

# Impaired short-term visual paired associative plasticity in patients with migraine between attacks

Chiara Abagnale<sup>a</sup>, Federico Ranieri<sup>b</sup>, Antonio Di Renzo<sup>c</sup>, Vincenzo Parisi<sup>c</sup>, Mariano Serrao<sup>a</sup>, Vincenzo Di Lazzaro<sup>d</sup>, Marco Lisicki<sup>e</sup>, Gianluca Coppola<sup>a,\*</sup>, Francesco Pierelli<sup>a,f</sup>

## Abstract

A common experimental neurophysiological method to study synaptic plasticity is pairing activity of somatosensory afferents and motor cortical circuits, so-called paired associative stimulation (PAS). Dysfunctional inhibitory and excitatory PAS mechanisms within the sensorimotor system were described in patients with migraine without aura (MO) between attacks. We have recently observed that the same bidirectional PAS rules also apply to the visual system. Here, we have tested whether dysfunctioning associative plasticity might characterize the visual system of patients with MO. In 14 patients with MO between attacks and in 15 healthy volunteers, we performed a previously validated visual PAS (vPAS) protocol by coupling 90 black-and-white checkerboard reversals with low-frequency transcranial magnetic stimulation pulses over the occipital cortex at 2 interstimulus intervals of  $-25/+25$  ms around the visual-evoked potential (VEP) P1 latency. We recorded VEPs (600 sweeps) before, immediately after, and 10 min after each vPAS session. We analysed VEP N1-P1 amplitude and delayed habituation. Although vPAS-25 significantly enhanced and vPAS + 25 reduced VEP amplitude habituation in healthy volunteers, the same protocols did not significantly change VEP amplitude habituation in MO between attacks. We provide evidence for lack of habituation enhancing and habituation suppressing visual PAS mechanisms within the visual system in interictal migraine. This finding, in combination with those previously obtained studying the sensorimotor system, leads us to argue that migraine disease-related dysrhythmic thalamocortical activity prevents the occurrence of physiological bidirectional synaptic plasticity induced by vPAS.

**Keywords:** Visual system, Depression, Potentiation, Associative learning, Habituation

## 1. Introduction

Changes in the cortical sensory information processing have been observed in migraine predominantly through the use of cortical-evoked potentials, in the form of habituation deficits,<sup>12</sup> or by recording peripheral responses to cortical neuromodulating magnetic stimuli, in the form of paradoxical amplitude response.<sup>18,19</sup> All these findings seem to vary greatly according to the migraine cycle, being evident mostly between, and not during, the attacks.<sup>8,13,18,20</sup>

Overall, these rapid variations of the neurophysiological state of the migraineur's brain are indicative of altered mechanisms of synaptic plasticity and are probably part of the pathophysiological mechanisms of recurrence of migraine.

One of the experimental ways to test the integrity of synaptic plasticity of cortical circuits is to pair a peripheral sensory stimulus

with a low-frequency transcranial magnetic stimulation (TMS) stimulus delivered over the contralateral cortical representation of the hand. Consistently with the results obtained on cortical preparations in animal studies,<sup>1,2</sup> in healthy humans, paired associative stimulation (PAS) applied with an interstimulus interval (ISI) shorter than that required for the initial peripheral somatosensory stimulus to reach the cortex induces long-term depression (LTD) mechanisms, while at longer ISIs, it induces long-term potentiation (LTP) mechanisms.<sup>40,45</sup> Evidence of dysfunctional associative bidirectional synaptic plasticity of the sensorimotor system has been observed in migraine between attacks.<sup>34</sup>

Recently, we have proved the possibility to induce the same two-way inhibitory/excitatory paired associative synaptic plasticity in the visual system by analysing visual-evoked potential (VEP) amplitude and habituation as test response.<sup>36</sup> We coupled peripheral visual stimuli with TMS applied to the primary visual cortex (V1) using various ISIs from  $-50$  to  $+50$  ms added to the individual P1 VEP latency value and, more consistently, found that habituation across 6 blocks of 100 sweeps increased after visual PAS(vPAS)-25, but disappeared after vPAS + 25.<sup>36</sup>

By using the vPAS protocol, here, we aimed to test whether the dysfunctions of associative synaptic plasticity can characterize the visual system of subjects with episodic migraine without aura during the interictal period.

## 2. Methods

### 2.1. Participants

A group of 14 patients with migraine without aura was recruited and underwent VEP recordings during the interictal period, ie,

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

<sup>a</sup> Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome Polo Pontino, Latina, Italy, <sup>b</sup> Unit of Neurology, Department of Neuroscience, Biomedicine, and Movement Sciences, University of Verona, Verona, Italy, <sup>c</sup> IRCCS Fondazione Bietti, Rome, Italy, <sup>d</sup> Unit of Neurology, Neurophysiology, and Neurobiology, Department of Medicine, Campus Bio-Medico University, Rome, Italy, <sup>e</sup> Headache Department, Neuroscience Unit, Conci Carpinella Institute, Córdoba, Argentina, <sup>f</sup> Headache Clinic, IRCCS Neuromed, Pozzilli (IS), Italy

\*Corresponding author. Address: Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Polo Pontino, Corso della Repubblica 79, 04100 Latina, Italy. Tel.: +39 0773 6513337; fax: +39 0773 651230. E-mail address: gianluca.coppola@uniroma1.it (G. Coppola).

PAIN 162 (2021) 803–810

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<http://dx.doi.org/10.1097/j.pain.0000000000002085>

being at least 3 days after the last migraine attack and 3 days before the subsequent attack. The inclusion criteria were absence of any other primary or secondary headache and any personal or family history of psychiatric or neurological disorders other than migraine (eg, chronic sleep deprivation, systemic hypertension, diabetes, other metabolic disorders, and autoimmune diseases) (Table 1). A group of 15 healthy volunteers (HVs) was also recruited. The data in this study are part of a larger study of which the results of the neurophysiological recordings from the HVs appear elsewhere.<sup>36</sup> The inclusion criteria were absence of personal or family history of migraine or other types of primary headaches or any other overt medical condition.

All participants received a full study description and signed informed consent. The project was approved by the Ethics Committee of the Sapienza University of Rome and was performed in accordance with the Declaration of Helsinki and of the World Medical Association. Participants taking daily therapy, except for the estrogen-progestin, were excluded from the study, as were those who had visual acuity < 9/10. To avoid hormonal interference in female participants, we managed to record participants during the midmenstrual cycle.

## 2.2. Visual-evoked potentials

We recorded VEPs according to methods described elsewhere.<sup>36</sup> In brief, we used a full-field checkerboard visual pattern (contrast 80%, mean luminance 200 cd/m<sup>2</sup>, and 3.1/second reversal rate) generated on a screen, with the viewing distance of 114 cm (single check edges subtended a 15° angle). The visual stimulation was monocular (right eye), with the contralateral eye covered by a patch.

Visual-evoked potentials were acquired from the scalp through Ag-AgCl cup electrodes, using the following positions: Oz (active electrode), Fz (reference electrode) locations of the 10/20 EEG International System, and a ground electrode placed on the right forearm.

For each participant, six-hundred consecutive trials of 300-ms duration (sampling rate of 4000 Hz) were collected. All acquired traces were low-pass 100 Hz filtered and analysed off-line. Signal artefact rejection tool automatically rejected artefacts if the signal amplitude exceeded 200 μV; the rejection rate was below 5%. After having corrected the signal offline for DC drift, trials were partitioned into 6 sequential averaged blocks. Thereafter, we

**Table 1**  
Demographic data of study participants and headache profile of patients.

|                                  | HV (n = 15) | MO (n = 14) |
|----------------------------------|-------------|-------------|
| Women (n)                        | 9           | 9           |
| Age (y)                          | 28.9 ± 5.8  | 31.6 ± 9.6  |
| Duration of migraine history (y) |             | 18.0 ± 12.7 |
| Migraine attacks/month (n)       |             | 2.1 ± 1.9   |
| Intensity of headache (0-10)     |             | 6.8 ± 0.8   |
| Nausea/vomiting (n)              |             | 11          |
| Photophobia (n)                  |             | 14          |
| Phonophobia (n)                  |             | 14          |
| Pulsating (n)                    |             | 14          |
| Resting motor threshold (%)      | 58.5 ± 8.3  | 60.3 ± 9.7  |

The data are expressed as mean ± SD.  
HV, healthy volunteer; MO, migraine without aura.

identified the 3 prominent VEP latencies: N1, identified as the negative peak at approximately 75 ms, P1 as the positive peak after N1 at approximately 100 ms, and N2 as the negative peak after P1 at approximately 135 ms.<sup>32</sup> We measured the N1-P1 and P1-N2 peak-to-peak amplitudes that we used to calculate the habituation as the slope of the linear regression line for the 6 blocks using the Microsoft Excel function SLOPE (amplitude values and block numbers), based on the following equation:  $slope = \sum(a - A)(b - B) / \sum(b - B)^2$  (with  $a$ : amplitude values;  $A$ : average of  $a$  values;  $b$ : block numbers; and  $B$ : average of  $b$  values).

## 2.3. Transcranial magnetic stimulation

Transcranial magnetic stimulation was delivered through a MagStim rapid stimulator (The Magstim Company Ltd, Whitland, South West Wales, United Kingdom), which is able to generate a monophasic magnetic pulse with a maximal stimulator output (MSO) of 1.2 T. The intensity of the stimulation was then expressed as percentage of the MSO. The MagStim apparatus was connected to a figure-of-eight coil (9 cm of external diameter). Since some participants do not perceive phosphenes to TMS delivered over the visual area, even at the MSO,<sup>5</sup> we decided to adjust the stimulation intensity to 120% of the individual resting motor threshold (RMT).<sup>36</sup> For the determination of the RMT, we used the same procedure described by Di Lazzaro et al. in the 2009<sup>26</sup>. In brief, we delivered single TMS pulses over the left motor cortex searching for the hot spot of the first dorsal interosseous muscle of the hand. Thereafter, we placed the center of the TMS coil over the Oz position, ie, over V1, in a vertical orientation (its handle pointing upward), using a stimulation intensity of 120% RMT.<sup>3,22</sup> A previous study has observed how this particular orientation of the coil is capable of generating a posterior-to-anterior-induced current across the interhemispheric fissure.<sup>36</sup>

## 2.4. Visual paired associative stimulation

Here, we adopted a vPAS protocol already validated elsewhere in healthy controls.<sup>36</sup>

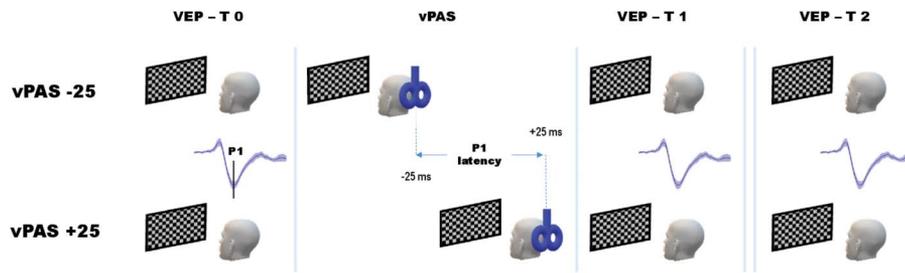
In brief, the vPAS protocol consists of 90 black-and-white checkerboard reversals, each followed by a TMS pulse over the V1 delivered at a frequency of 0.2 Hz. On the basis of our previous methodological article,<sup>36</sup> we a priori chose 2 interstimulus intervals between checkerboard reversal and TMS: the latency of P1 peak minus 25 ms (vPAS-25) and the latency of P1 peak plus 25 ms (vPAS + 25) (Fig. 1). We have chosen the latency value P1 because it is the prominent peak that shows relatively little variation between subjects, minimal interocular within-subject differences, and minimal variations with the repetition of the measurement over time.<sup>32</sup>

We analysed VEP N1-P1 and P1-N2 amplitudes and habituation before (T0), immediately after (T1), and 10 minutes (T2) after each vPAS procedure. For each participant, the 2 vPAS sessions (vPAS-25 and vPAS + 25) were performed in random order at ≥ 1-week intervals.

All recordings were performed in the afternoon (between 14:00 and 18:00).

## 2.5. Statistical analysis

All recordings were analysed offline by a single investigator who was blinded for the diagnosis, but not blind to the order of the blocks. Data were analysed using Statistica for Windows v8.0 (StatSoft Inc, Tulsa, OK). Sample size calculations were based on our previous



**Figure 1.** The experimental design of the study protocol was schematically represented. For each participant, the vPAS protocol consists of 90 black-and-white checkerboard, each followed by a TMS pulse over the V1 delivered at a frequency of 0.2 Hz. We a priori chose 2 interstimulus intervals between checkerboard reversal and TMS: the latency of P1 peak minus 25 ms (vPAS - 25) and the latency of P1 peak plus 25 ms (vPAS + 25). We analysed VEP N1-P1 and P1-N2 amplitudes and habituation before (T0), immediately after (T1), and 10 minutes (T2) after each vPAS procedure, performed in random order at ≥ 1-week intervals. vPAS, visual paired associative stimulation.

study in HVs with the same protocol: with a desired power of 0.80 and an alpha error of 0.05; 13 per group subjects (standardized effect size of 0.8559) are needed to disclose a significant difference in the habituation slope between 2 dependent VEP slopes (T0 vs T1).

A Kolmogorov–Smirnov test confirmed the Gaussian distribution for latencies and amplitudes of each VEP component. Repeated-measures analysis of variance (RM-ANOVA) was performed to analyse the effects on the amplitude of N1-P1 and of P1-N2 VEP components and on the slope of the linear regression line of amplitudes over the 6 blocks of 100 averaged traces, with “time” (3 levels: T0, T1, and T2) and “condition” (2 levels: vPAS - 25 and vPAS + 25) as independent variables. We verified the assumption of sphericity by using the Mauchly sphericity test and, in the case of violation, Greenhouse–Geisser (G-G) epsilon ( $\epsilon$ ) adjustment was used. In RM-ANOVA, the effect size was quantified using partial eta-squared (partial  $\eta^2$ ). Post hoc analysis was performed using the Dunnett test with baseline (T0) values as the control group.

The Pearson correlation test was used to search for correlations between first N1-P1 and P1-N2 VEP amplitudes and their VEP amplitude slopes and clinical features of migraine (duration of migraine history [years], mean monthly attack frequency [n], mean monthly attack duration [hours], number of days since the last migraine attack [n], severity of migraine headache on a 0 to 10 visual analogue scale [n]). *P* values of less than 0.05 were considered statistically significant.

### 3. Results

#### 3.1. Basic visual-evoked potential parameters

Assessable VEP recordings were acquired from all participants, with none of them reporting adverse events related to the TMS procedure.

The RM-ANOVA models using VEP N1, P1, or N2 latencies calculated on the first block as dependent variables did not show any significant effect for variable “group,” “time,” “condition,” and for their interaction (“group” × “time” × “condition”).

#### 3.2. Effects of visual paired associative stimulation on visual-evoked potential parameters

Visual-evoked potential amplitudes and slopes in the different experimental conditions are reported in **Table 2** and **3** and in **Figures 2** and **3**. Representative recordings of VEP habituation at T0, T1, and T2 after vPAS - 25 and vPAS + 25 in an HV and an MO are shown in **Figures 4** and **5**, respectively.

The RM-ANOVA model using first block N1-P1 VEP amplitude as dependent variable showed significant effect for the variable “group” ( $F_{1,54} = 22.747, P < 0.0001$ ), “time” ( $F_{2,108} = 7.363, P = 0.001$ ), and for the “group” × “time” ( $F_{2,108} = 3.562, P = 0.0318$ ) interaction, but not for the “group” × “time” × “condition” interaction effect ( $F_{2,108} = 2.438, P = 0.092$ ).

The RM-ANOVA model using first block P1-N2 VEP amplitude block as dependent variable showed significant effect for the variable “group” ( $F_{1,54} = 33.899, P < 0.0001$ ), but not for the “time” ( $F_{2,108} = 0.28, P = 0.756$ ), “group” × “time” ( $F_{2,108} = 1.25, P = 0.291$ ), or for the “group” × “time” × “condition” interaction effects ( $F_{2,108} = 0.22, P = 0.799$ ).

Subsequently, we analysed the behaviour of the VEP amplitudes along the 6 blocks of 100 responses to study the repetition effect, ie, the habituation.

The RM-ANOVA model using the N1-P1 VEP amplitude slope as dependent variable showed significant effect for the variable “group” ( $F_{1,54} = 13.367, P = 0.0005$ ) and for the “time” × “condition” ( $F_{2,108} = 9.446, P = 0.00017$ ), the “time” × “group”

**Table 2**  
**N1-P1 VEP component amplitude (μV) and habituation slope of healthy volunteers (HV) and of patients with migraine without aura (MO) at different time points, in the different experimental conditions.**

|                     | HV (n = 15)  |             | MO (n = 14)  |              | HV (n = 15)  |              | MO (n = 14) |  |
|---------------------|--------------|-------------|--------------|--------------|--------------|--------------|-------------|--|
|                     | T0           |             | T1           |              | T1           |              | T2          |  |
| <b>vPAS - 25</b>    |              |             |              |              |              |              |             |  |
| 1st amplitude block | 9.5 ± 5.0    | 4.5 ± 1.5   | 9.0 ± 5.1    | 4.1 ± 1.6    | 9.1 ± 4.8    | 3.8 ± 1.5    |             |  |
| Habituation slope   | -0.19 ± 0.43 | 0.05 ± 0.22 | -0.46 ± 0.39 | -0.11 ± 0.14 | -0.18 ± 0.22 | 0.06 ± 0.24  |             |  |
| <b>vPAS + 25</b>    |              |             |              |              |              |              |             |  |
| 1st amplitude block | 9.9 ± 4.5    | 5.2 ± 2.1   | 7.7 ± 4.5    | 4.9 ± 1.7    | 9.1 ± 5.2    | 4.6 ± 2.3    |             |  |
| Habituation slope   | -0.20 ± 0.34 | 0.01 ± 0.15 | 0.15 ± 0.28  | -0.15 ± 0.16 | -0.37 ± 0.30 | 0.003 ± 0.37 |             |  |

Data are expressed as mean ± SD.  
vPAS, visual paired associative stimulation.

**Table 3**

**P1-N2 VEP component amplitude ( $\mu\text{V}$ ) and habituation slope of healthy volunteers (HV) and of patients with migraine without aura (MO) at different time points, in the different experimental conditions**

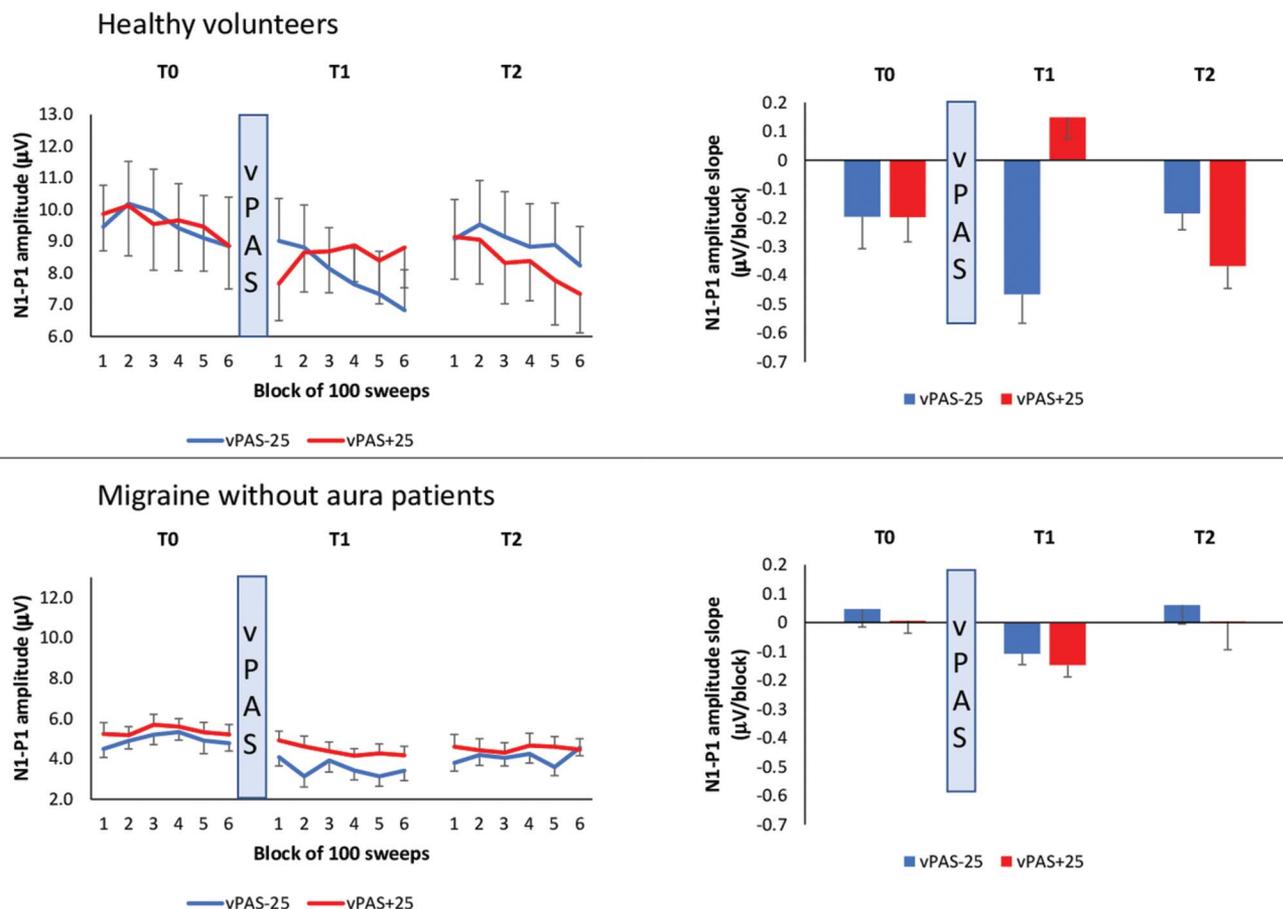
|                     | HV (n = 15)<br>T0 | MO (n = 14)<br>T0 | HV (n = 15)<br>T1 | MO (n = 14)<br>T1 | HV (n = 15)<br>T2 | MO (n = 14)<br>T2 |
|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| vPAS -25            |                   |                   |                   |                   |                   |                   |
| 1st amplitude block | 9.0 $\pm$ 4.0     | 3.9 $\pm$ 2.2     | 8.2 $\pm$ 4.1     | 3.8 $\pm$ 2.6     | 8.2 $\pm$ 4.0     | 3.9 $\pm$ 2.5     |
| Habituation slope   | -0.35 $\pm$ 0.47  | -0.03 $\pm$ 0.25  | -0.38 $\pm$ 0.47  | -0.12 $\pm$ 0.26  | -0.18 $\pm$ 0.59  | 0.02 $\pm$ 0.25   |
| vPAS +25            |                   |                   |                   |                   |                   |                   |
| 1st amplitude block | 9.6 $\pm$ 3.3     | 4.4 $\pm$ 3.9     | 8.6 $\pm$ 3.1     | 5.1 $\pm$ 2.4     | 8.5 $\pm$ 2.9     | 3.9 $\pm$ 2.9     |
| Habituation slope   | -0.44 $\pm$ 0.23  | -0.08 $\pm$ 0.28  | -0.21 $\pm$ 0.49  | -0.16 $\pm$ 0.20  | -0.25 $\pm$ 0.41  | 0.10 $\pm$ 0.31   |

Data are expressed as means  $\pm$  SD.

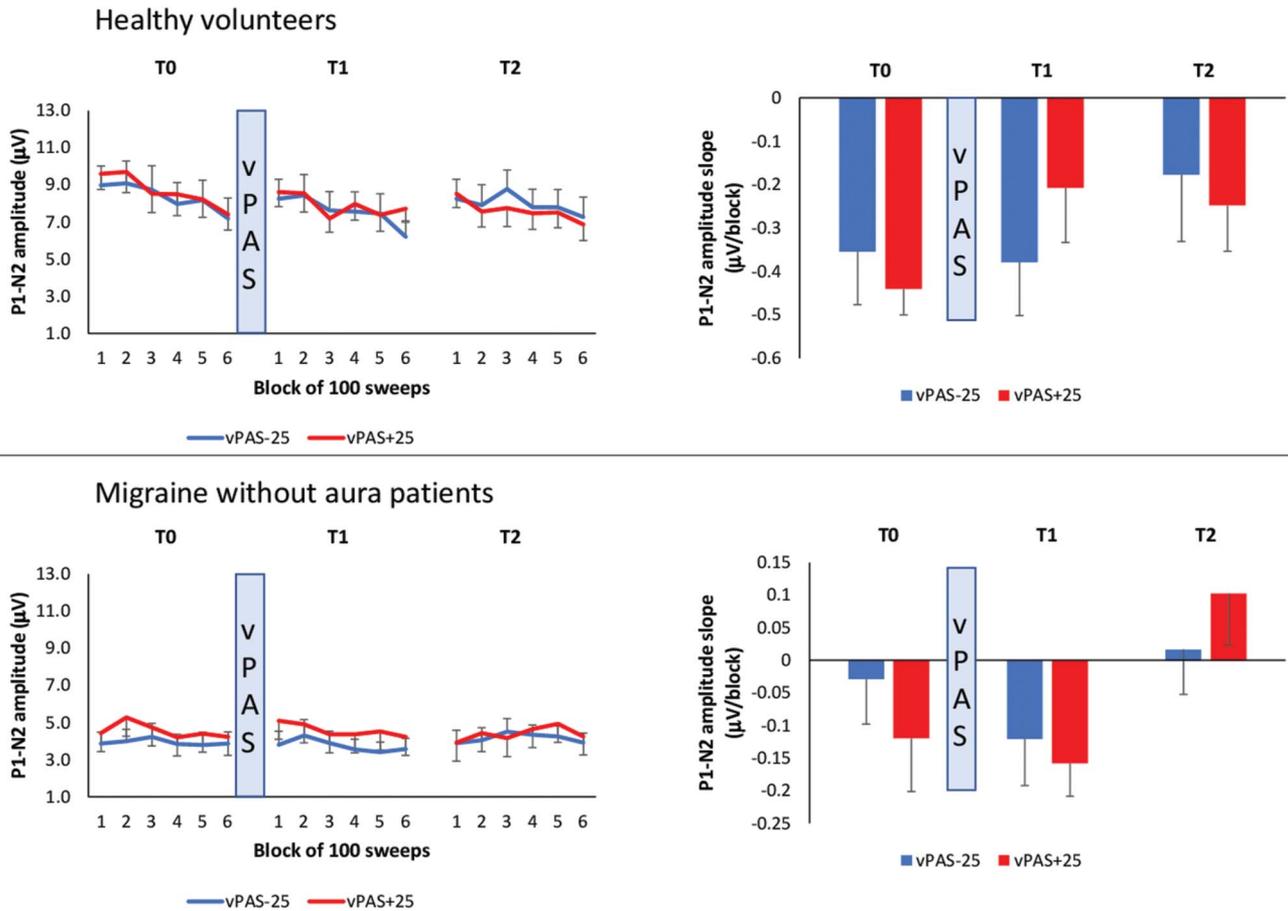
HV, healthy volunteers; vPAS, visual paired associative stimulation.

( $F_{2,108} = 4.269$ ,  $P = 0.016$ ), and the “time”  $\times$  “group”  $\times$  “condition” interaction effect ( $F_{2,108} = 8.936$ ,  $P = 0.00026$ ). The latter significant interaction was confirmed by the univariate RM-ANOVAs (Mauchly sphericity test:  $P = 0.699$ ;  $F_{2,108} = 8.93$ ,  $P = 0.0002$ , partial  $\eta^2 = 0.142$ ,  $op = 0.969$ ). Post hoc analysis revealed that, before both vPAS-25 and vPAS + 25 interventions (T0), N1-P1 VEP amplitudes linear trends were decremental, ie, habituated normally, in HVs (vPAS-25 =  $-0.196 \mu\text{V}/\text{block}$ ; vPAS + 25 =  $-0.197 \mu\text{V}/\text{block}$ ), while they were incremental, ie, lacked habituation, in patients with migraine (vPAS - 25 =  $+0.046 \mu\text{V}/\text{block}$ , vPAS + 25 =  $+0.014 \mu\text{V}/\text{block}$ ). After vPAS-25 intervention (T1), the N1-P1 VEP slope significantly increased

in HVs ( $-0.465$  vs  $-0.196 \mu\text{V}/\text{block}$ ,  $P = 0.046$ ), while the linear trend nonsignificantly changed from positive to negative in subjects with MO ( $-0.108$  vs  $+0.046 \mu\text{V}/\text{block}$ ,  $P = 0.1351$ ). After vPAS + 25 intervention (T1), the N1-P1 VEP slope significantly decreased in HVs ( $+0.148$  vs  $-0.197 \mu\text{V}/\text{block}$ ,  $P = 0.004$ ), while the linear trend nonsignificantly changed from positive to negative in subjects with MO ( $-0.147$  vs  $+0.014 \mu\text{V}/\text{block}$ ,  $P = 0.0927$ ). During the T2 recording session, the N1-P1 VEP habituation slope was not different from that at T0 in both HV (vPAS-25:  $-0.185 \mu\text{V}/\text{block}$ ,  $P = 1.0$ ; vPAS + 25:  $-0.366 \mu\text{V}/\text{block}$ ,  $P = 0.435$ ) and MO groups (vPAS-25:  $+0.060 \mu\text{V}/\text{block}$ ,  $P = 1.0$ ; vPAS + 25:  $+0.003 \mu\text{V}/\text{block}$ ,  $P = 1.0$ ).



**Figure 2.** Visual-evoked potential N1-P1 amplitudes over the 6 blocks of 100 responses (on the left) and the slope of the linear regression line of the 6 block amplitudes (on the right) during the 2 visual paired associative stimulation (vPAS) protocols (vPAS - 25 in blue and vPAS + 25 in red) in healthy volunteers (upper part) and patients with migraine without aura (lower part). Error bars indicate SEM.

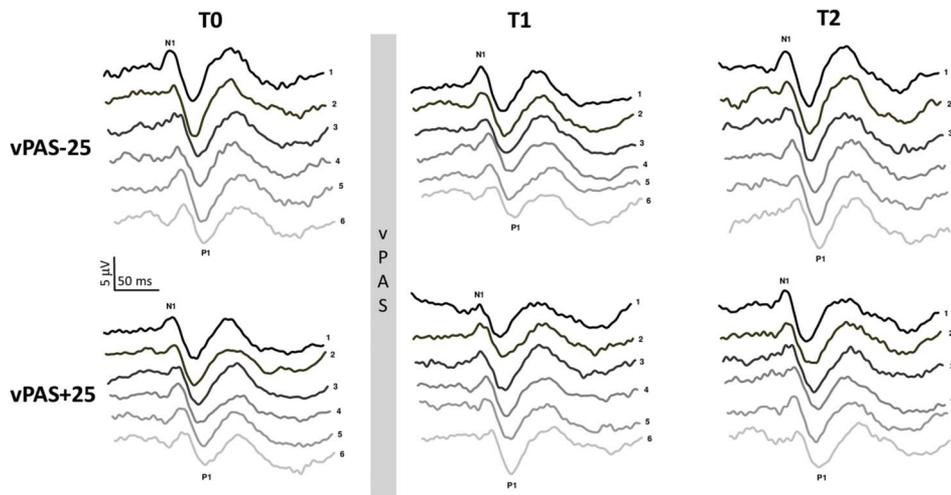


**Figure 3.** Visual-evoked potential P1-N2 amplitudes over the 6 blocks of 100 responses (on the left) and the slope of the linear regression line of the 6 block amplitudes (on the right) during the 2 visual paired associative stimulation (vPAS) protocols (vPAS – 25 in blue and vPAS + 25 in red) in healthy volunteers (upper part) and patients with migraine without aura (lower part). Error bars indicate SEM.

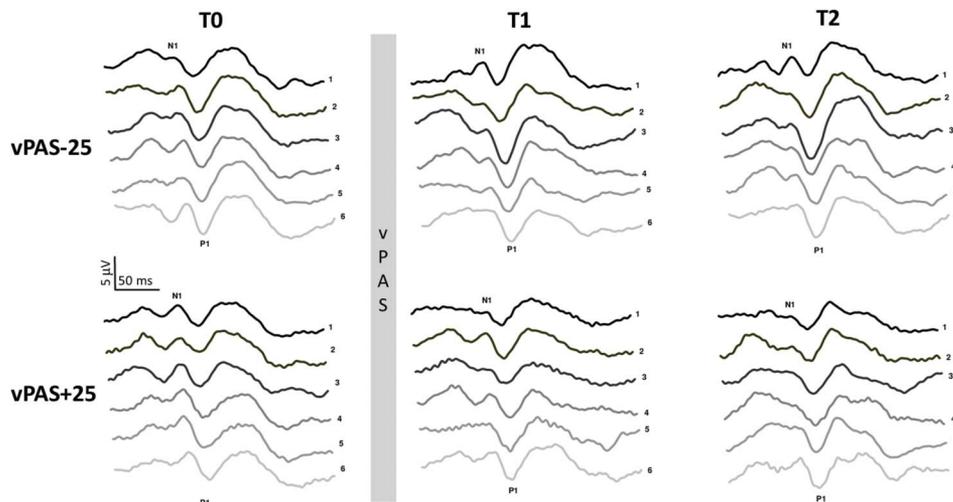
The RM-ANOVA model using the P1-N2 VEP amplitude as dependent variable showed significant effect for the variable “group” ( $F_{1,54} = 14.876, P = 0.0003$ ), and for the variable “time” ( $F_{2,108} = 3.506, P = 0.033$ ), but not for the “time” × “group” ( $F_{2,108} = 0.88, P$

$= 0.419$ ), and for the “time” × “group” × “condition” interaction effect ( $F_{2,108} = 0.97, P = 0.382$ ).

The Pearson correlation test in the patients’ group disclosed no correlation between the clinical features of migraine, the



**Figure 4.** Typical recordings of visual-evoked potential (VEP) habituation (6 blocks) at T0, T1, and T2 after visual paired associative stimulation (vPAS) –25 and vPAS + 25 in a healthy volunteer.



**Figure 5.** Typical recordings of visual-evoked potential (VEP) habituation (6 blocks) at T0, T1, and T2 after visual paired associative stimulation (vPAS) –25 and vPAS + 25 in a patient with migraine without aura.

first block VEP amplitudes, and the slopes of VEP amplitude linear trends.

#### 4. Discussion

Our data show, for the first time, that patients with migraine, during the pain-free phase, have altered mechanisms of associative synaptic plasticity within the visual system. In fact, in contrast to what we previously observed in healthy subjects, in our patients with migraine, we found that both habituation enhancing and habituation suppressing visual PAS has no effect on the basically deficient habituation process.

Following the Hebbian principles of synaptic plasticity,<sup>24</sup> PAS protocols were originally used to study the sensorimotor system by repetitive coupling of somatosensory peripheral stimulation with TMS of M1.<sup>26</sup> Modified PAS protocols were also used to study visuomotor integration, pairing visual with M1 stimulation,<sup>41</sup> and visuotactile integration, pairing observation of bodily tactile stimulations with S1 stimulation.<sup>46</sup>

Coherently with previous findings,<sup>36</sup> in our healthy subjects, we confirmed a bidirectional effect of vPAS protocol, pairing peripheral visual stimulation with TMS of V1, on VEP habituation: N1-P1 VEP habituation was enhanced by a vPAS paradigm in which the TMS pulse is delivered 25-ms previous V1 activation by peripheral visual input, while it is reduced by a vPAS in which the TMS pulse is delivered 25 ms after V1 activation. These vPAS-induced effects were short-lasting, observed only in the first 5 minutes after vPAS conditioning. We speculated that the neuromodulatory effects of both vPAS protocols on VEP act on the tendency of visual cortical neurons to easily run into phenomena of activity-dependent synaptic plasticity, ie, the learning phenomenon of habituation.<sup>36</sup> According to the findings obtained in experimental models, PAS is able to act through short- and long-term changes of the synaptic strength induced by activating presynaptic terminals while postsynaptic glutamatergic neurons are altered in its membrane polarization (hyperpolarized or depolarized).<sup>23,39</sup> The end product of these synaptic changes is the induction of LTD/LTP into excitatory intracortical glutamatergic synapses between cortical neurons.<sup>26,45</sup> However, we found that in our group of patients with migraine without aura, neither visual

PAS – 25 nor PAS+25 was able to significantly change the visual-evoked response habituation. And so, they have not been able to induce LTD- and LTP-like mechanisms.

Aberrant PAS mechanisms were previously detected in migraine. In fact, in a classical sensorimotor PAS paradigm, a lack of PAS-induced LTD and LTP effects during the interictal period of patients with migraine without aura was observed.<sup>34</sup> Therefore, present data, in association with those obtained previously with sensorimotor PAS, indicate a malfunction of the synaptic plasticity mechanisms at the cortical level between the attacks in migraineurs, which probably underlies a dysfunction in the synchronous regulation of glutamatergic synaptic excitement into the visual and sensorimotor cortices.<sup>42</sup>

Glutamate is known to be the most important excitatory neurotransmitter of thalamocortical processing.<sup>37</sup> Interestingly, in a subgroup of subjects, PAS-induced plastic modifications were negatively related to the degree of thalamocortical activation<sup>34</sup>; as such, we reasoned that malfunction in PAS-induced effects in migraine might suggest abnormal thalamic control of the cortical activation, preventing short-term and longer-term changes in cortical synaptic effectiveness. Animal models support this interpretation because it has been observed that the associative learning is influenced by changes in the sensitive afferents to the thalamus and in its connections with the brainstem nuclei.<sup>21,30</sup>

This information is relevant to our study because an increasing amount of evidence suggests the presence of an abnormal crosstalk between the thalamus and cortex in migraine, especially between attacks.<sup>17,35</sup> The oscillatory activity of the visual cortex,<sup>7,27</sup> that more directly reflect the degree of cortical activation in relation to thalamic control, is altered in migraine, and in correlation with the activation of peculiar inhibitory systems at the cortical level.<sup>8,13</sup> This general cortical thalamocortical network dysexcitability results in an increased cortical response to repeating stereotyped stimuli, ie, promotes an interictal habituation deficit of the VEPs, as confirmed by our present findings at T0. All this evidence suggests that migraine is part of the spectrum of thalamocortical dysrhythmia syndromes,<sup>28</sup> as also suggested by numerous studies of both functional and structural neuroimaging.<sup>15,16,29,38,43,44</sup>

## 5. Conclusions

Present data, taken together with the fact that other interventions acting through the modulation of thalamic activity such as experimentally induced visual deprivation<sup>10</sup> and tonic pain,<sup>11</sup> were also unable to modify VEP habituation, indicating that the migraine disease-related dysrhythmic thalamocortical activity disallows the occurrence of physiological bidirectional synaptic plasticity induced by vPAS.

It remains to verify whether these aberrant synaptic plasticity mechanisms in migraine, by reorganizing neural maps in the downstream cortical networks, might consequently alter all the modulatory processes at the basis of sensorial perceptions. Evidence obtained either from the animal model or from humans suggests that the information coming from the periphery is able to modulate the activity of specific sets of neurons of the trigeminovascular system and specific thalamic nuclei and is conveyed to multiple cortical areas that in turn can exert a feedback control, protective or compensatory, restraining subcortical feedforward afferent drives.<sup>6,25,31,33</sup> The chronic, probably genetically determined, dysfunction of short- and long-term learning mechanisms, such as those at the basis of the phenomenon of habituation, could counteract the normal balance of feedback and feedforward mechanisms between the subcortical and cortical structures restraining adaptation and protection from the overload of multisensory information.<sup>27</sup>

Malfunctioning in these physiologic learning mechanisms could lead to maladaptive changes, photophobia and phonophobia, sometimes presenting also interictally, compromised trigeminovascular functions, and consequently to changes in subjective perception of pain, which eventually include the occurrence of “central sensitization” process.<sup>14</sup>

Other studies are necessary to verify whether the same abnormalities are also present in patients with migraine with aura where dysfunctions in the mechanisms of habituation<sup>9</sup> and plasticity<sup>4</sup> are often more evident.

## Conflict of interest statement

The authors have no conflicts of interest to declare.

## Acknowledgements

The authors declare no competing interests related to the content of this manuscript. The contributing authors associated with the IRCCS—Fondazione Bietti were supported by the Italian Ministry of Health and the Fondazione Roma.

The data that support the findings of this study are available from the corresponding author, GC, upon reasonable request.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Article history:

Received 16 April 2020

Received in revised form 10 September 2020

Accepted 16 September 2020

Available online 16 September 2020

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