above 15 Hz during chirp stimulation while EEG photic drive remained present. Mutant mice also showed stronger responses to 14-Hz stimulation in the frequency-shift paradigm (with shifts between 8 and 14 Hz). Together, these findings indicate more pronounced frequency-following features in response to light flashes in FHM1 mutant compared to WT mice, that may reflect visual system hyperexcitability in the mutant mice.

Transgenic FHM1 mutant mice can be used to unravel mechanisms underlying migraine susceptibility that are difficult to study in humans, whereby we consider VEPs a powerful translational tool to assess migraine-related changes in visual network responsivity. The paradigm-specific alterations in visual network responsivity we observed in the present study indicate frequency-dependent enhancement of visual system excitability in FHM1 mutant mice. This may help understand how sensory hypersensitivity is brought about in migraine patients. The possibility to use VEPs in longitudinal studies, in freely behaving animals, thereby yields novel opportunities for translational studies on mechanisms and effects of attack-modulatory triggers or migraine drugs.

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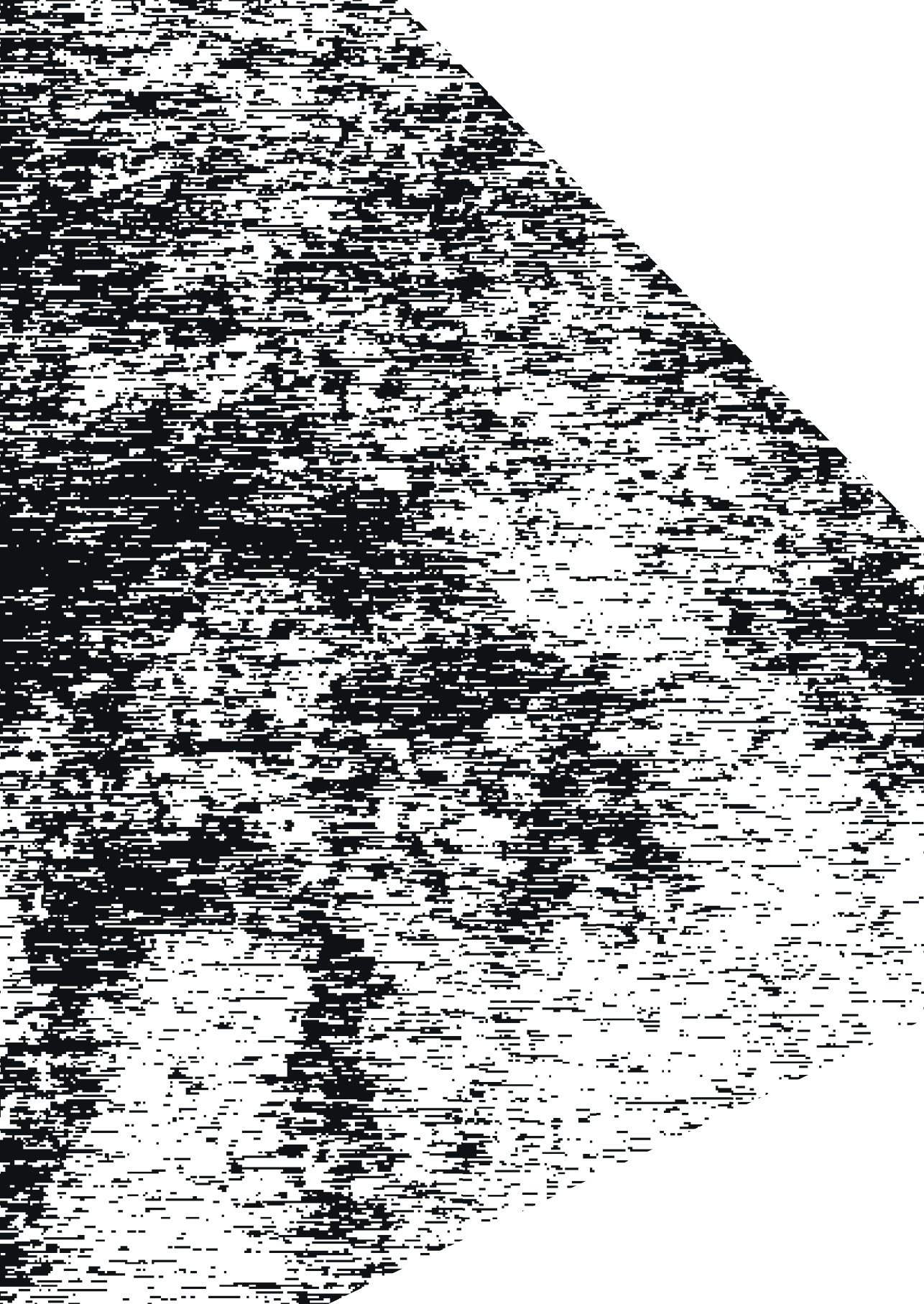
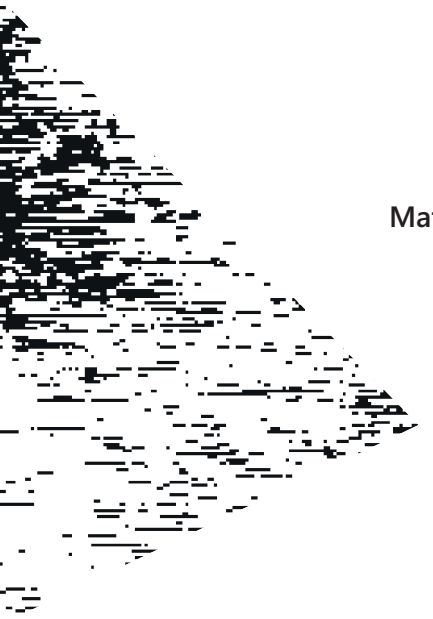


Chapter 4

Enhanced pre-ictal cortical responsivity in migraine patients assessed by visual chirp stimulation



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Abstract

Migraine is associated with altered sensory processing and cortical responsivity that may contribute to susceptibility to attacks by changing brain network excitability dynamics. To gain better insight into cortical responsivity changes in migraine we subjected patients to a short series of light inputs over a broad frequency range ("chirp" stimulation), designed to uncover dynamic features of visual cortex responsivity.

EEG responses to visual chirp stimulation (10–40 Hz) were measured in controls (n = 24) and patients with migraine with aura (n = 19) or migraine without aura (n = 20). Average EEG responses were assessed at (i) all EEG frequencies between 5 and 125 Hz, (ii) stimulation frequencies, and (iii) harmonic frequencies. We compared average responses in a low (10–18 Hz), medium (19–26 Hz) and high (27–40 Hz) frequency band.

Responses to chirp stimulation were similar in controls and migraine subtypes. Eight measurements (n = 3 migraine with aura; n = 5 without aura) were assigned as "pre-ictal", based on reported headache within 48 hours after investigation. Pre-ictally, an increased harmonic response to 22–32 Hz stimulation (beta band) was observed ($p < 0.001$), compared to interictal state measurements.

We found chirp responses to be enhanced in the 48 hours prior to migraine headache onset. Visual chirp stimulation proved a simple and reliable technique with potential to detect changes in cortical responsivity associated with the onset of migraine attacks.

Introduction

Migraine is a common paroxysmal brain disorder characterized by recurrent disabling attacks of severe headache with associated features such as nausea, vomiting, and enhanced sensitivity to sound and light.[1] It remains an enigma exactly why and when attacks strike. It has been suggested that the initiation of an attack may involve variations in cortical responsivity to sensory inputs such as light,[2,3] presumably as result of fluctuations in cortical excitability.[4] Such dynamics in cortical responsivity may provide functional biomarkers of relevance for attack prediction. There is evidence pointing to the visual cortex as an area of the brain where changes in cortical responsivity in migraine are most apparent. Responsivity to light in migraineurs was particularly enhanced for the visual cortex as assessed in neuroimaging studies,[5,6] and in some was reported to be most pronounced for migraine with aura.[7,8]

Cortical responsivity to light can be assessed by frequency-specific steady-state stimulation, using a series of flash light stimulation.[9] When combined with electroencephalography (EEG), the phenomenon of 'photic driving' is observed, which is the frequency-following response measured by EEG at the visual cortex. Photic driving is not only evident as the EEG response in the range of the stimulated frequencies, but also occurs at multiples of these frequencies, the so called higher-order 'harmonics'.[9] Using steady-state visual stimulation in between attacks, some studies (but not all[10]) reported enhanced photic driving for different stimulation frequencies in migraine patients,[6,11–14] and displayed enhanced harmonic activity that could result from altered cortical excitability.[15,16]

Changes in photic driving may relate to attack initiation, since frequency-following responses to flash light stimulation at 12 Hz were found to increase prior to the headache phase.[10] The use of relatively long stimulation series at different frequencies, however, make steady-state stimulation less suitable for assessing dynamic changes in frequency-dependent cortical responsivity over the migraine cycle. To this end, we set out to investigate responses in migraine patients to a short 'visual chirp' stimulation paradigm, from which the visual cortex EEG response at driving and harmonic frequencies can be assessed within a very short time period. Visual chirp stimulation is a quick and easy-to-apply paradigm to assess photic

driving which uses a single, short-duration, flash light stimulation paradigm consisting of increasing stimulation frequencies within a 6-second period.[17] When visual chirp stimulation was applied interictally in migraine patients without aura, responses were found to be more pronounced compared to controls, for stimulation frequencies between 18 and 26 Hz.[18] Given the association between migraine with aura and visual cortex responsivity,[7,8] we here aimed to assess visual chirp responses in the two main migraine subtypes. High-density EEG was used to test the specificity of cortical responses to chirp stimulation by determining the optimal recording location above the visual cortex. In addition, we compared interictal and pre-ictal recordings to investigate whether cortical responsivity to chirp stimulation may change towards an upcoming attack.

Methods

Participants, aged 18 to 65 years, were recruited from our Leiden University Medical Center Migraine Neuro Analysis (LUMINA) database.[19] Pre-screened non-headache controls and patients with migraine with aura or migraine without aura were included in the study. Exclusion criteria for all participants were: (i) psychiatric or neurological disorder (except migraine for participants with migraine); (ii) use of chronic medication (other than oral contraceptives), including migraine prophylactics, in the four weeks preceding the measurements; (iii) a history of malignancy. Patients with migraine were diagnosed according to the ICHD-III-beta criteria,[1] and were to have an attack frequency of at least one attack per month, for the six months prior to the measurement day. Controls, and their first-degree relatives, were not allowed to have migraine or any form of trigeminal autonomic cephalalgia. In addition, controls were not allowed to have any other form of headache on more than one day per month. Patients were contacted by telephone interview at least three days after the experiment to verify migraine status at the time of measurement. A measurement was considered interictal in case the participant was measured at least three days after the last migraine attack and three days before the next attack. A measurement was *a priori* defined as pre-ictal (i.e., before the onset of headache) when the measurement was performed within 72 hours before the next migraine attack. In the actual measurements, the pre-ictal group had received EEG

recordings between 0.5 to 48 hours prior to the migraine attack. The Medical Ethics Committee of the Leiden University Medical Center approved this study and all participants provided written informed consent.

Experimental protocols

All participants underwent EEG recordings during visual flash stimulation. Two experimental setups (occipital and cortex-wide) were used to record potentials in different experiments. Occipital responses were recorded with seven Ag-AgCl electrodes placed at 10-20 locations; that is, Fz, Cz, C3, C4, Oz, O1 and O2, and online referenced to electrodes at C3 and C4 (EEG-1200; Nihon Kohden, Tokyo, Japan). Data were sampled at 1,000 Hz and online band-pass filtered between 0.08 and 300 Hz. Cortex-wide responses were recorded with high-density-EEG cap using 126 Ag/AgCl electrodes (WaveGuard; ANT, Enschede, The Netherlands) arranged according to the 10-5 system. Data were recorded with a common average reference and sampled at 2,048 Hz using the 126-channel Refa system (TMSi, Oldenzaal, The Netherlands). A separate ground electrode was placed at the left mastoid, while cap mastoid electrodes at M1 and M2 were left unconnected. All recordings were performed at the Department of Clinical Neurophysiology of the Leiden University Medical Center between 9 a.m. and 5 p.m.

Participants lay on a bed with their eyes closed in a darkened room. Spontaneous EEG was recorded for ~10 minutes before visual stimulation started. Binocular red-light LED goggles (Synergy Plinth; Medelec International, Pleasanton, CA, USA) with a light intensity of 2.64 log cd/m^2 (438 lux) at wavelength 654 nm were controlled via custom-written scripts in Matlab (Mathworks, Natick, MA, USA). Goggles were placed on both eyes and taped to the temples on both sides of the head. Chirp stimulation consisted of single-flash stimuli with an increasing frequency between 10 and 40 Hz in 1-Hz incrementing steps, according to Gantenbein et al.[18] At each frequency, four flashes were presented, resulting in 124 flashes and stimulation duration of 5.7 seconds (Figure 1A). In total, 10 repetitions were presented at inter-repetition intervals of 10 to 15 seconds. Trigger pulses at the start of each chirp repetition were simultaneously recorded for post-processing.

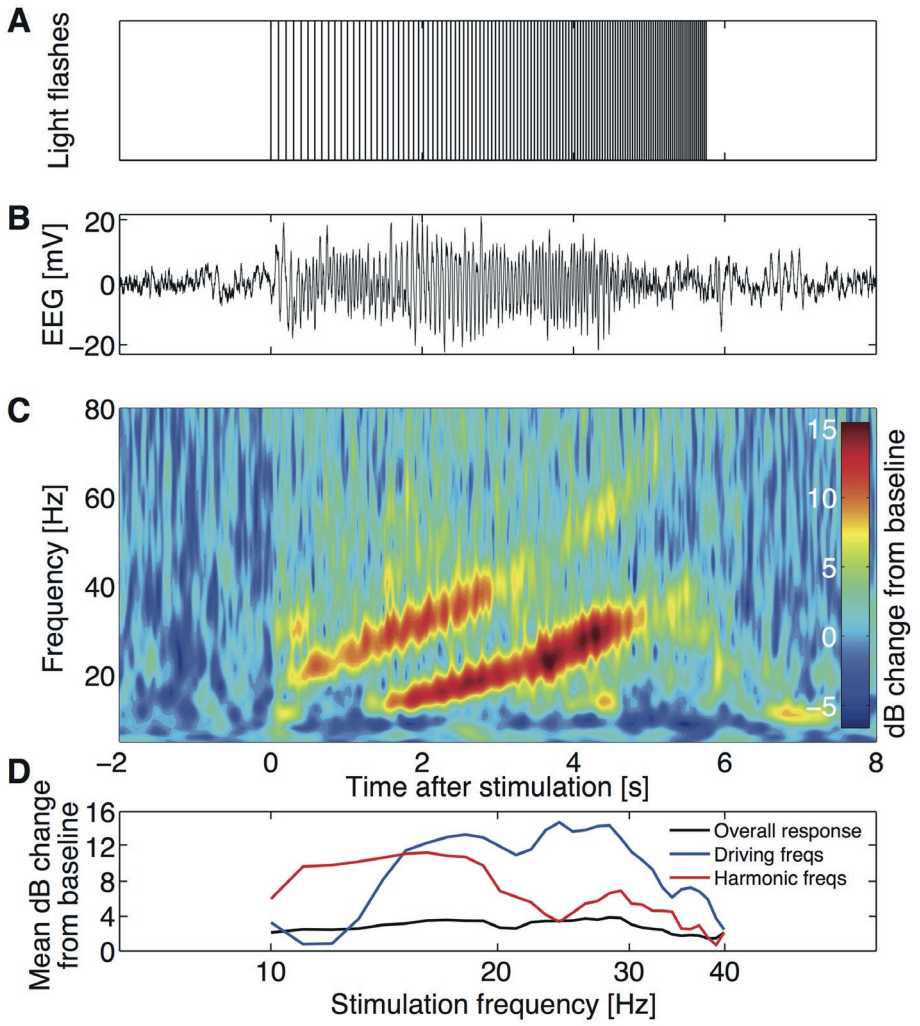

Figure 1. (A) The chirp stimulus consisting of four light flashes per frequency between 10 and 40 Hz resulting in a total duration of ~6 seconds. (B) Example trace of an averaged EEG response (average of 10 responses) at electrode Oz of a control subject. (C) Time-frequency representation of the averaged response with baseline correction, displayed as decibel (dB) change from baseline. Distinct responses at the driving frequency (between 10 and 40 Hz) and at the harmonic frequencies (between 20 and 80 Hz) are present. (D) Example trace of the mean dB change in overall power (response at 5–125 Hz; black line), driving frequencies (response at stimulation frequency; blue line) and harmonic frequencies (responses at twice the stimulation frequency; red line) from baseline per stimulation frequency. Responses are analyzed with respect to EEG power per frequency for the duration of the four flashes plus 100 milliseconds afterwards, for the overall response, driving and harmonic frequencies.

Data pre-processing and analysis

All data analyses were performed in Matlab (Version R2013b), performed independently by two researchers that were blinded to group assignment. The EEG response to chirp stimulation was processed per repetition, from 2 seconds before to 8 seconds after stimulation onset. Time-frequency (TF) spectra were calculated using morlet wavelets between 5 and 125 Hz in 1-Hz incrementing steps with wavelet cycles logarithmically increasing between 3 and 10 cycles for the lowest and the highest frequency as time-frequency accuracy trade-off. Spectra were averaged over repetitions and mean baseline power per frequency was calculated between 1.6 and 0.1 seconds before stimulation onset (Figure 1C). The stimulation response per participant was dB-converted with respect to mean baseline power. For each stimulation frequency between 10 and 40 Hz (31 frequencies), response power over all frequencies (between 5 and 125 Hz) was averaged in a predefined time window, resulting in 31 total power values per participant (Figure 1D). The time window used depended on the stimulation frequency, and consisted of the time period between the starts of subsequent four flashes plus 100 milliseconds, to take into account possible after-effects. The distinct response components at driving frequencies (EEG responses between 10 and 40 Hz) and harmonic frequencies (responses between 20 and 80 Hz) were analysed separately by averaging the TF response power at the frequencies between –1 and +1 Hz of the driving frequency, and at the stimulation frequency times two ('harmonic frequency').

Three frequency bands of interest were defined based on previous work[18]: (i) stimulation frequencies between 10 and 18 Hz (low frequencies); (ii) frequencies between 19 and 26 Hz (medium frequencies); and (iii) frequencies between 27 and 40 Hz (high frequencies). Averages were calculated within these bands based on overall power (5–125 Hz) and for driving and harmonic frequencies separately.

To determine the electrode showing the strongest response relative to noise level, the signal-to-noise ratio of the high-density EEG recordings was calculated for each of the 126 electrodes. Per electrode, the power between 5 and 45 Hz of the averaged chirp response (calculated by Fast Fourier Transform) was divided by the variance of the frequency domain response, and scaled by the number of repetitions,[20] to study the distribution of the overall response power over the cortex. The specific topographic distribution of the response at driving and harmonic frequencies was

also studied. For each electrode, the overall response amplitude was calculated separately for the driving frequency and the harmonic frequencies by summation of the photic driving response per frequency.

Statistical analysis

Test-retest reliability was calculated using the intraclass correlation coefficient (ICC; model ICC(2,1)) per outcome variable. Spearman's correlations examined the shared association between repeated experimental sessions. Between-group differences per outcome variable (mean dB-change from baseline, for low, medium and high frequencies) were analyzed using one-way Analyses of Variance (ANOVA) with three groups: (i) controls, migraine with aura (interictal), and migraine without aura (interictal); or (ii) controls, interictal migraine, and pre-ictal migraine. To examine a possible effect of time of day and gender on the results of the two interictal migraine groups (with and without aura) and controls, a three-way ANOVA was conducted additionally, including interactions between the three main factors (time of day (am/pm), gender (male/female) and group (control, migraine with aura interictal, and migraine without aura interictal). As each frequency band was analyzed independently, results were considered significant after compensating for multiple comparisons ($p = 0.05/3 = 0.017$). *Post hoc* analyses with respect to specific frequency responses were carried out with Bonferroni correction, with results considered significant at the 5% level ($p < 0.05$). The relationship between specific frequency responses – determined using post hoc analyses – and the number of days between the measurement and attack onset was tested using linear regression with four groups: interictal migraine, and three pre-ictal migraine groups (measured either 2 days before, 1 day before, or on the same day as the migraine attack). Statistical analyses were conducted in SPSS version 25 for Windows (IBM, Armonk, NY, US).

Results

EEG responses to visual chirp stimulation (Figure 1A) were measured in controls and migraine patients to investigate visual cortex responsivity to light inputs over a broad frequency range. A total of 100 measurements with chirp stimulation were conducted in 63 participants (controls (n = 24), migraine without aura (n = 20),

migraine with aura (n = 19)) (Table 1). All participants showed clear EEG photic driving in response to chirp stimulation (see example in Figure 1B).

Table 1. Baseline characteristics of controls and migraine subgroups.

	7-channel recordings			126-channel recordings		
Variable	Controls (n = 17)	Migraine without aura (n = 20)	Migraine with aura (n = 19)	Controls (n = 15)	Migraine without aura (n = 9)	Migraine with aura (n = 6)
Female (N (%))	14 (82)	16 (80)	15 (75)	12 (80)	8 (89)	5 (83)
Age (years)	38.4 ± 13.7	38.9 ± 10.2	38.7 ± 12.0	42.7 ± 11.3	39.3 ± 12.0	40.2 ± 12.4
Age at onset migraine	-	18.6 ± 6.9	16.0 ± 9.0	-	18.4 ± 6.5	12.7 ± 2.3
Migraine duration (years)	-	20.4 ± 10.5	22.7 ± 14.3	-	20.9 ± 12.1	27.5 ± 11.8
Migraine attacks per month	-	2.2 ± 1.7	1.5 ± 1.0	-	2.1 ± 1.1	1.7 ± 1.1
Migraine days per month	-	3.3 ± 2.3	2.1 ± 1.9	-	3.7 ± 2.3	1.8 ± 1.0
Use of triptans (N (%))	-	10 (50)	9 (47)	-	6 (67)	1 (17)
Attacks with aura (%)	-	-	75 ± 34	-	-	79 ± 39
Duration of aura (min)	-	-	49 ± 53	-	-	60 ± 33

Note: Values are presented as mean with standard deviations, or number with percentage.

Test-rest reproducibility using 7-channel EEG

To study reproducibility of the chirp responses we performed retest measurements in 13 participants, that is, controls (n = 7), migraine without aura (n = 3, of whom one was measured in the pre-ictal phase during both measurements), and migraine with aura (n = 3). Retest measurements were conducted 1 to 42 days (median 11 days) after the initial experiment. Repeatability of responses at electrode Oz in the bands of interest was good (ICC ≥ 0.68, significant r_s) for EEG power at the driving frequencies between 10–40 Hz (Table 2). The response at harmonic frequencies

showed moderate repeatability (ICC 0.41–0.62), with significant r_s for stimulation at low (10–18 Hz) and medium (19–26 Hz) frequencies, but not for stimulation at high frequencies (27–40 Hz) (Figure 2). EEG response power over all frequencies (between 5 and 125 Hz) showed no significant reproducibility, indicating low reliability (all ICC < 0.52, no significant r_s).

Table 2. Test-retest reliability parameters for overall response power, and power at driving and harmonic frequencies, grouped per stimulation band of interest.

	10–18 Hz			19–26 Hz			27–40 Hz		
	ICC	r_s	p	ICC	r_s	p	ICC	r_s	p
Overall response	0.51	0.51	0.08	0.34	0.18	0.55	0.10	0.21	0.49
Driving frequencies	**0.77**	**0.79**	**0.002**	**0.68**	**0.74**	**0.006**	**0.72**	**0.74**	**0.005**
Harmonic frequencies	**0.62**	**0.72**	**0.008**	**0.55**	**0.68**	**0.013**	0.41	0.18	0.57

ICC: Intraclass correlation coefficient; r_s: Spearman's rho. Note: Boldfaced values indicate significant association between measurements, with moderate to good repeatability.

Interictal occipital recordings of chirp responses in migraine with and migraine without aura

Occipital responses following chirp stimulation were recorded using 7-channel EEG in 56 participants; that is, controls (n = 17), migraine without aura (n = 20), and migraine with aura (n = 19). Eight measurements (five in four migraine without aura patients; three in three migraine with aura patients) were classified as pre-ictal since patients were retrospectively identified to have experienced a migraine headache within 72 hours from the time of investigation. In those cases, the time to the start of the headache ranged between 0.5 to 48 hours (median 24 hours). The other 32 measurements were classified as interictal (16 migraine without aura and 16 migraine with aura patients). No differences with respect to age, gender, migraine years, attack frequency or migraine days were present between interictal and pre-ictal measurements (independent t-tests; all $p > 0.05$).

To examine the interictal photic driving response between migraine subtypes, we compared interictal chirp responses for migraine without aura, migraine with aura and control groups in the pre-defined frequency bands based on Gantenbein et al.[18] Responses to low (10–18 Hz), medium (19–26 Hz) and high (27–40 Hz) frequency stimulation were not different (Figure 3) for: (i) overall EEG response power (between 5 and 125 Hz; low: $F(2,46) = 0.34$, $p = 0.71$; medium: $F(2,46) = 0.05$, $p = 0.95$; high: $F(2,46) = 0.16$, $p = 0.85$); (ii) EEG power at driving frequencies (low: $F(2,46) = 1.78$, $p = 0.18$; medium: $F(2,46) = 0.77$, $p = 0.47$; high: $F(2,46) = 0.29$, $p = 0.75$); nor (iii) EEG power at harmonic frequencies (low: $F(2,46) = 2.08$, $p = 0.14$; medium: $F(2,46) = 0.16$, $p = 0.86$; high: $F(2,46) = 1.44$, $p = 0.25$).

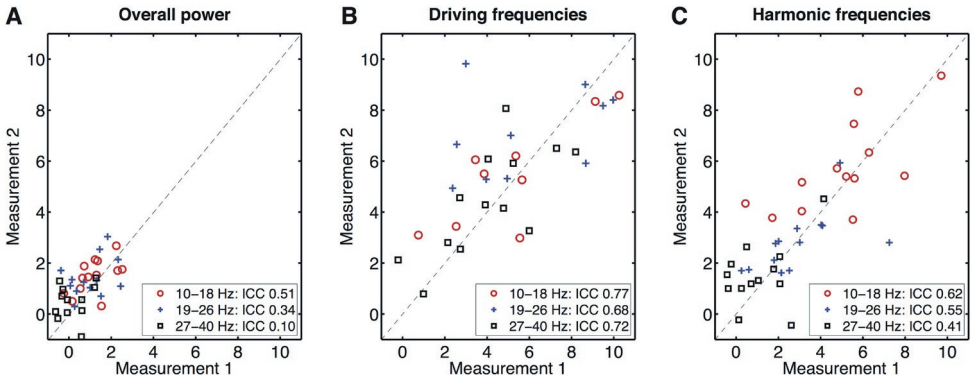

Figure 2. Test-retest reliability of chirp responses (based on 7-channel EEG, electrode Oz) in control and migraine groups is moderate to good at driving and harmonic frequencies. (A) Overall response power in the three bands of interest (red circles: 10–18 Hz; blue crosses: 19–26 Hz; black squares: 27–40 Hz) for measurement 1 and measurement 2 (1 to 42 days after measurement 1). Intraclass correlation coefficients (ICC) are between 0.10 and 0.51, indicating low to moderate reproducibility for the overall power. Dashed line indicates a perfect reproducibility between measurements. (B) Idem for the response at driving frequencies (10–40 Hz), with ICC between 0.68 and 0.77, indicating good reproducibility. (C) Idem for the response at harmonic frequencies (20–80 Hz), with ICC between 0.41 and 0.62, indicating moderate reproducibility.

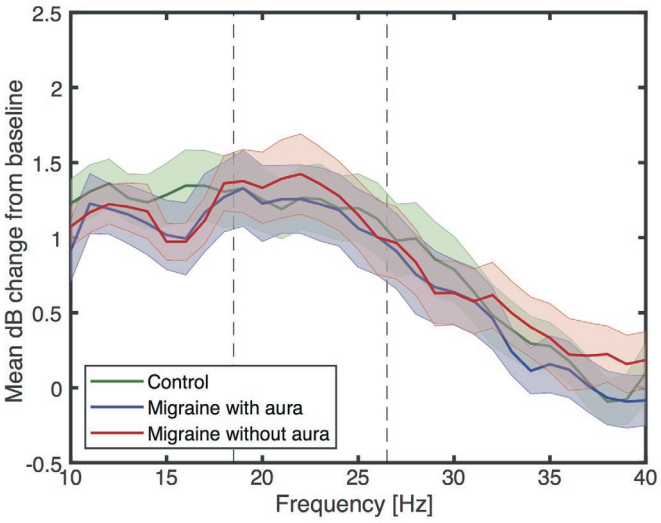

Figure 3. Overall response power (between 5 and 125 Hz), assessed per stimulation frequency as mean (± standard error) decibel (dB) change from baseline, for the different chirp stimulation frequencies. No differences in EEG power at electrode Oz (7-channel EEG) are present between controls and migraine with and without aura subjects, measured interictally, in the three pre-defined bands-of-interest (10–18 Hz, 19–26 Hz and 27–40 Hz; borders indicated by dashed lines).

An additional analysis was performed to assess possible effects of gender and the time of day at which the measurements were performed. Gender (controls: n = 3 male, n = 14 female; migraine with aura: n = 5 male, n = 11 female; migraine without aura: n = 3 male, n = 13 female) or time-of-day (controls: n = 9 a.m., n = 8 p.m.; migraine with aura: n = 7 a.m., n = 9 p.m.; migraine without aura: n = 10 a.m., n = 6 p.m.) did not have a significant effect on overall EEG response power, EEG power at driving frequencies or at harmonic frequencies (main effects for group all $p > 0.14$, time-of-day all $p > 0.22$ and gender all $p > 0.04$; interaction group and time-of-day all $p > 0.02$, interaction group and gender all $p > 0.05$, interaction time-of-day and gender all $p > 0.11$, interaction group, time-of-day and gender all $p > 0.07$). Female migraine patients with aura showed a tendency to a more pronounced response to chirp stimulation compared to males with respect to overall EEG power, while this distinction was not evident in the migraine without aura and control groups (Figure 4). However, as indicated above, gender differences in response across groups were not statistically significant.

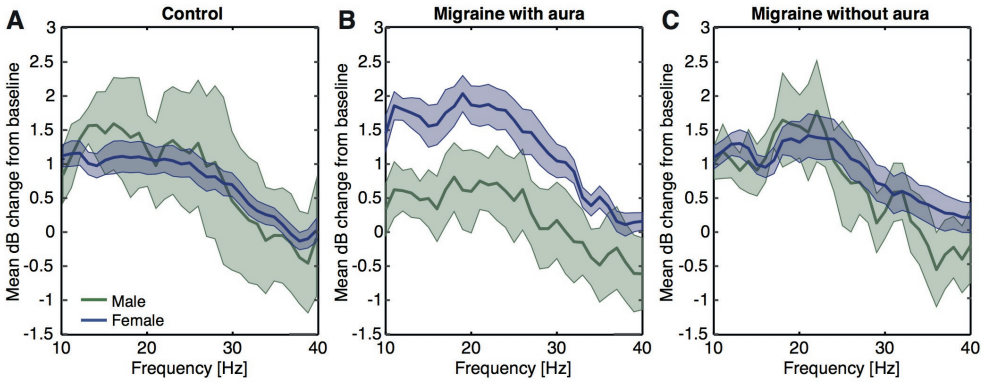

Figure 4. Gender effect within the overall response power, assessed per stimulation frequency as mean (± standard error) decibel (dB) change from baseline, for controls (A), migraine with aura (B) and migraine without aura (C) subjects. Female migraine patients with aura showed a tendency to more pronounced response to chirp stimulation compared to males. However, this difference did not reach statistical significance (interaction group and gender with respect to low, medium and high frequency windows, all $p > 0.06$).

Topographic distribution of cortical responses

As no interictal differences in the photic driving response to chirp stimulation were found between migraine and control groups, contrary to Gantenbein et al,[18] we assessed the optimal recording location at the visual cortex for measuring responses to chirp light stimulation. Cortex-wide responses were determined using high-density 126-channel EEG in a number of participants from the various groups. Chirp stimulation was performed in 30 participants; that is, in controls (n = 15), of which seven did not undergo the occipital recordings; migraine without aura (n = 9), and migraine with aura (n = 6), who all underwent the occipital recordings. Nine frontal electrodes (i.e. channels Fp1, Fpz, Fp2, AF7, AF8, FT9, FT10, AFp3h and AFp4h) were discarded from further analyses due to excessive noise in most participants. Cortical activation patterns (topoplots in Figure 5) did not show differences between the migraine and control groups, neither in signal-to-noise (SNR) ratio over the complete chirp response, nor in location of driving or harmonics responses. The response pattern was clustered at the occipital lobe, with highest SNR for both groups at Oz and POz. Maximum response amplitude showed a slight parietal shift for harmonic (maximum at POz) compared to driving responses (maximum at Oz). Responses at Oz to low, medium and high frequency bands were not different

between combined migraine (with and without aura) and control groups for these recordings, comparable to the interictal recordings with seven electrodes.

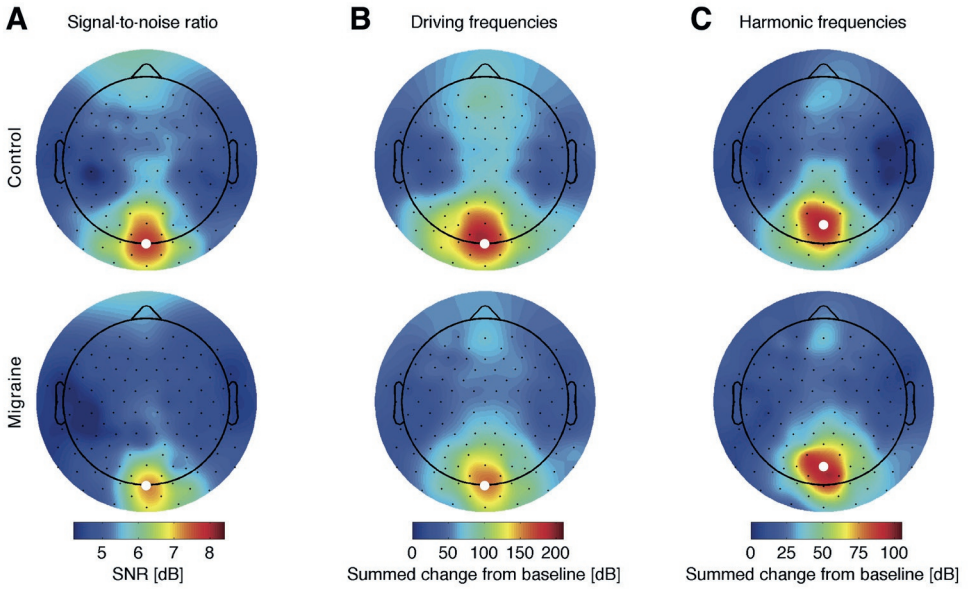

Figure 5. Topographical distribution of signal-to-noise ratio (SNR) of 126-channel EEG responses between 5 and 45 Hz (A) and summed responses at driving (B) and harmonic frequencies (C) as change from baseline in decibel (dB). Highlighted channel (white dot) indicates the channel with maximum response per group and parameter (Oz for SNR and driving frequencies, POz for harmonic frequencies).

Photic driving response in the pre-ictal phase

To assess the photic driving response to chirp stimulation in the pre-ictal phase, comparisons were made between the migraine group (n = 8 pre-ictal and n = 32 interictal) and control group (n = 17). Because we found no interictal difference between migraine subtypes, the interictal data from migraine with and without aura patients were combined. Overall EEG response power was not different between controls, interictal and pre-ictal migraine patients (low: $F(2,54) = 0.36$, $p = 0.70$; medium: $F(2,54) = 0.56$, $p = 0.57$; high: $F(2,54) = 0.38$, one-way ANOVA $p = 0.68$; Figure 6A), neither was the power at driving frequencies (low: $F(2,54) = 1.10$, $p = 0.34$; medium: $F(2,54) = 0.74$, $p = 0.48$; high: $F(2,54) = 0.34$, $p = 0.72$; Figure 6B). Instead, response power between groups was divergent for the harmonics of the high

stimulation frequencies ($F(2,54) = 5.74$, $p = 0.005$). The difference in harmonic response power for medium stimulation frequencies just failed to reach significance after compensating for multiple comparisons ($F(2,54) = 4.33$, $p = 0.02$). The harmonic responses to the low stimulation frequencies did not differ between groups ($F(2,54) = 2.17$, $p = 0.12$). *Post hoc* analyses for the high frequency response harmonics revealed higher power in the pre-ictal compared to the interictal state in migraine patients as well as to controls (all $p < 0.02$, Bonferroni-corrected; Figure 6C). The most pronounced increase in power in the pre-ictal period was found for the harmonics of stimulation frequencies between 22 and 32 Hz. An additional one-way ANOVA for this 22–32 Hz frequency band revealed a significant effect of the group ($F(2,54) = 7.37$, $p = 0.001$), with post hoc analysis demonstrating a statistically significant difference between pre-ictal measurements and both the interictal and control measurements (all $p < 0.004$, Bonferroni corrected). Harmonic response power in this frequency band increased from interictal responses to pre-ictal responses as the time (in days) to the next migraine attack onset decreased ($R^2 = 0.21$, $F(1,38) = 10.58$, $p = 0.002$).

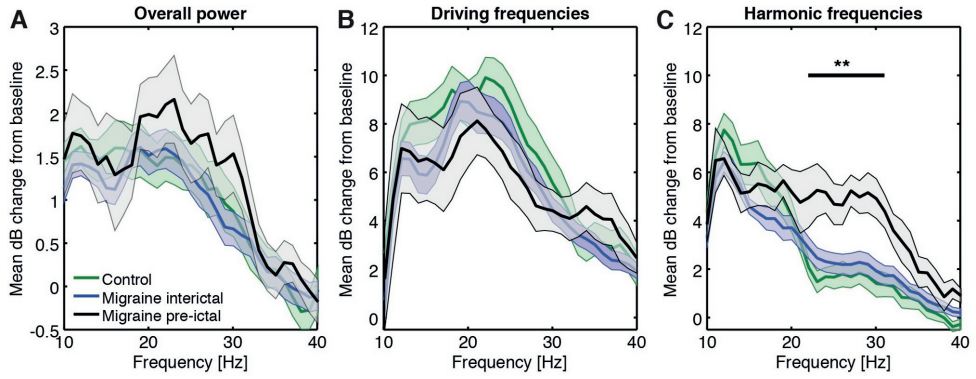

Figure 6. Response at electrode Oz (7-channel EEG) for the different chirp stimulation frequencies for control, interictal and pre-ictal migraine groups, showing an increase of EEG power for the harmonics of the stimulation frequencies between 22–32 Hz during the pre-ictal phase. (A) Overall response power (between 5 and 125 Hz), assessed per stimulation frequency as mean decibel (dB) change from baseline, was not different between groups for the defined EEG bands of interest. (B) Similarly, EEG responses at driving frequencies showed no differences between groups. (C) For the harmonics of the stimulation frequencies, an increased power was present for the pre-ictal group for high frequency band (27–40 Hz). A one-way ANOVA confirmed group differences across frequency bands (between 22–32 Hz),

with post hoc analyses showing an increase in power in the pre-ictal measurement compared to the interictal and control measurements (significance indicated: $^{**}p < 0.004$ for all frequencies, Bonferroni corrected). Shown per line: mean ± standard error. Note the different y-axis scaling for panel B and C compared to panel A.

Discussion

Here we used visual 'chirp' stimulation as a tool to measure the photic driving response and to assess cortical responsivity dynamics in migraine patients with and without aura compared to controls. Chirp responses showed good test-retest reliability over days within participants and could be measured with a few scalp electrodes over the occipital cortex. Interictally, no differences in cortical responses were observed between migraine patients, regardless of migraine subtype, and controls. However, in a group of migraine patients that were measured in a pre-ictal time window, 1 to 48 hours before an attack, the harmonic EEG response to stimulation in the higher beta band (22–32 Hz) was enhanced compared to measurements outside an attack or compared to controls.

Our high-density EEG recordings indicated the specificity of visual cortex activation by chirp light stimulation. This result is in line with earlier visual chirp recordings performed in healthy controls using 32-channel EEG.[17] Using 7-channel EEG, we demonstrated in the present study that interictal chirp-induced photic driving responses in subgroups of migraine patients with or without aura were not different from responses in controls. This contrasts with a previous report using chirp stimulation interictally in migraine patients without aura showing an increased overall response power between 18 and 26 Hz,[18] as well as the enhanced 'H-response' between 18 and 24 Hz reported interictally for migraine with and without aura.[13,14] An enhanced response in the 18–24 Hz range has not been a consistent finding, as migraine patients were also shown to have attenuated EEG responses in this frequency window.[10,12] In earlier studies into the H-response, controls seemed to have a lack of EEG response instead of an attenuated response compared to migraineurs,[13,14] while healthy subjects have been reported to be able to respond to flashing light stimulation up to 100 Hz.[14] It thus remains unclear if the responses of controls in the present study are particularly enhanced. Differences in migraine attack frequency between studies may contribute to this discrepancy, as we only

included patients with at least one headache per month, or could be due to variations in stimulation paradigm in e.g. length, waveform and used device in those studies. With respect to the use of chirp stimulation there also is a methodological difference as we used red light whereas Gantenbein et al.[18] used white light for chirp stimulation. However, as the color of flash light stimulation was shown to have little effect on visual evoked potentials in migraine patients,[21] we would not expect the color difference to explain the absence of an enhanced interictal chirp response in our study. Although the visual cortex was suggested to show particularly enhanced excitability in migraine with aura patients,[7,8] our data did not reveal differences in the chirp response between migraine with and without aura in the interictal phase.

In patients with migraine, in a pre-ictal time window less than 48 hours prior to reported headache, we observed increased power of the harmonic EEG responses to chirp stimulation. Based on previous literature,[18] initial analysis was performed with respect to three driving frequency bands (10–18 Hz, 19–26 Hz and 27–40 Hz). Only the harmonic responses to stimulation in the highest frequency window showed a statistically significant difference. Harmonic responses to the medium stimulation frequencies just failed to reach significance, possibly due to the small number of pre-ictal measurements, and inherent variance between measurements as well as within the frequency bands. The pre-ictal increase of harmonic EEG responses was largest for stimulation in the higher beta band, for frequencies between 22–32 Hz, and increased when the number of days to the next attack onset decreased. This frequency band overlaps and extends the 18–26 Hz frequency band reported in relation to interictal hyperresponsivity of the visual cortex.[13,14,18] A longitudinal EEG study in migraine with and without aura patients was the first to report enhanced pre-ictal photic driving responses within 72 hours before the migraine attack, showing an increased response to steady-state stimulation at 12 Hz, but not at beta band frequencies.[10] Discrepancy between enhanced H-responses reported interictally in earlier studies and changes at 12 Hz in pre-ictal patients was attributed to possible inclusion of pre-ictal patients in the interictal studies.[10]

Enhanced cortical responsivity towards a migraine attack as observed in our chirp data is suggestive of cortical hyperexcitability underlying attack initiation, a concept largely supported by preclinical findings.[22] In transgenic models of familial hemiplegic migraine type 1 (FHM1) in which cortical excitation-inhibition balance

is disturbed,[23–25] susceptibility to cortical spreading depolarization (CSD, the correlate of the migraine aura) is enhanced.[26–28] Our (preliminary) observation that overall EEG responsivity in between attacks appeared larger for females than males in the migraine with aura group is of interest given the female preponderance of migraine,[29] and in line with data from FHM type 1 mutant mice that show most pronounced CSD susceptibility in females.[28] Photic driving to flash light stimulation was reported to be variably enhanced for female migraineurs.[30] As we did not design our study to investigate gender differences, a follow-up study with more participants of both genders should assess whether visual responsivity towards an attack may indeed be more pronounced in females.

The chirp visual stimulation paradigm was quickly applicable within an experimental timeframe of less than three minutes. This will reduce bias that may be caused by habituation to long-duration steady-state visual stimulation paradigms,[31,32] which is of particular relevance when comparing migraine patients for whom habituation to visual stimulation has been reported to be abnormal.[2] Our test-retest measurements indicated that predominantly responses at driving frequencies and harmonic responses, but not the overall EEG power, were reproducible over days to weeks. Responses to steady-state visual stimulation are mainly expressed at the driving and harmonic frequencies,[9,16] and not at other frequencies. Therefore, to increase the reproducibility of the visual chirp response, outcome measures based on responses at driving and harmonic frequencies are preferential over the overall EEG response.

Our results are supportive of the hypothesis that in migraine patients, cyclic changes in cortical excitability result in higher harmonic frequency output before an attack.[33] Our dataset did not allow for a pair-wise comparison between interictal and pre-ictal phases. As a next step, within-patient longitudinal studies should substantiate whether the chirp-induced photic driving response can be a suitable marker of an impending migraine attack. The reliable chirp readouts from repeated measurements on different days support implementation of visual chirp stimulation in patients to assess day-to-day fluctuations in photic driving response over the migraine cycle. With a short-duration paradigm like chirp stimulation and using a minimum of two occipital EEG electrodes, longitudinal tests of visual cortex

responsivity seem feasible and may eventually lead to a predictive measure of an impending migraine attack.

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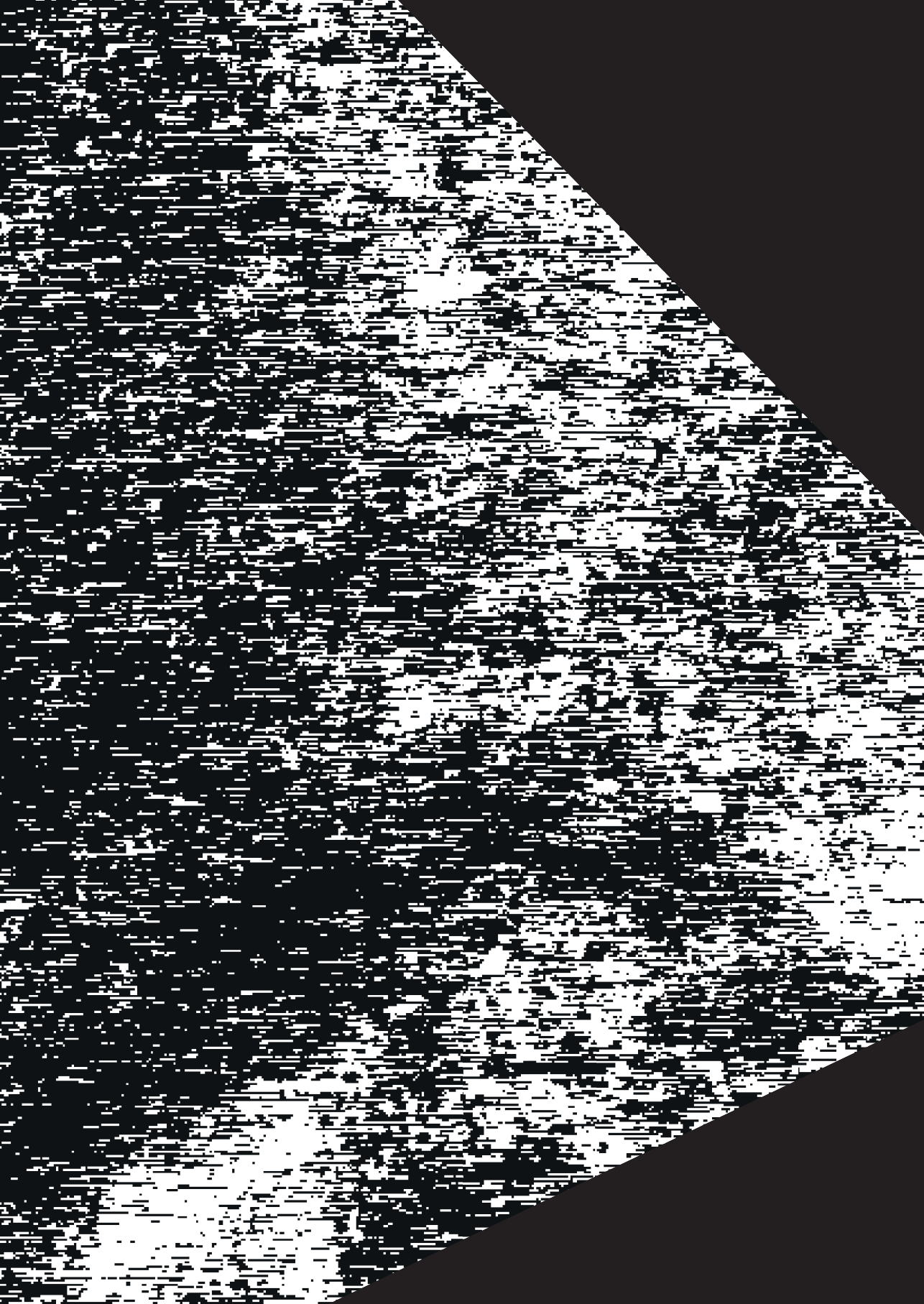
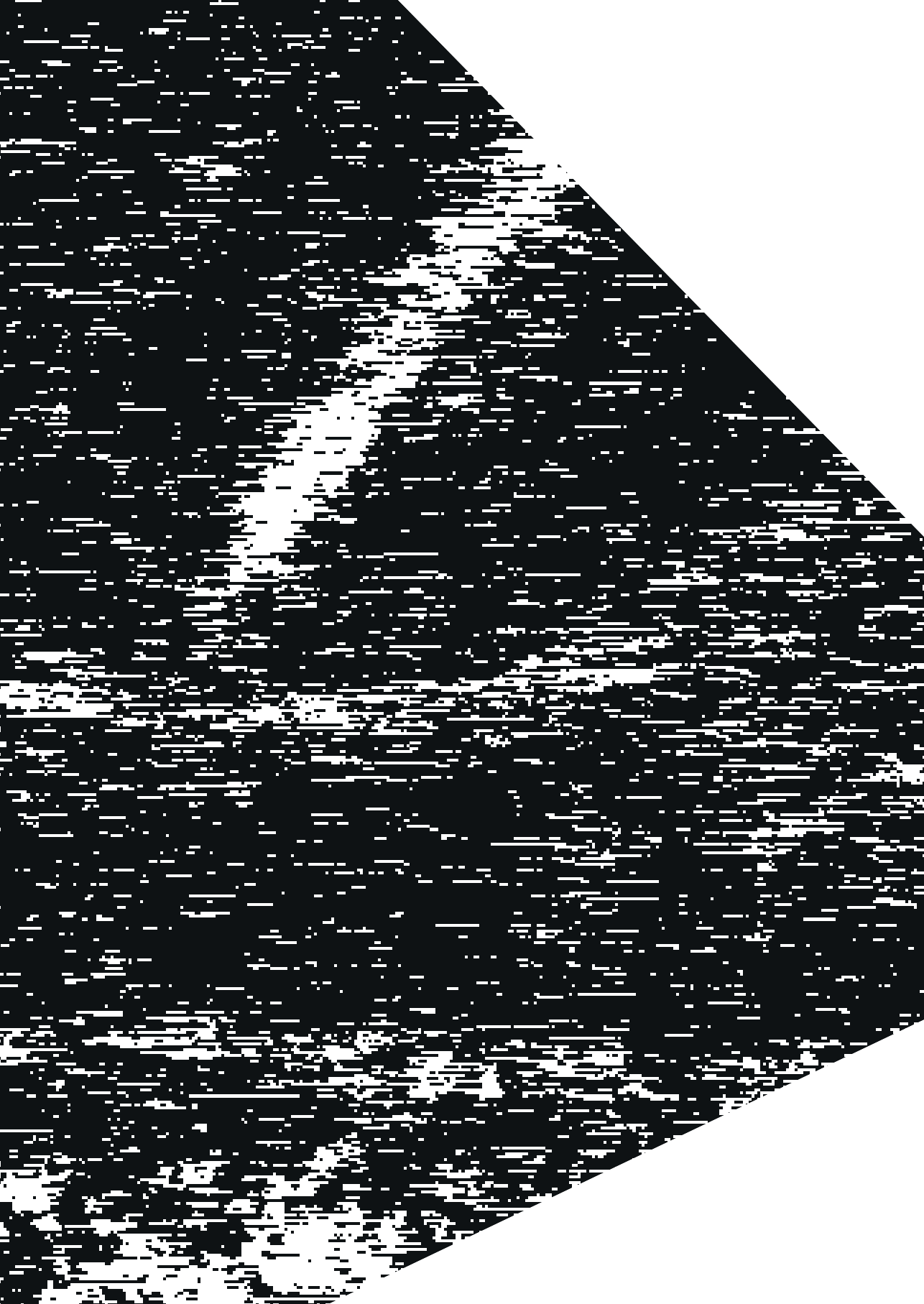


Part II

TMS-EEG, a novel method to measure cortical excitability in migraine



Chapter 5

TMS-evoked EEG potentials demonstrate altered cortical excitability in migraine with aura

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Submitted

Abstract

Migraine is associated with altered sensory processing, that may be evident as changes in cortical responsivity due to altered excitability, especially in migraine with aura. Cortical excitability can be studied directly by combining transcranial magnetic stimulation with electroencephalography (TMS-EEG). We measured TMS evoked potential (TEP) amplitude and response consistency as these measures have been linked to cortical excitability but were not yet reported in migraine.

We recorded 64-channel EEG during single-pulse TMS on the vertex interictally in 10 subjects with migraine with aura and 10 controls matched for age, gender and resting motor threshold. On average 160 pulses around resting motor threshold were delivered in clockwise and counterclockwise direction. Trial-averaged TEP responses, frequency spectra and phase clustering (over the entire scalp, as well as in frontal, central and occipital midline electrode clusters) were compared between groups.

In migraine with aura, TEP responses showed reduced amplitude around the frontal and occipital N100 peaks. Migraine and control groups had a similar distribution of TEP waveforms over the scalp. Responses over the entire scalp were affected by current direction, specifically over the primary motor cortex, somatosensory cortex and sensory association areas; but not for frontal, central or occipital midline clusters.

This study provides direct evidence of altered cortical responsivity in-between attacks in migraine with aura. Decreased TEP responses around the N100 peak are indicative of reduced cortical GABA-mediated inhibition and expand observations on enhanced cortical excitability from earlier migraine studies using more indirect measurements.

Introduction

Migraine is a brain disease characterized by recurring attacks of severe headaches, accompanied by other neurological symptoms like nausea, vomiting and sensitivity to light and sound.[1] Visual aura before the headache phase, experienced by about one third of people with migraine, is a transient focal symptom likely due to cortical spreading depolarization in the visual cortex.[2] People with migraine report increased visual sensitivity between and during attacks compared to healthy controls,[3,4] which appears most prominent in those with visual aura symptoms.[5] Altered visual cortex responsivity,[6] that could be caused by changes in cortical network excitability may explain these symptoms. However, both hyperexcitability[7,8] and hypoexcitability have been suggested as underlying mechanism,[6] largely based on indirect measures of cortical excitability.

Transcranial magnetic stimulation (TMS) has been one of the methods used to study cortical excitability in migraine, using subjective or indirect readouts.[9] Magnetophosphene induction, by applying TMS over the occipital cortex while registering the reported threshold of perceived visual responses, is a direct but subjective measure of visual cortex excitability.[10] A meta-analysis suggested decreased phosphene thresholds in migraine with and without aura compared to controls when a large circular coil was used. More localized stimulation using a figure-of-eight coil resulted in increased phosphene prevalence in subjects with aura, and not in those without aura or controls.[11] Studies on motor cortex excitability have used the muscle response to single pulse TMS as indirect readout by determining a resting motor threshold (RMT). This threshold does not reflect cortical excitability exclusively, as subcortical pathway excitability will also affect muscle responses.[12] Using this method, no changes were demonstrated between migraine with or without aura in-between attacks and controls.[9] Stimulus response curves of the motor response recorded by varying stimulation intensity showed contradictory patterns in migraine as well, with indications of motor cortex hyperexcitability at high stimulus intensities.[13,14] Motor responses to short-burst repetitive TMS differed over the migraine cycle for migraine with and without aura, which relates TMS-induced measures to cyclic changes in cortical excitability.[15]

Advances in electroencephalography (EEG) amplifier technology allow direct recordings of the cortical network response to TMS.[16] Using TMS-EEG, magnetically evoked cortical responses can be evaluated as direct and objective markers of cortical responsivity, and provide information on changes in network excitation or inhibition.[17] Single pulse stimulation at one location generates responses measurable over the entire scalp, enabling comparison of cortical excitability across cerebral regions.[18] The TMS-evoked potential (TEP) follows a specific pattern, of which peak amplitudes are altered by neuroactive drugs that modulate excitatory of inhibitory neurotransmission.[19,20] TEP amplitudes are also affected in conditions such as epilepsy and schizophrenia in which altered cortical excitability is implicated.[21,22] TEPs, however, have not yet been assessed in the context of migraine. In addition to amplitude characteristics, the phase of frequency components in evoked potentials[23] and ongoing EEG[24] also contains relevant information on cortical excitability. Occipital phase clustering of visually evoked responses between repetitions is predictive of a photoparoxysmal response in photosensitive epilepsy,[25] suggesting a relation between consistency of phase responses across stimulation trials and excitability levels.

We aimed to study possible alterations in cortical excitability directly using TMS-EEG in subjects with migraine with aura (in-between migraine attacks) and controls. Using a circular TMS coil, we induced broad, scalp-wide, activation while not limiting the study to a predefined local stimulation site. The combination with EEG allowed us to exploratively investigate local alterations in cortical excitability over the whole scalp based on local changes in TEP responses as direct measure of cortical excitability. We compared TEPs over the entire scalp to study the distribution, amplitude and phase characteristics of response patterns at frontal, central and occipital electrode clusters along the midline. These readouts could provide objective parameters on cortical excitability and allow identification of migraine-specific changes in excitability across cerebral regions including the visual cortex.

Methods

Participants (aged 18 or over) were recruited locally through digital and paper adverts and through the LUMINA study population of the Leiden University Medical Center.[26] Matching controls were selected from a cohort of 38 healthy controls that have been described elsewhere.[27] Migraine diagnosis was based on the International Classification of Headache Disorders (ICHD-3-beta) criteria.[28] Subjects with migraine headache preceded by visual aura in at least 30% of the attacks were included. Participants had to have at least 1 migraine attack per year, at least one in the year preceding the study and no more than eight attacks or 15 headache days per month (thus excluding chronic migraine). Experimental sessions were performed at least 72 hours after a migraine attack. Sessions that were followed by a migraine attack within 72 hours, verified by follow-up, were excluded.

Participants with migraine were matched with controls based on age, gender and RMT. Matching on RMT was performed to correct for effects of stimulation intensity and thereby prevent possible differences in threshold between groups to confound TEP readouts. Only controls without a history of epilepsy or migraine were included. Participants (with migraine and controls) with contra-indication to TMS, pregnant women and people with diabetes mellitus, psychiatric conditions and people using medication that could affect cortical excitability (such as psychoactive drugs and beta-blockers) were excluded. We established that participants did not smoke, used drugs or drank alcohol or coffee in the 12 hours preceding the measurement and to maintain a normal sleep pattern the night prior to the measurement. Written informed consent was obtained from all individual participants included in the study. The study was approved by Ethical Committee of Erasmus University Medical Center, Rotterdam, the Netherlands, and conducted according to the Declaration of Helsinki.

Recording setup

Transcranial Magnetic Stimulation

TMS was performed with a MagPro X100 magnetic stimulator (Magventure, Denmark), a 14 cm diameter parabolic circular coil (type MMC-140) using biphasic pulses with a width of 280 μs, to activate a large region of the cortex, including the

motor cortex,[29] or a sham coil (type MCF-P-B65). Measurements were conducted between 09.00 a.m. and 04.00 p.m. and distributed evenly between a.m. and p.m. in both participant groups. Soft foam earplugs were used to dampen the TMS-induced 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). 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 (WaveguardTM cap and ASAlabTM software, ANT Neuro, Enschede, The Netherlands), a sampling frequency of 4 kHz and a common average reference. Electrode impedance was kept below 5 kOhm during the experiment. Participants were seated in a comfortable chair with their eyes open and arms in supine position. Prior to stimulation, baseline EEG was recorded for 10 minutes with eyes open (5 min) and closed (5 min).

Single pulse TMS protocol

To be suitable for clinical settings, the stimulation protocol we employed was designed to be short while yielding maximum information at once.[27] The stimulation procedure was performed using counterclockwise (right hemisphere) and clockwise (left hemisphere) stimulation and repeated with the sham coil. With the center of the circular coil on electrode position Cz (vertex) the RMT, defined as lowest stimulation intensity evoking motor evoked potentials larger than 50 μV in 50% of the trials,[30] was determined. Then, a semi-automated, in-house designed stimulation protocol (created in Matlab® (release 2007b, The MathWorks, Natick, MA)) was used to deliver stimuli with a frequency of 0.5 Hz.[31] Stimulation started at a stimulator output value of RMT minus 10% and increased in 2% steps until a reproducible motor evoked potential (>200 μV) was seen after every stimulus (±110-120% RMT). At each intensity 20 stimuli were given and aggregated for TEP analyses to limit the participant's exposure to TMS stimuli.

Data analysis

Data pre-processing

Off-line analyses were performed in Matlab® (release 2015a) using custom-written scripts and the FieldTrip Matlab toolbox.[32] A TMS-EEG artefact removal pipeline[31] was used to eliminate ringing, decay, muscle and eye movement artefacts. Only trials performed at stimulation intensities between +0% and +6% stimulator output relative to the averaged RMT of two hemispheres were pooled and used for further analyses. All the datasets, both active and sham stimulation, were split in trial epochs starting 1 s before and ending 1 s after the TMS pulse. Ringing artefacts were segmented out from 0 to 6 ms relative to the time of stimulation and baseline corrected using the window from -200 ms to -1 ms relative to the start of the stimulus. Electrodes showing contaminated activity (e.g. excessive line noise) over the averaged trials were removed for each participant (average: 1 channel per participant, range: 0-4 channels). EEG data were then re-referenced to the common grand average of all non-interpolated EEG channels.

Next, independent component analysis (ICA) was used to remove exponential decay artefacts, recharge artefacts, eye blinks, eye movements and line noise. A maximum of 63 components were extracted from the data (number of components equal to the number of non-interpolated EEG channels minus 1), on average 8 components were removed in the first round of ICA (range: 3-18 components). The ICA decomposition was back-projected to the channel level after removal of the independent components containing the artifacts. Trial epochs were shortened to windows starting 200 ms before and ending 600 ms after the TMS pulse, followed by a second round of ICA to remove muscle related artefacts and remaining line noise artefacts (average of 8 components, range: 4-15). After reconstruction of the channel level data the split trials were re-combined. To completely remove residual time-locked muscle artefacts not captured by ICA, cubic interpolation was used from 1 ms to 15 ms around the stimulus. Next, some additional pre-processing steps were performed that are dependent on the type of analysis (time-amplitude or time-frequency).

Time-amplitude processing

Individual trials were baseline corrected and band-pass filtered between 1 and 80 Hz using a 3th order Butterworth filter. Removed electrodes were spherically interpolated. Trials were visually inspected and those who still showed contaminated activity were discarded. The resulting dataset consisted of 80 trials per current direction per participant (excluding removed trials). The TEP waveform was averaged over all trials and per current direction for each electrode. Also, an average TEP waveform was calculated over both current directions.

Time-frequency processing

Frequency spectra and phase clustering index[33] were calculated at all electrodes using Morlet wavelets. Three cycles/frequency were used for high temporal resolution, in 1 Hz frequency steps between 5 and 80 Hz and 5 ms time steps. To limit the number of comparisons (time-frequency versus time-frequency-electrode points) and to especially study occipital responses in migraine with aura, we compared the frequency spectra and phase clustering index for the three a priori defined frontal, central and occipital electrode clusters. Phase clustering index values vary between 0 (random phase clustering between trials) and 1 (all trials have equal phase clustering) per time-frequency point.

Statistical analysis

Magnetic or sham stimulation responses were compared between migraine and control groups for the window from 20 to 200 ms after stimulation (720 samples per channel at 4000 Hz sampling rate with a total of 62 channels). Within this time window, N100 and P180 peaks are present in all channels, allowing time-electrode cluster analysis of evoked activity. Statistical analysis was performed over all channels within the specified window for the combined dataset (with pooling of both current directions). To investigate consistency of the results, we also repeated the statistical analysis for each current direction separately.

TEPs were compared between groups using dependent t-tests (using the matched case-control design) at all samples within the pre-defined time window for the electrodes. In addition, we identified three regions of interest a priori: frontal (electrodes F1, F2, Fz, FCz, AF4, AF3), central (Cz, C1, C2, CPz, CP1, CP2) and

occipital (Oz, O1, O2, POz, PO3, PO4), to limit the number of comparisons and to especially study occipital responses in migraine with aura. Exact *p*-values were calculated by enumeration using cluster-based permutation testing to correct for multiple comparisons and the small sample size[34] using the FieldTrip Matlab toolbox.[32] Clusters based on adjacency in time and electrode space were formed using samples with a cluster-alpha of 0.10 (independent *t*-test). This threshold allows for detection of larger clusters in the time-electrode space, without selection of separate clusters of single time-electrode points detected at $p < 0.05$ as a cut-off.[34] Within each cluster, *t*-values (for both time samples and electrodes) were summed and compared to a dataset of all possible combinations of the original data (1024 combinations using the matched pair design). Clusters were considered significantly different between groups when their summed *t*-values where lower or higher than 2.5% ($p < 0.025$) of all permuted clusters.

Results

Ten individuals with migraine were assessed (9 females, 1 male; mean age 41 years, range 21–62; 3 left-handed), who were also included in previous research.[27] The migraine attack frequency was between 0.3 and 2 per month (average of 0.9 attacks). Ten controls were included (Table 1). All participants tolerated the experimental sessions. No migraine attacks were reported in the 72 hours following the experiment.

Effect of current direction

First, possible differences between clockwise and counterclockwise stimulation trials were analyzed within all subjects (60–80 trials per participant), combining migraine and control groups. Averaged TEP waveforms did not differ between polarities over time and electrodes for frontal (p =0.28), central (p = 0.20), and occipital waveforms (p = 0.30; Figure 1), but differed when analyzed over the entire scalp (p = 0.004; Suppl. Figure 1A). The difference clusters were present over primary motor and somatosensory cortices (Suppl. Figure 1B), likely due to the relationship current direction and preferential activation.[35] Clockwise and counterclockwise trials were grouped for further analyses, and, as secondary outcome, also analyzed per current direction. Although the averaged TEP waveforms differed between

current direction over the scalp in response to stimulation at Cz, they were of similar shape at the same electrode locations for migraine and control groups (Figure 2).

Table 1. Demographic, clinical and experimental data for healthy controls and migraine patients with aura reported as mean (± SD) or number.

	Control	Migraine with aura
No. (female / male)	10 (9/1)	10 (9/1)
Age [years]	39.8 (±11.1)	41.0 (±12.6)
Age at onset [years]	-	17.8 (±4.5)
Attack frequency [/month]	-	0.9 (±0.6)
Mean headache duration [hrs]	-	25 (±19)
Aura frequency [% of attacks]	-	86 (±28)
RMT [%]	41.1 (±6.6)	41.3 (±4.4)
Number of pulses	298 (±29)	293 (±35)
Removed ICA components	8.1 (±2.7)	7.4 (±1.9)

RMT: resting motor threshold; ICA: independent component analyses.

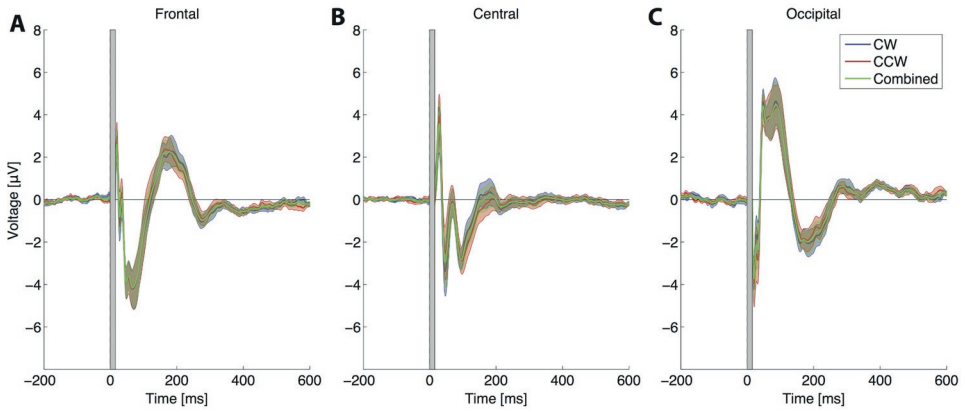

Figure 1. In controls and people with migraine, grand-averaged TEP responses show no overt differences and comparable peak distributions between clockwise (blue line) and counterclockwise (red line) current direction and when both current directions are combined (green line) for (A) frontal electrodes (F1, F2, Fz, Fpz, AF4, AF3), (B) central electrodes (Cz, C1, C2, CPz, CP1, CP2) and (C) occipital electrodes (Oz, O1, O2, POz, PO3 and PO4). Plots show mean and patched standard error, the grey bars indicate the spherically interpolated parts of the EEG traces (-1 to 15 ms). CW: clockwise; CCW: counterclockwise.

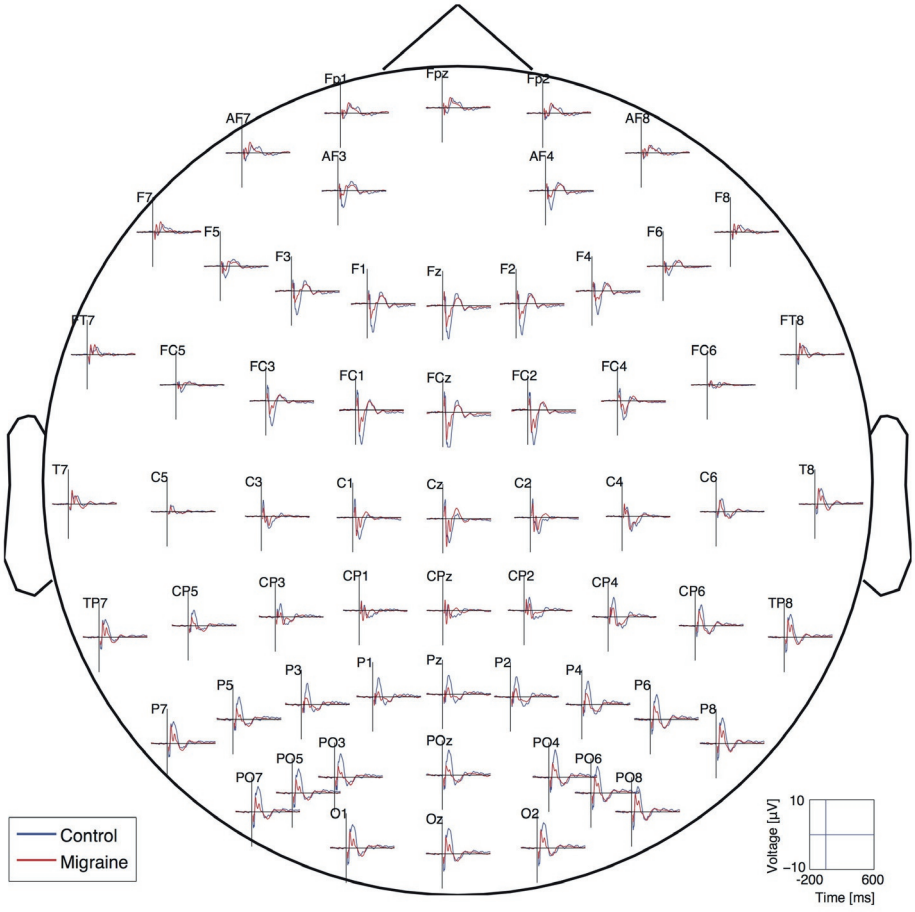

Figure 2. Distribution of average TEP waveforms over the scalp for control and migraine groups. Note the similarities in waveform between groups (e.g. direction and delay of the N100 and P180 peaks).

TMS evoked potentials

Cluster-based permutation analysis of TEP amplitudes over time and electrodes showed a significant difference in the a priori selected time interval between 20 and 200 ms after stimulation (p = 0.012 for combined polarities, p = 0.013 for CW stimulation and p = 0.018 for CCW stimulation) for people with migraine compared to controls. The revealed cluster was grouped around the N100 peak, between 60 and 120 ms after stimulation, and located mainly at the occipital cortex (Figure 3).

When analysed in the predefined electrode groups (frontal, central and occipital), no statistically significant difference was present at the central electrodes (p = 0.050 for combined polarities, p = 0.06 for CW stimulation and p = 0.025 for CCW stimulation). The N100 peak, however, was smaller in the migraine group at the frontal electrodes (p = 0.009 for combined polarities, p = 0.019 for CW stimulation and p = 0.009 for CCW stimulation). The largest difference in the frontal cluster (4.9±0.9 μV) was present at 77 ms after stimulation (Fig. 4A). Also at the occipital cortex, the N100 peak was decreased in people with migraine compared to controls (p = 0.008 for combined polarities, p = 0.009 for CW stimulation and p = 0.005 for CCW stimulation). Here, the largest difference (5.9±0.9 μV) was found at 78 ms after stimulation, similar to the frontal cluster (Figure 4B).

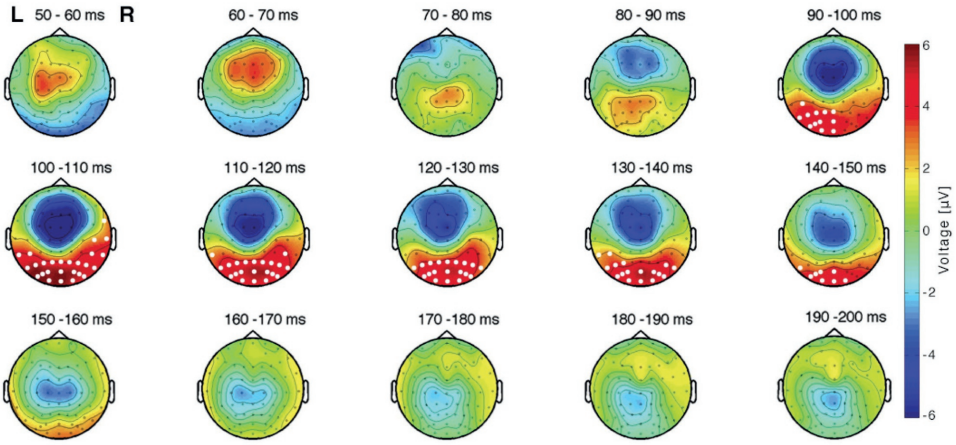

Figure 3. Topographical plots of difference in TEP amplitude between controls and migraine subjects show one distinct difference component. Plots display the averaged difference (control minus migraine) in 10 ms windows between 50 and 200 ms (NB, statistical analyses were carried out per ms, results are pooled in 10 ms bins for visualization purposes only). The significant cluster is highlighted over time with white dots at the significantly different electrode positions, mainly located over the occipital cortex between 90 and 150 ms.

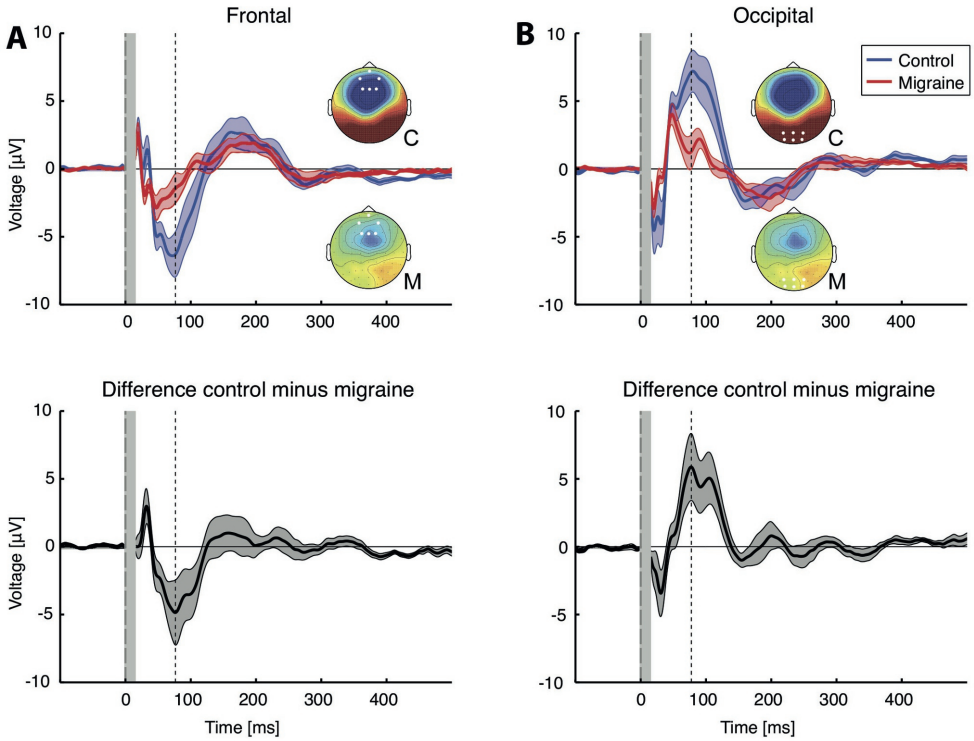

Figure 4. Grand-averaged TEP responses and difference waveform (control minus migraine) at frontal (F1, F2, Fz, Fpz, AF4, AF3) and occipital (Oz, O1, O2, POz, PO3 and PO4) electrodes show differences in TEP peaks between controls and migraine subjects. Two separate components of a negative cluster were found using exact cluster-based permutation testing (enumeration). (A) Migraine group (red line) shows decreased frontal activity around the TEP N100 peak compared to control group (blue line), with largest difference of -4.9 μV at 77 ms after stimulation (dashed line). Bottom plot shows the difference between migraine and control groups (standard error of the mean calculated using 10.000 bootstraps over both groups). (B) Occipitally, the TEP N100 peak decreased as well in migraine, with largest difference of -5.9 μV at 78 ms after stimulation (dashed line). Bottom plot shows the difference between migraine and control groups. Insets show topographical distribution in control (C) and migraine (M) at the time point of maximal difference with electrodes highlighted in white dots. Traces show grand-averaged mean with patched standard error. The grey bars indicate the spherically interpolated parts of the EEG traces (-1 to 15 ms).

Frequency spectra

To assess differences in time-frequency composition of TEPs between migraine and controls, cluster-based permutation analyses were conducted for the frequency

spectra of the averaged responses (20–200 ms, 5–80 Hz) for frontal, central, and occipital regions. No differences in power spectra were found in any of the predefined electrode clusters: frontal $p = 0.09$ (combined polarities), $p = 0.29$ (CCW), $p = 0.04$ (CCW)), central ($p = 0.12$, $p = 0.34$, $p = 0.08$) nor occipital ($p = 0.29$, $p = 0.35$, $p = 0.11$).

Phase clustering over trials

Consistency of TEP responses over trials was compared between groups using phase clustering analyses in the time-frequency domain. Statistical cluster-based permutation analyses were conducted for phase clustering over trials within the time-frequency domain over frontal, central and occipital electrode groups. There were no differences in phase clustering in migraine compared to controls, for none of the electrode groups and irrespective of current direction (frontal electrodes $p = 0.17$ (combined polarities), $p = 0.33$ (CCW), $p = 0.13$ (CCW); central electrodes $p = 0.23$, $p = 0.11$, $p = 0.47$; occipital electrodes $p = 0.17$, $p = 0.089$, $p = 0.18$).

Sham stimulation evoked potentials

Evoked responses induced by sham stimulation (averaged over 80 trials) showed a clear N100-P180 auditory complex[36] (Suppl. Figure 2) in both healthy controls and participants with migraine. Averaged waveforms after sham stimulation did not differ between migraine and controls over time and all electrodes ($p = 0.59$) nor over the predefined electrode groups (all $p > 0.28$). Frequency spectra were not different between groups for the electrode clusters (all $p > 0.13$). Phase clustering over trials did not include significantly different time-frequency clusters for the three electrode groups (all $p > 0.23$).

Discussion

Our data show altered cortical EEG responses to transcranial magnetic stimulation in-between attacks in migraine with aura compared to controls. We demonstrated that TEP amplitude waveforms in migraine with aura are distinct from those in healthy controls, by showing a reduced amplitude around the frontal and occipital N100 peak. These findings suggest that TEP features can be suitable markers of cortical excitability changes in migraine.

Analyses of TEP waveforms showed two distinct regions in which the N100 amplitude responses were decreased in migraine with aura: i) at the level of the frontal cortex, and ii) at the level of the occipital cortex. Our finding of a decreased N100 peak may reflect decreased cortical inhibition at the level of the frontal and occipital cortex. Increased N100 peak amplitude may reflect increased inhibitory $GABA_B$ mediated receptor activation.[19,37] A larger N100 peak in epilepsy was attributed to increased activation of inhibitory circuits as a possible result of the use of anti-epileptic drugs, which could have enhanced GABA-ergic activity.[22] The physiological underpinnings of various TEP peaks are, however, not straightforward.[38] While some studies report a linearly dependency of the $GABA_B$-ergic effect on N100 and P180 TEP peak amplitudes,[39,40] other studies only report a direct effect of $GABA_B$ on the N100 peak, but not the P180 peak amplitude.[19,37]

The frontal cortex was suggested to play a role in controlling pain processing in migraine. Reduced EEG-based activation of the anterior-medial prefrontal cortices during contact-heat stimuli in migraine with aura was interpreted as a heightened state of readiness to anticipated pain, compared to controls.[41] Also, the dorsolateral pre-frontal cortex (DLPFC) inhibits cortical as well as subcortical pain pathways.[42] If decreased DLPFC cortical inhibition represents reduced DLPFC inhibitory output, it could contribute to enhanced pain perception in migraine. Alternatively, if decreased DLPFC cortical inhibition represents reduced intracortical inhibition within the DLPFC, this would be expected to result in disinhibition of the inhibitory control from the DLPFC on cortical and subcortical pain processing, as possible protective mechanism against recurrent headaches in episodic migraine. Indeed, modulating DLPFC activity using high-frequency repetitive TMS decreased the number of monthly attacks in chronic migraine.[43] This suggests a role for the frontal cortex in migraine susceptibility, although the precise contribution of GABAergic inhibition remains unclear.

A decrease in cortical GABA-ergic inhibition could also explain the observed decreased occipital TEP waveform around the N100 peak in migraine patients, as indicated by TEP studies in healthy subjects.[19] With repeated visual stimulation in migraine, a decrease in habituation was attributed to lateral inhibitory processes in the thalamocortical network that could be mediated by GABA-ergic neurons in the occipital cortex.[44] Preclinically, single pulse TMS applied in rodents increased the

threshold for inducing cortical spreading depolarization,[45] the neurobiological correlate of the migraine aura.[46] However, $GABA_{A/B}$ antagonists reversed this effect, which indicates that TMS can suppress cortical neuronal activity by influencing GABA-ergic circuits.[45] Paired pulse TMS to study short-interval intracortical inhibition,[47] could be used to further investigate the role of GABA-ergic networks in altered cortical responsivity in migraine.

In patients with Huntington's Disease, a decreased N100 peak was related to disrupted phase coherence.[40] We found no altered phase clustering in people with migraine while the N100 TEP amplitude was decreased compared to controls. However, our approach of full TEP waveform analyses instead of peak amplitude extraction limited the possibility of a direct comparison. The electrode clusters and time windows of interest as highlighted by our exploratory approach could be used in future studies to further explore such relationship between TEP amplitude and phase coherence.

There are some methodological considerations. Firstly, TEP N100 and P180 peaks have been associated with auditory evoked responses,[36] and somatosensory activation.[38] With realistic sham stimulation at different locations on the scalp, activation patterns similar to TEPs have been measured with prominent N100 and P180 peaks.[38] However, especially the N100 peak has been related to cortical excitability using direct intervention with benzodiazepines.[19] As sensory processing of different modalities, including differences in the processing of auditory stimuli, appears altered in migraine,[48] the sound of the coil click during stimulation could partially explain observed differences (all participants, however, wore soft foam earplugs during real and sham stimulation). We controlled for this by using sham stimulation, which produces a coil click and mechanical vibrations but a severely dampened magnetic field. The fact that sham stimulation did not show differences between cases and controls, suggests that altered processing of auditory stimuli in people with migraine does not contribute to the observed TEP amplitude differences.

Secondly, to improve artefact removal using independent component analysis, we combined trials at suprathreshold stimulation intensities and both current directions. The signal-to-noise ratio of our waveforms, frequency spectra and phase

clustering readouts also benefitted from the larger number of trials. The pooling of trials at multiple stimulation intensities shortens the stimulation protocol,[27] and is supported by the relatively similar TEP waveforms in the small range of stimulation, between 100-110% of RMT.[18] The within-subject comparison of the effect of current direction over all electrodes revealed significant clusters located over the centroparietal electrodes corresponding to the primary and somatosensory motor cortex, probably due to the preferential activation of a hemisphere with clockwise and counterclockwise current direction.[35] Comparison of the frontal, central and occipital electrode clusters, however, revealed no significant difference between current directions. We therefore used the combined trials for the primary endpoints in the group comparisons. The independence of our results from the current direction was demonstrated by the separate analyses per current direction, which showed no differences to the results for the combined trials.

Thirdly, with non-focal stimulation over the vertex using a circular coil, we achieved diffuse activation of the cortex.[29] This contrasts the use of focal magnetic stimulation with a figure-of-eight coil.[49] As our goal was to identify region-specific differences in TEP responses between migraine and control groups, we assessed evoked responses over the entire scalp. This allowed comparison of responses in various cortical regions, despite limiting the physiological interpretation of our findings. TEP waveforms induced by circular coil stimulation were similar to focally induced waveforms in research with figure-of-eight coils (e.g. [19,38]). Localization of responses was limited to their scalp distribution, as we have not implemented source localization. In future studies, probing the here identified regions, i.e., the frontal and occipital cortices, with focal stimulation with similar readouts would be a way to verify the present findings.

Fourthly, we cannot exclude a possible neuromodulatory effect of the repeated stimulation procedure. However, the number of stimuli (at maximum 160) and stimulation frequency (0.5 Hz), was based on TMS-EEG literature where no neuromodulatory effects were reported.[21,31,36] A much more elaborate stimulation of 1200 stimuli presented at 1 Hz over the motor cortex in healthy controls revealed a regional inhibitory effect of prolonged stimulation, limited to the motor cortex and not affecting the visual cortex.[50] The differences between migraine and control

groups reported here are therefore unlikely to result from neuromodulatory effects due to prolonged single pulse TMS.

Lastly, our exploratory study is limited by a small sample size. To increase comparability between groups, we matched the subjects with migraine to healthy controls based on age, gender, and RMT. Matching cases and controls on RMT is not a standard approach, but we believe that this reduces the possibility of bias. The stimulation intensity was based on the RMT and matching on RMT ensures that the stimulation intensity was comparable for both groups and diminishes the effect of high RMT inter-individual variance[51,52] on our readouts. Although matching based on RMT resulted in similar variance in both groups, we cannot exclude a possible effect of the migraine cycle on RMT variance.[53] We used exact permutation-based tests by enumeration, an approach known to remain robust with relatively small sample sizes.[34] To increase the robustness of our statistical results, we compared the exact enumeration statistics with Monte Carlo permutation tests, which yielded similar results. Instead of performing peak-only analyses, our analyses were strengthened by analyzing the data for differences over time-electrode clusters (TEPs). The finding of statistically significant differences in frontal and occipital TEP amplitudes, despite the small number of study participants, indicates robust results with a large effect and only little inter-individual variation. Still, generalizability of our findings to the general migraine population may be limited due to the sample size and by the inclusion of only participants with migraine with aura. Future studies including larger numbers of participants with migraine with and without aura should therefore determine the reproducibility and generalizability of our observations.

In conclusion, people with migraine with aura show distinct cortical EEG responses to magnetic stimulation compared to controls in the periods in between attacks. Peak amplitude differences suggest that the changes are the result of reduced cortical inhibition. Our findings are in line with reports of altered interictal cortical excitability in migraine that were based on indirect measures, using e.g. visual or somatosensory inputs, or magnetic stimulation with peripheral readouts. In our study, all participants tolerated the TMS-EEG experimental procedure well and no induced migraine attacks were reported. This opens up possibilities for similar TMS studies in subjects without aura or with exclusive aura, and for longitudinal TMS-

EEG studies during the migraine cycle. Such studies could strengthen the specificity of our findings for migraine with aura, and provide insight in changes of cortical excitability related to the onset of a migraine attack.

Acknowledgements

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Supplementary material

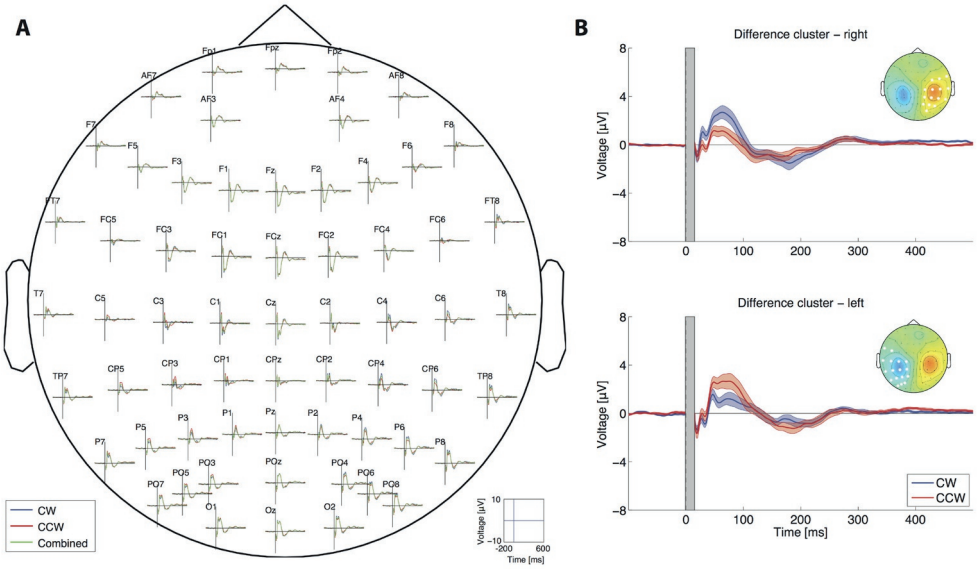


Figure S1. (A) Distribution of average TEP waveforms over the scalp for clockwise (CW), counterclockwise (CCW) and combined polarities, averaged over all participants. Note the similarities in waveform between current directions (e.g. direction and delay of the N100 and P180 peaks). (B) Waveforms differ between CW and CCW stimulation over the primary and somatosensory motor cortices, with the side of the difference depending on current direction. Inserts show topoplots of the TEP difference waveform distribution averaged between 70-80 ms after stimulation, where the mirrored activation between hemispheres is clearly visible.

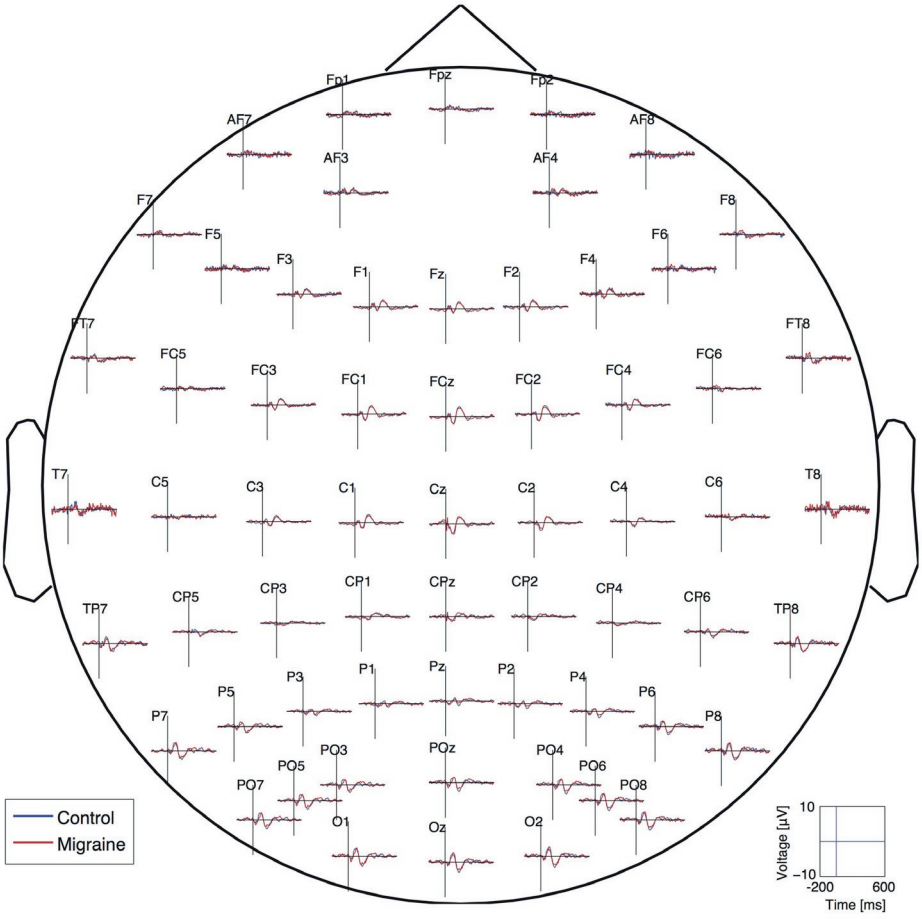
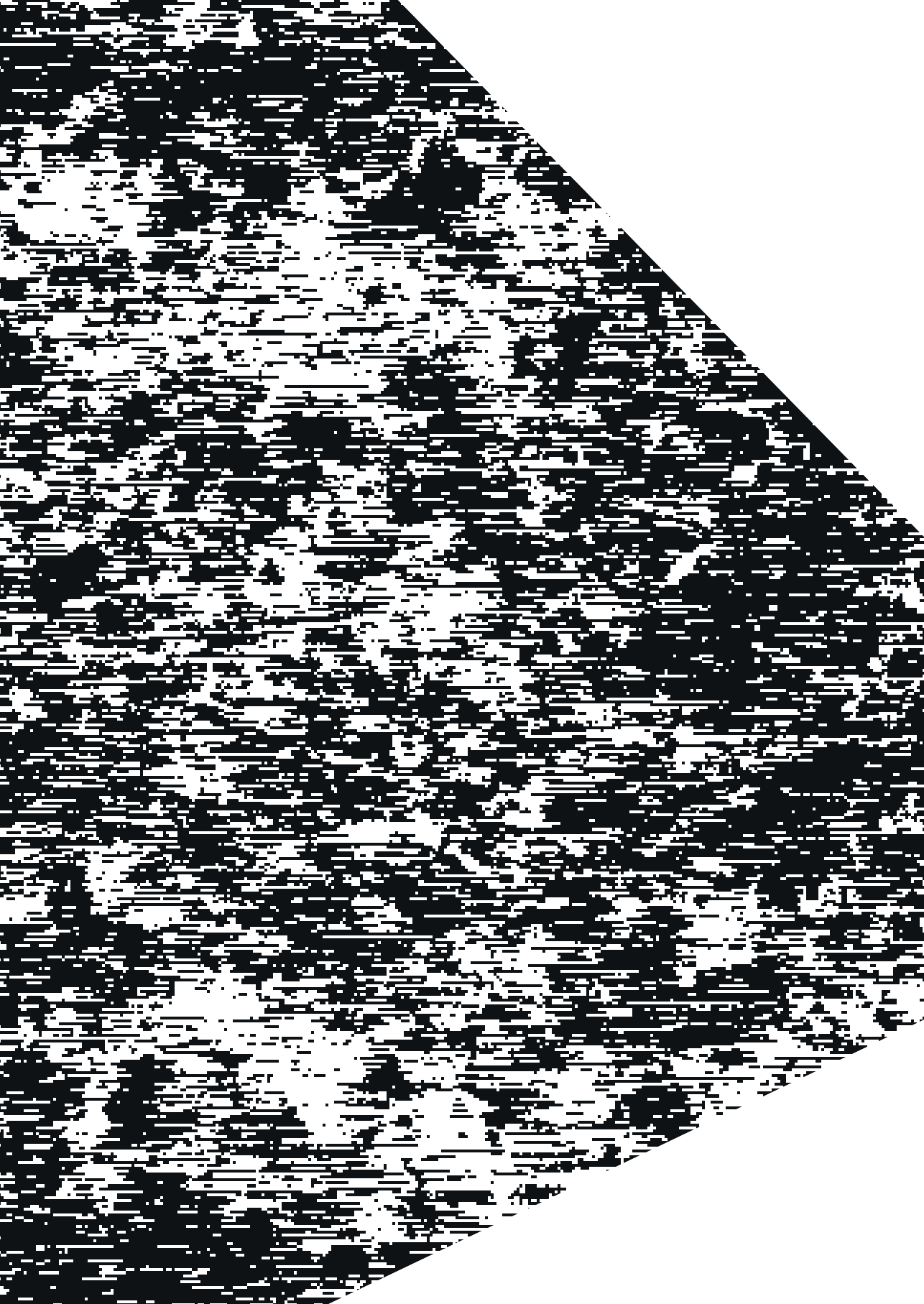


Figure S2. Distribution of sham waveforms over the scalp for control and migraine groups. Amplitude of the sham waveforms is much smaller compared to TEP waveforms (same y-axis limits are used as in Figure 2). Note the similarities in waveform between groups, like direction and delay of the sham-coil induced peaks around 100 and 180 ms.



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.

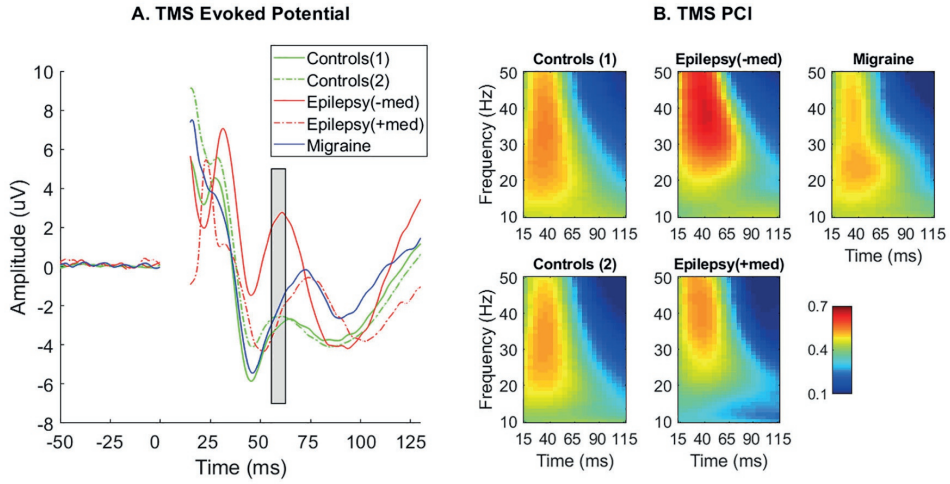

Figure 1. (A) TMS-evoked potential over the central electrode cluster for control, group with epilepsy, and group with migraine. Evoked responses averaged over a central electrode cluster, consisting of electrode Cz (the TMS target) and the neighboring electrodes surrounding electrode Cz. The gray area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

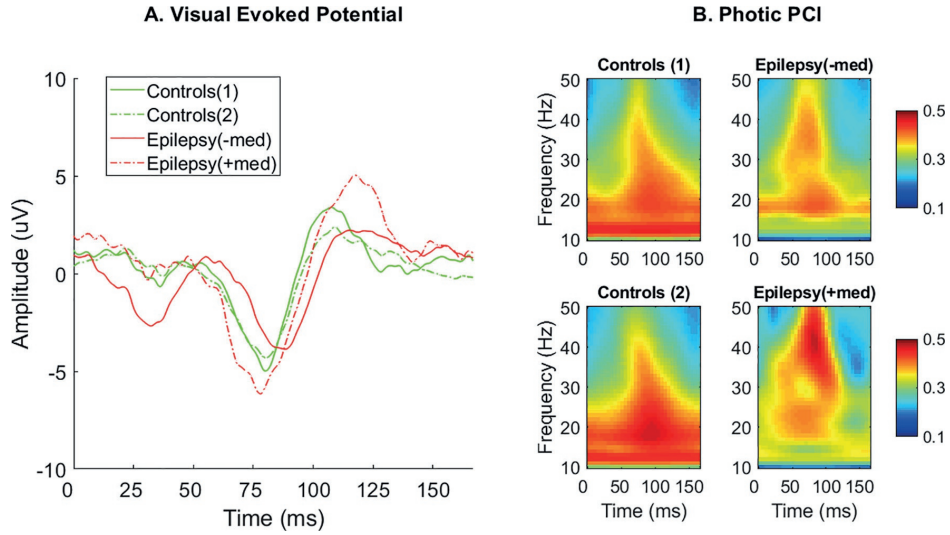

Figure 2. (A) Visual-evoked potential averaged over the occipital electrode cluster. Evoked photic response for the occipital electrode cluster consisting of Oz and the neighboring

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.

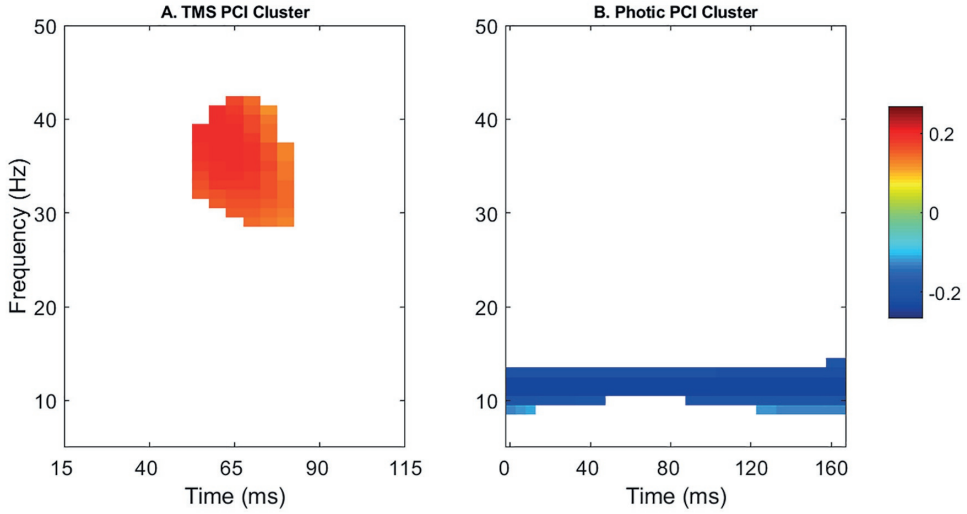

Figure 3. Monte Carlo permutation testing revealed significant differences in TMS (A) and photic stimulation (B) for the epilepsy(−med) versus controls(1) group comparison. Monte Carlo permutation testing with 2500 permutations, a cluster-alpha of 0.01 and significance of 0.025 revealed a significant 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.

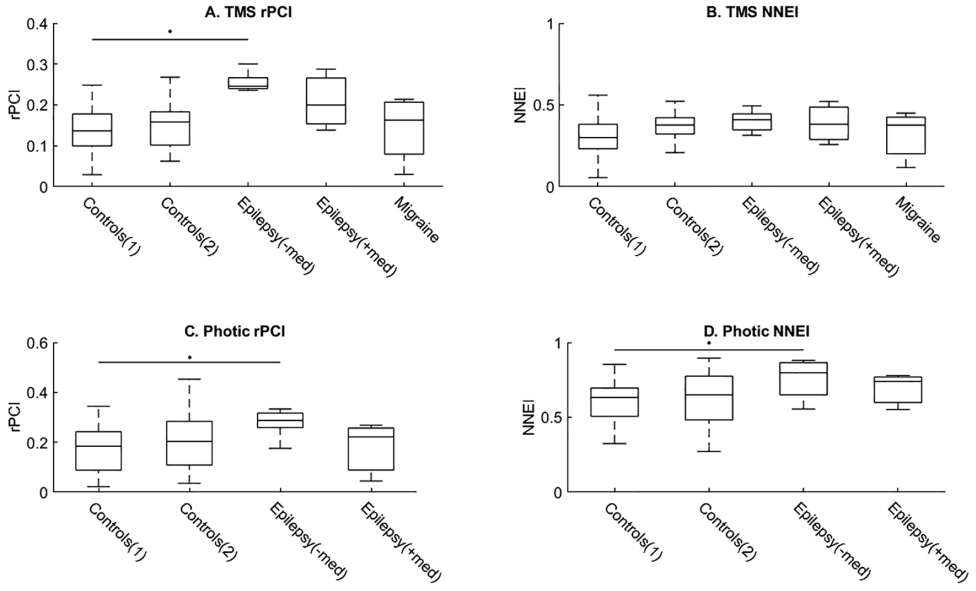

Figure 4. Excitability biomarker boxplots for all groups. Median TMS polarity-compensated relative phase clustering index (rPCI) and neural network excitability index (NNEI) for all groups and stimulation modalities. The boxes show the 25–75th percentiles, the line in the box is the sample median. The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

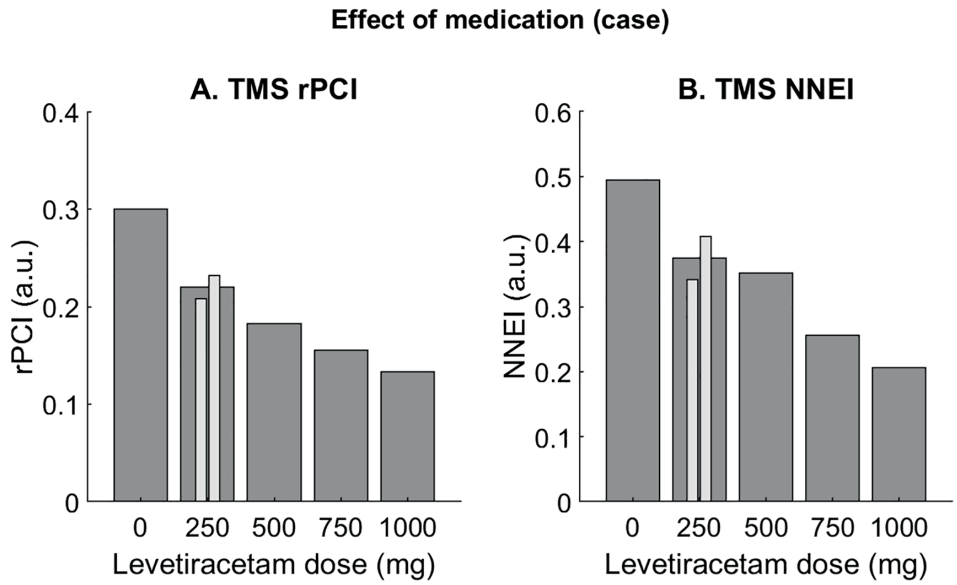

Figure 5. Effect of medication (levetiracetam) on rPCI and NNEI for one participant with juvenile myoclonic epilepsy. For case E3 of Table 1, the evolution of the polarity-compensated rPCI and NNEI against the levetiracetam dose is depicted. This is the only participant in whom several measurements were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

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

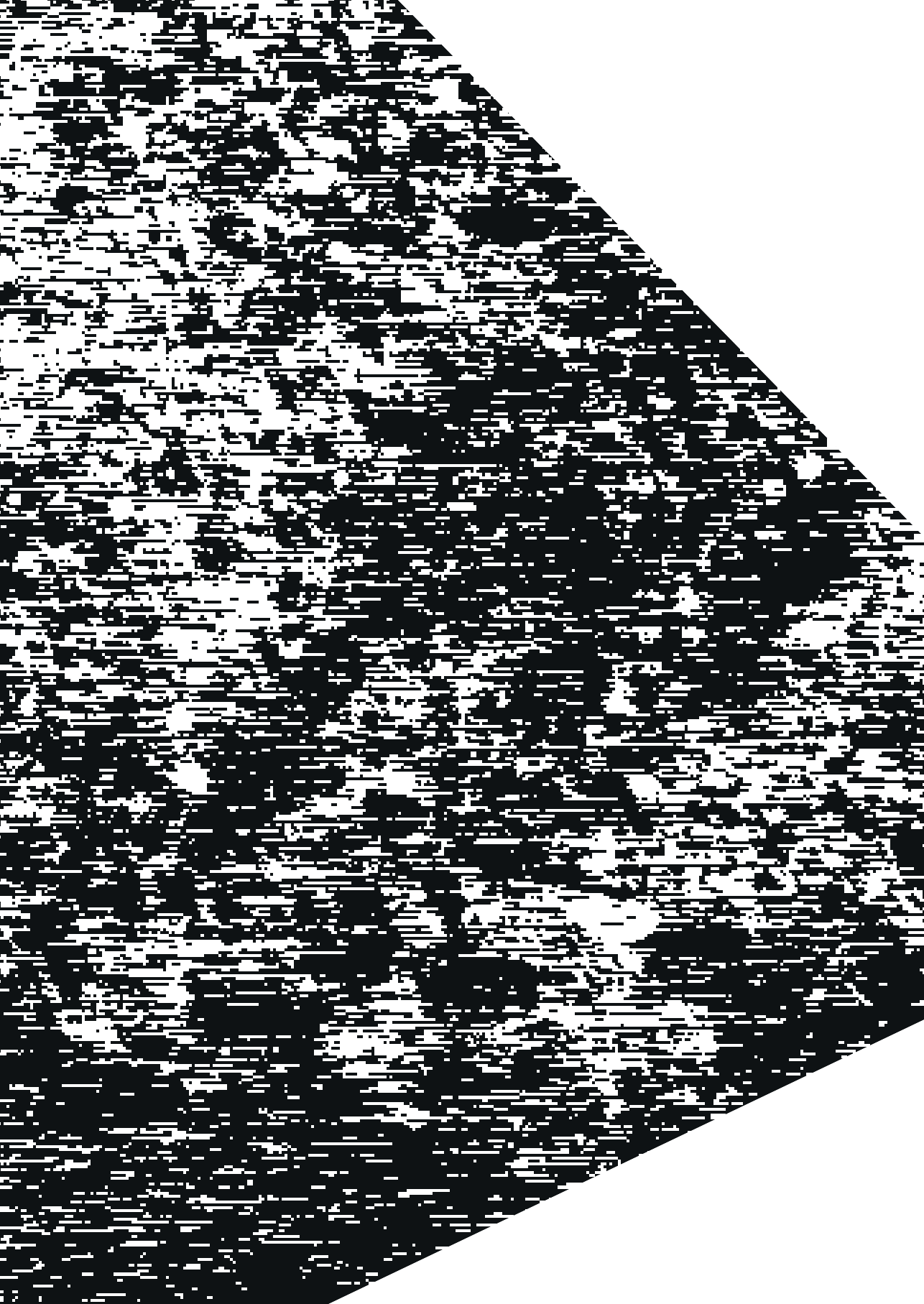
This form is different from earlier publications [28,47]. While the results calculated in both ways are similar, this norm allows for a better pathophysiological interpretation of the underlying mechanism.

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.



Chapter 7

General discussion

General discussion

The ability to predict the timing of a migraine attack would reduce the burden of migraine substantially, and open up new horizons for short-term preventive therapies. The fact that only few studies have focused on identifying functional markers of attack initiation in migraine is probably a reflection of the challenges to measure brain activity from a migraine patient while he or she develops a spontaneous attack. Migraine patients often indicate that certain *external* triggers specifically enhance the chance of developing an attack.[1,2] When tested in a clinical laboratory setting, however, it has always proven difficult to initiate an attack with supposedly reliable, patient-specific triggers like visual stimulation or physical exercise.[3,4] Elaborate neuroimaging and neurophysiology studies that aimed to dissect *internal* mechanisms contributing to the initiation of a migraine attack identified specific rises in brain activity during early phases of migraine attacks in the hypothalamus,[5] sensory cortex,[6] or visual cortex.[7] Applying such measurements on a daily basis for an early warning of an impending attack, preferably in a home setting, seems impracticable or even impossible.

The original research idea for this thesis was to combine longitudinal neurophysiological recordings with visual stimulation or transcranial magnetic stimulation, with the aim to identify neurophysiological features with predictive value for an upcoming migraine attack. This idea was based on the theory that in several biological systems, the speed by which a system recovers from a short perturbation is reduced when the system is nearing a tipping point, i.e., in our case a migraine attack onset.[8] However, we first had to develop a 'toolbox' of functional methods that are easy to apply over the migraine cycle with sufficient reproducibility, that allow measuring changes in brain excitability. Only after development of such methods, it is feasible to identify neurophysiological features indicative of an impending migraine attack. To this end, we developed and tested different techniques that allow to measure longitudinal changes in (cortical) responsivity in migraine. Firstly, we developed and applied the Leiden Visual Sensitivity Scale (**Chapter 2**). Secondly, we tested the applicability of several visual stimulation paradigms, including the visual chirp stimulation, in combination with EEG recordings, in a migraine mouse model (**Chapter 3**), and next validated the

usefulness of the same visual chirp stimulation in migraine patients (**Chapter 4**). Lastly, we studied transcranial magnetic stimulation evoked potentials (TEPs) and EEG phase clustering in migraine with aura and epilepsy (**Chapters 5 and 6**).

In this discussion, the findings presented in this thesis are placed in a broader context and suggestions are provided for future work aimed at identifying and understanding functional features of migraine attack onset.

Visual sensitivity and cortical excitability

In this thesis we identified features of abnormal visual processing in the migraine brain, evidenced from (i) the increased visual sensitivity in migraine, especially in the ictal state and further enhanced in migraine with aura and chronic migraine, as measured by the L-VISS questionnaire (**Chapter 2**), (ii) altered interictal TEP responses at the visual cortex (**Chapter 5**), and (iii) specific pre-ictal enhancement of visual responsivity in response to chirp stimulation (**Chapter 4**). Our findings point to hyperexcitability of visual cortical networks, and using a subjective outcome instrument as the L-VISS may provide a more simple approach to assess (clinical symptoms of) abnormal visual cortical excitability. In contrast to the used VEP and TMS measures, however, our L-VISS measures do not provide direct insight in underlying neuronal network mechanisms. Subjective measures of visual sensitivity in its different forms (like perception of luminance, contrast, color, motion and orientation[9]) are not a substitute for objective, but more elaborate, measures of cortical excitability using neuroimaging or neurophysiology. Indications of enhanced visual system activation during the interictal period based on subjective measures of visual sensitivity with questionnaires (**Chapter 2**), or psychophysical tests concerning visual motion, contrast or orientation sensitivity in migraine,[10,11] will have increased value when being supported by findings from neurophysiology and neuroimaging studies.

With magnetic resonance imaging, photophobia as measured by questionnaires positively correlated with blood oxygen-level dependent activation in the visual cortex interictally,[12,13] and positron emission tomography activation levels were also extra enhanced in migraine patients experiencing photophobia compared to patients without this symptom.[14] With a psychophysical approach, enhanced

contrast perception in the interictal period in migraine has been related to excessive cortical GABA-ergic inhibition in between attacks, which would reduce in the pre-ictal phase, thus resulting in more excessive excitation.[15]. Also in schizophrenia patients, performance in a psychophysical contrast increment tasks has been correlated to levels of the inhibitory neurotransmitter GABA as measured with magnetic resonance spectroscopy.[16] To further explore the underlying mechanisms of visual sensitivity as measured by the L-VISS questionnaire or other psychophysical measures, combined research including neuroimaging or neurophysiology would be a next step.

After publication, the L-VISS questionnaire has been applied in other patient groups with possible alterations in cortical excitability.[17,18] People with Visual Snow Syndrome, a condition where continuous visual distortions like TV-static are present in the entire visual field, report a level of visual burden (as measured by L-VISS) comparable to migraine patients during the attack, irrespective of comorbid migraine.[17] Other studies had shown that contrast and luminance increment thresholds are altered in those patients, suggestive of elevated visual cortex excitability in Visual Snow Syndrome.[19] In patients with the chronic pain syndromes fibromyalgia or chronic regional pain syndrome (CRPS), visual discomfort as measured by L-VISS was increased with regards to controls or patients with other chronic pain like back pain or ostheoarthritis.[18] In CRPS and fibromyalgia, but not in the other pain conditions studied, hypersensitivity to bright light and flashing stimuli was previously reported, possibly as a result of central sensitization.[18] The increased levels of visual sensitivity in those disorders could be another, still indirect, hint of the link between visual sensitivity and cortical excitability.

Recently, a couple of longitudinal studies employed measurements of visual sensitivity across the migraine cycle using on-screen image presentations. Afterimage duration was increased in individual migraine patients during the attack compared to interictal measurements, while 48 and 24 hours before attack onset the averaged afterimage duration showed a non-significant positive trend.[20] The detection of contrast increments, but not luminance increments, improved in patients two days (but not one day) before the attack.[15] This effect was mainly reported on a group level, but was also highly patient-specific; contrast perception was enhanced either before or during the attack depending on the subject. Lastly,

the threshold for detecting vertical coherent motion improved from two days before to two days after the attack.[9] These findings, based on subjective psychophysical experiments, illustrate that within-patient differences in visual processing may lead to individual 'attack predictors'. With a questionnaire like the L-VISS, such patient-specific differences in visual sensitivity could also be tracked. The subjective nature of such instruments, however, still allows for the presence of a learning effect and personal bias in the interpretation of these tests. Parallel future neurophysiological and neuroimaging studies would be required to identify whether patient-specific changes in visual processing occur towards and during attack onset.

Translational research for identification and understanding of migraine attack biomarkers

In this thesis, we studied the visual chirp stimulation, a promising neurophysiological method for migraine attack prediction, in a migraine mouse model (**Chapter 3**) as well as in migraine patients (**Chapter 4**). A translational approach helps to unravel the mechanistic underpinnings of functional differences between migraine patients and headache-free control subjects, and has identified neuronal hyperexcitability as key feature contributing to migraine-related functional changes.[21,22] The influence of genetic background on neurophysiological findings in migraine,[23] is controlled for in animal experiments by studying comparable stimulation paradigms as are used in patient studies. Also, more invasive recordings at *in vitro* (e.g., neuronal) and *in vivo* level (e.g., single cell, local fields potentials and intracranial EEG) could be conducted with relevant mouse models.

The mutant mice used for the VEP experiments in **Chapter 3** harbor an R192Q mutation that in patients causes familial hemiplegic migraine type 1 (FHM1). The mutation causes a gain of presynaptic neuronal $Ca_V2.1$ channel function that was demonstrated to lead to enhanced glutamatergic neurotransmission in the cortex.[24,25] Those Ca^{2+} channels play a key role in thalamocortical oscillatory activity, as absence of $Ca_V2.1$ channels showed reduced gamma-band power in *in vitro* and *in vivo* experiments.[26] FHM1 mutant mice showed entrainment of cortical oscillatory activity up to 40 Hz, as evidenced by an enhanced EEG response power to chirp stimulation in beta- and lower gamma-band (**Chapter 3**). This observation

adds to the literature pointing towards enhanced thalamocortical excitability in a migraine-susceptible brain.[27] In our mouse study, especially the combination of local field recordings and subdural EEG recordings allowed us to point to a role of neural network interactions outside of the visual cortex, as local neuronal activity during chirp stimulation was absent above 15 Hz, whereas in the EEG recordings entrainment was present up to 40 Hz (**Chapter 3**). In the same mouse model, females were shown to be most susceptible to induction of cortical spreading depolarization (CSD),[28] the neural correlate of the migraine aura. Our preliminary observation that EEG responses to visual chirp stimulation appeared larger (but not significantly so) interictally in female migraine patients (**Chapter 4**) is an interesting lead for further translational research.

In migraine patients, beta-gamma band responses to chirp stimulation were previously reported to be increased interictally,[29] whereas our experiments in migraine patients showed this enhancement only for pre-ictal recordings (**Chapter 4**). Our translational findings (**Chapter 3**) support the link between enhanced cortical excitability[22,30] and increased chirp responsivity in migraine, while our clinical findings (**Chapter 4**) suggest a transient pre-ictal, but not interictal, rise in responsivity. Compared to previous reports of interictal increases in EEG responsivity to visual stimulation in beta-gamma band frequencies,[29,31,32] our findings could differ due to selection of participants experiencing at least on migraine episode per month. As cortical excitability measured in migraine patients differs with the attack frequency, within episodic migraine[33] and between episodic and chronic migraine[34] (as also indicated in **Chapter 2**) a comparison between groups of patients with different attack frequencies could provide more insight into possible frequency related differences in EEG responses to visual chirp stimulation.

To further our mechanistic insights in brain disorders, the recent development of 'lab-on-a-chip' methods to study, e.g., the effect of ion channel deficiencies at a neuronal (and even vascular) level are exciting. *Ex vivo* cellular models using brain slices or neuronal cultures based on stem-cells derived from migraine patients can help to elucidate the role of glutamatergic versus GABA-ergic inhibitory neurons in altered brain excitability in migraine[35] and, by comparison to *in vivo* animal models, roles of larger-range brain connectivity. Cortical dynamics are being studied with 'brain-on-a-chip' models with thalamic and hippocampal input,[36] and epileptic

seizures could already be modelled with a modular approach to mimic functionally connected (human) neural networks.[37] On the side of visual input, several advances are made in 'eye-on-a-chip' models, with focus on retinal or cornea models to study ophthalmic disorders.[38] The combination of these different models can provide a first step towards unraveling functional features of the entire chain of visual processing, in line with future visions of a 'human-on-a-chip'.[39] In migraine research, however, factors like individual external attack triggers and the combination of systemic fluctuations possibly underlying an attack, are highly unlikely to be mimicked in *ex vivo* research. In this light, *in vivo* animal research will remain an important link between *ex vivo* research in cellular models, and human patient studies, to bridge the knowledge gaps between single cell responses, local interactions and visual system responsivity.

The value of TMS-EEG measurements in migraine attack prediction and prevention

Transcranial magnetic stimulation as a method to probe cortical excitability has been applied in migraine and epilepsy in various ways. Here, we focused specifically on the direct measurement of cortical responses using concomitant EEG recordings. In **Chapter 5**, we showed frontal and occipital decreases in the N100 peak of the TMS evoked potentials for migraine patients with aura in the interictal phase compared to controls, but no differences in phase clustering over stimuli for any of the studied EEG response frequency bands. In people with epilepsy, relative phase clustering was enhanced when no anti-epileptic medication (typically directed at reducing excitability) was used (**Chapter 6**), but not when such medication was used, nor in controls or migraine patients with aura. In line with the findings at the group level, in a single subject with epilepsy, we demonstrated an inversely proportional relationship between medication dosage and phase synchronization.

In contrast to our TMS-EEG findings in migraine patients (with aura; **Chapters 5 and 6**), altered EEG phase synchronization in relation to visual stimulation was reported for migraine (with and without aura) patients, indicating altered excitability of the visual cortex.[40,41] The effect, however, was not similar in both subgroups of migraine; phase synchronization was enhanced in the alpha band in

migraine without aura, whereas it was decreased in the beta band in migraine with aura.[40] In photosensitive epilepsy, increased phase synchrony to flash stimuli was measured in the EEG gamma band right before the occurrence of the light-induced epileptic discharge.[42] This synchronization could be indicative of an increased propensity to neural entrainment. Although we measured a similar propensity of enhanced synchronization involving the gamma-band using visual chirp stimulation in migraine (**Chapter 4**), direct stimulation (over electrode Cz at the center of the scalp) of cortical neurons by magnetic stimulation did not result in entrainment in the visual cortex (**Chapters 5 and 6**). The absence of such synchronization effects with TMS in migraine with aura points to different underlying mechanisms, including region and frequency specific effects, and between subgroups of patients. Future studies incorporating groups of migraine with and without aura (compared to controls), and utilizing multiple stimulation frequencies and synchronization measures are necessary to provide additional insight in phase clustering and its relationship to cortical excitability in migraine.

Over the migraine cycle, various motor responses after TMS, measured by electromyography (EMG), were altered pre-ictally compared to interictal measurements,[33,43] and also differed during and after an attack.[33] Those responses were also altered in interictal migraine patients compared to controls, in line with our findings obtained by EEG for cortical instead of motor activity (**Chapter 5**). In epilepsy, seizure susceptibility is suggested to be reflected in changes in TMS-induced motor responses up to 24 hours before the seizure.[44,45] TMS-EEG could be an addition to this repertoire of tests not only for epilepsy but also for migraine. Unlike visual stimulation (**Chapter 4**), such tests involving daily magnetic stimulation seem currently only suitable in a clinical setting and are thus mainly of interest for research purposes rather than supporting individual attack prediction.

The observed effect of medication which alters brain excitability on TMS induced phase clustering in one epilepsy patient (**Chapter 6**), emphasizes that another potential clinical application of TMS-EEG in migraine is the prediction of an individual's response to preventive medication.[46,47] Clinically, medication responsiveness can only be established when a period of at least three times the usual interval between attacks has passed without attacks. In epilepsy, attacks are usually easy to count (unless the patient is unaware of seizures) and are preventively treated

even when their frequency is low (e.g., two per year), hence this may require a long period of observation. In migraine, other issues hamper the clinical evaluation of preventive medication. The number of attacks per month is more difficult to register for migraine patients, as attacks vary in severity and duration. Furthermore, epilepsy preventives induce complete remission more often than migraine preventives. Therefore, migraine patients find it difficult to assess the overall effectiveness of their preventive medication. Objective measures to predict medication efficacy by assessing the inhibitory/excitatory balance in the brain could therefore be of great clinical benefit when preventing migraine attacks. In epilepsy, an increased TMS-EMG resting motor threshold, induced with anti-epileptic medication, was positively correlated with seizure reduction after one year.[48] With TMS-EEG, based on single and paired-pulse stimulation a distinction between anti-epileptic medication responders and non-responders could be made with 80% accuracy (compared to 92% accuracy for differentiating patients from controls).[49] In migraine, some older studies associated altered in phosphene thresholds with the prophylactic effects of e.g. beta-blockers or anti-epileptic medication on cortical excitability.[50,51]

Predicting migraine attacks requires longitudinal studies

Most studies in migraine focus on differences between measurements in migraine patients during the interictal phase and control subjects, with a wide variety of measurement modalities and readouts. Methods from neuroimaging, neurophysiology, neurochemistry and psychophysiology are applied to provide insights in the 'trait' of migraine, i.e., in which way differs the physiology (and psychology) of a migraine patient compared to people without the disease.[22,52] For insight in the start of the migraine attack, we are more interested in the 'state' of the migraine patient, i.e., in which way differs the physiology (and psychology) of an individual with migraine during the different phases of the migraine attack.

Different theories and frameworks regarding the onset of the migraine attack co-exist in scientific literature, most of which focus either on changes in cortical excitability,[8,53] or changes in subcortical brain activation levels.[5,54] The onset of the migraine attack is hypothesized to be related to a 'critical transition' in brain

dynamics.[8] In a similar way, EEG-based signatures of epileptic seizure susceptibility showed paroxysmal critical transitions before an attack based on the concept of critical slowing down over short (minutes) and longer (hours to days) timescales.[55] As the tipping point of this transition is approached, the migraine attack threshold lowers and smaller triggers are sufficient to start the attack.[8] Based on the theory of early-warning signals for critical transitions,[56] the recovery rate to small perturbations (like flashes of light) decreases as the tipping point (the migraine attack onset) is eminent.[57] Our findings support the view that brain excitability, including that of the cortex, fluctuates over the migraine cycle (**Chapters 2 and 4**). This fluctuation combines with (and may be caused by) effects of other physiological rhythms that are related to factors like (lack of) sleep, stress and hormonal levels. The combined impact of these changes on brain function could cause a migraine patient to have a temporarily lower attack threshold. A trigger like a flashing light, a change in external stressors or intake of certain food that normally would not initiate a migraine attack, could in case of a lower attack threshold start a cascade of brain activation leading to the migraine headache.[8] For instance, a reduction in stress level (i.e., relief after stress) appeared to be a specific trigger for headache initiation in certain patients.[58] The concept that relief after chronic stress could lower the attack threshold has been supported by pre-clinical findings in the FHM1 mouse model.[59] In the cortex, a lower threshold as result of hyperexcitability can also result in a migraine aura by initiation of a cortical spreading depolarization.

For longitudinal studies over the migraine cycle, the reproducibility of EEG read-outs within a participant should be high for consistent *intra*-individual comparisons (**Chapter 4**). Several findings of altered interictal cortical excitability, however, could not be reproduced in other, blinded, study designs.[60,61] Multiple reasons for this lack of *inter*-individual reliability are proposed, like differences in stimulation parameters (e.g., intensity, frequency and duration) and read-out parameters (e.g., block amplitude, synchronization, habituation, et cetera), but also timing with respect to the previous or next migraine attack, differences in medication or comorbidities, and (relatively) low number of patients in studies.[22,61] It could be possible that for different individuals, different stimulation paradigms and read-outs need to be combined to meet this criterium. Therefore, already during the development of biomarkers that could be used as early-warning signals for an

impending migraine attack, this longitudinal reproducibility should be taken into account by repeatedly measuring the same patients – preferably over multiple attacks – before conclusions can be drawn. As outlined in the next paragraphs several promising recent developments including home EEG recordings and the rise of data analysis using artificial intelligence are leading the way towards such studies.

Future directions in migraine attack prediction

The prediction of an impending migraine attack with a simple home test would be valuable for patients on several levels.[8,62] It provides patients with the possibility to manage their lives around the paroxysmal nature of the disease, and helps in timing the use of pre-emptive prophylactic medication to avert attacks, as well as acute medication to suppress or shorten the headache phase. From a research perspective, new avenues for therapeutic targets and drug development could open up when it becomes easier to study patients with more elaborate methods like neuroimaging in research labs or hospitals during the premonitory or early headache phase.

Home EEG recordings

Recording brain signals in a home environment used to be limited to small-scale, often recreative, EEG systems with a limited number of electrodes in e.g. a headband. Systems developed for this purpose demonstrated the potential for research applications by recording event-related potentials after auditory stimulation, although especially the signal-to-noise ratio warrants improvement.[63] More elaborate scalp EEG systems with a cap with 10 or more electrodes and direct connection to a smartphone could provide more information, although home application could be more bothersome due to the number of electrodes. Comparing data obtained with an open source smartphone-based system to a standard clinical EEG system, both used for recordings in a hospital setting, showed that epileptiform abnormalities were correctly captured when the smartphone-based EEG recordings were analyzed manually by neurologists, albeit with lower sensitivity than with the standard EEG recordings in the same patients.[64] To train patients to use comparable systems themselves in a home setting with the aim of consistent, longitudinal data generation is one of the challenges that have to be overcome. Easier-to-use EEG

systems could mitigate the inconsistent application of for instance the EEG cap, with mobile in-ear EEG electrodes integrated in a headphone providing a possible solution.[65]

In migraine, a promising longitudinal application of home EEG applying a commercial device with electrodes over the frontal cortex was recently published.[62] Resting state brain waves and image-induced event-related potentials were recorded daily for 14 days, with patients doing all the necessary setup themselves. Based on diary input, recordings were categorized into interictal, pre-ictal (<24 hrs before an attack), ictal or post-ictal (<48 hrs after an attack) phases. Decreased theta power, increased relative beta power, and decreased event-related potential amplitude were present in the 24 hours before an attack and during the attack, compared to interictal recordings in the same patients. In another pilot study, patients were recorded at least five times per week over several weeks with a similar commercial EEG device, while receiving an auditory oddball task. Prediction of attack likelihood improved with one or two (short) tests in the pre-ictal phase, where induced EEG responses differed from *a priori* defined template EEG activity.[66] The relationship between the changes in EEG features observed in these longitudinal recordings and possible underlying changes in cortical excitability remains to be determined.

To efficiently implement longitudinal home recordings of EEG activity in patients, several difficulties that were indicated in those recent longitudinal studies with large, at-home, patient involvement (like setting up the EEG recordings without help of a researcher)[62,66] need to be addressed in further research. Firstly, no within-patient repeatability could be tested, and when multiple recordings in the same phase were available in the same patient only the measurement with highest quality of data was used. Secondly, for a single daily measurement participants had to record at least 20 minutes of brain activity, which could be a large burden in a home setting. Still, data quality was relatively low, as just 20-30% (7-11 out of 35, depending on the paradigm) of participants had enough artifact-free recordings in all migraine phases.[62] A quicker stimulation with high signal to noise ratio, like the visual chirp stimulation (**Chapter 4**) could improve data quality issues. Thirdly, the division of phases in 24 hours blocks for the statistical analysis still averages brain activity that might change on an hour-level towards an impending attack. Daily neuroimaging measurements showed that up to 48 hours before an attack the brain's activity is

already altered.[5] Especially an easy-to-use method like EEG could shed further light on the relevant time scale of attack prediction, possibly in combination with psychophysical visual tests,[9] or questionnaires (**Chapter 2**).

Resting-state recordings of brain activity and artificial intelligence

In this thesis, we studied the brain's response to external perturbations. From the ongoing EEG activity (so-called 'resting-state' EEG), however, a wealth of additional information about the brain's functioning including disease propensity could also be extracted. Standard quantitative EEG analyses in migraine did not provide a clear biomarker for disease presence or phase of the migraine cycle.[67] With the advent of artificial intelligence methods to be applied to large EEG datasets, several new directions of study become available.[68]

Firstly, with machine learning methods, classification patterns that otherwise may go undetected could be distinguishable, by combining multiple – hundreds – of EEG-based features, and also data from e.g. questionnaires and patient headache diaries, in a single model.[69] For example, when classifying pain phenotypes using standard quantitative EEG features, multiple comparison correction limited the amount of features that could be taken into account. With a machine learning classification algorithm, hundreds of EEG features over multiple frequency bands and electrode locations were combined in one model, demonstrating the possibility of pain phenotype classification that was not feasible with the traditional combination of statistics and feature extraction.[70] Due to the number of features and the non-linear nature of the model, it was unfortunately not possible to study which parameters carried the largest distinctive load.[70]

Secondly, non-standard features can be extracted automatically from the raw EEG signal using a subset of machine learning, i.e., deep learning methods like convolutional neuronal networks. Using such methods, the gender of a subject could be predicted based on EEG signals only; reverse-engineering of the sex-specific features revealed that fast beta activity and its spatial distribution were main attributes.[71] With a large database of EEG recordings of people with migraine and controls, similar big data analyses could yield insights that are not attainable in smaller studies like presented in this thesis. The clinical relevance of the predictive

ability of newly detected EEG features should be in balance with the amount of intra- and interpatient recordings necessary to detect the feature(s). As observed in longitudinal recordings in people with epilepsy, features indicative of an impending migraine attack with a large individual effect size (high predictive value within a patient) could be more relevant than standard clinical neurophysiological features with a larger group effect size but smaller individual effect.[72]

Thirdly, 'deep learning' methods could aid in the prediction of migraine attacks by building patient-specific models that take into account individual variation in activity within and across brain areas. Deep learning methods are able to extract ('learn') relevant features from large datasets with examples, like EEG recordings with the corresponding migraine phase, without explicit definition of EEG features by a researcher. Within-patient epileptic seizure detection using longitudinal scalp EEG, recorded with a wearable setup, indicated that patient-specific models can be effective for individual seizure prediction.[73] Interestingly, a more general seizure prediction model, developed on EEG data from multiple patients, could easily be adapted to a personalized attack prediction model using transfer learning (i.e., adapting a general model by partly retraining it with a smaller amount of extra data).[73] After an initial model development phase on a large dataset containing longitudinal EEG data from many patients (20 or more), for other patients personalized predictive models could possibly be developed. Short EEG recordings (up to a couple of minutes) could then suffice in training such individualized predictors based on the general model, instead of needing multiple patient-specific, longitudinal EEG recordings (resting state and/or evoked EEG responses) to build a personalized model.[73] Applying such an approach to migraine attack prediction could direct future studies towards the development of a general EEG-based attack onset model, that is adaptable to individual differences in brain activity (including responses to triggers) towards the next attack.

Multidisciplinary research in a university medical center

A multidisciplinary approach towards migraine attack prediction, as described in this thesis, with a combination of clinical and translational studies with a focus on state-of-the-art data analytics, is important to bring together knowledge in the

different fields studying the origins of migraine. Within a university medical center, there is wide-spread clinical, medical and biological expertise; the addition of technical expertise provides opportunities in, amongst others, data analytics, and hardware and software design for stimulation and recording. With the emergence of overlapping, multidisciplinary study fields like biomedical engineering, clinical technology and technical medicine, researchers working as intermediaries between patients, clinicians, biologists and engineers will be better equipped to balance different visions on e.g. patient burden, clinical and biological relevance, technical implementation and, preferably, also make the sum more than its parts. Challenges in patient recruitment and measurements, stimulus design and data analysis would benefit from such a combined approach by selecting the right discipline for each step – while maintaining oversight of all developments.

The success of a multidisciplinary approach is not a given. Complex research questions, like the origin of the migraine attack, have to be solved at the cross-borders between the patient, the doctor, and a technical environment.[74] By being open to each other's viewpoints and qualities, expectations between researchers, medical doctors and patients can be managed and resources allocated; the so-called 'discipline openness' challenge.[74] For instance, while many recordings and burdensome stimulations might improve the availability of (EEG) data, the patient's involvement will probably be more difficult to ensure. As seen in migraine research, new methods to perturb and probe the brain's activity emerge on a regular basis, shining new light (sometimes literally) on the enigma at hand. Where this tendency to accumulate, by adding more techniques to a toolbox with each newly involved discipline, possibly leads to more publications, the step to better integrate and compare those techniques might lead to more insight.

While connecting people, data and health systems,[75] all involved disciplines should put the patient first. Especially in migraine, with a disease burden that stretches beyond the headache phase, a home test for an impending attack should be easy to do for the patient – possibly by subtracting as much technology as possible to get a simple yet effective home test.[76] How much technology could be subtracted is one of the next challenges.

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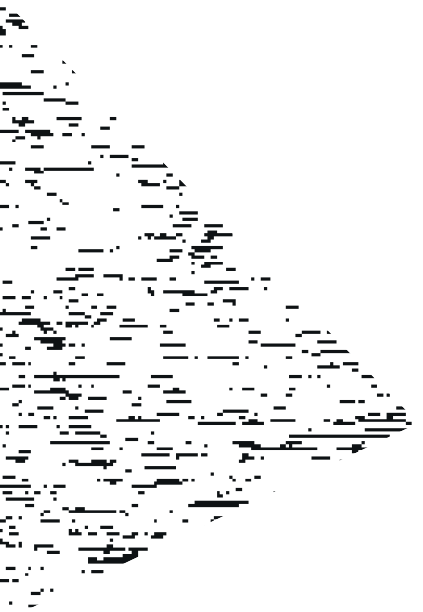
Appendices



Summary

The aim of this thesis was to identify functional biomarkers for migraine attack prediction based on neurophysiological readout parameters. Migraine is a paroxysmal brain disorder, whereby attacks of headache and associated neurological symptoms like nausea, vomiting and enhanced sensitivity to light and sound, are separated by periods without attacks. About one-third of people with migraine experience visual aura features, like expanding fortification spectra and/or scotoma's, before and during the start of the headache. In the International Classification of Headache Disorders, the number of headache and migraine days per month determines if a patient is considered as either episodic or chronic. Patients experiencing at least 15 headache days per month, of which at least 8 are migraine days, are classified as suffering from chronic migraine. It remains an enigma exactly when and why migraine attacks start. It has been hypothesized that episodic alterations in brain excitability may be an important factor in the initiation and cyclic recurrence of migraine attacks. The main focus of this work, therefore, was on the development of methodologies to measure brain excitability over the migraine cycle, with special emphasis on identifying changes in excitability of the visual system and the occipital cortex. Applying such measures over the course of a migraine cycle could help elucidate factors that initiate the migraine attack, and might lead to better (or better timing of) preventive measures. The research described in this thesis is divided into two parts. The **first part** reports on the development and application of several methodologies to measure excitability of the visual system including the cortex in migraine patients and a migraine mouse model. The **second part** consists of two studies employing transcranial magnetic stimulation (TMS) in combination with concurrent electroencephalography (EEG) recordings to provide direct measures of cortical excitability in migraine and epilepsy.

Part I focuses on visual system excitability as target for readouts that could help in predicting or indicating an upcoming migraine attack. Migraine patients often report (inter)ictal hypersensitivity to light, and visual pseudo hallucinations are the dominant symptom of a migraine aura. This suggests that migraine attack initiation

may involve fluctuations in responsivity of the visual system including the occipital cortex. Several methods can be used to examine occipital cortical excitability. Visual stimulation with flashes of light could be used to perturb the visual system in humans but also rodents, whereby the evoked potentials (VEPs) are evident in EEG recordings from the occipital cortex. In migraine patients, such measurements can be combined with subjective assessments of visual sensitivity. We developed a questionnaire to quantify self-reported sensitivity to light and patterns. In addition, for humans and for mouse migraine models, we developed several visual stimulation paradigms to be combined with EEG measurements.

Enhanced sensitivity to light (photophobia) and patterns is common in migraine and can – certainly when it is reported as painful – be regarded as visual allodynia. **Chapter 2** describes the development, validation and application of the Leiden Visual Sensitivity Scale (L-VISS), a 9-item questionnaire to assess sensitivity to light and patterns, with its content based on scientific literature and patient interviews. Construct validity (i.e., does the questionnaire measure what it aims to be measuring) was confirmed by comparing L-VISS scores to two behavioral tests. The light discomfort threshold was lower, whereas the pattern glare score was higher, with increased L-VISS scores. Comparing migraine subtypes (with versus without aura, chronic versus episodic) and states (during or outside an attack) between and within large groups of participants showed that L-VISS scores were increased for migraine with aura versus migraine without aura, for chronic versus episodic migraine, and during versus in between attacks. This pattern of increased visual sensitivity may reflect dynamics in cortical hyperexcitability between migraine subtypes and states, as also indicated by neurophysiological studies. The L-VISS has potential to be used in large-scale longitudinal assessments of sensitivity to light and patterns in patients, as it is quick to apply and not dependent on any recording technology. Besides, it could be used in conjunction with more elaborate neurophysiological recordings of visual cortex activity to provide a subjective assessment of changes in visual system excitability over the migraine cycle.

EEG studies in migraine patients show conflicting results indicating hypo- or hyperexcitability of the visual system. This can be caused by large inter-interindividual variation, differences in visual stimulation techniques and paradigms used, or intra-individual dynamics within the migraine cycle. To

understand the neuronal mechanisms underlying previously observed EEG features in patients, we strived to bridge the gap between the indirect measurements of visual system excitability by scalp EEG in patients and precise neurophysiological measurements in rodent models. In **Chapter 3** we studied EEG responses to visual stimulation in mice by combining local intracortical recording electrodes with simultaneous cortical surface EEG recordings. For clinical translation, we used transgenic mice carrying the human pathogenic R192Q missense mutation in the *Cacna1a* gene that causes familial hemiplegic migraine type 1 (FHM1). The *Cacna1a* gene encodes the α_{1A} subunit of presynaptic voltage-gated $Ca_V2.1$ Ca^{2+} channels, with the FHM1 R192Q mutation resulting in enhanced glutamatergic transmission and hyperexcitability. In freely-behaving transgenic and wild-type (control) mice, we investigated common clinical and newly developed visual stimulation paradigms consisting of flashes of light. FHM1 mutant mice displayed faster visual evoked potential responses following stimulation at varying intensities. The initial negative peak had a decreased amplitude with less neuronal suppression compared to controls. Flash light stimulation consisting of increasing stimulation frequencies between 10 and 40 Hz (the 'chirp' paradigm) showed enhanced photic drive in the beta-gamma bands (15–40 Hz). These results revealed a context-dependent enhancement of visual cortex excitability in the FHM1 mouse model. We hereby demonstrated that measurement of VEPs in transgenic mice can be applied to better understand changes in visual system responsivity in migraine.

One technique to study visual cortex excitability is the photic driving response in the EEG, as we also applied in the FHM1 mouse model in Chapter 3. In **Chapter 4**, we explore the use of the same 'chirp' visual stimulation paradigm to assess visual system responsivity including cortical excitability in migraine patients. Measurements were made in between attacks (interictal) and just before the next attack (pre-ictal). Using light flashes at increasing stimulation frequency, 'chirp' stimulation allows comparison of responsivity at various driving frequencies and related harmonic frequencies, which emerge in the EEG at multiples of the stimulation frequencies. This method thereby provides a quick way to examine photic driving over a range of stimulation frequencies. Our results showed that chirp readouts were repeatable over days to months, as demonstrated by repeated within-subject measurements. Interictally, responses to chirp stimulation were comparable

between controls and patients with migraine with and without aura. The 8 pre-ictal measurements (3 with, 5 without aura), which were recorded within 48 hours of an impending migraine attack, demonstrated an increased harmonic response in the beta band (22–32 Hz). Visual chirp stimulation proved a simple and reliable technique with potential to detect changes in visual cortex responsivity associated with the onset of migraine attacks.

Part II focuses on direct measurements of cortical excitability in migraine patients, in contrast to the indirect measurements with the L-VISS questionnaire or VEP recordings applied in the patient study in Part I. Visual stimulation is processed not only in the visual cortex, but also in pathways involving the retina, thalamus and superior colliculi. As such, VEP readouts are not only processed cortically, but also subcortically, thereby reflecting the excitability of the visual system as a whole. By employing transcranial magnetic stimulation (TMS) over the scalp with concordant EEG recordings, cortical excitability can be evaluated directly by studying TMS-evoked cortical responses. The TMS evoked potential (TEP) has been shown to be affected in conditions with implied underlying changes in cortical excitability like epilepsy and schizophrenia. **Chapter *5*** describes the first study investigating TMS evoked potentials in patients with migraine. Stimulation with a circular coil over the vertex, at stimulation intensities around the resting motor threshold, was applied to migraine patients with aura (in between attacks) and controls matched on sex, gender and resting motor threshold. Sham coil stimulation was employed to control for possible confounding effects of auditory and somatosensory activations by TMS. In migraine with aura, TEP waveforms were decreased in amplitude around the N100 peak at frontal and occipital electrodes. Decreased N100 peak amplitude is indicative of reduced cortical $GABA_B$-ergic inhibition, expanding previous – indirect – observations of cortical hyperexcitability in migraine.

Migraine and epilepsy are comorbid paroxysmal neurological disorders associated with altered cortical excitability. In **Chapter *6***, we investigated EEG phase clustering indices in response to transcranial magnetic stimulation in migraine with aura and juvenile myoclonic epilepsy patients, to identify potential functional biomarkers related to migraine with aura, epilepsy, or both disorders. Phase clustering in

response to TMS was significantly different between epilepsy (without medication) and controls. In one participant with epilepsy, the strength of phase clustering was inversely correlated with the dosage of antiepileptic medication. In migraine with aura, phase clustering did not differ from controls, indicating that the tendency for altered phase clustering is not shared between migraine and epilepsy.

Chapter 7 provides a general discussion of this thesis, with considerations for future clinical and preclinical translational research into migraine attack prediction using neurophysiological methods.

Nederlandse samenvatting

Het doel van dit proefschrift was het identificeren van functionele biomarkers voor de voorspelling van een migraineaanval, op basis van neurofysiologische parameters. Migraine is een paroxysmale hersenaandoening, waarbij aanvallen van hoofdpijn en bijbehorende neurologische symptomen zoals misselijkheid, overgeven en verhoogde gevoeligheid voor licht en geluid worden afgewisseld met periodes zonder aanvallen. Ongeveer een derde deel van mensen met migraine ervaart een visueel aura, bestaande uit visuele verschijnselen zoals een fortificatiespectrum en/of scotomen in het gezichtsveld, zowel voorafgaand aan als tijdens de hoofdpijn. In de Internationale Classificatie van Hoofdpijnaandoeningen bepaalt het aantal dagen met hoofdpijn en migraine per maand of een patiënt episodische of chronische migraine heeft. Patiënten met minimaal 15 hoofdpijndagen per maand, waarvan minimaal acht dagen met migraine, lijden aan chronische migraine. Het blijft een raadsel wanneer en waarom een migraineaanval begint. Een hypothese is dat episodische veranderingen in de exciteerbaarheid ('prikkelbaarheid') van het brein een belangrijke rol spelen bij de start en cyclische terugkeer van migraineaanvallen. De focus van dit proefschrift ligt daarom op de ontwikkeling van methodes om de exciteerbaarheid van het brein te meten over de migrainecyclus, met nadruk op de identificatie van veranderingen in exciteerbaarheid in het visuele systeem en de visuele schors. Door het toepassen van zulke methodes over de migrainecyclus kunnen factoren die bijdragen aan de initiatie van de migraineaanval worden bepaald, wat mogelijk leidt tot betere (tijdsbepaling van) preventieve medicatie. Het onderzoek in dit proefschrift is onderverdeeld in twee delen. Het **eerste gedeelte** beschrijft de ontwikkeling en toepassing van diverse methodes om de exciteerbaarheid van het visuele systeem (inclusief de hersenschors) te meten, in migrainepatiënten en een migraine muismodel. Het **tweede gedeelte** bestaat uit twee studies die transcraniële magnetische stimulatie (TMS) inzetten in combinatie met elektro-encefalografie (EEG) om een directe meting van corticale exciteerbaarheid te bereiken, in migraine en epilepsie.

Deel I is gericht op de inzet van metingen van de exciteerbaarheid van het visuele systeem om te helpen met het voorspellen of aanduiden van de start van een migraineaanval. Migrainepatiënten rapporteren vaak (inter)ictale overgevoeligheid voor licht, en visuele pseudohallucinaties zijn een belangrijk symptoom van de migraine-aura. Dit wekt de suggestie dat het begin van een migraineaanval samenhangt met fluctuaties in de gevoeligheid van het visuele systeem, inclusief de visuele schors. Diverse methodes kunnen worden gebruikt om de exciteerbaarheid van de visuele schors te meten. Visuele stimulatie met lichtflitsen kan worden ingezet om het visuele systeem te verstoren in zowel mensen als diermodellen, resulterend in opgewekte EEG-potentialen over de visuele schors ('visual evoked potentials' of VEPs). In migrainepatiënten kunnen zulke metingen worden gecombineerd met subjectieve bepalingen van visuele gevoeligheid. Wij ontwikkelden een vragenlijst om gevoeligheid voor licht en patronen uit te vragen. Daarnaast ontwikkelden wij, voor gebruik in mensen en diermodellen, diverse manieren van visuele stimulatie om te combineren met EEG-metingen.

Verhoogde gevoeligheid voor licht ('fotofobie') en patronen komt vaak voor in migraine en kan, zeker als het als pijnlijk wordt ervaren, beschouwd worden als visuele allodynie. In **Hoofdstuk 2** wordt de ontwikkeling, validatie en toepassing omschreven van de *Leiden Visual Sensitivity Scale* (L-VISS), een vragenlijst met negen vragen om de gevoeligheid voor licht en patronen te bepalen; de vragen zijn bepaald aan de hand van een literatuurstudie en interviews met patiënten. Constructvaliditeit (oftewel, 'meet de vragenlijst wat het beoogt te meten') werd bevestigd door de vergelijking van L-VISS scores met twee gedragstesten. De drempel van lichtgevoeligheid was lager, terwijl de patroongevoeligheid ('pattern glare') hoger was, bij hogere scores op de L-VISS. De vergelijking van diverse subtypes van migraine (met en zonder aura, chronisch en episodisch) en momenten in de cyclus (tijdens of tussen aanvallen) tussen en binnen grote groepen deelnemers toonde aan dat L-VISS scores verhoogd waren voor migraine met aura ten opzichte van migraine zonder aura, voor chronische ten opzichte van episodische migraine, en tijdens ten opzichte van tussen aanvallen. Dit patroon van verhoogde visuele gevoeligheid is mogelijk vergelijkbaar met patronen in verhoogde corticale exciteerbaarheid in subtypes van migraine, en momenten in de cyclus, zoals

gemeten in neurofysiologische studies. De L-VISS kan gebruikt worden in grootschalige, longitudinale metingen van gevoeligheid voor licht en patronen in patiënten, omdat de vragenlijst eenvoudig en onafhankelijk van specifieke meettechnieken te gebruiken is. De L-VISS kan eveneens gebruikt worden in combinatie met meer uitvoerige neurofysiologische metingen van hersenfunctie inclusief activiteit van de visuele schors, als subjectieve bepaling van veranderingen in de exciteerbaarheid van het visuele systeem gedurende de migrainecyclus.

EEG-studies in migrainepatiënten laten tegenstrijdige resultaten zien, indicatief voor zowel verlaagde als verhoogde exciteerbaarheid van het visuele systeem. Deze tegenstrijdigheid kan worden veroorzaakt door grote inter-individuele variatie, verschillen in technieken en patronen van visuele stimulatie, of intra-individuele dynamiek binnen de migrainecyclus. Voor beter begrip van de neuronale mechanismen die aan de basis staan van de geobserveerde EEG-resultaten in patiënten, hebben wij de kloof overbrugd tussen indirecte metingen van activiteit van de visuele schors met EEG op de schedel (bij patiënten) en precieze neurofysiologische metingen in diermodellen. In **Hoofdstuk 3** bestudeerden wij hiertoe EEG-responsen bij visuele stimulatie in een muismodel met zowel directe metingen middels lokale elektrodes in de visuele schors, als met meer indirecte metingen op het hersenoppervlak. Voor de klinische vertaalslag gebruikten wij een transgeen migraine muismodel waarin muizen de humane pathogene R192Q mutatie in het *Cacna1a* gen dragen, wat in patiënten familiaire hemiplegische migraine type 1 (FHM1) veroorzaakt. Het *Cacna1a* gen codeert de α_{1A} subunit van presynaptische voltage-gestuurde $Ca_V2.1$ Ca^{2+} kanalen, waarbij de FHM1 R192Q mutatie resulteert in een verhoogde glutamaterge overdracht en verhoogde exciteerbaarheid. In vrij bewegende transgene en wildtype (controle) muismodellen onderzochten wij bestaande en nieuw ontwikkelde visuele stimulatiepatronen bestaande uit lichtflitsen. FHM1 mutante muizen vertoonden snellere EEG-potentiaalverandering na stimulatie op verschillende flitsfrequenties. De eerste negatieve EEG-potentiaalpiek na licht-stimulatie had een verlaagde amplitude en was geassocieerd met minder neuronale suppressie vergeleken met controlemuizen zonder de FHM1 mutatie. Flitsstimulatie met oplopende stimulatiefrequenties tussen 10 en 40 Hz (de 'chirp' stimulatie) toonde verhoogde 'photic drive' in beta-

gamma EEG frequenties (15–40 Hz). Deze resultaten wijzen op een context-afhankelijke verhoging van exciteerbaarheid van de visuele schors in het FHM1 muismodel. Wij demonstreerden dat het meten van VEPs in transgene 'migraine' muizen gebruikt kan worden om beter inzicht te verkrijgen in veranderingen van de gevoeligheid van het visueel systeem in migraine.

Een van de technieken om exciteerbaarheid van het visuele systeem te bestuderen, is de 'photic drive' respons in het EEG, zoals toegepast bij het FHM1 muismodel in Hoofdstuk 3. In **Hoofdstuk 4** onderzoeken wij het gebruik van dezelfde 'chirp' visuele stimulatie om de gevoeligheid van het visuele systeem (inclusief corticale exciteerbaarheid) te bepalen in migrainepatiënten. We verrichtten hiervoor metingen *tussen* aanvallen (interictaal) en in de periode *voorafgaande aan* een aanval (pre-ictaal). Met lichtflitsen op oplopende stimulatiefrequenties biedt 'chirp' stimulatie de mogelijkheid om EEG-responsen te vergelijken op verschillende frequenties en de gekoppelde harmonische frequenties die ontstaan in het EEG op veelvouden van de stimulatiefrequenties. Deze methode geeft een snelle mogelijkheid om de 'photic drive' te bepalen over meerdere stimulatiefrequenties. Onze intra-individuele metingen toonden dat chirp-responsen herhaalbaar zijn over dagen tot maanden. Daarnaast waren, interictaal, de chirp-responsen vergelijkbaar tussen controles en migrainepatiënten (zowel met als zonder aura). Bij acht pre-ictale metingen (3 met, 5 zonder aura), die waren gemeten binnen 48 uur voor een aanstaande migraineaanval, was een toegenomen harmonische respons zichtbaar in de EEG betaband (22–32 Hz). Visuele chirpstimulatie blijkt hiermee een simpele en betrouwbare techniek om veranderingen te meten in gevoeligheid van de visuele schors die geassocieerd worden met het begin van een migraineaanval.

Deel II is gericht op directe metingen van corticale exciteerbaarheid in migrainepatiënten, in tegenstelling tot de indirecte metingen met de L-VISS vragenlijst en VEP-metingen in de patiëntstudies in Deel I. Visuele stimuli worden niet alleen verwerkt in de visuele schors, maar ook in de retina, thalamus en superieure colliculi. Visueel opgewekte veranderingen in hersenactiviteit worden niet alleen corticaal maar ook subcorticaal verwerkt in het brein, en zijn daarom een

maat van de exciteerbaarheid van het gehele visuele systeem. Door de combinatie van transcraniële magnetische stimulatie (TMS) over de schedel met gelijktijdige EEG-metingen kan corticale exciteerbaarheid direct bestudeerd worden, middels het meten van zogeheten TMS-opgewekte EEG-potentialen ('TEPs'). De TEP is aangedaan in ziektebeelden met veronderstelde onderliggende veranderingen in corticale exciteerbaarheid, zoals epilepsie en schizofrenie. In **Hoofdstuk 5** beschrijven wij de eerste studie die TMS-opgewekte EEG-potentialen onderzoekt in migrainepatiënten. Stimulatie met een cirkelvormige spoel over de vertex, gebruikmakend van stimulatie-intensiteiten rondom de motordrempel, is gebruikt in migrainepatiënten met aura (tussen aanvallen) en controles die zijn geselecteerd op geslacht, leeftijd en motordrempel. Schijnstimulatie ('sham'-stimulatie) is gebruikt om te controleren voor mogelijke invloeden van auditieve en somatosensorische activatie door TMS. In migraine met aura hadden TEP-responsen een verlaagde amplitude rond de N100 potentiaalpiek voor frontale en occipitale elektrodes. Verlaagde N100 piekamplitude is gerelateerd aan een verminderde corticale $GABA_B$-erge inhibitie, en deze resultaten zijn in lijn met – indirecte – observaties van verhoogde corticale exciteerbaarheid in migraine.

Migraine en epilepsie zijn comorbide paroxysmale neurologische aandoeningen, beiden geassocieerd met veranderingen in corticale exciteerbaarheid. In **Hoofdstuk 6** onderzochten wij indices voor faseclustering in het EEG na transcraniële magnetische stimulatie in patiënten met migraine met aura en patiënten met juveniele myoclonische epilepsie met als doel het identificeren van mogelijke functionele biomarkers gerelateerd aan migraine met aura, epilepsie, of beide aandoeningen. Faseclustering in respons op TMS was verschillend tussen epilepsie (zonder medicatie) en controles. In een deelnemer met epilepsie was de mate van faseclustering omgekeerd evenredig met de gebruikte dosis antiepileptica. In migraine met aura was de faseclustering niet verschillend van controles, wat er op wijst dat de tendens voor verschillen in faseclustering niet gedeeld wordt tussen migraine en epilepsie.

Hoofdstuk 7 bevat een algemene discussie van de onderzoeken in dit proefschrift, met overwegingen over toekomstig klinisch en preklinisch translationeel onderzoek naar de voorspelling van migraineaanvallen met neurofysiologische methodes.

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**/[#] Authors contributed equally*

Curriculum Vitae

Matthijs Johannes Lambertus Perenboom (given name: Thijs) was born February 15th 1988 in Nijmegen, the Netherlands. He attended Canisius College secondary school in Nijmegen (2000-2006), and moved to Delft to start the Bachelor program in Aerospace Engineering at Delft University of Technology. In 2009-2010, he was a visiting minor student at Utrecht University for a year of courses in cognition, psychology and neuroscience. In 2010, he received his Bachelor degree and started with a Master program in Biomedical Engineering (track: biomechatronics) at TU Delft. During his Master studies, he performed a scientific internship into brain mapping using transcranial magnetic stimulation (TMS) at the University of Birmingham, School of Sports and Exercise Sciences, under supervision of dr Michael Grey and dr Mark van de Ruit. His Master graduation research into the cortical involvement in stretch reflexes using TMS and high-density electromyography was carried out at the Leiden University Medical Center (LUMC), in the movement laboratory of the department of Rehabilitation under supervision of prof. dr Frans van der Helm and dr Alfred Schouten (TU Delt), and dr Jurriaan de Groot and dr Carel Meskers (LUMC). Both projects resulted in peer-reviewed scientific publications, and sparked the interest for further work in the academic world. In May 2013, he started his PhD research at the department of Neurology (LUMC), supervised by prof. dr Michel Ferrari, dr Else Tolner and dr Hans Carpay. The results of this research are described in this PhD thesis. Since May 2018, Thijs has been working as data consultant and technical lead at the Rotterdam-based company 52impact, combining artificial intelligence methods with spatial data like satellite imagery to help companies and governments work more sustainably.

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Cover sculpture

Sculpture: Regie I
Artist: Ludo Broos
(1965 – 2022)

Front view

Side view