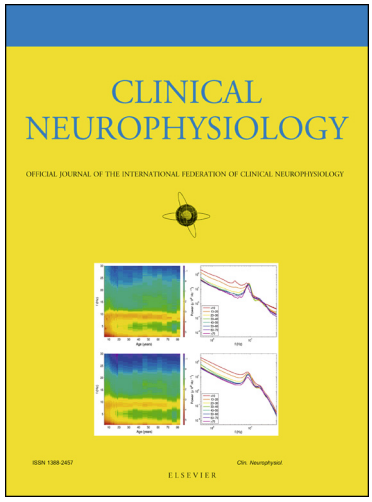

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Visual evoked potentials in migraine: Is the "neurophysiological hallmark" concept still valid?

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Highlights

- Habituation measured by visual evoked potentials (VEPs) were not significantly different between persons with and without migraine.
- Lack of VEP habituation is not a reliable "neurophysiological hallmark" in interictal migraine
- Strict focus on methodology, including blinding during the recording and assessment of VEP, is important to avoid overestimation of effect sizes in future studies.

Abstract

Objective

Lack of habituation is considered a neurophysiological hallmark of migraine. However, the results of visual evoked potential (VEP) studies have been discrepant, possibly because of different stimulation parameters and lack of blinding. Hence, there is a need for independent confirmation of lack of VEP habituation in migraine. In this blinded study we applied 16' checks to supplement our previous findings with 8', 31', 62' and 65' checks.

Methods

VEPs in 41 interictal migraineurs and 30 controls were compared. VEPs were recorded in six blocks of 100 single responses. Linear N70-P100 amplitude change over blocks (habituation slope) was compared with an independent samples Student's t-test.

Results

Amplitude decline over blocks was observed in both groups. Habituation slope was not significantly different between controls (-0.43 ± 0.54 μV/block) and migraineurs (-0.29 ± 0.35 μV/block) ($p = 0.33$).

Conclusion

VEP habituation with 16' checks did not differ in migraineurs and controls. This is in agreement with previous findings with other stimulation parameters. It is therefore unlikely that use of different stimulation parameters could explain the discrepant results of previous studies. No studies that applied blinding during recording of VEP have found lack of habituation in migraineurs.

Significance

Lack of VEP habituation cannot be considered a reliable neurophysiological hallmark in migraine.

Keywords: Visual evoked potentials; habituation; migraine.

1. Introduction

Most migraineurs experience visual symptoms including increased sensitivity to light both during and between attacks (Headache Classification Committee of the International Headache Society, 2013; Vanagaite et al., 1997). Moreover, many studies have measured visual evoked potentials (VEPs) in migraine to see if objective alterations in visual function are present (e.g. Kennard et al., 1978; Polich et al., 1986; Shibata et al., 1997).

VEP amplitude declines during repeated stimulation in controls (Omland et al., 2011; Omland et al., 2013). This decrement is often referred to as habituation (Bednar et al., 2014). This interpretation is also used in the present study, although there are other possible explanations for the amplitude decrement (Omland et al., 2011). Some groups have reported that VEP amplitudes do not decline in migraineurs in the period between attacks (interictally) (Bednar et al., 2014; Coppola et al., 2009; Ozkul and Bozlar, 2002). Migraineurs may therefore lack habituation. Some authors of review papers suggest that lack of habituation is a neurophysiological hallmark of migraine (Brighina et al., 2009; Magis et al., 2013), and others considered it the most reproducible abnormality in migraine interictally (Coppola et al., 2007a; Nappi and Moskowitz, 2011).

These claims are disputed for VEP because lack of habituation has not been reproduced in several VEP studies (Oelkers-Ax et al., 2005; Oelkers et al., 1999; Omland et al., 2013; Sand et al., 2008; Vigano et al., 2013). Interestingly, studies that reported lack of habituation in migraine applied 3.1 reversals per second (rps), while most other studies applied slower reversal rates. In addition, previous studies have also used different check sizes (see Table 5 in Omland et al., 2013). It has therefore been hypothesized that the use of different stimulation parameters could explain the discrepant findings (Sand et al., 2008). This was not confirmed in a study that applied 1.9 rps and 31' and 62' checks (Sand et al., 2009), nor in a study that applied 3.0 rps and 8' and 65' checks (Omland et al., 2013). However, most recent VEP habituation studies applied 14-16' checks (Bednar et al., 2014; Coppola et al., 2007b; Coppola et al., 2011; Coppola et al., 2010b; Coppola et al., 2010a; Coppola et al., 2013b). This check size may be preferable because habituation is more pronounced with smaller check sizes (e.g. 8' and 16' checks) than larger check sizes (e.g. 65') in both controls and migraineurs (Omland et al., 2011; Omland et al., 2013), whereas very small check sizes (e.g. 8') may result in a low signal to noise ratio in some subjects (Sand and Vingen, 2000). 14-16' checks are also recommended by the International Federation of Clinical Neurophysiology (Holder et al., 2010).

Most studies that reported lack of habituation in migraineurs were by one internationally collaborating group of authors (Afra et al., 1998; Afra et al., 2000b; Bohotin et al., 2002; Coppola et al., 2007b; Coppola et al., 2011; Coppola et al., 2010b; Coppola et al., 2010a; Coppola et al., 2013b; Di Clemente et al., 2005; Fumal et al., 2006; Schoenen et al., 1995; Vigano et al., 2013). As far as we know, lack of VEP habituation been independently confirmed in two studies (Bednar et al., 2014; Ozkul and Bozlar, 2002), while two other groups have not managed to reproduce this finding (Oelkers-Ax et al., 2005; Oelkers et al., 1999; Omland et al., 2013; Sand et al., 2008). Furthermore, studies that applied blinding of diagnosis when recording VEPs have not found lack of habituation in migraine (Omland et al., 2013; Sand et al., 2008).

In this study we investigated whether VEP habituation is different in migraineurs and controls. We applied 16' checks to supplement our previous findings with 8', 31', 62' and 65' checks. As far as we know, this has not been investigated previously with a blinded design. In addition, independent confirmation is essential if lack of habituation shall be considered a reliable neurophysiological hallmark of migraine.

2. Methods

2.1 Subjects

81 subjects were recruited by advertisements at the Intranet of the university and hospital in Trondheim, advertisements in a regional newspaper and from the blood donation centre in Trondheim. Among 50 of these a diagnosis of migraine were confirmed by neurologist according to the ICHD-II criteria (Headache Classification Committee of the International Headache Society, 2004). Migraineurs with 2-6 attacks per month and at most 10 migraine days per month were included. They were allowed to use symptomatic medication during attacks, while migraine prophylactic drugs were not permitted within 4 weeks before the tests. The remaining 31 subjects were controls. Demographical data of the subjects are shown in Table 1.

Exclusion criteria were previous history of migraine headache for controls, frequent episodic (1-14 days/month for controls and 7-14 days/month for migraineurs) or chronic (≥ 15 days/month) tension-type headache, neurological or psychiatric diseases, sleep disorders, active infectious diseases, connective tissue diseases, metabolic, endocrine or neuromuscular diseases, other clinically relevant painful conditions including recent injuries, malignancy, previous craniotomy or cervical spine surgery, heart disease, cardiopulmonary or cerebrovascular diseases, pregnancy, medication for acute or chronic pain, neuroleptic drugs, antidepressant drugs, antiepileptic drugs, or other drugs

that may influence neuronal, vascular or muscular function, alcohol or drug abuse, ferromagnetic implants and prophylactic allergy treatment. None of the subjects had any visual system disorder.

The study was approved by the Regional Ethics committee and followed the declaration of Helsinki. Subjects signed a written informed consent.

2.2 Recording of visual evoked potentials

16' (47 mm) checks and 3.0 rps were applied. Corrected visual acuity was measured on Snellen's chart at distance of 5 meters (Table 1). It was ensured that all subjects could see the 16' checks clearly at a distance of 1 meter. Subjects used their regular optical refraction if they had any. Additional optical refraction was not necessary in any subjects. The pattern contrast was 93 %. The distance between subject's eye and screen was 1 meter. The screen size was 17^{o} x 13^{o} (30.7 cm x 22.5 cm). Responses were averaged from the midline occipital (MO) (5.0 cm above the inion) to the midline frontal (Fz) deviation (defined by the International 10/20 system).

The subjects sat comfortably in a chair and had a patch over the left eye. All subjects received the same instructions before the examination. They were asked to focus on the red dot in the centre of the checkerboard pattern. Rejection rate was set to 90 % of amplifier input (± 100 μV). 0.2 % of responses were rejected. 600 uninterrupted responses (rejections not included) were divided into 6 consecutive blocks, each consisting of an average of 100 single responses. The same number of blocks and stimuli have been applied in most recent VEP studies (see Table 5 in Omland et al., 2013).

Recordings were performed at approximately 08:30, 10:00, 12:00 or 13:30 in both controls and migraineurs. VEPs were recorded four times in migraineurs with an average interval of 6.7 days (SD 1.9, range 3-15) between each examination. Recordings in each migraineur were performed at the same time of day. VEPs were recorded once in controls.

Investigators were blinded to whether the subjects were migraineurs or not during the first examination, as well as to how long time had passed since the last migraine attack on the later examinations.

2.3 Handling of recordings and exclusions

One migraineur withdrew consent. The recordings from this subject were therefore not included in the analysis. In another migraineur, recordings of VEPs could not be completed because the subject found the experiment uncomfortable. 182 VEP recordings were performed in the remaining 48 migraineurs. Four examinations were performed in 42 migraineurs. One, two and three examinations

respectively, were performed in three migraineurs because they did not show up for all appointments. In another subject, the second and third recordings were not performed because she feared that the VEP examination could trigger a migraine attack. Two recordings could not be performed in two subjects because of technical difficulties.

Recordings in migraineurs were divided into periods depending on relation to the nearest migraine attack. Recordings that were made > 2 days before the next and > 2 days after the previous migraine attack were consider interictal and were included in the analysis. In subjects with more than one interictal recording, only the first recordings were included. Seventy-eight recordings were interictal, and 41 migraineurs had at least one interictal recording. VEP recordings made ≤ 2 days before/after the closest migraine attack were not included in the present analysis, but will be reported elsewhere.

Two interictal recordings in different migraineurs were excluded from the analysis because of drowsiness (subjects were observed drowsing during the examination). These two subjects had other interictal recordings that were included in the analysis. One control was excluded because of drowsiness. Exclusions made on the first visit were done without knowledge of diagnosis. Exclusions of migraineurs on the remaining visits were done without knowledge of relation to nearest migraine attack.

2.4 Data analysis and statistics

N70, P100 and N145 peaks were visually determined by an investigator blinded to diagnosis and block number. The investigator also determined whether the quality of each recording was adequate while blinded to diagnosis and block number, but it was not deemed necessary to exclude any of the recordings.

Peak-to-peak N70-P100 and P100-N145 amplitudes and P100 latencies were measured. All amplitudes were square root transformed to improve normality of the distribution.

Habituation slope, the linear decrement of amplitude over blocks, was calculated for each recording by least squares method. Habituation slope of the early N70-P100 amplitude component was chosen as primary efficacy parameter because N70-P100 habituation slope has been analysed by most recent VEP habituation studies (see Table 5 in Omland et al., 2013). Block ratio, the ratio between amplitude of the last and the first block, was also calculated because this habituation measure has

been used in previous studies (see Table 5 in Omland et al., 2013). The habituation slopes and block ratios of controls and migraineurs were compared with independent samples Student's t-test.

Repeated measures ANOVA, with amplitude or P100 latency as the dependent variable, block as within subject factor and group as between subject factor was used to check for amplitude and P100 peak latency differences between groups, and latency and amplitude changes over blocks. ANOVA degrees of freedom were adjusted for non-sphericity with the Huynh–Feldt method. Amplitudes and P100 peak latency of block 1 and block 6 were compared with paired samples Student's t-test post hoc.

P-values < 0.05 were considered significant. A two sample t-test with 71 subjects has a 80 % power to detect a group difference of 0.68 SD. Differences in habituation between migraineurs and controls have been reported to be about 1 SD in most previous studies (Omland et al., 2013).

3. Results

N70-P100 and P100-N145 habituation slopes and block ratios were not significantly different in controls and interictal migraineurs ($p \geq 0.33$, Table 2). Repeated measures ANOVA revealed no amplitude differences or differences in amplitude changes over blocks between controls and interictal migraineurs (group factor, $p = 0.71$, block x group interaction, $p > 0.31$, Table 3). Amplitudes in the first block were similar in controls and migraineurs (Table 4).

Significant effects of block for both N70-P100 and P100-N145 amplitudes were found ($p < 0.001$ and $p = 0.017$ respectively, Table 3), indicating a significant amplitude decline over blocks in both groups (Tables 2 and 4, Figure 1 and 2). The N70-P100 amplitude were lower in block 6 compared to block 1 ($p < 0.001$). P100-N145 amplitude in block 6 tended to be lower than in block 1 ($p = 0.08$).

Repeated measure ANOVA revealed no P100 latency differences between groups (group factor, $p = 0.38$). There were no significant differences between P100 latency in block 1 (100.1 ± 5.9 ms) and block 6 (100.5 ± 5.2 ms) ($p = 0.29$).

4. Discussion

4.1 Habituation measured by visual evoked potentials in migraine

We could not reproduce the reported lack of habituation in interictal migraineurs. N70-P100 habituation slope was chosen as primary efficacy parameter because many previous studies only report N70-P100 and not P100-N145 amplitude habituation (Afra et al., 2000b; Coppola et al., 2007b; Coppola et al., 2011; Coppola et al., 2010a; Coppola et al., 2013b; Di Clemente et al., 2005; Ozkul and Bozlar, 2002). Neither N70-P100 nor P100-N145 habituation measures were significantly different between groups. Independent confirmation of the habituation-deficit has been achieved only by two groups (Bednar et al., 2014; Ozkul and Bozlar, 2002), whereas it has not been confirmed in five studies by two other groups (Oelkers-Ax et al., 2005; Oelkers et al., 1999; Omland et al., 2013; Sand et al., 2009; Sand et al., 2008). In addition, lack of habituation was not observed in subset of migraineurs in study by a third group (Khalil et al., 2000). Taken together, in contrast with the view expressed in some recent reviews (e.g. Brighina et al., 2009; Coppola et al., 2013a; Coppola et al., 2009; de Tommaso et al., 2014; Magis et al., 2013), the actual evidence suggests that lack of pattern VEP habituation cannot be considered a reliable finding in interictal migraine. In addition, we could neither reproduce lack of habituation with 3.0 rps and 16' checks in the present study, nor with 3.0 rps and 8' and 65' or 1.9 rps and 31' and 62' checks in previous studies (Omland et al., 2013; Sand et al., 2009; Sand et al., 2008). It is therefore unlikely that use of different stimulation parameters can explain the discrepant findings in previous VEP studies.

Few VEP habituation studies have applied a blinded procedure during the recording of VEPs, and none of the blinded studies found lack of habituation (Omland et al., 2013; Sand et al., 2008). Evidence from several other fields show that lack of blinding may result in overestimation of differences between groups (Bebarta et al., 2003; Crossley et al., 2008; Jadad et al., 1996; Lijmer et al., 1999; Schulz et al., 1995). In spite of that, blinded procedures have not become the standard in neurophysiological migraine research (Sand, 2014). Some recent studies have applied a blinded *assessment* of VEPs (Bednar et al., 2014; Coppola et al., 2013b). However, this will only reduce some sources of bias. Blinding during the *recording* of VEPs is necessary to ensure that subjects are treated in the same way, and to avoid differences in the recoding procedure between groups.

The guidelines of the International Federation of Clinical Neurophysiology do not state what instruction should be made when recording VEP (Holder et al., 2010). This is also rarely specified in VEP studies of migraine. However, it is well known from clinical experience and the literature (Chiappa, 1990) that decreasing attention and/or inability to focus on the screen may cause declining VEP amplitudes. Hence, in clinical practice it is important that the technician helps the patient to remain alert and cooperative during a VEP recording. In research, different approaches may be chosen depending on the purpose of the study. In habituation studies, talking to the subjects during

the stimulation should be avoided, as this may cause dishabituation according to the habituation paradigm (Rankin et al., 2009). However, using a blinded design ensures that similar instructions are given regardless of subject group. Hence, bias caused by expectations, e.g. regarding differences in block amplitudes in migraine and controls, is avoided if blinding of the technician is applied during VEP-recording.

Several previous VEP habituation studies seem underpowered compared to the present study (Coppola et al., 2011; Coppola et al., 2010b; Coppola et al., 2010a; Di Clemente et al., 2005; Fumal et al., 2006; Vigano et al., 2013). Significant findings in small studies with low power are less likely to reflect a true effect, and low power is associated with other sources of bias that may overestimate effect sizes (Button et al., 2013).

Most VEP habituation studies did not aim to confirm lack of habituation in migraineurs. Several studies merely investigated how lack of VEP habituation in migraineurs is affected by interventions, such as coloured glass (Afra et al., 2000a), fluoxetine (Ozkul and Bozlar, 2002), repetitive transcranial magnetic stimulation (Bohotin et al., 2002; Fumal et al., 2006), cold pressor test (Coppola et al., 2010a), hyperventilation (Coppola et al., 2010b), light deprivation (Coppola et al., 2011) and direct current stimulation (Vigano et al., 2013). Other studies investigated how lack of VEP habituation relates to other measures, such as personality tests (Wang et al., 1999), cortical auditory evoked potentials (Afra et al., 2000b) and nociceptive blink reflex (Di Clemente et al., 2005). The appropriate sample size, analysis and presentation of results in a study depend on its primary aim. This may explain the low sample size seen in some studies, as well as why many studies only report the early N70-P100 amplitude component and not the late P100-N145 amplitude component (Afra et al., 2000a; Afra et al., 2000b; Coppola et al., 2007b; Coppola et al., 2011; Coppola et al., 2010a; Coppola et al., 2013b; Di Clemente et al., 2005; Fumal et al., 2006; Ozkul and Bozlar, 2002). However, it is often difficult to evaluate if these studies show that migraineurs lack habitation. For instance, several studies have not reported amplitude changes over blocks, but only a habituation summary-measure (i.e. block ratio or habituation slope) (Afra et al., 2000b; Fumal et al., 2006; Ozkul and Bozlar, 2002; Vigano et al., 2013; Wang et al., 1999). In these studies it is not possible for a reader to assess if the applied habituation measures are adequate and consistent.

Of the 182 VEP recordings of migraineurs in the present study, only 78 recordings (i.e. 2/5) occurred > 2 days before and after a migraine attack. This is not surprising since we included migraineurs with 2-6 attacks per months, and by definition 2 preictal days, 2 postictal days and at least 1 ictal day for each attack. Cross-sectional migraine studies often include migraineurs that have on average 2-3

attacks per month, and define the interictal period as > 2 or > 3 days before and after the nearest migraine attack (Coppola et al., 2010a; Di Clemente et al., 2005). Yet, most studies have not reported exclusions of preictal, ictal or postictal recordings, or how these recordings were handled (Afra et al., 1998; Afra et al., 2000b; Bohotin et al., 2002; Coppola et al., 2007b; Coppola et al., 2011; Coppola et al., 2010b; Coppola et al., 2010a; Coppola et al., 2013b; Di Clemente et al., 2005; Fumal et al., 2006; Ozkul and Bozlar, 2002; Schoenen et al., 1995; Vigano et al., 2013). Systematic exclusion of subjects may influence study results and it is therefore unfortunate that most studies do not disclose sufficient details regarding exclusions.

Disease duration (headache-history), migraine-frequency and usual headache duration were similar in the present study and in several studies that reported lack of habituation in migraine (Afra et al., 2000b; Bohotin et al., 2002; Coppola et al., 2010b; Coppola et al., 2010a; Ozkul and Bozlar, 2002). It is therefore unlikely that the migraineurs in those studies had a more severe disorder and therefore more pronounced VEP alterations.

When comparing measurements in subjects with and without migraine it is important that they do not differ considerably in other aspects. Some studies had considerable differences in age and gender distribution between migraine and control groups (e.g. Afra et al., 1998; Bohotin et al., 2002; Di Clemente et al., 2005). In studies that reported lack of habituation the migraineurs were outpatients from specialized headache clinics while the controls were hospital employees, laboratory staff, medical students and/or health care professionals (Afra et al., 1998; Afra et al., 2000b; Bednar et al., 2014; Bohotin et al., 2002; Coppola et al., 2011; Coppola et al., 2013b; Ozkul and Bozlar, 2002; Schoenen et al., 1995; Wang et al., 1999). VEPs may be influenced by mood (Joost et al., 1992), attention (Torriente et al., 1999), fatigue (Kremlacek et al., 2007), and how the subjects concentrates (Di Russo and Spinelli, 2002) and focus (Hoshiyama and Kakigi, 2001) on the visual stimuli. These factors may differ in outpatients and for instance staff members. In addition, outpatients treated in headache speciality clinics may have more comorbidity, which could influence the results because lack of habituation also has been reported in other conditions, including chronic back pain (Flor et al., 2004) and tinnitus (Walpurger et al., 2003).

4.2 Visual evoked potential amplitude in migraine

Several review articles state that migraineurs have a tendency to have lower first block VEP amplitude (Brighina et al., 2009; Coppola et al., 2009; Magis et al., 2013). As far as we know, only one study has reported lower first block amplitude in migraineurs (Afra et al., 1998). Two studies have reported increased first block amplitude in migraineurs (Oelkers et al., 1999; Sand et al., 2009), while

most studies, including the present study, found no significant differences (Afra et al., 2000a; Coppola et al., 2007b; Coppola et al., 2011; Coppola et al., 2010b; Coppola et al., 2010a; Coppola et al., 2013b; Di Clemente et al., 2005; Oelkers-Ax et al., 2005; Ozkul and Bozlar, 2002; Sand and Vingen, 2000; Schoenen et al., 1995; Vigano et al., 2013; Wang et al., 1999).

Many VEP studies measured average (or global) VEP amplitude and not only how amplitude changes during the stimulation. Several studies reported increased VEP amplitude in migraineurs (Diener et al., 1989; Khalil et al., 2000; Oelkers et al., 1999; Sand et al., 2008; Shibata et al., 1997, 1998), while other studies could not confirm this (Coppola et al., 2007b; Kennard et al., 1978; Mariani et al., 1988; Oelkers-Ax et al., 2005; Polich et al., 1986; Sand and Vingen, 2000; Sener et al., 1997). In other words, the results of VEP measurements in migraine are contradictory in general, and not just regarding habituation.

4.3. Progressive amplitude changes during recoding of VEP

Both habituation slope and the repeated measures ANOVA indicated that amplitude declined during the VEP recording. These measures take data from all blocks into account, and are therefore more reliable than comparing only the first and the last block.

This study was designed to measure VEP habituation using a similar protocol to that of contemporary migraine studies. The amplitude reduction during stimulation was therefore interpreted to reflect habituation. This interpretation is in line with numerous evoked potential studies that have applied similar protocols (e.g. Afra et al., 1998; Ambrosini et al., 2003; Brodsky et al., 2013; Chen et al., 2009; Coppola et al., 2013b; Ozkul and Uckardes, 2002; Sand et al., 2008; Schoenen et al., 1995; Siniatchkin et al., 2006; Valeriani et al., 2003). The present study did not aim to investigate if the amplitude decline during VEP stimulation is caused by habituation in the central nervous system. To investigate this further, pattern electroretinography (Holder, 2001) could be measured simultaneously with pattern-reversal VEP. This setup could determine the site of amplitude reduction.

The underlying mechanism of habituation is not completely understood (de Tommaso et al., 2014) and the amplitude reduction during VEP stimulation may not only be caused by habituation. For instance, in some studies the amplitude reduction is considered a result of sensory adaptation (Heinrich and Bach, 2001; Heinrich and Bach, 2002). The relative effect of sensory adaptation and habituation cannot be separated with the methods commonly applied in migraine habituation studies (Omland et al., 2011). It is also likely that changes in concentration could influence VEP

amplitudes, although VEP amplitude decrement during stimulation unrelated to lack of attention and alertness have been reported (Skuse and Burke, 1992).

Reduced accommodation could cause lower amplitude in the later blocks. However, reduced accommodation should also cause increased latency (Bartel and Vos, 1994). As P100 peak latency was stable from the 1st to the 6th block, it is therefore unlikely that amplitude decrement can be explained by changes in accommodation during the stimulation. It is unlikely that changes in accommodation and concentration during the recording of VEP should affect persons with and without migraine differently in a blinded study. Therefore these factors should not affect the main finding of the present study, i.e. that VEPs are not different in persons with and without migraine.

4.4 Strengths and limitation

A major strength of the present study is the blinded design, although the investigators were blinded for diagnosis only on the first visit. On later visits they were blinded according to the nearest migraine attack. This was a necessary compromise because of the longitudinal study design.

Since migraine is hereditary, some authors argue that subjects with first-degree relatives with migraine should not be included in the control group in VEP habituation studies (Coppola et al., 2013a). As far as we know there are no published data showing that first-degree relatives of migraineurs have altered VEP habituation compared to other controls. These subjects (n = 4) were therefore not excluded from primary analysis of the present study (removing these subjects did not affect the results).

4.5 Conclusion

We could neither reproduce lack of VEP habituation in migraine, nor find differences in VEP amplitude between migraineurs and controls. As discussed above and elsewhere (Omland et al., 2013; Sand, 2014), there are several methodological issues with previous habituation studies, and the weight of available evidence suggests that lack of VEP habituation is not a reliable "neurophysiological hallmark" in interictal migraine. It is possible that small relative differences in VEP habituation between controls and migraineurs do exist, but the clinical relevance of any such differences is uncertain. As described in a recent review by de Tommaso et al (2014), impaired habituation has been shown for other sensory modalities than VEP. Lack of habituation may therefore still be a potential biomarker in migraine with other sensory modalities.

Strict focus on methodology, including recruitment of similar subject groups and description of handling and exclusion of all participants, is important in future studies to avoid overestimation of effect sizes. Most importantly, this also includes a study design with blinding both during the recording and assessment of VEP and other potential neurophysiological markers of the pathophysiology of the migraine disorder.

Conflict of Interest

None of the authors have potential conflicts of interest to be disclosed.

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Figure legends

Figure 1: N70-P100 habituation slopes (mean) and block amplitudes (mean ± SD). Neither N70-P100 habituation nor amplitudes were significantly different in controls and interictal migraineurs (p = 0.33 (independent samples Student's t-test) and p = 0.71 (repeated measures ANOVA group factor) respectively).

Figure 2: P100-N145 habituation slopes (mean) and block amplitudes (mean ± SD). Neither P100-N145 habituation nor amplitude were significantly different in controls and interictal migraineurs (p = 0.80 (independent samples Student's t-test) and p = 0.56 (repeated measures ANOVA group factor) respectively).

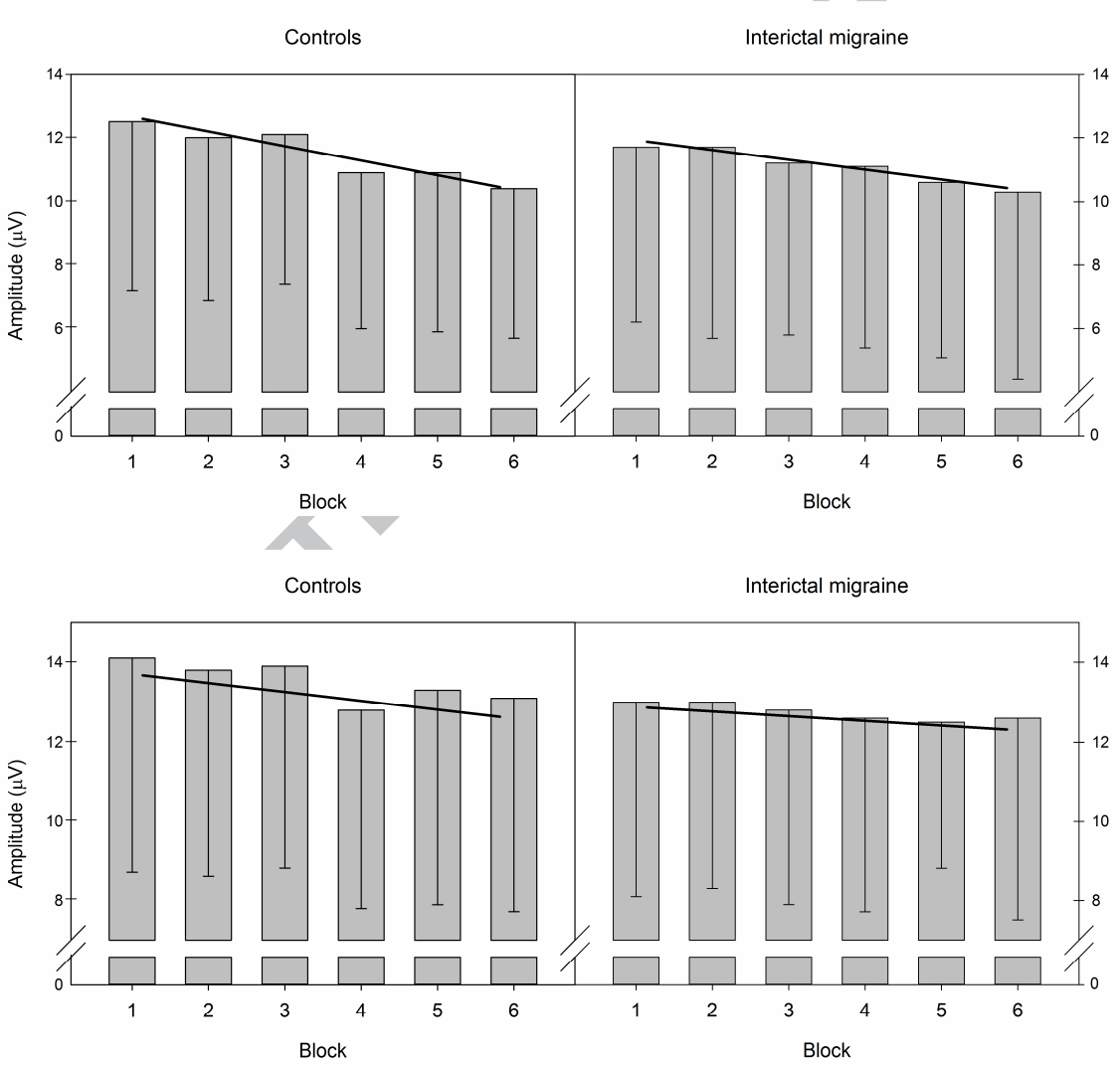

Table 1. Demographic and clinical data for controls and migraineurs included in analysis (Mean ± SD or no).

	Controls	Migraineurs
Total number of subjects	31	50
Exclusions	1	1
Subjects included in analysis	30	41[1]
Age (mean ± SD)	37.8 ± 11.2	38.5 ± 9.6
Age (range)	21-59	19-56
Women/Men	25/5	36/5
Days since start of last menstrual period	16.9 ± 6.7	16.4 ± 9.3
Visual acuity	1.1 ± 0.2	1.1 ± 0.1
Migraine without aura (ICDH-II)	NA	24
Migraine without and with aura (ICDH-II)	NA	15
Migraine with aura (ICDH-II)	NA	2
Headache-history (years)[2]	NA	20.1 ± 9.6
Migraine-frequency (1-4)[3]	NA	1.8 ± 0.6
Migraine-intensity (1-4)[4]	NA	2.6 ± 0.6
Usual headache-attack duration (hours)	NA	15.1 ± 19.7

Demographical and clinical data for subject with valid recordings
[1]Recordings from migraineurs in the interictal period (> 2 days before and after nearest migraine attacks).
[2]Headache-history: Years since first appearance of headache.
[3]Migraine-frequency: Number of headache days/month, 0: < 1. 1: 1-3. 2: 4-7. 3: 8-14. 4: > 14.
[4]Migraine-intensity: 1: Mild, 2: Moderate, 3: Severe and 4: Extreme.
NA: Not applicable.
ICDH-II: 2nd edition of the international classification of headache disorders.

Table 2. N70-P100 and P100-N145 habituation slopes and block ratios in interictal migraineurs and controls.

	Controls (n = 30)	Interictal migraine (n=41)	P-value[1]
N70-P100			
Habituation slope[2]	-0.43 ± 0.54	-0.29 ± 0.35	0.33
Block 6/Block 1 ratio[3]	0.91 (0.78, 1.07)	0.92 (0.81, 1.11)	0.46
P100-N145			
Habituation slope[2] (mean ± SD)	-0.21 ± 0.75	-0.11 ± 0.36	0.80
Block 6/Block 1 ratio[3]	0.92 (0.85, 1.09)	0.98 (0.87, 1.11)	0.56

[1]Independent samples Student's t-test comparing habituation slopes in controls and interictal migraine.

[2]Habituation slopes (mean ± SD) calculated by least squares method from block amplitudes in each recording.

[3]Block ratio mean with lower (16%) and upper (84%) percentile limits in parenthesis (calculated from the logarithm of the ratio ± SD and retransformed to the ratio scale).

Table 3. Repeated measures ANOVA F-values in models with amplitude as dependent variable, block as within-subject factor and group (controls, interictal migraine) as between-subject factor.

Comparison	Block	Block x group	Group
N70-P100	16.3**	1.193	0.143
df[1]	4.1	4.1	1
P100-N145	3.1*	0.421	0.332
df[1]	4.0	4.0	1

[1]Huynh–Feldt correction was applied.
* p = 0.017. ** p <0.001

Table 4. N70-P100 and P100-N145 peak-to-peak amplitudes in µV (mean ± SD) for the first and the last block in controls and interictal migraine: post-hoc contrasts.

N70-P100	Controls (n = 30)	Interictal migraine (n=41)
Block 1[1]	12.5 ± 5.3	11.7 ± 5.5
Block 6[2]	10.4 ± 4.7	10.3 ± 5.9
Overall amplitude	11.2 ± 4.7	10.8 ± 5.5
P100-N145		
Block 1[1]	14.1 ± 5.4	13.0 ± 4.9
Block 6[3]	13.1 ± 5.4	12.6 ± 5.1
Overall amplitude	13.2 ± 4.9	12.3 ± 4.7

Statistics for the complete block 1-6 data set is summarized in Table 3. [1]Block 1 ampitude was not significantly different in controls and interictal migraineurs (Independet samples t-test ($p > 0.46$). [2] A post hoc paired t-test showed significant lower N70-P100 amplitude in block 6 compared to block 1 ($p < 0.001$). [3] A trend towards lower P100-N145 amplitude in block 6 compared to block 1 ($p = 0.08$). Block x group interactions were non-significant, as shown in Table 3.