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Headache related alterations of visual processing in migraine patients.

--Manuscript Draft--

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Abstract:	Migraine is characterized by an increased sensitivity to visual stimuli that worsens during attacks. Recent evidence has shown that feedforward volleys carrying incoming visual information induce high frequency (gamma) oscillations in the visual cortex, while feedback volleys arriving from higher order brain areas induce oscillatory activity at lower frequencies (theta/alpha/low-beta). We investigated visually induced high (feedforward) and low (feedback) frequency activations in healthy subjects and various migraine patients. Visual evoked potentials from 20 healthy controls and 70 migraine patients (30 inter-ictal and 20 ictal episodic migraineurs, 20 chronic migraineurs) were analysed in the frequency domain. We compared power in the theta-alpha-low beta and gamma range between groups, and searched for correlations between the low-to-high frequency activity ratio and number of monthly headache and migraine days. Compared to healthy controls, inter-ictal migraine patients had increased visually induced low frequency (feedback) activity. Conversely, ictal and chronic migraine patients showed an augmented gamma band (feedforward) power. The low-frequency-to-gamma (feedback/feedforward) activity ratio correlated negatively with monthly headache days and tended to do so with migraine days. Our findings show that visual processing is differentially altered depending on migraine cycle and type. Feedback control from higher order cortical areas predominates interictally in episodic migraine while migraine attacks and chronic migraine are associated with enhanced incoming afferent activity, confirming their similar electrophysiological profile. The presence of headache is associated with proportionally higher gamma (feedforward) activities.

Reviewers' comments:

Reviewer #2:

The authors present a manuscript exploring differential visual processing in healthy controls, migraine (ictal v's inter-ictal), and chronic migraine patients. They demonstrate that during the ictal phase and in chronic migraine patients there was an alteration in the gamma band (feedforward) as oppose to low frequency alterations in the inter-ictal patients compared to healthy controls. The results add an interesting dimension to the current body of literature on visual processing abnormalities in migraine and I only have minor comments.

1. Why were the same patients not recorded for the ictal v's inter-ictal phases as this would have made the findings more robust. While it is accepted and highlighted as a limitation the rationale for not doing it is not clear.

In agreement with the referee's comment, we acknowledge in the discussion that recording the same patients both during and outside of an attack would have made our findings more robust and should be planned in future studies (limitations on page 10/ line 18-20). This, however, is logistically more demanding for the patient and the investigator, as 2 recording sessions are necessary in each subject and the migraine attack is not programmable. We chose therefore, like in several other studies.[1,2], to perform most ictal recordings in patients who consulted our headache clinic during an ongoing migraine attack.

1. Chen WT, Wang SJ, Fuh JL, et al. Persistent ictal-like visual cortical excitability in chronic migraine. Pain 2011; 152: 254–258.
2. Coppola G, Iacovelli E, Bracaglia M, et al. Electrophysiological correlates of episodic migraine chronification: evidence for thalamic involvement. J Headache Pain 2013; 14: 76.

2. This reviewer does not agree that the results support that chronic migraine is a "never-ending migraine attack", in fact the results show that abnormalities correlate better with headache (not migraine) frequency and ultimately given the high frequency of attacks in CM patients would always be in the 72 hour attack free zone used to define inter-ictal phase and as such this bold statement, while likely correct is not substantiated.

We agree with the reviewer that reporting that our results support the concept that chronic migraine is like a "never-ending migraine attack" may be an overstatement. We have therefore made it clear in the abstract, main body and discussion that the similarity between ictal recordings in episodic migraine and chronic migraine is only for the electrophysiological profile with proportionally increased gamma band activity (feedforward). As pointed out by the referee, we have now underscored that headache (not necessarily migraine headache) is associated with this profile and that in chronic migraine most recordings are more likely to occur within 72h of a headache. We have also mentioned (page 9 / lines 23 - 30) that the similar electrophysiological profile of a qualified migraine attack and a tension-type like headache in migraineurs favours the concept that both headache types have the same pathophysiological underpinning.

3. ICHD3 is no longer a beta version an is available in full, cite the updated version.

We know that the ICHD-3 is now available in its final version. However, at the time our study was conducted it was not, and we used the beta version to diagnose and select patients.

4. Did the authors attempt to see if photophobia impacted on the results, given this data was collected in the diary?

The headache diaries we used for this study, which are routinely used in our headache clinic, are intended to be diagnostic and follow-up tools based on the ICHD criteria. They therefore only question about presence or absence of photophobia and phonophobia during a headache. Unfortunately, they do not allow a more detailed analysis of photophobia, which would have been an added value for the study. We have now mentioned this in the " limitations" section of the manuscript (page 10 / lines 26 -27).

5. Where a non-normally distributed result was being further studied for correlation should the Spearmans coefficient have been used?

We have now performed more adequate, non-parametric, Spearman's test suggested by the reviewer. . The manuscript has been modified accordingly in the "methods" and "results" sections. Figure 3 has also been corrected.

6. In areas the P value is noted with either a comma or a decimal point. Please update all to decimal points.

P values are now all shown with decimal points.

7. Figure 5 is of limited value as is and could be turned into a more graphical abstract representation of the results that would be better received.

Thank you for your suggestion. Figure 5 has now been modified in an attempt to provide a more graphical explanatory scheme of the results.

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Reviewer #3:

Overall the study is well designed and well presented. The strength of the manuscript is the comparisons between episodic migraine patients during and between attacks, chronic migraine patients and healthy controls.
The association of gamma activity to pain may already be known to the literature, however the potential suppressive role of low-frequency (feedback) activity on the pain related gamma activity and the association of the ration to migraine and headache attacks are

interesting.

Major concerns:
1) The authors will need to explain more the following conclusion: "Our findings support the notion that from an electrophysiological perspective chronic migraine can be conceptualized as a 'never-ending migraine attack'."

It appears that between attacks there is an increase in the low-frequency activity but there is no increase in gamma activity. The attacks and chronic migraine conditions are characterized by increase in gamma activity. Do the authors mean that the ongoing Gamma activity is suppressed by the low frequency activity and therefore it is not increase between attacks?

As mentioned above, we agree that reporting that our results support the concept that chronic migraine is like a "never-ending migraine attack" may be an overstatement. We have therefore made it clear in the abstract, main body and discussion that the similarity between ictal recordings in episodic migraine and chronic migraine is only for the electrophysiological profile with proportionally increased gamma band activity (feedforward). As suggested by the reviewer and mentioned in the text, the feedback inhibitory drive may well suppress between attacks the feedforward gamma activity in a homeostatic perspective (page 9 / lines 4 - 14). This however would be overtaken by an increased gamma drive during a headache both during an attack in episodic migraine but permanently in chronic migraine, which may favour a rupture of metabolic homeostasis. Hence the notion that chronic migraine is similar to a migraine attack from an electrophysiological point of view. We have now underscored that headache (not necessarily migraine headache) is associated with this profile and that in chronic migraine most recordings are more likely to occur within 72h of a headache. We have also mentioned (page 9 / lines 23 – 30) that the similar electrophysiological profile of a qualified migraine attack and a tension-type like headache in migraineurs favours the concept that both headache types have the same pathophysiological underpinning.

2) The low-frequency activity level may be affected by the time elapsed since the last migraine attack. It would be helpful if the authors shown this association and correlation between the activity and time since last painful headache episode

We agree with the referee that the cyclic nature of migraine reflects in cerebral electrophysiological responses and we have confirmed this in our previously published studies. This is the reason why interictal recordings are performed at an interval of at least 72h after the last and before the next attack, as mentioned in the "methods" section. The most prominent electrophysiological changes occur periictally within this time window. We have therefore not paid attention in this study to changes that might occur at longer time intervals. As mentioned by the reviewer, such (subtle) changes might nonetheless occur and reflect slow fluctuations in thalamo-cortical rhythms. Capturing them needs ideally a longitudinal study with multiple recordings in the same patients, which is certainly of great interest for future studies This has been added to the "limitations" section of the manuscript (page10 / lines 20 - 23).

Minor concerns:
1) "The low-frequency-to-gamma (feedback/ feedforward) activity ratio correlated more strongly with total monthly headache days than with

migraine specific days"
Please explain what non-specific headache episodes were included in this analysis.

Only headaches fulfilling the diagnostic criteria for a migraine attack (ICHD-3 beta code 1.1) (unless they had been treated with a triptan) were considered migraine specific headaches. All other episodes of head pain were coded as unspecific headaches. This is clarified on page 5.

2) "three of the ictal group had it not more than 48 hours before the test"

Were those patients results in line with the patients that had the recording during the attack?

Yes; the averaged low-frequency-to-gamma activity ratio in this small subset of patients is the same as that of the whole ictal group (0.67).

3) "Participants did not take any medication on a daily basis and, at the time of recordings, migraine patients had not taken any preventive anti-migraine treatment for at least 3 months."
Medication taking status is not clear: Did the patients take any other medication during the attack / chronic pain such as triptans or NSAIDs ? If some of them did, were the results different from those that did not take medications.

Patients were not under any preventive treatment, nor had they been for the 3 preceding months.
For the sake of clarity, this has been corrected in the manuscript on page 5 lines 8 - 9.

Were the patients instructed not to take medications during the migrainous attack?

For ethical reasons, patients were not instructed not to take medications during the attack. However, recordings are not possible in patients with very severe attacks, and hence, the intensity of attacks was most often moderate at the time of the recordings, explaining why, with rare exceptions, patients withheld acute therapy until the end of recordings.

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Reviewer #4:

Migraine headaches are associated with various visual phenomenon that are poorly understood but strongly associated for diagnosis and consequently treatment. This study "Headache related alterations of visual processing in migraine patients" is targeted in this important but relatively less understood area of headaches. The manuscript is well written. The tables and figures are appropriate and help assimilate data easily. The study certainly poses interesting questions and attempts to uncover fundamental

neurophysiological underpinnings using EEG and VEP.

Major comments:

1. A. In page 5, Line 20, authors say "The investigators in this study were not blinded to the diagnosis, but all electrophysiological analyses were full automated." Is there a reason why investigators could not be blinded. I would ask them to include 1-2 lines why they decided to unblind the investigators.

The reason for not blinding the investigators in this study is explained by the available human resources: the researchers in charge of data collection also had to analyse the data. This limitation was partially overcome by performing automated analyses. Nonetheless we agree with the referee that a blinded protocol would have been advantageous. We have now included a statement concerning this limitation on page 10 / lines 16 – 17 of the revised version.

B. In discussion, the last paragraph about limitations, I would request the authors to included that the study not being blinded is a limitation.

We have now included a statement concerning this limitation 10 / lines 16 – 17 of the revised version.

2. In page 5, Line 1 the authors mention "Epochs whose amplitude exceeded a two standard deviations from the channel mean amplitude limit were considered artefacted and rejected (<6% of epochs). In EEG analysis, 2 standard deviations above the baseline on power spectral analysis is considered seizure in epilepsy EEG literature.

A. Is that established parameter in EEG analysis for headaches that 2 standard deviations from channel mean is an artifact ? Please include source/reference for that established precedent.

The selected threshold is not headache specific, and was empirically chosen in order to separate artefacted from non-artefacted epochs on the basis of the law of normal distribution. We presupposed that bad epochs (e.g. blink artefacts, movement…) would behave as outliers in terms of amplitude. This threshold has been useful for us in previous studies but, as the referee correctly points out, it needs to be specifically tested as a suitable parameter. As mentioned below, this has now been added to the revised version of the manuscript.

B. If not, please include in discussion as a limitation of the study.

We have now included the artefact rejection method as a limitation of our study on page 10 / lines 9 – 12.

Minor comments:

1. Table 1, the authors use commas (,) instead of (.) for p-values. Please correct.

Thank you for this observation. This error has been corrected in the revised version.

2. Table 2, the authors again use commas (,) for every value in the table. Is that accurate ?

Thank you for this observation. This error has been corrected in the revised version.

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Reviewer #5:

Aim of this study was to investigate low (feedback) and high (feedforward) frequency visually induced activations in healthy subjects and migraine patients during the ictal and inter-ictal periods.

Visual evoked potentials from 90 participants (30 inter-ictal and 20 ictal episodic migraine patients, 20 chronic migraine patients and 20 healthy controls) were analysed in the frequency domain. Power in the theta-alpha-low beta (feedback) and gamma (feedforward) peaks was compared between groups. In addition, correlations between the feedback/feedforward activity ratio and number of monthly headache attacks were examined.
The findings support the notion that from an electrophysiological perspective chronic migraine can be conceptualized as a 'never-ending migraine attack'. All headaches, and not only those with typical migraine features, were associated to alterations in cortical visual processing.

The issue is surely of great interest and the study is well organised and written and provides an incremental increase in knowledge relatively to its purpose.

The only objection that could be raised is the fact - mentioned by the authors themselves- that different patients were included in the ictal and interictal episodic migraine groups while it would have being preferable to compare the same patients in and outside of an attack.

In agreement with the referee's observation, we acknowledge in the manuscript that recording the same group of patients during both the ictal and inter-ictal periods would have made our findings more robust and should be performed in future studies (limitations on page 10/ line 18-20Due to logistic constraints, we conducted this experiment under a design that resulted more feasible for us, in a similar way to that of prior studies.[1,2] (see comment to referee 1).

1. Chen WT, Wang SJ, Fuh JL, et al. Persistent ictal-like visual cortical excitability in chronic migraine. Pain 2011; 152: 254–258.
2. Coppola G, Iacovelli E, Bracaglia M, et al. Electrophysiological correlates of episodic migraine chronification: evidence for thalamic involvement. J Headache Pain 2013; 14: 76.

Headache related alterations of visual processing in migraine patients.

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Running title:

Feedback & feedforward visual activity in migraine

Disclosures:

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Conflict of Interest

The authors of this study have no relevant conflict of interest to declare.

Abstract:

Migraine is characterized by an increased sensitivity to visual ~~stimulus~~ stimuli that worsens during ~~the~~ attacks. Recent evidence has shown that ~~while~~ feedforward volleys carrying incoming visual information induce high frequency (gamma) oscillations in the visual cortex, while feedback volleys arriving from higher order brain areas induce oscillatory activity at lower frequencies (theta/alpha/low-beta).

We investigated ~~–~~visually induced high (feedforward) and low (feedback) ~~and high (feedforward)~~ frequency ~~visually induced~~ activations ~~in~~ in healthy subjects and various migraine patients ~~during the ictal and inter-ictal periods~~. Visual evoked potentials from 20 healthy controls and ~~90~~ 70 ~~participants~~ migraine patients (30 inter-ictal and 20 ictal episodic migraineurs ~~patients~~, 20 chronic migraineurs ~~patients and 20 healthy controls~~) were analysed in the frequency domain. We compared p~~P~~ower in the theta-alpha-low beta ~~(feedback)~~ and gamma ~~(feedforward) peaks~~range ~~was compared~~ between groups, and ~~. In~~searched ~~addition,~~for correlations between the low-to-high frequency ~~feedback/feedforward~~ activity ratio and number of monthly ~~headache~~ headache and migraine days~~attacks were examined~~.

Compared to healthy controls, inter-ictal migraine patients had ~~an~~ increased visually induced low frequency (feedback) activity. Conversely, ictal and chronic migraine patients showed an augmented gamma band (feedforward) power. The low-frequency-to-gamma (feedback/ feedforward) activity ratio correlated ~~more strongly~~negatively with ~~total~~ monthly headache days and tended to do so ~~than~~ with migraine ~~specific~~ days.

Our findings show that visual processing is differentially altered depending on migraine cycle and type. Feedback control from higher order cortical areas predominates interictally in episodic migraine while migraine attacks and chronic migraine are associated with enhanced incoming afferent activity, confirming their similar electrophysiological profile. The presence of headache is associated with proportionally higher gamma (feedforward) activities. ~~support the notion that from an electrophysiological perspective chronic migraine can be conceptualized as a 'never ending migraine attack'. All headaches, and not only those with typical migraine features, are associated to alterations in cortical visual processing.~~

Perspective:

This study provides an insight into the pathophysiology of migraine headache fro~~or~~m the perspective of cortical sensory processing dynamics. Patients with migraine present

alterations in feedback and feedforward visual signalling that differ ~~between the~~with the presence of headache ~~and headache-free periods.~~.

Keywords:

Visual evoked potentials, spectral analysis, episodic migraine, chronic migraine, feedback, feedforward.

Introduction

It is well established in healthy humans that marked changes in brain rhythmic oscillatory activity over a wide range of frequency bands are related to pain processing.[30,32] This also applies for head pain associated to migraine. Several electrophysiological studies have shown that migraine is a brain disorder characterized by an abnormal cortico-subcortical oscillatory activity that fluctuates along the migraine cycle, differs between the ictal and inter-ictal intervals,[7,12,34,35,46] and remains persistently altered as the disease chronifies.[8]

According to available experimental evidence, oscillations in the alpha and gamma frequency bands can be used as direct, objective, experimentally stable, and interrelated measures of cognitive and sensory brain tasks. During on-going pain alpha power is reduced and gamma power is increased in several brain regions,[17,18,45] including posterior cortical areas.[4,5] Similar modifications correlate with active selection and integration of relevant unattended visual information, resulting from the balance between feedforward volleys reaching the visual cortex from the lateral geniculate nucleus (fast gamma oscillations) and feedback activity ~~in~~coming from higher order visual areas (low frequency (theta/alpha/low-beta) oscillations).[24,28] Spectral analysis allows to easily identify these two main frequency peaks (theta/alpha/low-beta and gamma) in common scalp-recorded visual evoked potentials (VEPs), as confirmed by recent intracortical recordings in non-human primates as well as magnetoencephalographic studies in humans.[24,28]

In this study we analysed the previously described fluctuations of visual processing in migraine[23,39] from the perspective of visually induced feedback (theta/alpha/low-beta) and feedforward (gamma) activations. We also tested whether these alterations in visual signalling were specifically associated ~~to~~ with the ~~number~~ frequency of full-blown migraine attacks, or if they were ~~as well~~also related to ~~recurrent episodes~~the presence of mild tension-type like headaches, ~~–~~often present in migraineurs, particularly in those suffering from ~~.—~~chronic migraine.

Subjects and methods

Subjects

The study involved 90 participants: 20 healthy volunteers (HV), 30 episodic migraine without aura patients recorded during a headache-free interval (minimum 72 hours before or after an

attack) verified on a headache diary and/or by a telephone call (EM), 20 ictal episodic migraineurs recorded during an attack (IM, 17 during the headache phase, 3 within 48 hours of the headache), and 20 chronic migraine patients without medication overuse (CM). Diagnoses were made in accordance with The International Classification of Headache Disorders 3rd edition beta version (ICHD3 beta).[20] Healthy volunteers did not report any first degree relative suffering from recurrent headaches of any type. Participants were consecutively recruited amongst University students or their families and via our headache clinic. Specifically, an announcement was posted in the University's intra-net, and headache patients attending the consultation were personally invited to take part. Patients were not under any preventive treatment, nor had they been for the 3 preceding months. ~~Participants did not take any medication on a daily basis and, at the time of recordings, migraine patients had not taken any preventive anti-migraine treatment for at least 3 months.~~ To ascertain the diagnosis, attack occurrence, and headache attacks severity, patients filled in a paper diary for ≥ 30 days in which headache intensity, associated symptoms (nausea, vomiting, photo-, phonophobia) and acute medication intake were registered. As in recent therapeutic trials,[41] only headaches fulfilling the diagnostic criteria for a migraine attack (ICHD3 beta code 1.1) (unless they had been treated with a triptan) were considered migraine specific headaches. All other episodes of head pain were coded as unspecific headaches. None of the participants that initially agreed to participate were excluded afterwards. The study was approved by the Hospital's ethics committee (Centre Hospitalier Régional de la Citadelle, Liège, Belgium – protocol n°1422) and conducted following the principles of the Declaration of Helsinki. All participants gave written informed consent.

Visual Evoked Potentials (VEP) recordings and analysis

VEP recordings were performed in the electrophysiology laboratory of the Headache Research Unit (Neurology Department, Centre Hospitalier Régional de la Citadelle, Liège, Belgium). All participants were studied in the morning, between 9 a.m. and noon. Subjects were sitting on a comfortable armchair, in a quiet room with dimmed light. A patch was placed over the left eye, and needle recording electrodes were introduced in the scalp at Oz (active) and Fz (reference) based on the 10-20 EEG system. During the recordings, subjects were instructed to maintain fixation on a red dot in the centre of a screen which displayed a black and white reversing checkerboard pattern (contrast of 80%, mean luminance 50 cd/m2). Temporal and spatial stimulating frequencies employed were 1.55 Hz (3.1 reversals/second) and 68' respectively. Six hundred epochs, each lasting 250ms, were continuously recorded at

a sampling rate of 5.000 Hz using a CED™ power 1401 device (Cambridge Electronic Design Ltd, Cambridge, UK). After DC subtraction, recordings were exported to EEGLAB,[13] an open-source MATLAB (The MathWorks Inc.) toolbox for electrophysiological signal processing, where they were band-pass filtered (low pass 100 Hz, high pass 1 Hz). Epochs whose amplitude exceeded a two standard deviations from the channel mean amplitude limit were considered artefacted and rejected (<6% of epochs). The Fast Fourier Transform was applied on each epoch to compute spectral decomposition. Log-transform of single-trial spectral power was performed before averaging. Data were zero-padded in order to increase frequency resolution to steps of 1Hz. As in previous studies,[28] the two most prominent peaks of the spectrogram were observed in the theta/alpha/low-beta (1) and gamma (2) frequency band ranges. To estimate power at these frequencies, the area under the curve (trapezoidal numerical integration; MATLAB function 'trapz') of activity at each peak and nearby surrounding frequencies (4 to 16 Hz for theta-alpha-low beta and 40 to 60 Hz for gamma) was calculated for each individual (Fig 1). Considering the recent evidence showing that alpha-beta and gamma activity embedded in visually-induced cortical responses convey different information,[24,28] and that abnormal visual responsiveness in migraine is the result of a complex process involving several cortical areas,[27] we calculated the low frequency-to-gamma activity ratio as a measure of the interaction between simultaneous volleys reaching the visual cortex. In addition, considering the overlap between visually induced cerebral gamma activity and the frequency spectrum of different possible sources of contamination of the signal (muscular artefacts, AC line noise) we performed a supplementary analysis of event related spectral perturbations which permits to visually inspect changes in the power spectrum throughout time. Investigators in this study were not blinded to diagnosis, but all electrophysiological analyses were fully automated.

Statistical analysis

Statistical analyses and graphs were performed in Prism version 6.00 for Windows (GraphPad Software, La Jolla, California, USA). The assumption of normal distribution was assessed using the Shapiro-Wilk normality test. Continuous variables were compared using ANOVA or Kruskal-Wallis tests (in case of non-normal distributions or violations in the assumption of homoscedasticity evaluated using Bartlett's test), followed by post-hoc comparisons between groups (corrected for multiple comparisons using Dunn's multiple comparison test). Correlation analyses between spectral power ratios and monthly number of headache or migraine days were performed using Spearman's rank correlation test corrected for multiple

comparisons by applying a Bonferroni correction. Because alterations in the power spectrum of patients from the ictal migraine group are likely to be transient,[1,39] these patients were not included in correlation analyses. The significance level for all tests was set at $p<0.05$.

Results

There were no significant between-group differences in mean ages or gender ratio in the whole subject sample, nor between disease duration amongst migraine sub-groups (Table 1).

The results of spectral analyses are displayed in table 2. Mean low-frequency (theta-alpha-low beta) power was significantly higher in headache-free episodic migraine patients compared to healthy controls (Kruskal-Wallis test H= 8.330, p=0.040; Dunn's multiple comparisons test (episodic migraine patients vs healthy controls) p= 0.030, adjusted for multiple comparisons). Conversely, gamma power was higher in both ictal and chronic migraine patients (Kruskal-Wallis test H= 14.00, p< 0.003; Dunn's multiple comparisons tests: chronic migraine vs healthy controls, p= 0.023; ictal migraine vs healthy controls, p= 0.013, both adjusted for multiple comparisons) (Fig 1 & 2). The low-frequency-to-gamma activity ratio was significantly smaller in ictal and chronic migraine patients compared to headache-free episodic migraine patients, and in ictal migraine patients compared to healthy controls (Kruskal-Wallis test H= 16.33, p= 0.001); Dunn's multiple comparisons tests: episodic vs chronic, p= 0.032; episodic vs ictal, p= 0.012; healthy volunteers vs ictal, p = 0.024 (all adjusted for multiple comparisons). A similar trend was observed between chronic migraine patients and healthy controls, but it did not reach statistical significance (p= 0.055). (Fig 2). The low-frequency-to-gamma activity ratio was negatively correlated with the total number of monthly headache days (pr= -0.3734; p= 0.009015) and), but not with the total number of migraine specific days (pr= -0.2925; p=0.04208) days, although the latter correlation did not withstand correction for multiple comparisons (Fig 3). A partial correlation (controlling for age) between the low-frequency / gamma activity ratio and the monthly headache days was also significant (r= -.33; p= 0.02). The N1-P1 amplitude of the broad-band VEP did was not significantly different significantly between the groups (healthy controls: 5.088µV ± 1,444; headache free migraine patients: 5.860 µV ± 2.361; chronic migraine patients: 5.368 µV ± 2.281; ictal migraine patients: 6.396 µV ± 2.436; (one-way ANOVA $F_{(3,86)} = 1.399$; p=0.249). Supplementary event-event related spectral perturbations analysis (Fig 4) showed that gamma activity exhibited temporal fluctuations, as one would expect from a neural

signal, rather than being constant over time, (as would be 50Hz power line noise or other possible sources of signal contamination).

Discussion

We measured power of low (theta/alpha/low-beta) and high (gamma) frequency oscillations embedded in pattern-reversal visual evoked potentials (PR-VEP) in healthy controls, episodic migraine patients (both headache-free and during or in between an attacks), and chronic migraineurs. The results show that, during headache, gamma power patients haveis an increasedgreater in patients gamma power compared tothan in healthy subjects. In By contrast, in the absence of headache, episodic migraine patients exhibit anhave increased power at low frequencyies power (theta/alpha/low-beta). Concordantly, the low-frequency-to-gamma activity ratio was significantly higher in headache--free patients compared to patients experiencingthan during a migraine attack or in chronic migraineurs. andFurthermore, this ratio was negatively correlated with the monthly number of headache days.

We have previously found a decreased habituation of late visual induced gamma components studied visual induced gamma activity in headache-free interictal episodic migraine patients and mainly found a decreased habituation of late gamma components.[7] In the present study we focused on total gamma power and its relation with the low-frequency power spectrum analysed from ain the frequency-domain perspective, which is better suited to evaluate high frequency oscillations. There is strong evidence showing that , in the visual pathway, feedforward (afferent) volleys incoming from the lateral geniculate nucleus induce oscillations within the gamma frequency range in the primary visual cortex (Fig. 45). This frequency range has been associated with the efficiency of stimulus processing by thalamocortical networks [15,36,40] and with the translation of the stimulus features into coherent perception (for a review, see Gray and Singer, 1995 [42] ; Tallon-Baudry and Bertrand, 1999 [44]). Therefore, increased visually induced gamma (feedforward) activity during migraine attacks and in chronic migraine may reflect augmented efficiency within the thalamo-cortical circuit. This is in line with previous electrophysiological,[8,9,23,43] and functional neuroimaging[11] studies supporting showing that thalamo-cortical network activity is decreased in migraineurs during the headache-free interval, but increaseds during an migraine attack and with migraine chronification.

On the other hand, it is known that pain is accompanied by widespread enhancement of gamma activity in the brain (prefrontal, mid-cingulate, and primary somatosensory cortices

and insula)[19] associated ~~to~~ with contralateral alpha power reductions,[32] which suggests that the former reflects tonic pain processing while the latter may be related to a top-down cognitive process linked to attention.[4,5,17,18,45] Reciprocal anatomical and functional connections between the visual and the trigeminal systems are well documented in animals and human beings.[3,25,31,37] In particular, convergence of nociceptive trigeminal and visual afferents in the posterior thalamus[30] may explain how head pain can amplify visually induced thalamocortical activity, and thus gamma power in PR-VEP.

~~By contrast,~~As opposed to feedforward afferent activity that generates gamma oscillations in the primary visual cortex, feedback volleys from higher order visual areas (V2-V4) induce oscillatory activity ~~in the primary visual cortex~~ within the theta/alpha/low-beta frequency range (Fig. 5) ~~which~~ that notably plays a role ~~, for instance, io~~n focusing attention to salient unattended stimuli.[24,28] Such ~~Evidence shows that~~ feedback volleys reaching the visual cortex are ~~capable of~~able to modulate~~ing~~ the response to ~~feedforward~~ visual afferents ~~.~~[14,21,23] ~~Indeed, low-frequency (feedback) activity is thought to~~ by exert a selectively inhibiting ~~inhibitory effect on~~ high frequency (gamma~~),~~ feedforward~~)~~ oscillations, and thus to exert ~~suggesting~~ a possible 'gating' ~~role of the former~~process.[22] The sensory processing profile of migraine patients makes them vulnerable to sensory overload,[2,16] and therefore, in need of compensatory protective mechanisms. Between attacks, repetitive photic stimulation causes whole-brain alpha hyper-synchronization,[46] indicative of a diffuse cortical deactivation,[33] which may be favoured by the lower interictal activity in thalamocortical networks.[8] Our finding of increased theta/alpha/low-beta power during the interictal phase of episodic migraine may thus reflect an increased feedback inhibition restraining thalamo-cortical feedforward afferents as a protective (or compensatory) mechanism. ~~Accordingly~~Concordantly, short-range lateral inhibition in the visual cortex of episodic migraineurs was found ~~to be initially~~ increased at the beginning of a sustained visual stimulation ~~protocol~~, but ~~subsequently~~ decreased ~~as~~ with subsequent persistent stimulus presentation ~~persisted~~.[10] This phenomenon likely contributes to the lack of habituation of ~~common~~ broad-band ~~pattern-reversal visual evoked potentials~~PR-VEP, and ~~underscores~~ supports the ~~possibility~~ hypothesis that ~~its~~ the protective mechanism against sensory overload in migraine patients may at some point become ~~overwhelmed~~overtaken.

The ratio between low ~~-~~frequency and ~~-to-~~gamma power was negatively correlated with disease activity, but more so with headache days than with qualified migraine days. ~~activity ratio that we used to measure the relation between feedback and feedforward activity in this~~

~~study showed marked disparities between the groups.~~ Its ~~relative~~ lower value in ~~reduction observed~~ in chronic ~~migraineurs~~ migraineurs could be due to the higher frequency of headache days in these patients rendering them ~~and to the fact that patients with frequent headaches are~~ more likely to be recorded in close temporal relation to an attack. ~~Interestingly, this ratio was remarkably similar between ictal episodic migraine patients and chronic migraineurs; a resemblance that has been described for other electrophysiological features in the past.[6] Such similarity strongly supports the notion that, from an electrophysiological perspective, chronic migraine can be conceptualized as a 'never-ending migraine attack'.[38]~~

~~In addition, results from our study show that the negative correlation between this low-frequency-to-gamma (feedback/feedforward) activity ratio and the number of all headache days was stronger than that of specific migraine days.~~ The pathophysiological distinction between archetypal migraine attacks and episodes of mild headache that co-occur in migraine patients is ~~to date~~ a matter of debate. ~~Indeed, previous p~~Clinical studies~~harmacological studies~~ have shown that these mild headaches ~~without migrainous features~~with a tension-type like phenotype respond just like full-blown migraine attacks to specific anti-migraine drugs like triptans.[26] ~~Given that in o~~Our findings might suggest that most headaches in migraine patients, with or without migrainous features, have a similar pathophysiological underpinning. This hypothesis merits further studies ~~study the total number of headache days exhibited the strongest electrophysiological correlation with the (feedback/feedforward) activity ratio (and not the number of migraine-specific days), one possible interpretation of our findings would be that, in migraine patients, all recurrent headaches constitute manifestations of the same pathophysiological phenomenon that present with different degrees of intensity.~~ b~~B~~ecause of ~~the~~ its potential implications ~~of this conjecture~~ in the diagnosis of chronic migraine[49]. ~~we consider that this hypothesis merits further study.~~

Interestingly, ~~this~~e feedback/feedforward ratio was remarkably similar between ictal episodic and chronic migraine patients. Such similarity was also reported for other electrophysiological features ~~and chronic migraineurs; a resemblance that has been described for other electrophysiological features in the past~~.[6] and confirms that~~Such similarity strongly supports the notion that, from an electrophysiological perspective,~~ chronic migraine ~~can be conceptualized as~~resembles a 'never-ending migraine attack' as far as cortical electrophysiology is concerned.[38]

~~This~~ Our ~~experiment~~ study has ~~some~~ several limitations ~~worth to mention~~. Analysis of gamma band activity does not allow notch filtering ~~utilisation~~ at the frequency of the power line (AC) and one cannot exclude that the gamma band power was to ~~therefore, despite our biggest efforts,~~ some degree ~~of~~ contaminated by the power line oscillations. However, as mentioned, gamma activity exhibited temporal fluctuations in our study, which would be expected from a neural signal, and was not constant over time, as would be 50Hz power line noise. ~~contamination of the signal is probable.~~ Also, artefact rejection with single channel recordings is restricted, and ~~because of that,~~hence subtraction of ~~muscular~~ muscle activity[29,47] or miniature ocular saccades[48] was not possible. Moreover, the two standard deviations from the channel mean amplitude limit that we employed for artefact rejection was empirically chosen and, although apparently adequate, needs to be experimentally corroborated. ~~Furthermore~~Of note, since our analysis was limited to a single derivation (Oz), it ~~and therefore~~ lacks spatial resolution. Multi-channel recordings using high-density EEG would allow to perform ~~a spatial decomposition which would permit an~~ anatomical segregation of neural activity and much better artefact suppression. Analysing pre-stimulus spectral power, and the influence of different temporal frequencies of the visual stimulus would also be worthwhile. Likewise, although signal analyses were automated, blinding the investigators would have been advantageous. With regards to ~~methodologic issues~~subjects, different patients were included in the ictal and interictal episodic migraine groups. In future studies, it would be preferable to compare the same patients in and outside of an attack, which would allow a more powerful paired analysis. For some episodic migraine patients, the next attack following the VEP recordings occurred after the 30-~~-~~day headache diary registry had ended and thus we were unable to correlate their electrophysiological results with time elapsed before/after the most proximal attack. ~~Additionally~~~~G~~given that our sample of migraine patients was entirely ~~comprised by~~composed of migraine ~~patients~~ without aura patients, ~~our~~ the results cannot be readily extrapolated to migraine with aura patients before further testing. P~~p~~hotophobia was not quantitatively assessed, which impeded us from correlating this clinical symptom with electrophysiological data. Finally, in the future it would be of interest to explore the dynamic, ~~(~~intra-individual~~)~~ fluctuations of the low -frequency-to-gamma ratio over the migraine cycle, and its correlation with PR-VEP habituation, the most common neurophysiological abnormality in migraine.

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Figure legends:

Fig 1. Power (μV^2) in the various frequency bands (Hz). Median power (bold line) ± standard error (shaded area) is depicted for each group. Healthy volunteers (HV-blue) showed the lowest mean power at all frequencies. Episodic migraine patients (EM-orange) have the highest alpha power values, while gamma power is greatest amongst chronic migraine patients (CM-red), followed by ictal episodic migraine patients (IM-magenta).

Fig 2. Pattern-reversal visual evoked potential spectral analyses showing Alpha (left) and Gamma (middle) area under the curve (x102μV2/Hz) and Alpha/Gamma area under the curve ratio (right) by subject group. Asterisks (*) indicate significant differences between groups ($p<0.05$ corrected for multiple comparisons). HV: healthy volunteers; EM: episodic migraine patients; CM: chronic migraineurs; IM: episodic migraineurs during an attack.

Fig 3. Correlation between the visually induced alpha/gamma power ratio and the monthly number of migraine days (left) or non-specific headache days (right). (*) $p<0.05$. Ictal migraine patients were not included in this analysis.

Fig 4. Event related spectral perturbations in the gamma frequency range. Gamma activity is dynamically modulated throughout time. Areas delimited by a discontinuous line show the time and frequency range where gamma suppression reaches its maximum in healthy controls and episodic migraine patients in the inter-ictal period. See colour-scale on the right.

Fig 5. Schematic representation of feedback and feedforward signalling towards the primary visual cortex. Feedforward (green) signals reaching the primary visual cortex from the lateral geniculate nucleus induce oscillations in the gamma band frequency range. Feedback signals (red) originating in higher order visual areas (V2-V4) induce activity in the primary visual cortex within the alpha frequency band. Asterisks denote statistically significant differences. ~~The box on the right shows how the distinct volleys reach different layers of the primary visual cortex.~~

Table 1. Participants' characteristics. Mean monthly migraine days and headache days did not differ significantly between episodic migraine patients in the inter-ictal and ictal periods.

Table 2. Alpha and Gamma power (x102μV2/Hz) and their ratio in the 4 subject groups: HV: healthy volunteers (n=20); EM: interictal episodic migraineurs (n=30); CM: chronic migraineurs (n=20); IM: ictal episodic migraineurs (n=20). Symbols: $p<0.05$ corrected for multiple comparisons, (*) as compared to controls, (†) as compared to interictal episodic migraine patients.

Headache related alterations of visual processing in migraine patients.

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Conflict of Interest

The authors of this study have no relevant conflict of interest to declare.

Abstract:

Migraine is characterized by an increased sensitivity to visual stimuli that worsens during attacks. Recent evidence has shown that feedforward volleys carrying incoming visual information induce high frequency (gamma) oscillations in the visual cortex, while feedback volleys arriving from higher order brain areas induce oscillatory activity at lower frequencies (theta/alpha/low-beta).

We investigated visually induced high (feedforward) and low (feedback) frequency activations in healthy subjects and various migraine patients. Visual evoked potentials from 20 healthy controls and 70 migraine patients (30 inter-ictal and 20 ictal episodic migraineurs, 20 chronic migraineurs) were analysed in the frequency domain. We compared power in the theta-alpha-low beta and gamma range between groups, and searched for correlations between the low-to-high frequency activity ratio and number of monthly headache and migraine days.

Compared to healthy controls, inter-ictal migraine patients had increased visually induced low frequency (feedback) activity. Conversely, ictal and chronic migraine patients showed an augmented gamma band (feedforward) power. The low-frequency-to-gamma (feedback/feedforward) activity ratio correlated negatively with monthly headache days and tended to do so with migraine days.

Our findings show that visual processing is differentially altered depending on migraine cycle and type. Feedback control from higher order cortical areas predominates interictally in episodic migraine while migraine attacks and chronic migraine are associated with enhanced incoming afferent activity, confirming their similar electrophysiological profile. The presence of headache is associated with proportionally higher gamma (feedforward) activities.

Perspective:

This study provides an insight into the pathophysiology of migraine headache from the perspective of cortical sensory processing dynamics. Patients with migraine present alterations in feedback and feedforward visual signalling that differ with the presence of headache.

Keywords:

Visual evoked potentials, spectral analysis, episodic migraine, chronic migraine, feedback, feedforward.

Introduction

It is well established in healthy humans that marked changes in brain rhythmic oscillatory activity over a wide range of frequency bands are related to pain processing.[30,32] This also applies for head pain associated to migraine. Several electrophysiological studies have shown that migraine is a brain disorder characterized by an abnormal cortico-subcortical oscillatory activity that fluctuates along the migraine cycle, differs between the ictal and inter-ictal intervals,[7,12,34,35,46] and remains persistently altered as the disease chronifies.[8]

According to available experimental evidence, oscillations in the alpha and gamma frequency bands can be used as direct, objective, experimentally stable, and interrelated measures of cognitive and sensory brain tasks. During on-going pain alpha power is reduced and gamma power is increased in several brain regions,[17,18,45] including posterior cortical areas.[4,5] Similar modifications correlate with active selection and integration of relevant unattended visual information, resulting from the balance between feedforward volleys reaching the visual cortex from the lateral geniculate nucleus (fast gamma oscillations) and feedback activity coming from higher order visual areas (low frequency (theta/alpha/low-beta) oscillations).[24,28] Spectral analysis allows to easily identify these two main frequency peaks (theta/alpha/low-beta and gamma) in common scalp-recorded visual evoked potentials (VEPs), as confirmed by recent intracortical recordings in non-human primates as well as magnetoencephalographic studies in humans.[24,28]

In this study we analysed the previously described fluctuations of visual processing in migraine[23,39] from the perspective of visually induced feedback (theta/alpha/low-beta) and feedforward (gamma) activations. We also tested whether these alterations in visual signalling were specifically associated with the frequency of full-blown migraine attacks, or if they were also related to the presence of mild tension-type like headaches, often present in migraineurs, particularly in those suffering from chronic migraine.

Subjects and methods

Subjects

The study involved 90 participants: 20 healthy volunteers (HV), 30 episodic migraine without aura patients recorded during a headache-free interval (minimum 72 hours before or after an attack) verified on a headache diary and/or by a telephone call (EM), 20 ictal episodic

migraineurs recorded during an attack (IM, 17 during the headache phase, 3 within 48 hours of the headache), and 20 chronic migraine patients without medication overuse (CM). Diagnoses were made in accordance with The International Classification of Headache Disorders 3rd edition beta version (ICHD3 beta).[20] Healthy volunteers did not report any first degree relative suffering from recurrent headaches of any type. Participants were consecutively recruited amongst University students or their families and via our headache clinic. Specifically, an announcement was posted in the University's intra-net, and headache patients attending the consultation were personally invited to take part. Patients were not under any preventive treatment, nor had they been for the 3 preceding months. To ascertain the diagnosis, attack occurrence, and headache attacks severity, patients filled in a paper diary for ≥ 30 days in which headache intensity, associated symptoms (nausea, vomiting, photo-, phonophobia) and acute medication intake were registered. As in recent therapeutic trials,[41] only headaches fulfilling the diagnostic criteria for a migraine attack (ICHD3 beta code 1.1) (unless they had been treated with a triptan) were considered migraine specific headaches. All other episodes of head pain were coded as unspecific headaches. None of the participants that initially agreed to participate were excluded afterwards. The study was approved by the Hospital's ethics committee (Centre Hospitalier Régional de la Citadelle, Liège, Belgium – protocol n°1422) and conducted following the principles of the Declaration of Helsinki. All participants gave written informed consent.

Visual Evoked Potentials (VEP) recordings and analysis

VEP recordings were performed in the electrophysiology laboratory of the Headache Research Unit (Neurology Department, Centre Hospitalier Régional de la Citadelle, Liège, Belgium). All participants were studied in the morning, between 9 a.m. and noon. Subjects were sitting on a comfortable armchair, in a quiet room with dimmed light. A patch was placed over the left eye, and needle recording electrodes were introduced in the scalp at Oz (active) and Fz (reference) based on the 10-20 EEG system. During the recordings, subjects were instructed to maintain fixation on a red dot in the centre of a screen which displayed a black and white reversing checkerboard pattern (contrast of 80%, mean luminance 50 cd/m2). Temporal and spatial stimulating frequencies employed were 1.55 Hz (3.1 reversals/second) and 68' respectively. Six hundred epochs, each lasting 250ms, were continuously recorded at a sampling rate of 5.000 Hz using a CED™ power 1401 device (Cambridge Electronic Design Ltd, Cambridge, UK). After DC subtraction, recordings were exported to EEGLAB,[13] an open-source MATLAB (The MathWorks Inc.) toolbox for electrophysiological signal

processing, where they were band-pass filtered (low pass 100 Hz, high pass 1 Hz). Epochs whose amplitude exceeded a two standard deviations from the channel mean amplitude limit were considered artefacted and rejected (<6% of epochs). The Fast Fourier Transform was applied on each epoch to compute spectral decomposition. Log-transform of single-trial spectral power was performed before averaging. Data were zero-padded in order to increase frequency resolution to steps of 1Hz. As in previous studies,[28] the two most prominent peaks of the spectrogram were observed in the theta/alpha/low-beta (1) and gamma (2) frequency band ranges. To estimate power at these frequencies, the area under the curve (trapezoidal numerical integration; MATLAB function 'trapz') of activity at each peak and nearby surrounding frequencies (4 to 16 Hz for theta-alpha-low beta and 40 to 60 Hz for gamma) was calculated for each individual (Fig 1). Considering the recent evidence showing that alpha-beta and gamma activity embedded in visually-induced cortical responses convey different information,[24,28] and that abnormal visual responsiveness in migraine is the result of a complex process involving several cortical areas,[27] we calculated the low frequency-to-gamma activity ratio as a measure of the interaction between simultaneous volleys reaching the visual cortex. In addition, considering the overlap between visually induced cerebral gamma activity and the frequency spectrum of different possible sources of contamination of the signal (muscular artefacts, AC line noise) we performed a supplementary analysis of event related spectral perturbations which permits to visually inspect changes in the power spectrum throughout time. Investigators in this study were not blinded to diagnosis, but all electrophysiological analyses were fully automated.

Statistical analysis

Statistical analyses and graphs were performed in Prism version 6.00 for Windows (GraphPad Software, La Jolla, California, USA). The assumption of normal distribution was assessed using the Shapiro-Wilk normality test. Continuous variables were compared using ANOVA or Kruskal-Wallis tests (in case of non-normal distributions or violations in the assumption of homoscedasticity evaluated using Bartlett's test), followed by post-hoc comparisons between groups (corrected for multiple comparisons using Dunn's multiple comparison test). Correlation analyses between spectral power ratios and monthly number of headache or migraine days were performed using Spearman's rank correlation test corrected for multiple comparisons by applying a Bonferroni correction. Because alterations in the power spectrum of patients from the ictal migraine group are likely to be transient,[1,39] these patients were not included in correlation analyses. The significance level for all tests was set at $p<0.05$.

Results

There were no significant between-group differences in mean age or gender ratio in the whole subject sample, nor between disease duration amongst migraine sub-groups (Table 1).

The results of spectral analyses are displayed in table 2. Mean low-frequency (theta-alpha-low beta) power was significantly higher in headache-free episodic migraine patients compared to healthy controls (Kruskal-Wallis test H= 8.330, p=0.040; Dunn's multiple comparisons test (episodic migraine patients vs healthy controls) p= 0.030, adjusted for multiple comparisons). Conversely, gamma power was higher in both ictal and chronic migraine patients (Kruskal-Wallis test H= 14.00, p< 0.003; Dunn's multiple comparisons tests: chronic migraine vs healthy controls, p= 0.023; ictal migraine vs healthy controls, p= 0.013, both adjusted for multiple comparisons) (Fig 1 & 2). The low-frequency-to-gamma activity ratio was significantly smaller in ictal and chronic migraine patients compared to headache-free episodic migraine patients, and in ictal migraine patients compared to healthy controls (Kruskal-Wallis test H= 16.33, p= 0.001); Dunn's multiple comparisons tests: episodic vs chronic, p= 0.032; episodic vs ictal, p= 0.012; healthy volunteers vs ictal, p = 0.024 (all adjusted for multiple comparisons). A similar trend was observed between chronic migraine patients and healthy controls, but it did not reach statistical significance (p= 0.055) (Fig 2). The low-frequency-to-gamma activity ratio was negatively correlated with the total number of monthly headache days (ρ= -0.34; p= 0.015), but not with the total number of migraine specific days (ρ= -0.25; p=0.08) (Fig 3). A partial correlation (controlling for age) between the low-frequency / gamma activity ratio and the monthly headache days was also significant (r= -.33; p= 0.02). The N1-P1 amplitude of the broad-band VEP was not significantly different between the groups (healthy controls: 5.088µV ± 1,444; headache free migraine patients: 5.860 µV ± 2.361; chronic migraine patients: 5.368 µV ± 2.281; ictal migraine patients: 6.396 µV ± 2.436; (one-way ANOVA $F_{(3,86)}$ = 1.399; p=0.249). Supplementary event-related spectral perturbations analysis (Fig 4) showed that gamma activity exhibited temporal fluctuations, as one would expect from a neural signal, rather than being constant over time, as would be 50Hz power line noise or other possible sources of signal contamination.

Discussion

We measured power of low (theta/alpha/low-beta) and high (gamma) frequency oscillations embedded in pattern-reversal visual evoked potentials (PR-VEP) in healthy controls, episodic

migraine patients during or in between attacks, and chronic migraineurs. The results show that, during headache, gamma power is greater in patients than in healthy subjects. By contrast, in the absence of headache, episodic migraine patients have increased low frequency power (theta/alpha/low-beta). Concordantly, the low-frequency-to-gamma activity ratio was significantly higher in headache-free patients than during a migraine attack or in chronic migraineurs and negatively correlated with the monthly number of headache days.

We have previously found a decreased habituation of late visual induced gamma components in headache-free interictal episodic migraine patients .[7] In the present study we focused on total gamma power and its relation with the low-frequency power spectrum analysed in the frequency-domain, which is better suited to evaluate high frequency oscillations. There is strong evidence showing that feedforward (afferent) volleys coming from the lateral geniculate nucleus induce oscillations within the gamma frequency range in the primary visual cortex (Fig. 5). This frequency range has been associated with the efficiency of stimulus processing by thalamocortical networks [15,36,40] and with the translation of the stimulus features into coherent perception (for a review, see Gray and Singer, 1995 [42] ; Tallon-Baudry and Bertrand, 1999 [44]). Therefore, increased visually induced gamma (feedforward) activity during migraine attacks and in chronic migraine may reflect augmented efficiency in the thalamo-cortical circuit. This is in line with previous electrophysiological,[8,9,23,43] and functional neuroimaging[11] studies showing that thalamo-cortical network activity is decreased in migraineurs during the headache-free interval, but increased during an attack and with migraine chronification.

On the other hand, it is known that pain is accompanied by widespread enhancement of gamma activity in the brain (prefrontal, mid-cingulate, and primary somatosensory cortices and insula)[19] associated with contralateral alpha power reductions,[32] which suggests that the former reflects tonic pain processing while the latter may be related to a top-down cognitive process linked to attention.[4,5,17,18,45] Reciprocal anatomical and functional connections between the visual and the trigeminal systems are well documented in animals and human beings.[3,25,31,37] In particular, convergence of nociceptive trigeminal and visual afferents in the posterior thalamus[30] may explain how head pain can amplify visually induced thalamocortical activity, and thus gamma power in PR-VEP.

As opposed to feedforward afferent activity that generates gamma oscillations in the primary visual cortex, feedback volleys from higher order visual areas (V2-V4) induce oscillatory activity within the theta/alpha/low-beta frequency range (Fig. 5) that notably plays a role in

focusing attention to salient unattended stimuli.[24,28] Such feedback volleys reaching the visual cortex are able to modulate the response to visual afferents [14,21,23] by selectively inhibiting high frequency (gamma) feedforward oscillations, and thus to exert a possible 'gating' process.[22] The sensory processing profile of migraine patients makes them vulnerable to sensory overload,[2,16] and therefore, in need of compensatory protective mechanisms. Between attacks, repetitive photic stimulation causes whole-brain alpha hyper-synchronization,[46] indicative of a diffuse cortical deactivation,[33] which may be favoured by the lower interictal activity in thalamocortical networks.[8] Our finding of increased theta/alpha/low-beta power during the interictal phase of episodic migraine may thus reflect an increased feedback inhibition restraining thalamo-cortical feedforward afferents as a protective (or compensatory) mechanism. Concordantly, short-range lateral inhibition in the visual cortex of episodic migraineurs was found increased at the beginning of a sustained visual stimulation, but decreased with subsequent persistent stimulus presentation.[10] This phenomenon likely contributes to the lack of habituation of broad-band PR-VEP, and supports the hypothesis that the protective mechanism against sensory overload in migraine patients may at some point become overtaken.

The ratio between low frequency and gamma power was negatively correlated with disease activity, but more so with headache days than with qualified migraine days. Its lower value in in chronic migraineurs could be due to the higher frequency of headache days in these patients rendering them more likely to be recorded in close temporal relation to an attack. The pathophysiological distinction between archetypal migraine attacks and episodes of mild headache that co-occur in migraine patients is a matter of debate. Clinical studies have shown that these mild headaches with a tension-type like phenotype respond just like full-blown migraine attacks to specific anti-migraine drugs like triptans.[26] Our findings might suggest that most headaches in migraine patients, with or without migrainous features, have a similar pathophysiological underpinning. This hypothesis merits further studies because of its potential implications in the diagnosis of chronic migraine[49]. Interestingly, the feedback/feedforward ratio was remarkably similar between ictal episodic and chronic migraine patients. Such similarity was also reported for other electrophysiological features [6] and confirms that, chronic migraine resembles a 'never-ending migraine attack' as far as cortical electrophysiology is concerned.[38]

Our study has several limitations. Analysis of gamma band activity does not allow notch filtering at the frequency of the power line (AC) and one cannot exclude that the gamma band

power was to some degree contaminated by the power line oscillations. However, as mentioned, gamma activity exhibited temporal fluctuations in our study, which would be expected from a neural signal, and was not constant over time, as would be 50Hz power line noise. Also, artefact rejection with single channel recordings is restricted, and hence subtraction of muscle activity[29,47] or miniature ocular saccades[48] was not possible. Moreover, the two standard deviations from the channel mean amplitude limit that we employed for artefact rejection was empirically chosen and, although apparently adequate, needs to be experimentally corroborated. Of note, since our analysis was limited to a single derivation (Oz), it lacks spatial resolution. Multi-channel recordings using high-density EEG would allow to perform anatomical segregation of neural activity and much better artefact suppression. Analysing pre-stimulus spectral power, and the influence of different temporal frequencies of the visual stimulus would also be worthwhile. Likewise, although signal analyses were automated, blinding the investigators would have been advantageous. With regards to subjects, different patients were included in the ictal and interictal episodic migraine groups. In future studies, it would be preferable to compare the same patients in and outside of an attack, which would allow a more powerful paired analysis. For some episodic migraine patients, the next attack following the VEP recordings occurred after the 30-day headache diary registry had ended and thus we were unable to correlate their electrophysiological results with time elapsed before/after the most proximal attack. Given that our sample of migraine patients was entirely composed of migraine without aura patients, the results cannot be readily extrapolated to migraine with aura patients before further testing. Photophobia was not quantitatively assessed, which impeded us from correlating this clinical symptom with electrophysiological data. Finally, in the future it would be of interest to explore the dynamic, intra-individual fluctuations of the low frequency-to-gamma ratio over the migraine cycle, and its correlation with PR-VEP habituation, the most common neurophysiological abnormality in migraine.

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Figure legends:

Fig 1. Power (μV^2) in the various frequency bands (Hz). Median power (bold line) ± standard error (shaded area) is depicted for each group. Healthy volunteers (HV-blue) showed the lowest mean power at all frequencies. Episodic migraine patients (EM-orange) have the highest alpha power values, while gamma power is greatest amongst chronic migraine patients (CM-red), followed by ictal episodic migraine patients (IM-magenta).

Fig 2. Pattern-reversal visual evoked potential spectral analyses showing Alpha (left) and Gamma (middle) area under the curve (x102μV2/Hz) and Alpha/Gamma area under the curve ratio (right) by subject group. Asterisks (*) indicate significant differences between groups ($p<0.05$ corrected for multiple comparisons). HV: healthy volunteers; EM: episodic migraine patients; CM: chronic migraineurs; IM: episodic migraineurs during an attack.

Fig 3. Correlation between the visually induced alpha/gamma power ratio and the monthly number of migraine days (left) or non-specific headache days (right). (*) $p<0.05$. Ictal migraine patients were not included in this analysis.

Fig 4. Event related spectral perturbations in the gamma frequency range. Gamma activity is dynamically modulated throughout time. Areas delimited by a discontinuous line show the time and frequency range where gamma suppression reaches its maximum in healthy controls and episodic migraine patients in the inter-ictal period. See colour-scale on the right.

Fig 5. Schematic representation of feedback and feedforward signalling towards the primary visual cortex. Feedforward (green) signals reaching the primary visual cortex from the lateral geniculate nucleus induce oscillations in the gamma band frequency range. Feedback signals (red) originating in higher order visual areas (V2-V4) induce activity in the primary visual cortex within the alpha frequency band. Asterisks denote statistically significant differences.

Table 1. Participants' characteristics. Mean monthly migraine days and headache days did not differ significantly between episodic migraine patients in the inter-ictal and ictal periods.

Table 2. Alpha and Gamma power (x102μV2/Hz) and their ratio in the 4 subject groups: HV: healthy volunteers (n=20); EM: interictal episodic migraineurs (n=30); CM: chronic migraineurs (n=20); IM: ictal episodic migraineurs (n=20). Symbols: $p<0.05$ corrected for multiple comparisons, (*) as compared to controls, (†) as compared to interictal episodic migraine patients.

Highlights

- Visually-induced feedback and feedforward signalling is altered in migraine
- Inter-ictal episodic migraine patients show increased feedback to the visual cortex
- Ictal episodic and chronic migraine patients show augmented feedforward activity
- Electrophysiologically, chronic migraineurs resemble ictal episodic migraine patients
- All headaches in migraine patients have a similar electrophysiological background

Figure 1

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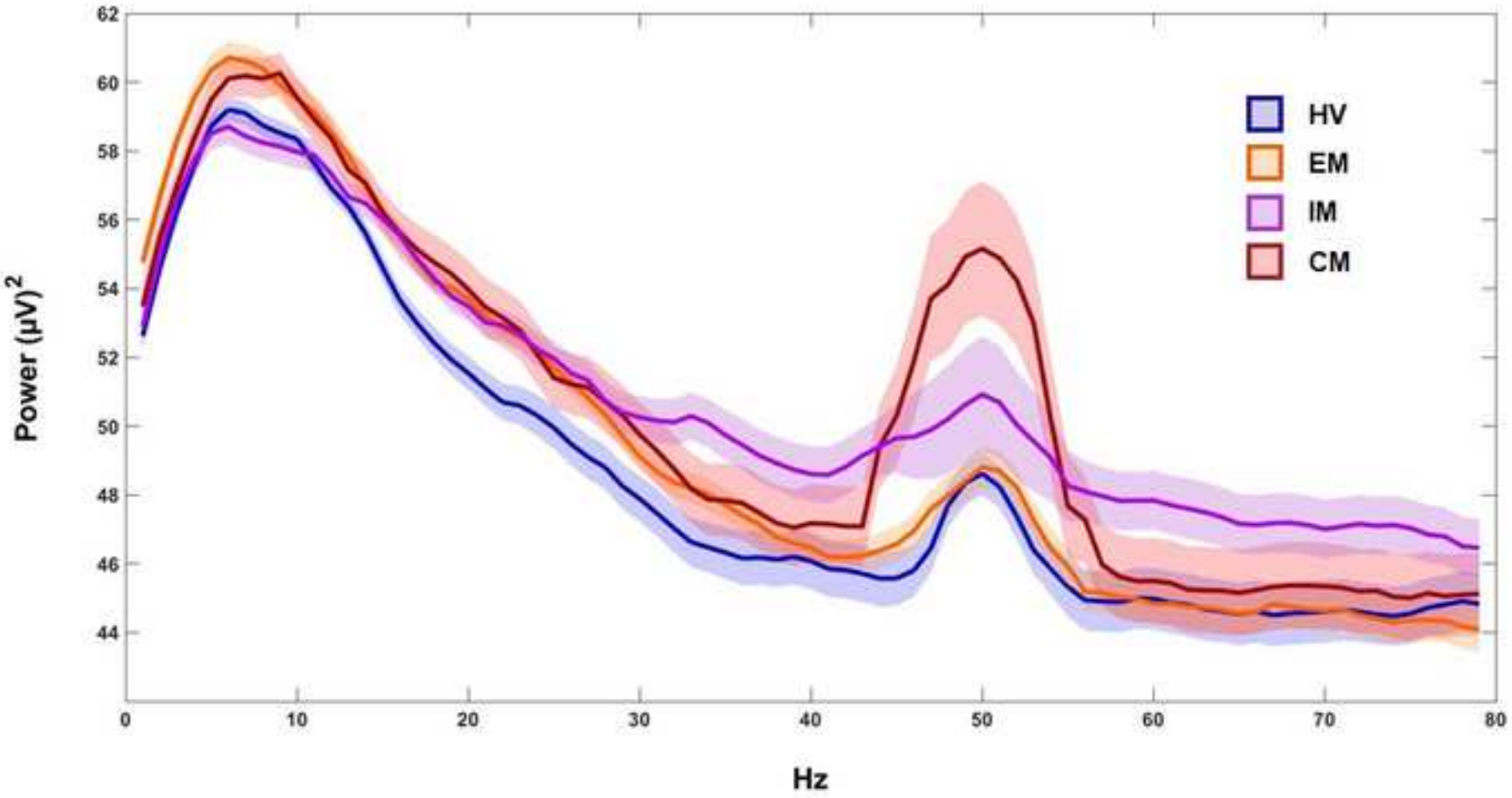

Figure 2

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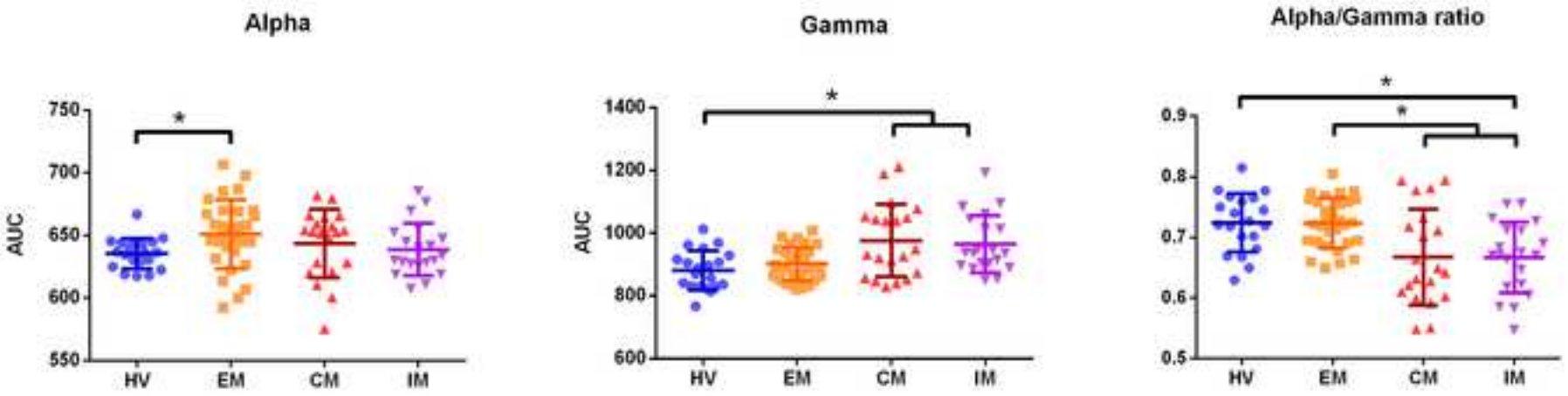

Figure 3

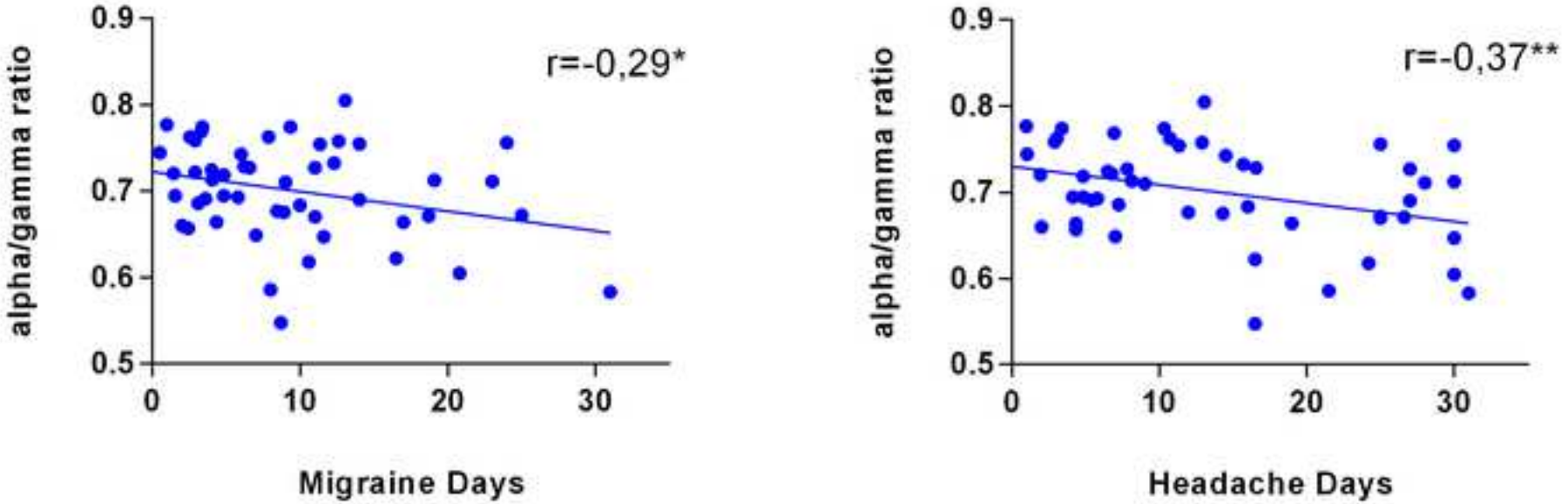

Figure 4

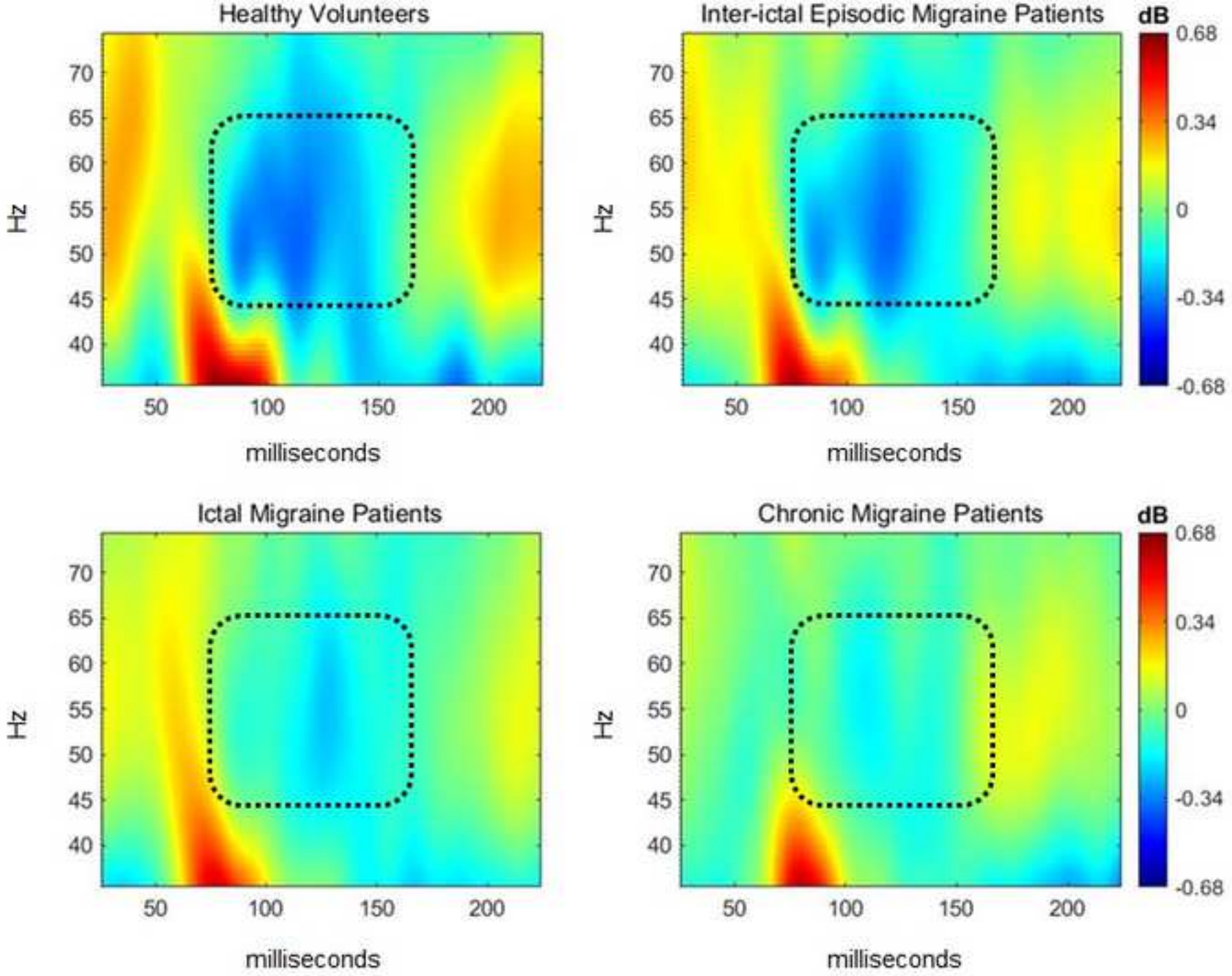

Figure 5

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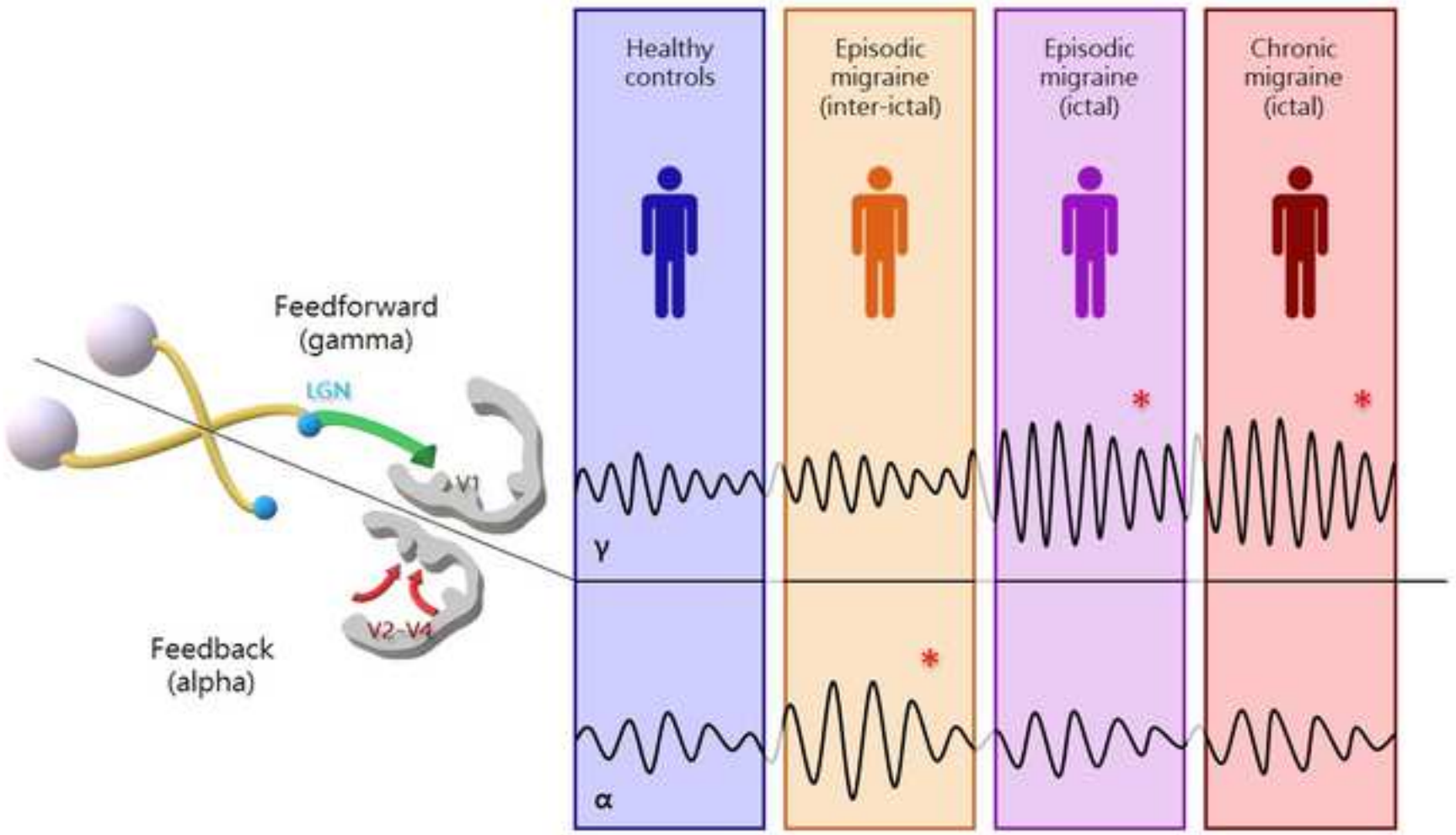

Table 1

	Healthy volunteers		Inter-ictal episodic migraine		Ictal episodic migraine		Chronic migraine		p-value
Age (mean ± SD)	36.1	11.4	33.3	11.9	32.7	9.1	40.3	12.7	p = 0.126
Female percentage	75%		90%		100%		95%		p = 0.051
Disease duration (mean ± SD)			14.6	9.4	15.7	11.8	18.75	11.8	p = 0,430
Monthly migraine days (mean ± SD)			5.5	3.5	5.9	3.6	15.8	6.4	p < 0,001
Monthly headache days (mean ± SD)			7.3	4.1	8.6	6.6	23.9	5.7	P < 0,001

Table 2

	HV		EM		CM		IM	
	mean	SD	mean	SD	mean	SD	mean	SD
Low frequency	635,6	± 12,1	651,0*	± 27,5	643,4	± 27,4	638,7	± 21,0
Gamma	881,9	± 62,7	901,9	± 53,4	976,6*	± 116,6	965,7*	± 91,2
Ratio	0,72	± 0,05	0,72	± 0,04	0,67†	± 0,08	0,67*†	± 0,06

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page # where this item is located:
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3-4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3-4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the	

		number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	n/a

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	3-5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	n/a
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	3-4
		(*e*) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3-4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3-4
		(b) Indicate number of participants with missing data for each variable of interest	3-4
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	*6*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	

	(*b*) Report category boundaries when continuous variables were categorized
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	4-5
Discussion			
Key results	18	Summarise key results with reference to study objectives	6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-9
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

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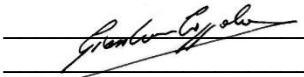
Signature (1)		Print Name: Gianluca Coppola	Date:December 11, 2018
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