Ulg – UNIVERSITY OF LIEGE
Faculty of Medicine

Interrelations between the cerebral cortex and the trigeminal system: studies in healthy subjects and in migraine patients; therapeutic perspectives.

by
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Declaration of Authorship

I declare that this thesis and the all the work presented in it are my own and confirm that when I have quoted the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work. All rTMS and light-stimulation experiments, data analysis, statistics and graph have been done by myself, some parts of them with the collaboration of my co-workers Dr. Roberta Baschi and Dr. Anna Cosseddu. Signal scripts used were written jointly with Victor de Pasqua. StimLux building was performed with Gino Mancini. Prof. Jean Schoenen conceived the general topic of this thesis, helped discussing the results and revised the manuscript. Prof. Alain Maertens de Noordhout helped correcting the discussion of some results and the manuscript.

Contents

Acknowledgements

Abbreviations

Abstract

First Part: Background

1. Introduction
   1.1. History of migraine
   1.2. Clinical features of migraine
   1.3. Epidemiology of migraine

2. Migraine Pathophysiology
   2.1. Migraine aura
   2.2. Migraine headache
   2.3. Is there any link between CSD and migraine headache?
   2.4. Migraine generator
   2.5. Genetic predisposition
   2.6. Contribution of electroneurophysiology to migraine pathophysiology
      2.6.1. Generalities
      2.6.2. Differences in evoked potentials between migraineurs and healthy subjects
         2.6.2.1. Visual evoked potentials (VEP)
         2.6.2.2. Auditory evoked potentials (AEPs)
         2.6.2.3. Somatosensory evoked potentials (SSEP)
         2.6.2.4. Contingent negative variation (CNV)
         2.6.2.5. Nociceptive evoked activities: laser-evoked potentials (LEPs), nociceptive blink reflex (nBR) and contact-heat evoked potentials (CHEPs)
      2.6.3. Habituation and sensitization: the "dual process" theory
         2.6.3.1. Characteristics of habituation
         2.6.3.2. Characteristics of sensitization
         2.6.3.3. Habituation and sensitization in migraine
         2.6.3.4. Significance of the habituation deficit on migraine pathophysiology

3. Photophobia
   3.1. Mechanisms of photophobia in animal models
   3.2. Mechanisms of photophobia in humans

4. Migraine therapies
4.1. Pharmacotherapy
4.2. Neurostimulation
4.2.1. Peripheral nerve stimulation
4.2.2. Repetitive transcranial magnetic stimulation (rTMS)
4.2.3. Transcranial direct current stimulation (tDCS)

5. Aim of this thesis & hypotheses

Second part: Personal contribution

6. Subjects and methods
6.1. Subjects
6.2. Materials and methods
6.2.1. Spontaneous blink rate (SBR)
6.2.2. Nociceptive blink reflex (nBR)
6.2.3. Visual evoked potentials (VEP)
6.2.4. Contact-heat evoked potentials (CHEPs)
6.2.5. Repetitive transcranial magnetic stimulation (rTMS)
6.2.6. Transcutaneous Occipital Nerve Stimulation (t-ONS)
6.2.7. Flash light stimulation – Micromed
6.3. Data processing and statistical analysis

7. Variation of spontaneous blink rate (SBR) in light and dark in ictal and interictal episodic migraine patients compared to healthy subjects
7.1. Introduction
7.2. Subjects and methods
7.3. Results
7.4. Discussion

8. Effects of visual cortex modulation by repetitive transcranial magnetic stimulation on the nociceptive blink reflex in healthy subjects and in migraine patients
8.1. Introduction
8.2. Subjects and methods
a) rTMS studies in healthy subjects
b) Control studies in healthy subjects
c) rTMS studies in migraine patients
8.3. Results
a) rTMS studies in healthy subjects
b) Control studies in healthy subjects
c) rTMS studies in migraine patients

8.4. Discussion
a) Results in healthy subjects
b) Results in migraine patients

9. Effects of low or high frequency rTMS over the motor cortex on the nociceptive blink reflex in healthy subjects
9.1. Introduction
9.2. Subjects and methods
9.3. Results
9.4. Discussion

10. Effects of visual cortex activation by flash light stimulation on nociceptive blink reflex in healthy subjects and migraine patients
10.1. Introduction
10.2. Subjects and methods
10.3. Results
10.4. Discussion

11. Visually induced analgesia in healthy subjects and migraine patients
11.1. Introduction
11.2. Subjects and methods
11.3. Results
11.4. Discussion

12. StimLux: an alternative device to assess photophobia
12.1. Introduction
12.2. Why do we need a new stimulator?
12.3. Technical characteristics of the StimLux
12.4. StimLux Software
12.5. Assessment of photophobia using StimLux in healthy subjects and migraine patients.
12.5.1. Objective
12.5.2. Subjects and methods
12.5.3. Results
12.5.4. Discussion

13. Effects of frequency, colour and intensity of light stimulation on the nociceptive blink reflex in healthy subjects.
13.1. Introduction
13.2. Subjects and methods
13.2.1. *Part 1. Varying stimulation frequency*
13.2.2. *Part 2. Varying colour*
13.2.3. *Part 3. Varying intensity*
13.3. Results

13.3.1. *Part 1. Effects on trigeminal nociception of varying stimulation frequency*
13.3.2. *Part 2. Effects on trigeminal nociception of varying colour*
13.3.3. *Part 3. Effects on trigeminal nociception of varying intensity*
13.4. Discussion

14. Using the top-down inhibitory control of the visual cortex on trigeminal nociception to treat migraine: a proof-of-concept trial.
14.1. Introduction
14.2. Subjects and methods
14.3. Results
14.4. Discussion

Third part: Conclusions

15. General discussion and conclusion
15.1. Summary of results
15.2. Pathophysiological relevance
15.3. Perspectives

References

Annexes

Dubium sapientiae initium.
(René Descartes)

Imagination is more important than knowledge.
(Albert Einstein)

Imagination is innovation.
(Marcel Bazié)

Innovation distinguishes between a leader and a follower.
(Steve Jobs)

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The dream begins with a teacher who believes in you, who tugs and pushes and leads you to the next plateau, sometimes poking you with a sharp stick called 'truth'.

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Thank you all!

To my daughter Diane

Believe it's possible.
Sometimes it's just that simple.
Yet, some people will never truly believe.
Have faith. But just because it's possible,
doesn't mean it will be easy.
Know that whatever life you want, grades you want,
job you want, reputation you want,
friends you want…that it's possible.
Nothing is off limits.
Everything is in reach.
Anything can be.

(Shel Silverstein)

Abbreviations

- 5-HT: 5-hydroxytryptamine, serotonin
- AEPs: auditory evoked potentials
- AUC: area under the curve
- BOLD: Blood Oxygen Level-Dependent
- BR: blink reflex
- CBF: Cerebral blood flow
- CGRP: calcitonin gene relater peptide
- CHEPs: contact-heat evoked potentials
- CM: chronic migraine
- CNV: Contingent negative variation
- CSD: Cortical Spreading Depression
- CTTH: chronic tension-type headache
- dLGN: dorsal lateral geniculate nucleus
- DLT: dorsolateral thalamus
- DNIC: diffuse noxious inhibitory controls
- EEG: electroencephalogram
- EM: episodic migraine
- FHM: familial hemiplegic migraine
- fMRI: Functional magnetic resonance imaging
- HFOs: high frequency oscillations
- ICHD: International Classification of Headache Disorders
- IDAP: intensity dependence of auditory evoked potentials
- IHS: International Headache Society
- ipRGCs: intrinsic photoreceptive retinal ganglion cells
- LDLPFC: left dorsolateral prefrontal cortex
- LEPs: laser-evoked potentials
- LGN: lateral geniculate nucleus
- MA: migraine with aura
- MEP: motor evoked potentials
- MO: migraine without aura
- MOH: Medication overuse headache
- MT: motor threshold
- nBR: nociceptive blink reflex
- NRM: nucleus raphe magnus
- OPN: olivary pretectal nucleus
- PAG: periaqueductal grey matter
- PET: Positron Emission Tomography
- PhT: phosphene threshold
- PRF: the pontine reticular formation
- PR-VEP: pattern-reversal visual evoked potential
- PSP: progressive supranuclear palsy
- PWI: perfusion-weighted imaging
- REM: rapid eye movements
- rMS: repetitive magnetic stimulation
- rTMS: repetitive transcranial magnetic stimulation
- RVM: rostral ventromedial medulla
- SAD: seasonal affective disorder
- SBR: Spontaneous blink rate
- SN: Substantia nigra
- SP: Substance P
- SSEP: Somatosensory evoked potentials
- SSN: superior salivatory nucleus
- TCC: trigeminocervical complex
- TNC: trigeminal nucleus caudalis
- tDCS: Transcranial direct current stimulation
- tONS: Transcutaneous occipital nerve stimulation
- TRN: thalamic reticular nucleus
- tSNS: transcutaneous supraorbital nerve stimulator
- TTH: Tension-type Headache
- tVNS: Transcutaneous vagus nerve stimulation
- VC: visual cortex
- VEP: visual evoked potentials
- VIA: visually-induced analgesia
- vlPAG: ventrolateral periaqueductal grey
- YLDs: Years lived with Disability

Abstract

Understanding migraine pathophysiology is probably the most challenging point in migraine management, since an efficient acute and preventive treatment should rely on clear pathophysiological bases. Migraine is characterized interictally by a lack of habituation of evoked responses, possibly due to a decreased preactivation level of sensory cortices. By contrast, during an attack and in chronic migraine, the preactivation level increases and habituation normalizes. New neurostimulation techniques could be useful to durably modify the activation of the underlying cortex, decreasing the repetition of attacks, giving also insight on the pathophysiology of migraine.

The visual cortex plays a pivotal role in migraine pathophysiology, but its effect on the trigeminal nociceptive system remains poorly understood. On the other hand migraine attack is often associated to photophobia, but the pathophysiological relation between headache and the discomfort to the light, during the ictal but also the interictal phase, is unclear.

This thesis puts a new insight into the relation between the visual cortex modulation and the response of the trigeminal nociceptive system, showing a possible inhibitory functional interrelation between these structures, via thalamic modulation.

The hypothesis is based on our first finding investigating the modulation of the visual cortex by the repetitive transcranial magnetic stimulation on the nociceptive blink reflex in healthy subjects and migraine patients.

This role of the activation of the visual cortex is also better understood by using the flash light stimulation, and thanks to the conception of a new device of flash light stimulation, we performed several protocols in healthy subjects and migraine patient with the final result a proof-of-concept trial using the flash light stimulation in migraine patients.

Abstract

Comprendre la physiopathologie de la migraine est probablement le point le plus difficile dans la gestion de la migraine, car un traitement aigu et préventif efficace doit reposer sur des bases physiopathologiques claires. La migraine est caractérisée dans la phase inter-critique par un déficit d'habituation des réponses évoquées, peut-être en raison d'un niveau de préactivation réduit au niveau du cortex sensoriels. En revanche, lors d'une attaque et dans la migraine chronique, le niveau de préactivation augmente et l'habituation se normalise. De nouvelles techniques de neurostimulation pourraient être utiles pour modifier durablement l'activation du cortex sous-jacent, en diminuant ainsi la répétition des attaques d'une part, et d'autre part donnant un aperçu sur la physiopathologie de la migraine.

Le cortex visuel joue un rôle central dans la physiopathologie de la migraine, mais son effet sur le système nociceptif trigéminal reste peu clair. La céphalée migraineuse s'accompagne souvent de photophobie, mais la relation physiopathologique entre les deux symptômes est inconnue.

Cette thèse propose un nouvel aperçu dans la relation entre la modulation du cortex visuel et la réponse du système nociceptif trigéminal, montrant une corrélation fonctionnelle inhibitrice possible entre les deux structures, par l'intermédiaire de la modulation thalamique.

Cette hypothèse est basée sur nos résultats de modulation fonctionnelle du cortex visuel par la stimulation magnétique transcrânienne répétitive sur le réflexe de clignement nociceptif chez des sujets sains et chez les migraineux.

Le rôle de l'activation du cortex visuel est mieux investigué en utilisant une stimulation lumineuse intermittente, grâce aussi à la conception d'un nouveau stimulateur testé chez des sujets sains et des migraineux avec le résultat final d'un essai-pilote utilisant la stimulation lumineuse intermittente comme traitement de fond de la migraine.

First Part: Background

1. Introduction

1.1. History of migraine

Headache is one of the most frequent symptoms in the general population.

Descriptions of migraine attacks and proposed treatments can be found since the earliest historical records.

Trepanation has been practiced as headache treatment since the Neolithic age. In fact recently it was found a trepanned skull of a woman in central Italy, dating 7000 years ago. What is interesting in this discovery is the microscopic evidence of a new bone growth, proof that the patient survived to the intervention. Trepanation has been used as a treatment for several years, and more frequently in the Middle Ages.

In 4000 B.C. a Sumerian poem claimed these verses "*Take the hair of a virgin kid. Let a wise woman spin it on the right side and double it on the left. Then perform the incantation of Eridu. Bind therewith the head of the sick man. Bind therewith the neck of the sick man. Bind therewith the life of the sick man. Cast the water of the incantation over him that the headache may ascent to heaven*."

In the Ancient Egypt it was suggested to "*take a crocodile made of clay, with sacred grain in its mouth, and an eye of faience.* [The Physician] *should bind it to the head of the patient with a strip of fine linen upon which was written the name of the Gods. And the physician should pray*."

The Ebers papyrus which mentioned migraine, shooting pain and neuralgia, dates to 1552 B.C.:

"Headache roameth over the desert, blowing like the wind.

Flashing like lightning, it is loosed above and below.

It cutteth off like a reed him who feareth not his god

like a stalk of henna, it slitteth his thews.

It wasteth the flesh of him who hath not protecting goodness.

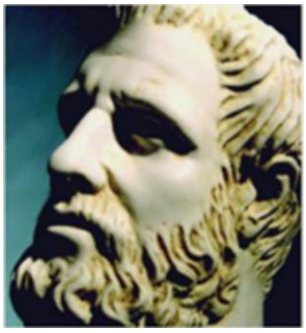
Flashing like a heaventhly star, it commeth like the dew:

it standeth hostile against the wayfarer, scorching him like the day.

This man is hath struck and like one with heart disease he staggereth.

Like one bereft of reason he is broken."

In Mesopotamia features of a sufferer from migraine were: "*the head is bent with pain gripping his temples, and his eyes are afflicted with dimness and cloudiness*."



Hippocrates

In 460 B.C., Hippocrates, the most famous Greek physician described a migraine with aura attack: "*Most of the time he seemed to see something shining before him like a light, usually in part of the right eye. At the end of a moment, a violent pain supervened in the right temple, then in all the head and neck, where the head is attached to the spine. Vomiting, when it became possible, was able to divert the pain and render it more moderate*." He thought that migraine and other type o headaches came from "humors" – some fluids or vapours circulating between the liber and the brain, whose action might be controlled by the exercise and the sexual activity.

In particular the presence of nausea and vomiting during a headache attack was due to the yellow bile (the choler humor).

The idea that migraine was a digestive tract problem induced Hippocrates to propose to vomit in order to decrease the intensity of pain. He also made for the first time a relation between vomiting attacks of childhood and "bilious attacks" that develop in a migraine attack in the adult life; in fact we know now that a recurrent vomiting can

be an episodic syndrome that may be associated to migraine, described also in the International Headache Classification. To treat headache Hippocrates used the Willow Bark (from which aspirin is made).

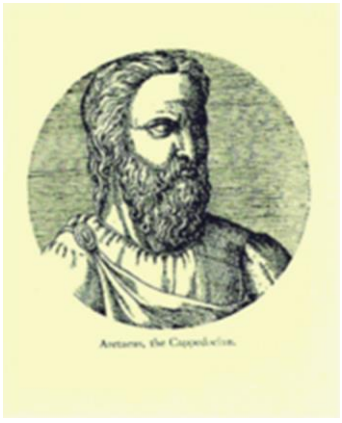

Thomas Willis

Aretaeus of Cappodocia (30-90 A.D.) was the first that well described the syndrome of migraine and called it *heterocrania*, the composition of "*hetero*" → different, and "*kranion*"→ skull.

Later Galen (131-201 A.D., Asia Minor) modified this definition in *emicrania*. The term changed to *hemigranea* and after this to *migrainea* and so on to *migrana.*

AbuAli Sina, known by the Latins with the name of Avicenna (980-1037) wrote: "*small movements, drinking and eating, and sounds provoke the pain; the patient cannot tolerate the sound of speaking and light. He would like to rest in darkness alone*."

In the Middle Ages in Europe it was used to treat migraine attack with poultices applied to the scalp: the vinegar first to open the pores of the scalp and after that an opium solution. Another treatment was to apply some hot irons on the head.


Aretaeus of Cappodocia

Thomas Willis (1621-1675), an English anatomist and physician, made accurate observations on migraine features and in particular on the hereditary link and trigger factors of the migraine attack. He thought that the cause of migraine was related to a vasospasm that leads to abandon the method of trepanation for headache. Willis wrote "*I think the opening of the Scull will profit nothing*."

The first vascular theory advanced by Willis was widely accepted.

In 1850s Brown-Sequard and Claude Bernard asserted that the active headache phase of migraine was due to sympathetic deficit of vasodilatation.

William Richard Gowers (1845-1915) divided the treatment of migraine in prophylactic and acute, but he made an interesting "Gowers mixture" combining Nitroglycerin and alcohol to treat the acute phase.

In the same period migraine aura became also an inspiration for authors like Reverend Charles Dodgson (Lewis Carroll as pen name, 1832-1898) who wrote "*Alice's Adventures in Wonderland*", for painters like Vincent Van Gogh (1853-1890) in the painting "*Starry Night*", for philosophers such as Friedrich Nietzsche (1844-1900), for novelists as H.G. Wells (1866-1946), James Joyce (1882-1941), Emily Dickinson (1830-1886).

Nowadays migraine is not only one of the most frequent neurological diseases but also a source of inspiration of a new artistic movement named "migraine art".

1.2. Clinical features of migraine

The diagnosis of migraine is essentially a clinical one.

Migraine is characterized by the repetition of attacks where headache is the principal, but not the only, symptom.

We can distinguish two main types of migraine that can occur in the same patient: migraine without aura and migraine with aura. The knowledge on the pathophysiology of migraine has increased in the last 30 years, even if we don't know yet if these two types of migraine are different entities or not.

Before 1988 the taxonomy of headaches was seldom based on precise criteria. In 1988 the International Headache Society (IHS) established a classification of headache that became rapidly the first approach to diagnose headaches not only in the clinical field but also in research.

A new edition was published in 2004 and the last one was issued 2013 (ICHD 3Beta, 2013).

The classification represents an incredible step forward in the codification of headache disorders. The guiding principles for the classification were:

1) To standardize terminology in order to overcome the obstacles in the communication between physicians all over the world.
2) To set up a hierarchical system of clinical manifestations.
3) To provide precise and specific diagnostic criteria for headache disorders.
4) To provide a useful tool for specialists, researchers and general practitioners.

The ICHD – 3 Beta Version (2013) includes 85 different type of headache and 196 subtypes.

Anamnesis is the crucial phase to perform a diagnosis of migraine, in particular because the majority of migraineurs has a normal neurological and general examination, and has normal neuroimaging.

In this thesis are reported only diagnostic criteria of the following types of headaches: migraine without aura, migraine with aura and chronic migraine.

Table 1.1. Diagnostic criteria of migraine without aura (code 1.1):

A. At least 5 attacks1 fulfilling criteria B-D
B. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)2;3;4
C. Headache has at least two of the following characteristics:
   1. unilateral location
   2. pulsating quality
   3. moderate or severe pain intensity
   4. aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
D. During headache at least one of the following:
   1. nausea and/or vomiting
   2. photophobia and phonophobia
E. Not better accounted for by another ICHD-3 diagnosis.

Table 1.2. Diagnostic criteria of migraine with aura (code 1.2):

A. At least two attacks fulfilling criteria B and C
B. One or more of the following fully reversible aura symptoms:
1. visual
2. sensory
3. speech and/or language
4. motor
5. brainstem
6. retinal

C. At least two of the following four characteristics:
   1. at least one aura symptom spreads gradually over 5 minutes, and/or two or more symptoms occur in succession
   2. each individual aura symptom lasts 5-60 minutes
   3. at least one aura symptom is unilateral2
   4. the aura is accompanied, or followed within 60 minutes, by headache
D. Not better accounted for by another ICHD-3 diagnosis, and transient ischaemic attack has been excluded.

Table 1.3. Diagnostic criteria of chronic migraine (code 1.3):

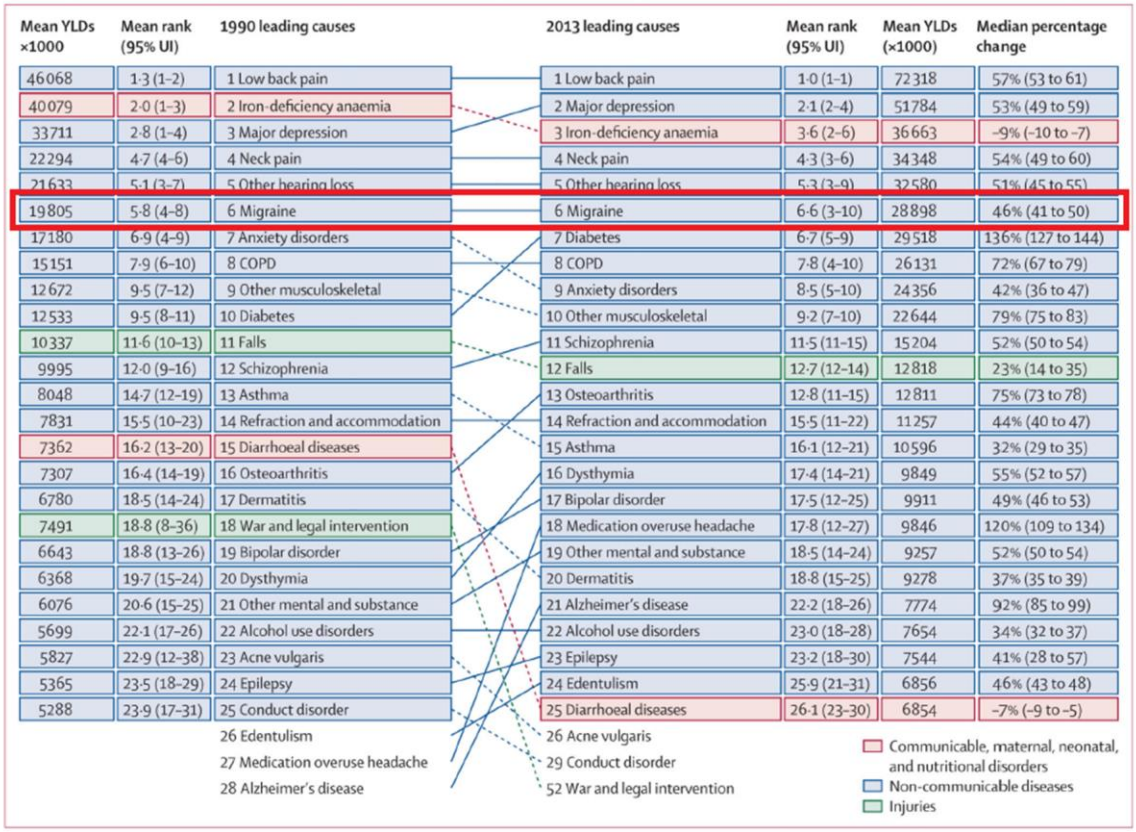
A. Headache (tension-type-like and/or migraine-like) on 15 days per month for >3 months and fulfilling criteria B and C
B. Occurring in a patient who has had at least five attacks fulfilling criteria B-D for 1.1 Migraine without aura and/or criteria B and C for 1.2 Migraine with aura
C. On 8 days per month for >3 months, fulfilling any of the following:
   1. criteria C and D for 1.1 Migraine without aura
   2. criteria B and C for 1.2 Migraine with aura
   3. believed by the patient to be migraine at onset and relieved by a triptan or ergot derivative
D. Not better accounted for by another ICHD-3 diagnosis.

1.3. Epidemiology and Burden of migraine

In general population the most represented type of headache is firstly Tension-type Headache (TTH), and then migraine and medication overuse headache (MOH).

According to the last published Global Burden of Diseases Study (Lancet 2015), which updates evidences about levels and trends in disease and injury incidence, prevalence, and years lived with disability (YLDs) in 188 countries, from 1990 to 2013, migraine ranks 6th in the Top 25 causes of global Years Lived with Disability (YLDs), a ranking that remained stable over the last 20 years (*Fig 1.1*).

Figure 1.1: *From the Global Burden of Diseases Study 2013 (Lancet 2015).*



The global prevalence of individuals with recurrent migraine, for more than 3 months, has increased in 2013 (848,366,000 cases) compared to 1990 (581,025,000 cases). The augmentation can be explained by the growing population and of course by better diagnostic tools and increased attention for migraine.

Among neurological diseases, migraine ranks 2nd regarding prevalence, after tension-type headache (1,561,446,000 cases), and followed in 3rd position by medication overuse headache (62,899,000 cases). However, the percentage of YLDs in migraine has increased by 46.1% from 1990 to 2013, while the gain is more conspicuous in other neurological disorders such as multiple sclerosis (116.1%), Alzheimer's disease (91.8%) and Parkinson's disease (81.2%).

The prevalence is estimated at 14.7% in adults in Europe (8% in men and 17.6% in women) (Stovner et al., 2010). In children and youth people the prevalence is estimated in 10%, and in elderly people (64-75 years old) is between 6 and 11%.

There is a huge disparity in prevalence between Europe and USA on the one hand, and Africa and Asia on the other hand, maybe due to the difficulty in diagnosing and treating it and also because of some cultural differences in migraine perception.

In Wallonia-Belgium the one-year prevalence of migraine is 25.8% (Streel et al., 2015), and overall costs for migraine in Belgium are estimated at 860,000,000 Euros per year (Schoenen et al., 2006).

2. Migraine Pathophysiology

In the last decades, remarkable strides forward have been made; mainly brought about by advanced imaging techniques which have helped to shine light upon the underlying causes of primary headache disorders.

At first migraines were thought to have a vascular pathogenesis; this was overshadowed by the conception that a "neurovascular phenomenon" seemed to be the permissive triggering factor in migraines and in cluster headaches. Neuronal structures are involved in the pathogenesis of migraine, in which the vasodilatation is only an epiphenomenon.

However, the exact pathogenic process of migraine remains to be determined.

Since the 1940s the evolution in the concept of migraine pathophysiology has seen the opposition of two main theories: the vascular hypothesis and the integrated neurovascular model. Harold G. Wolff, a pioneer of the vascular theory of migraine, suggested that the aura phase was caused by vasoconstriction and the headache by rebound vasodilatation (Wolff et al., 1963). Lashley's experience of his own visual aura led him to the concept that the Cortical Spreading Depression (CSD) of Leão was the primary cause (Lashley et al., 1941), and advanced the neural theory of migraine (Leão et al., 1944), where the vascular changes are a consequence and not directly a cause to the headache. Laurintzen (1994) and Olesen (1981) confirmed that during the migraine aura there is a hypovascularisation spreading in the parieto-occipital regions and changes in the vascularisation can persist also during the cephalic phase. Curtrer et al. (1998) demonstrated that the occipital cortex, contralateral to the visual aura, was involved by a decrease in the cerebral flow using Positron Emission Tomography (PET), at a level that didn't reach the ischemic threshold. On the other hand vascular changes do not occur in all migraine sufferers (Pietrobon et al., 2003).

Nonetheless, the relation between migraine aura and headache remains controversial, and so also the "primum movens" of migraine without aura, which is the most frequent type of headache.

Below we will summarize the main knowledge on the pathophysiology of migraine; in particular those aspects that are the most closely related to our research.

We will distinguish the pathophysiology of the migraine attack from the interictal phase. Bearing in mind that migraine attacks occur periodically, factors that precipitate attacks are pivotal in the pathophysiology of the disorder.

2.1. Migraine aura

The clinical features of the aura have been described since a long time, but it is only since 1941 that researchers have tried to explain this phenomenon.

The first was Lashley (1941) who posited that the aura was caused by a neuronal dysfunctioning that is composed firstly of an activation phase (phosphenes), followed by an inhibition phase (scotoma). This process spreads over the visual cortex at a velocity of 3-5 mm per minute. The same characteristics were observed also in the animal model of cortical spreading depression (CSD) by Leão (1944): CSD is a wave of brief, intensive neuronal depolarisation followed by a wave of depolarisation block. CSD is considered to be associated with the migraine aura phenomenon and can be triggered by various physical and chemical stimuli. In lissencephalic animals such as rodents the propagation of CSD is facilitated by the absence of circumvolutions in the brain, whereas in gyrencephalic animals a sulcus or a fissure can interrupt CSD. During the inhibitory phase of CSD there is also a decrease in cerebral blood flow (CBF) by 20-30%, but interestingly the area of the propagation of this oligemia is independent from the vascular territories.

The visual cortex is the region where the CSD is facilitated because of a high neuronal density and neuron to astrocyte ratio. Astrocytes are able to buffer extracellular

potassium and glutamate released during CSD and hence are crucial in CSD termination.

SPECT studies during visual auras triggered by the injection of xenon 133 into the carotid artery were first to show an oligemia comparable to that recorded during experimental CSD (Olesen et al., 1981 and 1991). While CSD has not yet been demonstrated as such in humans, metabolic changes characteristic of CSD have been shown in migraine patients (Cao et al., 1999; Hadjikhani et al., 2001). Using Blood Oxygenation Level-Dependent (BOLD) Functional magnetic resonance imaging (fMRI) during migraine aura induced by visual stimulation with red/green checkerboard, the authors found a decrease in the signal BOLD in the visual cortex that preceded the triggered headache even if patients did not experience a visual change. The question was further investigated by Hadjikhani et al. (2001) with fMRI during spontaneous auras that showed similar changes as those found by Cao et al. Occipito-parietal spreading oligemia was also reported in a migraine-without-aura patient undergoing visual stimulation during $H2O^{15}$-PET (Woods et al. 1994). Nevertheless, the precise link between the spreading events that accompany migraine attacks and CSDs recorded in animals events is not clear (Brennan et al., 2007). Using MRI perfusion-weighted imaging (PWI), Sanchez de Rio et al. (1999) observed a reduction in the CBF in the occipital cortex but not in other areas during spontaneous migraine auras and did not find such CBF reduction in migraine without aura patients. Similarly no CBF change was found with $H2O^{15}$- PET during attacks of migraine without aura (Weiller et al., 1995).

A study conducted in our Headache Research Unit using quantitative EEG brain mapping during the ictal phase in migraineurs without aura showed depression in posterior alpha power, ipsilateral to the pain that was interpreted as a possible reflection of reduced neuronal activity (Schoenen et al., 1987) reminiscent of the decrease in alpha EEG activity reported during experimental CSDs by Leão's (1945).

2.2. Migraine headache

The fact that the migraine pain involves trigeminal nerve afferents, in particular the trigeminovascular system located around meningeal vessels, has been well documented and well accepted for several decades now.

Trigeminovascular small calibre myelinated Aδ and unmyelinated C axons surrounding blood vessels of the pia and dura mater can release vasoactive peptides producing a sterile inflammatory reaction (Moskovitz, 1984). These structures taken together are termed the "Trigeminovascular System". Afferent impulses from the trigeminovascular system converge on 2^{ary} nociceptors in the trigeminal nucleus caudalis (TCN), situated in C1 and C2, that also receive somatic afferents from the somatic portion of the ophthalmic nerve and C2 dermatoma. This is probably why the head pain in migraine is often localized in the fronto-orbital or cervico-occipital region (Arbab et al., 1986, Kerr et al., 1972), and can be considered a "referred pain". Neurons in the Gasserian ganglion contain calcitonin gene-related peptide (CGRP) or substance P (SP) (Uddman et al., 1985). The stimulation of the trigeminal ganglion induces the release of vasodilatatory peptides such as CGRP, substance P, neurokinin A and nitric oxide (Moskowitz et al., 1992 and 1993). The reaction termed "neurogenic inflammation" may lower the nociceptive threshold required to stimulate meningeal sensory fibers (Moskowitz et al., 1990) and also act on vascular tissues to cause vasodilatation, plasma protein extravasation, endothelial changes, platelet aggregation, subsequent release of serotonin (5-hydroxytryptamine, 5-HT) and other mediators, white-cell adhesion and inflammation. In turn the cranial vasodilatation stimulates the trigeminal endings, and the latter further reinforces the release of vasodilator peptides. Neurogenic inflammation has not been demonstrated in migraine patients and recent imaging studies show that migraine attacks are not associated with significant vasodilatation of extra- or intracerebral arteries (Amin et al., 2013). By contrast, there is undisputable evidence for the ictal release of CGRP from meningeal 1^{ary} nociceptors as shown by the studies of CGRP levels in external

jugular vein blood during migraine attacks (Goadsby et al., 1990). Specific antimigraine drugs, including sumatriptan, are able to supress plasma extravasation produced by the antidromic stimulation of trigeminal nerve terminals in rodent meninges (Moskowitz et al., 1991) and more interestingly, antagonists of CGRP-receptors, such as olcegepant and telcagepant, and more recently monoclonal antibodies against CGRP or its receptor (Yao et al., 2013) are effective for migraine treatment although they have no vascular effects (Petersen et al., 2005).

2.3. Is there a link between CSD and migraine pain?

While we can produce CSD in anesthetized or evoked animals, anaesthesia may have profound effects on the mechanisms underlying the CSD and clearly the behavioural response is absent. Therefore studies in awake animals seem more appropriate to investigate CSD-associated behavioural changes. A single CSD has been shown to evoke freezing and reduce motor activity in rodents (Akcali et al., 2010; Fioravanti et al., 2011).

Moskowitz et al. (1993) found that CSD in animals is associated with an increased ipsilateral expression of c-fos, considered a surrogate marker of pain, in trigeminal nucleus neurons and he postulated that the CSD is the "primary" event of a migraine attack, leading to the neurogenic inflammation. In 2002 Bolay et al. provided evidence in animal subjects that CSD activates trigeminal afferents causing inflammatory changes (vasodilatation, oedema and protein extravasation) in the meninges (Bolay et al., 2002), lending support to Leão's theory. The authors also investigated, after resection of trigeminal nerve, how the expression of c-fos was increased in lamina I and II in the nucleus caudalis after CSD.

At the same time this conclusion was criticized by two studies based on the direct measure of trigeminal second-order neurons firing after CSD (Lambert et al., 1999;

Ebersberger et al., 2001) both were critical of Moskowitz's theory for methodological reasons.

A recent study showed that the propagation of the CSD also involves the thalamic reticular nucleus (TRN) visual sector (Tepe et al., 2015) and this effect can be blocked by the acute administration of valproic acid, one of the most effective prophylactic treatments in migraine. These findings may well lead to new developments in the treatment for migraine that focuses on the role of thalamic nuclei and its integrative role in the pain matrix.

2.4. Migraine generator

The two studies cited before (Lambert et al., 1999; Ebersberger et al., 2001) sought to tackle the as yet unsolved question of what causes migraine attacks.

Considering migraine as the derangement of a complex network of neural structures involved in pain processing can provide an answer to some extent. Pain can be considered as an integrative, modelled, and multidimensional sensation, with the aim to localise and discriminate the nature and the intensity of the threat in order to induce the most appropriate emotional and cognitive processing of the stimulus and thus lead to the best behavioural defensive response. The process inevitably involves numerous cerebral areas, those of somatic sensation, emotion and cognitive modulation, vegetative and motor action. Inhibitory and facilitatory mechanisms for controlling pain signals are involved in the so-called pain matrix.

In migraine the first generator seems to be the brainstem, where the trigeminocervical complex (TCC), composed of the TCN and the neurons of the C1 and C2 spinal cord, projects. Even if the pivotal role of the brainstem was postulated several decades ago, the first evidence was published in 1995 by Weiller and colleagues: using $H2O^{15}$-PET they demonstrated in 9 migraine patients during right-sided migraine attacks an increased blood flow in the contralateral brainstem. Similar studies confirmed these

results by showing an activation in the dorsal rostral brainstem in a patient with migraine without aura (Bahra et al., 2001), the red nucleus and substantia nigra in a patient with migraine with aura (Welch et al., 1998) and the dorsal lateral pons in 24 migraine patients (Afridi et al., 2005).

Brainstem activation, however, does not seem to be migraine-specific: it was also found in other chronic pain conditions (Kupers et al., 2000).

The question to be elucidated is how the brainstem can cause enhanced responses in the nociceptive trigeminovascular system? Two complementary theories address this question: a low level of descending inhibition or a high level of descending facilitation. The final result is hyperexcitability of 2nd order trigeminovascular neurons.

Interestingly, during migraine attacks the brainstem activation persists after injection of sumatriptan with complete clinical relief not only of the headache but also of associated symptoms such as photophobia and phonophobia (Weiller et al., 1995) and the nucleus cuneiformis remains hypofunctional between migraine attacks (Moulton et al., 2008). The brainstem could thus be the link connecting the pathophysiology of the migraine attack to the interictal phase.

2.5. Genetic predisposition

Migraine has been known as a familial disorder for a long time. Genetic studies have contributed significantly to the understanding of migraine pathophysiology. Migraine is known to be 50% more common among 1st degree relatives of sufferers than in matched controls. The risk is higher for those with more disabling symptoms than for those with lighter symptoms, and higher for those with migraine with aura than for those with migraine without aura. Studies have also shown a higher rate of concordance for monozygotic than dizygotic twins, and this effect is greater in females than males (Stewart et al., 1996). Concordance in monozygotic twins is

nevertheless under 100% (Larsson et al., 1995). Migraine is clearly genetically complex, with a non-Mendelian mode of inheritance and mutations in multiple genetic loci. Mutations are likely to affect changes in the threshold of susceptibility to migraine attacks.

In familial hemiplegic migraine (FHM) type 1, a rare autosomal dominant form of migraine with prolonged hemiplegic aura, various pathogenic mutations have been discovered in single genes. The first mutations (FHM1) were found in the CACNA1A gene, a P/Q voltage gated calcium channel gene (Ophoff et al., 1996). Other mutations in 2 other genes have also been documented to cause related phenotypes (ATP1A2 in FHM2 (De Fusco et al., 2003) and SCN1A in FHM3 (Dichgans et al., 2005). ATP1A2 codes for an alpha 2 subunit of the Na/K ATPase pump, and mutations cause changes in the sodium gradient across the cell membrane, with associated changes in synaptic neurotransmitter levels. Similarly, mutations in SCN1A affect transmembrane sodium flux.

There is a known association between FHM1 and basilar-type aura symptoms, and between FHM1 and chronic progressive cerebellar ataxia in 50% of families.

The FHM mutations are not found in the common forms of migraine with or without aura. In the latter genome-wide association studies have identified up to now 14 genetic loci (single nucleotide polymorphisms), each of which contributes only to a small percentage of migraine susceptibility (Anttila et al., 2010). A number of other genetic associations have been reported in migraine, including with polymorphisms in MTHFR, ACE, ETA, and PGR genes (Lee et al., 2007; Rubino et al., 2009; Tzourio et al., 2001). The relative contribution of each has yet to be verified and quantified in different populations.

Although family and twin studies indicate involvement of genetic factors in the aetiology of migraine, the exact contribution of genes and the mode of inheritance of such factors remain unknown. Overall, the genetic studies indicate that the common forms of migraine are complex genetic disorders with a multifactorial inheritance,

combining genetic and environmental factors that set the "migraine threshold" and thus can lead to the development of a repetitive pattern of attacks.

2.6. Contribution of electroneurophysiology to migraine pathophysiology

2.6.1. Generalities

The electroneurophysiologic techniques (evoked potentials, electroencephalography, and electromyography) allow the recording of central and peripheral responses of the nervous system.

During the last three decades electroneurophysiology was widely used to explore migraine pathophysiology. Among the different techniques, evoked potentials have been the most studied. Differences have been found in the latency and amplitude of evoked potentials, but one of most reproducible alterations found in migraineurs compared to healthy subjects is a "deficit of habituation", confirmed in studies using visual (Schoenen et al., 1995), auditory (Wang et al., 1996; Ambrosini et al., 2003), somatosensory (Ozkul et al., 2002), cognitive (Siniatchkin et al., 2000, 2006 and 2007; Kropp et al., 1993 and 1995; Schoenen et al., 1993) and nociceptive stimuli (Valeriani et al., 2003, de Tommaso et al., 2005; Di Clemente et al., 2005 and 2007).

We will first discuss the data concerning latencies and amplitudes of evoked potentials and thereafter the results on habituation, including a short explanation of the phenomenon of habituation/sensitization.

2.6.2. Differences in latency and amplitude of evoked potentials

2.6.2.1. Visual evoked potentials (VEP)

In early VEP studies the responses were evoked by flash light stimulation: the main result was increased amplitude in migraineurs compared to controls (Lehtonen et al., 1974; Connolly et al., 1982; Brinciotti et al., 1986), except in one study (Richey et al., 1966).

Using pattern-reversal visual evoked potential (PR-VEP), results are more heterogeneous. VEP amplitude was found to be normal, or increased between attacks and during the pre-ictal phase, whilst some studies reported decreased amplitudes. PR-VEP latencies were also found increased in some studies but not in others (see review by Ambrosini et al., 2006). While in one study the decreased PR-VEP amplitude was correlated to the duration of the disease (Khalil et al., 2000), it wasn't in another (Yucesan et al., 2000). It seems that there is no difference between migraine with and without aura, except for P100 amplitude, which was found to be reduced in one study (Tagliati et al., 1995) but increased in another (Shibata et al., 1997) in migraine with aura.

These contradictory findings are probably due to differences in methodology and in patients' diagnosis.

Results using PR-VEP are more convincing when changes in successive blocks are considered, i.e. habituation that will be discussed below.

2.6.2.2. Auditory evoked potentials (AEPs)

Several studies of brainstem AEPs did not find any significant difference in latencies between healthy subjects and migraineurs in the interictal phase, but as with the VEP recordings, the results are moot and sometimes the authors found an increased

latency of the 5th component (Bussone et al., 1985; Drake et al., 1990) in particular during the ictal phase of migraine with aura (Schlake et al., 1990).
Cortical long-latency AEPs did not show significant differences between groups of normal subjects and patients regarding latencies or amplitudes of N1, P2 and N2 (Drake et al., 1989; Sand et al., 2000).

2.6.2.3. Somatosensory evoked potentials (SSEP)

Overall, no significant abnormalities were demonstrated using the standard SSEP after median nerve or index-finger stimulation in migraine patients, except for a prolonged N13 latency in the interictal phase, a prolonged N19 and reduced amplitudes during the aura phase (see review by Ambrosini et al., 2006).

2.6.2.4. Contingent negative variation (CNV)

CNV, an event-related potential obtained during a reaction time task, showed increased amplitude in migraineurs during the interictal phase that was more pronounced in MO patients (Schoenen et al., 1985; Maertens de Noordhout et al., 1986; Böcker et al., 1990; Kropp et al., 1993). A positive correlation between CNV and risk for developing migraine based on 1st degree relatives affected by the disease was also documented (Siniatchkin et al., 2000 and 2001).

2.6.2.5. Nociceptive laser-(LEPs) or contact heat-(CHEPs) evoked potentials and nociceptive blink reflex (nBR)

LEPs are likely generated by the cingulate cortex (Bentley et al., 2003), which belongs to the limbic system and is involved in the emotional aspect of pain. Studies by de Tommaso et al. (2005) showed increased N2-P2 amplitude when the supraorbital area or the dorsum of the hand were stimulated in migraine patients during an attack. Oral administration of nitroglycerin increased LEP P2 amplitude (de Tommaso et al., 2004) whereas almotriptan or lycine-acetylsalicytate reduced P2 amplitude (de Tommaso et al., 2005).

Amplitude of the nBR (R2 component) was found increased and its latency decreased in the ictal phase but not in sinusitis pain (Katsarava et al., 2002; Kaube et al., 2002). The abnormality disappeared after acute migraine treatment (Kaube et al., 2002).

A recent study using CHEPs showed enhanced amplitudes in both MA and MO (Lev et al., 2013), but not in another study where the amplitude was decreased (Beese et al., 2015).

2.6.3. Habituation and sensitization: the "dual process" theory

The first description of the phenomena of habituation and sensitization, and the coining of these terms, dates back to Thompson and Spencer in 1966. The authors presented nine main behavioural characteristics of habituation that are common to many different species.

In the early stage of a repeated stimulation, there is initially an increased response, so-called sensitization, while the response decreases as the stimulation continues, so-called habituation. Considered together these two phenomena are known as "dual process" theory (Groves et al., 1970). The theory was revised in 2009 (Rankin et al.,

2009) when a 10th characteristic was added; we will be dealing only with the recent version.

"Habituation is defined as a behavioural response decrement that results from repeated stimulation and that does not involve sensory adaptation/sensory fatigue or motor fatigue." – from Rankin et al., 2009. Behavioural responses that follow the habituation process involve every type of stimulus, including reflexes and hormone release.

Habituation is the most elementary form of learning, but even if the neurophysiological mechanisms underlying this process are not clear.

2.6.3.1. Characteristics of habituation (Adapted from Rankin et al., 2009)

1. "Repeated application of a stimulus results in a progressive decrease in some parameter of a response to an asymptotic level. This change may include decreases in frequency and/or magnitude of the response."
2. "If the stimulus is withheld after response decrement, the response recovers at least partially over the observation time (''spontaneous recovery")."
3. "After multiple series of stimulus repetitions and spontaneous recoveries, the response decrement becomes successively more rapid and/or more pronounced (this phenomenon can be called potentiation of habituation)."
4. "Other things being equal, more frequent stimulation results in more rapid and/or more pronounced response decrement, and more rapid spontaneous recovery (if the decrement has reached asymptotic levels)."
5. "Within a stimulus modality, the less intense the stimulus, the more rapid and/or more pronounced the behavioural response decrement. Very intense stimuli may yield no significant observable response decrement."
6. "The effects of repeated stimulation may continue to accumulate even after the response has reached an asymptotic level (which may or may not be zero, or no response)."

7. “Within the same stimulus modality, the response decrement shows some stimulus specificity.”

8. “Presentation of a different stimulus results in an increase of the decremented response to the original stimulus. This phenomenon is termed ‘‘dishabituation”.”

9. “Upon repeated application of the dishabituating stimulus, the amount of dishabituation produced decreases (this phenomenon can be called habituation of dishabituation).”

10. “Some stimulus repetition protocols may result in properties of the response decrement (e.g. more rapid rehabituation than baseline, smaller initial responses than baseline, smaller mean responses than baseline, less frequent responses than baseline) that last hours, days or weeks. This persistence of aspects of habituation is termed long-term habituation.”

2.6.3.2. Characteristics of sensitization

Sensitization is also a learning phenomenon: it is characterized by the increasing response to many types of stimuli, normally considered harmless, when the subject receives a painful stimulus. In other words it is the capacity to evoke a response to stimuli with an intensity under-threshold or to produce an excessive response during painful stimulation.

The clinical manifestation of sensitization is allodynia or hyperalgesia. In some studies cutaneous allodynia was found in 79% of migraine patients (Burstein et al., 2000).

One can distinguish peripheral and central sensitization. Albeit the former is sustained by peripheral nociceptors, and the latter by trigeminal neurons projecting to brainstem nuclei, these two phenomena are chronologically correlated: between 5 and 20 minutes after the beginning of the headache the subject develops a peripheral sensitization; between 20 and 120 minutes the central sensitization starts and reaches

a peak between 120 and 240 minutes (Strassman et al., 1996). The clinical manifestation of the allodynia begins when central sensitization occurs, suggesting that changes involve central and not peripheral mechanisms.

Moreover sensitization can be persistent, in particular when the painful stimulus is repeated over a longer period e.g. several days, weeks, months or years, a repetition of pain that may occur in migraine because of the repetition of attacks.

2.6.3.3. Habituation and sensitization in migraine

Electroneurophysiology allows to study habituation and sensitization by recording response amplitude in sequential blocks of averagings during continuous stimulation and analysing its change between the 1st and the last block of responses. In migraine the characteristic feature is a lack of habituation in interictal phase, as opposed to normal habituation during the attack (*Fig. 2.1*). This abnormal response pattern was found for all stimulation modalities and might be genetically determined.

The first study showing that habituation is decreased in migraine patients was conducted by Schoenen et al. (1985) using CNV: the early component was the most modified comparing healthy subjects and migraineurs and was confirmed in studies using visual or auditory oddball paradigms (see review by Coppola et al., 2007).

PR-VEP were widely used to investigate the process of habituation in migraineurs and showed that the amplitude of N1-P1 and P1-N2 decreases, and thus habituates, during repetitive stimulation in healthy subjects but not in migraineurs between attacks (Schoenen et al., 1995; Afra et al., 1998; Wang et al., 1999). During the attack, the deficit of habituation normalizes (Afra et al., 2000). However the interictal deficit of habituation interictally was not confirmed by others (Oelkers et al., 1999 and 2005; Sand et al., 2000), which could be related to geographical genetic or environmental differences (Ambrosini et al., Cephalalgia 2016 in press).

Moreover the habituation deficit was also found in related parent-child pairs of migraineurs but not in unrelated pairs (Sándor et al., 1999). The deficit of habituation is normalized by preventive treatment with beta-blockers (Sándor et al., 2000), which also normalizes the increased amplitude of grand average VEPs (Diener et al., 1989), and by fluoxetine (Ozkul et al., 2002). High frequency repetitive transcranial magnetic stimulation (rTMS), that activates the underlying cortex, produced a normalisation of the deficit of habituation in migraineurs when applied over the occipital region, while low frequency rTMS supposed to inhibit the cortex, induced a potentiation (Bohotin et al., 2002). After 5 daily sessions of rTMS these effects last days or weeks both in healthy subjects and migraine patients (Fumal et al., 2006).

Transcranial direct current stimulation (tDCS) over the visual cortex increases transiently habituation of the N1-P1 VEP component (Viganó et al., 2013). The late component of high-frequency oscillations in the gamma band (GFO 20-60 Hz) of VEPs also lack habituation both in MO and MA (Coppola et al., 2007).

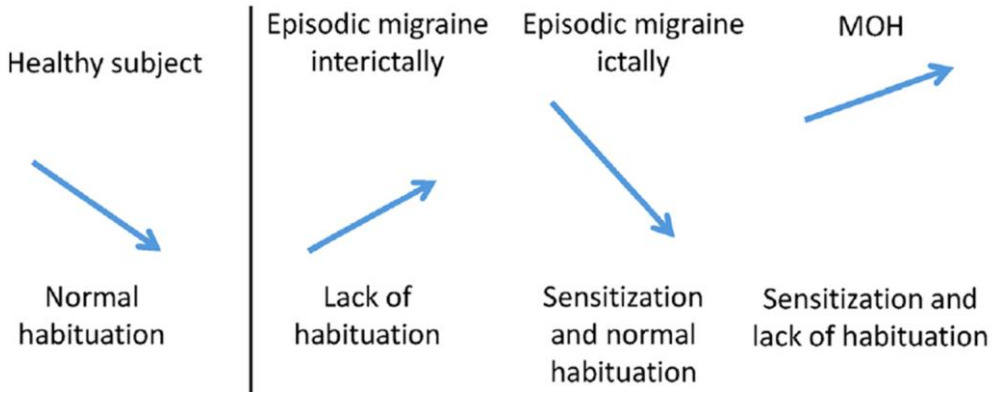
The amplitude-stimulus slope of AEPs is steeper in migraine patients compared to healthy subjects (Wang et al., 1996), though not in all studies (Sand et al., 2000), and this is attributed to a lack of habituation of the responses during high intensity stimuli (Ambrosini et al., 2003). When two auditory stimuli are delivered at an interval of 500 ms, the second response (P50) is reduced in healthy subjects but not migraine patients reflecting a deficient gating mechanism (Ambrosini et al., 2001).

As for SSEPs Ozkul et al. (2002) found a potentiation of the N20 component after stimulating the median nerve, and the recovery cycle of SSEPs in children with migraine without aura was higher than in the controls (Valeriani et al., 2005), probable because of potentiation in the somatosensory cortex.

Pain-evoked potentials equally lack habituation in migraineurs. The N2-P2 LEPs component showed potentiation in migraine patients (Valeriani et al., 2003, de Tommaso et al., 2005), but contrary to VEPs, the deficit of habituation persists during the attack (de Tommaso et al., 2005).

Three studies showed that the nBR undergoes potentiation in migraine patients between attacks while it habituates in healthy controls (Di Clemente et al., 2005 and 2007; Katsarava et al., 2003); nBR and VEP abnormalities were correlated in the same patients suggesting a common underlying mechanism (Di Clemente et al., 2005).
Lack of habituation was also reported for CHEPs in migraineurs (Lev et al., 2010 and 2013; Beese et al., 2015).

Figure 2.1: Summary of the pattern of habituation/sensitization for all evoked potentials and the main pattern of responses during repetitive stimulation, painful or not, in healthy subjects, episodic migraineurs in the interictal phase, episodic migraineurs ictally and MOH patients (from Coppola et al., 2013).



The other facet of the "dual process" theory, sensitization, is also suggested by studies in migraine, particularly when using noxious stimuli and in chronic migraine. During the migraine attack the area under the curve (AUC) of the nBR R2 is increased on the affected side compared to the non-affected side (Kaube et al., 2002). Similar results were also found in the N2-P2 components of LEPs (de Tommaso et al., 2005), suggesting ictal sensitization. In medication overuse headache (MOH) the first block of the SSEP N20-P25 component had greater amplitude than in episodic migraine patients or healthy controls and this correlated with the duration of disease. This was interpreted as a reinforcement and perpetuation of central sensitization due to the medication intake and the repetition of headache attacks. This is supported by the

study of Ayzenberg et al. (2006) showing that in MOH the increased amplitude of trigeminal pain-related evoked potentials (PREP) normalizes after drug withdrawal.

2.6.3.4. Significance of the habituation deficit on migraine pathophysiology

Taken together, the electroneurophysiological results are key to advance toward the now accepted theory that the demonstrated changes are the reflection of altered cortical preactivation, probably due to an abnormal subcortical control by monoaminergic afferents (Schoenen et al., 1995). This notion was not forthwith understood, and authors, several years ago, thought the primary culprit was "cortical hyperexcitability" possibly due to decreased intracortical inhibition. This hypothesis was not confirmed by a series of studies performed by Schoenen and Coppola et al., (2007, 2009 and 2010) showing that migraine can most likely be considered as a thalamo-cortical dysrhythmia where an insufficient thalamo-cortical drive results both in initial low preactivation of sensory cortices and during stimulus repetition to lack of habituation, explaining why the cortex is hyper-responsive without being hyperexcitable. What the precise role of the thalamo-cortical dysrhythmia and cortical hyper-responsivity in migraine pathogenesis is remains to be determined. Since migraine patients are biochemically characterized between attacks by a decreased mitochondrial energy reserve and ATP synthesis, cortical hyper-responsivity could favour a rupture of metabolic homeostasis leading to activation of the pain-signalling trigeminovascular system and hence the migraine attack (see Schoenen et al., 1994, De Tommaso et al., 2014).

3. Photophobia

Most migraineurs have photophobia as an associated symptom during an attack, but they are also overall more sensitive to light between attacks (Drummond et al., 1986; Friedman et al., 2009), in particular those suffering from migraine with aura (Hay et al., 1994). In the ICHD ictal photophobia combined with phonophobia is part of the diagnostic criteria for migraine, though not mandatory.

Photophobia is not a migraine-specific symptom; in other diseases, neurological or not, photophobia may occur, among them blepharospasm (Hallet et al., 2008), ocular pathologies (Lebensohn et al., 1951), tumoral lesions compressing the anterior visual pathways (Kawasaki et al., 2002), trigeminal neuralgia (Gutrecht et al., 1994), and fibromyalgia (Martenson et al., 2015).

The neuronal circuit involved in the pathophysiology of photophobia is poorly understood. However, recent evidence from animal and human studies provided some insight in possible pathophysiological mechanisms.

3.1. Mechanisms of photophobia in animal experiments

The control of light tolerance is located in a complex circuit involving the retina, the trigeminal ganglion, the periaqueductal grey matter (PAG), the rostral ventromedial medulla (RVM) and particularly the nucleus raphe magnus (NRM), the olivary pretectal nucleus (OPN), the superior salivatory nucleus (SSN), the dorsolateral thalamus (DLT) and the visual cortex (VC).

Some studies in animals have shown possible interactions between these structures during high intensity light stimulation.

In 1927, Crozier and Pincus observed that neonatal rats, even prior to the opening of their eyes, turn away from a localized light source, which he termed "negative phototaxis". Interestingly this primitive reflexive behaviour occurs at a point when the image-forming photoreceptors are not yet functional within the retina.

More recent studies have put forward possible explanations for the exacerbation of photophobia in migraine in the animal model.

Okamoto et al. (2009) submitted anesthetized rats to light stimulation while recording trigeminal nucleus caudalis neurons. They found that during light exposure firing of these neurons was increased and the response was suppressed after injection of lidocaine into the ocular globe or the trigeminal ganglion, indicating that both structures were involved in the light-evoked nociceptive discharge. They also showed that the circuit involves the olivary pretectal nucleus (OPN), and inhibition blocked completely light-evoked trigeminal nucleus caudalis neural activity and tear formation (Okamoto et al., 2010).

Further evidence comes from studies by Noseda et al. (2010). The authors injected a viral tracer into the globe of rats and found a direct connection between the intrinsic photoreceptive retinal ganglion cells (ipRGCs) and the posterior, the lateral posterior and the intergeniculate thalamus all three of which are not believed to be associated with the classical visual pathway. The same thalamic nuclei were also activated by stimulation of the dura, demonstrating convergent input from the retina and from trigeminal nociceptors. From the thalamus, afferents reach the cortex, including the visual cortex. Interestingly the posterior and lateral posterior thalami both receive direct projections from forebrain structures like the nucleus of the diagonal band of Broca, the dopaminergic cell groups of the hypothalamus, the ventromedial and the ventral tubero-mamillary nucleus of the hypothalamus (Kagan et al., 2013).

A third possible circuit excludes the role of the optic nerve to explain how the light stimulus links to the nociceptive trigeminal system. Dolgonos et al. (2011) showed that in rats after optic nerve section, the amplitude of the blink reflex remains increased during high intensity light stimulation.

In cats, light stimulation increases the response of trigeminal nociceptors in nucleus caudalis via inhibition of raphe magnus (NRM) serotonergic neurons (Lambert et al., 2008).

Concerning the role of the rostral ventromedial medulla (RVM), where the NRM is located, it has been extensively studied in pain processing since the 1980s. The RVM contains two different nociceptive cell populations: ON-cells, which enhance the perception of pain and OFF-cells that have an inhibitory control on the nociceptive information (Fields et al., 1985). A shift in the balance between these two populations can lead to increased or diminished pain. In a recent study (Martenson et al., 2015) it was found that ON-cells and OFF-cells in the RVM are also activated by light. When these authors exposed rats to light stimulation, they found that the pain threshold for heat stimuli was lowered, suggesting a pro-nociceptive effect of intense light on the pain sensation. Moreover they recorded the RVM ON- and OFF-cells during heat and light stimulation while using lidocaine to selectively block the trigeminal ganglion, the posterior thalamus or the olivary pretectal nucleus. The blocking of the trigeminal ganglion and the posterior thalamus did not affect the response of ON- and OFF-cells in the RVM, while OPN inactivation led to attenuated neuronal responses to light but not to heat.

Mice with increased sensitivity to CGRP (calcitonin-gene-related-peptide) (nestin/hRAMP1 mice) show light aversion to intracerebro-ventricular CGRP injection (Recober et al., 2009 and 2010). However, wild-type animals show the same light aversion as long as the dose of CGRP is high enough and this effect is reduced by administration of rizatriptan, showing how 5-HT1B/D agonists (triptans) can have an effect on ictal photophobia and may act by a mechanism distinct from inhibition of CGRP release (Kaiser et al., 2012).

In summary, experimental studies in animals indicate various mechanisms by which light stimulation can induce discomfort and aversion. However, these results cannot be transposed to humans without reservation because rats are more active during the

night as opposed to humans who sleep during night and in whom the circadian rhythm plays a crucial role in the homeostasis of the organism. The pathophysiology of photophobia in humans may thus be underpinned by different functional connections.

3.2. Mechanisms of photophobia in humans

In humans it is more difficult to directly test the effect of light on the descending/ascending control of pain; the integration of results from electroneurophysiology and neuroimagery can nevertheless contribute to have an overview of this matter.

During migraine attack the brainstem, in particular the dorsolateral pons is activated (Weiller et al., 1995; Bahra et al., 2001), and activation of the trigeminal system was also reported in a photophobic pain-free migraine patient (Moulton et al., 2009). A PET study conducted after the implantation of sub-occipital electrodes in chronic migraine patients, who experienced pain relief, showed activation in the rostral pons, in the anterior cingulated cortex, cuneus and left pulvinar during the electrical stimulation (Matharu et al., 2004). Furthermore in four patients who had received laser-assisted in-situ keratomileusis (LASIK) to treat myopia, with intense photophobia as a side effect, BOLD fMRI showed a greater activation of visual associative cortices during photic stimulation in the symptomatic eye compared to the non-symptomatic eye (Malecaze et al., 2001), suggesting a connection between the visual cortex and the pain networks.

That in migraine the visual cortex is hyper-responsive is supported by several studies using electroneurophysiological methods (*see Chapter 2*).

Migraineurs between attacks have more fMRI activation in the occipital cortex than controls at low and medium light intensities (Martin et al., 2011). Similarly, Boullouche et al. (2010) showed with PET that migraineurs have increased activation

of the visual cortex to light stimulation and that this activation is potentiated during painful stimulation in the trigeminal territory. Interestingly, in healthy subjects the visual cortex was significantly activated only when the subjects underwent trigeminal pain stimulation.

Vanagaite et al. (1997) have proposed convergence of retinal and trigeminal nociceptive afferents as a possible explanation for photophobia. Direct proof of their hypothesis in humans is yet to be demonstrated, but in one case where the subject was photophobic due to corneal irritation caused by contact lenses, Moulton et al. (2009) found light-induced fMRI activation of various structures of the nociceptive trigeminal pathway, including the thalamus and anterior cingulate cortex.
A reciprocal relation between visual input and trigeminal nociception is suggested by the decreased tolerance to light after painful stimulation of the ophthalmic branch of the trigeminal nerve (Drummond et al., 1993). Migraine patients display lowered pain thresholds after light stimulation (Drummond et al., 1997; Kowacs et al., 2001). In functional MRI studies, the top-down inhibitory effect of vision on pain evoked by laser-heat applied to the hand, i.e. vision-induced analgesia, is associated with lower activation in the somatosensory cortex SI and the operculo-insular cortex but not in the anterior cingulate cortex (Longo et al., 2012).

The majority of studies on photosensitivity compared healthy subjects and migraine patients, but one study also included patients affected by blepharospasm (Adams et al., 2006). The difference in light discomfort to an increased light stimulation was not significant between the two groups of patients; however, the subjective perception of discomfort, tested using a questionnaire, was higher in blepharospasm than in migraine patients. The analogy with blepharospasm is not due to ophthalmologic variation of levels of xanthophyll carotenoids lutein and zeaxanthin in the retinas (Frandsen et al., 2012), which are lower in blepharospasm patients and higher in migraine patients than in the controls.

It has been stated that the use of a questionnaire to investigate the degree of photophobia is useful in clinical practice (Choi et al., 2009). However, questionnaires are biased by subjectivity and recall bias. Direct photosensitivity assessment with a light stimulus of increasing intensities is more reliable and would allow more objective comparisons between centres and studies. Unfortunately, there is no consensus at present on which type of light stimulation should be used (Klein et al., 2015).

4. Migraine therapies

4.1. Pharmacotherapy

Migraine treatment has two facets: prophylactic or abortive. Abortive therapies used for acute treatment include simple analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and specific anti-migraine drugs such as ergots and triptans. Simple analgesics are frequently the first choice for mild to moderate attacks (MacGregor et al., 2003). Ergots have been used with some success for many years, but they may induce severe side effects and based on efficacy/adverse effect profile triptans should be preferred (Linde et al., 2006). The latter, selective 5-HT1B/D receptor agonists, have been proven effective in numerous studies (Ferrari et al., 2001 and 2004), particularly for severe attacks. Frequent use of abortive migraine medications is associated with medication-overuse headache resulting in daily or near daily headache (Linde et al., 2006). Preventative treatments include β-adrenergic blockers devoid of intrinsic sympathomimetic activity, certain calcium channel antagonists, serotonin antagonists, and the anticonvulsants topiramate and valproic acid (Silberstein et al., 2000). Most of these treatments can produce cumbersome side effects such as sleepiness, exercise intolerance, impotence, nightmares, dry mouth, weight gain, tremor, hair loss, or fetal deformities (Goadsby et al., 2006). The *Table 4.1* below, adapted from Goadsby et al. (2006), lists some of the potential side effects of commonly used preventative treatments.

Table 4.1: Side effects of pharmachological treatments for migraine prevention

Agent	Side Effects
Pizotifen (antihistamine)	Weight gain, drowsiness, fatigue, nausea, unusual weakness, dizziness, headache, dry mouth
Propranolol (β blocker)	Reduced energy, tiredness, postural symptoms, contraindicated in asthma
Tricyclics (inhibit noradrenaline and serotonin uptake)	Drowsiness
Anticonvulsants: Valproate	Drowsiness, weight gain, tremor, hair loss, fetal abnormalities, haematological or liver abnormalities
Topiramate	Paraesthesiae, cognitive dysfunction, weight loss, renal stones, glaucoma
Gabapentin	Dizziness, sedation
Methysergide	Drowsiness, leg cramps, hair loss, retroperitoneal fibrosis (1 month drug holiday required every 6 months)
Flunarizine	Drowsiness, weight gain, depression, parkinsonism

The overall efficacy rate of prophylactic migraine treatments does not exceed 60%. Other treatments have fewer side effects, but also somewhat lower efficacy rates like: riboflavin, feverfew, petasites or magnesium supplementation. Botulinum toxin type A is useful only in chronic migraine (Silberstein et al., 2002). Non-pharmacological treatments include cognitivo-behavioural therapies, massage, diets or acupuncture. Unfortunately, despite its high prevalence, migraine is frequently not diagnosed by a medical practitioner, and migraineurs therefore often resort to taking over-the-counter medications rather than prescription drugs (Silberstein et al., 2000).

4.2. Neurostimulation

Due to the inefficiency of available preventative drugs and their side effect profile, neurostimulation methods have raised great interest in recent years because of technological and scientific advances allowing, for some of them, a pathophysiologically-based rationale in headache treatment. Neurostimulation can be applied to peripheral (pericranial) nerves or to central structures (the cerebral cortex). The pivotal limitation for peripheral neurostimulation trials is the difficulty to control with sham stimulation because of the sensations caused by the real stimulation.

There are two types of neuromodulation techniques: invasive and non-invasive. Invasive methods are restricted to very disable chronic migraine patients. In this thesis we will limit the discussion to the non-invasive methods that can be used in all migraine patients.

4.2.1. Peripheral nerve stimulation

The analgesic effects of TENS (transcutaneous electrical nerve stimulation) are known since a long time (Cruccu et al., 2007), and the potential benefit of TENS in headache therapy has been proposed since 1985 (Solomon et al., 1985), but limitation in trials designs were pinpointed in a Cochrane review (Bronfort et al., 2004).

The effectiveness of a portable transcutaneous supraorbital nerve stimulator (tSNS) (Cefaly®) in episodic migraine (EM) prophylaxis was proven in a randomized double-blind sham-controlled trial (Schoenen et al., 2013) and is supported by the fact that amongst 2,313 subjects in the general population who rented the device for 60 days via the internet, 53.7% were satisfied and decided to buy it (Magis et al., 2013). Cefaly® could also be useful during the migraine attack and in chronic migraine, but RCTs are lacking.

Side effects are limited to the stimulation site and consist of paraesthesia and painful sensation with the high intensity stimulation. This is the principal reason interrupting the stimulation.

New devices thought to stimulate transcutaneously the vagus nerve (tVNS) were developed recently and their efficacy as acute and preventive treatment of primary headaches is being evaluated. Preliminary results suggest that the cervical stimulator could help some CM patients (Magis et al., 2013).

4.2.2. Repetitive transcranial magnetic stimulation (rTMS)

rTMS can induce long-lasting changes of cortical excitability: low stimulation frequencies (1 Hz) have an inhibitory effect (Chen et al., 1997) whereas high frequencies (≥10 Hz) are excitatory (Pascual-Leone et al., 1994). In healthy volunteers and migraine patients, rTMS is able to durably modify excitability of the visual cortex, and hence to reverse the abnormalities of evoked potentials found in many migraineurs (Fumal et al., 2006; Coppola et al., 2012).

In patients suffering from EM with aura, two single TMS pulses over the visual cortex within an hour after aura onset resulted in a pain-free response rate at 2 h of 39%, compared to 22% for the sham stimulation (Lipton et al., 2010).

The efficacy of rTMS for CM prevention was investigated only in a few small studies. Based on the hypothesis that the left dorsolateral prefrontal cortex (LDLPFC) is hypoactive in chronic pain disorders, Brighina et al. (2004) studied the effect of excitatory high frequency (20 Hz) rTMS over the LDLPFC in 11 chronic migraineurs. After 12 sessions of rTMS, attack frequency, headache index, and acute medication intake were reduced for up to 2 months, while there was no significant improvement in the 5 patients receiving the sham stimulation. These results were not confirmed by another study where high frequency (10 Hz) rTMS over the LDLPFC in 13 CM patients turned out to be less effective than placebo (Conforto et al., 2013).

The principal risk of rTMS is to trigger an epileptic seizure, and this risk is directly proportional to the frequency of stimulation. Subjects with a history or at risk of epilepsy have thus to be excluded from such studies, as recommended for studies on rTMS (Belmaker et al. 2003).

4.2.3. Transcranial direct current stimulation (tDCS)

tDCS uses weak currents to modify the cells' resting membrane potential, leading to focal modulation of cortical excitability. Like in rTMS, two opposite effects can be obtained: cathodal stimulation inhibits neuronal firing whereas anodal stimulation increases it. In healthy volunteers, tDCS is able to modulate resting EEG and event-related potentials (Keeser et al., 2011), and functional connectivity of cortico-striatal and thalamo-cortical circuits (Polania et al., 2011).

Anodal tDCS over the visual cortex (2 weekly sessions for 8 weeks) significantly reduces attack frequency and duration in EM (Viganó et al., 2013).

Anodal tDCS over the primary motor cortex in 13 CM patients for 4 weeks produced a beneficial delayed effect on pain intensity and duration (120 days after stimulation) that was attributed to slow modulation of central pain-related structures (Dasilva et al., 2012).

Central side effects of tDCS are not known. Mild and transient paraesthesia at the stimulation site on the scalp may occur, but blinding is usually not a problem in sham-controlled trials.

5. Aims of this thesis & hypotheses

Understanding migraine pathophysiology is crucial for progress in migraine management, since an efficient acute and preventive treatment should rely on clear pathophysiological bases. Migraine is characterized interictally by a lack of habituation of evoked responses, possibly due to a decreased preactivation level of sensory cortices. By contrast, during an attack and in chronic migraine, the preactivation level increases and habituation normalizes. New neurostimulation techniques could be useful to durably modify the activation of the underlying cortex, decreasing the repetition of attacks, giving also insight on the pathophysiology of migraine.

The visual cortex plays a pivotal role in migraine pathophysiology, but its effect on the trigeminal nociceptive system remains poorly understood. On the other hand, the migraine attack is associated with photophobia and even between attacks migraineurs are more sensitive to light, but the pathophysiological relation between migraine headache or discomfort and light stimulation is not well understood.

To clarify this features within the complexity of migraine pathophysiology and to extend our knowledge on the mechanisms of photophobia in humans, we designed experimental protocols with the following purposes:

1) To analyse the spontaneous blink rate in healthy subjects and migraine patients during and outside the attack, in a dark and in a lit room, and to understand how light, and thus activation of the visual cortex, influences spontaneous blinking.
2) To modulate visual cortex activity using rTMS in healthy subjects and interictal migraine patients, and to study the effect on activity in the trigeminal nociceptive system indexed by the nociceptive blink reflex, in order to disentangle a possible functional connection between the two structures.
3) To test the reliability of results using sham rTMS and repetitive magnetic stimulation over the greater occipital nerve in healthy subjects.

4) To compare the results on modulation of the visual cortex with those found modulating the motor cortex in healthy subjects, knowing that the motor cortex activation is implied in central pain control and a target for neurostimulation in other neurological and pain disorders.
5) To search for visually induced analgesia in the trigeminal area in healthy subjects and migraine patients using Contact-Heat-Evoked-Potentials, which could reveal a possible role of the visual cortex in the subjective perception of pain.
6) To assess photophobia using a custom-built flash light stimulator.
7) To compare the effects of changing frequency, colour and intensity of light stimulation on the nociceptive blink reflex in healthy subjects in order to find the most effective stimulation pattern on the trigeminal activity.
8) To test flash light stimulation as a possible preventive treatment in episodic and chronic migraine in a proof-of-concept trial.

Second part: Personal contribution

6. Subjects and methods

6.1. Subjects

All projects were reviewed and approved by the Ethics Committee of the CHR Citadelle Hospital, Faculty of Medicine, University of Liège, Belgium, and conformed to the Declaration of Helsinki. All participants gave their written informed consent.

Healthy subjects (HS) were recruited among the students of the Faculty of Medicine, the staff of the Citadelle Hospital of Liège and from the general public through notice-boards. They were devoid of any medical condition and had no personal or family history of neurological disorder, especially migraine and epilepsy. The same inclusion criteria were employed for the light stimulation study, in order to decrease the risk of photosensitive epileptic seizures. All subjects were adults, except in one study (*Chapter 8*) where we included two healthy participants of 14 and 16 years old, whose parents gave written informed consent.

Episodic migraine (EM) without aura patients (MO), migraine with aura patients (MA) and chronic migraine patients (CM) were recruited in our outpatient clinic and diagnosed according to the ICHD-3β criteria (2013).

The "interictal phase" was defined as the absence of a headache attack for 72 hours before and after the recordings. The latter was checked by telephone.

The "ictal phase" was defined as the presence of a headache on the day of recording or a maximum of 12 hours before the recording if the subjects used an abortive treatment. The persistence or not of the ictal phase after the recordings was also checked by telephone.

The majority of studies were conducted on EM without a prophylactic treatment. CM patients had a stable prophylactic treatment for at least one month.

All patients accurately completed their calendar, in particular during the therapeutic study.

To avoid hormonal interferences (Smith et al., 1999 and 2002; Inghilleri et al., 2004), women were recorded outside of menses. We checked for use and type of birth-control pill.

6.2. Materials and methods

6.2.1. Spontaneous blink rate (SBR)

Blinking protects the conjunctiva from drying and other possible injuries. External conditions such as humidity and fumes from smoking increase the blink rate (Ponder et al., 1928; Karson et al., 1988). Humans have an average rate of approximately 14-19 blinks per minute when looking straight ahead (Doughty et al., 2001; Karson et al., 1981), and make about 14,000 spontaneous blinks during a waking day.

Variation in the SBR may be due to an ophthalmic cause: the blink rate increases with ocular irritation and decreases with corneal anaesthesia (Ponder et al., 1928; Tsubota et al., 1995 and 1996; Nakamori et al., 1997; Zaman et al., 1998; Schlote et al., 2004; Naase et al., 2005; Borges et al., 2010), but corneal and conjunctival anaesthesia does not eliminate spontaneous blinking (Naase et al., 2005), suggesting central physiologic mechanisms.

The SBR is considered to be an indicator of dopaminergic activity and its regulation differs depending on pathological condition. Reduction in the SBR has been found in Parkinson's disease (Karson et al., 1982), in progressive supranuclear palsy (Pfaffenbach et al., 1972), and in subjects taking dopamine receptor blockers (Karson et al., 1981). An increase of blinking has also been noted amongst some patients with Huntington's disease (Karson et al., 1984) or blepharospasm (Karson et al., 1988 and 1984; Valls-Sole et al., 1991).

The anatomical pathways and physiological connections involved in the central control of blinking include the parapontine reticular formation and the lateral geniculate nucleus, with a facilitatory effect on SBR (Karson et al., 1988; Cohen et al., 1968). The cerebellum seems to inhibit blinking (Karson et al., 1988), and evidence comes from the fact that after removing the cerebellum the SBR increases in rats (Karson et al., 1984).

The basal ganglia play a pivotal role in regulating the frequency of eye blinks (Karson et al., 1983), confirmed by a decrease of the SBR in patients with Parkinson's disease in which the diminished SBR correlates with the duration of the disease (Karson et al., 1982).

Cortical processes are also involved because cognitive states modify the SBR: the number of blinks while silent is a mean of 19 per minute in healthy subjects; it can increase during speech or listening to 24-27 per minute and decrease during reading to 12 per minute (Karson et al., 1981). Moreover, the SBR can be modulated by task demand (Fogarty et al., 1989), mental and visual workload (Fournier et al., 1999; Veltman et al., 1998) and position of gaze (Cho et al., 2000).

In animals the SBR is lower in nocturnally than in diurnally active animals, being one-tenth lower in nocturnal versus diurnal mammals and birds (Stevens et al., 1978; Tada et al., 2013).

Vertical and horizontal electro-oculograms (EOG) were recorded on a Viking and Synergy EMG system. Ag-AgC1 electrodes were placed 3 cm above and 2 cm below the subject's right eye (Barbato et al., 1993 and 2000). Eye blink was defined as a sharp, high-amplitude wave ≥100μV and < 200 ms in duration. For the eye blink recording, subjects were asked to sit silently in front of a blank, neutral wall. Each subject had 3 minutes to adjust to the recording environment. SBR was defined as the number of blinks in one minute following a 3-minute adjustment period of which the subjects were unaware (Barbato et al., 1993).

6.2.2. Nociceptive blink reflex (nBR)

The blink reflex (BR) is a brainstem reflex and its advantage is to provide valuable information on the functional integrity of the brainstem through the afferent and efferent pathways.

In clinical practice BR recording is helpful to exclude structural lesions or to localize more accurately lesions within the brainstem. In the extreme case, the reflex can be abolished due to structural abnormalities, such as tumours or infarcts of the brainstem. Studies of the various components of the BR provide information about segmental and supra-segmental control mechanisms and may help to differentiate between the segmental and supra-segmental origin of abnormalities.

The BR was described for the first time by Overend in 1896 by tapping one side of the forehead. Kugelberg (1952) analyzed the blink reflex electromyographically by electrically stimulating the supraorbital nerve. The best response is produced when the subject is alert, when the stimulus is delivered at intervals of 7 seconds or longer (Boelhouwer et al., 1977) and when it is recorded simultaneously over the inferior portion of the right and left orbicularis oculi muscle.

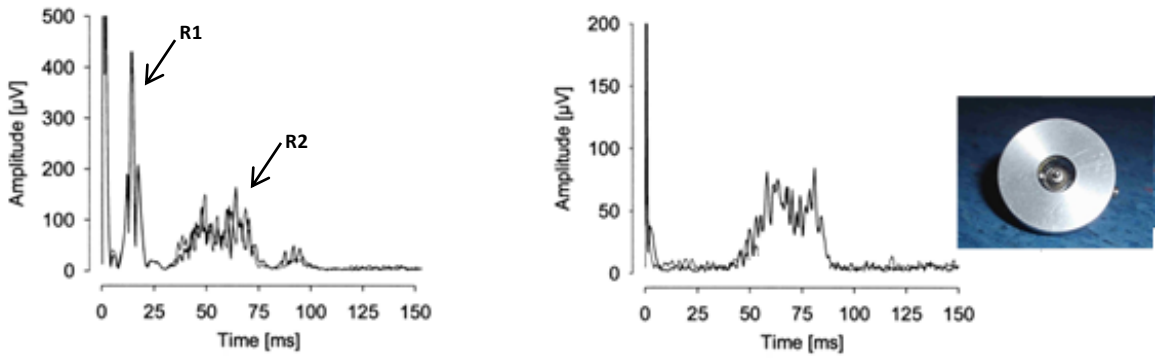
The afferent limb is located in the ophthalmic division of the trigeminal nerve (Kugelberg et al., 1952; Cruccu et al., 1987) and the efferent limb in the facial nerve.

The electrical stimulation of the supraorbital nerve elicits two responses: the first or early response, R1, is a brief ipsilateral response that occurs with a latency of about 10 milliseconds (ms); the second or late response, R2, has a latency of about 30 ms and occurs bilaterally (*Fig. 6.1*). The R1 response is regarded as delayed if its latency exceeds 13 ms and R2 is regarded as delayed if its latency exceeds 41 ms. A latency difference between the two sides exceeding 1.5 ms for R1 and 5.0 ms or 8.0 ms for R2 is also considered abnormal (Kimura et al., 1969; Ongerboer de Visser et al., 1974).

Afferent impulses for the R2 response run through the descending spinal tract of the trigeminal nerve in the pons and the medulla oblongata before they reach the caudal spinal trigeminal nucleus (Kimura et al., 1972; Ongerboer de Visser et al., 1978). From

there, impulses are relayed to the facial nuclei in the pons. The R2 response, ipsilateral to the electrical stimulation, originates at the level of the medulla oblongata and the contralateral one in an ascending trigeminofacial connection that crosses the midline at the level of the lower third of medulla oblongata (Aramideh et al., 1997).

Figure 6.1: Exemple of a nBR elicited with a routine bipolar electrode (left) and with the concentric electrode (insert) (right)



In clinical practice, the supraorbital routine stimulation electrode activates Aβ, Aδ and probably C fibers and elicits two components, an early R1 and a late R2. We were only interested in the nociceptive component of the BR (nBR), i.e. R2, and used therefore a custom-built stimulation electrode.

During BR recordings, subjects were asked to relax in a comfortable armchair in an illuminated room and to keep their eyes open.

The nociceptive-specific blink reflex (nBR) (*Fig. 6.1*) was elicited according to the method described by others (Kaube et al., 2000; Katsarava et al., 2002), before and immediately after the neuromodulation session.

We used a custom-made planar concentric electrode (central cathode: 1 mm D; insert: 8 mm; anode: 23 mm OD) placed on the forehead close to the supraorbital foramen on the right side. The concentric electrode has the advantage of preferentially exciting

Aδ fibres (Kaube et al., 2000; Katsarava et al., 2002; Di Clemente et al., 2005 and 2007), but at the same time C-fibres and Aβ fibres may also be recruited (de Tommaso et al., 2011).

Recording electrodes were placed below the orbit (active) over the orbicularis oculi muscle and lateral to the orbit (reference) on both sides. A ground electrode was placed at the root of the nose. The signal was recorded with a sampling rate of 5000 Hz and sweep duration of 150 ms (1401, Signal Averager, Cambridge Electronic Design).

The electrical stimulus consisted of monophasic square pulses with duration of 0.2 ms. We first determined perception and pain thresholds by using ascending and descending sequences of 0.2 mA intensity steps.

To elicit the nBR, the final stimulus intensity was set at 1.5 times the initial individual pain threshold. Interstimulus intervals varied pseudo-randomly between 15 and 17 s. We recorded 16 rectified EMG responses that were averaged off-line. As previously described, the first response of each nBR recording session was excluded from the signal analysis to avoid contamination with startle responses (Kaube et al., 2000; Di Clemente et al., 2005 and 2007). The remaining 15 sweeps were averaged in 3 sequential blocks of 5 responses. For each averaged block, the amplitude of the R2 reflex was expressed as its area under the curve (AUC). To minimize R2 AUC variability due to inter-individual threshold differences we used the ratio between the area and the square of the stimulus intensity (AUC/i2) to express nBR amplitudes, as recommended by Sandrini et al. (2002). Habituation of the nBR R2 was defined as the percentage change of the R2 area between the 1st and the 3rd block of averages or as the slope of R2 area changes over the three blocks.

6.2.3. Visual evoked potentials (VEP)

We studied broadband VEP elicited by reversal of a checkerboard pattern (B-B PR-VEP) and digitally filtered between 1 and 35 Hz (Barlett-Hanning window, 701 filters' coefficients).

The VEP is composed of three peaks, identified according to their respective latencies (*Fig. 6.2*): N1 is the most negative peak between 60 and 90 ms after the stimulus, P1 the most positive peak following N1 at a latency of 80–120 ms and N2 the second negative peak between 130 and 160 ms. The peak-to-peak amplitude of N1–P1 and P1-N2 was measured.

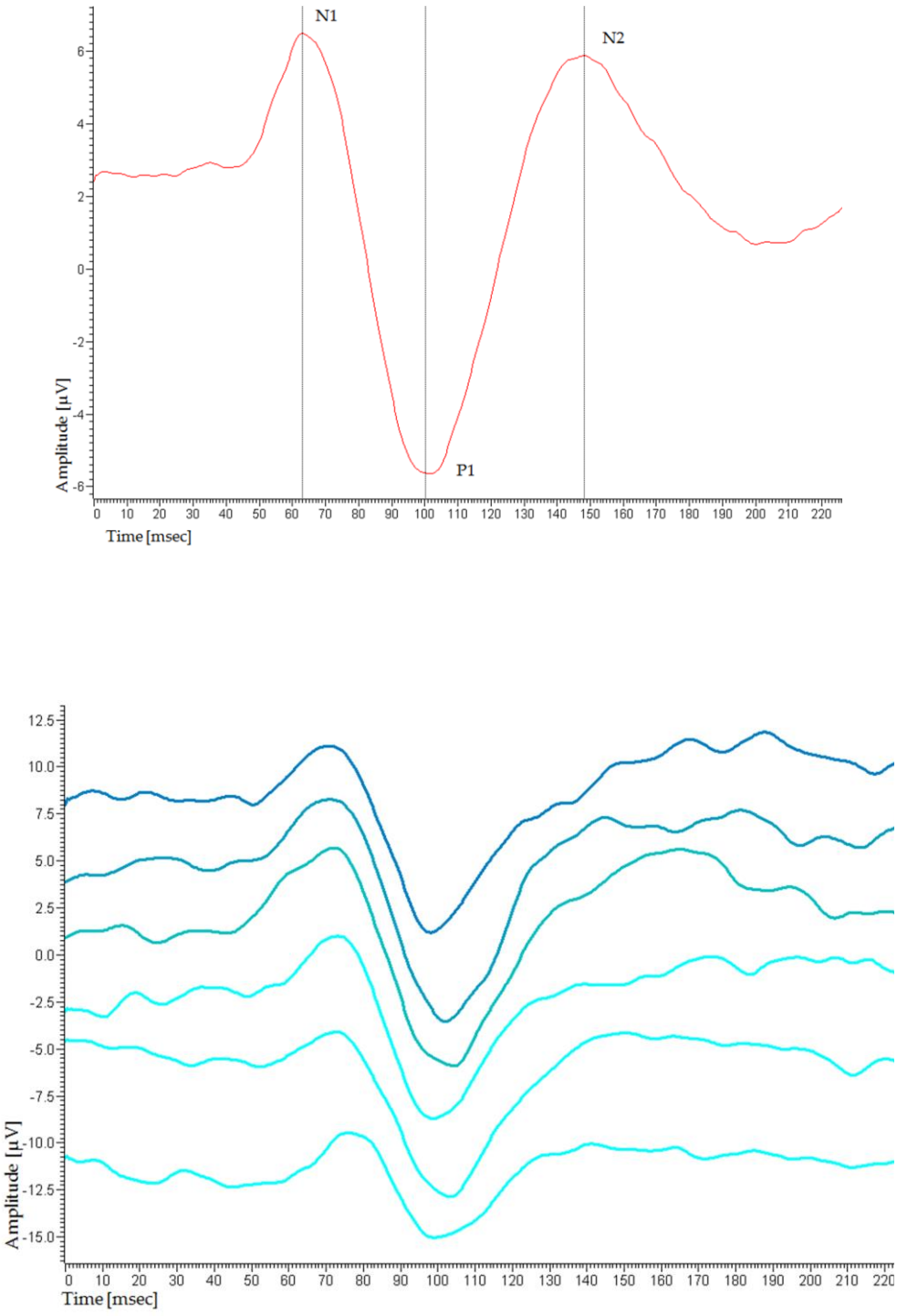
Subjects were seated in a semi-dark acoustically-insulated room in front of the display surrounded by a uniform field of luminance of 5 cd/m². Prior to the recording, each subject was allowed to adapt to the ambient light in the room for 10 minutes to obtain a constant pupil diameter. Stimulation was monocular (right side) after occlusion of the other eye. Visual stimuli consisted of full-field checkerboard patterns (contrast 80%, mean luminance 250 cd/m2) generated on a TV monitor and reversed in contrast at a rate of 3.1/s. At the viewing distance of 80 cm, the single check edges subtended 15 min of visual angle. Subjects were instructed to fix their gaze upon a red dot in the middle of the screen with the left eye covered by a patch to maintain stable fixation. The bioelectric signal was recorded from the scalp by means of pin electrodes positioned at Oz (active electrode) and at Fz (reference electrode, 10/20 system); a ground electrode was placed on the right forearm.

The evoked potential signals were amplified by CEDTM 1902 preamplifiers (band-pass 0.05–2000 Hz, Gain 1000) and recorded by a CEDTM 1401 device (Cambridge Electronic Design Ltd, Cambridge, UK). During uninterrupted stimulation 600 sweeps of 200 ms duration were sampled at 4000 Hz.

The recordings were divided into six sequential blocks of 100 responses (*Fig. 6.2*), of which at least 85 artefact-free sweeps were averaged off-line ('block averages') using the Signal™ software package version 4.11 (CED Ltd). Habituation was defined as the

percentage change of N1-P1 or P1-N2 amplitudes between the 1st and the 6th block of averages or as the slope of amplitude changes over the six blocks.

Figure 6.2: Exemple of a VEP recording composed of 600 average responses (above) and the recordings of 6 successive blocks of 100 responses showing habituation (below)



6.2.4. Contact-heat evoked potentials (CHEPs)

The use of contact heat has several unique advantages for pain research (Chen et al., 2001). It activates small-calibre noxious thermal afferents, in the C-fibre range, in addition to mechano-thermal Aδ-fibres. It elicits a diffuse nagging pain at a sufficient intensity. Sometimes, it can produce double pain sensations: that is to say both sharp and dull pain (Magerl et al., 1999; Price et al., 1996).

The reliability and suitability of CHEPs to study the function of small fibres have been well documented (Itskovich et al., 2000; Chen et al., 2001; Valeriani et al., 2002; Granovsky et al., 2005).

The so-called CHEPs device (Medoc Ltd., Ramat Yishai, Israel) is composed of a thermode, used to elicit heat pain, applied to the skin and covering a cutaneous area of 572.5 mm^2 (diameter 27 mm). The CHEPs thermode is comprised of two layers of stimulators working together. The external layer consists of a heating foil, and the lower layer is a Peltier element with two thermistors (electronic thermal sensors). The heating thermofoil (Minco Products, Inc., Minneapolis) is covered with a 25-μm layer of thermo-conductive plastic (Kapton® [DuPont, Wilmington, DE]; thermal conductivity at 23°C of 0.1–0.35 W/m/K) that separates the external foil from the skin. Two thermocouples (electronic thermal sensors) are embedded at 10 μm within this conductive coating, which comes into direct contact with the skin, thus providing an estimate of skin temperature at the thermode surface (Valeriani et al., 2002; Granovsky et al., 2005). The external thermofoil allows the thermode to reach a very rapid heating rate of 70°C/s, and the Peltier element allows the CHEPs thermode to reach a fast cooling rate of up to 40°C/s. It has a temperature range of 30-55°C. Cooling begins immediately after the thermode reaches its target stimulus temperature, which is set by the manufacturer's algorithms.

Subjects were seated in a comfortable armchair, and we tried to avoid interference with photophobia by dimly lighting the room.

The device allowed us to keep a constant baseline skin temperature of 35°C. Twenty brief heat stimuli were delivered (peak set at 53°C, the increment speed was of 70°C/s whereas the decrement was of 40°C/s, for a total stimulus duration of 707 ms) with a randomized interstimulus interval of 10-22 seconds. The evoked cortical responses to heat, i.e. the CHEPs themselves, were recorded using pin-electrodes: the active electrode was inserted at Cz and was referenced to Fz (according to the 10–20 system as aforementioned), with a band pass of 0.15-100-Hz (CED™ 1902 preamplifier and CED™ Micro1401 converter; Cambridge Electronic Design Ltd, Cambridge, UK). The ground electrode was fixed to the right hand. The impedance for all electrodes was kept below 5 kΩ. Twenty responses were averaged off-line and partitioned into 5 blocks of 4 responses using Signal™ software version 4.11 (Cambridge Electronic Design Ltd). The latencies (in ms) and the Area under the curve (AUC) P1-P2 (in μVxms) of each block were measured. P1 is the first most positive point around a latency of 200 ms for the wrist and 150 ms for the face, N2 the following negative peak around 280 ms for the wrist and 250 ms for the face and P2 was the second most positive point around 400 ms for the wrist and 350 ms for the face. Habituation was defined as the AUC change of P1- P2 over the five successive blocks and the slope of the linear regression line of amplitude changes for the five blocks. An analysis in percentage between the 5th and the 1st block of 4 sweeps was also performed to measure habituation.

6.2.5. Repetitive transcranial magnetic stimulation (rTMS)

We used a Magstim Rapid magnetic stimulator (Magstim Co. Ltd, Whitland, Dyfed, UK), connected to a 2 x 7 cm figure-of-eight coil, with a maximal stimulator output of 1.2 T.

Using single pulses over the visual cortex, we first identified the phosphene threshold, defined as the lowest stimulation intensity (expressed as a percentage of

the maximal stimulator output) able to evoke phosphenes in at least three out of five pulses (Bohotin et al., 2002).

The coil was placed in a vertical position (its handle pointing upward) on the inion-nasion line, with its inferior limit 1 cm above the inion. Stimulation was applied initially at 30% of stimulator output. The intensity of the stimulation was increased by 2%-steps until the subject reported phosphenes. Increasing and decreasing the intensity in 1%-steps then refined the threshold.

In participants who did not report phosphenes at the 100% intensity level, the procedure was repeated with the coil placed 1 or 2 cm higher or lower and, if necessary, to the right or to the left, before accepting the absence of phosphenes. In this case, we placed the coil over the left motor area and determined the motor threshold. In accordance with recommended safety guidelines (Chen et al., 1997), stimulus intensity was set to the phosphene threshold (PhT) or to 110% of the motor threshold, if no phosphenes were elicited.

We used two different stimulation frequencies in a randomised order: 1 Hz (low frequency rTMS) and 10 Hz (high frequency rTMS) with at least a 24 hour-interval between the 2 sessions, as recommended by others (Wu et al., 2000). 1 Hz rTMS was applied in a single train without interruption for 15 minutes. 10 Hz rTMS was applied in 20 trains of 40 pulses with inter-train intervals of 10 seconds. For both frequencies the same number of 800 pulses was delivered.

6.2.6. Transcutaneous occipital nerve stimulation (tONS)

tONS was performed using a Cefaly® device with suboccipital stimulation electrodes. The device attains a maximal intensity of 20 mA, but due to the progressive increase in intensity at the beginning of the stimulation, the mean intensity was 17.4 mA corresponding to an electric charge of 1.56 microCoulomb (μC). The frequency of stimulation was 100 Hz.

6.2.7. Flash light stimulator

A microflash MF 9607178 stimulator (Micromed & Co., Mogliano Veneto, IT) for flash light stimulation was placed in front of the subjects at a distance of 15 cm; they were asked to look at the stimulator throughout the whole session. The stimulation was at 27.8 lux (0.63 cd/m^2) and the flash colour was yellow.

To minimize any attenuation of light perception due to continuous stimulation without spatial or temporal contrast (Chapman et al., 1991; Hubel et al., 1990), the flash frequency was set at 8 Hz for 4 minutes in a quiet room with dimmed light.

6.3. Data processing and statistical analysis

All statistics were performed using STATISTICA for Windows version 8.0 (StatSoft, Inc. Tulsa, OK, USA). Wilcoxon's test was applied to compare the differences between pre- and post-stimulation. Mann-Whitney's test was used to compare the differences between groups. Spearman's test was used for the correlation analysis. All results were considered significant at the 5% level ($p < 0.05$).

7. Variation of the spontaneous blink rate (SBR) in light and dark in ictal and interictal episodic migraine patients compared to healthy subjects

7.1. Introduction

The first step for this thesis was to measure the spontaneous blink rate (SBR) in healthy subjects and migraine patients during the ictal and interictal phase, in a lit or dark environment.

The SBR had not been measured in migraine patients before. It is known that its variation principally relies upon a dopaminergic pathway (Karson et al., 1982) and there is circumstantial evidence for a role of dopamine in migraine pathophysiology (Charbit et al., 2010; Barbanti et al., 2013). The modulation of SBR is also dependent on cortical and subcortical controls, in which the occipital cortex may play a role. In fact, it has been shown that a visual task diminishes the SBR in healthy subjects (Karson et al., 1983) and the decrease is proportional to the difficulty of the task (Phelps et al., 1981). The involvement of the visual cortex in migraine pathophysiology is suggested by numerous studies using electrophysiology or neuroimaging as well as animal models (*see Chapter 2*).

In this study we searched for modulation of the SBR in the presence/absence of light.

7.2. Subjects and methods

We enrolled a total of 38 subjects:

- 7 healthy subjects (HS) (4 females and 3 males, mean age 38.42 ± 12.23 [SD] years old), without any familiar of personal history of headache and without any other neurological disease.

- 12 interictal episodic migraineurs (EM) without any prophylactic treatment (9 females and 3 males, mean age 27.08 ± 14 [SD] years old; 7 patients suffering from migraine without aura). They had a mean of 4.2 ± 4.18 [SD] headache days per month, duration of each attack of 39 ± 31.18 [SD] hours and a disease history of 15.8 ± 15.28 [SD] years.
- 10 ictal EM without any prophylactic treatment (8 females and 2 males, mean age 34.4 ± 8.2 [SD] years old; 8 patients suffering from migraine without aura), with a mean of 7.2 ± 3.93 [SD] headache days per month, a duration of each attack of 32.4 ± 17 [SD] hours and a disease history of 18.5 ± 17.63 [SD] years.

For more details on the subjects' recruitment see *Chapter 6.1.*

The SBR was measured as described in *Chapter 6.2*, in a room lit at a luminance intensity of 145 Lux or in almost total darkness, 12 Lux (*Fig. 7.1*). The low level of persistent light was due to the screen of the recording device itself.

The subjects were in a seated position and asked to relax in particular their jaw muscle in order to avoid chewing or swallowing artefacts. They were also instructed to fix their gaze on a point in front of them on a neutral wall. After 3-minutes of adjustment to allow the subjects to get used to wearing electrodes around the right eye, we started the recordings. The latter lasted 2 minutes (without artefacts) or longer if muscle artefacts were present. Thereafter the lights were turned off. An adaptation period of 3 minutes followed, during which the subject was allowed to reposition, to chew and to swallow. When the subject was ready, instruction was given to relax and the recording in the dark began. For the recording in the dark we employed the same timing as during light.

7.4. Results

In the lightened ambiance the SBR in HS was 15.7 ± 8.8 [SD] per minute, in interictal EM patients 23.5 ± 15.8 [SD] per minute and in ictal EM patients 21.3 ± 9.75 [SD] per minute (*Fig. 7.2*). There was no statistical difference between groups at baseline.

By contrast, in the dark we counted 10.6 ± 6.9 [SD] blinks/minute in HS, 22.6 ± 13.1 [SD] blinks/minute in interictal EM and 16.4 ± 10.1 [SD] blinks/minute in ictal EM. We found a significant difference between HS and interictal MO ($p=0.05$), but not between interictal and ictal MO (*Fig. 7.2*).

The percentage of variation between light and dark was -36.71 ± 22% [SD] in HS; 1.9 ± 43.98% [SD] in interictal EM and -18.7 ± 34.74% [SD] in ictal EM. There was thus a decrease of SBR in the dark in HS and ictal EM. Instead, in interictal EM the modulation induced by the presence/absence of the light was minimal. The difference was significant in HS ($p = 0.017$).

Figure 7.1: Examples of SBR recordings in a healthy subject and an ictal migraine patient in lightened and dark room.

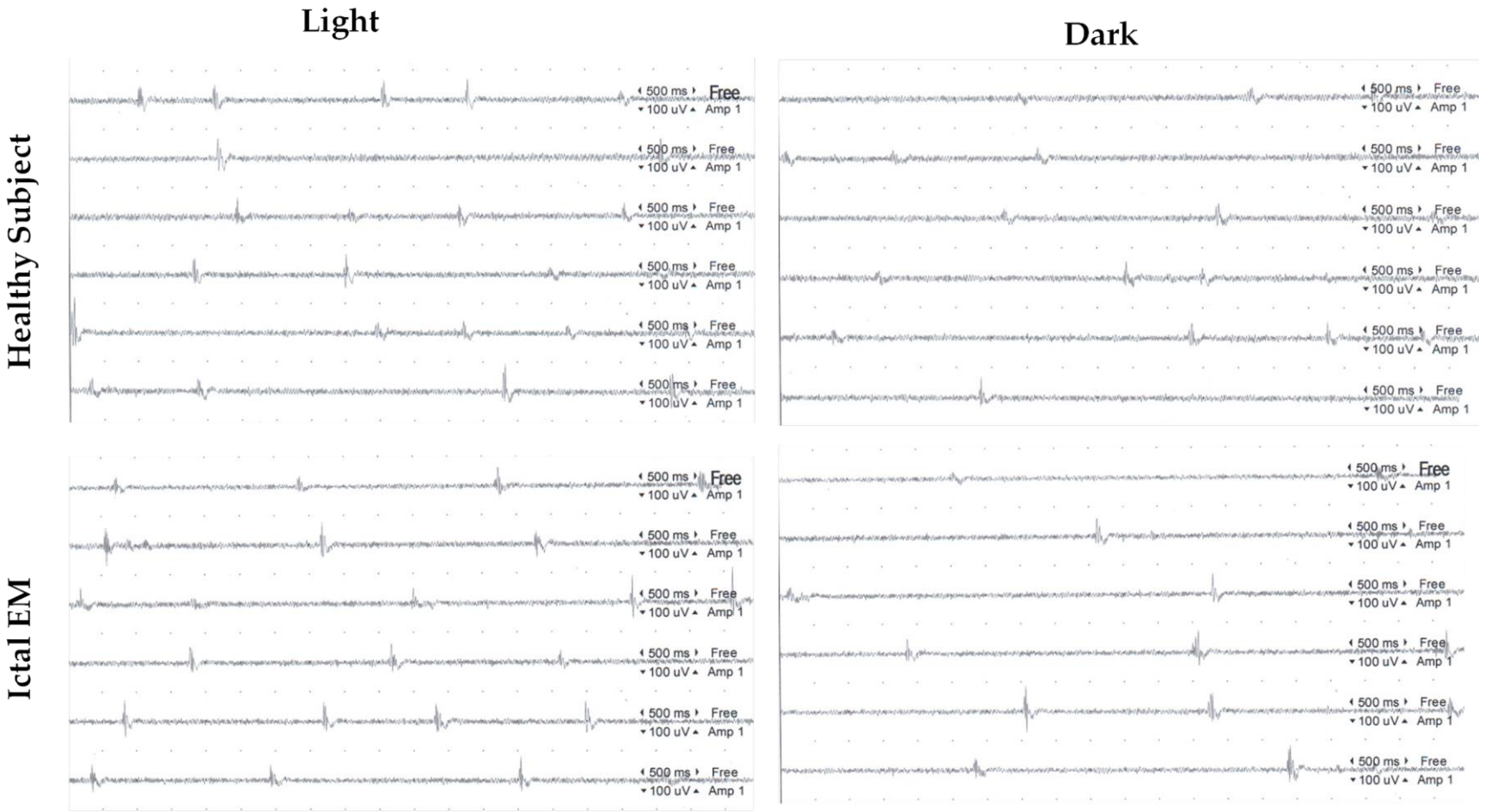
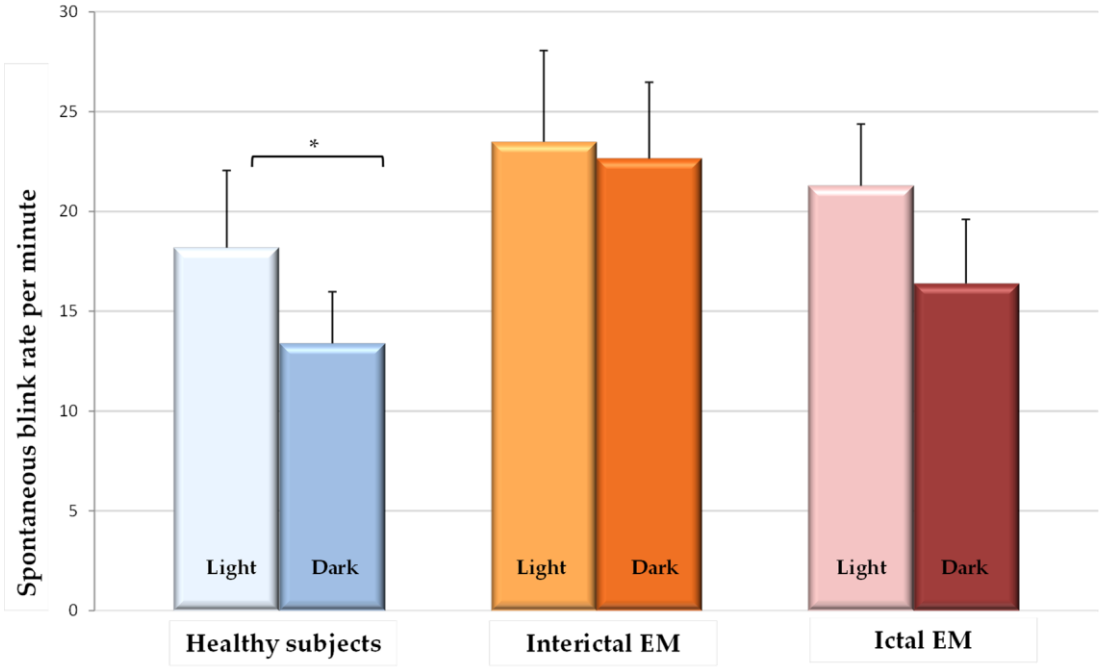


Figure 7.2: Spontaneous blink rate (SBR) in healthy subjects (blue), interictal episodic migraineurs (EM) (orange) and ictal EM without a preventive treatment (rose), in a lightened ambiance (light colour) and in the dark (dark colour). The SBR decreased significantly in the dark in HS, but not in interictal EM.



7.5. Discussion

The principal result of this study is that the SBR is not different between HS and EM in a lit environment, but in the dark the SBR decreases both in HS and in ictal EM patients, while in interictal EM patients there was no change.

In humans, as in other diurnal species, in which the circadian rhythm plays a pivotal role in guaranteeing the homeostasis of the organism, the SBR decreases in the dark, as found in our healthy subjects. There may be several explanations for this finding: 1) in the dark the attention of the subject is heightened due to a possible feeling of threat that the subject attempts to detect by increased concentration on the environment; 2) in the dark the SBR can also be decreased because of lowered corneal fatigue and dryness; 3) the dark is associated in humans with being a less active period and it is not necessary to excite the visual pathways by frequent blinking.

Spontaneous blinks are influenced by several factors, engaging peripheral and central factors. Peripheral factors are essentially ophthalmic causes for increasing or decreasing SBR (*see Chapter 6.2.1*). Our subjects were free of any ophthalmic disease. Hence the results are probably related to central mechanisms.

The generators of spontaneous blinking are located in the pons: a study conducted on subhuman primates demonstrated a triphasic discharge occurring in the pontine reticular formation (PRF) before each spontaneous blink (Cohen et al., 1968). From this region, the information is transmitted to the orbicularis oculi muscles via the 7th cranial nerve; the evidence for this is that unilateral facial palsy abolishes ipsilateral blinks.

However, an afferent contingent is also sent to the lateral geniculate nucleus (LGN) that discharges after a blink (Cohen et al., 1968).

Substantia nigra (SN) is certainly involved in the generation and maintenance of a normal SBR and the evidence comes from studies in Parkinson's disease patients (Karson et al., 1984), in which the number of blinks per minute is lower compared to healthy controls. SN seems to facilitate blinking, and the cellular loss in this region, as

in Parkinson's disease, is one of the causes of a decrease in the SBR (Adams et al., 1981).The fact that we found no difference in SBR between migraineurs and healthy subjects does not favour a significant failure of dopaminergic mechanisms in migraine.

Also gliosis in the periaqueductal grey matter (PAG), in the superior colliculus and in the pretectal area may cause decreased blinking as seen in progressive supranuclear palsy (PSP) (Adams et al., 1981). Among these structures the superior colliculus is strongly implicated in visual and visuo-motor functions (Denny-Brown et al., 1976). Its involvement is demonstrated by the fact that "nystagmus retractorius", due in the majority of cases to a pineal mass lesion, is associated with a decrease in the SBR.

The cerebellum acts as an inhibitory control on the SBR: cerebellectomized rats have an SBR four times higher than that of controls (Freed et al., 1981).

The occipital cortex is believed to be involved in the SBR due to the fact that blinking is reduced during visual fixation and increased during a visual task (Phelps et al., 1981). Moreover, the decrease is more significant if the visual task is more difficult. The reciprocal relation also exists: during blinks the electrical visual activity in the occipital cortex is suppressed (Volkmann et al., 1979; Buisseret et al., 1982).

A functional link between the EEG alpha activity in the occipital cortex and the SBR may exist (Karson et al. 1990). Studies report an inverse correlation between the SBR and alpha EEG power measured in the occipital cortex; in fact after sleep deprivation subjects exhibited a decrease in alpha EEG and an increase in the SBR (Barbato et al. 2000).

Stevens et al. (1978) hypothesized that blinking during waking and rapid eye movements (REM) during sleep may both serve to periodically excite the visual pathway through the contrast produced by opening and closing the eyes. This is supported by the fact that diurnal species exhibit an increased SBR and decreased REM duration whereas nocturnal species have increased REM duration and decreased SBR (Stevens et al., 1978). Another study supports this relation (Doughty et al., 2013): a progressive increase of luminance (the dark in this study was not tested)

does not significantly affect the SBR, while a sudden increase in luminance doubles the SBR. This may be due to the induction of a transient photophobic reaction.

Interestingly, in our study, interictal and ictal migraineurs, of whom the latter are known to be the most photophobic, have different responses: during attacks migraineurs react like HS whereas between attacks they display a SBR decrease during the dark session.

The observation that functional alterations, found during the interictal phase of migraine, normalize during the ictal phase is not new. It is well documented that another abnormality found in migraine during the interictal phase, the deficit of habituation of cortical evoked potentials, is normalized during the migraine attack (Afra et al., 2000). However, the two phenomena are thought to involve different pathways and different networks as explained above (*see also Chapter 2*).

The possible relation between the SBR and EEG activity in the occipital cortex (*see above*) is reminiscent of one of the first EEG abnormalities described in migraineurs: the so-called "H response", i.e. an increased photic driving at high flash stimulation frequencies (Golla et al., 1959; Fogang et al., 2015). This response is more pronounced during the ictal phase than in the interictal phase (Bjørk et al., 2011).

To sum up, our results showing that the SBR is higher in the dark in migraine patients between attacks compared to healthy subjects confirms that the visual cortex is dysfunctioning in the interictal phase, but tends to react normally during the migraine attack.

8. Effects of visual cortex modulation by repetitive transcranial magnetic stimulation on the nociceptive blink reflex in healthy subjects and in migraine patients

8.1. Introduction

In the previous study we explored the functional relation between the occipital cortex and the brainstem nuclei involved in spontaneous eye blinking and its difference between HS and EM patients. In the present experiment we focus attention on the relation between the visual cortex and more specifically the nociceptive trigeminal system.

This study aims at answering the following questions:

1. Does the visual cortex modulate the subjective perception of pain in the trigeminal area and/or the nociception-specific blink reflex in healthy subjects?
2. Is the modulation of trigeminal nociception by the visual cortex different in migraine patients?

8.2. Subjects and methods

a) rTMS studies in healthy subjects

We evaluated the subjective perception of pain in the first division of the trigeminal nerve, the ophthalmic nerve, using electrical stimulation applied to the right supraorbital area. Both the sensory threshold (ST) and the pain threshold (PT), defined as the moment where the sensation became uncomfortable and/or painful, were measured.

In addition, we measured the nBR, an objective index of a reflex activity in the trigeminal nociceptive system.

Subjective and objective measurements were performed before and immediately after applying the rTMS at low or high frequency over the visual cortex in two different sessions. Low frequency rTMS (0.5-5 Hz) is supposed to have an inhibitory effect on the cortex while high frequency rTMS (> 5 Hz) is excitatory (Chen et al., 1997). The stimulation intensity was set to the phosphene threshold or to the 110% of motor threshold if the subject did not report phosphenes.

More detailed information on the methods can be found in *Chapters 6.2.2 and 6.2.5*.

We recruited 21 HS (12 females, 9 males, mean age 25.9 ± 8.03 [SD] years old), all naïve for rTMS. For more details on HS enrolling *see Chapter 6.1.*

b) Control studies in healthy subjects

In order to verify that the effects observed after rTMS were due to a genuine modulatory action on the visual cortex and not to a placebo effect or to possible activation of superficial nerves or muscles, we performed a series of control experiments.

For these experiments, we enrolled 30 HS who were partitioned in three different sessions:

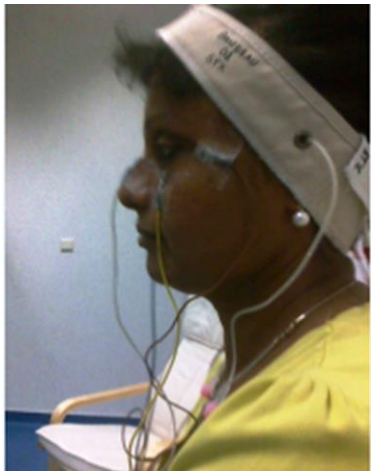
- 13 HS (8 females, 5 males, mean age 25.38 ± 11.18 [SD] years old) received a 10 Hz rTMS sham stimulation over the occipital cortex;
- 7 HS (5 females, 2 males, mean age 29 ± 10.59 [SD] years old) received repetitive magnetic stimulation (rMS) over the greater occipital nerve;
- 10 HS (6 females, 4 males, mean age 25.5 ± 10.21 [SD] years old) underwent to a session of 1h of tONS.

First we used a sham stimulation paradigm. 10 Hz rTMS sham stimulation was delivered with the coil placed at a 90° angle to the occipital region, with its anterior border pressed against the scalp. The rTMS intensity was fixed at the intensity of the phosphene threshold or 110% of the motor threshold. Twenty trains of 40 pulses with an inter-train interval of 10 seconds were delivered for 5 minutes. In the sham situation, there is an acoustic perception of the stimulation, but no brain activation occurs (Klein et al., 1999).

In the 2nd experiment we applied rMS over the right greater occipital nerve. We performed 1 Hz and 10 Hz rMS over the right GON by placing the figure-of-eight coil over the emergence of the GON just beneath the superior nuchal line. We considered as optimal the location where the sensation induced by the magnetic pulse radiated to the parietal region of the head. To make a comparable control protocol, the patterns of 1 Hz or 10 Hz stimulation were the same as those applied over the visual cortex (*see Chapter 6.2.5*).

In the 3nd control experiment we used transcutaneous occipital nerve stimulation (tONS) (*see Chapter 6.2.6*). tONS was performed using a Cefaly® device (*Fig. 8.1*), applied to the occipital region. The device attains a maximal of intensity of 20 mA, but due to the progressive increasing in intensity at the beginning of the stimulation, the mean of intensity received was of 17.4 mA and a dose of stimulation of 1.56 microCoulomb (μC). The frequency of stimulation was of 100 Hz.

Figure 8.1: tONS installation



Before and immediately after each type of stimulation we measured sensory and pain thresholds in the supra-orbital area and the nBR (*see Chapter 6.2.2*).

c) rTMS studies in migraine patients

We recruited a total 32 episodic migraine patients:

- 23 migraine without aura patients (MO) (14 females and 9 males, mean age 29.08 ± 9.39 [SD] years old).
- 9 migraine with aura patients (MA) (5 females and 4 males, mean age 30.33 ± 8.77 [SD] years old)

All patients in this experiment were recorded in the interictal phase (*see Chapter 6.1*). *Table 8.1* summarizes the demographic characteristics of included patients.

Table 8.1: Demographic characteristics of included subjects. Mean ± SD

Demographic data of episodic migraine patients		
	rTMS 1 Hz and 10 Hz	
	MO (n=23, 14F/9M)	**MA (n=9, 5F/4M)**
Mean age (years)	29.08 ± 9.39	30.33 ± 8.77
Attacks frequency/month (n)	4.10 ± 2.55	1.28 ± 0.92
Attacks duration/attack (h)	34.60 ± 23.98	8.55 ± 10.51
Disease hystory (years)	10.63 ± 8.74	6.11 ± 2.61

We used the same protocol as in HS to measure trigeminal pain perception and nBR before and after 1Hz or 10Hz rTMS over the visual cortex.

8.3. Results

a) rTMS studies in healthy subjects

The results are synoptically presented in *Table 8.2*.

During TMS over the visual cortex 12 participants out of 21 (57.14%, 3 males and 12 females) reported phosphenes. The phosphene threshold (expressed as a percentage of the maximal stimulator output) was 66 ± 4.7% [SD]. The motor threshold was determined in the remaining 9 participants (42.86%, 7 males and 2 females) and was 58 ± 8% [SD] of the maximal stimulator output.

We observed a significant relation between the presence of phosphenes and female gender (*p=0.04*). There was no correlation between intensity of rTMS and the effect on the nBR.

After 1 Hz rTMS over the visual cortex, the supraorbital pain threshold was significantly decreased (*p=0.001*) (*Fig.8.2*), while the sensory threshold remained unchanged.

Moreover, 1 Hz rTMS significantly increased amplitude of the 1st nBR block expressed as AUC/i2 both ipsi- and contralaterally to the supraorbital stimulation (*p=0.024* and *p=0.036* respectively) (*Fig. 8.3*). By contrast, habituation was significantly potentiated contralaterally to the stimulated side (*p=0.0002*) (*Fig 8.4*).

After 10 Hz rTMS we found no significant variation of sensation or pain thresholds, nor of nBR amplitude and habituation (*Fig.8.2, 8.3, 8.4*).

Table 8.2: Means of electrophysiological data in HS

	Number	Age (y)		ST (mA)	PT (mA)	AUC 1°block ipsilateral (µV*ms)	AUC 1° block contralateral (µV*ms)
rTMS 1 Hz visual cortex	21	27.45 ± 10.68	before	0.67 ± 0.19	5.85 ± 2.28	0.027 ± 0.034	0.019 ± 0.024
			after	0.71 ± 0.19	4.69 ± 2.58	0.031 ± 0.033	0.025 ± 0.027
			p	0.17	***0.001***	***0.024***	***0.036***
rTMS 10 Hz visual cortex	21	27.45 ± 10.68	before	0.67 ± 0.23	5.61 ± 2.52	0.031 ± 0.042	0.026 ± 0.038
			after	0.72 ± 0.23	5.64 ± 3.21	0.023 ± 0.023	0.015 ± 0.015
			p	0.71	0.07	0.32	0.1

Figure 8.2: Pain threshold before and immediately after rTMS over the visual cortex in HS

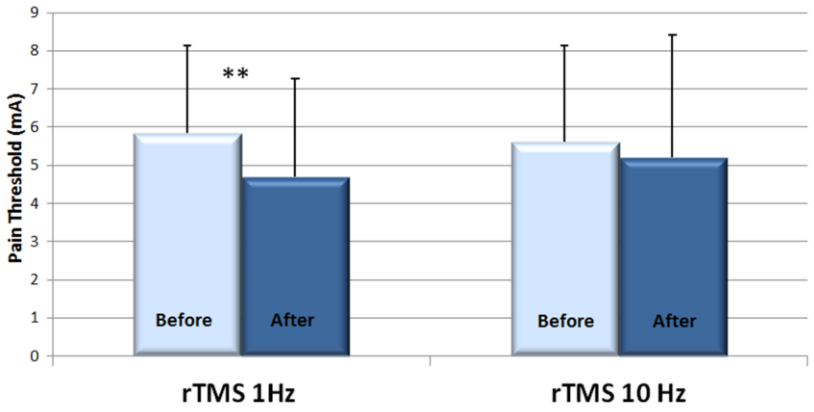


Figure 8.3: First block of 5 ispilateral (blue) and contralateral (green) nBR responses (area under the curve in mVxms ± sem) before (light bars) and after (dark bars) 1 Hz rTMS and 10 Hz rTMS over the visual cortex in HS. * *p< 0.05*.

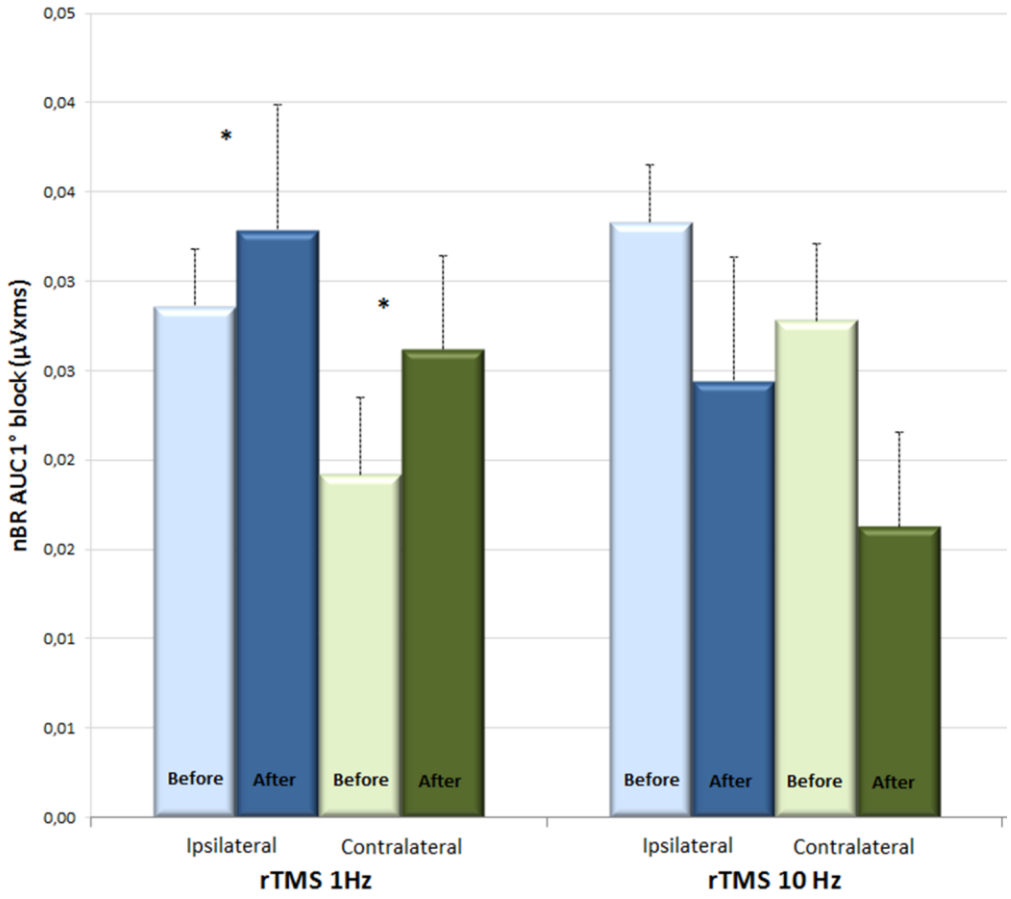
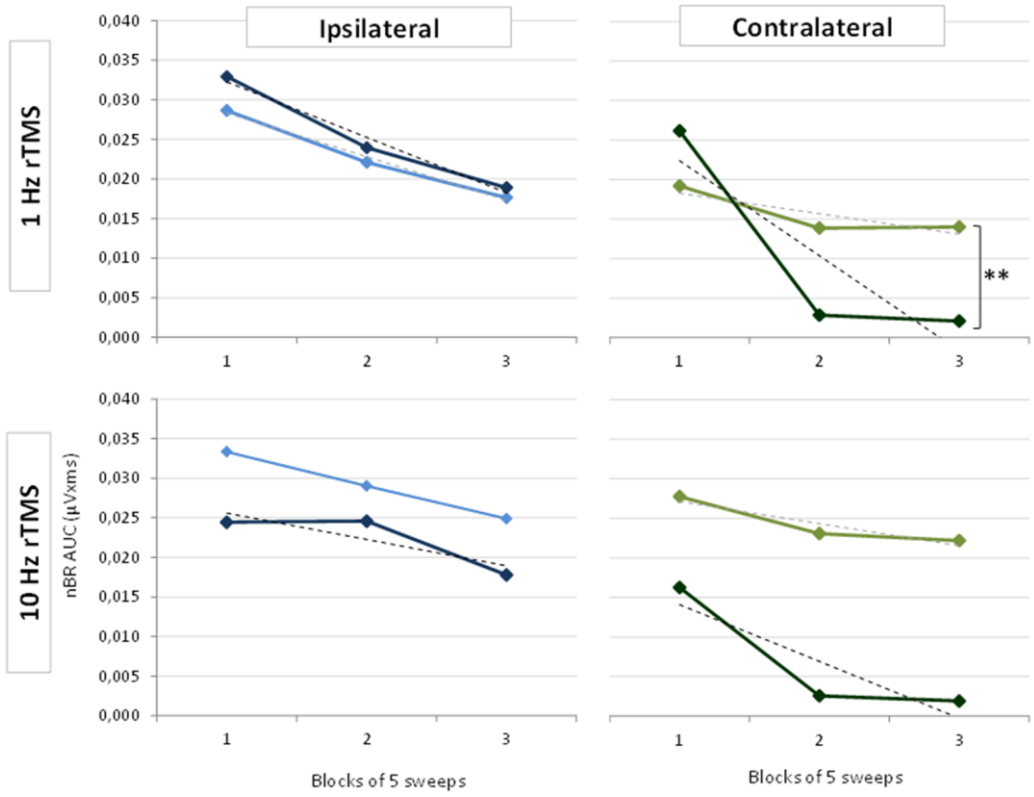


Figure 8.4: Area under the curve of ipsilateral (blue) and contralateral (green) nociceptive blink reflexes in 3 successive blocks of 5 averaged responses before (light lines) and after (dark lines) 1 Hz rTMS or 10 Hz rTMS over the visual cortex in HS. Vertical brackets indicate significant differences before and after stimulation. ** *p< 0.01*.



b) Control studies in healthy subjects

There were no significant changes in the ST, PT, 1st block AUC of the R2 responses and habituation on nBR after the rTMS sham stimulation over the visual cortex, nor the rMS over the occipital nerve, or of tONS over the occipital area.

c) rTMS studies in migraine patients

14 MO out of 23 (60.86%, 8 females and 6 males) stimulated with TMS over the visual cortex reported phosphenes. The phosphene threshold (expressed as a percentage of the maximal stimulator output) was 62%. The motor threshold was determined in the

remaining 9 participants (39.13%, 6 females and 3 males) and was 66% of the maximal stimulator output.

Table 8.3: Means of electrophysiological data in migraine patients without aura (MO) and with aura (MA).* *p< 0.05;** p < 0.01.*

		rTMS 1 Hz		rTMS 10 Hz	
		MO	**MA**	**MO**	**MA**
PT (mA)	*Before*	6.59±3.93	7.47±4.04	6.28±4.05	5.51±1.89
	After	6.17±3.87	7.61±3.77	6.36±3.13	5.61±2.05
1° block nBR Ipsilateral (μV*ms)	*Before*	0.018±0.018	0.017±0.01	0.017±0.017	0.019±0.013
	After	0.023±0.022	0.016±0.012	0.018±0.02	0.017±0.014
1° block nBR Contralateral (μV*ms)	*Before*	0.019±0.028	0.015±0.009	0.032±0.1	0.017±0.013
	After	0.02±0.026	0.011±0.008	0.015±0.021	0.012±0.007
Slope 3 blocks Ipsilateral	*Before*	-0.00034±0.0002	-0.0013±0.002	-0.001±0.004	-0.0024±0.005
	After	-0.0039±0.006	-0.0013±0.002	-0.0038±0.006	-0.0012±0.003
Slope 3 blocks Contralateral	*Before*	**-0.0008±0.004**	**-0.002±0.001**	**-0.009±0.047**	-0.002±0.007
	After	**-0.0097±0.012****	**-0.0053±0.003***	**-0.0074±0.009****	-0.0058±0.003

After 1 Hz and the 10 Hz rTMS there was no significant change neither in trigeminal sensory and pain threshold (*Fig. 8.5*), nor in the AUC of the 1st nBR block in migraine patients. Results were similar in migraine with or without aura (*Table 8.3*).

The slope of amplitude changes over the 5 blocks of averaged responses was significantly modified after rTMS only for the contralateral R2 response: it increased after 1 Hz rTMS (*p=0.0006),* but decreased after 10 Hz rTMS in MO (*p=0.001*); it increased after 1 Hz rTMS in MA (*p=0.049*) (*Fig. 8.6*).

Figure 8.5: Pain threshold (mA) in migraine without aura (MO) (yellow) and migraine with aura (MA) (claret-red) patients before (light colour) and after (dark colour) 1 Hz rTMS and 10 Hz rTMS. No significant changes were found.

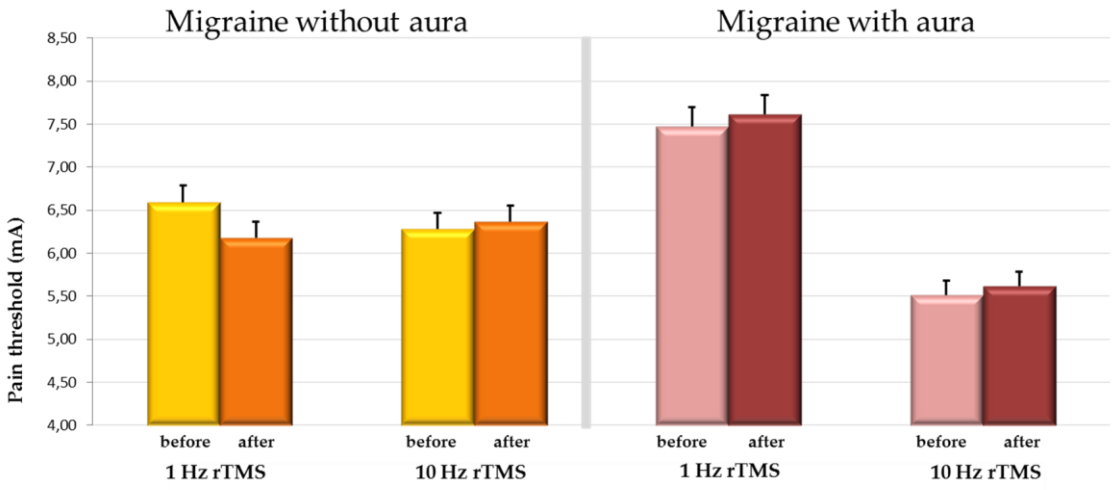
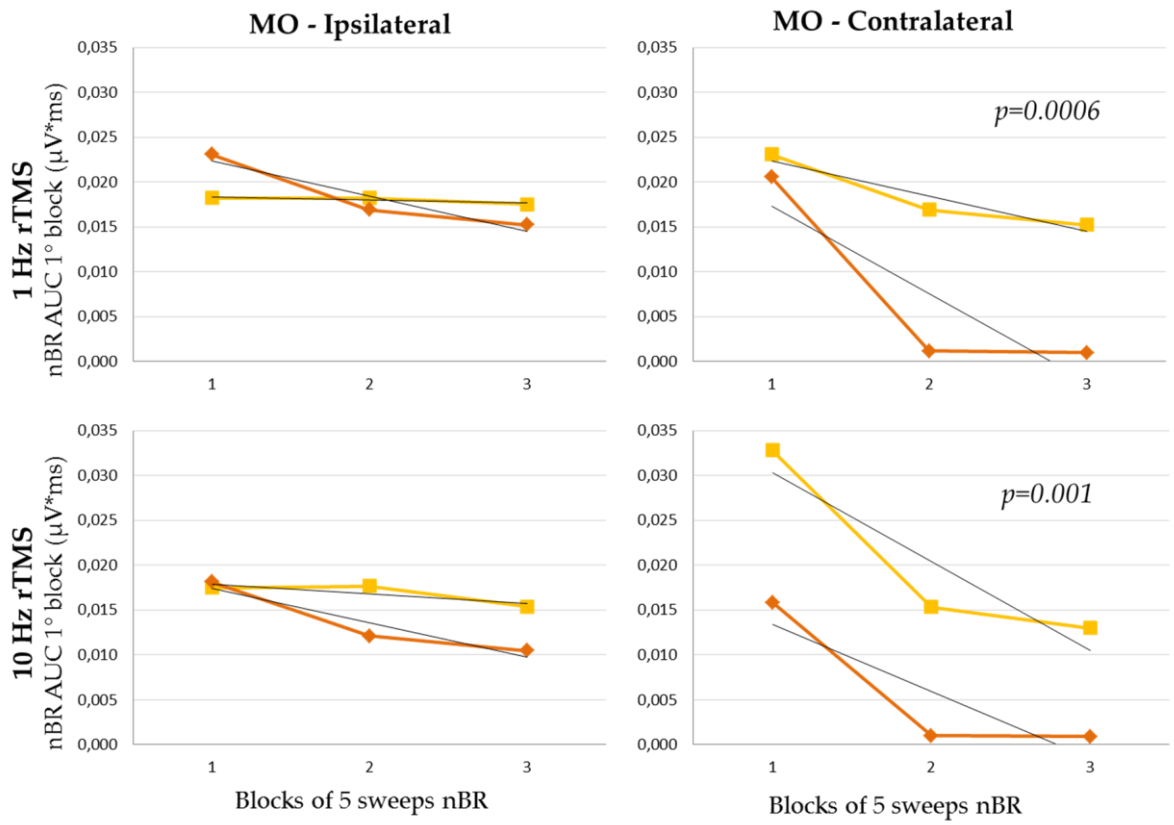


Figure 8.6: Habituation of ipsilateral (left) and contralateral (right) of nBR R2 AUC over 3 blocks in migraine without aura patients (MO), before (light orange) and after (dark orange) 1 Hz rTMS (above) and 10 Hz rTMS (below) over the visual cortex.



8.4. Discussion

a) Results in healthy subjects

This experiment supports the existence in healthy subjects of a functional relation between the visual cortex and the trigeminal nociceptive system, as assessed by nBR. The relation seems to be inhibitory: when we apply inhibitory rTMS over the visual cortex there is a reduction in the pain threshold, and thus an increased perception of pain, and, as a corollary a facilitation of the nBR; when we apply excitatory rTMS over the visual cortex, the effect tends to be opposite, but does not reach the level of statistical significance.

The differential effect of low and high frequency of rTMS was investigated in several studies. rTMS at 0.9 Hz over the motor cortex reduces the amplitude of motor evoked potentials (MEP) (Chen et al., 1997), whereas high-frequency rTMS (5 to 20 Hz) increases MEP amplitude and lowers MEP thresholds (Pascual-Leone et al., 1994). Occipital low-frequency rTMS was found to increase the threshold for double pulse TMS-induced phosphenes and to decrease visual imagery performance, suggesting inhibition of the visual cortex (Kosslyn et al. 1999; Boroojerdi et al., 2000). On the other hand the effect of 10 Hz rTMS is more controversial. When applying it over the motor cortex, some authors found a facilitation of MEP (Maeda et al., 2000), others no effect (Peinemann et al., 2000), especially when the train was longer than 2 minutes. In our paradigm there was similarly no detectable effect of high frequency rTMS. A similar effect of rTMS over the visual cortex was found in a study of visual evoked potentials (VEP) in healthy subjects: 1 Hz rTMS reduced amplitude of the 1st VEP block, while 10 Hz rTMS had no effect (Fumal et al., 2003). As a possible explanation for these different results, it was postulated that in normal subjects the cortical baseline activation level is close to the ''ceiling'', i.e. the upper limit of the cortical activation range, hence it cannot be further activated by excitatory 10 Hz rTMS but it can be decreased by the inhibitory 1 Hz rTMS. *A contrario* this hypothesis is supported by the finding that in migraine patients who may have a lowered cortical

baseline activation level of the visual cortex and a decrease in 1st block VEP amplitude at baseline, 10 Hz rTMS increases 1st block VEP amplitude whereas 1 Hz has no effect (Bohotin et al., 2002).

The intensity of stimulation can influence the effect of rTMS on the underlying cortex (Modugno et al., 2001). In accordance with previous studies, we decided to set the stimulation intensity at the phosphene threshold or 110% motor threshold (Bohotin et al., 2002; Fumal et al., 2003). We found no difference in the effect on the nBR between subjects who had no phosphenes and subjects who reported them.

After 1Hz rTMS we found an increased habituation of the contralateral nBR, whereas 10 Hz rTMS had no effect. The result is surprising, as we expected to find sensitization and not habituation with an increase in perceived pain. The lack of sensitization over the three blocks after 1 Hz rTMS testifies to the complexity of the mechanisms of habituation/sensitization in HS. Cognitive processes may interfere: the subjects read the informed consent form, were aware of the recording and stimulation procedure, and may have anticipated the progression of the session.

The sensory branches of the greater occipital nerve lie between the coil of the magnetic stimulator and the cranium and occipital cortex. The electro-magnetic pulses could activate some of these peripheral afferents of the C2 dermatoma that are known to project centrally on the trigeminal sensory system. Aβ afferents, if activated, could decrease activity of 2nd order trigeminal nociceptors via the gate control. Aδ afferents from C2 are known to converge on the spinal trigeminal nucleus, but they are most likely not activated, since the magnetic is usually not perceived as painful.

The results of our control experiments show that the rTMS-induced effects on trigeminal pain perception and nociceptive reflexes are not due to activation of superficial nervous structures or muscles nor to a placebo effect.

The rationale to use pericranial nerve stimulation to treat headaches is that trigeminal and cervical afferents converge on second-order nociceptors in the spinal trigeminal nucleus (Bartsch et al., 2003). Invasive occipital nerve stimulation (ONS) seems beneficial in refractory chronic cluster headache but less so in chronic migraine (Magis et al., 2012). In a recent sham-controlled study transcutaneous ONS (tONS) was found effective in chronic migraine and chronic tension-type headache patients, but only in those who were not allodynic (Bono et al., 2015).

In our group, a study of daily tONS for 2 months in 23 CM patients showed after treatment a 17% decrease in total monthly headache days, a 22% decrease in monthly migraine days and 42% of patients with at least 30% reduction in migraine days. As in HS, there was no significant change of nBR after tONS, but VEP habituation reversed to an episodic migraine pattern (Schoenen et al., 2016, Neurology, abstract AAN 2016).

b) Results in migraine patients

In migraine patients both 1 and 10 Hz rTMS failed to induce a significant change of pain perception in the trigeminal V1 area and of the nBR. However, habituation of the contralateral nBR response was enhanced after 1 Hz rTMS in MO and MA patients and reduced after 10 Hz rTMS. We will discuss these results in sequence.

As mentioned before, it was shown previously in our research unit that 1Hz rTMS of the visual cortex has differential effects on VEP in HS and migraine patients (Bohotin et al., 2002): 1Hz rTMS decreases significantly the amplitude of the 1stVEP block only in HS but not in MO patients. Conversely, in the same study after 10 Hz rTMS, the 1st VEP block was not modified in HS but it was significantly increased in MO patients. This result was interpreted as suggesting that the preactivation excitability level of the visual cortex is reduced in migraineurs between attacks and cannot be further decreased by inhibitory rTMS, while in HS who have a normal preactivation level it is difficult to further activate the cortex with excitatory rTMS. Along the same line, it

was shown that the therapeutic effect of high frequency rTMS over the dorsolateral prefrontal cortex in depressive patients is related to hypoactivation of this area during a mental task (Eschweiler et al., 2000).

Since low frequency rTMS over the visual cortex is not able to modify functional responses, i.e. VEP, of the occipital cortex itself in migraine patients, it is not surprising that the same stimulation was unable to change trigeminal pain perception and amplitude of nociceptive reflexes.

In the present study we were not able confirm the differences in magnetophosphene prevalence and threshold reported previously in our group by Afra et al., 1998b. This could be due to the lower number of patients studied here.

Di Clemente et al. (2007) in our group have shown that habituation of the nBR is decreased in migraine patients between attacks. Although our study was not designed to search for baseline nBR differences between HS and migraineurs, the comparison of *Figures 8.4* and *8.6* suggests that nBR habituation is induced lower in migraine patients than in HS.

Excitatory rTMS (Bohotin et al., 2002) and tDCS (Viganó et al., 2013) over the visual cortex are able to reverse the interictal deficit of VEP habituation in migraineurs, which may explain their therapeutic potential in EM. If, as discussed above, one accepts the concepts of a top-down inhibitory control of the visual cortex over the trigeminal nociceptive system in the brainstem and of an inverse relation between habituation and the preactivation level of the relevant neural system, one might not be surprised by our findings, both in HS and MO patients, that inhibitory visual rTMS increased nBR habituation while excitatory rTMS decreased it. The fact that 10Hz rTMS had a significant effect on nBR habituation in migraineurs but not in HS may be related to the difference in visual cortex preactivation levels between the two groups, as discussed above.

In conclusion these results show that:

1. There is a functional connection between the visual cortex and the trigeminal nociceptive system in HS, as evidenced by the effect of inhibitory rTMS.

2. This connection seems to be top-down inhibitory, not only on subjective measurements of trigeminal pain perception, but also on an objective measure, the nBR.
3. Contrary to HS, rTMS of the visual cortex in migraine patients is not able to significantly modify trigeminal pain perception and nBR amplitude, which we attribute to a different state of cortical responsivity in migraine between attacks.

We hypothesize therefore that inhibition of the visual cortex allows a facilitatory discharge in the brainstem activating the trigeminal nociceptive system. By contrast, exciting the visual cortex may lead to inhibition of the trigeminal nociceptive system. Unfortunately this inhibitory effect is inconspicuous when excitatory rTMS is used. This could be in part due to the fact high frequency rTMS, though being an interesting experimental tool and therapeutic approach, activates directly the underlying visual cortex, and not the thalamus that may play an important role in the physiological connection between vision and trigeminal pain, as well as in migraine pathophysiology. Regarding the latter, our group has suggested indeed that, based on electrophysiological studies, the core of migraine pathophysiology could be a thalamo-cortical dysrhythmia (Coppola et al., 2005). rTMS might thus not be optimal for studying the relation between vision and trigeminal pain in the context of migraine. It might be of major interest to use a more physiological activation of the visual cortex via the retino-thalamo-cortical pathway. This is the reason why we have studied the effect of flash light stimulation in a subsequent chapter.

On the other hand, the visual cortex might not be the best rTMS target to modify trigeminal pain. In clinical pain management, stimulation of the motor cortex is known since a long time to be an effective treatment for chronic pain. We have therefore explored the possible effects of motor cortex rTMS on the same nociceptive tests used in the study of the visual cortex.

9. Effects of low or high frequency rTMS over the motor cortex on the nociceptive blink reflex in healthy subjects

9.1. Introduction

It is well established that stimulation of the motor cortex has analgesic properties (Osenbach et al., 2006; Galhardoni et al., 2015) including in facial pain (Henderson et al., 2006).

We felt therefore that it would be instructive to compare our results on the changes of trigeminal nociception induced by rTMS modulation of the visual cortex with those obtained after rTMS of the motor cortex.

For this purpose we studied the effects of high and low frequency rTMS over the motor cortex on trigeminal pain sensation and the nociceptive blink reflex.

9.2. Subjects and methods

We recruited 15 HS for the rTMS experiments of the motor cortex. All 15 subjects (8 females, 7 males, mean age 28.73 ± 10.87 [SD] years old) had 1 Hz rTMS while 13 of them also underwent 10 Hz rTMS in a separate session (6 females, 7 males, mean age 27.30 ± 10.06 [SD] years old).

For more details on recruited subjects *see Chapter 6.1*.

Sensory thresholds (ST) and pain thresholds (PT) to the electrical supra-orbital stimulation were determined before the nBR was recorded (*see Chapter 6.2.2*).

For each subject we determined the motor threshold, defined as the percentage of rTMS output necessary to produce a motor evoked potential (MEP) higher than 50 μV amplitude in at least 4 out 5 pulses, recorded in the abductor pollicis brevis

muscle with surface electrodes. The stimulation intensity was set to 110% of the motor threshold. The stimulation protocols were similar to those used for visual cortex modulation (*see Chapter 6.2.5 & 8*).

9.3. Results

The TMS threshold to evoke a contraction of the abductor pollicis brevis in HS was 60.53 ± 6% [SD] of the maximal output of the Magstim stimulator.

Following low or high rTMS over the motor cortex we found no significant difference in sensory threshold (ST), pain threshold (PT) (*Fig. 9.1*) or AUC of the 1st block of the ipsi- or contralateral nBR R2 response (*Fig. 9.2*).

However, habituation of the contralateral nBR over the three blocks of averages increased after 1 Hz rTMS (*p=0.01*) and even more so after 10 Hz rTMS (*p=0.004*) (*Fig. 9.3*), while habituation of the ipsilateral reflex was not significantly changed.

Figure 9.1: Pain Threshold (mA) in HS before and after 1 Hz rTMS (left) and 10 Hz rTMS (right) over the motor cortex. No significant changes are found.

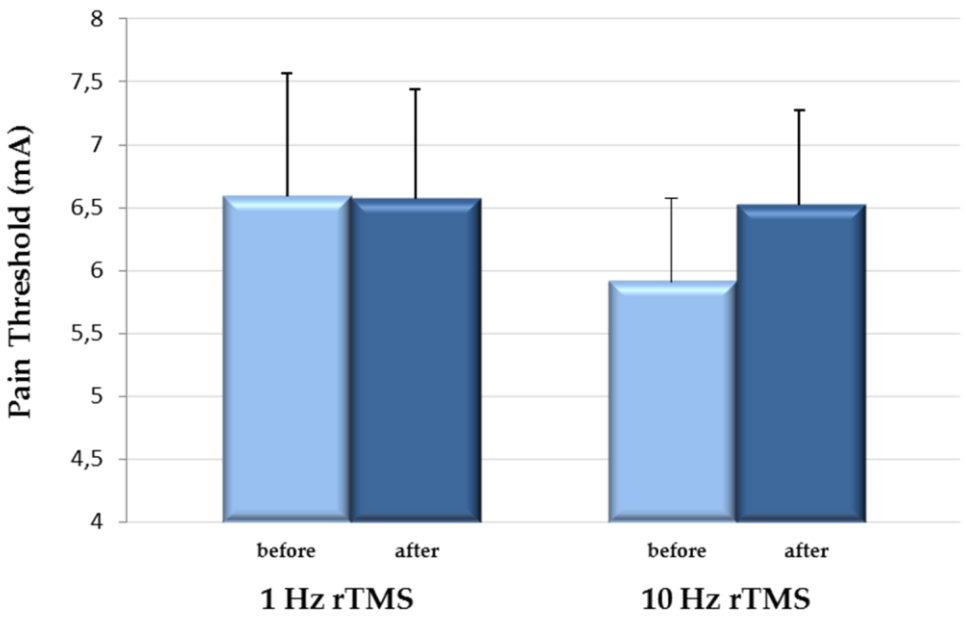


Figure 9.2: First block of ipsilateral (blue) and contralateral (green) nBR R2AUC in HS after 1 Hz rTMS and 10 Hz rTMS over the motor cortex in HS. No significant changes are found.

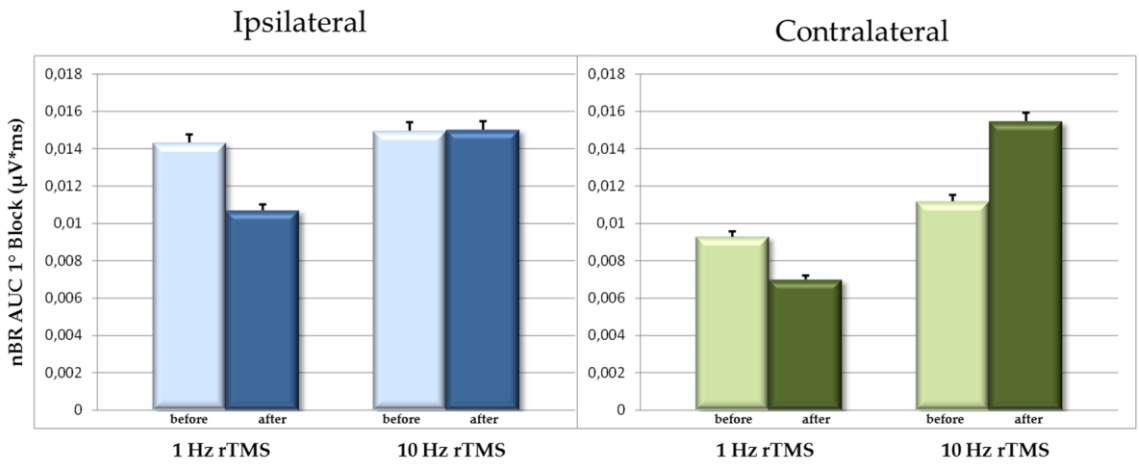
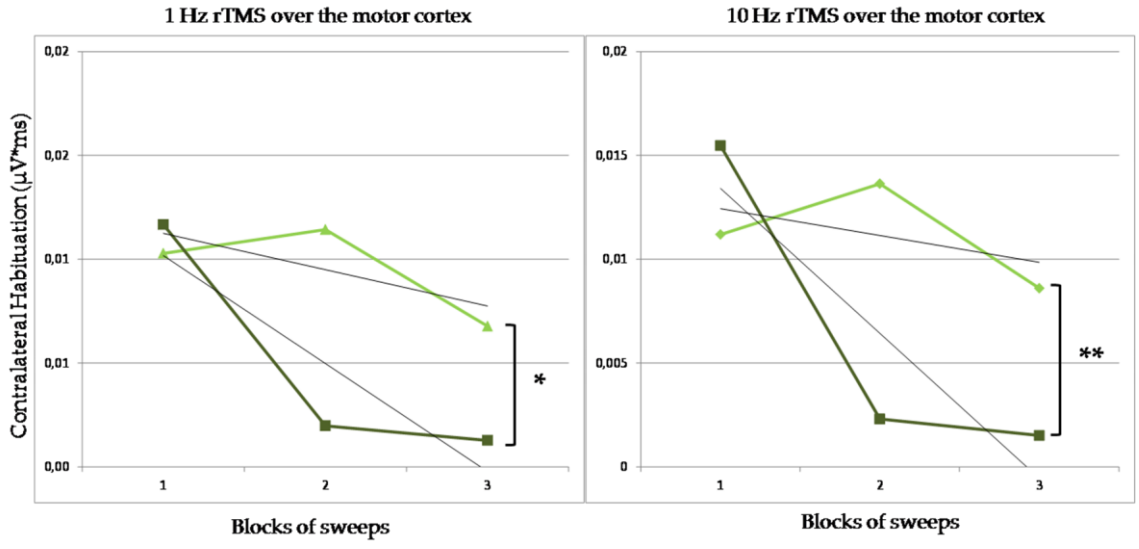


Figure 9.3: Habituation of the contralateral nBR R2 response before (light green) and after (dark green) 1 Hz rTMS (left) and 10 Hz rTMS (right) over the motor cortex. * *p< 0.05*; ** *p < 0.01*.



9.4. Discussion

The study was conceived as a comparator for our previous experiment on the effects of rTMS over the visual cortex (*Chapter 8*). Its main result is that rTMS over the motor cortex does not cause the same changes of the nBR and pain perception as visual cortex modulation. Nonetheless, the motor cortex seems to increase habituation of the contralateral nBR R2 response, but this effect is similar with low or high frequency rTMS.

The lack of effect of motor cortex stimulation on trigeminal pain perception and nociceptive reflex activity contrasts with the inhibitory effect of motor cortex rTMS on cortical potentials evoked by laser heat stimulation of the hand (Lefaucheur et al., 2010) and its well-established beneficial effects on neuropathic facial pain (Lefaucheur et al., 2001, 2006 and 2008). Stimulation of the motor cortex increases cerebral blood flow in the ipsilateral thalamus, the orbito-frontal and the cingulate gyri and in the upper brainstem (Peyron et al., 1995). The motor cortex could thus exert its analgesic effects both by influencing the affective–emotional component of chronic pain (cingulate and orbitofrontal cortices) and activating descending pain control centres in the upper brainstem (Garcia-Larrea et al., 1999).

It is possible that the methods we have used are not sensitive enough in healthy subjects to detect mild changes in trigeminal nociception.

It is not clear how rTMS stimulation of the motor cortex increases habituation of the contralateral nBR. Cortical mechanisms are known to modulate blink reflexes. In migraine patients, there is, on the one hand, a lack of habituation of the nBR between attacks (Di Clemente et al., 2007). Furthermore, cortical evoked potential studies suggest that the preactivation level of the cerebral cortex is decreased in migraineurs (Schoenen et al., 2003) and the habituation deficit of visual evoked potentials and that of the nBR are correlated in the same migraine patients (Di Clemente et al., 2005). One might thus speculate that rTMS-induced activation of the motor cortex could have the opposite effect on nBR habituation, i.e. enhance it.

That excitability of the motor cortex can be abnormal in migraine patients has been shown in several studies. The first studies using TMS were performed in our Headache Research Unit (Maertens de Noordhout et al., 1992) and found that the motor threshold was significantly increased on the affected cortical side of patients suffering from migraine with aura (MA) compared to HS. In migraine without aura patients (MO) the same results were also found in the interictal phase, in the ictal phase and in menstrual migraine (Bettucci et al., 1992). Increased MT and decreased MEP amplitude were also found in familial hemiplegic migraine (FHM) patients interictally (van der Kamp et al., 1997). Afra et al. (1998) searched for differences in MT in MA and confirmed that MT was higher in the patients than in the controls. Intracortical facilitation was more pronounced in migraine patients than in the controls (Siniatchkin et al., 2007) and it was enhanced after 1 Hz rTMS (Brighina et al., 2005).

The major confirmation of this study is that it appears more appropriate to target the visual rather than the motor cortex in migraine therapy.

10. Effects of visual cortex activation by flash light stimulation on nociceptive blink reflex in healthy subjects and migraine patients

10.1. Introduction

An abnormal rhythmic activity between thalamus and cortex, namely thalamo-cortical dysrhythmia, may be the pathophysiological mechanism subtending abnormal information processing in migraine (Coppola et al., 2013).

Increasing the thalamo-cortical drive may induce a beneficial on trigeminal pain perception and brainstem excitability. We have shown in the previous chapters that the visual cortex can have a top-down inhibitory effect on the trigeminal nociceptive system. However, this could be demonstrated only in healthy subjects, but not in migraine patients using rTMS of the visual cortex. In order to activate the global visual pathway including its thalamic relays, we chose therefore to study the effect on trigeminal pain perception and the nociceptive blink reflex (nBR) of short flash light stimulation. We compared the results between healthy subjects (HS) and episodic migraine patients (EM).

10.2. Subjects and methods

We enrolled a total of 41 subjects:

- 22 HS (12 females, 10 males, mean age 26.59 ± 9.29 [SD] years old).
- 19 EM (15 without aura and 4 migraine with aura patients, 15 females and 4 males, mean age 30.42 ± 8.26 [SD] years old)

EM patients were recorded during the interictal phase and had the following clinical characteristics: number of attacks per month: 3.5 ± 2.92 [SD]; mean attack duration: 26.52 ± 20.03 [SD] hours; disease duration 12.53 ± 9.07 [SD] years.

For more details on subjects' recruitment *see Chapter 6.1*.

Sensory threshold (ST), pain threshold (PT) and nBR were measured as described in the previous chapters (*see Chapters 6.2.2 and 6.2.7*) before and after 4 minutes of a flash light stimulation at 8 Hz. All recordings started after 5 minutes of adaptation to the dimmed light in the recording room.

10.3. Results

The flash light stimulation increased the pain threshold in HS (*p=0.008*) and in migraine patients (*p=0.034*) (*Fig. 10.1*). It decreased AUC/i² of the 1st nBR block in HS (*p=0.004* ipsilateral; *p=0.001* contralateral) and in migraineurs (*p=0.0006* ipsilateral; *p=0.0008* contralateral) (*Fig. 10.2*) and increased habituation of the contralateral nBR in both groups (*p=0.002* in HS and *p=0.036* in EM) (*Fig. 10.3*).

We found no significant variation of sensory thresholds using the photic stimulation.

Figure 10.1: Pain threshold in healthy subjects (HS) (blue) and episodic migraine patients (orange) before (light colour) and after (dark colour) the flash light stimulation at 8 Hz.

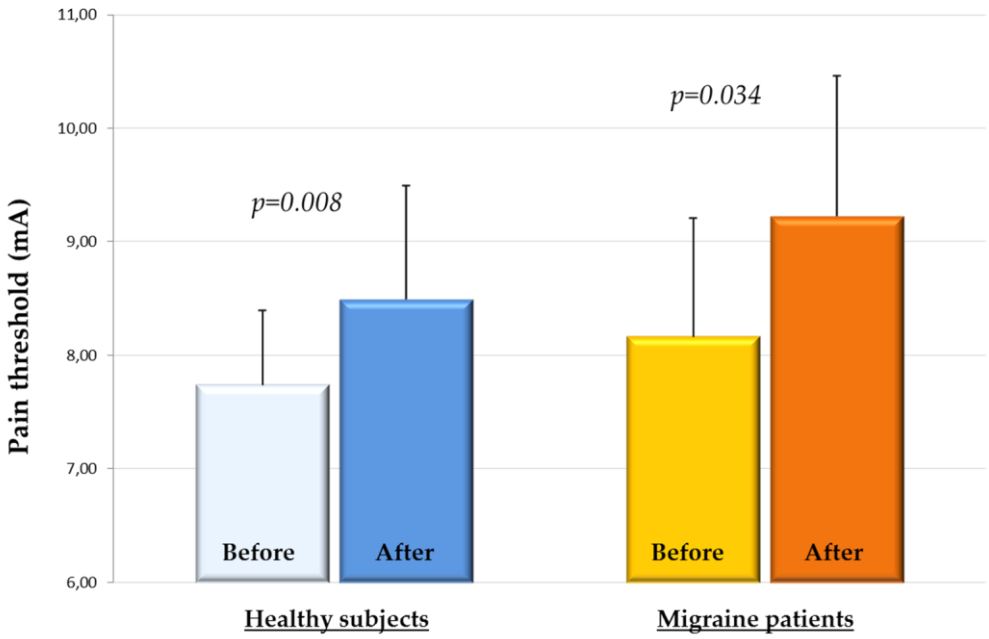


Figure 10.2: Ipsilateral (above) and contralateral (below) 1st block AUC nBR in healthy subjects (blue) and episodic migraine patients (orange) before (light colour) and after (dark colour) the flash light stimulation at 8 Hz.

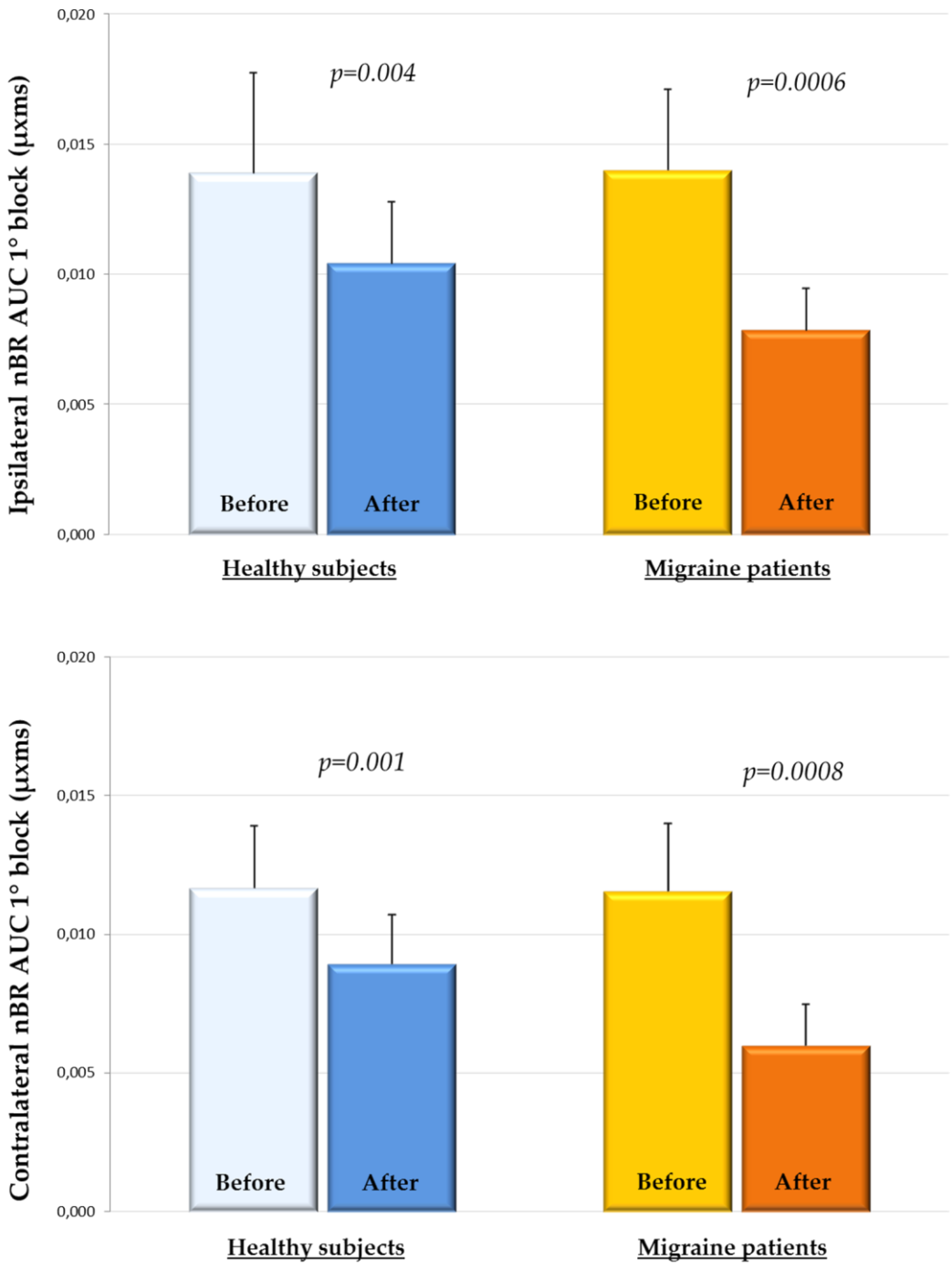
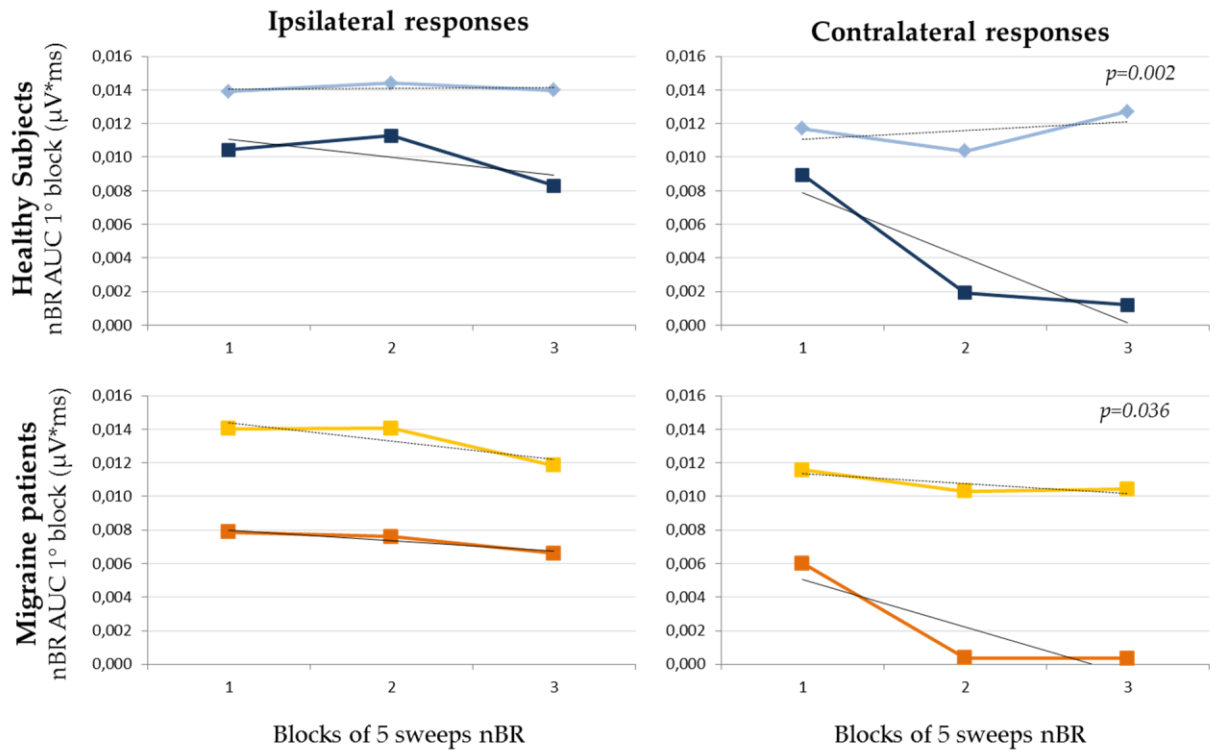


Figure 10.3: Habituation of ipsilateral (left) and contralateral (right) nBR over three blocks in healthy subjects (blue) and episodic migraine patients (orange) before (light colour) and after (dark colour) the flash light stimulation at 8 Hz.



10.4. Discussion

The main findings of this study are that flash light stimulation decreases pain perception (as assessed by the increased pain threshold), reduces nBR amplitude, and favours habituation of the contralateral nBR in both HS and migraine patients.

They confirm our results with rTMS in HS suggesting an inhibitory control of the visual system on trigeminal nociception. The major difference is that this inhibitory with flash light stimulation is demonstrable in migraineurs, in whom we could not demonstrate it with excitatory rTMS of the visual cortex. The effect of flash light stimulation seems thus more robust on both trigeminal pain perception and the nociceptive blink reflex.

The mechanisms of the flash light-induced inhibition of trigeminal nociception must a priori be sought in the retino-geniculo-cortical pathway of vision. However, the connections between retina, thalamus, trigeminal system and cortex are complex and involve various other structures that are not part of the classical visual pathway, but are modulated by light.

The visual cortex is undoubtedly involved in migraine pathophysiology (*see Chapter 2*); it is the area in the brain where cortical spreading depression (CSD) is supposed to begin (*see Chapter 2.1 and 2.3*). If, as indicated by our results, the visual cortex exerts a tonic inhibitory effect on trigeminal nociception, one may hypothesize that the long-lasting inhibition of cortical neurons during CSD may reduce this descending inhibition and hence release activation in trigeminal nucleus caudalis favouring the migraine headache. Admittedly, this would be plausible only in migraine with aura where CSD is well accepted to explain the aura symptoms. In migraine without aura, there is no convincing data showing that CSD may occur, unless one accepts the concept of "silent auras". Nevertheless, posterior spreading oligemia was reported by Woods et al. (1994) in a 21-year-old migraine without aura patient during visual stimulation and PET scanning. The patient developed a throbbing headache accompanied by nausea, mild vertigo, and photophobia, but had no obvious aura symptoms.

The inhibitory effect of flashing light on trigeminal nociception found in our study was not expected based on the studies by Lambert et al. (2008) who showed in cat that such stimulation increases stimulus-induced activation of 2nd order trigeminovascular nociceptors in the spinal trigeminal nucleus via inhibition of dorsal raphe neurons. These authors found the same results after experimental CSD. Several differences may explain these opposite findings. First, the authors did not record the activity in the visual system, and thus the effect of the repetitive flash light stimulation on the visual cortex was not assessed. The visual stimuli could have

modified trigeminal nociception via non-visual pathways (see above) or they might have induced CSDs, which could have disinhibited trigeminal nociceptors via the mechanism explained before. Multiple CSD are known to increase the expression of *c-fos* in TNC (Moskowitz et al., 1993); suggesting an activation induced by the cortical depression, like in our study. Therefore, if one accepts that such a spreading depression might have similar effects on the visual cortex and its connectivity as inhibitory 1 Hz rTMS used in our study, both the findings in cat and ours in humans would concord in showing that the visual cortex exerts a tonic descending inhibitory action on trigeminal nociceptors. Second, species differences in visuo-trigeminal interactions cannot be excluded considering the differences in vision between cats and humans.

The connexions between visual input and the trigeminal nociceptive system are complex and multiple. Okamoto et al. (2009) submitted anesthetized rats to light stimulation while recording trigeminal nucleus caudalis neurons. They found that during light exposure firing of these neurons was increased and the response was suppressed after injection of lidocaine into the ocular globe or the trigeminal ganglion, indicating that both structures were involved in the light-evoked nociceptive discharge. They also showed that the circuit involves the olivary pretectal nucleus (OPN), of which inhibition blocked completely light-evoked trigeminal nucleus caudalis neural activity and tear formation (Okamoto et al., 2010).

Further evidence comes from studies by Noseda et al. (2010). The authors injected a viral tracer into the globe of rats and found a direct connection between the intrinsic photoreceptive retinal ganglion cells (ipRGCs) and the posterior, the lateral posterior and the intergeniculate thalamus all three of which are not believed to be associated with the classical visual pathway. The same thalamic nuclei were also activated by stimulation of the dura, demonstrating convergent input from the retina and from trigeminal nociceptors. From the thalamus, afferents reach the cortex, including the visual cortex. Interestingly the posterior and lateral posterior thalami both receive

direct projections from forebrain structures like the nucleus of the diagonal band of Broca, the dopaminergic cell groups of the hypothalamus, the ventromedial and the ventral tubero-mamillary nucleus of the hypothalamus (Kagan et al., 2013).
A third possible circuit excludes the role of the optic nerve to explain how the light stimulus links to the nociceptive trigeminal system. Dolgonos et al. (2011) showed that in rats after optic nerve section, the amplitude of the blink reflex remains increased during high intensity light stimulation.

It is important to note that all studies cited above investigated the effect of provoked photophobia. Consequently, light stimuli were very intensive and of short duration (maximum 30 seconds). It is not surprising that high luminance light stimulation evokes a photophobic reaction. All of us can experience such a phenomenon when stepping into a sunny environment out of the dark. The photophobic reaction is associated with eye blinking as a protective mechanism. In studies investigating the mechanism of photophobia, this is the expected reaction in animals and humans and the objective is to assess the mechanisms induced by intense light.
In our study we were primarily interested in assessing subjective and objective trigeminal pain perception when applying well-tolerated light stimuli. The areas involved within the central nervous system are likely the same, but their modulation may change. The light stimulation can probably inhibit or activate the same area depending on the stimulation protocol. This is well known for non-invasive neuromodulation techniques such as transcranial magnetic stimulation that has an excitatory or an inhibitory effect depending on stimulation frequency.

That vision is able to reduce limb pain in humans is known since several years (Longo et al., 2009) and was called “visually-induced analgesia”. The functional visuo-trigeminal connection described in our study could play a role in this phenomenon. We wondered therefore whether visually-induced analgesia can be demonstrated in the trigeminal territory and, in case it can, whether it is abnormal in

migraine patients knowing that they have functional abnormalities of both visual cortex and trigeminal pain processing. This led us to perform the study presented in the next chapter.

11. Visually induced analgesia in healthy subjects and migraine patients

11.1. Introduction

The visual cortex is involved in the complex process of pain processing through both sub-cortical and cortico-cortical projections.

The term "visually-induced analgesia" (VIA) defines a phenomenon in which viewing one's own body part during its painful stimulation decreases the perception of pain (Haggard et al., 2013; Longo et al., 2008, 2009, 2011 and 2012; Mancini et al., 2011 and 2012).

The analgesic effect induced by vision occurs during direct vision but also when indirectly seeing the stimulated body part reflected in a mirror. VIA is absent when viewing someone else's corresponding body part. To the best of our knowledge, VIA has never been studied in the face, i.e. in the trigeminal area, where it could be relevant for the control of headache.

The aim of this study was to investigate VIA in healthy subjects and migraine patients by using, as an index of pain, contact heat-evoked potentials (CHEPs) elicited after thermal stimulation of the right wrist or the right side of the forehead with or without viewing the stimulated body part in a mirror.

11.2. Subjects and methods

We recruited 11 healthy women (HS, mean age 29.45 ± [SD] 10.25 years) and 14 patients with migraine without aura according to the ICHD 3 beta criteria (ICHD 3β, 2013) (MO, 14 females, mean age 26.4 ± [SD] 4.55 years) (*Table 11.1*). MO patients had

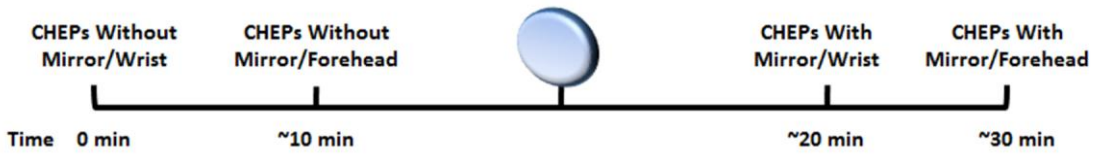
no prophylactic treatment and were recorded during the interictal phase. For more details on recruitment *see Chapter 6.1*.

Table 11.1: Mean demographic data

	HS *(n = 11)*	MO *(n = 14)*
Women *(n)*	11	14
Age *(years)*	29.45 ± 10.25	26.5 ± 4.55
Duration of history of migraine *(years)*		11.07 ± 4.9
Attack frequency/month *(n)*		2.02 ± 1.52
Attack duration *(h)*		21.57 ± 17.85

CHEPs were obtained by stimulating the right wrist and thereafter the right supra-orbital area, first while the subjects were fixing their gaze on a neutral point on the wall in front of them, then while they viewed the stimulated area in a mirror (*Fig. 11.1*).

Figure 11.1: Timing for recordings without and with mirror.



For more details on the CHEPs technique and recordings *see Chapter 6.2.4*.

To obtain a more reliable result, we chose to measure the area under the curve (AUC) of CHEPs components P1-P2 by multiplying amplitude (in mV) x duration (in ms). We compared measures of AUC with those of peak amplitudes of P1-N2 and N2-P2 and found no difference between the two types of analysis. Habituation of CHEPs amplitude over the five blocks of averaged responses was calculated between the 1st

and the 5th block. A visual analogue scale (VAS, 0-10) was used to compare the thermal pain perception without and with the mirror.

11.3. Results

Table 11.2 displays all electrophysiological data.

All CHEPs recordings obtained from the right forehead were analysable, while recordings from the wrist of 2 subjects (1 HS and 1 MO) were excluded because of poor signal quality.

During right wrist stimulation, we found a decrease of 1st block AUC P1-P2 in HS when they were seeing their wrist reflected in the mirror compared to the control recording (*p=0.036*) (*Fig. 11.2*), but this was not the case in MO patients. In the latter the VAS pain score increased viewing the reflected wrist (*p=0.04*) (*Fig. 11.4*).

Seeing their forehead reflected in the mirror induced a significant increase of N2 latency in HS, as well as a decrease of 1st block CHEPs P1-P2 AUC both in HS (Wilcoxon's test *p=0.007*) and MO groups (*p=0.03*) (*Fig. 11.2*).

Habituation of CHEPs amplitude over the five blocks of averaged responses did not change, neither for the wrist, nor for the forehead stimulation (*Fig. 11.3*).

Table 11.2: Mean electrophysiological results (± Standard deviations) in HS and MO for wrist and forehead stimulations, without and with seeing the stimulated body part in a mirror.

Right Wrist						
	HS = 10			MO = 13		
	Without mirror	With mirror	*p*	Without mirror	With mirror	*p*
P1 latency (ms)	208.74 ± 31.36	212.01 ± 48.40	0.76	203.28 ± 39.26	192.29 ± 44.19	0.64
N2 latency (ms)	272.75 ± 39	272.97 ± 42.82	0.77	278.54 ± 54.25	273.35 ± 43.47	0.55
P2 latency (ms)	404.15 ± 27.79	388.48 ± 27.70	0.18	422.61 ± 75.14	403.11 ± 74.88	0.08
1st block AUC P1P2 (µV*ms)	**2.17 ± 1.38**	**1.63 ± 1.37**	***0.03***	1.47 ± 0.66	1.47 ± 0.86	0.97
Slope P1P2 5 Blocks	-0.27 ± 0.18	-0.20 ± 0.17	0.33	-0.16 ± 0.15	-0.12 ± 0.25	0.50
VAS (0-10)	5.90 ± 2.28	5.10 ± 2.54	0.12	**5.58 ± 1.57**	**6.25 ± 1.51**	***0.04***
Right Forehead						
	HS = 11			MO = 14		
	Without mirror	With mirror	*p*	Without mirror	With mirror	*p*
P1 latency (ms)	165.09 ± 12.13	160.94 ± 31.36	0.87	164.62 ± 9.64	162.24 ± 11.09	0.53
N2 latency (ms)	**247.31 ± 19.02**	**244.37 ± 31.45**	***0.04***	253.52 ± 36.26	248.70 ± 29.95	0.85
P2 latency (ms)	376.14 ± 29.69	373.24 ± 29.98	0.24	395.68 ± 39.5	376.46 ± 45.83	0.12
1st block AUC P1P2 (µV*ms)	**2.60 ± 1.55**	**1.63 ± 0.7**	***0.007***	**2.75 ± 1.97**	**2.23 ± 1.42**	***0.03***
Slope P1P2 5 Blocks	-0.18 ± 0.33	-0.17 ± 0.14	0.86	-0.20 ± 0.38	-0.21 ± 0.27	0.47
VAS (0-10)	5.91 ± 1.92	5.00 ± 2.88	0.26	5.92 ± 2.6	6.38 ± 1.74	0.47

Figure 11.2: 1st block of CHEPs P1-P2 (AUC: mean±sd) in healthy women (blue) and migraine women (orange) after stimulation of the the wrist (left) or the forehead (right) without (light colour) and with (dark colour) mirror.

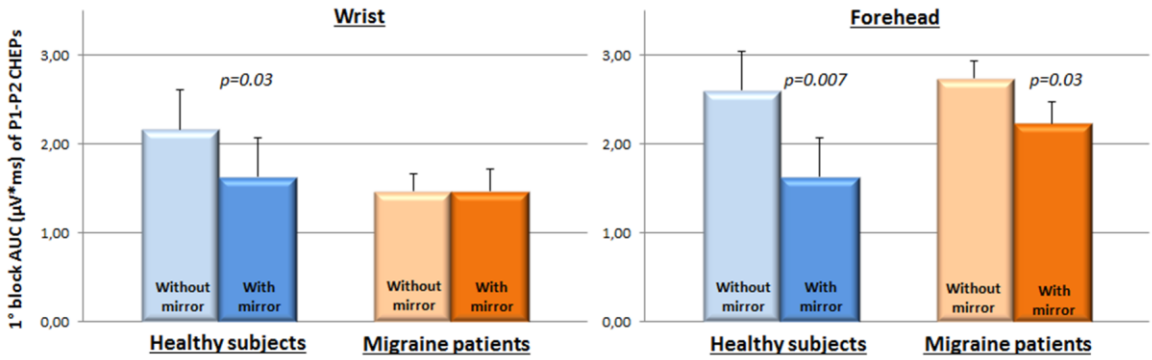


Figure 11.3: Habituation of CHEPs P1-P2 (AUC)over 5 blocks of 4 averaged responses in healthy women (blue) and migraine women (orange) after stimulation of the wrist (left) or the forehead (right) without (light colour) and with (dark colour) mirror. There was no statistical difference.

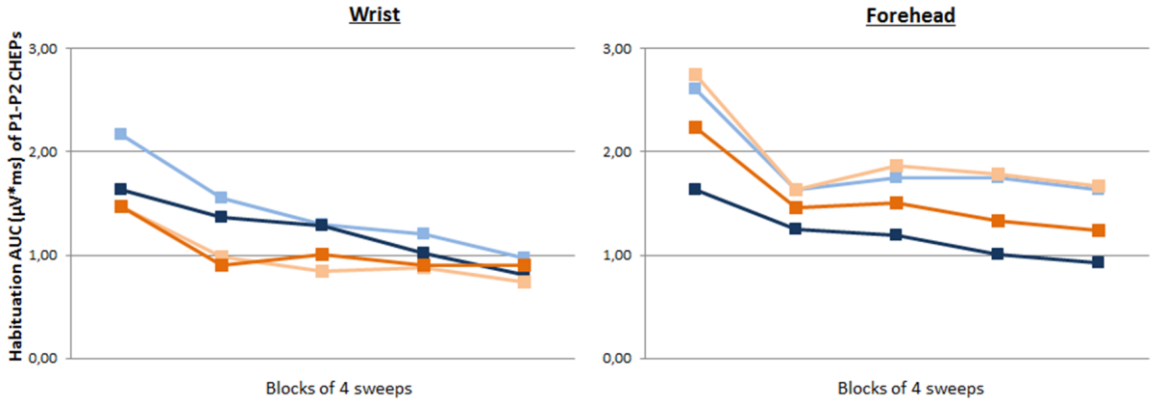
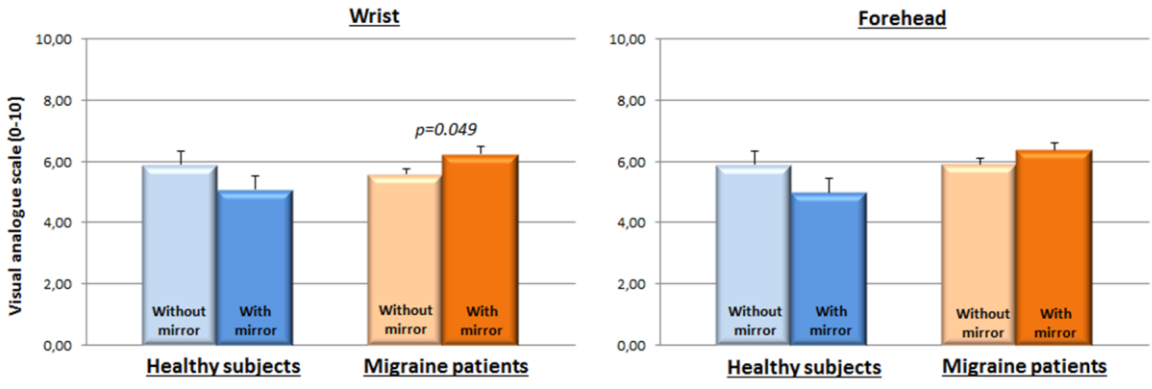


Figure 11.4: Pain scores (VAS: mean±sd) in healthy women (blue) and migraine women (orange) after stimulation of the wrist (left) or the forehead (right) without (light colour) and with (dark colour) mirror.



11.4. Discussion

This study shows for the first time that the phenomenon of visually-induced analgesia (VIA) can be demonstrated in healthy subjects in the trigeminal area, like in the upper limb, as far as it is assessed by contact heat evoked potentials. However, the reduction of subjective pain scores, though numerically detectable, is not significant and habituation of CHEPs amplitude is not modified.
In migraine patients, VIA seems normal in the cephalic area, but abnormal changes with the mirror-viewing are seen at the extracephalic stimulation site: no detectable decrease in CHEPs amplitude and an increase in pain scores.

Visually-induced analgesia is a complex phenomenon for which there are several physiological explanations. A conflict between visual and proprioceptive informations could possibly induce visual analgesia, based on the suggestion that the experience of viewing one's own body involves multiple dissociable elements (Longo et al., 2008), including the sense of ownership (i.e. "that is my body"). An fMRI study with infrared laser stimulation showed that VIA did not involve an overall reduction of the cortical response elicited by the painful stimulus, but that it increased connectivity between the brain's pain network (formerly the "pain matrix") and posterior brain areas activated by the visual perception of the body (or "visual body network"), resulting in modulation of the experience of pain (Longo et al., 2012).
From a therapeutic point of view, well-known studies have suggested that vision of the body was able to reduce chronic phantom limb pain (Ramachandran and Rogers-Ramachandran, 1996; Chan et al., 2007). In this disorder the reflection of the damaged body part given by the mirror helped to reorganize and integrate the mismatch between the subject's proprioception and the actual visual feedback, and thus, help to relieve phantom limb pain, probably via slow neuroplastic changes (Weeks et al., 2010; Kawashima et al., 2013; Foell et al., 2014). Recently, the mirror therapy has been used not only for patients with phantom limb pain, but also for patients with complex

regional pain syndrome and strokes (Rothgangel et al., 2011; Ezendam et al., 2009; Thieme et al., 2013).

The reason why we found no statistical difference in VAS pain scores in our HS group contrary to Longo et al. (2012) may in part be due to the fact that all of our subjects were females, whereas, Longo's cohort of 14 HS comprised only 3 females. Gender differences in the experience of pain are known and well documented; women report higher pain sensitivity associated with various types of noxious stimuli (e.g., ischemic, pressure, electrical, and thermal) (Berkley et al., 1997; Fillingim 1996 and 2000; Riley et al., 1998; Shinal et al., 2007). The magnitude of these effects varies from moderate to high depending on sample size, the nature of the stimulus and whether pain sensitivity is indexed by non-verbal behaviours (i.e., certain body movements, facial grimace) or by verbal behaviours such as pain threshold and tolerance reports (Berkley et al., 1997; Fillingim et al., 2009; Riley et al., 1998; Shinal et al., 2007).
The reason for enrolling only women in our study was that we wanted to compare HS with migraine patients who are predominantly females. As a weakness of our study, we must mention that we did not correct VAS results for certain features like social context, BMI and height, which can have an effect on pain perception (Vigil et al., 2015).

That MO patients have a decrease in CHEPs amplitude when viewing the stimulated body part after supra-orbital but not after wrist stimulation is surprising, as we rather expected to find the opposite given the known sensitisation of the trigeminal nociceptive system in migraine. In fact, although migraine patients may remain hypersensitive during the interictal phase, whole body allodynia is more pronounced during an attack (Burstein et al., 2000). In our study, extracephalic pain perception seems to be more resistant to reduction by the VIA phenomenon than trigeminal pain. One could hypothesize that between attacks in episodic migraine there might be

a “compensatory” suppression of head pain by central control mechanisms, which would not be the case in extracephalic territories where increased pain sensitivity would remain.

The relation between vision and somatosensory perception is complex. VIA involves chiefly the primary somatosensory cortex (SI) and the operculo-insular cortex (Longo et al., 2012). Visuo-tactile stimulation increases the activation of the somatosensory cortex more than does touch alone (Dionne et al., 2010). The role of the somatosensory cortex is thus more complex than being a simple collector of somatosensory input. For example its activation through cutaneous peripheral stimulation can produce a transient suppression of EMG responses evoked by TMS over the motor cortex. This function is not surprising if we bear in mind that via the same mechanism epileptic patients are able to extinguish a Jacksonian seizure by vigorous cutaneous stimulation (Maertens de Noordhout et al., 1992).

The connection between visual and somatosensory cortices is probably modulated by the thalamus. This is supported by a study (Imbert et al., 1965) showing in thalamectomized cats that visual stimulation activates the orbito-frontal but not the somatosensory cortex, suggesting that projections from the occipital cortex have to pass through the thalamus to be conveyed to the somatosensory cortex. Interestingly in this study, the lateral geniculate nucleus was intact, showing that it is not an obligatory relay for visuo-somatosensory connections.

More recent studies have focused on the role of the extrastriate cortex in VIA (Mancini et al., 2012). Interestingly in the latter study excitatory anodal tDCS over extrastriate areas increased the VIA phenomenon, while cathodal tDCS had no effect. These results are in line with our previous study where excitatory flash light stimulation increased the pain threshold.

In our study of healthy and migraine subjects using heat stimulation of the forehead there was a discordance between a visually-induced decrease of CHEPs but no

change of VAS pain scores that were even numerically increased in migraineurs. This could be explained by the fact that pain perception engages a complex multifocal interconnected network in the brain, while CHEPs more simply reflects activity in the cingulate gyrus.

CHEPS habituation did not change while viewing the stimulated body part in our study neither in HS nor in MO and there was no baseline difference between the two groups of subjects. This is in line with another study that found a deficit of CHEPs habituation in migraine with aura but not in migraine without aura that was the exclusive migraine type enrolled in our study (Lev et al., 2013).

To conclude, this study adds to the available knowledge on visually-induced analgesia and extends this phenomenon to the facial area as far as contact heat-evoked potentials are used as indices of central pain processing. Facial VIA is within normal limits in migraine without aura, but absent at the wrist, suggesting that between attacks control of extracephalic pain perception could be dysfunctioning.

12. StimLux: an alternative device to assess photophobia

12.1. Introduction

Photophobia assessment is always subjective. One can assess the degree of photophobia using a questionnaire or using the illuminance of the light stimulation (measured in Lux) for a more objective analysis. In any case the point at which a subject perceives the light as uncomfortable is subjective. The problem is similar when pain thresholds are measured using available devices (electrical, magnetic, algometric, cutaneous, etc.).

A questionnaire to assess photophobia that is much discussed in the literature was published by Choi et al. (2009), with interesting results: it detected photophobia in 82.5% of migraine patients.

Tolerance to continuous light was determined in several studies (Drumond et al. 1986; Vanagaite et al. 1997; Kowacs et al. 2001): they found that tolerance, as expected, is lower in migraine patients compared to HS, and lower during than between attacks. Using continuous light is interesting but not comprehensively adapted to migraine, knowing migraine patients have an abnormal photic EEG drive to flickering light (Bjørk et al., 2011).

12.2. Why do we need a new stimulator?

The StimLux (*Fig. 12.1*) is a prototype light stimulator, non-commercialized and custom-made by the principal inventor, Simona Liliana Sava, with the help of an IT technician, Gino Mancini.

The reason for the conception of this stimulator was to render the light stimulation more precise and more flexible by allowing to vary all its main physical parameters: intensity, frequency and wavelength, as well as to perform a sequence of stimulations

while externally measuring light illumination with an integrated luxmeter. We shall call it 'intensity' in the rest of the text.

Figure 12.1. : StimLux

12.3. Technical characteristics of the StimLux

The StimLux is a stimulator that allows the transmission of flashes of colour, intensity, frequency, and exposure time defined by the investigator using independent owner software conceived of by the inventor. The colour is chosen with indices ranging from 0 to 255 permitted by three LEDs, red, green, and blue. This also determines the radiation power.

It is able to produce 16.7 million different colours.

To adjust the frequency the StimLux uses an electronic card, Arduino STK 2560, that varies the rate at which the LEDs switch on and off. The frequency may vary from 0 Hz (continuous light) to 30 Hz maximum.

The intensity of the stimulation is measured by a small luxmeter placed on the exterior of the stimulator, close to the subject's eyes. The luxmeter continuously analyses the intensity in lux, calculating the mean intensity of the flash light stimulations, and thanks to a mathematic paradigm it includes in this calculation also the "non-exposure time" interval between flashes. The luxmeter was put externally and not internally in the stimulator to avoid the reduction in light intensity due to the various internal supports interposed between the LEDs and the subject's eyes.

We chose as a maximum power of 4200 lux has been made so as to avoid any collateral effects to the retina. In the literature other authors have used stimulators up to 10000 lux of intensity without any ophthalmologic side effects; they were unable to provoke a migraine attack if the subject was stimulated only by light, even if the stimulation was very intense and uncomfortable (Hougaard et al., 2013).

The StimLux is connected to a PC by a USB port. We can also connect it to another trigger such as Signal™, by a 5V input, in order to synchronize the light stimulation and evoked potentials recordings. The trigger is able to switch on the StimLux but the sequencing of the stimulation has to be introduced into the StimLux software. StimLux can have an input trigger but it cannot itself trigger any other stimulator. To connect the StimLux to Signal we used a Jack connector 3.5V, necessary only if the 5V connector is not available or not recognised by the trigger machine.

The StimLux has a steel support with two main arms that allows the regulation of the distance and the height of the stimulator to position it just in front of the subject's eyes. The anterior part of the support's base is weighted with steel to ensure stability when the superior arm is at its maximum extension. The posterior part of the main arm is also reinforced with steel for the same reason. StimLux is on wheels to be mobile, but it still is a prototype that can be used only in the laboratory and is not a portable stimulator.

The stimulation affects both eyes and the device does not permit to restrict the stimulation to a defined portion of the visual field.

12.4. StimLux Software

To set the exposure time the user is able to encode into the software the desired time in seconds. In the software we chose to have 2 principal windows: the first to encode the exposure time only if the colour of the stimulation does not change during the session; the second window allows the possibility to make consecutive sequences of stimulations, during which the colour, the frequency, the intensity and the duration can be varied as required. The maximum number of different sequences is 10 per session.
The StimLux device allows to modify frequency, colour and intensity of the light stimulation utilising dynamic sequencing.

A possible adverse effect of photic stimulation is induction of an epileptic fit. It is therefore of uttermost importance to exclude subjects with a personal or familial history of epilepsy during recruitment for StimLux studies. The risk of epileptic seizures increases with the increase of stimulation frequency.
In case of a worrisome adverse effect during the stimulation an "Emergency power shut-off button" in the software allows to immediately switch off the stimulator. To protect the stimulator from short circuits it has an internal automatic power breaker. This function is also activated if the user would introduce excessive parameters of stimulation that could compromise the integrity of the stimulator. In this case the sequence of stimulation is not accepted and the StimLux remains switched off.

12.5. Assessment of photophobia using StimLux in healthy subjects and migraine patients.

12.5.1. Objective

The aim of this study was to determine photophobia during flash light stimulation in HS and episodic migraine patients (EM) during the ictal and interictal phases, using the StimLux at low (5 Hz) and high (20 Hz) frequency and at the two ends of the visual spectrum, blue (~470 nm) and red (~720 nm). The choice of colours was made knowing that ipRGCs are predominantly activated by blue light (*see above*).

12.5.2. Subjects and methods

We enrolled a total of 36 subjects:

- 7 HS (3 females, 4 males, mean age 33.71 ± 10.43 [SD] years old).
- 10 EM during the interictal phase (7 females, 3 males, mean age 36.4 ± 16.65 [SD] years old, 8 without any prophylactic treatment and 2 with a stable preventive treatment).
- 19 EM during the ictal phase (17 females, 2 males, mean age 37.26 ± 10.07 [SD] years old, 7 without any prophylactic treatment and 12 with a stable preventive treatment).

Before the stimulation, all subjects completed the photophobia questionnaire adapted from Choi et al. (2009) (*Table 12.1*).

Table 12.1: Questionnaire of photophobia, adapted from Choi et al. (2009)		
Q1) During your headache, do you feel a greater sense of glare or dazzle in your eyes than usual by bright lights?	Yes	No
Q2) During your headache, do flickering lights, glare, specific colours or high contrast striped patterns bother you or your eyes?	Yes	No
Q3) During your headache, do you turn off the lights or draw a curtain to avoid bright conditions?	Yes	No
Q4) During your headache, do you have to wear sunglasses even in normal daylight?	Yes	No
Q5) During your headache, do bright lights hurt your eyes?	Yes	No
Q6) Is your headache worsened by bright lights?	Yes	No
Q7) Is your headache triggered by bright lights?	Yes	No
Q8) Do you have any of the above symptoms mentioned even during your headache-free interval?	Yes	No
Q9) During your headache, how much is the unconfort resulting from the exposure to an intense bright light? (From 0 to 10, where 0 is no discomfort and 10 it is the maximum intensity of the discomfort)	Yes n/10	No
Q10) Outside of migraine attacks, how much is the unconfort resulting from the exposure to an intense bright light? (From 0 to 10, where 0 is no discomfort and 10 it is the maximum intensity of the discomfort)	Yes n/10	No

After 4 minutes of adaptation in a dark room, subjects were seated in front of the light stimulator, at 5 cm of distance from the eyes.

We tested 4 dynamic sequences, 2 colours and 2 flicker frequencies with a progressive increase in intensity by steps of 50 Lux, beginning at 50 Lux, each step lasting 5 seconds. The 4 sequences (Blue 5 Hz; Blue 20 Hz; Red 5 Hz; Red 20 Hz) were delivered in random chronological order.

The subjects were asked to tell us to stop the stimulation when he perceived an uncomfortable sensation. To assess if that moment was really the threshold point, the stimulation continued for the following sequence that in all subject became to be painful and induced a photophobic reaction of closing eyes. There was a 2-minute rest period between successive sequences.

12.5.3. Results

We found no difference in scores of the photophobia questionnaire or in sensitivity to light stimulation sequences between patients with or without preventative treatment. We therefore combined the data of these patients (*Fig. 12.2*).

The Mann-Whitney's U Test disclosed a significant difference between the three subjects groups in the total score of the questionnaire ($p=0.0001$), in the 5 Hz Blue sequence ($p=0.00009$), in the 20 Hz Blue sequence ($p=0.001$), in the 5 Hz Red sequence ($p=0.002$) and in the 20 Hz Red sequence ($p=0.004$).

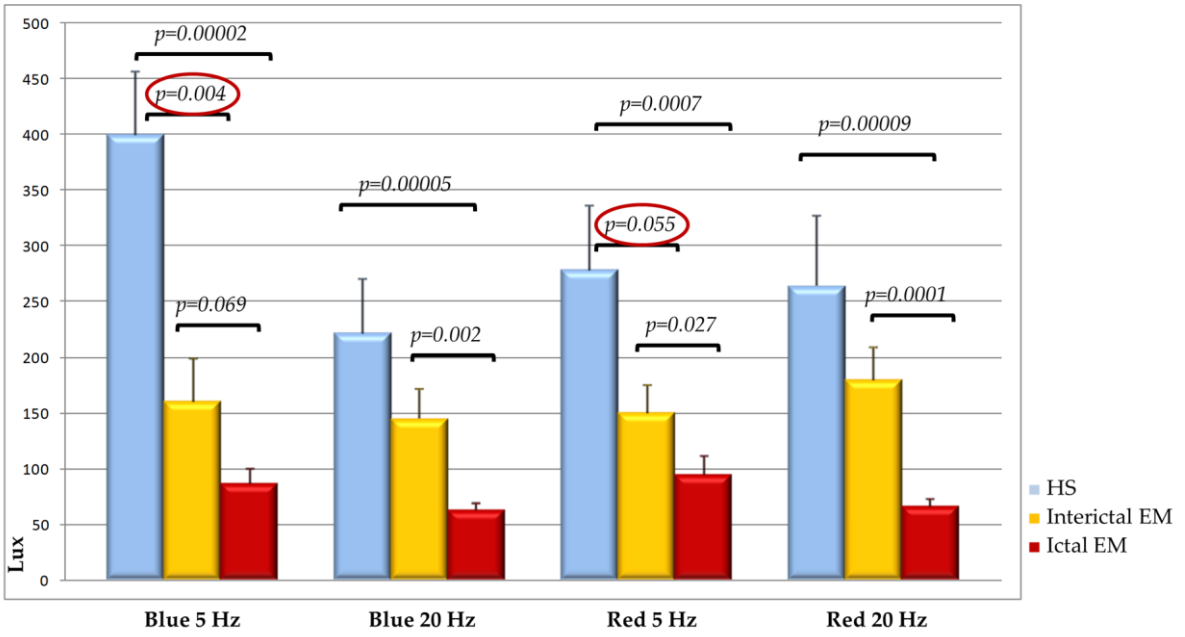
Compared to HS, interictal EM patients were significantly more light-sensitive using the questionnaire ($p=0.003$), in the 5 Hz Blue sequence ($p=0.004$) and tended to be so in the 5 Hz Red sequence ($p=0.055$), but were not significantly different during the 20 Hz Blue and Red sequences.

Compared to HS, EM patients during an attack had a greater sensitivity to light in the total score of the questionnaire ($p=0.0001$), in the 5 Hz Blue sequence ($p=0.00002$), in the 20 Hz Blue sequence ($p=0.00005$), in the 5 Hz Red sequence ($p=0.0007$) and in the 20 Hz Red sequence ($p=0.00009$).

Compared to interictal EM patients, ictal patients were significantly more light sensitive during the 20 Hz Blue sequence ($p=0.002$), the 5 Hz ($p=0.027$) and 20 Hz Red sequence ($p=0.00019$) and tended to be so for the 5 Hz Blue sequence ($p=0.069$), but there was no significant difference in the total score of the questionnaire.

We found no significant correlation between the photophobia assessment using StimLux and frequency of headache, mean duration of attacks or duration of the disease.

Figure 12.2: Photophobia assessment using StimLux in HS (Blue), Interictal EM (yellow) and Ictal EM (Red) during the four tested sequences : Blue 5 Hz, Blue 20 Hz, Red 5 Hz and Red 20 Hz. The circled p values indicate the differences between HS and interictal EM is significant for Blue 5 Hz sequences but just fails to be significant for Red 5 Hz while it is not significant for the 20 Hz sequences. The difference between interictal and ictal is significant for all sequences except Blue 5 Hz, whereas ictal EM significantly differ from ictal EM in all sequences.



12.5.4. Discussion

This study discloses various novel features. First we designed a light stimulator we called StimLux that allows to vary wavelength, frequency and intensity of light stimulation. A device allowing such a diversity of stimulation protocols is not commercially available. We used the StimLux to assess more precisely light sensitivity in healthy subjects and its abnormality in migraine patients. The device also allows determining which stimulation protocol is the most efficient in producing the inhibition of trigeminal nociception by light stimulation shown in *Chapter 10*.

The photophobia questionnaire is useful to distinguish healthy subjects from migraineurs, but it lacks sensitivity to detect the difference in the degree of photophobia between the interictal and ictal phases of migraine.

The main difference between HS and interictal EM is found at low frequencies of stimulation (5 Hz), while the main difference between interictal and ictal EM occurs at high frequencies (20 Hz). Tolerance to the light is less influenced by colour than by frequency of the stimulus.

The mean threshold for tolerance to the light is 50 Lux for EM patients during an attack, which is 3-5 times lower than the normal luminance of an artificially lit room and even more clearly lower than the natural ambient light on a sunny day.

Photophobia assessed by the questionnaire or by the flash light stimulation does not correlate with the clinical features of migraine. Finally, light tolerance is not modified by the preventive pharmacotherapy of migraine.

The use of a questionnaire to determine photophobia in clinical practice has been validated by several studies (Choi et al., 2009; Evans et al., 2008): an adequate and precise questionnaire on photophobia can detect 24% more photophobic migraineurs than the simple history taking. It seems, however, that the questionnaire should be more complete, especially regarding the persistence of photophobia between attacks.

This is the reason why we added two questions to the Choi et al.'s questionnaire with the possibility to enter the degree of discomfort on a scale from 0 to 10. Furthermore, in our experiment patients had some difficulties in answering "yes" or "no" to the first and second questions due to the multiple circumstances listed.

Our findings show nevertheless that the modified photophobia questionnaire is not able to distinguish between the ictal and the interictal phases of migraine, contrary to the graded flash light stimulation with the StimLux.

This is the first study that has used flash light stimulation to measure the degree of photophobia. In fact, the other three light stimulation studies (Drumond et al., 1986; Vanagaite et al., 1997; Kowacs et al., 2001) all used continuous light. The distinction seems particularly important since we show here that the subjects groups differed not only according to light intensity but to light frequency. Applying frequency as a variable appears therefore of greater interest than intensity. We decided to assess photophobia using different frequencies for several reasons: firstly in our previous study (*Chapter 10*) we used flash light stimulation at 8 Hz and we found that it is able to increase the facial pain threshold and to decrease the R2 response of the nociceptive blink reflex. Secondly, flash light stimulation, due to contrast, has a strong activating effect on the visual cortex.

The stimulation frequency influences the difference between HS and interictal patients and also between ictal and interictal EM. Low frequency proves more tolerable to HS, particularly if associated with the blue colour. High frequencies are associated with greater discomfort in HS.

The tolerance of interictal EM is around 150 Lux for all types of lights and that of ictal EM is around 50 Lux, compared to HS that showed a mean tolerance threshold around 300 Lux. Our results are in line with the study on photophobia by Drummond et al. (1986) in which all migraineurs reported discomfort at 153.5 Lux. Our study adds knowledge to the previous one by displaying the cut-off between ictal and interictal patients that Drummond et al. were unable to investigate because they used

only 5 intensities (1.1 Lux, 7.3 Lux, 36.3 Lux, 153.3 Lux and 500 Lux). We found that the tolerance threshold of ictal and interictal patients differs by 100 Lux (from 50 Lux to 150 Lux respectively).

The duration of each light-sequence was set at 30 seconds in Drummond's protocol, in our protocol it was of 5 seconds and in those of Vanagaite and Kowacs, 2 seconds. In the subjects' perception, having 2 seconds of continuous light and having 5 seconds at 20 Hz, i.e. a total of 100 pulses, is very different. The tolerance to light was higher in the abovementioned studies (Vanagaite et al., 1997; Kowacs et al., 2001). This could be due to the greater distance (40 cm; 5 cm in our protocol), which reduces the luminance. Moreover the authors interposed a heat-filtering barrier between the light source and the subject's eye in order to reduce the risk of corneal lesions; they probed intensities of 20000 Lux, far superior to the intensities we used. In addition our StimLux device produced binocular stimulation, which was found to causes greater discomfort than monocular stimulation (Vanagaite et al., 1997).

Blue and red were chosen as tested colours to investigate the extremities of the visual spectrum, because to the best of our knowledge no study has yet analysed the influence of colour on the photophobia threshold. The blue colour activates the ipRGCs more than the other spectral wavelengths (*see Chapter 10.4*).

An experiment performed in our research unit some years ago using five different coloured lenses (red, green, blue, yellow, grey) showed that VEP amplitude increased with red and green lenses in HS but not in migraine patients between attacks (Afra et al., 2000). This result was interpreted as reflecting a possible hypoexcitability of the visual cortex in migraine patients. Our findings clearly show that the red light is the most able to distinguish ictal from interictal photophobia thresholds, but the blue colour produces greater differences when comparing HS and EM.

Many studies have demonstrated an altered EEG pattern in the visual areas with flash light stimulation, especially at the higher frequencies (Bjørk et al., 2011). Photic

driving induced by flash light stimulation was found increased during the interictal phase in migraineurs and depressed during the attack (de Tommaso et al., 1998). Conversely, in another study the opposite pattern was found (Bjørk et al., 2011). Photic driving to higher frequencies might have a protective role to permit the tolerance of high frequencies during the interictal phase, a control that is probably impaired during the migraine attack where the subject habituates with greater difficulty to high frequency light stimulation. It would be interesting to study the photic driving using different colours.

In conclusion, this part of our thesis confirms that light sensitivity and tolerance differ between HS, interictal EM and ictal EM, and shows that the rate of the light stimulation can influence the photophobia threshold.

We have shown in *Chapter 10* that flashlight stimulation is able to reduce trigeminal nociception. It is thus of interest to determine which stimulation parameters are the most efficient in producing this effect. We have studied his in the next chapter using the StimLux device.

13. Effects of frequency, colour and intensity of light stimulation on the nociceptive blink reflex in healthy subjects

13.1. Introduction

Flash light stimulation of the visual cortex inhibits tonically the trigeminal nociceptive blink reflex and trigeminal pain sensitivity in healthy subjects (HS) and episodic migraine patients (EM) (*see Chapter 10*). In this study we explored how changing frequency, wavelength and intensity of the light stimulation influences these effects on the trigeminal nociceptive system in HS.

Its ultimate goal was to identify the flash light stimulation protocol that is most effective in reducing pain perception and nBRs in HS and could be used in a future trial as therapeutic strategy in migraine patients.

The study measures changes in nBR and trigeminal pain thresholds in three conditions: 1) variation of frequency alone; 2) variation of colour; 3) variation of intensity with fixed frequency and colour.

13.2. Subjects and methods

For this study we recruited 11 HS (8 females and 3 males, mean age 36.45 ± 11.29 [SD] years old).

One subject decided to interrupt the study and the effect of varying light intensity was thus assessed only in 10 HS.

For more details on the recruitment of HS *see Chapter 6.1*.

The protocol was divided into three sessions, separated by several days each.

In each session, we first measured the sensory threshold (ST) and the pain threshold (PT) and then we recorded the nBR at baseline (*see Chapter 6.2.2*).

The chronological sequence of types of frequency, colour and intensity was randomized and blinded to the subject and the main investigator (SLS); another colleague drew by lot the sequence. So each subject had a different chronological order of stimulation to avoid the possible bias of an order effect.

13.2.1. Part 1. Varying stimulation frequency

The first step to define the most effective stimulation parameter to inhibit trigeminal nociception was the variation in stimulation frequency.

As fixed wavelength we chose yellow (~580 nm) that was also used in the previous study of the effect of 8 Hz flash light on trigeminal nociception (*see Chapter 10*).

As fixed intensity we chose 2000 Lux, i.e. the mean intensity that the flash light prototype StimLux delivers. This choice was empiric.

The only variable was the frequency of stimulation. We tested five different frequencies: 8 Hz, 10 Hz, 12 Hz, 15 Hz and 20 Hz. We chose 20 Hz as the upper limit because the risk of inducing an epileptic fit increases at higher frequencies. The total duration of the flash light stimulation for each analysed parameter of light was 7 minutes.

The five sessions were performed on the same day, separated by at least 15 minutes.

13.2.2. Part 2. Varying colour

In this part of the experiment the only variable parameter was the colour of the light stimulation.

We tested six different colours: violet (~390 nm), blue (~470 nm), green (~530 nm), yellow (~580 nm), orange (~610 nm) and red (~ 730 nm).

As fixed parameters we chose a frequency of 12 Hz based on the results obtained in *Part 1* and intensity at 2000 Lux.

Five sessions were performed on the same day, separated by at least 15 minutes. For the yellow colour we used the data obtained in *Part 1* at 12 Hz.

13.2.3. Part 3. Varying intensity

As fixed parameters we used a 12 Hz frequency and violet (~390 nm) as colour.

As variable parameter we tested six different intensities: 500 Lux, 1000 Lux, 1500 Lux, 2000 Lux, 3000 Lux and 4000 Lux.

Five sessions were performed on the same day; separated by at least 15 minutes. For the 2000 Lux intensity we considered the data obtained in *Part 2*.

All subjects tolerated the flash light stimulation without any adverse effect and finished all sessions. A Visual Analogue Scale (VAS) from 0 to 10 cm was used in all experiments to evaluate the discomfort to the light.

Due to the randomized sequence for each session and for each HS, we compared all measures to the baseline recordings done at the beginning of each session.

13.3. Results

13.3.1. *Part 1. Effects on trigeminal nociception of varying stimulation frequency*

Table 13.1: Electrophysiological data (means ± sd) after variation of flash light **frequency**. Fixed parameters: Yellow colour (580 nm) as described *in Chapter 12*; 2000 Lux intensity. **p<0.05; **p<0.01*

	Baseline	8 Hz	10Hz	12 Hz	15 Hz	20 Hz
PT (mA)	10.41±5.42	**11.26±4.85****	**12.63±7.01****	**15.34±9.73****	**14.70±9.96****	**11.54±6.03***
1° block nBR Ipsilateral (µV*ms)	0.010±0.012	0.007±0.008	**0.007±0.008***	**0.007±0.013***	0.009±0.016	0.009±0.014
1° block nBR Contralateral (µV*ms)	0.011±0.016	**0.006±0.009***	**0.005±0.007***	**0.007±0.012****	**0.008±0.014****	**0.006±0.009***

Table 13.1 summarizes the effects of varying stimulation frequencies on pain threshold (PT) and 1st block amplitude of ipsi- and contralateral nociceptive blink reflexes.

After light stimulation at an 8 Hz frequency we found a significant increase in the supraorbital pain threshold (PT) (*p=0.004*) (*Fig. 13.1*), a decrease of contralateral 1st block nBR (AUC) (*p=0.03*) (*Fig. 13.2*) and a trend for increased habituation of contralateral nBR expressed as the slope of amplitude changes over the five averaged blocks of 5 responses (*p=0.09*).

After 10 Hz stimulation there was an increased PT (*p=0.007*), a decreased AUC of the 1st block of ipsilateral (*p=0.05*) and contralateral nBR (*p=0.02*) and a trend for increased habituation of the contralateral nBR (*p=0.09*).

The 12 Hz frequency had the greatest effect on the nBR: it increased the sensory threshold (ST) (*p=0.021*) and the PT (*p=0.008*) and decreased the AUC of the 1st block of ipsilateral (*p=0.04*) and contralateral (*p=0.003*) nBR, but had no effect on habituation.

After the 15 Hz frequency we found an increased ST (*p=0.021*) and PT (*p=0.007*) and a decrease of the AUC of the 1st block of contralateral nBR (*p=0.003*).

The 20 Hz frequency increased the PT (*p=0.04*) and decreased the AUC of the 1st block of contralateral nBR (*p=0.05*).

Figure 13.1: Pain Threshold (mA) changes with the variation of frequency in HS. Data are shown as mean ± standard error. **p<0.05; **p<0.01*

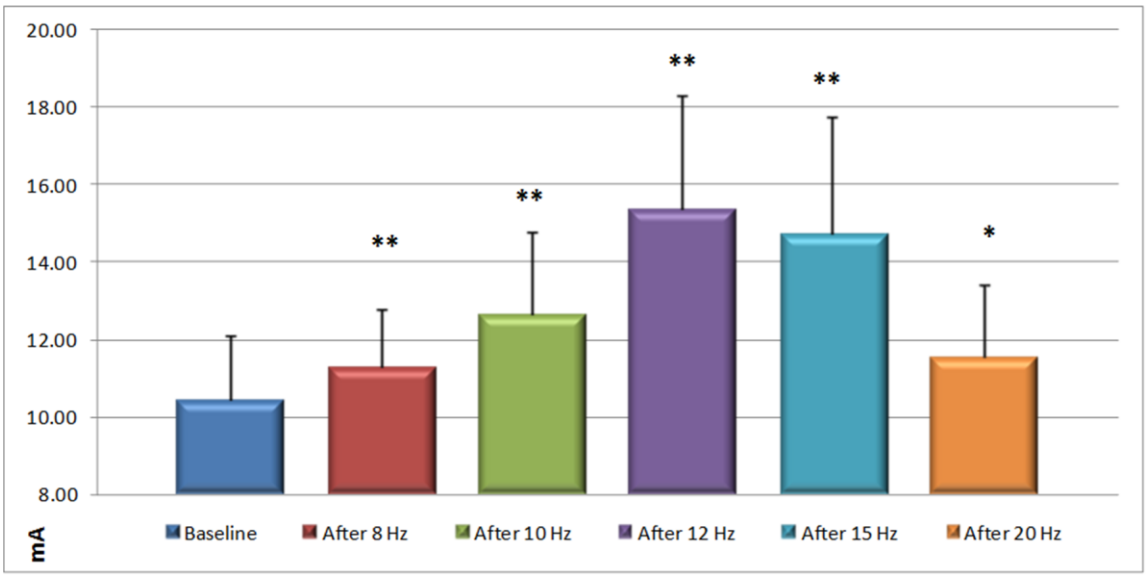
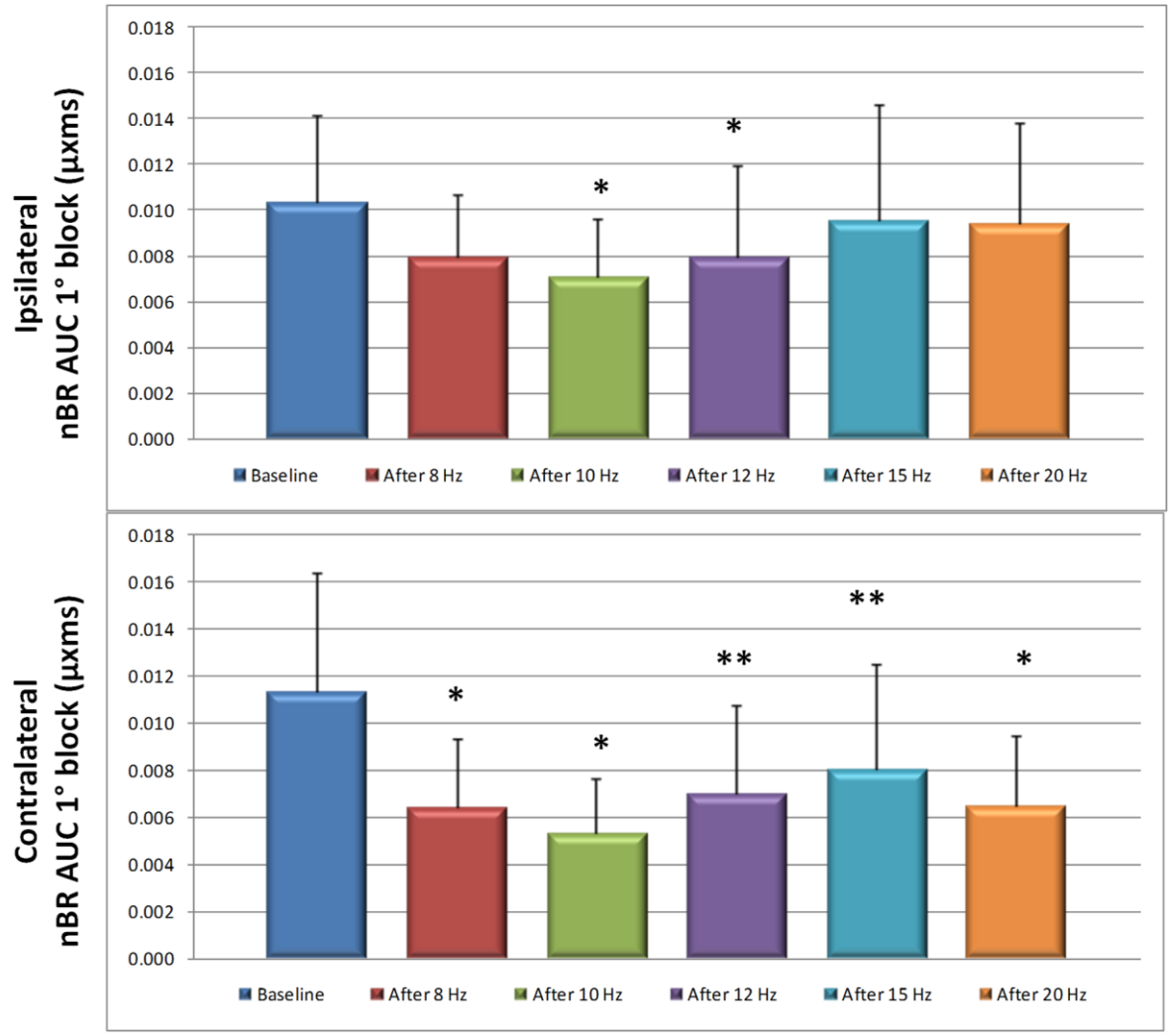


Figure 13.2: Changes in 1st block AUC R2 nBR with variation of frequency in HS. Data are shown as mean ± standard error. *$p<0.05$; **$p<0.01$.



13.3.2. *Part 2. Effects on trigeminal nociception of varying colour*

Table 13.2: Electrophysiological data (means ± sd) after variation of flash light **wavelength**. Fixed parameters: 12 Hz frequency; 2000 Lux intensity.*$p<0.05$; **$p<0.01$. **NB: For yellow session see Chapter 13.3.1. at 12 Hz**

	Baseline	Violet	Blue	Green	Orange	Red
PT (mA)	13.59±11.06	**22.29±26.08****	**25.93±28.89****	**23.18±24.26****	**30.01±39.06****	**22.32±29.67****
1° block nBR Ipsilateral (µV*ms)	0.007±0.009	0.004±0.006	**0.002±0.004***	**0.003±0.004****	**0.004±0.006***	0.006±0.01
1° block nBR Contralateral (µV*ms)	0.005±0.007	0.003±0.004	**0.002±0.002****	**0.002±0.002****	0.003±0.005	0.004±0.007

Table 13.2 summarizes the changes of PT and nBR amplitudes observed by modifying wavelength of the flash light stimulation.

The shows the modulation of the Pain Threshold on the supraorbital area after varying colour of stimulation.

The violet stimulation markedly increased PT (*p=0.005*) (*Fig. 13.3*) and induced a negative correlation between the VAS score and nBR habituation: the higher the discomfort score the smaller the nBR habituation. The other colours tested did not correlate inversely with the VAS.

The blue colour increased the PT (*p=0.003*) and decreased the AUC of the 1st block of 5 nBR responses ipsilaterally (*p=0.02*) and contralaterally (*p=0.009*).

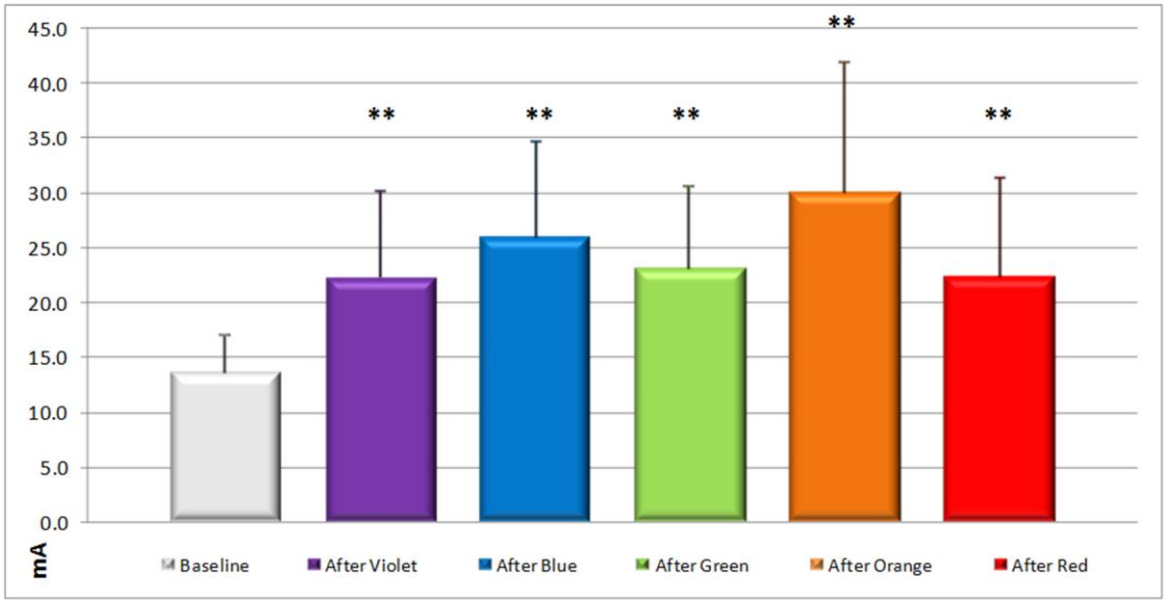
The green stimulation similarly increased the PT (*p=0.003*) and decreased the amplitude of ipsilateral and contralateral nBR (*p=0.005* both).

The yellow colour (*see Chapter 13.3.1*) increased the sensory threshold (ST) (*p=0.021*) and the PT (*p=0.008*) and decreased the 1st block AUC of ipsilateral (*p=0.04*) and contralateral nBR (*p=0.003*), without any effect on habituation.

The orange stimulation increased the pain threshold (PT) (*p=0.004*) and decreased the AUC of the 1st nBR block only ipsilaterally (*p=0.03*).

The red light increased the PT (*p=0.006*) but had no significant effect on nBR.

Figure 13.3: Pain Threshold (mA) changes with the variation of colour in HS. Data are shown as mean ± standard error. *$p<0.05$; **$p<0.01$. **NB: For yellow session see paragraph 13.3.1 at 12 Hz.**



13.3.3. *Part 3. Effects on trigeminal nociception of varying intensity*

Table 13.3: Mean electrophysiological data (± standard deviations) after variation of flash light **intensity**. Fixed parameters: 12 Hz frequency; Violet (390 nm). *$p<0.05$; **$p<0.01$. **NB: For 2000 Lux session see paragraph 13.3.2. Violet.**

	Baseline	500 Lux	1000 Lux	1500 Lux	3000 Lux	4000 Lux
PT (mA)	18.72±13.59	**27.55±25.53***	**27.63±27.02***	**33.58±41.14***	**33.42±44.54***	**35.28±44.95***
1° block nBR Ipsilateral (µV*ms)	0.005±0.009	**0.003±0.005***	**0.003±0.005***	**0.003±0.005***	0.002±0.003	**0.003±0.004***
1° block nBR Contralateral (µV*ms)	0.006±0.01	**0.002±0.005****	**0.002±0.005***	**0.003±0.005***	**0.001±0.002***	**0.004±0.007***

Table 13.3 summarizes the PT and nBR amplitude changes by different intensities of violet flash light stimulation.

With a stimulation intensity of 500 Lux we found an increased pain threshold (PT) (*p=0.021*) (*Fig. 13.4*) and a decrease of the 1st nBR block AUC ipsilaterally and contralaterally (*p=0.012* and *p=0.006*) (*Fig. 13.5*).

After the 1000 Lux intensity stimulation the PT increased (*p=0.024*) and the AUC of the 1st block nBR decreased ipsilaterally and contralaterally (*p=0.036* and *p=0.021*).

The intensity of 1500 Lux increased PT (*p=0.02*), decreased of AUC of ipsilateral (*p=0.046*) and contralateral (*p=0.036*) 1st block nBR and tended to increase the habituation over the three blocks (*p=0.07*).

2000 Lux increased only the PT significantly (*p=0.005*) but left unchanged the nBR.

After the 3000 Lux intensity the PT increased significantly (*p=0.036*) and the AUC of the 1st block nBR decreased contralaterally (*p=0.05*).

The 4000 Lux intensity increased PT (*p=0.021*) and decreased of the 1st block AUC of ipsilateral (*p=0.036*) and contralateral (*p=0.048*) nBR but left the habituation slope unchanged.

As expected, 3000 Lux and 4000 Lux induced high discomfort scores on the VAS. Many subjects had difficulties to finish the session at these intensities.

Figure 13.4: Pain Threshold (mA) changes with the variation of intensity in HS. Data are shown as mean ± standard error. **p<0.05; **p<0.01*. **NB: For 2000 Lux session see paragraph 13.3.2 Violet**

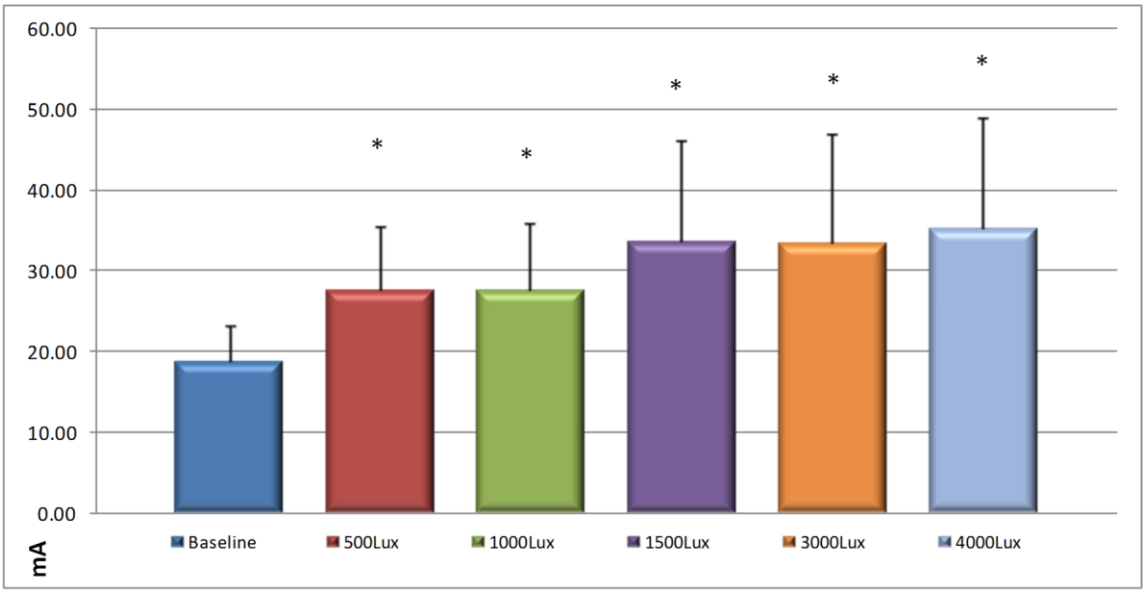
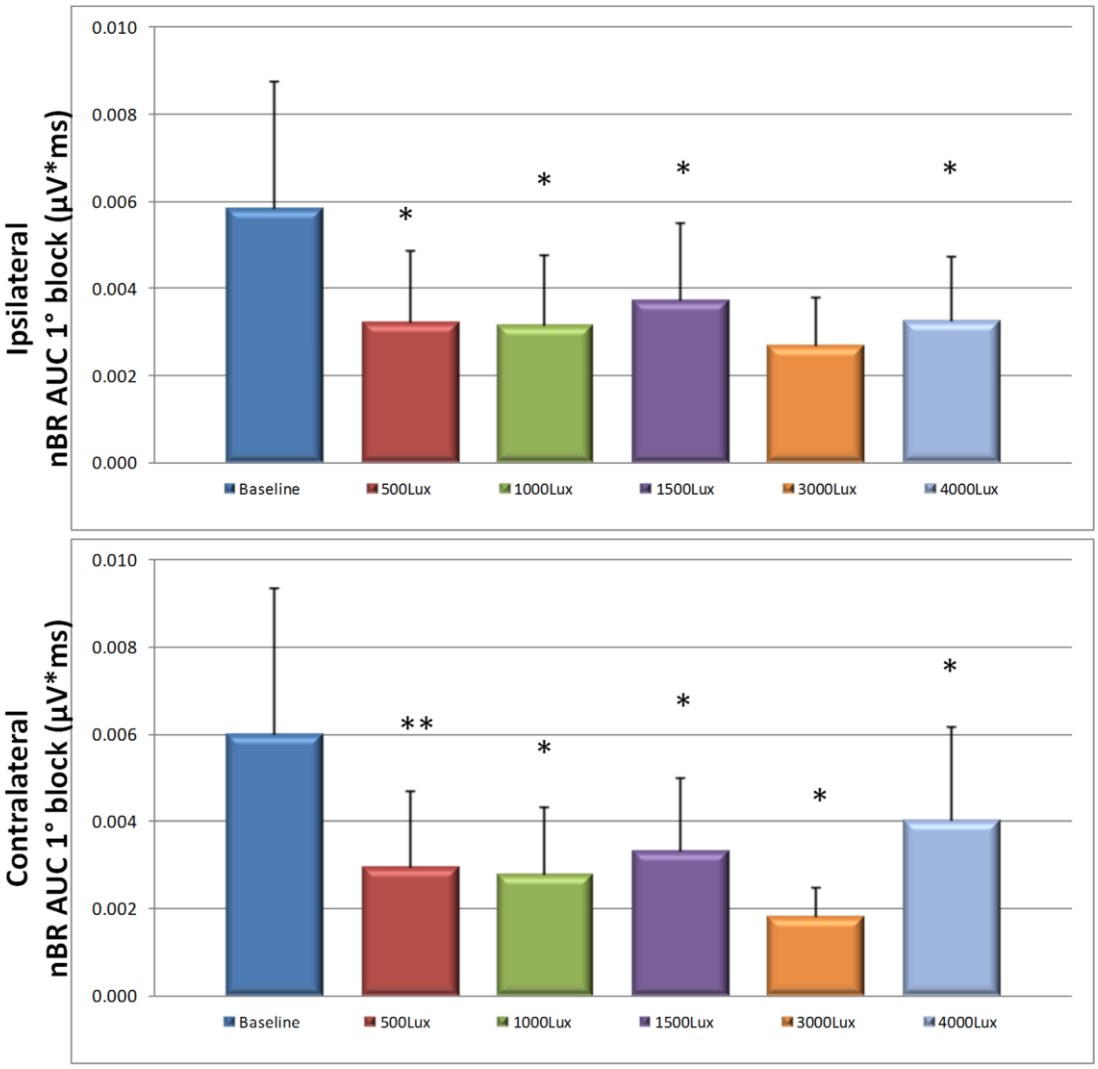


Figure 13.5: AUC changes of the 1st R2 nBR block with variation of intensity in HS. Data are shown as mean ± standard error. *$p<0.05$; **$p<0.01$. **NB: For 2000 Lux session see paragraph 13.3.2 Violet**



13.4. Discussion

The first goal of this experiment was to confirm our finding in *Chapter 10* that flash light stimulation is able to increase the electrical pain threshold (PT) in the supra-orbital area and to decrease the amplitude of the nociceptive blink reflex (nBR). In HS, who overall, underwent 160 measurements, the PT was increased whichever stimulation frequency, wavelength or intensity were used. By contrast, changing these light parameters had a differential influence a more objective measure of trigeminal nociceptive processing, the nBR.

For the first time this study allowed analysing the effect of the main physical characteristics of light in the same healthy subjects, which was rendered possible thanks to the conception and development of the StimLux device (*see Chapter 12*).
During each session of this experiment subjects had five or six light stimulation sessions in the same day, which in theory could increase the risk of inducing corneal lesions. We must point however that no adverse effect was reported by the subjects in any session, except for some colour illusions at the end of each 7-minute stimulation that disappeared after less than 1 minute of time. Several instances of colour illusions were detected in *Part 2* of the experiment: subjects experienced a transient alteration of the perception of colour at the end of the stimulation caused probably by a loss of the capacity of cones to be stimulated by the same wavelength for 7 minutes. This explanation is supported by the fact that the colour illusion was characterised by the perception of the opposite wavelength on the visual spectrum. For instance, after violet stimulation the subjects experienced a transient red perception of the surrounding environment while after the green session they saw the world in a pink hue for a few seconds.

The 20 Hz frequency increased pain thresholds less than the other frequencies, possibly because it induced a greater light discomfort. The ipsilateral 1st nBR block

was decreased only by the 10 Hz and 12 Hz stimulation rates, and not by the other frequencies. This is in line with previous studies of light-induce changes of electrical brain activity. de Tommaso et al. (2005) studied the synchronisation of alpha band EEG during stimulations of different frequencies. They analysed the effects of low (3, 6 Hz), frequencies (9, 12 Hz) and high frequencies (15, 18, 21, 24, and 27 Hz) comparing HS and migraine patients. They found that in migraine patients 9 Hz induced hypersynchronisation in migraine patients but not in controls. The same effect was observed at 24 and 27 Hz. We chose to limit the frequency to 20 Hz to reduce the risk for adverse effects of the light stimulation, the more so that the duration of stimulation in our protocol was longer (7 minutes) than in de Tommaso et al.'s study (between 30 and 120 seconds).

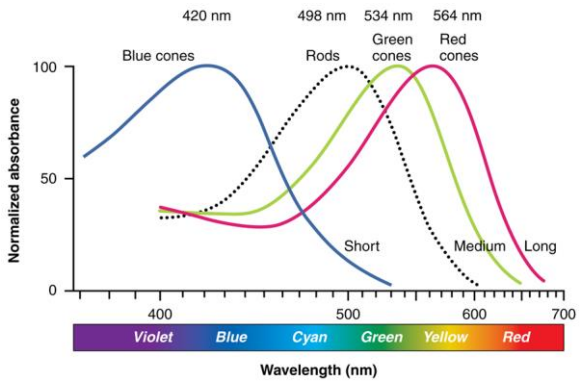
We tested six different colours at precise wavelengths. However, as the stimulator doesn't produce polarized light, for each colour there was at the same time a small amount of the rest of the visual spectrum. We chose not to use polarized light because we wanted to explore the effects of natural light, as much as possible.

Other authors have studied the influence of colour using cortical evoked potentials. In particular Afra et al. (2000) found that the interposition of red or green filters increased VEP amplitudes in HS but not in migraine with aura patients. Interestingly, while the red wavelength is more effective to activate the visual cortex (Zerbe et al., 1979) and to elicit photoconvulsive responses or photic driving responses in patients suffering from epilepsy (Takahashi et al., 1976 and 1981), in our study the red colour influenced the nBR response less than the lower wavelengths. It is a well-established fact that the energy value per quantum of light is inversely related to wavelength. This may explain why we found such a significant effect on the nBR with lower wavelengths and why red filters amplify less VEPs in migraineurs who have a decreased cortical preactivation excitability between attacks (Afra et al., 2000).

For the low intensities we chose those that can be encountered in a conference room (~500 Lux), for high luminance those we can find in large convention centres with more than 20000 people to illuminate (~2000 - 4000 Lux).We tested only HS, but others tried to provoke a migraine attack in patients, using 10000 Lux of continuous light, without success (Hougaard et al., 2013). Using the external luxmeter we were reliably informed on the luminance upon the subjects' eyes.

Taking together, these results suggest that the 12 Hz rate and the 1500 Lux of intensity are the most efficient in increasing the PT and decreasing the 1st block nBR AUC. Regarding wavelengths, the only colour that was associated with an inverse correlation between the VAS discomfort score and nBR habituation is violet, although blue, green and orange all were able to decrease nBR amplitude. Colour seems to be the parameter that is associated with the greatest variability. The reason may be found in the visual spectrum. Using non-polarized light the main wavelength peaks at a fixed value of nanometres, but each of the three cone populations overlap in the respective logarithmic wavelength distribution and responds also to adjacent wavelengths (*Fig. 13.6*), which is confounding factor.

Figure 13.6.: Visual spectrum and wavelengths



Given the results found in this study and in *Chapter 12*, we designed a flash light stimulation protocol using the StimLux susceptible to have a therapeutic benefit in migraine patients and tested it in a proof-of-concept trial described in the next chapter.

14. Using the top-down inhibitory control of the visual cortex on trigeminal nociception to treat migraine: a proof-of-concept trial.

14.1. Introduction

In the previous study we demonstrated that flash light stimulation at 12 Hz and 1500 Lux is more effective in HS at increasing the electrical pain threshold in the supra-orbital area and to decrease the 1st block nBR amplitude. Blue, green and orange were most effective on the nBR, however violet correlated inversely to the VAS score of discomfort perceived by the subject due to the flash light stimulation.

The next step was thus to investigate the effect of this inhibitory pathway in episodic (EM) and chronic patients (CM).

In this experiment we extended the duration of stimulation sessions from 7 to 20 minutes and they were applied 5 days per week for two weeks.

The effect of low wavelengths and in particular of blue light is well documented on seasonal affective disorder (SAD) (Golden et al., 2005), and also in non-seasonal mood disorders associated with circadian rhythm disturbances (jet lag, shift work or dementia), sleep disorders, bulimia nervosa and adult attention-deficit/hyperactivity disorder (Pail et al., 2011). In an unpublished study conducted in our centre, the effect of daily treatment with continuous blue light stimulation was tested in migraine patients using the Luminette® device. The results were inconclusive.

In order to stay within the low wavelength range and to submit the patients to the less uncomfortable light stimulation, we chose to use the violet colour (~390 nm).

14.2. Subjects and methods

In the first part of this study, we applied 20-minute light stimulations of violet colour, at a frequency of 12 Hz and an intensity of 1500 Lux in 10 HS (8 females, 2 males, mean age 37.55 ± 10.70 [SD] years old). Before and after the stimulation we measured the electrical pain threshold (PT), nBR and VEP amplitude and habituation.

In the second part, we recruited 20 migraine patients: 11 with episodic migraine (EM) (8 females and 3 males, mean age 40.89 ± 9.66 [SD] years old) and 9 with chronic migraine (CM) (8 females and 1 male, mean age 48.56 ± 13.77 [SD] years old), diagnosed according to ICHD 3β criteria (2013). EM patients had no a prophylactic treatment, while CM patients had a stable prophylactic treatment since at least one month before the study. Patients were recruited in our outpatient headache clinic. The patients had to fill in a headache diary to be eligible for the study.

EM and CM patients had baseline recordings of nBR and VEP, where after they were stimulated with the StimLux device in the hospital during 2 consecutive weeks for 20 minutes daily, 5 days a week. At the end of the 2-week treatment they underwent another recording of nBR and VEP.

Therapeutic outcome measures were total days of headache/month; migraine days/month; headache days/month; mean attack duration in hours/month; NSAID intake/month; triptan intake/month; number of days/month with headache of severe intensity (grade 3 on a 0 to 3 categorical scale); number of days/month with moderate headache (grade 2) and number of days/month with mild headache (grade 1).

We compare these outcomes measures between the 1-month baseline before the beginning of the therapeutic protocol and the 1-month period that followed the 1st stimulation session and comprised the 2 weeks of light simulation and 2 subsequent weeks of follow-up without stimulation. During this period CM patients were not allowed to modify their preventive anti-migraine drug treatment.

Other two CM patients dropped out because they had found it difficult to come daily to the hospital for the stimulation sessions.

For more details *see Chapter 6.1*; *6.2.2*; *6.2.3* and *13*.

14.3. Results

In HS the flash light session significantly increased the supra-orbital PT (*p=0.007*) (*Fig.14.1*), tended to decrease the AUC of the 1st nBR block ipsilaterally (*p=0.06*) and decreased significantly AUC of contralateral nBR (*p=0.05*). It had no influence on the nBR habituation (*Fig.14.2*).

In the post-stimulation VEP recordings we found a numerical decrease of the 1st block N1-P1 and P1-N2 amplitudes that did not reach significance, and no effect on habituation.

Figure 14.1: Pain Threshold (mA; means ±standard errors) in HS before and after the 20-minute flash light stimulation.

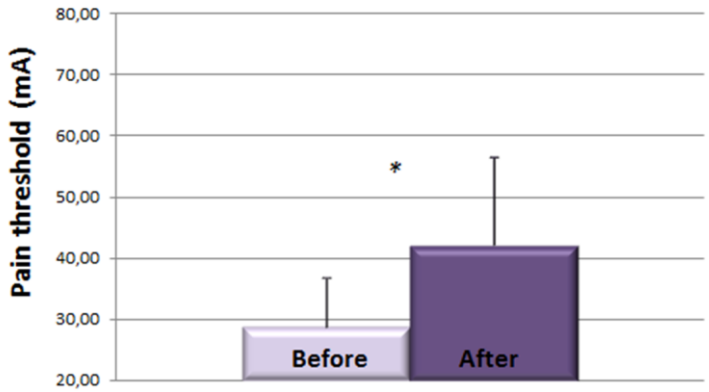


Figure 14.2: Ipsilateral and contralateral nBRbefore (light violet) and after (dark violet) the 20-minute flash light stimulation in HS. (means ± standard errors).

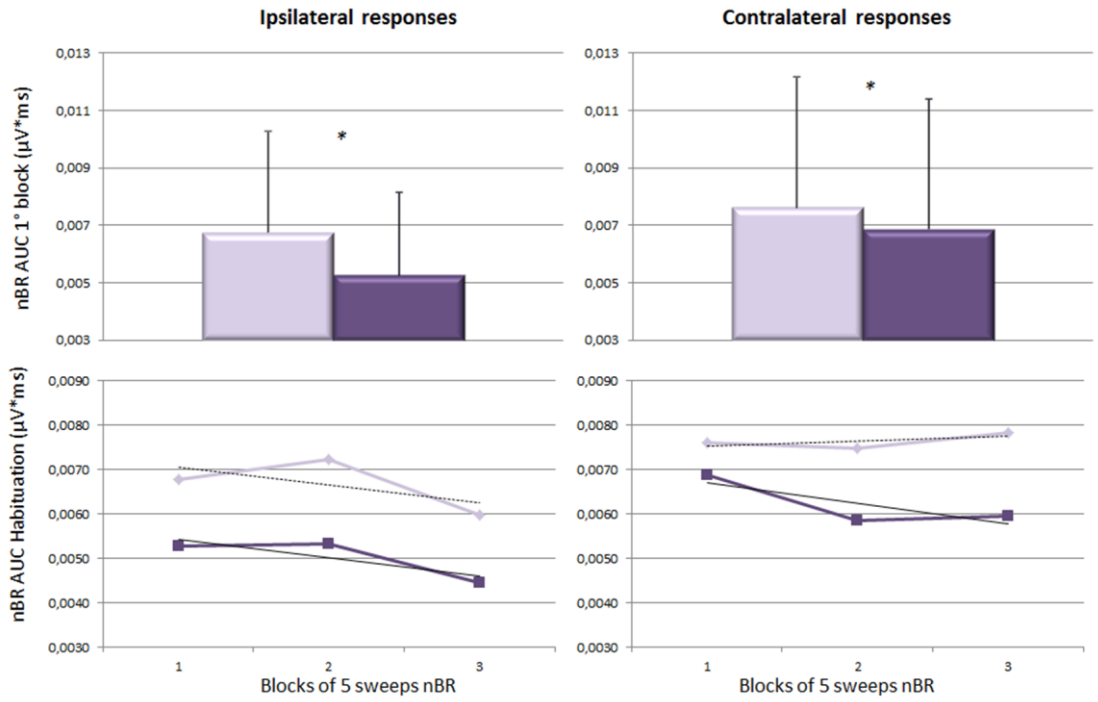


Table 14.1 shows in migraine patients the change in clinical outcome measures assessed with the headache diary during the month preceding the trial and during the subsequent month comprising 2 weeks of light stimulation and 2 weeks of follow-up without stimulation.

Table 14.1: Clinical outcome measures (means ± standard deviationin episodic and chronic migraine patients as assessed by the migraine calendar during the 1-month baseline before stimulation and during the month following the 1st stimulation session comprising 2 weeks of light therapy and 2 weeks follow-up without stimulation.

Episodic Migraine n = 11						
	Before StimLux	**After StimLux**	***Effect %***	***p***	**Resp.50%**	**Resp.30%**
Total Headache days/month (n)	9.36±4.37	8.64 ± 4.95	↓7.69%	.48	27.3%	27.3%
Migraine days/month (n)	8.45 ± 4.32	6.45 ± 5.35	**↓23.66%**	**.03**	**36.4%**	**45.5%**
Headache days/month (n)	0.91 ± 1.30	2.27 ± 1.90	**↑149.4%**	**.01**	---	---
Duration in hours/month (mean)	80.64 ± 60.44	58.27 ± 66.33	**↓27.74%**	**.03**	**54.5%**	**54.5%**
NSAID Intake/month (n)	11.73 ± 14.70	5.00 ± 5.81	**↓57.37%**	**.05**	**54.5%**	**63.4%**
Triptans Intake/month (n)	4.09 ± 6.93	3.09 ± 5.28	↓24.44%	.40	27.3%	27.3%
Days/month severe intensity (n)	2.73 ± 4.20	1.36 ± 1.63	↓50.18%	.47	45.5%	45.5%
Days/month moderate intensity (n)	3.55 ± 3.47	3.36 ± 2.23	↓5.35%	.48	27.3%	36.4%
Days/month mild intensity (n)	3.09 ± 3.39	3.82 ± 2.23	↑23.62%	.30	---	---

Chronic Migraine n = 9						
	Before StimLux	**After StimLux**	***Effect %***	***p***	**Resp.50%**	**Resp.30%**
Total Headache days/month (n)	23.33 ± 5.72	12.78 ± 8.32	**↓45.22%**	**.01**	**44.4%**	**66.6%**
Migraine days/month (n)	15.56 ± 4.42	6.67 ± 5.52	**↓57.13%**	**.01**	**77.7%**	**77.7%**
Headache days/month (n)	7.78 ± 6.30	6.11 ± 7.01	↓21.46%	.47	55.5%	66.6%
Duration in hours/month (mean)	212.67 ± 54.70	114.78 ± 89.88	**↓46.02%**	**.007**	**55.5%**	**77.7%**
NSAID Intake/month (n)	15.44 ± 24.65	8.78 ± 6.59	↓43.11%	.86	22.2%	22.2%
Triptans Intake/month (n)	6.56 ± 11.39	3.09 ± 5.28	↓52.89%	.14	33.3%	33.3%
Days/month severe intensity (n)	10.00 ± 10.46	4.89 ± 5.33	↓51.10%	.15	55.5%	55.5%
Days/month moderate intensity (n)	6.89 ± 5.51	2.67 ± 2.69	**↓61.24%**	**.02**	**55.5%**	**55.5%**
Days/month mild intensity (n)	6.33±7.37	5.00 ± 6.76	↓21.01%	.49	44.4%	44.4%

Fig. 14.3 shows the change of clinical outcome measures at three time points: before light stimulation, after 2 weeks of light stimulation and during the 2-week follow-up period after light therapy.

In EM patients, the 50% responder rate for migraine days was 36.4%; there was a significant decrease in the mean duration of headache attacks and NSAID intake.

CM patients had a significant decrease in total headache days/month, migraine days/month, mean duration of attacks and days per month with moderate headache. 77.7% of CM patients had at least a 50% reduction in monthly migraine days.

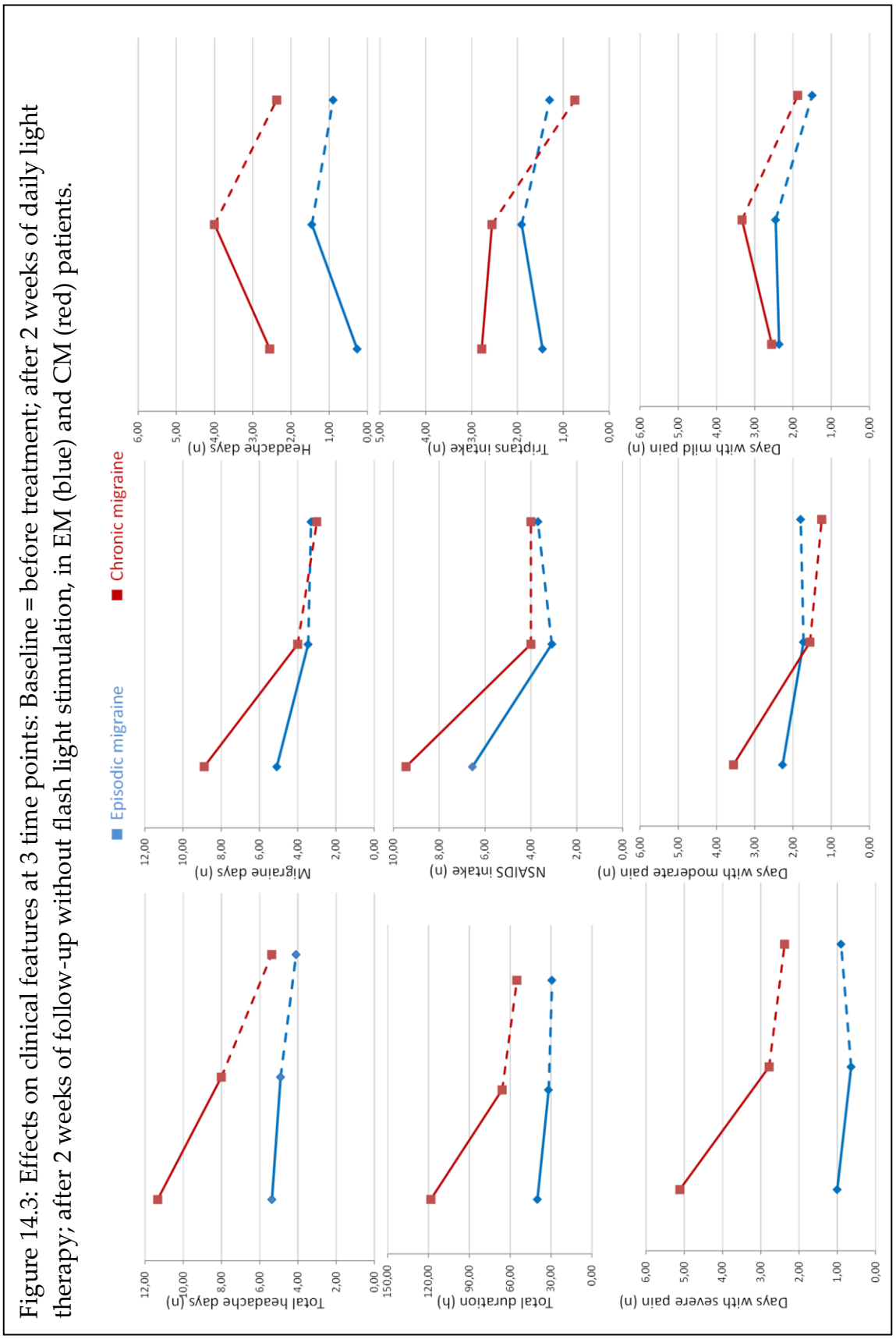
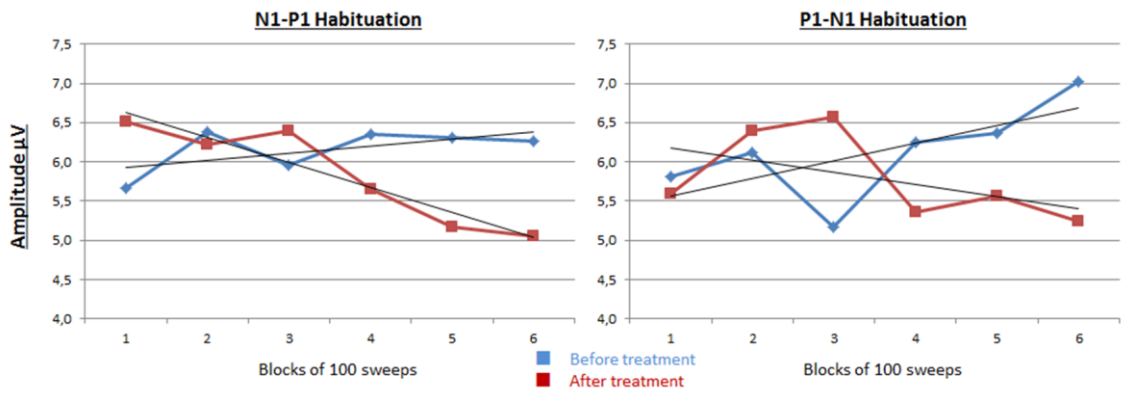


Figure 14.3: Effects on clinical features at 3 time points: Baseline = before treatment; after 2 weeks of daily light therapy; after 2 weeks of follow-up without flash light stimulation, in EM (blue) and CM (red) patients.

After the 2-week light treatment we found that habituation of VEP N1-P1 was significantly increased in 50% CM responders (*p=0.017*) while habituation of the P1-N2 component tended to increase (*p=0.09*) (*Fig 14.4*). There was no change in nBR parameters.

Figure 14.4.: VEP N1-P1 and P1-N2 habituation in 50%CM responders



14.4. Discussion

Confirming our previous studies on flash light stimulation (*Chapter 10*), one session of violet flashing light (12 Hz, 1500 Lux, duration 20 minutes) increased the supra-orbital pain threshold and decreased nBR 1st block amplitude in healthy subjects. As we hypothesized on basis of this anti-nociceptive effect in the trigeminal system, daily stimulations for 2 weeks using the same protocol had a therapeutic effect in migraine patients, as suggested by our pilot trial. The effect size seems to be greater in CM than in EM with a significant decrease in total number of headache days, migraine days, in duration of attacks and total number of days with moderate headache. The only significant electrophysiological change was an increase of VEP habituation in 50% CM responders.

To the best of our knowledge, this pilot trial is the first study using flash light stimulation in migraine as a prophylactic treatment. Because many migraine patients are light sensitive during and, to a lesser degree, between attacks, one might, indeed, at first thought not envisage treating them with light stimulation.

Our findings raise several fundamental questions. Is light stimulation able to desensitize the nociceptive trigeminovascular system and its activation by light and is this the underlying mechanism for the clinical improvement in our pilot trial? Does daily light stimulation change responsiveness of the visual cortex in migraineurs and hence reduce photophobia as well as disease activity? Is it worthwhile pursuing the investigations on therapeutic effects of light stimulation, for instance by a sham-controlled trial and can such a trial be blinded?

It is unquestionable that migraine patients have photophobia during attacks (Choi et al., 2009), and also, though less so, in the interictal phase of the migraine cycle. The validation study of the ID Migraine® diagnostic tool suggests that photophobia is a specific symptom that allows to diagnose migraine in 98% of cases when associated with disability and nausea (Lipton et al., 2003). However, photophobia is not a migraine-specific symptom and it is associated with many other ocular, neurological and sometimes psychiatric pathologies. In ocular diseases photophobia occurs in anterior segment diseases such as iritis, cyclitis, and blepharitis, where the mechanism is presumably direct irritation of the trigeminal afferents that innervate the cornea and eye. Photophobia is a classical symptom in meningitis (Lamonte et al., 1995), sub-arachnoid haemorrhage (Welty et al., 1990) or pituitary tumours or apoplexy (Kawasaki et al., 2002) due to irritation of the primary meningeal nociceptive afferents, predominantly belonging to the visceral part of the ophthalmic nerve (Trobe et al., 2002). In traumatic brain injury photophobia may persist for several weeks after the initial trauma and patients with post-concussive syndrome retain an increased sensitivity to light (Bohnen et al., 1991). In blepharospasm the cause of photophobia is unknown but thought to be due to an excitation/inhibition

imbalance in the brainstem blink reflex pathways (Berardelli et al., 1985). Finally photophobia can also be associated with progressive supranuclear palsy (PSP), where it seems to be more frequent than in corticobasal degeneration or Parkinson's disease (Cooper et al., 2009; Hills et al., 2008). Finally, photophobia can accompany depression and anxiety (Bossini et al., 2009). In fact, photophobia tends to accompany many chronic pain disorders such as fibromyalgia (Martenson et al., 2015).

The cardinal stimulus for photophobia is light; thus, afferent light pathways and their respective projection areas must be involved. Interestingly, photophobia can be experienced without image formation, as documented in some blind patients (Zaidi et al., 2007; Amini et al., 2006; Noseda et al., 2010 and 2011).

Okamoto et al. (2009) recorded activation of the trigeminal nucleus caudalis (TNC) neurons in rats while shining light on their eyes. The responsible circuit for the observed effect starts in retinal photodetectors (whether rod, cone, or ipRGC is unclear), activates the superior salivatory nucleus, which in turn induces ocular vasodilation and activation of pain-sensing neurons on blood vessels. Noseda et al. (2010) identified a completely different circuit in animals: projections from retinal ganglion cells to the posterior thalamus, from where via the caudate-putamen and the external capsule they reach multiple cortical regions, including the binocular area of the primary visual cortex. Martenson et al., (2015) excluded the posterior thalamic relay after light exposure in rats, and showed a possible functional connection permitted by the olivary pretectal nucleus.

In humans Moulton et al. (2009) performed BOLD fMRI recordings in an individual with photophobia associated with the overuse of contact lenses. During the photophobic state, activation of the trigeminal ganglia, the trigeminal nucleus caudalis, the ventro-postero-medial thalamus and the anterior cingulate gyrus was observed, while these structures were not activated when photophobia was absent. In a series of patients with LASIK-induced photophobia, Malecaze et al. (2001) found that light-induced BOLD fMRI activation was increased in the visual association

cortex, compared to controls. Emoto et al. (2010) used 18-fluorodeoxyglucose positron emission tomography (18-FDG-PET) to compare patients suffering from blepharospasm with or without photophobia to controls. They found that blepharospasm patients with photophobia had significantly increased metabolic activity in the thalamic ventral anterior (VA) and ventral lateral (VL) nuclei and in the superior colliculus.

Few studies have tried to treat photophobia and most of them employed tinted lenses, although in 1934 already Lebelsohn wrote "tinted glasses as a symptomatic remedy for chronic photophobia are to be condemned because of both their ineffectiveness and their habit forming tendency". Since that date, not much progress has been made to treat photophobia. The rationale of our study was to use the light stimulus that causes photophobia to treat migraine by assuming that this might desensitize the afferent or the efferent arm of the "photophobia circuit".

One clinically relevant finding was that none of our patients developed a migraine attack after the light stimulation. In fact, light seems to be able to trigger a migraine attack only when it is associated with other trigger factors such as physical exercise (Hougaard et al., 2013). CM patients experienced greater discomfort at the beginning of the session, but this uncomfortable sensation rapidly vanished during the stimulation. If the stimulation took place during the headache, in particular in CM patients, the headache intensity was attenuated during the flash light stimulation and even more so after the session. The repetitive nature of the light stimulation may explain these findings, as it contrasts with a sudden bright light stimulus that can cause additional discomfort in migraine patients. The flashing light probably allows the visual processing to habituate, hence increasing the tolerance to light. This is supported by the fact that in the CM group 50% responders had, after 2 weeks of flash light stimulation, an increase in VEP habituation. Activation of the visual cortex in migraineurs by anodal tDCS (Viganó et al., 2013) or high frequency rTMS (Bohotin

et al., 2002) was previously shown in our research unit to increase VEP habituation. Habituation is a complex neurobiological phenomenon that is not linearly related to cortical excitability. A recent study failed to find a correlation between VEP habituation and the magnetophosphene threshold, a direct measure of visual cortex excitability (Ambrosini et al., 2015), probably because habituation represents a dynamic response pattern of sensory cortices to repeated stimuli, reflecting the temporal profile of cortical reactions to incoming inputs.

Regarding the trigeminal nociceptive system, one 20-minute session of flash light stimulation with the StimLux device decreased pain perception and nBR amplitude in healthy subjects, which is concordant with the results found in *Chapters 10* and *13* with shorter stimulation sessions. Contrasting with the acute light-induced changes found in the previous chapters, however, we found here no significant modification of nBR parameters after 2 weeks of daily stimulations in migraine patients. This may be due to the low number of patients in each group, or to physiological factors related to adaptation processes over long periods of stimulation. This question cannot be answered in our study, as we did not test the effect on the nBR or VEP immediately after the first stimulation in migraine patients.
Whether the observed therapeutic effect of flash light stimulations is associated with an excitability change in the visual processing pathways and/or in the trigeminal nociceptive system needs therefore further studies.

It is difficult and probably premature to compare our results with those of other preventive neurostimulation methods in migraine for several reasons. First our patients were stimulated for only 2 weeks, while in the other trials neurostimulation treatments are administered for one to several months. Since the StimLux device is not portable, the patients had to be treated in the hospital, which caused some inconvenience to them and may have increased a placebo effect, but on the other hand ensured a perfect compliance. Our trial was intended to proof a concept and

hence not sham-controlled. However, sham-controlled studies with neurostimulation methods still remain scarce. Antal et al. (2011) performed a sham-controlled trial of inhibitory cathodal tDCS over the visual cortex for migraine prevention in 26 patients. They found no significant change in attack frequency, the primary efficacy measure, but there was a decrease in mean attack duration and intensity. A sham-controlled study of daily anodal tDCS over the motor cortex for 20 consecutive days in 42 EM patients (Auvichayapat et al., 2012) significantly reduced attack frequency and abortive medication use at weeks 4 and 8 after treatment. In another sham-controlled study of 10 anodal tDCS for 4 weeks over the motor cortex in 13 CM patients, pain intensity was significantly reduced at weeks 4, 8, and 12 (Dasilva et al., 2012). Another study of rTMS over the motor cortex reported a significant reduction of headache frequency (about 85% lower at 1st week after stimulation), headache severity, functional disability and analgesic intake (Misra et al., 2013).

A small (11 patients) study of 12 sessions of high frequency-rTMS over the left dorsolateral prefrontal cortex (LDLPFC) for preventive treatment in patients affected by chronic refractory migraine (Brighina et al., 2004) found a significant reduction of migraine attacks (about 57% lower), abortive medication use, headache index and migraine disability scores in the verum group.

External trigeminal neurostimulation with the Cefaly® decreased the number of migraine days by 2.06 and the frequency of monthly migraine attacks by at least 50% in 38% of patients after 3 months in a sham-controlled trial of 67 episodic migraine patients (Schoenen et al., 2013).

To sum up, large RCTs of neurostimulation therapies in migraine are rare. The results of our proof-of-concept suggest that it might be worthwhile to set up a RCT of flash light stimulation therapy in both EM and CM patients. As in all neurostimulation trials, except tDCS that induces only a very slight sensory perception, blinding could be a problem. This problem could nonetheless be minimized by randomizing the

sham group to a flash light stimulation of low intensity (< 15 lux), < 5 Hz frequency, and > 580 nm wavelength.

Such a RCT would be easier to organize with a portable StimLux device, but for obvious financial reasons this will probably not be feasible before the outcome of a larger RCT is known.

Third Part: Conclusions

15. General conclusion and discussion

15.1. Summary of results

This thesis investigates and extends the connection between the cortex and the trigeminal nociceptive system using neurostimulation technics in healthy subjects and migraine patients.

The main finding is the possible functional top-down inhibitory pathway between the visual cortex, the thalamus and the trigeminal nociceptive system that might protect constantly the brain from the onset of a new migraine attack.

This pathway is closely related to the presence of light, and a flash light stimulation seems to be interesting to desensitize the subject to the light discomfort and to decrease at the same time the frequency and the duration of attacks.

First of all, we measured the spontaneous blink rate (SBR) in healthy subjects and migraine patients during the ictal and interictal phase, in a lit or dark environment.

The SBR had not been measured in migraine patients before, and it is known that its variation principally relies upon a dopaminergic pathway (Karson et al., 1982) and there is circumstantial evidence for a role of dopamine in migraine pathophysiology (Charbit et al., 2010; Barbanti et al., 2013). The modulation of SBR is also dependent on cortical and subcortical controls, in which the occipital cortex may play a role. The principal result was that the SBR is not different between HS and EM in a lit environment, but in the dark the SBR decreases both in HS and in ictal EM patients, while in interictal EM patients there was no change, suggesting a dysfunction in the visual cortex in the interictal phase.

It is well known that the visual cortex is involved in the pathophysiology of migraine, but its connection with the trigeminal nociceptive system is unclear.

Our second study supports the existence in healthy subjects of a functional relation between the visual cortex and the trigeminal nociceptive system, as assessed by nBR.

The relation seems to be inhibitory: when we apply inhibitory rTMS over the visual cortex there is a reduction in the pain threshold, and thus an increased perception of pain, and, as a corollary a facilitation of the nBR; when we apply excitatory rTMS over the visual cortex, the effect tends to be opposite, but does not reach the level of statistical significance.

Notwithstanding, results in HS are not confounded by the stimulation of other peripheral regions, in fact the magnetic and transcutaneous stimulation over the occipital nerve did not have any effect on the PT and the nBR; as well as the sham stimulation over the visual cortex, performed putting the coil at 90° to the occipital region.

In migraine patients both 1 and 10 Hz rTMS failed to induce a significant change of pain perception in the trigeminal V1 area and of the nBR. However, habituation of the contralateral nBR response was enhanced after 1 Hz rTMS in MO and MA patients and reduced after 10 Hz rTMS. These results suggest that the visual cortex in migraine patients is not able to significantly modify trigeminal pain perception and nBR amplitude, which we attribute to a different state of cortical responsivity in migraine between attacks.

On the other hand, it is well established that stimulation of the motor cortex has analgesic properties (Osenbach, 2006; Galhardoni et al., 2015) including in facial pain (Henderson et al., 2006). This study was conceived as a comparator for our previous experiment on the effects of rTMS over the visual cortex. Its main result is that rTMS over the motor cortex does not cause the same changes of the nBR and pain perception as visual cortex modulation. Nonetheless, the motor cortex seems to increase habituation of the contralateral nBR R2 response, but this effect is similar with low or high frequency rTMS.

An abnormal rhythmic activity between thalamus and cortex, namely thalamo-cortical dysrhythmia, may be the pathophysiological mechanism subtending abnormal information processing in migraine (Coppola et al., 2013).

Increasing the thalamo-cortical drive may induce a beneficial on trigeminal pain perception and brainstem excitability. Using the flash light stimulation we found a decrease in pain perception (as assessed by the increased pain threshold), a diminution of the nBR amplitude, and facilitation of the habituation of the contralateral nBR in both HS and migraine patients.

The study confirms our results with rTMS in HS suggesting an inhibitory control of the visual system on trigeminal nociception. The major difference is that this inhibitory effect with flash light stimulation is demonstrable in migraineurs, in whom we could not demonstrate it with excitatory rTMS of the visual cortex. The effect of flash light stimulation seems thus more robust on both trigeminal pain perception and the nociceptive blink reflex.

That vision is able to reduce pain in humans is known since several years (Longo et al., 2009) and was called "visually-induced analgesia" (VIA). We showed for the first time that the phenomenon of visually-induced analgesia can be demonstrated in the trigeminal area in HS, as far as it is assessed by contact heat evoked potentials. The reduction of subjective pain scores, though numerically detectable, was however not significant and habituation of CHEPs amplitude is not modified.

In migraine patients, VIA seems normal in the cephalic area, but abnormal changes with the mirror-viewing are seen at the extracephalic stimulation site: no detectable decrease in CHEPs amplitude and an increase in pain scores.

The last part of this thesis was made possible thanks to the development of a new prototype of light stimulation, called "StimLux" and built by the investigators.

Using this device, we search to evaluate photophobia in healthy subjects and migraine patients during and outside the migraine attack, comparing the subjective discomfort to the flash light stimulation to a photophobia questionnaire. We found that the photophobia questionnaire is useful to distinguish healthy subjects from migraineurs, but it doesn't evaluate the photophobia degree between the interictal

and ictal phases of migraine. The main difference between HS and interictal EM is found at low frequencies of stimulation (5 Hz), while the main difference between interictal and ictal EM occurs at high frequencies (20 Hz). Sensitivity to the light is less influenced by colour than by frequency of the stimulus.

The ultimate goal was to identify the flash light stimulation protocol that is most effective in reducing pain perception and nBRs in HS and could be used in a future trial as therapeutic strategy in migraine patients.

Taking together, these results suggest that the 12 Hz rate and the 1500 Lux of intensity are the most efficient in increasing the PT and decreasing the 1st block nBR AUC. Regarding wavelengths, the only colour that was associated with an inverse correlation between the VAS discomfort score and nBR habituation is violet, although blue, green and orange all were able to decrease nBR amplitude. Colour seems to be the parameter that is associated with the greatest variability.

We designed a flash light stimulation protocol using the StimLux susceptible to have a therapeutic benefit in migraine patients and tested it in a proof-of-concept trial. The effect size seems to be greater in CM than in EM with a significant decrease in total number of headache days, migraine days, in duration of attacks and total number of days with moderate headache. The only significant electrophysiological change was an increase of VEP habituation in 50% CM responders. This was not a controlled trial and its results have to be interpreted with caution.

15.2. Pathophysiological relevance

Taking into account published data from human studies and animal experiments, we will examine step by step the various anatomical relays that are possibly involved in the (patho-)physiology of photophobia and relevant for its role in migraine and novel therapeutic approaches.

The retina

Retinal cells that are directly sensitive to light are rods (for black-white vision), cones (for colour vision) and intrinsically photosensitive retinal ganglion cells (ipRGCs). The latter are excited by light even if rods and cones are blocked and contain the photopigment melanopsin (Provencio et al., 2000; Lucas et al., 2001; Hattar et al., 2003; Qiu et al., 2005) that is preferentially excited by blue light in the visual spectrum (~480 nm). ipRGCs represent no more than 1% of retinal cells (Berson et al., 2003) and they have a role in non-image-forming functions.

The non-image-forming visual circuits play a role in the synchronisation of circadian rhythm through a retino-hypothalamic tract to the suprachiasmatic nucleus of hypothalamus and in pupillary control through the activation of the olivary pretectal nucleus in the midbrain; finally they lead to the release of melatonin from the pineal gland via the sympathetic system. ipRGCs also project to the contralateral dorsal lateral geniculate nucleus (dLGN), implying a contribution to more conventional aspects of visual discrimination. Projections from ipRGCs were also found in the ciliary marginal zone (Semo et al., 2014) and in the iris (Rupp et al., 2013). Interestingly, these projections interfere with those ocular regions that are highly innervated by the trigeminal nerve. Noseda et al. (2010) advanced the hypothesis that ipRGCs are implicated in the mechanism of photophobia, through a connection between meningeal and retinal afferents in the dorsal and dorso-lateral thalamus.

Another role of ipRGCs is to regularize the general activity of the organism, the so-called “masking” effect, i.e. the disruption of overt rhythms by external factors

occurring for example during the day in in diurnal species. In fact several studies have shown that despite removing rods or cones or melanopsin, masking still occurs (Mrosovsky et al., 2001; Panda et al., 2002). By contrast, the removal of all three types of photoreceptors and particularly of ipRGCs eliminates masking (Hattar et al., 2003; Panda et al., 2003; Goz et al., 2008; Guler et al., 2008; Hatori et al., 2008).

Diseases causing loss of rods and cones do not cause photophobia, such as in X-linked cone-rod dystrophy or in Leber's congenital amaurosis.

Is thus likely that light aversion is connected to ipRGCs and a melanopsin pathway, although it can also be induced by morphine in rats through a non-melanopsin pathway (Matynia et al. 2012).

Animal models of photoallodynia, bradyopsia and corneal surface damage all manifest light aversion (Recober et al., 2009). Disorders affecting the anterior segment of the eye such as uveitis, iritis, cyclitis, blepharitis and corneal damage or inflammation can cause photophobia (Digre et al., 2012). Melanopsin gene mutations are linked to seasonal affective disorder (Roecklein et al., 2009) and in glaucoma, where photophobia is common, ipRGCs can be lost if inflammation from the disease or its medication, or ischemic tissue damage are present (Feigl et al., 2011; Kankipati et al., 2011).

From animal studies it has been suggested that the retina itself can activate trigeminal neurons in response to bright light through a parasympathetic circuit (Okamoto et al., 2010) and in humans ipRGCs project directly to the areas of the "pain matrix" (Maleki et al., 2012) just as in the animal model (Hattar et al., 2006) (*Fig. 15.1*). To support this hypothesis it has been shown that photophobia is present in blind migraineurs who are capable of light perception (cone/rod degeneration), but not in those who are totally blind due to complete damage of the optic nerves (Noseda et al., 2010).

On the other hand corneal damage activates trigeminal pathways (Moulton et al., 2009); the cornea is one of the most densely innervated structures in the body, and its innervation comes from the first branch of the trigeminal nerve.

Even if eye damage, including retinal damage, such as achromatopsia, may imbalance the homeostatic equilibrium and cause photophobia, it does not cause headache. The close relationship between migraine headache and photophobia implies that the mechanism is central and not ocular.

The Thalamus

The thalamus is obviously involved in the pathogenesis of photophobia and in migraine. In the thalamus, information carried by the optic nerve, the "visual pathway", reaches the lateral geniculate nucleus (LGN) and from there to the striate cortex. The extra-striate pathway involves the superior colliculus, the pulvinar and the extrastriate cortex.

We will focus our attention on the "non-visual pathway" and particularly on the thalamic regions involved in the retino-trigemino-visual pathways.

That trigeminal information converges in the thalamus, especially from the meningeal and also from the ocular structure, is well known animals (Davis et al., 1988; Zagami et al., 1990; Angus-Leppan et al., 1995; Shields et al., 2005, Noseda et al., 2010 and 2011).

The dorsal and dorso-lateral thalamus seems to be involved in a new light-activated pathway. By studying rats Noseda et al. (2010) traced the path from the ipRGCs stimulated by light, and found that there is a contingent that conveys both retinal and dura-sensitive spinal trigeminal nucleus information (*Fig. 15.1*).

In humans Maleki et al. (2012) demonstrated, by MR tractography, a direct connection between the optic nerve and the pulvinar (*Fig. 15.1*). Moulton et al. (2009) showed with fMRI in a subject with photophobia induced by contact lenses, that the ventro-postero-medial thalamus was activated during the photophobic state and that after recovery no activation occurred.

The thalamus is one of the possible localisations of the origin of cephalic and extracephalic allodynia, not only for photo-allodynia: in rats stimulated by mechanical and thermal skin stimuli, the thalamus exhibited a long-lasting

hyperexcitability; in migraine patients, undergoing migraine with whole-body allodynia, acute thalamic activation to extracephalic brush or heat stimuli has been found using fMRI (Burstein et al., 2010).

The Brainstem

In the three regions that compose the brainstem we can find structures activated by light: the trigemino-cervical complex, the olivary pretectal nucleus and the raphe magnus.

The brainstem is considered to be the generator of migraine attacks, and its involvement is well accepted in their repetition (*see Chapter 2*). In this part of the central nervous system is located the trigeminal nociceptive system. The activation of the latter by light-induced information has been documented in the animal model.

Okamoto et al. (2009) showed that intermittent exposure to light can activate neurons in laminae I and II at the Vc/C1 junction and in the nucleus tractus solitaris (NST). The same group one year later (Okamoto et al., 2010) hypothesized that the activation of the trigeminal nociceptive system may occur thanks to the interposition of the olivary pretectal nucleus (OPN). Increased parasympathetic outflow, permitted by the activation of the superior salivatory nucleus, allows the transmission of light to connect to the Vc/C1 junction. It is known that the OPN is necessary for several light-induced responses, such as the pupillary light reflex, eye blink and circadian rhythms: the inhibition of OPN blocked light-evoked Vc/C1 neural activity and tear formation. The authors also demonstrated that bright light also caused a prompt increase in ocular blood flow, and the intensity of firing by neurons in the trigeminal complex is dependent on vascular changes in the eye (Okamoto et al., 2012). Moreover, the above light-induced responses are inhibited by stimulating the posterior hypothalamus, acting through a sympathetic action (Katagiri et al., 2013) (*Fig. 15.1*). These findings open the possibility that the autonomic nervous system plays a critical role in mediating light-evoked trigeminal brainstem neural activity. The involvement of the OPN is also demonstrated by the group of Martenson et al.,

(2015): the blockage of this structure can inhibit the discharge of ON and OFF-cells (*see Chapter 3*) without requirement of a trigeminal or posterior thalamic relay.

Another structure involved regarding light information is the raphe magnus. The first evidence was published in 2008 (Lambert et al., 2008) showing that the raphe magnus in cats is interjected between a top-down excitatory relationship between the visual cortex and the trigeminal nociceptive system. The function of the raphe magnus is to supress the activity of the trigeminal activation through a serotoninergic mechanism (*Fig. 15.1*).

The Limbic system

The activation of the limbic pathways can superimpose an emotional processing of discomfort leading to light avoidance. The amygdala is the most involved structure, a principal site for processing fear and anxiety, and importantly, it also relays and modulates nociceptive information. Retinal ipRGCs also project to the amygdala (Hattar et al., 2006) (*Fig.15.1*) and a study conducted on mouse pups showed that light induced a response in the posterior thalamus and in amygdala, but not in the trigeminal nucleus, maybe due to the observations being made at too late a stage i.e. the CNS being too mature (Delwig et al., 2012). Interestingly CGRP-containing neurons project to the amygdala where they mediate pain responses (Han et al., 2010).

The Visual cortex

Our findings add to the existent literature on mechanisms of photophobia the possible role of the visual cortex as an inhibitory sustained control on the trigeminal nociceptive system. In the light of our results, we have no possibility to trace accurately this circuit but we can speculate on possible explanations.

On the one hand, and more probable, the visual cortex can modulate the trigeminal response by the interposition of the thalamus, in particular of the dorsal and dorso-lateral thalamic nuclei (*Fig. 15.1*) (Noseda et al., 2010 and 2011, Maleki et al., 2012).

Following this point of view, our results support the theory of a thalamo-cortical dysrhythmia in migraineurs that we can modulate by the exposition to a flash light stimulation during several sessions.

On the second hand, it is not excluded (and not confirmed yet, too) that the visual cortex is directly connected with the trigeminal nociceptive system and acts through a targeted control. This possibility fails to find in the existent literature other supporting studies, and further investigations, not only in humans but also in animals, are necessary to answer to the question. One possibility should be to analyse if the exposure to the light modulates the trigeminal response in thalamectomized animals, monitoring at the same time the visual cortex and the 2nd order neurons discharge in the brainstem.

On the third hand, the role of the visual cortex should be only an epiphenomenon of the activation of the thalamus, which should lead simultaneously to the activation of the visual cortex and to the inhibition of the trigeminal nociceptive system. In this case, one should find the photophobia generator in the thalamic nuclei and not in the visual cortex. However, the «cognitive" role in the elaboration of the nociceptive information induced by the visual cortex, as we found testing the "visually induced analgesia" in migraineurs, suggests that the visual cortex plays a central role in the inhibitory control to the brainstem.

On the fourth hand, one of these three explanations does not exclude the other one, the three circuits can coexist.

For the moment, these questions are unresolved, and many further studies are need for the understanding of the photophobia with migraine headache.

15.3. Perspectives

The results presented in this thesis provide perspectives for a better understanding of photophobia and migraine pathophysiology, as well as for migraine therapy.

To extend knowledge in these areas, several studies have to be performed.

The first one is to detect the prevalence in the general population of isolated photophobia, by a detailed neurological and ophthalmological examination.

Many studies are suggested by our results both in animals and in humans: one of the most important would be to investigate the role of a prolonged flash light stimulation on the trigeminal system in animals and verify if the same pattern can be found as the one we have in humans.

In humans, we can ameliorate our knowledge on the effects of light stimulation by using the same light stimulator in in experimental studies in order to favour reliability and comparability of results. The present use of different types of stimulators adds a technical confounding factor that can be overcome.

Using StimLux, our next step is to perform a "sham-controlled" trial, which is not easy to design, but can be implemented by choosing as sham the light stimulus parameters that in our studies had the smallest effect on sensitivity scores, nociceptive blink reflexes and visual evoked potentials.

The device should be ameliorated by installing one efficient program of stimulation in a portable stimulator, easy to use and adapted for a prolonged, home-based treatment (two or three months akin to the neurostimulation protocols).

Moreover, it will be also be of great interest to couple EEG recordings and coloured flash light stimulation, in order explore the effect of colour on the visual cortex activity.

In conclusion, this thesis opens several doors for the pathophysiological and therapeutic research in migraine, but in particular for the study of photophobia.

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ANNEXES

- Curriculum Vitae

- **Sava SL**, de Pasqua V, Magis D, Schoenen J. Effects of visual cortex activation on the nociceptive blink reflex in healthy subjects. PLoS One. 2014 Jun 17;9(6):e100198. doi: 10.1371/journal.pone.0100198. eCollection 2014.

- Magis D, Viganó A, **Sava S**, d'Elia TS, Schoenen J, Coppola G. Pearls and pitfalls: electrophysiology for primary headaches. Cephalalgia. 2013 Jun;33(8):526-39. doi: 10.1177/0333102413477739.

- Magis D, **Sava S**, d'Elia TS, Baschi R, Schoenen J. Safety and patients' satisfaction of transcutaneous supraorbital neurostimulation (tSNS) with the Cefaly® device in headache treatment: a survey of 2,313 headache sufferers in the general population. J Headache Pain. 2013 Dec 1;14:95. doi: 10.1186/1129-2377-14-95.

- Viganò A, D'Elia TS, **Sava SL**, Auvé M, De Pasqua V, Colosimo A, Di Piero V, Schoenen J, Magis D. Transcranial Direct Current Stimulation (tDCS) of the visual cortex: a proof-of-concept study based on interictal electrophysiological abnormalities in migraine. J Headache Pain. 2013 Mar 11;14:23. doi: 10.1186/1129-2377-14-23.

- Piquet M, Balestra C, **Sava SL**, Schoenen J. Supraorbital transcutaneous neurostimulation has sedative effects in healthy subjects. BMC Neurol. 2011 Oct 28;11:135. doi: 10.1186/1471-2377-11-135.

- Coppola G, Currà A, Di Lorenzo C, Parisi V, Gorini M, **Sava SL**, Schoenen J, Pierelli F. Abnormal cortical responses to somatosensory stimulation in medication-overuse headache. BMC Neurol. 2010 Dec 30;10:126. doi: 10.1186/1471-2377-10-126

- Coppola G, Currà A, **Sava SL**, Alibardi A, Parisi V, Pierelli F, Schoenen J. Changes in visual-evoked potential habituation induced by hyperventilation in migraine. J Headache Pain. 2010 Dec;11(6):497-503. doi: 10.1007/s10194-010-0239-7. Epub 2010 Jul 13.

Curriculum Vitae

Personal information

First names / Surname	**Simona Liliana / Sava**
Private Address	Avenue de L'Observatoire 154, boite 31, 4000 Liège, Belgium
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Telephone	+32 498 75 58 06
E-mail	simonaliliana.sava@gmail.com
Nationality	Italian, Rumanian
Date of birth	22.11.1984
Gender	F
Civil Status	Divorced
Children	One daughter
Occupational field	**Medical Doctor, PhD Student, Resident in Neurology**

Work experience

Dates	01.08.2010 – 31.09.2011
Occupation	Clinical Researcher, Neurology Resident
Name and address of employer	Prof. Jean Schoenen, Headache Research Unit. Department of Neurology & GIGA - Neurosciences. Liège University, CHU-Sart Tilman, 4000 LIEGE. Belgium
Dates	01.10.2011 – (30.09.2016)
Occupation	Medical Doctor, Resident in Neurology and PhD Student
Name and address of employer	CHU-Sart Tilman, CHR-Citadelle, University of Liège (Ulg), 4000 Liège, Belgium
Sector	Neurology

Education and training

Dates	23.07.2010
Title of qualification awarded	Diploma in Medicine and Surgery to the University of Rome "Sapienza", Italy, with the thesis "Study of the recovery cycle of the somatosensory evoked potentials in migraine patients", final degree **110/110** ***cum laude***.

Languages

Rumanian: Speaking: Mother Tongue; Listening: Mother Tongue; Writing: Mother Tongue; Reading: Mother Tongue.

Italian: Speaking: As a native/perfect; Listening: As a native/perfect; Writing: As a native/perfect; Reading: As a native/perfect.

French: Speaking: Very Good/fluent; Listening: Very Good; Writing: Very good; Reading: Very Good

English: Speaking: Very Good/fluent; Listening: Very Good; Writing: Very good; Reading: Very Good

Awards

1. Proclamation by the President of the Italian Republic C.A. Ciampi as "Alfiere" for the best academic results in the all region of Lazio, and 1 of 20 best students in Italy (2004)
2. Award for the best academic results by the University of Rome "Sapienza", Italy (2004 till 2010)
3. Award for the thesis abroad by the University of Rome "Sapienza", Italy (2009)
4. Distinction for "the younger researcher presenting a poster" by the SINC, Italy (2010)
5. Travel Grant by the Belgian Neurological Society (2011)
6. Travel Grant by the Belgian Neurological Society (2012)
7. Travel Grant by the Belgian Neurological Society (2013)
8. Travel Grant by the International Headache Society for the participation to iHead Meeting (2014)
9. Price for the research project CIRF, Citadelle Hospital (2014)
10. Travel Grant by the International Headache Society (2015)

Congress active participation

1. I regional congress SISC Lazio-Molise (08.11.2008) – Ospedale pediatrico Bambino Gesù, Roma, Italy.
2. Management of the patient with migraine (15.11.2008) – ICOT, Latina, Italy.
3. Stem cell and Tissue engineerings symposium (16.03.2009) – Latina, Italy.
4. Parkinson disease and demence (17.04.2010) – Orvieto, Italy
5. National congress of the Italian Society of the Clinical Neurophysiology – SINC (13-15.05.2010), Siena, Italy – distinction « the younger researcher presenting a poster »
6. Belgian Brain Congress (BBC) (17-18.09.2010), Bruxelles, Belgium
7. European Headache and Migraine Trust International Congress (EHMTIC), Nice, France (28-31.10.2010)
8. 15th Congress of the International Headache Society (IHS), Berlin, Germany (23-26.06.2011)
9. 7th Congress of the European Federation of IASP Chapters (EFIC), Hamburg, Germany (21-24.09.2011)
10. 11th Congress of the French Society of the study and treatment of pain (SFETD), Paris, France (16-19.11.2011)
11. International Headache Academy (IHA), Copenhagen, Denmark (18-20.05.2012)
12. Belgian Brain Congress (BBC), Liège, Belgium (27.09.2012)
13. XXI World Congress of Neurology (WCN), Vienna, Austria (21-26.09.2013)

14. Joint Congress of European Neurology (EFNS-ENS), Istanbul, Turkey (31.05-03.06.2014)
15. Belgian Brain Congress (BBC), Ghent, Belgium (04.10.2014)
16. iHead Meeting (International Headache Society), Leiden, the Netherlands (31.10-02.11.2014)
17. 17th Congress of the International Headache Society (IHS), Valencia, Spain (14-17.05.2015)
18. Masterclass, Leading Excellence for Stroke Prevention in Non-Valvular Atrial Fibrillation, Rome, Italy (16-17.10.2015)
19. InterUniversity Certificate Postgraduate studies in algology Course Week 2015, Spa, Belgium (25-30.10.2015)

Publications

1. Coppola G, Currà A, **Sava SL**, Alibardi A, Parisi V, Pierelli F, Schoenen J. Changes in visual-evoked potential habituation induced by hyperventilation in migraine. J Headache Pain. 2010 Dec;11(6):497-503.
2. Coppola G, Currà A, Di Lorenzo C, Parisi V, Gorini M, **Sava SL**, Schoenen J, Pierelli F. Abnormal cortical responses to somatosensory stimulation in medication-overuse headache. BMC Neurol. 2010 Dec 30;10:126.
3. Piquet M, Balestra C, **Sava SL**, Schoenen JE. Supraorbital transcutaneous neurostimulation has sedative effects in healthy subjects. BMC Neurol. 2011 Oct 28;11(1):135.
4. Viganò A, D'Elia TS, **Sava SL**, Auvé M, De Pasqua V, Colosimo A, Di Piero V, Schoenen J, Magis D. Transcranial Direct Current Stimulation (tDCS) of the visual cortex: a proof-of-concept study based on interictal electrophysiological abnormalities in migraine. J Headache Pain. 2013 Mar 11;14(1):23
5. Magis D, Vigano A, **Sava SL**, Sasso d'Elia T, Schoenen J and Coppola G. Pearls and pitfalls: electrophysiology for primary headaches. Cephalalgia 2013 33(8):526-39.
6. Magis D, **Sava SL**, d'Elia TS, Baschi R, Schoenen J. Safety and patients' satisfaction of transcutaneous supraorbital neurostimulation (tSNS) with the Cefaly® device in headache treatment: a survey of 2,313 headache sufferers in the general population. J Headache Pain. 2013 Dec 1;14:95
7. **Sava SL**, de Pasqua V, Magis D, Schoenen J. Effects of visual cortex activation on the nociceptive blink reflex in healthy subjects. PLoS One. 2014 Jun 17;9(6)

Publications in press/ Submitted articles

Abstracts

1. Coppola G, **Sava SL**, Porretta E, Alibardi A, Gorini M, Parisi V, Currà A, Pierelli F. Ciclo di recupero dei potenziali evocati somatosensoriali in pazienti affetti da emicrania senz'aura. *Clinical Neurophysiology* (SINC, 2010).
2. Coppola G, Porretta E, **Sava SL**, Gorini M, Alibardi A, Parisi V, Currà A, Pierelli F.

Alterazione dello sviluppo temporale dell'inibizione laterale nel sistema visivo in pazienti affetti da emicrania senz'aura. *Clinical Neurophysiology* (SINC, 2010).

3. **Sava SL**, Coppola G, De Pasqua V, Pierelli F, Schoenen J. La stimolazione magnetica transcranica ripetitiva sulla corteccia visiva modula il sistema nocicettivo trigeminale. *Clinical Neurophysiology* (SINC, 2010).

4. Coppola G, Currà A, Gorini M, Davassi C, **Sava SL**, Pierelli F. Cortical silent period duration in medication overuse headache changes according to the drug overused. *Clinical Neurophysiology* (SINC, 2010).

5. **Sava SL**, Coppola G, De Pasqua V, Pierelli F, Schoenen J. The visual system influences the nociceptive trigeminal system: possible mechanism underlying photophobia? *The Journal of Headache and Pain* (SISC, 2010)

6. Coppola G, Di Lorenzo C, **Sava SL**, Gorini M, de Micco M, Davassi C, Pierelli F. Trigeminal activity in episodic cluster headache. *The Journal of Headache and Pain* (SISC, 2010)

7. Currà A, Coppola G, Di Lorenzo C, Gorini M, Davassi C, de Micco M, **Sava SL**, Pierelli F. Cortical silent period duration in medication oveuse headache changes according to the drug overused. *The Journal of Headache and Pain* (SISC, 2010)

8. Coppola G, Currà A, **Sava SL**, Di Lorenzo C, Gorini M, de Micco M, Davassi C, Parisi V, Pierelli F. Effects of 3 minutes forced hyperventilation on viaul evoked potential habituation in migraine. *The Journal of Headache and Pain* (SISC, 2010)

9. Coppola G, **Sava SL**, De Pasqua V, Pierelli F, Schoenen J. Trigeminal nociceptive pathway is modulated by repetitive transcranial magnetic stimulation over the visual cortex. *Neurological Sciences,* (SIN, 2010).

10. Coppola G, **Sava SL**, Di Lorenzo C, Gorini M, de Micco M, Davassi C, Parisi V, Currà A, Pierelli F. Impairement of temporal development of the visual system short-range lateral inhibition in migraine without aura patients. *Neurological Sciences,* (SIN, 2010).

11. Currà A, Coppola G, **Sava SL**, Di Lorenzo C, Gorini M, Davassi C, de Micco M, Parivi V, Pierelli F. Effects of experimentally induced hyperventilation on visual evoked potential habituation in migraine. *Neurological Sciences,* (SIN, 2010).

12. **Sava SL**, Coppola G, De Pasqua V, Pierelli F, Schoenen J. Repetitive transcranial magnetic stimulation over the visual cortex modulated trigeminjal nociceptive pathways. *The Journal of Headache and Pain* (EHMTIC, 2010).

13. Coppola G, **Sava SL**, Di Lorenzo C, Gorini M, de Micco M, Davassi C, Parisi V, Currà A, Pierelli F. Impairement of temporal development of the visual system short-range lateral inhibition in migraine without aura patients. *The Journal of Headache and Pain* (EHMTIC, 2010).

14. Coppola G, Currà A, Di Lorenzo C, Gorini M, Davassi C, de Micco M, **Sava SL**, Pierelli F. Cortical silent period duration in medication oveuse headache changes according to the drug overused. *The Journal of Headache and Pain* (EHMTIC, 2010).

15. **Sava SL**, Coppola G, De Pasqua V, Pierelli F, Schoenen J. Repetitive TMS of the visual cortex modulates the nociceptive blink reflex in healthy volunteers. *Acta Neurologica Belgica* (BBC, 2010).

16. **Sava SL**, Vigano A, De Pasqua V, Magis D, Schoenen J. No effect of a 1 hour sub-occipital transcutaneous stimulation on the nociceptive blink reflex in healthy subjects. *Cephalalgia* (IHS, 2011).

17. **Sava SL**, Coppola G,Vigano A, De Pasqua V, Magis D, Schoenen J. Repetitive transcranial magnetic stimulation modulates the nociceptive-specific blink reflex in healthy

subjects but not in migraineurs. *Cephalalgia* (IHS, 2011).

18. **Sava SL**, de Pasqua V, Magis D, Schoenen J. Changes in Visual Evoked Potentials induced by supraorbital electrical stimulation or capsaicin application in healthy volunteers. *European Journal of Pain* (EFIC, 2011).

19. **Sava SL**, de Pasqua V, Magis D, Schoenen J. Can vision influence trigeminal nociception: a study of the effect of visual cortex activation on the nociceptive blink reflex in healthy subjects and migraine patients. *Acta Neurologica Belgica* (BBC, 2012).

20. Sasso d'Elia T, Vigano A, **Sava SL**, Auvé M, Schoenen J and Magis D. Theta burst and quadripulse repetitive Transcranial Magnetic Stimulation (rTMS) may have therapeutic potentials in migraine prevention: a proof-of-concept study in healthy volunteers and a pilot-trial in migraine patients. *Acta Neurologica Belgica* (BBC, 2012).

21. Sasso d'Elia T, Vigano A, **Sava SL**, Auvé M, Schoenen J, Magis D. Anodal transcranial direct current stimulation over the visual cortex as a preventive treatment of migraine: a proof-of-concept study. *Acta Neurologica Belgica* (BBC, 2012).

22. Vigano A, Magis D, **Sava SL**, De Pasqua V, Auvé M, Giuliani A, Colosimo A, Di Piero V, Schoenen J. Anodal transcranial direct current stimulation of the visual cortex for migraine prevention: a proof-of-concept study. *Journal of Headache and Pain* 2012, Suppl, p144: P194.

23. **Sava SL**, de Pasqua V, Magis D, Schoenen J. Does trigeminal nociception influence the visual cortex: a study of the effects of supraorbital electro- or chemo-nociceptive stimulation. *Journal of Neurological Sciences* (WCN, 2013).

24. **Sava SL**, Roberta B, de Pasqua V, Magis D, Schoenen J. Does trigeminal nociception influence the visual cortex: a study of the effects of supraorbital electro- or chemo-nociceptive stimulation. *Journal of Neurological Sciences* (WCN, 2013).

25. Magis D, Rigaux P, Mignolet JY, **Sava SL**, Sasso d'Elia T, Schoenen J. Safety and efficiency of supraorbital transcutaneous neurostimulation with the Cefaly® device for headache treatment: outcome of a prospective registry on 2313 patients. *Cephalalgia* (IHS, 2013).

26. Fataki M, Sasso d'Elia T, De Pasqua V, **Sava SL**, Magis D, Schoenen J. Thermosensitivity in migraine between attacks: a study of quantitative thermo-sensory testing and contact heat evoked potentials. *Cephalalgia* (IHS, 2013).

27. Sasso d'Elia T, Fataki M, **Sava SL**, De Pasqua V, Schoenen J, Magis D. Effect of anodal transcranial direct current stimulation over the visual cortex on thermosensitivity. *Cephalalgia* (IHS, 2013).

28. **Sava SL**, Baschi R, La Salvia V, de Pasqua V, Magis D, Schoenen J. Contact heat-evoked potentials (CHEPs) in healthy subjects and patients with episodic or chronic migraine. European Journal of Neurology (EFNS-ENS, 2014).

29. **Sava SL**, Baschi R, La Salvia V, de Pasqua V, Schoenen J, Magis D. Visually-induced facial analgesia effect on thermonociceptive cortical evoked responses in healthy subjects and migraine patients. *Acta Neurologica Belgica* (BBC, 2014).

30. **Sava SL**, Baschi R, La Salvia V, de Pasqua V, Schoenen J, Magis D. Differences in contact heat-evoked potentials (CHEPs) between healthy subjects and patinets with episodic or chronic migraine. *Acta Neurologica Belgica* (BBC, 2014).

31. Baschi R, **Sava SL**, La Salvia V, de Pasqua V, Schoenen J, Magis D. Transcranial direct current stimulation in chronic migraine: a pilot trial combining cathodal visual and anodal DLPFC stimulation. *Acta Neurologica Belgica* (BBC, 2014).

32. Baschi R, Vecchio E, **Sava SL**, de Pasqua V, Schoenen J, Magis D. Neurophysiological

study of tDCS effects in healthy volunteers. *Acta Neurologica Belgica* (BBC, 2014).

33. **Sava SL,** Baschi R, Coddeddu A, D'Ostilio K, de Pasqua V, Schoenen J, Magis D. Thermal pain threshold in migraine: comparaison between episodic or chronic migraine patients and healthy volunteers using QST. *Cephalalgia* (IHS, 2015).

34. **Sava SL,** Baschi R, D'Ostilio K, de Pasqua V, Schoenen J, Magis D. Modulation of the nociceptive blink reflex by repetitive transcranial magnetic stimulation in healthy volunteers: comparison of visual or motor cortex stimulation. *Cephalalgia* (IHS, 2015).

35. D'Ostilio K, Thibaut A, Laureys S, Cosseddu A, **Sava SL**, Schoenen J, Magis D. Cerebral FDG uptake changes after supraorbital transcutaneous electricl stimulation with the Cefaly device in patients with migraine. *Cephalalgia* (IHS, 2015)

36. Magis D, D'Ostilio K, Cosseddu A, Nonis R, **Sava SL**, Schoenen J. Anodal trascranial direct current stimulation (tDCS) targeting the anterior cingulated gyrus for the treatment of chronic cluster headache: a proof of concept trial. Submitted to *Neurology* (AAN, 2016)

37. Schoenen J, D'Ostilio K, Cosseddu A, Nonis R, **Sava SL**, Magis D. Trasncranial direct current stimulation and transutaneous occipital nerve stimulation in chronic migraine: a pilot-comparaison of therapeutic and electrophysiological effects. Submitted to *Neurology* (AAN, 2016)

38. Schoenen J, D'Ostilio K, Nonis R, **Sava SL**, Magis D. Non-invasive vagus nerve stimulation with GammaCore in healthy subjects:is there electrophysiological evidence for activation of vagal afferents? Submitted to *Neurology* (AAN, 2016)

Invited speaker and oral presentations

1. SFETD, Paris, France (16-19.11.2011): "Interrelations between visual cortex and the nociceptif trigeminal system"

2. WCN, Vienna, Austria, (21-26.09.2013): "Can vision influence trigeminal nociception: A study of the effect of visual cortex activation on the nociceptive blink reflex"

3. EFNS-ENS Istanbul, Turkey (31.05-03.06.2014): "Visual induced analgesia in the face in healthy subjects and migraineurs"

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Effects of Visual Cortex Activation on the Nociceptive Blink Reflex in Healthy Subjects

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Abstract

Bright light can cause excessive visual discomfort, referred to as photophobia. The precise mechanisms linking luminance to the trigeminal nociceptive system supposed to mediate this discomfort are not known. To address this issue in healthy human subjects we modulated differentially visual cortex activity by repetitive transcranial magnetic stimulation (rTMS) or flash light stimulation, and studied the effect on supraorbital pain thresholds and the nociceptive-specific blink reflex (nBR). Low frequency rTMS that inhibits the underlying cortex, significantly decreased pain thresholds, increased the 1st nBR block ipsi- and contralaterally and potentiated habituation contralaterally. After high frequency or sham rTMS over the visual cortex, and rMS over the right greater occipital nerve we found no significant change. By contrast, excitatory flash light stimulation increased pain thresholds, decreased the 1st nBR block of ipsi- and contralaterally and increased habituation contralaterally. Our data demonstrate in healthy subjects a functional relation between the visual cortex and the trigeminal nociceptive system, as assessed by the nociceptive blink reflex. The results argue in favour of a top-down inhibitory pathway from the visual areas to trigemino-cervical nociceptors. We postulate that in normal conditions this visuo-trigeminal inhibitory pathway may avoid disturbance of vision by too frequent blinking and that hypoactivity of the visual cortex for pathological reasons may promote headache and photophobia.

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Introduction

"Photophobia" is the clinical term to indicate discomfort to light. It is a common symptom of several neurological and ophthalmological disorders: blepharospasm [1], corneal abrasion, iritis [2], tumors compressing the anterior visual pathways [3], trigeminal neuralgia [4] and, most characteristically, migraine [5].

The pathophysiology of photophobia remains poorly understood.

Reciprocal relations between the visual system and centers involved in trigeminal nociception have been documented in animal studies. Acute exposure to bright light, for instance, increases the number of Fos-like immunoreactive neurons in superficial laminae of trigeminal subnucleus caudalis (Vc/C1) [6] and parasympathetic outflow to the eye [7]. On the one hand, the visual cortex is influenced by projections from the brainstem, especially from dorsal raphe and nucleus raphe magnus [8,9]. On the other hand, the visual cortex projects downward to brainstem nuclei, including nucleus raphe magnus [10] where it exerts an inhibitory effect [11] and to nucleus cuneiformis [12]. Interestingly, nucleus cuneiformis is part of the descending pain control system and was found hypoactive with fMRI in migraineurs during thermo-nociceptive stimulation [13].

Recently, a novel retino-thalamo-cortical pathway was proposed as a possible anatomo-functional substrate for exacerbation of migraine headache by light. This concept is based on the finding in rat of convergence of retinal afferents and trigeminovascular nociceptive afferents in the posterior and lateral posterior thalamic nuclei [14] whence dural-sensitive thalamic neurons project to various sensory cortical areas including the visual cortex [15]. In humans, MR DTI tractography has revealed a direct connection between optic nerve fibers and the pulvinar [16].

Vanagaite et al. [17] have previously proposed convergence of retinal and trigeminal nociceptive afferents as a possible explanation for photophobia. Direct proof of their hypothesis in humans is still missing, but in a photophobic subject due to corneal irritation by contact lenses, Moulton et al. [13] found light-induced fMRI activation of various structures of the trigeminal nociceptive pathway including thalamus and anterior cingulate cortex. In humans a reciprocal relation between visual input and trigeminal nociception is suggested by the decreased tolerance to light after painful stimulation of the ophthalmic branch of the trigeminal nerve [18] and the reduction of trigeminal pain thresholds after light stimulation in migraine patients [19,20]. In a PET study, continuous light stimulation induced a stronger activation of the visual cortex in migraine subjects than in healthy subjects, and, when it was combined with a painful stimulation in the trigeminal territory, the activation was markedly greater in migraine patients [21].

The aim of our study was to testing healthy volunteers the hypothesis that the visual cortex is able to modulate excitability in the trigeminal nociceptive system, which would be relevant for migraine-related photophobia and for migraine headache. As indices for excitability in the trigeminal sensory system we have chosen sensory and pain detection thresholds to supraorbital

electrical stimuli as well as amplitude of the nociceptive-specific blink reflex (nBR), a brain stem reflex modified by cortical and subcortical afferents [22,23,24,25]. To modulate the visual cortex, we used flash light stimulation or repetitive transcranial magnetic stimulation (rTMS) at high or low stimulation frequency [26,27]. As controls, we applied sham rTMS over the visual cortex and effective repetitive magnetic stimulation (rMS) over the greater occipital nerve.

Materials and Methods

Ethics Statement

The project was reviewed and approved by the Ethic Committee of the CHR Citadelle Hospital, Faculty of Medicine, University of Liège, Belgium, and was conform to the Declaration of Helsinki. All participants gave written informed consent prior to testing. 2 participants of 14 and 16 years old were included in our study, a written informed consent was given by their parents.

Subjects

The experiments were performed on 63 healthy subjects (HS) without a personal or family history of primary headache. We applied rTMS on the visual cortex, at low and high frequency, in 21 subjects (12 females, 9 males, mean age 25.9±8.03) and flash light stimulation in 22 subjects (12 females, 10 males, mean age 26.59±9.29). As controls for rTMS, we used occipital sham stimulation in 13 subjects (8 females, 5 males, mean age 25.38±11.18) and effective stimulation over the greater occipital nerve in 7 subjects (5 females, 2 males, mean age 29±10.59). As recommended for rTMS [28], all subjects were devoid of any medical condition and had no personal or family history of epilepsy. To avoid interference with changes of cortical excitability due to hormonal variations, females were recorded during mid-cycle. All subjects were naïve for rTMS.

Nociceptive Blink Reflex

Subjects were seated relaxed in a comfortable armchair in an illuminated room and were asked to leave their eyes open. The nociceptive-specific blink reflex was elicited according to the method described by others [29,30], before and immediately after the rTMS session or flash light stimulation.

We used a custom-made planar concentric electrode (central cathode: 1 mm D; insert: 8 mm; anode: 23 mm OD) placed on the forehead close to the supraorbital foramen on the right side. The concentric electrode has the advantage of exciting preferentially Aδ fibers [29,20,31,32], but at the same time C-fibers and Aβ fibers may also be recruited [33]. It seems that the recruitment of Aβ fibers may vary with regard to the site of stimulation, stimulus repetition rate and duration as well as penetration of the electrode in the skin [34].

Recording electrodes were placed below the orbit (active) over the orbicularis oculi muscle and lateral to the orbit (reference) on both sides. A ground electrode was placed on the root of the nose. The signal was recorded with a sampling rate of 5000 Hz and sweep duration of 150 ms (1401, Signal Averager, Cambridge Electronic Design).

We first determined perception and pain thresholds by using ascending and descending sequences of 0.2 mA intensity steps. The mean number of assessments per participant was 11±4 for sensory thresholds and 15±8 for pain thresholds. The electrical stimuli consisted of monopolar square pulses with 0.2 ms duration. To elicit the nBR, the final stimulus intensity was set at 1.5 times the initial individual pain threshold. Interstimulus intervals varied pseudo-randomly between 15 and 17 s. We recorded 16 rectified EMG responses that were averaged off-line. As previously described, the first response of each nBR recording session was excluded from the signal analysis to avoid contamination with startle responses [30,31,32]. The remaining 15 sweeps were averaged in 3 sequential blocks of 5 responses. For each averaged block, amplitude of the R2 reflex was expressed as its area under the curve (AUC). To minimize R2 AUC variability due to inter-individual threshold differences we used the ratio between the area and the square of the stimulus intensity (AUC/i^2) as an index of nBR amplitude changes, as recommended by Sandrini et al. [35]. Habituation of the nBR R2 was defined as the percentage change of the R2 area between the 1st and the 3rd block of averages.

Magnetic Stimulation

rTMS over the visual cortex. We used a Magstim Rapid magnetic stimulator (Magstim Co. Ltd, Whitland, Dyfed, UK), connected to a 2×7.0 cm figure-of-eight coil, with a maximal stimulator output of 1.2 T. Using single pulses, we first identified the phosphene threshold, defined as the lowest stimulation intensity (expressed as a percentage of the maximal stimulator output) able to evoke phosphenes in at least three out of five pulses [36]. The coil was placed in a vertical position (its handle pointing upward) on the inion-nasion line, with its inferior limit 1 cm above the inion. Stimulation was applied initially at 30% of stimulator output. The intensity of the stimulation was increased by 2% steps until the subject reported phosphenes. Increasing and decreasing the intensity in 1% steps then refined the threshold. In participants who did not report phosphenes at the 100% intensity level, the procedure was repeated with the coil placed 1 or 2 cm higher or lower and, if necessary, to the right or to the left, before accepting the absence of phosphenes. In this case, we placed the coil over the left motor area and determined the motor threshold. In accordance with recommended safety guidelines [28], stimulus intensity was set to the phosphene threshold (PT) or to 110% of the motor threshold, if no phosphenes were elicited.

We used two different stimulation frequencies in a randomised order: 1 Hz (low frequency rTMS) and 10 Hz (high frequency rTMS) with at least a 24 hour-interval between the 2 sessions, as recommended by others for stimulation of the motor cortex [37]. 1 Hz rTMS was applied in a single train without interruption for 15 minutes. 10 Hz rTMS was applied in 20 trains of 40 pulses with inter-train intervals of 10 seconds. For both frequencies, a same amount of 800 pulses was thus delivered.

Sham rTMS Over the Visual Cortex

In 13 subjects blinded to the stimulation protocol, 10 Hz rTMS sham stimulation was delivered with the coil placed at a 90° angle to the occipital region, with its anterior border pressed against the scalp. The rTMS intensity was fixed at the intensity of the phosphene threshold or 110% of the motor threshold. Twenty trains of 40 pulses with an inter-train interval of 10 seconds were delivered for 5 minutes. In the sham situation, there is an acoustic perception of the stimulation, but no brain activation occurs [38]. We decided to enrol only subjects completely naive to rTMS in order to ensure blinding.

rMS Over the Greater Occipital Nerve (GON)

We performed 1 Hz and 10 Hz rMS over the right GON in 7 HS by placing the figure-of-eight coil over the emergence of the GON just beneath the superior nuchal line. We considered as optimal the location where the sensation induced by the magnetic pulse radiated to the parietal region of the head. The rMS intensity was fixed at the phosphene threshold or 110% of the motor threshold found during the previous session of effective

rTMS, to make a comparable control protocol. The patterns of 1 Hz or 10 Hz stimulation were the same as those applied over the visual cortex.

Flash Light Stimulation

We used the Microflash MF 9607178 stimulator (Micromed & Co., Mogliano Veneto, IT) for flash light stimulation in 22 subjects. We placed the light stimulator in front of the subjects at a 15 cm distance, asking them to look at the stimulator during the whole session. The stimulation was at 27.8 lux (0.63 cd). To minimize attenuation of light perception due to continuous stimulation without spatial or temporal contrast [39,40], flash frequency was set at 8 Hz for 4 minutes in a quiet room with dimmed light.

Data Processing and Statistical Analysis

STATISTICA for Windows version 8.0 (StatSoft, Inc. Tulsa, OK, USA) was used for all statistical analyses. Wilcoxon's test was applied to compare the differences between pre- and post-stimulation in perception and pain thresholds, AUC of the 1stnBR block and slope of amplitude changes over 3 consecutive blocks of nBR averagings, ipsilaterally and contralaterally. Mann-Whitney's test was used to compare the differences between stimulation methods. Spearman's test was used for the correlation analysis. All results were considered significant at the 5% level ($p<0.05$).

Results

Transcranial magnetic stimulation – visual cortex. 12 participants out of 21 (57.14%, 3 males and 12 females) stimulated with TMS over the visual cortex reported phosphenes. The phosphene threshold (expressed as a percentage of the maximal stimulator output) was 66±4.7%. The motor threshold was determined in the remaining 9 participants (42.86%, 7 males and 2 females) and was 58±8% of the maximal stimulator output. We observed a significant relation between the presence of phosphenes and female gender ($p = 0.04$). There was no correlation between intensity of rTMS and the effect on the nBR. After 1 Hz rTMS over the visual cortex, the supraorbital pain threshold was significantly decreased ($p = 0.001$) (Table 1), while the sensory threshold remained unchanged.

Moreover, 1 Hz rTMS significantly increased amplitude of the 1st nBR block expressed as AUC/i^2 both ipsi- and contralaterally to the supraorbital stimulation ($p = 0.024$ and $p = 0.036$ respectively) (Table 1, Fig. 1). By contrast, habituation was significantly potentiated contralaterally to the stimulated side ($p = 0.0002$) (Fig. 2).

We found no significant variation of sensation or pain thresholds, nBR amplitude and habituation after the 10 Hz rTMS session (Fig. 2) or after sham rTMS.

Magnetic stimulation – right GON. There was no significant change of sensory thresholds, nBR amplitude or habituation after stimulating the right GON, neither for 1 Hz rMS, nor for 10 Hz rMS (Table 1).

Photic stimulation. Figure 3 shows an illustrative recording of the nBR responses before and after flash light stimulation. The latter increased pain threshold ($p = 0.008$) (Table 1), decreased AUC/i^2 of the 1stnBR block ($p = 0.004$ ipsilateral; $p = 0.001$ contralateral) and increased habituation contralaterally ($p = 0.002$) (Fig. 1 and Fig. 2). Although both 10 Hz rTMS and flash light stimulation are known to activate the visual cortex, the effect on the nBR was significantly more pronounced after flash stimulation than after excitatory rTMS. This was the case in particular for ipsilateral ($p = 0.002$) and contralateral ($p = 0.027$) 1st nBR blocks and even more so for increase in habituation of ipsilateral ($p = 0.00008$) and contralateral responses ($p = 0.00000$) (Fig. 2).

Discussion

Our data add to the existent literature experimental evidence in humans for a functional connection between the visual cortex and 2ndorder nociceptors in spinal trigeminal nucleus.

As an objective marker of excitability in the trigeminal nociceptive system, we have chosen the nociceptive specific blink reflex (nBR). Ophthalmic nerve afferents, mainly Aδ fibers, mediate the R2 response and reach via the ponto-medullary descending spinal trigeminal tract wide dynamic range 2nd order nociceptors in caudal spinal trigeminal nucleus whence impulses ascend to the facial nuclei in the pons via a bilateral trigemino-facial pathway located in the lateral tegmental field [22,23,29,30].

We have found that sensation and pain thresholds of the supraorbital electrical stimulus as well as area under the curve (AUC) and habituation of the nBR are modulated differentially by excitatory or inhibitory repetitive transcranial magnetic stimulations (rTMS) over the visual cortex and by flash light stimulation. As controls for visual cortex rTMS, we used sham rTMS and repetitive magnetic stimulation (rMS) over the right greater occipital nerve (GON).

As can be seen from figure 2, habituation of the contralateral R2 response increases in our study whatever the experimental intervention is. During repeated stimulation with an inter-stimulus interval of 15–17sec as used here, nBR responses clearly habituate bilaterally in healthy subjects, but not in migraine patients [31]. The more pronounced habituation of contralateral responses could be related to the fact that 1st block amplitude is overall lower on the side opposite to the supraorbital stimulus, a relation that was also reported for visual evoked potentials [32].

We will discuss the changes induced by modulating visual cortex activity and thereafter the possible relevance of our findings for migraine pathophysiology.

Modulations of Visual Cortex Activity

The supraorbital pain threshold decreased after 1 Hz rTMS over the visual cortex but increased after flash light stimulation. Concordantly, amplitude of the 1st block of five averaged nBR responses increased bilaterally after the former and decreased after the latter. By contrast, 10 Hz rTMS over the visual cortex produced no significant changes, but it was followed by a numerical decrease of pain sensitivity and nBR amplitude. Taken together, these results may suggest that the visual cortex exerts at baseline a sustained top-down inhibitory effect on trigeminal nociception. Indeed rTMS at low frequency is supposed to inhibit the underlying cortex [26] while the flash stimulation excites visual areas. This is in line with a study showing in healthy volunteers a tendency for an increase of pain perception thresholds in the innervation territories of the trigeminal and greater occipital nerves after intense light stimulation [20]. We have found a similar difference between low and high frequency rTMS over the visual cortex in a study of visual evoked potentials (VEP) in healthy subjects: 1 Hz rTMS reduced amplitude of the 1st VEP block, while 10 Hz rTMS had no effect [41]. As a possible explanation for these differential results, we postulated that in normal subjects the cortical baseline activation level is close to the "ceiling", i.e. the upper level of the cortical activation range, hence it cannot be further activated by the excitatory 10 Hz rTMS but it can be decreased by the inhibitory 1 Hz rTMS. This explanation is supported *a contrario* by the finding that in migraine patients who

Table 1. Means of electrophysiological data.

	Number	Age		ST	PT	AUC 1°block ipsilateral	AUC 1° block contralateral
rTMS 1 Hz visual cortex	21	27.45±10.68	before	0.67±0.19	5.85±2.28	0.027±0.034	0.019±0.024
			after	0.71±0.19	4.69±2.58	0.031±0.033	0.025±0.027
			p	0.17	***0.001***	***0.024***	***0.036***
rTMS 10 Hz visual cortex	21	27.45±10.68	before	0.67±0.23	5.61±2.52	0.031±0.042	0.026±0.038
			after	0.72±0.23	5.64±3.21	0.023±0.023	0.015±0.015
			p	0.71	0.07	0.32	0.1
rTMS Sham	13	25.38±11.18	before	0.89±0.31	4.79±2.63	0.034±0.070	0.037±0.0889
			after	0.79±0.24	4.98±2.65	0.014±0.020	0.012±0.019
			p	0.06	0.47	0.19	0.27
rTMS 1 Hz Right GON	7	29±10.59	before	0.72±0.18	4.4±1.88	0.03±0.048	0.027±0.041
			after	0.90±0.16	4.73±2.1	0.031±0.045	0.037±0.056
			p	0.12	0.23	0.49	0.31
rTMS 10 Hz Right GON	7	29±10.59	before	0.74±0.25	4.78±2.8	0.037±0.052	0.036±0.056
			after	0.73±0.26	4.71±1.82	0.02±0.018	0.020±0.027
			p	0.83	0.61	0.17	0.31
Flash light	22	26.60±9.30	before	0.93±0.19	5.39±2.82	0.022±0.022	0.013±0.011
			after	1.0±0.17	6.11±2.69	0.009±0.010	0.008±0.008
			p	0.41	***0.008***	***0.004***	***0.001***

rTMS: repetitive transcranial magnetic stimulation; ST: sensory threshold; PT: pain threshold.

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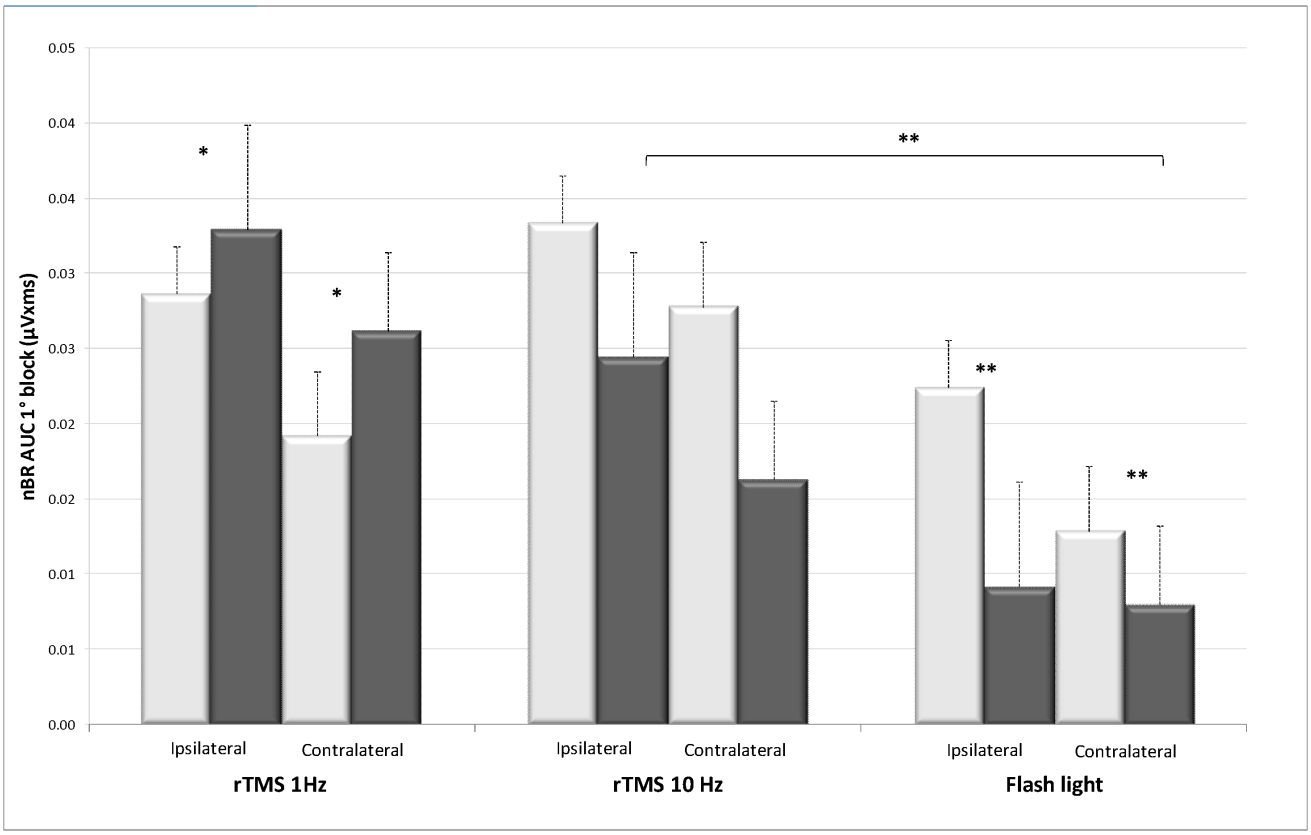

Figure 1. First block of 5 ispilateral and contralateral nBR responses (area under the curve in µVxms ± sem) before (light bars) and after (dark bars) 1 Hz rTMS, 10 Hz rTMS over the visual cortex, or flash light stimulation. ** $p<0.01$; * $p<0.05$. The inhibitory effect on the nBR is significantly stronger after flash light stimulation than after10 Hz rTMS over the visual cortex.
doi:10.1371/journal.pone.0100198.g001

may have a lowered cortical baseline activation level of the visual cortex and a decrease in 1st block VEP amplitude at baseline, 10 Hz rTMS increases 1st block VEP amplitude whereas 1 Hz has no effect [36]. The difference between 10 Hz rTMS and flash light stimulation in the present study is likely due to the fact that the former moderately increases the activation level of the visual cortex while the latter activates more robustly the visual areas via the retino-geniculo-cortical pathway of visual perception.

Extrageniculate visual pathways may provide an alternative explanation. In cat and monkey there is evidence for a pathway connecting the retina with the visual cortex via the pulvinar [42,43]. More recently, Noseda et al. [14] have demonstrated in animals projections from retinal ganglion cells to the posterior thalamus, whence via caudate-putamen and external capsule they reach multiple cortical regions, including the binocular area of the primary visual cortex. The authors suggest that this novel pathway may explain why even blind migraine patients experience photophobia. One may hypothesize that these extrageniculate pathways, if they exist also in humans, can induce an inhibitory top-down modulation of trigeminal nociceptors by thalamic neurons after flashing light but not after direct electro-magnetic activation of the visual cortex.

In migraine patients the photophobia threshold is lower than in healthy subjects after a painful stimulation applied on the forehead [18]. Along the same line, continuous light was shown to produce detectable oxygenation changes in the visual cortex of healthy subjects, only if combined with painful heat stimulation in the territory of the ophthalmic nerve [21]. The authors explain their finding by a "bottom-up" activation by the trigeminal nociceptive stimuli of visual areas rendering them responsive to a stimulus that normally produces no detectable activation because of its continuous nature and absence of any contrast pattern. Activation of visual areas by pain may not be specific to the trigeminal system, as it has also been found after pain applied to the hand [44,45]. In our study we assume that the cortical activation by the flickering light stimulation was sufficient to unravel an opposite "top-down" inhibitory control by the visual cortex of nociceptive trigeminal processing.

Sensory terminals of the greater occipital nerve are interposed between the coil of the magnetic stimulator and the occipital cortex. The electro-magnetic pulses could activate some of these peripheral neural structures and produce an afferent input that may at least in part reach the spinal trigeminal nucleus and modify its excitability. To exclude this possibility, we have positioned the coil over the greater occipital nerve underneath the upper nuchal line in control experiments. Magnetic stimuli over the GON had no significant effect on the nBR, which suggests that putative activation of peripheral afferents is not a confounding factor in our rTMS results.

Gender may be a confounding factor in activation studies of the visual cortex. Magnetophosphenes are indeed more prevalent in females than in males in our study. A sexual dimorphism of magnetophosphenes was not studied or reported in previous studies. Such dimorphism is present in migraine and sex hormones are well known to modulate cortical excitability in humans and in animals [46]. The magnetic stimulation intensity to evoke

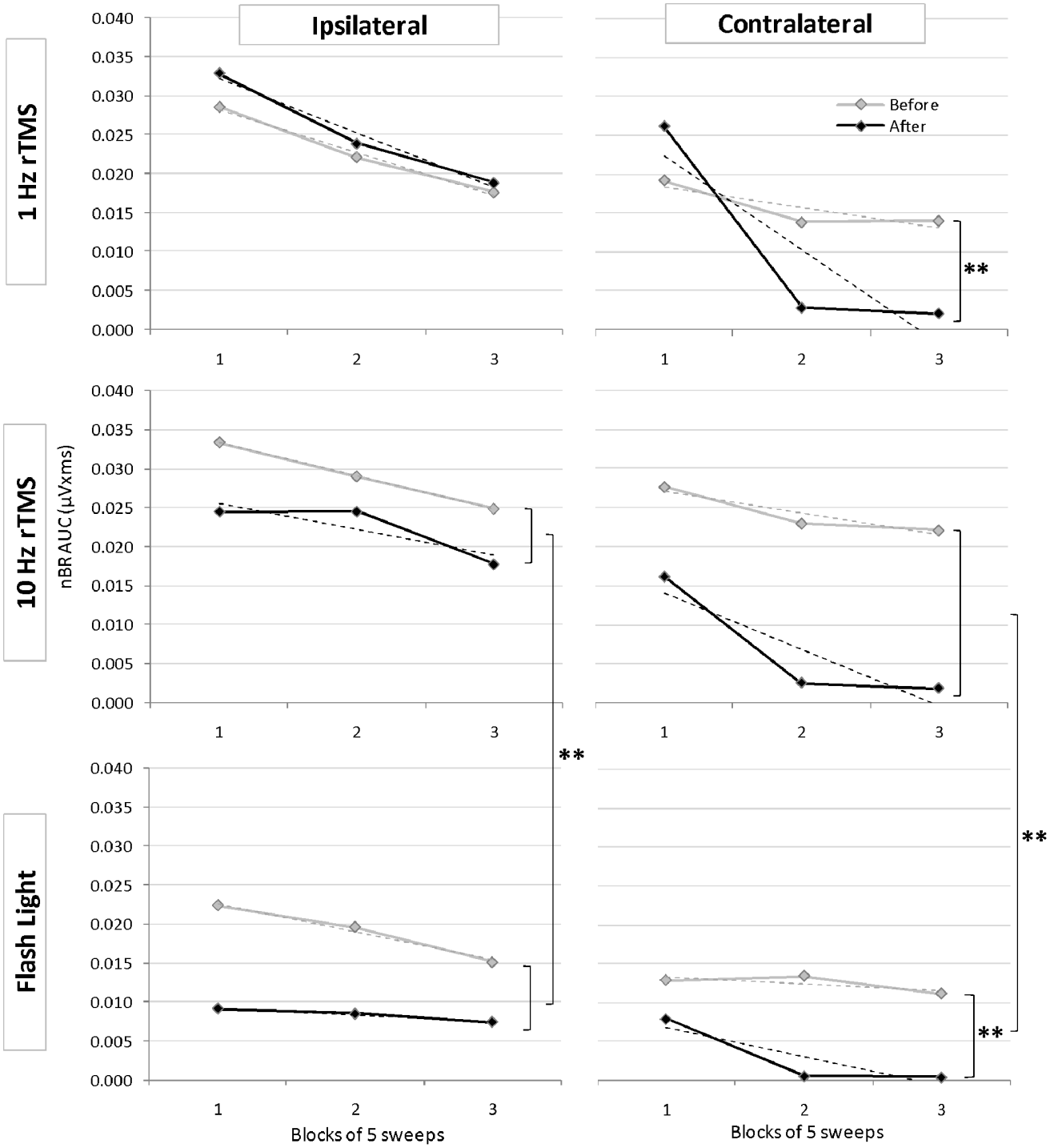

Figure 2. Area under the curve of ipsi- and contralateral nociceptive blink reflexes in 3 successive blocks of 5 averaged responses before (grey lines) and after (black lines) 1 Hz rTMS, 10 Hz rTMS over the visual cortex, or flash light stimulation. Vertical brackets indicate significant differences before and after stimulation, or between stimulation modalities. ** $p<0.01$; * $p<0.05$.
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phosphenes in our study is in line with that found in other studies [36]. In our study we did not use phosphene thresholds after rTMS to verify changes in excitability for several reasons. First, it is well established that rTMS is able to modify visual cortex excitability as indexed by visual evoked potentials (VEP) [41]. Second, although magnetophosphenes are easy to use as indicators of visual cortex excitability, they are not very reproducible and less reliable than VEPs [47]. Unfortunately, because of the design of the experimental protocol and the necessity to record blink reflexes as soon as possible after rTMS or flash stimulation, there was no sufficient time for VEP recordings.

Another confounding factor in our study could be a change in excitability of the facial nucleus motor neurons that contract orbicularis oculi muscles. Although we cannot exclude this

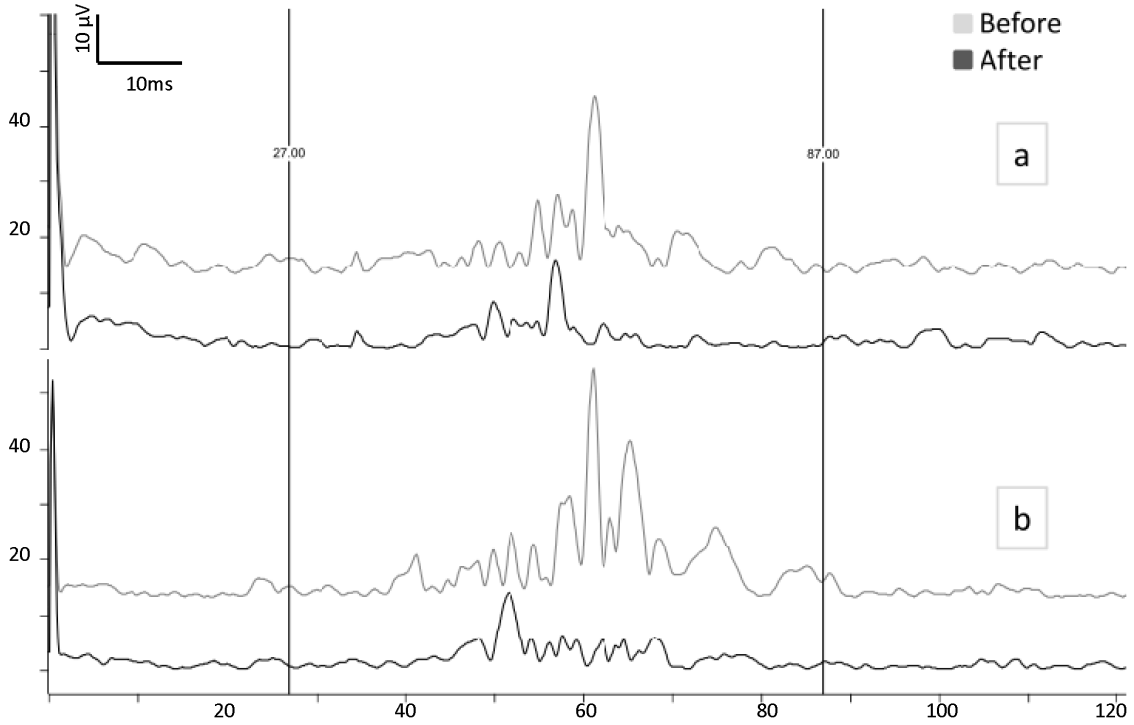

Figure 3. Averaged ipsi- (a) and contralateral (b) nociceptive blink reflex (rectified EMG) in a subject before (grey trace) and after (black trace) flash light stimulation.
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possibility, it is highly unlikely to have influenced our results significantly as the decrease of nBR amplitude was associated with an increase in pain thresholds after flashing light.

Possible Physiological and Pathophysiological Relevance

The top-down relation between the visual cortex and the trigeminal system may play a role in the pathophysiology of photophobia. In rodents bright light is able to activate neurons at multiple sites of the trigemino-cervical complex [6], which is associated with activity of the olivary pretectal nucleus and the superior salivary nucleus [7]. Given its role in saccades and blink [48], the superior colliculus is a possible relay for the effects we have observed. It receives indeed projections from the visual cortex [49] as well reticular and cervical spinal cord projections involved in eyelid movements during the blink reflex [50].

The top-down control we have shown here differs from the one reported in cats by Lambert et al. [11]. These authors found that cortical spreading depression (CSD) or light flash inhibits activity of neurons in nucleus raphe magnus (NRM) and hence disinhibits the responses of trigeminal nociceptors receiving dural input. Multiple waves of CSD antagonized the inhibitory effect of NRM stimulation on responses of trigeminal neurons to dural but not to skin mechanical stimulation. The apparent discrepancy between Lambert et al's [11] and our results may have several explanations. First, there are obvious methodological differences. Lambert et al. [11] used extracellular recordings in trigeminal nucleus caudalis as opposed to indirect assessment of the excitability of trigeminal neurons interposed in the nBR circuit in our study. Ten Hz flash light stimulation was applied for 10 minutes in the cats, while 8 Hz flashes were delivered for 4 minutes to our subjects. Moreover, species differences in visuo-trigeminal interactions cannot be excluded considering the differences in vision between cats and humans. Lastly, CSD, albeit starting with a brief depolarization of cortical neurons, chiefly induces a long-lasting depression of neuronal activity. If one accepts that such a depression might have similar effects on the visual cortex and its connectivity as inhibitory 1 Hz rTMS, both the study in cat and ours in humans would concord in showing that the visual cortex exerts a tonic descending inhibitory action on trigeminal nociceptors.

An inhibitory top-down control by the visual cortex of the trigeminal nociceptive system may have other implications in health and disease. In normal conditions it could contribute to avoid excessive blinking during visual attention. Viewing the stimulated site can decrease pain perception in peripheral limbs of healthy subjects, a phenomenon called "visual analgesia" [51,52,53]. In functional MRI studies, the top-down inhibitory effect of vision on laser-heat evoked pain in the hand is associated with diminished activation in somatosensory cortex SI and operculoinsular cortex but not in anterior cingulate cortex [52]. Our study would be in line with a similar effect of vision in the nociceptive trigeminal system, although a similar analgesic effect in the trigeminal territory by viewing the face remains to be demonstrated.

Tonic inhibition of trigeminal nociceptors by the visual cortex could also be relevant for the pathophysiology of the migraine headache. We have shown that between attacks most migraineurs are characterized by lack of habituation of VEPs [54] resulting in greater net activation of the visual cortex during repetitive stimulation (hyper-responsivity). By contrast VEP habituation normalizes just before and during the migraine attack [55] as well as in chronic migraine [26,56], which reduces net activation of the visual cortex. If our present findings are applied to the changes in cortical activity over the migraine cycle, the trigeminal nociceptive system would be rather inhibited at a distance from an attack because of visual cortex hyper-responsivity, while it would be disinhibited just before and during the attack as well as in chronic

migraine because of a decrease in cortical responsivity. The finding in migraine patients of a deficient habituation of the nBR in the interictal period and its normalization ictally [29,30,32] favours such an excitability cycle of trigeminal nociceptors, as habituation is inversely related to amplitude of the 1st block of responses and thus to baseline excitability.

In addition, the migraine aura is caused by CSD that, as mentioned above, comprises an initial brief neuronal depolarization front, followed by a long-lasting depolarization block of neuronal activity in the visual cortex. Applying our results to the migraine aura, the long-lasting inhibition may cause disinhibition of trigeminal nociceptors and contribute to the CSD-induced neuronal activation in trigeminal nucleus caudalis [57] and thus to the migraine headache.

Conclusion

Our study demonstrates in healthy subjects a functional relation between the visual cortex and the trigeminal nociceptive system, as assessed by the nociceptive blink reflex. Our results favour of a top-down inhibitory pathway from the visual areas to trigeminocervical nociceptors. This pathway may be functionally different from the one attributing to the visual cortex a disinhibitory role on nucleus raphe magnus-mediated inhibition of dural trigeminal nociceptors in cats. In normal conditions the top-down inhibitory pathway may avoid that too intensive blinking disturbs vision. In case of increased responsivity of the visual cortex, like during the interictal period in migraine, the visuo-trigeminal inhibitory pathway may reduce trigeminal nociception. By contrast, when visual cortex responsivity is decreased like during the migraine attack, or in chronic migraine, reduced activation of the visuo-trigeminal inhibitory pathway may increase excitability of trigeminal nociceptors and hence favour headache.

Author Contributions

Conceived and designed the experiments: SLS DM JS. Performed the experiments: SLS VdP. Analyzed the data: SLS DM JS. Contributed reagents/materials/analysis tools: SLS DM JS. Wrote the paper: SLS DM JS.

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Pearls and pitfalls: Electrophysiology for primary headaches

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Abstract

Background: Primary headaches are functional neurological diseases characterized by a dynamic cyclic pattern over time (ictal/pre-/interictal). Electrophysiological recordings can non-invasively assess the activity of an underlying nervous structure or measure its response to various stimuli, and are therefore particularly appropriate for the study of primary headaches. Their interest, however, is chiefly pathophysiological, as interindividual, and to some extent intraindividual, variations preclude their use as diagnostic tools.

Aim of the work: This article will review the most important findings of electrophysiological studies in primary headache pathophysiology, especially migraine on which numerous studies have been published.

Results: In migraine, the most reproducible hallmark is the interictal lack of neuronal habituation to the repetition of various types of sensory stimulations. The mechanism subtending this phenomenon remains uncertain, but it could be the consequence of a thalamocortical dysrythmia that results in a reduced cortical preactivation level. In tension-type headache as well as in cluster headache, there seems to be an impairment of central pain-controlling mechanisms but the studies are scarce and their outcomes are contradictory. The discrepancies between studies might be as a result of methodological differences as well as patients' dissimilarities, which are also discussed.

Conclusions and perspectives: Electrophysiology is complementary to functional neuroimaging and will undoubtedly remain an important tool in headache research. One of its upcoming applications is to help select neurostimulation techniques and protocols that correct best the functional abnormalities detectable in certain headache disorders.

Keywords

Headache, migraine, evoked potentials, blink reflex, nociception, neurostimulation, electrophysiology

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Introduction

Primary headaches are neurological syndromes that evolve in the absence of any underlying structural lesion, as defined by the 2nd edition of the International Classification of Headache Disorders (1). They are characterized by functional disturbances of the central nervous system at several levels, by a dynamic pattern over time (ictal/interictal) and by complex gene–environment interactions. There is no validated paraclinical diagnostic test, and the evaluation of these diseases in a pathophysiological perspective is difficult and tricky.

Electrophysiological surface recordings are an easy way to assess the spontaneous activity of the nervous system, or to evaluate its response to a stimulus. Basically, the different components of the nervous system (central nervous system or CNS, nerves, muscles) generate an electrical signal which is the result of summation of several action potentials. This signal can be recorded, most of the time with surface electrodes, and thereafter processed (amplification, filtering) in

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order to assess the global function of the underlying nervous structure.

In 1947, Dow and Whitty used electroencephalography (EEG) to detect interictal abnormalities in the brain function of their migrainous patients (2). Ever since, the usefulness of EEG in routine headache diagnosis has been controversial and is now only recommended in patients with atypical symptoms suggesting an underlying pathological process (such as thrombophlebitis, encephalitis, tumour) and especially epileptic phenomena. Evoked and event-related potential studies started in the late 1960s and have also demonstrated several abnormalities in headache patients, but because of high inter- and intraindividual variability, they do not have any usefulness in primary headache diagnosis. Nonetheless, like EEG, they can be helpful to exclude mimics in selected cases.

On the other hand, electrophysiological studies have widely contributed to a better understanding of headache pathophysiology, especially migraine. Electrophysiology continues to be part of the headache research armamentarium, and is complementary of more recent techniques like functional neuroimaging. In this article, we will review the main 'pearls' of the electrophysiological findings in headache, and afterwards describe their limitations. The last part of the review will discuss open questions and give suggestions for future studies.

Pearls

Electrophysiology is particularly suitable for the study of primary headaches that are functional disorders of the CNS. These techniques are non-invasive and the existence of portable devices can provide a high flexibility in patient recordings, for example in a recent study familial hemiplegic migraine patients were recorded at home throughout Denmark (3). Moreover, electrophysiological recordings are often technically simple to obtain for a trained physician, are harmless and can be repeated at many time points. The latter aspect is of high importance in diseases with such a dynamic pattern as headaches. Indeed, primary headaches are cyclic diseases characterized by the repetition of attacks that notably differ by their frequency, length and intensity. The biological mechanisms subtending this pattern are unknown, but thanks to the advantages mentioned above (flexibility etc.), electrophysiology appears particularly suitable to investigate the dynamics of primary headaches.

The majority of the following text will discuss electrophysiological findings in migraine, which is the best-studied headache type. We will not describe the different techniques reported here from a methodological point of view, as this had been the aim of a previous review article (see (4**) for more information).

Migraine

The most important electrophysiological studies performed in migraine demonstrate three functional characteristics of the disease, which are interrelated: 1. habituation modifications, 2. cortical dysexcitability and 3. abnormal functional connexions and circuits within the CNS.

Habituation modifications. Habituation is defined as a behavioural response decrement that results from repeated stimulations and does not involve sensory adaptation or fatigue, that is a decrease in peripheral receptor activity (5). The average habituation deficit to repetitive stimuli is probably the most reproducible and redundant hallmark of episodic migraine recordings in the interictal period, whatever the modality of stimulation, that is the neuronal population that is stimulated (see Figures 1 and 2). It is, however, not specific to migraine as it has been found in other diseases such as photosensitive epilepsy or tinnitus (6,7), and some psychiatric conditions. This interictal habituation deficit, sometimes resulting in potentiation, has been mainly demonstrated for visual evoked responses (VEP, Figure 1) (8**–10), but also for auditory (AEP) (11), somatosensory (SSEP, Figure 2) (12,13) pain (laser, LEP) (14,15) and event-related (contingent negative variation, CNV) responses (16,17). Moreover, it was also retrieved for the nociception-specific blink reflex (nsBR, Figure 1), a subcortical brainstem electromyographic (EMG) response that reflects trigeminal activity and is mediated by bulbopontine excitatory interneurons (18**). Besides this habituation deficit, migraineurs exhibit an increased intensity dependence of auditory evoked potentials (IDAP), which was found to be correlated to the lack of habituation and perhaps to be the consequence of it (19**). The habituation phenomenon has been extensively studied in migraineurs, and some characteristics have been drawn.

– First, the habituation deficit is not constant in migrainous patients. The studies showing a lack of habituation are based on the averaging of numerous patient recordings, compared with healthy volunteers. Therefore, the habituation deficit cannot be considered as a diagnostic criterion of migraine. In addition, it was shown that the degree of habituation depended on the stimulus properties, for example the temporal or spatial frequencies of a visual pattern, which may explain why some authors did not retrieve any habituation deficit in migrainous patients (20).

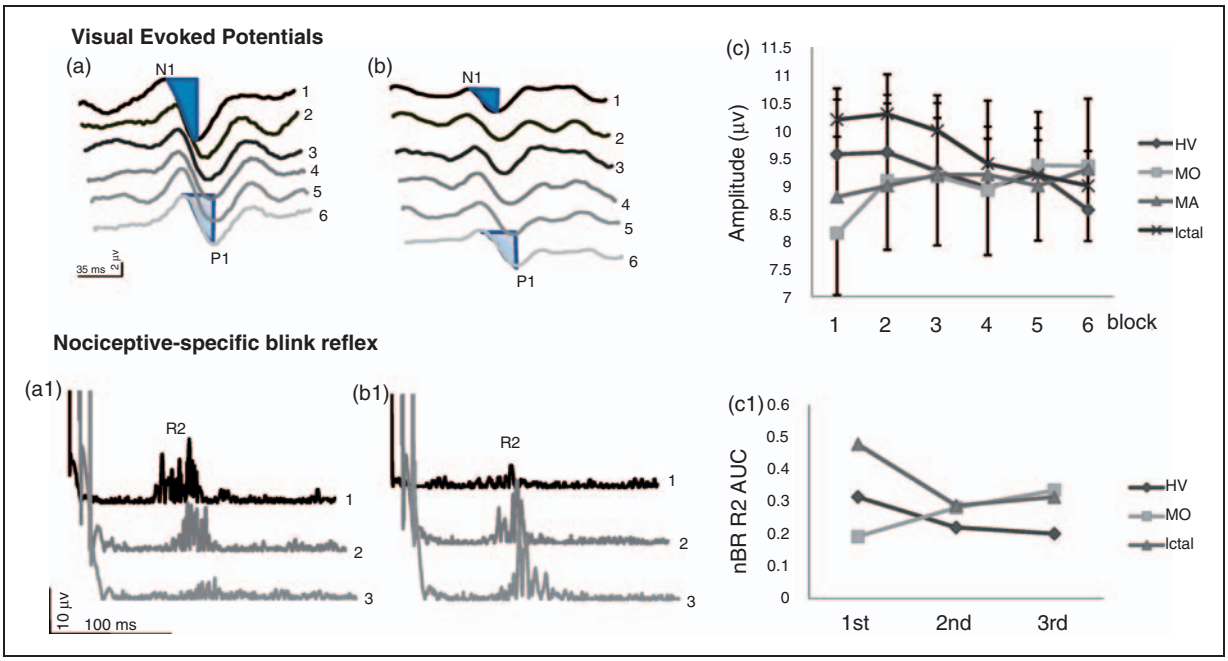

Figure 1. The upper part of the figure represents visual evoked potential (VEP) recordings in a healthy volunteer (HV, (a)) and a migraineur in the interictal period (b). The deep blue triangles highlight the mean VEP amplitude at the beginning of the recording (a block corresponds to the averaging of 100 individual sweeps) and the light blue triangles show mean VEP amplitude at the end of the recording (sixth or last block). There is an amplitude decrease with time in the HV, that is habituation (a), whereas this habituation lacks in the migraine patient, in whom the first block amplitude is also smaller than in HV, suggesting a reduced cortical preactivation level (b). Part (c) represents the evolution of VEP amplitudes (numerical data, mean ± standard deviations) with time in a healthy volunteer (HV), migraineurs with (MA) and without (MO) aura in the interictal period, and in a migraineur in the ictal period. Note that both reduced initial VEP amplitude and the lack of habituation found in MO and MA normalize during the attack. The lower part of the figure represents nociception-specific blink reflex (nsBR) recordings in a HV (a1) and a migraineur (b1) interictally. Note the lack of habituation and the reduced initial amplitude in the migraine patient. Part (c1) of the figure is the average nsBR area under the curve (AUC) evolution with time in a HV, a migraineur without aura (MO) and a migraineur recorded in the ictal period.

– Second, habituation is a dynamic parameter that provides interesting data about the current CNS information processing. In migraine, important peri-ictal changes were found in habituation. During the days preceding the attack, the habituation deficit (CNV, P300) becomes maximal (21,22). It increases with stress which is a known migraine-provoking factor (23), or in the pre-menstrual period (LEP, (24)). Interestingly, most of the sensory modalities showing an interictal lack of habituation then normalize 12–24 hours before and during the migraine attack (13,17,18**,21,22,25). It takes 24–48 hours to get back to the abnormal habituation pattern seen in the headache-free interval (25). These sequential recordings have thus demonstrated that the cortical dysfunction level varied with the migraine cycle. Along the same line, recent data revealed that patients suffering from chronic migraine and evolving to episodic migraine after successful prophylactic treatment exhibited a switch of visual responses from normal habituation to potentiation (26**), as if chronic migraine corresponded to a 'never ending attack' (27) and the treatment restored the interictal habituation deficit found in the episodic form. This is not the case in medication-overuse headache (MOH) where habituation of SSEP remains impaired, whereas the initial response amplitude is increased, suggesting a sensitization of somatosensory cortices in MOH patients (13). However, this phenomenon was dependent on the drug of overuse, as it is maximal in patients overusing NSAIDs and almost non-existent in those who overuse only triptans (25).
– Third, genetics appears to be a determinant factor of the interictal dysfunction leading to deficient habituation in migraine. Hence, Sàndor et al. studied VEP and AEP in migrainous pairs (parents and their children), and found that habituation was abnormal in both parents and children, with a stronger relationship between related pairs (28**). A lack of habituation was also demonstrated in healthy volunteers with a familial history of migraine in first-degree relatives (29,30). It could thus be an endophenotypic marker of a genetic predisposition to migraine, even

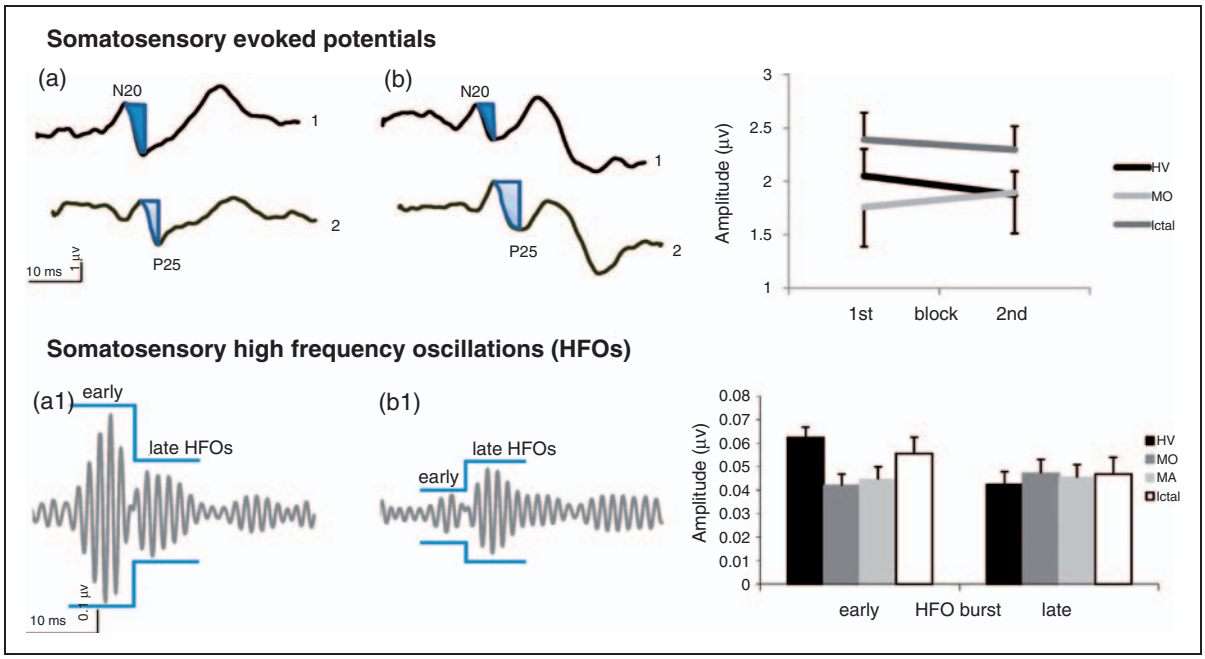

Figure 2. The upper part of the figure represents somatosensory evoked potentials (SSEPs) of the median nerve in a healthy volunteer (HV, (a, c)) and in migraineurs without (MO) and with aura (MA) (b, c). The typical electrophysiological abnormalities as mentioned previously are also retrieved and normalized in the ictal period (see Figure 1). The lower part of the figure represents the high frequency oscillations bursts (HFOs) extracted from broad-band SSEPs. Note that the early HFO burst is reduced interictally in the migraine patient (b1) compared with the HV (a1), reflecting reduced thalamocortical activity, whereas the late HFO burst remains normal at any time, suggesting a normal activity of cortical inhibitory interneurons.

if these conclusions cannot be applied to individuals. Finally, Di Clemente et al. found that VEP and nsBR habituation deficits were correlated in migraineurs, which argues in favour of a common underlying pathological mechanism (31). However, VEP habituation and IDAP slope are not correlated (32).

– Fourth, the habituation can be modulated by external interventions, especially drugs known to provoke or alleviate migraine attacks, or transcranial magnetic stimulation (see below). Hence, various studies demonstrated a normalization of the interictal habituation deficit with several established preventive drugs like beta-blockers (33) or topiramate (34). This habituation deficit reversal has also been shown in children treated with behavioural therapy (35). The relationship between the normalization of habituation and the clinical improvement is probably more complex, as for example fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is not an effective antimigraine drug but corrects the lack of habituation of VEP in patients (36), whereas riboflavin, which acts on mitochondrial metabolism, does not modify habituation (33). Moreover, the migraine-provoking agent nitroglycerin is able to induce nsBR and VEP changes similar to those found before and during a migraine attack when it is administered to healthy volunteers without any familial history of migraine (37).

Cortical dysexcitability. Assessment of cortical excitability by neurophysiological techniques has provided contradictory results that have long been debated. Interictal cortical dysexcitability has been indirectly suggested by two main neurophysiological variables: the cortical sensitivity to transcranial magnetic stimulation (TMS) and the reduced initial evoked potential (EP) amplitude, which is correlated to the lack of habituation described above.

– TMS is an easy and non-invasive way to study the excitability of the underlying cortical area. The main studies performed on migraine used single-pulse TMS (sTMS) to assess the visual or motor cortex activation thresholds, or repetitive TMS (rTMS) to inhibit (low frequencies, 1 Hz) or activate (high frequencies, 10 Hz) the underlying cortex. In sTMS, the motor threshold was found to be normal or increased (38–41), but the latter is less relevant than the magnetophosphene threshold (PT) to visual cortex TMS, as an abnormal excitability of the occipital cortex has been suspected in migraine

for a long time, especially with aura. The numerous trials on PT in migraine between attacks gave conflicting results, that is either increased PT suggesting cortical hypoexcitability (40–42), or decreased PT in favour of a hyperexcitable state (43–45). These discrepancies between various studies might be because of methodological differences (subject recruitment, proximity with a migraine attack, individual perception and description of phosphenes, etc.) (46). In a study with repetitive stimulations, Bohotin et al. revealed that 10 Hz (excitatory) rTMS was able to normalize the interictal deficient habituation, whereas 1 Hz (inhibitory) rTMS had no effect on VEP in migraineurs (47**). In apparent contradiction to these findings, Brighina et al. found that inhibitory rTMS increased subjective PT in healthy volunteers but surprisingly decreased it in migraine with aura (MA) patients (42). The authors suggest that the migrainous brain probably has a 'non-physiological' and paradoxical response to rTMS, which could be attributed to abnormal cortical processing. However, this paradoxical effect of modulating rTMS was also observed after stimulation of the motor cortex and not only by means of inhibitory (48,49), but also with the excitability enhancer rTMS. Short trains of 5 Hz (excitatory) rTMS delivered at 130% of resting motor threshold determined a significant depression of MEP size in MA patients rather than MEP facilitation as in controls (49). These paradoxical behaviours in response to rTMS point to altered synaptic plastic mechanisms that prevent the immediate and longer-lasting cortical changes reflecting adaptation to repeated stimulations. Further evidence comes from a long-term study showing that rTMS is able to induce VEP changes lasting up to several weeks in about 50% of healthy volunteers, whereas the effect lasts only several hours in most migraineurs (50).

– The initial amplitude of the evoked CNS responses to various sensory modalities is (or tends to be) lower in migraineurs recorded in the interictal period, that is VEP (8**,9,32,36,47**), AEP (19**,32), SSEP (12) and even the subcortical nsBR (29,31). In VEP and AEP, the initial amplitude is negatively respectively correlated with the potentiation and the IDAP (32), which suggests that a reduced cortical preactivation level might be responsible for the lack of habituation found in migraineurs (see next section: 'Abnormal functional connexions and circuits: the 'unifying' thalamic hypothesis').

Abnormal functional connexions and circuits: the 'unifying' thalamic hypothesis. The habituation modifications and cortical dysexcitability found in migraine were thus probably interrelated, but the origin of these phenomena per se remained obscure. Recent works have pointed out possible thalamocortical dysfunctional connexions that could provide an explanation for both abnormalities.

There are two main hypotheses subtending the lack of habituation found interictally in episodic migraine, a reduced intracortical inhibition or an increased cortical excitability, but neither has proved satisfactory as yet (51**).

– Light deprivation is supposed to decrease both excitatory and inhibitory processes within the cortex; however, it did not modify the habituation deficit found in migraineurs in a recent study, which argues against the reduced intracortical inhibition hypothesis (52).
– As for the hyperexcitability hypothesis, the results with TMS appear too contradictory from which to draw any conclusions (see above). Red glasses are known to increase the excitability of the human visual cortex. However, Afra et al. did not observe any significant modification of the VEP in MA patients wearing red glasses, whereas healthy volunteers had an increase of VEP amplitude (53). This result also disfavours the hyperexcitability hypothesis.

A third possibility arose from one of the more reproducible neurophysiological parameters, that is the finding of a recurrent reduced initial response of various sensory cortices. As stated previously, the correlation between this reduced initial response and the degree of habituation suggests that a reduced cortical preactivation level is responsible for the lack of habituation found in migraine.

Recent neurophysiological works have shed a new light on the possible pathophysiological mechanisms of this decreased cortical preactivation. Coppola et al. applied a specific filter to broad-band SSEP recordings in order to extract the high-frequency oscillations or HFOs (Figure 2). HFOs are thought to reflect thalamocortical cholinergic fibre activity (early component) and cortical inhibitory GABAergic interneuron activity (late component) (51**). Interictally, the early component of the HFO was significantly smaller in migraineurs than in healthy subjects, but became comparable between the two populations during the attack (Figure 2). The late component did not differ between the two groups at any time. Moreover, reduced early HFOs were associated with worsening of the clinical evolution of migraine (54). In a recent study, 10 Hz repetitive transcranial magnetic stimulation (rTMS)-induced activation of the sensorimotor cortex increased thalamocortical drive in migraineurs, because it was low at the baseline, and induced habituation of the

broad-band SSEP. This was not possible in healthy subjects probably because their thalamocortical activity and habituation were already maximal before the rTMS (55). Thus, the deficit of habituation found in migraineurs could be because of impaired thalamocortical activity, namely reduced cortical preactivation, and not because of decreased intracortical inhibition (51**). That the thalamus abnormally controls the cortex in migraine between attacks is further evident by the analysis of the high-frequency oscillatory components embedded in the visual EPs (gamma-band oscillations, GBO) (56). Investigators observed a significant habituation deficit of the late GBO components, supposed to be of cortical origin, in migraineurs relative to healthy controls, which was interpreted as indicative of a dysfunction in cortical oscillatory networks that could be because of an abnormal thalamic rhythmic activity, namely a 'thalamocortical dysrhythmia' (56). Coppola et al. stressed that this thalamocortical dysrythmia could result from a functional (or anatomical?) thalamic disconnection from its modulating afferences, for example the brainstem serotoninergic pathways (56). This explanation may reconcile the controversy between increased cortical excitability and deficient inhibition, as an insufficient thalamocortical drive, namely a low level of cortical preactivation, results in a dysfunction of both inhibitory and excitatory cortical neurons. Lower inhibition and cortical preactivation may thus not be mutually exclusive, as the latter can promote the former through a reduction of lateral inhibition. The final common pathway of both dysfunctions is a heightened cortical response to repeated stimuli, that is hyperresponsivity.

Tension-type headache

Electrophysiological data on tension-type headache (TTH) are scarce compared with those on migraine. Early neurophysiological studies analysed electromyography, as pain caused by TTH was believed to be the result of an abnormal myofascial activity. More recent works now suggest that this is true for episodic TTH (ETTH) but not chronic TTH (CTTH), in which central dysnociception mechanisms are more likely involved (57).

Electromyographic responses. More than 20 surface EMG activity studies on TTH are available (58), but results are contradictory, therefore EMG has no diagnostic indication in TTH. The most common finding between positive studies was a slightly increased EMG activity, but this was not correlated to the intensity of the headache.

The so-called exteroceptive suppression of temporalis muscle activity corresponds to the suppression of voluntary EMG of the temporalis muscle in response to a painful stimulus in the trigeminal area. Two successive silence periods (ES1 and ES2) can be identified. The duration of the late component ES2 is decreased in CTTH but not ETTH, migraine or cluster headache patients (59). Modulation of ES2 by various parameters (drugs, pain, TMS) has led to the hypothesis that ES2 reflects the excitability of interneurons in the pontomedullary reticular formation (57). In CTTH, the excitability of these interneurons would be impaired because of inadequate control by the descending control from the limbic system through the serotoninergic raphe magnus nuclei (59). Several studies have been published on ES2 duration in TTH, with some discrepancies as ES2 was either shorter (60**–65) or normal (66–68). Again, these discordant results might be attributed to methodological differences or to patient-related factors such as age, comorbidities and headache severity (57).

The blink reflex (BR) was mentioned above in the migraine section. In TTH, most studies involved the 'standard' BR, that is evoked in response to stimulation of large Aß myelinated fibre activation in the supraorbital nerve area, contrary to the nociception-specific BR or nsBR which is elicited by Aδ nociceptive afferents and has been mainly studied in migraine (58). The BR was normal in all forms of TTH (see (58) for a more detailed review), this was also the case of the sole nsBR study in CTTH (69). A single trial demonstrated a decrease of the R2 recovery cycle after double supraorbital stimulation in both ETTH and CTTH, suggesting a reduced excitability of brainstem interneurons (70).

The biceps femori flexion reflex (BFR) is a complex reflex mediated at both spinal and supraspinal levels in response to a nociceptive stimulus. In CTTH, studies found a lower RIII flexion reflex threshold which might suggest central sensitization of nociceptive circuits (71–73) and/or be because of impaired supraspinal descending inhibitory control (72).

Cortical responses. Few studies are available on electroencephalography in TTH and those few provide inconsistent results (58). In contrast to migraine, most evoked potential studies (VEP, LEP, CNV) performed in TTH did not demonstrate any recurrent abnormalities like reduced preactivation or lack of habituation (14,16,74,75). The only abnormality was found in LEP by de Tommaso et al., who demonstrated increased N2-P2 amplitude in CTTH after pericranial skin stimulation (76,77). This higher amplitude was correlated with the total pericranial tenderness and with anxiety scores (the latter was interpreted as a hypervigilance to painful stimuli), and decreased after treatment with amitriptyline (78). An interesting but unique study recorded

SSEP in response to intramuscular trapezius electrical stimulation using high-density EEG mapping, and found a significant reduction in magnitude of the dipolar source during and after induced tonic muscle pain in healthy volunteers but not in CTTH patients (79). They concluded that this lack of magnitude reduction might be because of impaired inhibition of the nociceptive input in CTTH patients, suggesting an abnormal supraspinal response to muscular pain (79).

Cluster headache

Electrophysiology could seem of modest importance to the understanding of cluster headache (CH) pathophysiology regarding other techniques like functional neuroimaging. However, it remains of high interest to study nociceptive spinal and supraspinal mechanisms, and to understand the mode of action of recent neuromodulation methods.

Subcortical electromyographic responses. A study of 'standard' BR found that the amplitude of the contralateral R2 response on the symptomatic side was lower than on the healthy side in the active cluster period (80). A further trial did not confirm these findings, but showed a decrease of R2 inhibition after supraorbital and peripheral conditioning stimuli in CH, the latter being partially reversed by naloxone IV (81). Another study demonstrated an R2 habituation deficit in untreated episodic CH patients during the cluster period, which was even more pronounced than in migraine patients (82). A more recent study with nsBR did not confirm these findings in a population of episodic and chronic CH patients on prophylactic medication, both during and outwith a bout (83). Finally, a study of nsBR found a decrease of latency ratio (cluster side vs. healthy side), as well as an increase of R2 area ratio in episodic CH patients during a bout (84). Overall, these findings suggest an impaired nociceptive processing at brainstem level in the CH period.

This impairment of pain control systems was also confirmed by the study of BFR, which exhibited a lower threshold in CH patients during (85) and outwith the bout period (86). Interestingly, the modifications of BFR have a circadian rhythm in ECH patients but not in CCH patients (86).

Evoked potentials. As in TTH, studies of evoked potentials are scarce in the CH population. Various abnormalities have been highlighted in sensory evoked potentials, but these are not as 'homogenous' as in migraine (84,87–93). The intensity dependence of auditory evoked potentials (IDAP, see before) is also increased in CH patients, during and outwith the bout, which might suggest a decreased serotoninergic activity in the raphe-hypothalamic pathways (94).

Neuromodulation in cluster headache: mechanism of action. Posterior hypothalamic deep brain stimulation (hDBS) and occipital nerve stimulation (ONS) have shown their efficacy in the symptomatic treatment of drug-resistant CCH (95–98). Electrophysiological measurements were performed in order to understand their mechanisms of action. The nsBR was not significantly modified after hDBS, but the latter decreased peripheral pain thresholds (95) and increased trigeminal cold detection and pain thresholds (99), suggesting subtle pain-modulating processes. In ONS, nsBR was paradoxically increased after treatment (97), which mirrors a more centrally located mode of action. That brief low frequency ONS does not modify nsBR in healthy volunteers, could also argue in favour of this suprasegmental mechanism (100). The latter was also proposed to explain occipital nerve steroid injection efficacy, after which CH patients have an R2 decrease, but that is not especially correlated to clinical improvement (101).

Pearls of headache electrophysiology: summary

The contribution of electrophysiology to the understanding of primary headache pathophysiology can be summarized as follows.

- In episodic migraine, there is an interictal lack of habituation of the brain to various sensory modalities, which is associated with a reduced cortical (and even subcortical) preactivation level suggesting an abnormal underlying cortical excitability. A recent hypothesis pointed out that the thalamus could play a key role in these phenomena: a thalamocortical dysrythmia (possibly because of a functional disconnection of the thalamus from the brainstem) would reduce the cortical preactivation level and thus impair the normal habituation process.
- In tension-type headache, available studies suggest that the chronic form would be associated with dysfunctioning supraspinal descending antinociceptive pathways coming from the limbic system through the serotoninergic raphe magnus nuclei to the interneurons of the pontomedullary reticular formation. This is not the case in the episodic form where an abnormal myofascial activity was retrieved but there were no signs of abnormal central antinociceptive control.
- Finally, in cluster headache, electrophysiological studies are scarce and their results are conflicting. Overall, impaired sensory and nociceptive processing can be suspected but no consistent underlying

pathophysiological hypothesis has been proposed unlike in migraine.

Neuroimaging correlates of the lack of habituation

In order to better understand the underlying mechanisms of the interictal abnormalities found during the electrophysiological recordings in migraine patients, several studies recently focused on their neuroimaging correlates, especially on habituation which was often indirectly evaluated. Only a few studies are available and differ by their methodology (neuroimaging type: functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), stimulation paradigms, etc.), leading to results discrepancies (102–105). With ^{3}H MR spectroscopy searching for occipital lactate changes during visual stimulation, Sándor et al. (106) reported increased baseline lactate levels in patients suffering from migraine with pure visual auras, whereas patients with complex neurological auras had normal baseline levels, but lactate increases, mimicking lack of habituation, during visual stimulation. In an fMRI study, an initial weaker blood-oxygen-level dependent (BOLD) signal was found during visual stimulation in 10 migraine patients (with and without aura). By contrast, a progressive increase of cortical occipital BOLD was found during sustained visual stimulation in migraineurs, a pattern resembling the VEP habituation deficit, whereas there was a habituation in healthy volunteers (107). More recently, Boulloche et al. (103) and Martin et al. (105) studied the visual cortex response in $H_2{}^{15}O$ PET (103) and fMRI-BOLD (105), using different stimulation paradigms in episodic migraineurs. Although the first authors indirectly found a lack of habituation (or a cortical hyperexcitability) to light, the second authors failed to demonstrate any lack of habituation to repetitive light stimuli in migraineurs. Aderjan et al. used a painful olfactive stimulation to study habituation over several days, unlike electrophysiological studies where the latter is often evaluated within minutes (102). The pain perception did not differ with time between healthy volunteers and migraine patients but the BOLD signal activity level of some antinociceptive structures (such as the prefrontal cortex, rostral cingulate cortex, red nucleus) decreased in migraineurs and increased in healthy volunteers, suggesting existing alterations of pain inhibitory circuits. Finally, another fMRI-BOLD trial designed with paired face stimuli speculated that the absence of haemodynamic refractory effects in migraineurs was the neurovascular correlate of the lack of habituation found in electrophysiology (104). However, habituation in face perception areas has never been studied in electrophysiological trials as it would require intracranial recordings (104). However, even recognizing that these interictal fMRI studies did not use completely comparable stimulus parameters to those typically used to demonstrate habituation with EPs, they confirm that cortical responsivity to repeated stimuli is abnormal in migraineurs.

Pitfalls

Methodological considerations

Methodological problems were extensively reviewed in a previous article (4**). Electrophysiological recordings can be easily contaminated by artefacts of various origins (external: alternative current etc., or internal/organic: ECG, EMG, drugs). There are also several recommendations in terms of signal sampling frequency and filtering, as well as stimulation frequencies for evoked responses. The latter have been pointed out as a possible explanation for discrepancies found in evoked potential studies, especially in migraine (46). Hence, there is a need for a better standardization, and some proposals for methodological optimization of recordings have been suggested before (4**).

Unfortunately, only part of the nervous system is accessible to non-invasive electrophysiological recordings. Deep structures are not easily reached (hypothalamus etc.), but indirect neurophysiological assessment methods can be found for some of them, for example analysis of HFOs as representing thalamocortical activity (51**,55). Moreover, not all structures provide a clear 'witness' of activation (for example the cerebellum, orbito-frontal cortex, hypothalamus) and knowing if they are being stimulated could be difficult.

Patient phenotypes

Differences between populations included in electrophysiological studies can also explain the variability of results and the lack of interindividual reproducibility. It is well known that evoked potential modifications can occur with age and coexisting comorbidities such as depression and anxiety. Between EP studies, inconsistencies can also be because of concomitant acute or preventive drugs, or even caffeine intake (82,83,108–110).

Intrinsic recording discrepancies may also happen. In women, the menstrual cycle affects pain perception and should be considered when recordings are performed (24). The dynamic electrophysiological pattern of migraine can be used to situate a patient in his/her migraine 'cycle' at the time of the recording (see before, proximity to the last/next attack), using headache diaries and phone calls. It is important to emphasize again here that even in the interictal period not all migraineurs exhibit a habituation deficit or reduced initial

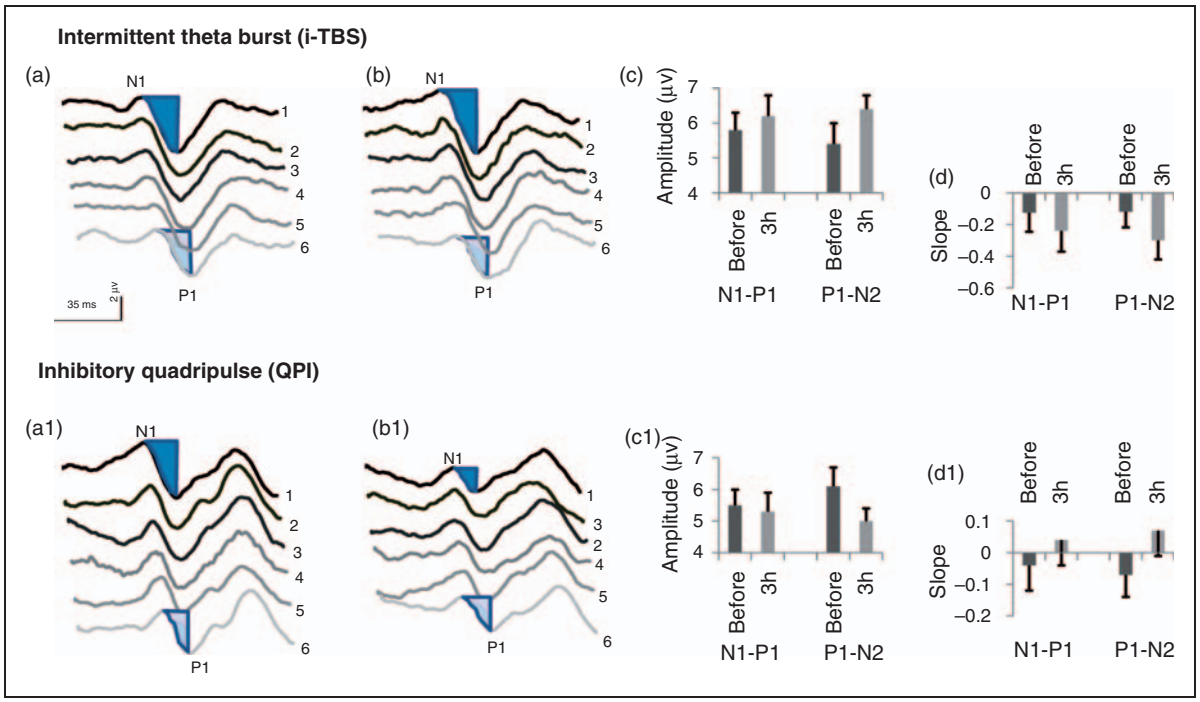

Figure 3. This figure presents two non-invasive neurostimulation techniques that are able to modify visual evoked potential (VEP) recordings in healthy volunteers (HV). VEP traces (six blocks of recordings) are represented before (a, a1) and after (b, b1) the application of intermittent theta burst stimulation (i-TBS, upper part of the figure) or inhibitory quadripulse (QPI, lower part) in one HV. Part (c, c1) shows average baseline N1P1 and P1N2 VEP amplitudes in 13 HV, and their evolution 3 hours after stimulation with i-TBS (upper part) or QPI (lower part). Part (d, d1) shows VEP habituation slopes values before and 3 hours after stimulation with i-TBS (upper part) or QPI (lower part). The degree of habituation is expressed as a negative slope, that is the more negative value, the higher the habituation.

response amplitudes, and that these electrophysiological traits were statistically demonstrated on averaged measures of several patients compared with healthy volunteers.

Heterogeneity of the disease

Headaches are heterogeneous polygenic diseases, and ICHD-II classification of primary headaches (1) is probably not accurate enough to classify patients into homogenous subgroups for electrophysiological studies. For example in migraine, the severity of the disease is an essential factor: studying a migraineur with high attack frequency in interictal period is a real challenge, and this patient cannot be reasonably considered as similar to another migraineur with one attack every other month. The extremity of this clinical spectrum is chronic migraine, now considered a 'never ending attack' (27), as patients exhibit similar electrophysiological patterns to those during the ictal state (111). In many older studies the chronic migraine subgroups included patients with and without various kinds of acute medication overuse; however, the electrophysiological profile of both patient types appears different, suggesting diverse mechanisms leading to headache chronification (13). Further subclassifications of headache patients, especially migraineurs, have been proposed according to associated symptoms like photophobia or vertigo. This method has already been employed in genetics (latent class analysis) but results were disappointing.

Conclusions: open questions and recommendations for future studies

Overall, the contribution of electrophysiology to the understanding of primary headache pathophysiology is more significant for migraine than for other primary headaches, where studies are comparatively rarer and often disclose a high variability of results for similar methods.

The reduced preactivation level of sensory cortices and the lack of habituation to sensory stimuli found in migraine could be the consequence of a thalamocortical dysrythmia as suggested by recent works (51**,55,56). A thalamic involvement in migraine pathophysiology is also suspected by other studies using different research methods (112–114). The activity of the thalamus itself is modulated by several afferences, among them inputs from the aminergic nuclei of the dorsal rostral pons.

– Future electrophysiological works must understand the role of each structure in the dynamic mechanisms that lead to the migraine cycle, from one attack to the next, and from episodic to chronic migraine.
– Given the heterogeneity of the disease, patients should be carefully selected as mentioned before, and perhaps classified according to their electrophysiological profile, which might subtend different underlying mechanisms.
– Moreover, further studies should focus on the connections between the cortex, the thalamus and the brainstem (trigeminal structures), and especially their modulations by excitatory and/or inhibitory stimuli.
– One of the upcoming applications of electrophysiology would be to help select neurostimulation techniques and protocols that would be able to correct the functional abnormalities detectable in certain headache disorders such as the lack of habituation in migraine (see example in Figure 3, (115)). Hence, previous results provided by electrophysiological measurements lead to therapeutic neurostimulation trials that gave encouraging results (115), these translational research protocols should be highly promoted in future.
– Conversely, electrophysiology remains a simple method to appreciate the mode of action of various pharmacological and non-pharmacological treatments.

Pearls, pitfalls and perspectives

Pearls

- Electrophysiology is a non-invasive and easy way to access the activity of the nervous system, and is therefore particularly suitable for the study of primary headaches which are CNS functional disorders characterized by a dynamic pattern (ictal/interictal).
- The most reproducible electrophysiological abnormality is the lack of habituation to repetitive stimuli found in migraine patients in the interictal period, whatever is the sensory modality. This lack of habituation could be the consequence of a thalamocortical dysrythmia resulting in a reduced preactivation level of sensory cortices.

Pitfalls

- As a result of high inter- and intraindividual variability, electrophysiological measurements cannot be used for diagnosis of primary headaches but can be helpful to rule out mimics in some cases (secondary headaches, epileptic syndromes).
- The discrepancies between electrophysiological studies might be because of methodological differences as well as patients' dissimilarities.

Perspectives

- Electrophysiology will remain an important tool in the headache research armamentarium. One of the upcoming applications of electrophysiology would be to help select neurostimulation techniques and protocols able to correct the functional abnormalities detectable in certain headache disorders such as the lack of habituation in migraine.

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Conflict of interest

None declared.

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Safety and patients' satisfaction of transcutaneous Supraorbital NeuroStimulation (tSNS) with the Cefaly® device in headache treatment: a survey of 2,313 headache sufferers in the general population

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Abstract

Background: Transcutaneous supraorbital nerve stimulation (tSNS) with the Cefaly® device was recently found superior to sham stimulation for episodic migraine prevention in a randomized trial. Its safety and efficiency in larger cohorts of headache sufferers in the general population remain to be determined.
The objective of this study was to assess the satisfaction with the Cefaly® device in 2,313 headache sufferers who rented the device for a 40-day trial period via Internet.

Methods: Only subjects using specific anti-migraine drugs, and thus most likely suffering from migraine, were included in the survey. Adverse events (AEs) and willingness to continue tSNS were monitored via phone interviews after the trial period. A built-in software allowed monitoring the total duration of use and hence compliance in subjects who returned the device to the manufacturer after the trial period.

Results: After a testing period of 58.2 days on average, 46.6% of the 2,313 renters were not satisfied and returned the device, but the compliance check showed that they used it only for 48.6% of the recommended time. The remaining 54.4% of subjects were satisfied with the tSNS treatment and willing to purchase the device. Ninety-nine subjects out of the 2,313 (4.3%) reported one or more AEs, but none of them was serious. The most frequent AEs were local pain/intolerance to paresthesia (47 subjects, i.e. 2.03%), arousal changes (mostly sleepiness/fatigue, sometimes insomnia, 19 subjects, i.e. 0.82%), headache after the stimulation (12 subjects, i.e. 0.52%). A transient local skin allergy was seen in 2 subjects, i.e. 0.09%.

Conclusions: This survey of 2,313 headache sufferers in the general population confirms that tSNS with is a safe and well-tolerated treatment for migraine headaches that provides satisfaction to a majority of patients who tested it for 40 days. Only 4.3% of subjects reported AEs, all of them were minor and fully reversible.

Keywords: Transcutaneous peripheral nerve neurostimulation; Preventive migraine therapy; Cefaly®

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Clinical relevance summary

Transcutaneous supraorbital neurostimulation with the Cefaly® device is a safe and satisfactory treatment modality for migraine headache sufferers in the general population who tested it for 40 days. Treatment failure may be partly due to poor compliance.

Background

Migraine is a highly prevalent primary headache disorder and one of the most disabling diseases worldwide according to the recent epidemiologic data [1]. Preventive anti-migraine drug therapies have incomplete efficacy and many of them have cumbersome side effects [2]. Blumenfeld et al. (2013) [3] have recently found that only 28.3% and 44.8% of subjects suffering respectively from the episodic and chronic forms of migraine (ICHD-III beta criteria 1.1, 1.2 and 1.3 [4]) were currently using a preventive medication [3]. The reasons for treatment discontinuation were lack of efficacy and side effects in an equal proportion. Furthermore, over the last decade hardly any novel migraine preventive drug has been marketed. Hence, there is a need for new preventive therapies with similar or better clinical efficacy, and most importantly fewer treatment-related side effects.

Peripheral nerve stimulation (PNS) has shown promising preventive properties in episodic and chronic migraine [5]. PNS conveys its effects by the electrical stimulation of peripheral nerves branches either sub- or percutaneously with implantable devices, or transcutaneously via superficial skin electrodes linked to external neurostimulators. Due to its invasiveness percutaneous PNS like occipital nerve stimulation (ONS) was used hitherto only in the most disabled migraine patients [5-7]. Transcutaneous PNS have the advantage of being non-invasive and thus applicable also in less severely disabled subjects suffering from episodic migraine.

We have shown previously in a randomized double-blind sham-controlled trial that transcutaneous supraorbital neurostimulation (tSNS) is effective in the preventive treatment of episodic migraine (the PREMICE trial, [8]). In this study, subjects were treated with an external ultra-portable and user-friendly tSNS device stimulating both supraorbital nerves, the Cefaly® device (CEFALY Technology, Herstal, Belgium). After daily 20 minutes tSNS sessions for 3 months, the 50% responder rate was 38.2% for active tSNS vs.12.1% for sham stimulation [8]. The effect was significant, and within the range of other migraine preventive therapies. Moreover, there were no side effects or drop-outs due to device-related adverse events.

However, the number of subjects included in this trial was limited to 67 patients recruited in tertiary headache clinics. It remains therefore to be studied how tSNS with the Cefaly® device performs in larger cohorts of headache sufferers in the general population. For this purpose, we have conducted a survey of subjects who rented the device via Internet for 40 days, in order to assess safety and satisfaction of tSNS in a large cohort of more than 2000 headache sufferers.

Methods

Subjects

A prospective registry of 2,573 headache sufferers who rented the tSNS Cefaly® device (CEFALY-Technology, Liège, Belgium) was established between September 2009 and June 2012. Most subjects were French or Belgian citizens, while a minority lived in Switzerland, three countries where subjects can directly rent and buy the device via the Internet without medical prescription. The device can be rented at a cost of 49€ for 40 days, where after the patient has to decide either to return the device or to keep it and pay the balance between its cost of 295€ and the rental fee.

Transcutaneous supraorbital neurostimulation

tSNS was delivered with an external self adhesive electrode placed on the forehead (Figure 1, Cefaly® device, CEFALY Technology, Liège, Belgium). The bipolar electrode (30 mm × 94 mm) covers bilaterally the origins of the supraorbital nerves (branches of 1st trigeminal

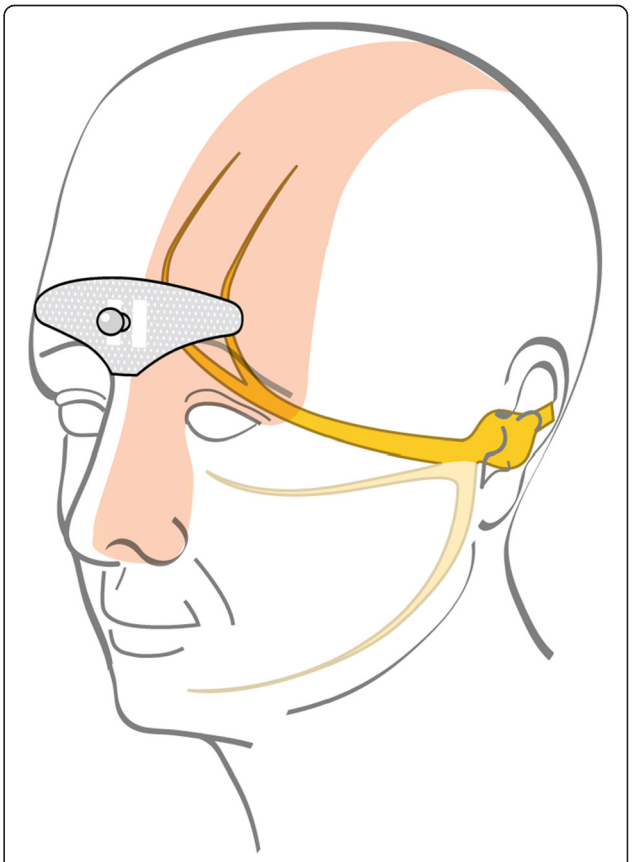

Figure 1 Area covered by the tSNS electrode.

division). A constant current generator (maximum skin impedance of 2.2 KΩ) generates biphasic rectangular impulses with an electrical mean equal to zero (preventive stimulation protocol: impulse width 250 μS, frequency 60 Hz, maximum intensity 16 mA). All subjects received an explicative leaflet advising to perform tSNS at least once daily in order to obtain a preventive antimigraine effect. As single sessions have a fixed duration of 20 minutes, the recommended minimal total time of use was 800 minutes in subjects renting the device for 40 days. A built-in electronic system allowed recording of the total time of tSNS use in subjects who returned then device to the manufacturer after the trial period.

Data collection and processing

The objectives of this survey were to record self-reported adverse events and to assess the satisfaction of subjects who received the tSNS Cefaly® device at home with its accessories for the rental period.

After the end of this rental period they were contacted to answer to the following questions:

1. Which kind of medication do you usually take to treat your headache attack?
2. Did you have side effects when using tSNS or any complaint or comment about the device?
3. Did you encounter technical issues with the device?
4. Are you satisfied with tSNS and do you want continue the treatment?

"Satisfied" subjects who wanted to keep on the treatment had to purchase the device (i.e. to pay 246€), whereas "unsatisfied" subjects sent it back by surface mail.

The devices collected from unsatisfied subjects were analyzed for the total time of tSNS use in order to estimate compliance.

A trained medical secretary was paid by the manufacturer to contact all subjects by phone or e-mail after the rental period. Phone contact was tried in the morning, at noon and in the afternoon; an e-mail was sent in case the person did not answer the phone. This was repeated for up to 2 weeks until a formal contact was achieved.

A total of 2,573 patients rented the device during the 29 months of the survey; 26 never responded to the phone calls or e-mails; 234 were not using triptans and were not included in the survey, as they were assumed not to suffer from migraine. In the three involved countries (Belgium, France and Switzerland) triptans are indeed only delivered and/or reimbursed with a medical prescription certifying that the patient has a diagnosis of migraine according to ICHD-II criteria [9].

The diagram in Figure 2 depicts the sequential steps of the survey.

According to European regulations on non-interventional studies with medical devices (CE directive 93/42 and ISO 13485) this survey did not require ethics committee approval.

Results

According to the triptan use selection criterion, 2,313 headache sufferers were included in the survey (age 14–87 years, 1641 females i.e. 70.95% and 672 males i.e. 29.05%): 1,208 (52.2%) from France, 999 (43.2%) from Belgium and 106 (4.6%) from Switzerland. The average rental period, computed from the day they received the device until they were actually contacted to answer the questions, was 58.2 ± 33.6 days.

Safety

Ninety-nine subjects reported at least one adverse event (AE) during tSNS therapy, i.e. 4.3% of all subjects. In the subgroup of unsatisfied patients the AEs rate was 5.48% (59 patients) and it was 3.24% (40 patients) in the subgroup of satisfied patients. Five patients reported more than one AE, one in the satisfied subgroup and four in the unsatisfied subgroup. Forty-six subjects, i.e. 2%, stopped tSNS because of an AE. None was serious and all were fully reversible. The most remarkable AE was a forehead skin allergy in 2 subjects (0.09%).

Table 1 is an exhaustive list of all AEs recorded.

The most frequent AE was intolerance to the paresthesia induced by the electrical stimulation (N = 31, 46% of all AEs), despite the fact that the subjects were allowed to interrupt the gradual intensity increase from 0 to 16 mA by pressing the "on" button as soon as the forehead sensation became uncomfortable. All subjects complaining of paresthesia intolerance stopped the treatment. Some other painful feelings were reported: 3 strong pressure feelings on the forehead, 2 dental and 2 cervical pains during the session. Two subjects felt paresthesia more on one side of the forehead. While the paresthesia stopped in most subjects at the end of the stimulation, 4 individuals reported that the forehead paresthesia persisted for several hours after the end of the stimulation.

Twelve subjects (0.52%) complained after the tSNS session of tension-type like headache that led to treatment interruption.

Arousal and sleep changes were the second most frequently reported AEs (19 subjects or 18.6% of all AEs). Among them, sleepiness during the stimulation was reported by 12 subjects, while 4 complained of insomnia. Three subjects (3.03%) complained of a feeling of stress during the tSNS session.

Three subjects had nausea and vomiting at the end of a session, but did not complain of headache.

Two subjects reported not being able to keep their eyes open during the stimulation. Two reported increased

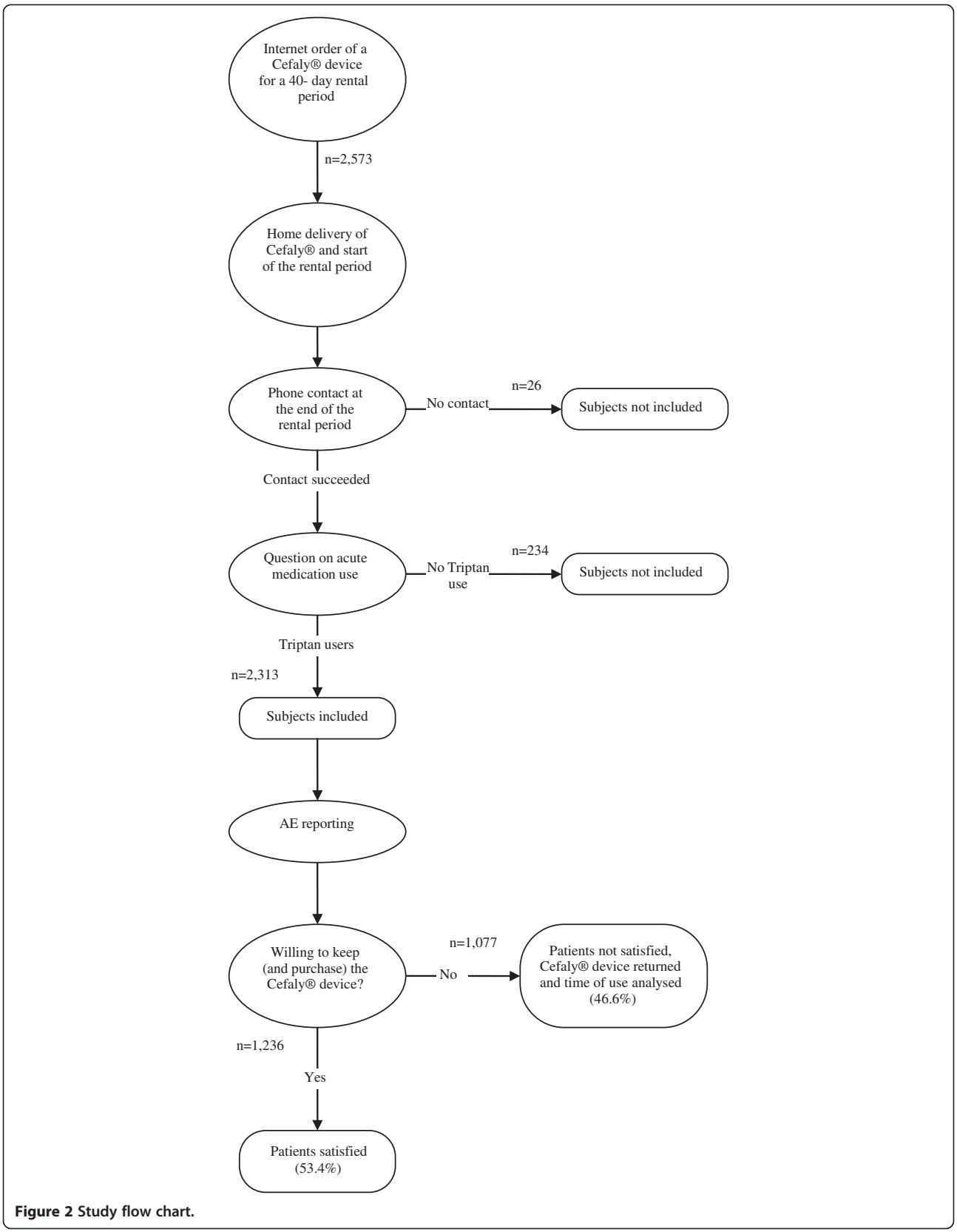

Figure 2 Study flow chart.

Table 1 AE reported by the patients within the trial period

	Number of patients	Percentage of AE	Percentage of patients
Do not like the feeling and do not want to continue using the device	29	29.29%	1.25%
Sleepiness during the Cefaly® session	12	12.12%	0.52%
Headache after a Cefaly® session	12	12.12%	0.52%
Reversible forehead skin irritation	5	5.05%	0.22%
Insomnia	4	4.04%	0.17%
Feeling of fatigue	3	3.03%	0.13%
Persistent forehead paresthesia for several minutes after the session	3	3.03%	0.13%
Feeling of stress during the session	3	3.03%	0.09%
Allergic skin reaction	2	2.02%	0.09%
Dental pain during the session or at the beginning	2	2.02%	0.09%
Inability to keep eyes open during sessions	2	2.02%	0.09%
Feeling of contusion on the forehead during a few days	2	2.02%	0.09%
Pre-existing tinnitus increased during the session	1	1.01%	0.04%
Tinnitus appearing during some sessions	1	1.01%	0.04%
Red eye after a session	1	1.01%	0.04%
Eyes weeping during a session	1	1.01%	0.04%
Wake up during night with a feeling of anxiety and tremor	1	1.01%	0.04%
Vertigo during the first session	1	1.01%	0.04%
Vomiting after a session	1	1.01%	0.04%
Forehead skin burning sensation during a session	1	1.01%	0.04%
Cervical pain during sessions	1	1.01%	0.04%
Cervical pain with nausea after the two first sessions	1	1.01%	0.04%
Short feeling of electrical shock	1	1.01%	0.04%
Slight pain at one eyebrow during the first session	1	1.01%	0.04%
Nausea and vertigo during sessions	1	1.01%	0.04%
Nausea during sessions	1	1.01%	0.04%
More head pain when using the device during a headache	1	1.01%	0.04%
Forehead and cranial anaesthesia feeling during a few hours after a session	1	1.01%	0.04%
Pressure feeling between the eyebrows during sessions	1	1.01%	0.04%
Numbness at the back of the head after a session	1	1.01%	0.04%
Stronger paresthesia feeling on the left side	1	1.01%	0.04%
Stronger paresthesia feeling on the right side	1	1.01%	0.04%
Subjective tachycardia during a session	1	1.01%	0.04%
Migraine feeling during sessions	1	1.01%	0.04%

Complaints reported by patients.

tinnitus during the session, one a red eye and another one tearing.

Five subjects (5%) complained about transient skin irritation and 2 subjects had a local cutaneous reaction, probably allergic to the electrode gel containing acrylate (2% of all AEs, and 0.09% of all subjects) (Figure 3). These patients did not report a history of allergy to adhesive tapes but one of them had previously suffered from an allergic skin reaction.

The four remaining AEs were single and mild: numbness at the back of the head, slight pain over one eyebrow, feeling of abrupt electrical variation, tachycardia during one session.

Satisfaction

Out of 2,313 subjects, 1,236 (53.4%) were satisfied with the tSNS therapy and wanted to continue the treatment. These subjects purchased the Cefaly® device. On the

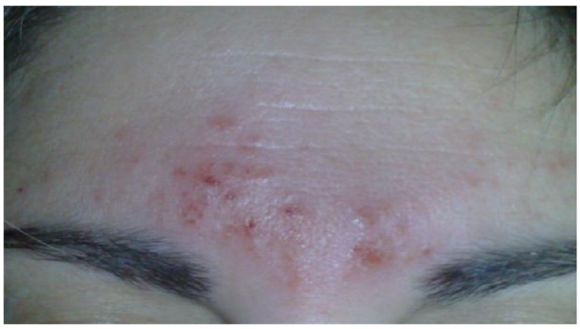

Figure 3 Allergic skin reaction.

opposite, 1,077 subjects (46.6%) were not satisfied with tSNS and sent back the device.

Compliance

The devices collected back from the 1,077 non-satisfied subjects who discontinued the therapy were analyzed for time of use (Table 2). A built-in electronic system in each device recorded the total time of use. In these 1,077 unsatisfied subjects the mean time of use was 583 ± 903 minutes, for an average rental period of 49.5 ± 26.7 days. As the recommended treatment schedule was one session of 20 minutes per day, their time of use was 58.8% of the recommended time.

Interestingly, 4.46% of "unsatisfied" subjects (N = 48, 2.08% of all subjects) did not even switch on the device, and 19.03% used it less than 60 minutes. Conversely, 40% of the discontinuers applied the tSNS for more than 400 minutes over the rental period (N = 431, 18.63% of all subjects), which was probably sufficient to achieve a therapeutic effect. If we exclude from the survey subjects who never switched on the device, i.e. who did not try the treatment at all, the percentage of satisfied subjects raises to 55.51%. Also, if one accepts that 400 minutes of treatment are necessary to obtain a treatment effect, only 18.63% of all subjects would be classified as non-responders; the compliance of the other discontinuers (N = 646) was not large enough to assess a treatment response.

Table 2 Compliance in the 1,077 unsatisfied subjects

Total time of use (minutes)	Number of subjects (percentage)*
0	48 (4,46%)
1 to 20	58 (5,39%)
21 to 40	46 (4,27%)
41 to 60	53 (4,92%)
60 to 100	78 (7,24%)
100 to 200	174 (16,16%)
200 to 400	189 (17,55%)
> 400	431 (40.02%)

*Number (and percentage) of unsatisfied subjects who used the device for the time indicated in the first column i.e. 48 "unsatisfied" subjects did not switch on the device, 58 used the device between 1 and 20 minutes, 46 between 21 and 40 minutes, etc.

Out of the 646 patients who used their device less than 400 minutes 56 reported AEs (8.64%), i.e. twice more the AE rate for all subjects. In patients who used the device at least 400 minutes the AE rate was 1.85%.

Discussion

This survey on a large cohort of 2,313 headache sufferers in the general population provides important data on tolerance and safety of tSNS with the Cefaly® device as well as some information about its performance.

First, it underscores the safety of tSNS and the low incidence of self-reported AEs (4.3% of 2,313 subjects). About half (2%) of these subjects discontinued the therapy because of an AE. In the PREMICE trial [8] the 34 subjects randomized to the effective tSNS (verum) arm reported no AE and none dropped out, which can be explained by the small number of patients. In the present survey the most frequent adverse effect was intolerance to forehead paresthesia that was perceived as painful burning sensations. As a matter of fact, paresthesia is a "normal" sensation linked to every PNS, and responsible for the difficulty in effectively blinding such studies. It is common experience, however, that a number of subjects in the general population do not tolerate the sensations induced by cutaneous electrical stimuli even at low intensities. This intolerance may be pronounced in migraine sufferers and might be related to the cutaneous allodynia that may persist in some of them between attacks [5]. Subjects reporting sleepiness confirm that tSNS can have sedative properties, as shown previously in a study of healthy volunteers [10]. Finally, the most remarkable and cumbersome AE was skin allergy under the forehead electrode (0.09%). Though very rare, such an allergic reaction is well known for self-adhesive electrodes and attributed to the acrylate component of the electrode gel [11]. It is fully reversible within 10 days after removing the electrode and can be avoided by using a newly developed hypoallergenic gel without acrylate.

Second, this survey indicates that 53.4% of subjects were satisfied with the tSNS after a trial period of on average 58 days, and decided to continue the treatment and to purchase the device. Although this is a purely subjective global assessment, patients' satisfaction could somehow parallel treatment effectiveness. In the PREMICE trial, 70.6% of episodic migraineurs were satisfied with the treatment (29.4% very, 41.2% moderately satisfied) [8]. The global rate of satisfaction is lower in the present survey, but one has to take into account that subjects had to pay 246€, i.e. the difference between the full price and the rental cost, to purchase the Cefaly® to keep the device for treatment continuation, and that the

subject population is far more heterogeneous. The compliance to tSNS therapy was 58.8% in subjects who discontinued the treatment while in the PREMICE study it was 61.7% in the total group of patients. This slight difference can be due to the fact that only the devices of unsatisfied subjects could be analyzed for time of use. Moreover, patients included in the PREMICE trial were recruited by established headache specialists and thus well educated in headache management including the use of headache diaries while the majority of subjects included in the present survey had no regular neurological follow-up.

If we exclude from the analysis those 48 unsatisfied subjects who never used the device, the rate of satisfied subjects raises to 55.5%. It is likely that non-satisfied subjects who used the device for less than 400 minutes (N = 646, 27.9% of the 2,313 subjects and 59.98% of the unsatisfied subjects) were not sufficiently dosed to expect a therapeutic effect, although they may have experienced adverse effects. Conversely, those 40% of unsatisfied subjects (18.6% of the 2,313 subjects) who applied tSNS for more than 400 minutes, i.e. for a potentially effective duration, are most probably genuine non-responders.

The survey presented here has several weaknesses. The major one is that we have no certainty about the precise diagnosis of included subjects, which is the reason why we have focused our analysis on safety and tolerance. We assume that a majority of them probably suffered from migraine because they were using triptans for the treatment of headache attacks. In the three involved countries (Belgium, France and Switzerland) triptans are not available over-the counter, but delivered and reimbursed only with a medical prescription certifying that the patient suffers from migraine according to ICHD-II criteria [9]. Triptan users are thus most likely to have been diagnosed as migraineurs by a general practitioner and/or a neurologist. Whether they suffer from episodic or chronic migraine, from migraine with or without aura cannot be determined in our survey. Possible diagnostic confounders are misdiagnosed headache, tension-type headache, medication overuse headache and cluster headache.

Other weaknesses are the absent control for concurrent drug treatment and for natural history of the headache disorder, as well as the outcome parameter and the time point at which it was assessed. As mentioned above, patients' satisfaction, the only parameter available here, is a composite subjective outcome measure combining efficacy, tolerance, adverse effects, expectations and, in this case, willingness to pay. It is not a recommended primary measure of efficacy, like the number of headache days, and it does not necessarily parallel a reduction in headache frequency. Despite its shortcomings, however, patients' satisfaction is considered to be valuable in pragmatic trials such as ours, according to the IHS guidelines for controlled trials of drugs in migraine [12]. The time point of about 60 days of tSNS at which the subjects' satisfaction was assessed may not be optimal. In the PREMICE trial the treatment period was 3 months and the reduction in migraine day frequency was maximal at the end of the 3rd month [8]. The tSNS efficiency may thus be underestimated in our survey, though this would probably concern only a minority of subjects, since the therapeutic advantage over sham stimulation was already significant at the end of the 2nd month of treatment in the PREMICE trial [8]. Finally, we cannot exclude the possibility that some individuals in whom the device was effective did not purchase it for financial reasons, which would have led to an overestimation of the proportion of non-satisfied subjects.

Because of these shortcomings no definitive conclusion about therapeutic efficacy can be drawn from this survey.

Conclusions

This survey of 2,313 headache subjects treated with tSNS is to the best of our knowledge the largest database available for a neuromodulation treatment in headache. Its major contribution is to confirm the safety and excellent tolerance of tSNS therapy with the Cefaly® device. Adverse events were reported by only 4.3% of subjects and they were all minor and reversible. The most frequent AE was intolerance to the local paresthesia, which is a common, though rare, reason for treatment interruption in every PNS therapy. About 2% of subjects stopped the tSNS therapy because of an AE, which is remarkably low compared to preventive anti-migraine drugs [3]. Although this survey does not allow reliable deductions about efficacy for methodological reasons, it provides some clinically useful indications about patients' satisfaction and compliance. Among the 2,313 subjects, 53.4% were satisfied with the treatment and the device, and decided to buy it. The mean time of tSNS use in those subjects who discontinued the therapy was 58.8% of the recommended time; 4.46% of "unsatisfied" subjects did not even switch on the device, and 19.03% used it for less than 60 minutes. Hence, low compliance to tSNS is an issue that might explain lack of efficacy in a number of subjects.

Abbreviations

AE: Adverse event; ICHD-II: International classification of headache disorders – 2nd edition; PNS: Peripheral nerve stimulation; PREMICE: PREvention of MIgraine by supraorbital transcutaneous neurostimulation using the CEfaly device; tSNS: Transcutaneous supraorbital neurostimulation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SS, TSD and RB participated in data collection, management and analysis. DM analysed the final data. DM and JS wrote the manuscript. All authors read and approved the final manuscript.

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Transcranial Direct Current Stimulation (tDCS) of the visual cortex: a proof-of-concept study based on interictal electrophysiological abnormalities in migraine

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Abstract

Background: Preventive pharmacotherapy for migraine is not satisfactory because of the low efficacy/tolerability ratio of many available drugs. Novel and more efficient preventive strategies are therefore warranted. Abnormal excitability of cortical areas appears to play a pivotal role in migraine pathophysiology. Transcranial direct current stimulation (tDCS) is a non-invasive and safe technique that is able to durably modulate the activity of the underlying cerebral cortex, and is being tested in various medical indications. The results of small open studies using tDCS in migraine prophylaxis are conflicting, possibly because the optimal stimulation settings and the brain targets were not well chosen. We have previously shown that the cerebral cortex, especially the visual cortex, is hyperresponsive in migraine patients between attacks and provided evidence from evoked potential studies that this is due to a decreased cortical preactivation level. If one accepts this concept, anodal tDCS over the visual cortex may have therapeutic potentials in migraine prevention, as it is able to increase neuronal firing.

Objective: To study the effects of anodal tDCS on visual cortex activity in healthy volunteers (HV) and episodic migraine without aura patients (MoA), and its potentials for migraine prevention.

Methods: We recorded pattern-reversal visual evoked potentials (VEP) before and after a 15-min session of anodal tDCS over the visual cortex in 11 HV and 13 MoA interictally. Then 10 MoA patients reporting at least 4 attacks/month subsequently participated in a therapeutic study, and received 2 similar sessions of tDCS per week for 8 weeks as migraine preventive therapy.

Results: In HV as well as in MoA, anodal tDCS transiently increased habituation of the VEP N1P1 component. VEP amplitudes were not modified by tDCS. Preventive treatment with anodal tDCS turned out to be beneficial in MoA: migraine attack frequency, migraine days, attack duration and acute medication intake significantly decreased during the treatment period compared to pre-treatment baseline (all $p < 0.05$), and this benefit persisted on average 4.8 weeks after the end of tDCS.

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Conclusions: Anodal tDCS over the visual cortex is thus able to increase habituation to repetitive visual stimuli in healthy volunteers and in episodic migraineurs, who on average lack habituation interictally. Moreover, 2 weekly sessions of anodal tDCS had a significant preventive anti- migraine effect, proofing the concept that the low preactivation level of the visual cortex in migraine patients can be corrected by an activating neurostimulation. The therapeutic results indicate that a larger sham-controlled trial using the same tDCS protocol is worthwhile.

Keywords: Migraine, Habituation deficit, tDCS, Treatment, Visual cortex

Background

Finding the 'right' migraine preventive treatment often remains a challenge in many patients. The drugs currently used in migraine prophylaxis (such as antiepileptics, beta blockers. . .) are not migraine-specific, unlike acute therapies like triptans or gepans, which were designed to treat headache. Moreover, they are not devoid of side-effects and their efficacy rarely exceeds 50-60% for the best of them [1]. Chronic migraine patients, i.e. the presence of at least 15 days of headache per month, of which at least 8 migraine attacks, represent almost the 2-3% of the population and they are particularly difficult to manage as their response to existing preventive therapies is often unsatisfactory [2,3]. There is thus a need for new effective and well-tolerated treatments in migraine prophylaxis. The latter should ideally be more disease-specific, i.e. designed to counteract the dysfunctions known to be involved in migraine pathogenesis.

Migraine is a complex and heterogeneous disorder, in which genetics and environment interact to generate dysfunctioning paths and loops at several levels of the central nervous system. These intricate phenomena are responsible for the multifaceted clinical features of the disease and especially its dynamics characterized by a cyclic ictal-interictal pattern and the repetition of attacks [4].

It has been known for a long time that the brain excitability is abnormal in migraine during the interictal period [5]. However many past studies on cortical excitability had provided conflicting results, and whether the brain was hyperexcitable [6-8] or hypoexcitable [9-12] remained extensively debated for years. A recent theory proposed a semantic modification that was able to unify these opposite hypotheses, i.e. that the brain cortex was not hyper*excitable* per se but hyper*responsive* to sensory stimuli in migraine between attacks [13]. A reproducible hallmark mirroring this hyperresponsiveness is the lack of habituation to repeated sensory or cognitive stimulations reported in both evoked potentials and neuroimaging trials (for review, see [14]). Habituation is defined as a behavioural response decrement that results from repeated stimulations and does not involve sensory adaptation or fatigue, i.e. a decrease in peripheral receptor activity [15]. According to Groves and Thompson, habituation relies on the balance of two opposite mechanisms, facilitation and depression of brain responses to a sensory stimulus.

In the interictal period of migraine, many evoked potentials studies to various sensory modalities found on average lower initial response amplitudes followed by a decreased habituation -or even a potentiation- of subsequent responses, whereas in healthy subjects a higher initial response preceded a more pronounced habituation. These results paved the way to the hypothesis that the lack of habituation was possibly due to a lower preactivation level of brain sensory cortices, according to the ceiling theory [16]. Recent studies suggested that this lower preactivation level could be the consequence of impaired functional thalamocortical loops, the so-called Thalamocortical Dysrhythmia, a dysfunction, which is also involved in other neurological diseases [17,18]. Further works demonstrated that the lack of habituation was not constant and normalized just before and during the migraine attack. Interestingly, it was recently shown that in chronic migraine patients habituation was normal [19] but evolved to potentiation when these chronic migraineurs went to remission towards episodic migraine [20], suggesting that chronic migraine could be considered as a "never-ending attack" [21].

In the last decade there has been an increasing interest for neuromodulation in migraine treatment [22]. Even if randomized controlled trials are scarce, some preliminary results are encouraging and peripheral and central neuromodulating techniques are considered as promising alternatives to pharmacological treatment. Among them, 2 central non-invasive techniques appear particularly suitable for migraine preventive treatment: repetitive transcranial magnetic stimulation (rTMS) and trancranial Direct Current Stimulation (tDCS). Both are able to durably modify the excitability of the underlying cortex and could potentially correct the functional abnormalities found in migraine patients. They were already applied in several other neurological diseases with some success [23]. High frequency (around 10 Hz) rTMS stimulation can increase brain excitability, while low frequency rTMS (about 1 Hz) is able to decrease it [23,24]. Anodal tDCS appears to increase brain excitability, while cathodal tDCS stimulation decreases it [23,25-27] though not all studies agreed on this point [28].

Few recent therapeutic trials applied rTMS and tDCS in migraine prevention, and their results were conflicting [29-31]. This could be due to dissimilarities in their stimulation protocols, as stimulated brain regions as well as stimulation frequencies, length and intensities were different and depended on the baseline pathophysiological hypothesis, mainly the belief that the migrainous brain was hyperexcitable or, on the contrary, hypoexcitable. Moreover, these trials did not assess the brain excitability before and after treatment. In a previous study, we had reported that a single 10 Hz excitatory rTMS session was able to restore normal habituation and initial amplitude of visual-evoked responses (VEPs) in migraineurs, and that this effect lasted at least 9 minutes. In a subsequent trial, this stimulation was applied on 5 successive days, but the VEPs normalization did not exceed several hours in most migraineurs. However, these results had not been applied in a preventative therapeutic study for now, and whether the normalization of habituation was associated to a clinical improvement remained unknown [12,32].

We therefore performed a pilot proof-of-concept study combining the two approaches for the first time, but we used anodal (i.e. excitatory) tDCS instead of 10 Hz-rTMS. This was a 2-step trial: we first repeated the electrophysiological study in healthy volunteers and migraineurs in order to ensure that anodal tDCS could modulate habituation and correct the impaired interictal excitability in migraineurs like rTMS, then in the second phase the same stimulation paradigm was converted into a preventive therapy for episodic migraine in a prospective pilot trial.

Methods

1. Subjects and clinical records

Eleven healthy volunteers (HV) were enrolled for the electrophysiological study (5 males and 6 females, mean age 25.8 ± SD 5.7 years). Exclusion criteria were: age below 18 or above 65 years, a personal history of recurrent headache or other neurological diseases especially seizures, familial history of recurrent headache, child migraine equivalents (motion sickness, cyclic vomiting or recurrent abdominal pain, somnambulism etc...), chronic pain syndromes, analgesics intake at the time of recording, and contra-indications to tDCS neurostimulation (metal prosthetics in the head or internal stimulation like a pacemaker). They were compared to 13 migraineurs without aura (MoA) according to the second International Classification of Headache Disorders (ICHD-IIR) criteria (2 males and 11 females, mean age 29.3 ± 5.1). Patients had more than 2 and less than 8 attacks/month and were not under preventive therapy for at least 3 months before the experimental day. All volunteers and patients were naive to any kind of neurostimulation, i.e. they never got this type of treatment before (central or peripheral neurostimulation), whatever the indication was. Patients were recruited in the outpatient clinic through headache-specialized consultations (DM and JS).

The therapeutic study involved 10 migraineurs suffering from episodic MoA (2 males and 8 females, mean age 38.4 ± 16.3) with a frequency ranging between 3 and 8 attacks/month, knowing that none of them fulfilled the criteria for chronic migraine. Only two of them were previously involved in the electrophysiological study. Intake of a drug preventive treatment was allowed in the therapeutic study only, but this pharmacological therapy had to be stable for at least 2 months. Five out of the 10 enrolled patients were under preventive therapy at the moment of the trial: one was taking riboflavin alone, two riboflavin associated with a beta-blocker (metoprolol or propranolol), the other two were under topiramate. All of them had treatment for several months and this treatment did not give them any satisfaction. The average time under prophylactic therapy at inclusion was 3.2 months (2 patients were under preventive therapy for 2 months, the other 3 for 4 months).

During the whole therapeutic study period the patients were asked to fill a headache diary to record migraine attacks, migraine and headache days, pain intensity in a scale from 1 (light) to 3 (severe), duration of attack (hours), medication intake, and associated symptoms (nausea, vomiting, photo- and phonophobia). This headache diary had to be completed at least 2 months before the treatment initiation, in order to have a 2-month pretreatment baseline.

All subjects participating in the electrophysiological and/or the therapeutic studies received detailed oral and written explanations of the whole experiment provided by the experimenter (AV or TSD) and gave written informed consent. This study was approved by the local Ethics Committee of the CHR Citadelle Hospital of Liège, Belgium.

2. Material and stimulation protocols

Electrophysiological study

For the electrophysiological study we recorder pattern reversal visual evoked potentials (PR-VEPs), as described before [33]. PR-VEPs were selected as they are one of the best studied electrophysiological responses in migraine, where a decreased preactivation level and a lack of habituation has been reported in many studies [34]. Briefly, subjects sat in a comfortable armchair in a quite dark room at a +/− 90 cm distance from the monitor. They were asked to relax and to fix a red sticker in the centre of the screen (Nicolet™; 24 × 18 cm) with their right eye, the left eye being covered by a patch. The visual stimulus was a checkerboard pattern of black and white squares (15 mm

side, 80% contrast, mean luminance 250 cd/m^2, colour temperature 9500 K) alternating at a frequency of 3.1 Hz. Pin-electrodes were used to record the signal: the active electrode was inserted at Oz and was referenced to Fz according to the 10–20 system [34]. The ground electrode was fixed to the right forearm. During uninterrupted stimulation, 600 cortical responses were recorded (CED™ 1902 preamplifier and CED™ Micro1401 converter; Cambridge Electronic Design Ltd, Cambridge, UK). Two hundred and fifty milliseconds of the poststimulus period were sampled at a rate of 4000 Hz.

Acquisitions were made at baseline (T0), immediately after (T1) and 3 hours after (T2) a single anodal tDCS session (see below). At the end of the first VEPs recording (T0), the place of the pin electrodes was marked with a pen, in order to ensure that their locations remained the same in the subsequent recordings (T1 and T2). Hence, after T1 the subjects had a 3-hour free time before coming back to the laboratory for T2 acquisition. During this period, they were not allowed to smoke, to drink alcohol or beverages containing caffeine or other energy drinks, and to take a nap. All recordings were distant from at least 72 hours of a migraine attack. The time of the last attack was checked on patient's diary and the absence of an attack occurrence within the next 72 hours after the experiment was checked by phone call. To avoid changes of cortical excitability due to hormonal variations, all female subjects performed the experiment in the first half of the menstrual cycle.

Anodal tDCS

Anodal tDCS stimulation was performed using a programmable DC stimulator (NeuroConn, Ilmeanu, Germany©) with 2 rubber electrodes (5x7cm). The anode was placed in the occipital region near Oz in order to stimulate the underlying visual cortex, and the cathode was fixed on the chin. We chose to put the cathode outside the cranial vault in order to avoid a concomitant inhibition of other cerebral cortices, for example the frontal cortex when Fz had been chosen as cathode. The subjects were stimulated at 1 mA intensity and each session lasted 15 minutes. To decrease their possible discomfort the stimulation increased gradually during the first 8 seconds and decreased progressively within the last 8 seconds of the tDCS.

Thus, the electrophysiological study comprised a single tDCS session and in the therapeutic pilot study anodal tDCS was applied twice a week for 8 weeks, i.e. 16 sessions, using the same tDCS parameters. The 2 weekly sessions were fixed, i.e. were always applied the same days during the whole treatment period of a single patient (for example, every Tuesday and Friday).

3. Data analysis and statistics.

In the electrophysiological study, the 600 PR-VEP responses were averaged off-line into six blocks of 100 responses using Signal™ software version 4 (Cambridge Electronic Design Ltd, bandpass 1–100 Hz). The peak-to-peak N1–P1 and P1-N2 amplitudes were measured, N1 being the most negative point around 70 ms latency after the stimulus (range 60–90), P1 the most positive around 100 ms latency (range 80–130) and N2 the most negative point following P1 between 90 and 200 ms. To visualize better the slope of N1P1 and P1N2 amplitude changes over the total duration of visual stimulation, a linear regression analysis of the mean amplitudes in the 6 blocks of 100 averages responses was performed and considered as the reflect of habituation degree (see Figure 1). Hence, a normal habituation gave a negative slope value, while potentiation gave a positive slope. We calculated means and standard deviations for the first block amplitude (first 100 averaged N1P1 VEP responses, ìV, which reflects cortical preactivation level – see above introduction) and N1P1 and P1N2 habituation slopes, at T0, T1 and T2, and compared them between HV and MoA.

In the therapeutic study we followed prospectively the evolution of migraine attack frequency, migraine days, mean pain intensity, attack duration and acute drugs intake during treatment with tDCS, compared to the baseline. We compared baseline clinical variables (2nd month) with those of the 2nd month of tDCS treatment, to study the cumulative effect of the repeated stimulation.

Statistical calculations were carried out using STATISTICA (version 7, StatSoft, Oklahoma, USA).

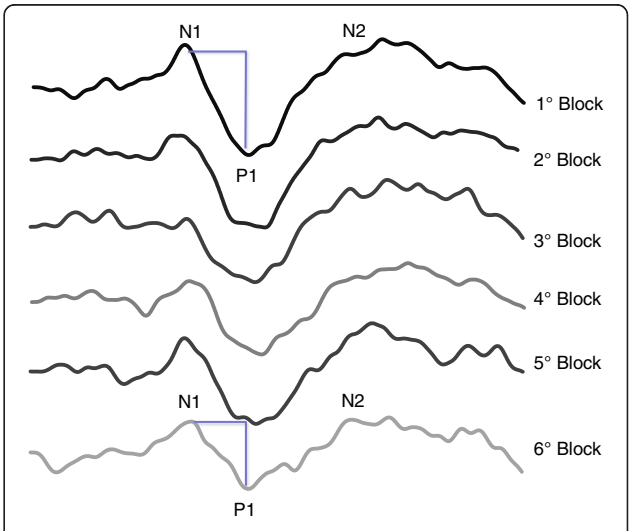

Figure 1 The time-dependent changes of N1P1 and P1N2 components of visual evoked potentials in a healthy subject. Over six blocks of 100 averaged single trial responses a reduction in amplitude of both components is shown, in the representative example.

We first used the Shapiro-Wilk test to assess the distribution of the variables. Since most of them did not fit the normal distribution, we used Wilcoxon signed-rank test (paired samples) to study modifications induced over time by tDCS within the same subjects, and we employed Whitney–Mann U-test to compare electrophysiological values between HV and MoA groups. The time-dependent changes in habituation were assessed with one-way analysis of variance (ANOVA) for repeated measures. We also did a post-hoc comparison with Wilcoxon signed-rank test. All results were considered significant at $p < 0.05$.

Results

Electrophysiological study

The results of the electrophysiological study are presented in Table 1 and Figure 2.

In baseline (T0), HV and MoA did not differ in first PR-VEP block amplitude, nor in N1P1 habituation slopes ($p > 0.05$). However, P1N2 habituation slope was significantly deeper in HV than in MoA (−0.23 in HV vs. -0.05 in MoA; $p = 0.04$), which mirrors a lack of habituation in MoA compared to HV.

In the HV group, anodal tDCS stimulation had no effect on PR-VEP first block amplitude (N1P1: 6.1 μV ±2.0 at T0 vs. 6.8 μV ±2.6 at T1; $p = 0.45$; P1N2: 6.6 μV ±2.1 at T0 vs. 6.5 μV ±2.0 at T1; $p = 0.49$), and did not modify the amplitude of subsequent blocks (Table 1). However, the habituation slope of N1P1 amplitude became more negative after tDCS stimulation, i.e. tDCS was able to strengthen habituation in HV at T1 ($p = 0.024$, Figure 2 Panel A) but this change in habituation did not persist after 3 hours (T2) where it returned on average to baseline values.

In the MoA group, anodal tDCS did not induce any significant effect on VEP amplitudes as well (Table 1). However, like in HV, N1P1 and P1N2 habituations increased immediately after anodal tDCS (T1), and for N1P1 slope this change was significant (−0.11 to −0.24 after tDCS, $p = 0.04$, Figure 2 Panel C), meaning that tDCS was also able to increase the habituation level in MoA. These changes did not last for a long time and returned to baseline at T2 as well.

Therapeutic study

The results of the pilot therapeutic study with anodal tDCS in MoA are presented in Figure 3 and are encouraging.

Hence, during the 8 weeks of anodal tDCS treatment, there was already on average a significant reduction of migraine frequency, which was decreased from 9.6 days in 2 months to 6.3 (34%,$p = 0.005$), while there was a remarkable reduction in the number of migraine days from 15 to 8 (47%, $p = 0.01$). The average cumulative attack duration over 2 months decreased from 184 to 119 hours (35%, $p = 0.043$), and the average acute treatment intake dropped from 18 tablets to 13 in two months ($p = 0.041$). The duration of each attack slightly decreased as well, but in a non-significant manner ($p = 0.70$).

We performed a further subanalysis where we only considered the outcome within the last 4 weeks of tDCS, which was compared to the baseline diary of the month preceding tDCS application, on the assumption that the clinical effect would improve with the repetition of tDCS sessions. Migraine frequency reduction was more pronounced during the second month of therapy, with a mean decrease from 5 to 3 attacks (−38%; $p = 0.03$), the number of migraine days also decreased from 8 to 4.3 (48%, $p = 0.002$), and noteworthy the average attack duration dropped from 88.5 to 33.2 (60%, $p = 0.02$). The drug intake tended to decrease from 9 pills/month to 6 pills/month (28%, $p = 0.06$).

To rule out a pure long-term pharmacological effect of the ongoing preventive therapy we then compared patients with (N = 5) and without (N = 5) migraine preventive treatment. The evolution under tDCS treatment was similar in both groups: patients without drug

Table 1 This table shows the results of the electrophysiological study: Pattern Reversal-VEP initial amplitudes (N1P1 and P1N2, μV), and habituation slopes in healthy volunteers (HV) and episodic migraineurs (MoA), before, just after and 3 h after anodal tDCS

Groups and VEP comparison	First block amplitude (μV)			Habituation slope (over six blocks)		
	Before	After	+ 3h	Before	After	+ 3h
Healthy volunteers						
(n=11)						
N1P1	6.1±2.0	6.8±2.6	6.3±2.2	-0.07±0.14	-0.21±0.14*	-0.08±0.14
P1N2	6.6±2.1	6.5±2.0	6.0±1.6	-0.18±0.19	-0.14±0.16	-0.12±0.25
Episodic migraineurs						
(n=13)						
N1P1	7.1±2.9	7.3±3.1	7.2±2.7	-0.10±0.11	-0.24±0.18*	-0.11±0.17
P1N2	6.6±2.6	6.4±2.9	6.8±2.5	-0.01±0.21	-0.17±0.24	-0.07±0.21

The * mark corresponds to a significant change ($p < 0.05$).

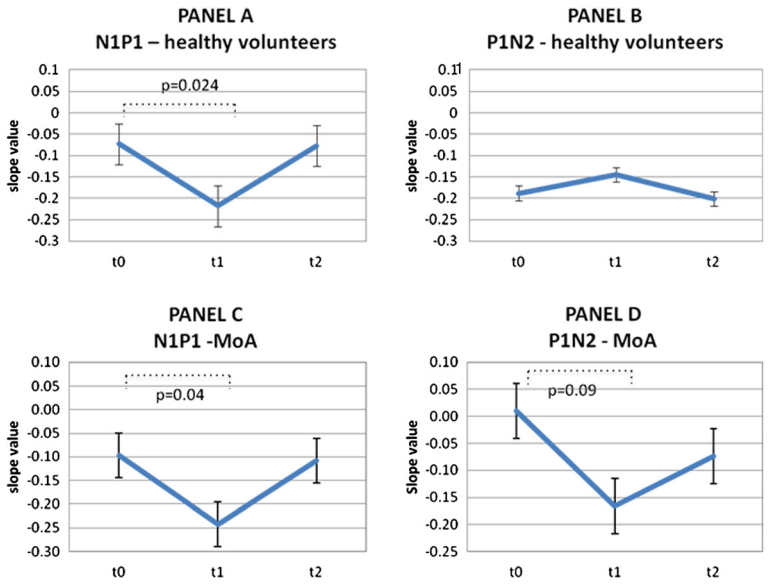

Figure 2 Time-dependent changes of habituation slope after anodal tDCS. From the up to the bottom of the table the changes on habituation slopes induced by anodal tDCS on N1P1 and P1N2 in healthy volunteers (HV, Panel **A** and Panel **B**) and episodic migraine patients (MoA, Panel **C** and Panel **D**) at T0, T1 and T2. The habituation value is expressed as the decrement of the response with stimulus repetition so a more negative value of the slope corresponds to a stronger habituation. The value of the slope at T0, T1, T2 was reported at every time point as it is obtained by the interpolation of mean values in all blocks by linear regression equation. The x axis corresponds to the time (T0 = baseline; T1 = immediately after the stimulation; T2 = after 3 hours).

therapy had a frequency reduction from 9.2 ±1.64 to 5.4 ± 2.19 attacks in two months ($p = 0.04$), while in patients under preventive therapy the frequency decreased from 10 ± 1.4 to 7.2 ± 1.8 attacks in two months ($p = 0.04$). No inter-group difference was found ($p = 0.45$). In addition, when we compared the sustained post-treatment benefit, we found no difference between the 2 groups in terms of attack recurrence: the group without any drug preventive therapy returned to the baseline migraine frequency 4.2 ± 3.8 weeks after the end of tDCS, while the group under prophylaxis returned to baseline migraine frequency after 5.4 ± 3.7 weeks ($p = 0.62$). Hence, a delayed effect due to the drug preventive treatment seems unlikely.

Adverse events

No adverse events were reported by patients, neither in the electrophysiological nor in the therapeutical tDCS study, but a light itching sensation that invariantly disappeared in few minutes after the end of stimulation.

Discussion

As we said before, the lack of cortical habituation to repetitive sensory stimuli is the more reproducible electrophysiological hallmark of the migrainous brain when recordings are made interictally. As far as we know, this is the first study using excitatory tDCS in order to modify habituation, especially to normalize it in migraineurs, and trying afterwards to translate these findings into a new kind of preventive therapy.

Electrophysiological study

The results of our electrophysiological study are in line with those found previously with rTMS, where an excitatory 10 Hz stimulation was able to increase the initial lower VEP response and restore normal habituation in migraineurs [12]. The latter supported the idea that the habituation deficit could be due to a lower preactivation level of the brain cortex, and suggested that transcutaneous central neurostimulation could have therapeutic potentials in migraine.

We chose to perform anodal, i.e. “excitatory” tDCS along the same line, in order to increase visual cortex preactivation and subsequently correct the lack of habituation in migraineurs. However we did not find any enhancement of the VEP initial amplitude, neither in healthy subjects nor in migraineurs, but surprisingly tDCS increased habituation of the second component of the VEP in both groups. Like in the rTMS [12] the duration of tDCS effect on habituation was brief and VEP recordings performed after 3 hours (T2) demonstrated that habituation slopes had come back to baseline values. The significant increase of habituation in absence of any initial amplitude modification, i.e. any cortical preactivation level enhancement with tDCS, is difficult to explain. It could be attributed to the different mechanisms of action of tDCS

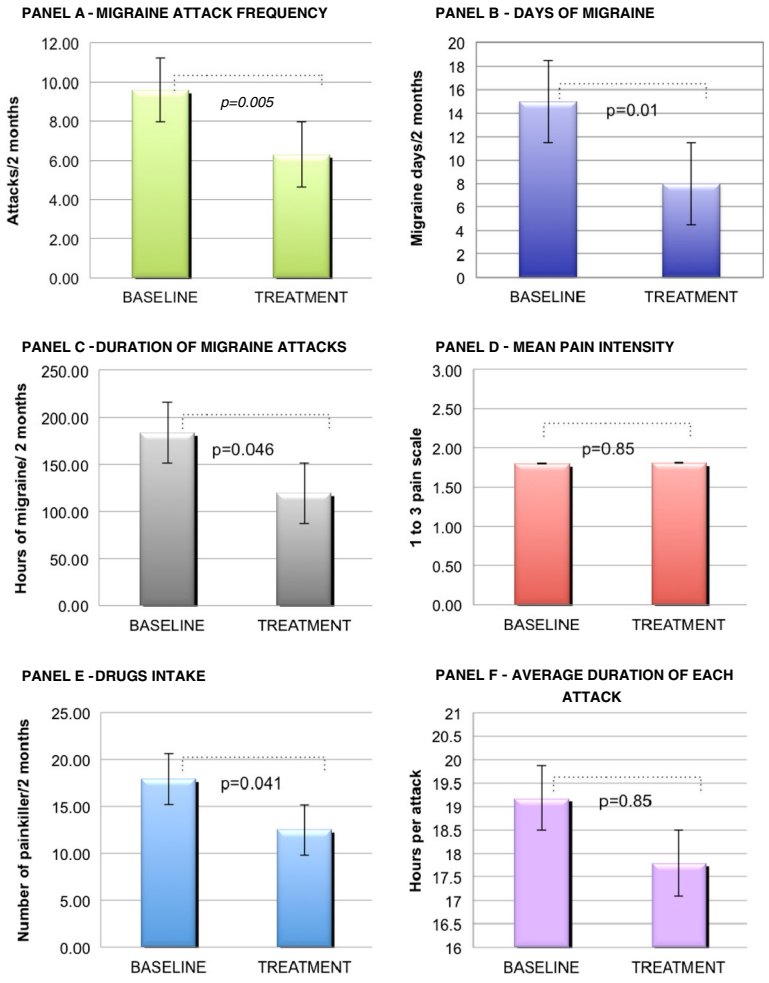

Figure 3 This figure shows the outcome of the therapeutic pilot trial. The averages and standard deviations (black lines) of the following clinical parameters are displayed at baseline and for the whole period of tDCS treatment: migraine frequency (Panel **A**), days with migraine (Panel **B**), cumulated duration of all headache attacks (Panel **C**), pain intensity per attack (Panel **D**), acute drug intake (Panel **E**) and duration of each single attack (Panel **F**).

and rTMS [23]. Moreover, some authors suggest that the cortical dysexcitability found in migraine could also be related to abnormal inhibitory circuits within the cortex, and that an impaired habituation does not necessary requires a lower preactivation level [35].

The relationship between the electrophysiological abnormalities and the patient clinical state is still obscure and complex; and whether the normalization of electrophysiological responses with neuromodulation could lead to a concomitant significant clinical improvement in migraineurs remains debated. Hence, we had shown a while ago that effective prophylaxis with betablockers was correlated to an average normalization of auditory evoked potentials (AEP), but not effective riboflavine therapy, which did not modify AEP, suggesting 2 distinct mechanisms [36]. In another study we had found similar electrophysiological abnormalities in healthy volunteers with a familial history of migraine, although they did not have any headache themselves at the time of the recordings [37]. A recent publication found that topiramate [38], one of the most effective drugs in migraine prevention, was able to normalize habituation in these patients. At baseline, episodic migraineurs showed a significant lack of habituation, which disappeared after 2 months of treatment with topiramate, and the individual improvement of habituation was positively correlated with the clinical benefit.

This underlined the need for a proof-of-concept clinical trial using a central neuromodulation technique able to normalize habituation, such as anodal tDCS.

Therapeutic study

The results of our pilot trial with anodal tDCS in only 10 MoA patients are encouraging and most clinical variables already significantly improved within 8 weeks of

treatment. Migraine frequency, migraine days, painkillers intake and attack duration decreased, and this improvement was even stronger in the second month of treatment (except for acute medications), which underlines that anodal tDCS preventive therapy sessions should be continued on a regular basis for at least 2 months, like drug prophylaxis or other non-invasive neurostimulation techniques, for example supraorbital nerve stimulation [39]. Migraine days and attack duration exhibited the strongest average improvement with respectively 48% and 60% reduction. However, we are aware that our study has some shortcomings. A placebo effect cannot be ruled out without a randomized controlled trial. Moreover, some patients might have a long-term response to drug prophylaxis, but the comparison between treated and untreated patients could argue against this hypothesis (both responded similarly to tDCS), as well as the attack recurrence observed in most patients after the end of tDCS, within a variable time interval. Finally, the improvement of patients under long-term tDCS therapy contrasts with the results of the electrophysiological study, where one single tDCS session over the visual cortex only induced a very short-term habituation modification (<3 h). However, the repetition of tDCS sessions over 8 weeks could have been responsible for neuroplastic changes and induce sustained modifications within the underlying visual cortex. Unfortunately, we did not record VEPs before and after the 8 weeks of tDCS therapy. These measures could be worthwhile in a next study.

In a pathophysiological point of view, these results emphasize that the lack of habituation is probably playing a key role in the genesis of migraine headache, even if other pathological mechanisms may also be involved.

There are few existing trials on migraine prevention using central non-invasive neurostimulation methods, i.e. rTMS or tDCS, and their stimulation paradigms differed according to the author's baseline pathophysiological hypotheses. Thus, in order to correct an eventual cortical hyperexcitability, Teepker et al. [30] and Antal et al. [31] applied inhibitory stimulations, respectively 1Hz-rTMS and cathodal tDCS over the vertex and the visual cortex, leading to minor or negative clinical results. This could eventually be due to an incorrect baseline assumption.

Chronic migraine management is often challenging and thus non-invasive neurostimulation could offer a new hope to these patients. The patients included in our clinical study did not fulfill the criteria for chronic migraine, and we stress that excitatory stimulations paradigms could even be counterproductive in these patients. Even if the excitatory 10 Hz-stimulation of the dorsolateral prefrontal cortex (DLPFC), known for its implication in pain control [40], was able to slightly improve chronic migraine patients [29], these results were also uncontrolled and there was a comorbid state of depression which might have been a major confounding factor. Hence, beyond depression, chronic migraine seems to differ from episodic migraine in terms of brain excitability. While habituation deficit is a hallmark of the disease in episodic migraine, in chronic migraine, surprisingly, habituation does not differ from control subjects [19]. Recent works suggest that in chronic migraine, the cerebral cortical excitability increases as the activity of cortical inhibitory interneurons decreases, which finally leads to a normal habituation, at least in visual areas (for details, see [41]). When the same chronic patients are successfully treated and evolve to episodic migraine, the lack of habituation reappears. These data support the idea that chronic migraine could be a "never-ending attack [20,21]. Thus, we believe that chronic migraine should paradoxically be treated using inhibitory stimulations unlike episodic migraine and that excitatory stimulations, like anodal tDCS reported in the present study, could be ineffective or even worsen these patients. More neurostimulation studies are warranted to confirm this assumption.

Conclusion

This study demonstrates for the first time that a 15-min session of anodal tDCS over the visual cortex is able to transiently increase habituation in healthy volunteers but also in episodic migraineurs. Its mechanism of action does not seem to involve cortical preactivation modifications as the initial amplitude of the visual evoked potentials is not modified.

The same excitatory paradigm applied twice a week during 8 weeks as preventive therapy in 10 episodic migraineurs results in a significant reduction of migraine attack frequency, migraine days, painkiller intake and attack duration. All positive effects seem to improve with time, suggesting that preventive therapy with anodal tDCS should be performed on a regular basis, and could involve additional slow neuromodulating processes.

These encouraging results need to be confirmed in a well-designed randomized controlled trial.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

AV has participated in the study design, has recorded patients, has analyzed the results and has drafted the manuscript. TSD has participated in the recordings of patients and the analysis of the results. SS has participated in the discussion of the results. MA helped AV for the recordings of patients. VDP has participated in the study design and the recordings of patients. AC and VDPi are senior supervisors of AV at the Sapienza University. JS has participated in the design of the study, results discussion and manuscript revision. DM has participated in the design of the study, patients recruitment, results discussion, manuscript drafting and revision. All authors read and approved the final manuscript.

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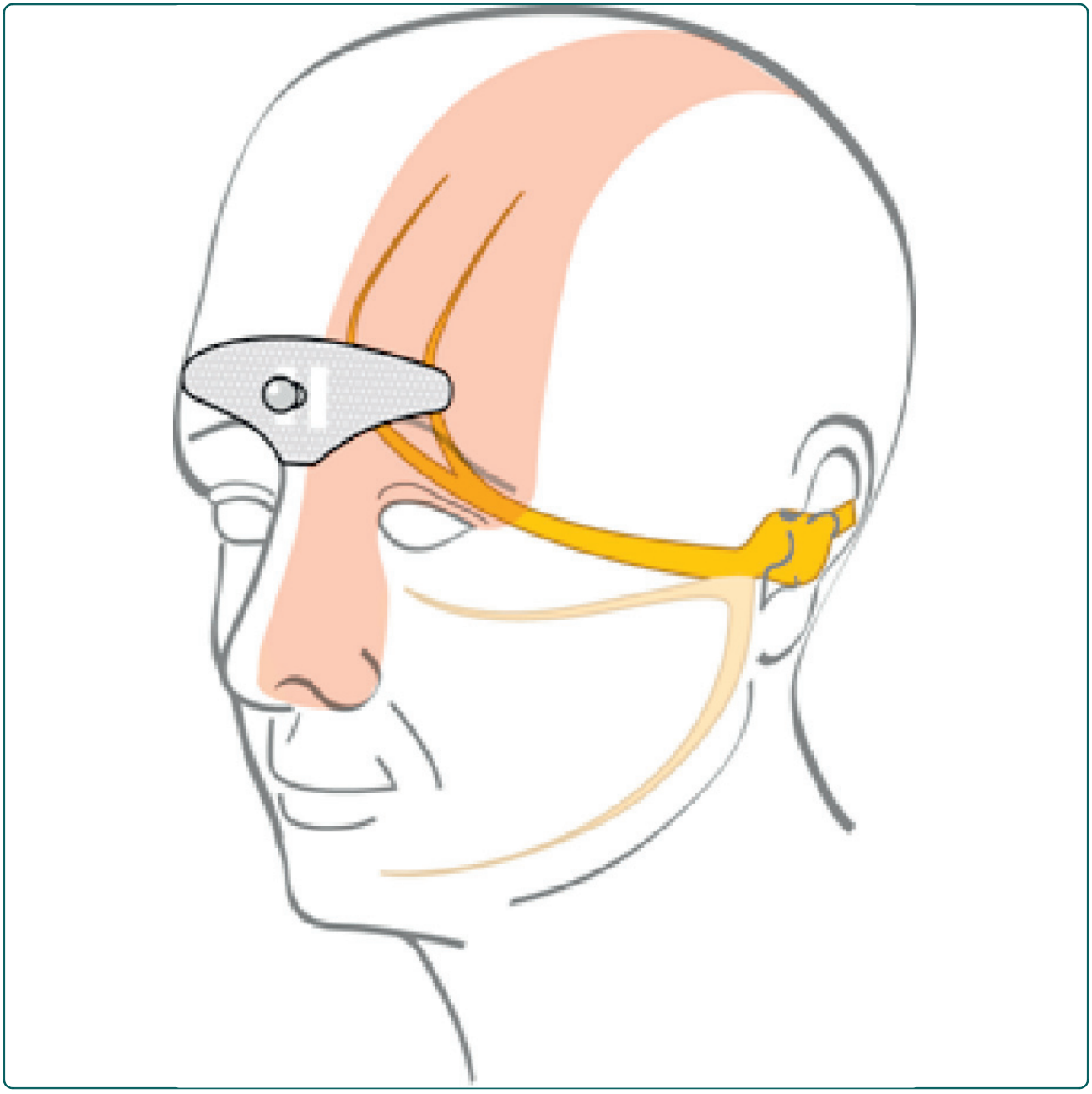

Supraorbital transcutaneous neurostimulation has sedative effects in healthy subjects

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RESEARCH ARTICLE Open Access

Supraorbital transcutaneous neurostimulation has sedative effects in healthy subjects

Maxime Piquet[1*], Costantino Balestra[1], Simona L Sava[2] and Jean E Schoenen[2]

Abstract

Background: Transcutaneous neurostimulation (TNS) at extracephalic sites is a well known treatment of pain. Thanks to recent technical progress, the Cefaly® device now also allows supraorbital TNS. During observational clinical studies, several patients reported decreased vigilance or even sleepiness during a session of supraorbital TNS. We decided therefore to explore in more detail the potential sedative effect of supraorbital TNS, using standardized psychophysical tests in healthy volunteers.

Methods: We performed a double-blind cross-over sham-controlled study on 30 healthy subjects. They underwent a series of 4 vigilance tests (Psychomotor Vigilance Task, Critical Flicker Fusion Frequency, Fatigue Visual Numeric Scale, d2 test). Each subject was tested under 4 different experimental conditions: without the neurostimulation device, with sham supraorbital TNS, with low frequency supraorbital TNS and with high frequency supraorbital TNS.

Results: As judged by the results of three tests (Psychomotor Vigilance Task, Critical Flicker Fusion Frequency, Fatigue Visual Numeric Scale) there was a statistically significant ($p < 0.001$) decrease in vigilance and attention during high frequency TNS, while there were no changes during the other experimental conditions. Similarly, performance on the d2 test was impaired during high frequency TNS, but this change was not statistically significant.

Conclusion: Supraorbital high frequency TNS applied with the Cefaly® device decreases vigilance in healthy volunteers. Additional studies are needed to determine the duration of this effect, the underlying mechanisms and the possible relation with the stimulation parameters. Meanwhile, this effect opens interesting perspectives for the treatment of hyperarousal states and, possibly, insomnia.

Background

Neurostimulation is a therapeutic method where action potentials are elicited by depolarizing nerve fibres with electrical impulses produced by a current generator device generally called neurostimulator. This method is used percutaneously with implantable neurostimulators and electrodes positioned over the spinal cord or peripheral nerves, or transcutaneously via superficial skin electrodes and external neurostimulators.

Percutaneous neurostimulation (PNS) of the spinal cord has been developed in the last decade for the management of intractable pain [1,2], but also for the treatment of several neurological disorders such as spasticity [3], parkinsonian tremor [4] or epilepsy [5], More recently, PNS has been explored for the treatment of intractable headaches [6-11].

Transcutaneous neurostimulation (TNS) is a classical technique which has demonstrated its efficacy in the treatment of pain [12,13] and is nowadays largely in use in pain clinics and physical therapy centres. It has the advantage of being non-invasive, safe and almost devoid of adverse effects contrary to PNS which needs a surgical intervention to implant the electrodes and the neurostimulator.

TNS at cephalic sites has been technically difficult and usually rather painful. STX-Med company has recently developed a headset for TNS of supratrochlear and supraorbital nerves, both branches of the ophthalmic division of the trigeminal nerve (V1), making the technique comfortable and easy to use [14]. Consequently, the utility of TNS in the treatment and prevention of headaches and migraine has been investigated [15] and several clinical trials are underway. Subjects enrolled in those

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trials have repeatedly reported that supraorbital TNS tended to affect vigilance and decrease attention with a tendency to fall asleep during the stimulation.

Cephalic electrical stimulation has been used many years ago to induce sleep or decrease anxiety. The method known as "Cranial Electrotherapy Stimulation (CES)", also called transcranial or transcerebral electrostimulation differs from TNS in that its objective is to generate different types of electrical currents through the head and not to specifically stimulate cranial nerves like TNS. For this purpose, CES uses generally an anterior frontal or a jaw electrode and a posterior electrode placed over the mastoid process [16,17]. CES was reported to have some effects on anxiety, depression and insomnia [18-20].

Given the anecdotal reports by patients of TNS-induced sedative effects, not hitherto reported in the literature, and the reported mental effects of CES, we decided to explore the effect on vigilance of supraorbital TNS with the headset developed by STX-Med in a double blind cross-over study.

Methods

We performed a double-blind crossover sham-controlled study of 30 subjects to assess the effect on vigilance of different protocols of supra-orbital TNS. Each subject was tested in 4 different experimental conditions: without neurostimulation device (blank control: BC), with a sham neurostimulation (Sham control: SC), with a low frequency neurostimulation (LFN) and with a high frequency neurostimulation (HFN). The study protocol was approved by the local ethics committee (CE B200-2010-074-2010-05-03).

Subjects

We included 30 healthy subjects: 15 men and 15 women ranging in age from 19 to 29 years (mean age = 23,9 +/- 2.4).

To be eligible, subjects had to be right-handed, drink no more than 1 cup of tea or coffee per day and no more than 2 glasses of alcohol per week. Exclusion criteria were a history of serious surgical, medical or psychiatric disease, smoking, and drug intake. Informed consent was obtained for all subjects prior to the study.

Neurostimulation

Supra-orbital neurostimulation was delivered with an external self adhesive electrode placed on the forehead (see Figure 1). The bipolar electrode is designed in order to cover the supratrochlear and supraorbital nerves bilaterally. Its dimensions are 30 mm × 94 mm.

The neurostimulator was a Cefaly® device (STX-Med, Liège, Belgium). It is a constant current generator for a maximum skin impedance of 2.2 KΩ. It generates biphasic rectangular impulses with an electrical mean equal to zero. The impulses have the following parameters: impulse width 250 μS, maximum intensity 14 mA. Low frequency neurostimulation (LFN) was delivered at a frequency of 2.5 Hz, high frequency neurostimulation (HFN) at 120 Hz. The neurostimulation lasted 20 minutes. For both LFN and HFN, the intensity reached was above perception threshold, so that all subjects experienced paresthesias and tingling under the stimulation electrodes. For sham neurostimulation (SC) we used a Cefaly® device with a low current intensity of 1 mA that was below the perception threshold and produced no sensation detectable by the subjects.

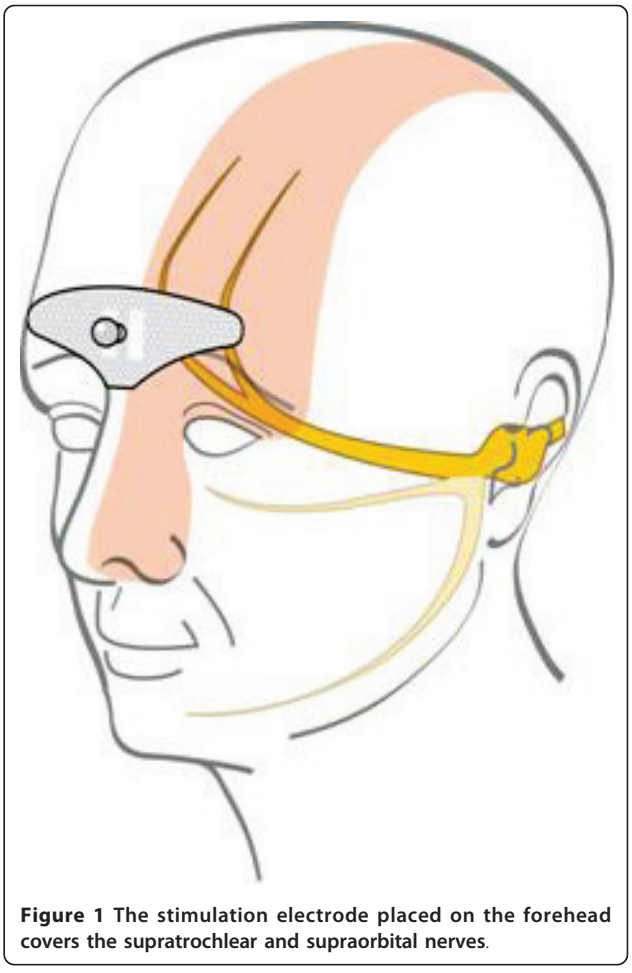

Figure 1 The stimulation electrode placed on the forehead covers the supratrochlear and supraorbital nerves.

Psychophysical measures

Four psychophysical tests were selected to detect sedative effects.

1) The *Psychomotor Vigilance Task* (PVT) was developed [21] to measure performance during mental fatigue. It is regarded as the gold standard for sleepiness.

We used the PEBL [22] implementation of the PVT (PPVT). Briefly, the subject sits in front of a black

computer screen. As soon as a red dot appears, the subject is supposed to hit the space bar of the computer keyboard. The reaction time is recorded in milliseconds. In total 12 reaction times measures are measured for each PVT test, separated randomly by intervals of 2 to 12 seconds. The results are expressed as the mean value of the 12 measures.

2) The *Critical Flicker Fusion Frequency* (CFFF) test is defined as "the highest or lowest temporal frequency, at a given percentage modulation, that can be resolved" [23], i.e. the frequency at which the subject is able to distinguish a flashing from a steady light. The CFFF decreases with fatigue. A portable device powered with a 9 V battery and equipped with a blue LED was used to vary flicker frequency by 0.5 Hz steps. The device starts with a steady light and the flicker frequency is decreased until the subject reports that the light is flashing. This frequency is recorded as the CFFF for that experiment.

3) The *d2 test* for attention and concentration [24] allows to assess visual attention and the ability to concentrate on a task. It consists of 14 lines of a combination of the letters "d" and "p" with one to four dashes placed above and/or below the letter. The objective is to mark all "d" with two dashes within 20 seconds for each line. Three scores are evaluated: GZ ("Gesamtzahl der bearbeiteten Zeichen") is the total number of letters marked; KL ("Konzentrationsleistungswert") is the number of correct letters marked minus the number of non correct letters; and F% ("Fehlerprozentwert") representing the percentage of errors compared to the number of characters marked (GZ). As this test can be biased by a learning effect, it is only presented once during the session without recording of a baseline.

4) For the subjective evaluation of fatigue we used the *Fatigue Visual Numeric Scale* (FVNS - Stanford Patient Education Research Centre [25]. This is a visual analogue scale where the subject scores fatigue from 0 (not tired at all) to 10 (very tired).

Procedures

Two groups of 8 subjects and two groups of 6 groups performed the experiments as depicted in Table 1. The sessions were separated by at least 6 hours as to ensure there was no remaining effect of the stimulation.

At the first session, each subject of the group is randomly assigned to one of the 4 experimental conditions:

- LFN, where the subjects get a Low Frequency Neurostimulation
- HFN, where the subjects get a High Frequency Neurostimulation
- SC, where the subjects get a sham neurostimulation (Sham Control)
- BC, where the subjects do not have a device (Blank Control)

Two subjects are assigned to each condition. In the subsequent sessions, the same subjects are re-assigned to another condition in order for each of them to have been through each condition after the 4 sessions.

The subjects are sitting comfortably in a chair in front of a wall to avoid any distraction. Once the session has started, each subject fills in the FVNS and performs the PPVT test where after the CFFF is determined. After these baseline tests, the neurostimulation is started for all subjects assigned to conditions LFN, HFN and SC while no neurostimulation is applied for the subjects assigned to condition BC. After 10 minutes of stimulation for LFN, HFN and SC or a 10-minute waiting time for BC, the subjects perform the d2 test that lasts 280 s. Thereafter they score FVNS once more, redo the PPVT test and finally have the CFFF measured again. The psychophysical tests are thus studied in the same sequence under every experimental condition.

This means in practice that we have a set of results for FVNS, PPVT and CFFF as measured before the application of the neurostimulator. A second set of results is obtained while the neurostimulator is applied since ± 15 minutes. The results can therefore also be expressed as a percentage of the measurement during the neurostimulation compared to the baseline value recorded before the neurostimulation.

Statistical Analysis

We compared the results of the psychophysical tests for each of the 4 experimental conditions: LFN, HFN, SC and BC. For FVNS, PPVT and CFFF we used the variation in percentage between pre- and perstimulation values to verify the effects of the 4 conditions. Since the

Table 1 Schedule of the experiments for each group

	First Experiment	Second Experiment	Third Experiment	Fourth Experiment
Group I	Tuesday 8 AM	Tuesday 2 PM	Thursday 8 AM	Thursday 2 PM
Group II	Tuesday 9 AM	Tuesday 3 PM	Thursday 9 AM	Thursday 3 PM
Group III	Tuesday 10 AM	Tuesday 4 PM	Thursday 10 AM	Thursday 4 PM
Group IV	Tuesday 11 AM	Tuesday 5 PM	Thursday 11 AM	Thursday 5 PM

results did not have a Gaussian distribution, we used the Wilcoxon test to measure the significance of the variation observed.

For the d2 test, we compared GZ, KL and the F% between the 4 conditions (as there was no control values to compare with). We have used the Mann-Whitney test to verify the significance of the differences observed.

Results

PPVT Test

The mean reaction times (RT) for the PPVT (N = 30) before the session was 339 ms±176 for LFN, 304 ms ± 37 for HFN, 294 ms ± 44 for SC and 306 ms ± 46 for BC. Reaction time increased during HFN, while it was stable for the LFN, SC and BC conditions (Figure 2).

As explained in the methods section, for FVNS, PPVT and CFFF the statistical analysis was performed on the ratio (in percentage) between the mean value during and before the experimental condition for each subject. The mean percentage increase in RT is significant only during the HFN condition ($p = 0.0002$).

CFFF Test

The mean values for CFFF (N = 30) before the session was 38.2 Hz ± 2.5 for LFN, 39.7 Hz ± 2.7 for HFN, 39.9 Hz ± 3.3 for SC and 38.2 Hz ± 2.2 for BC. During HFN there was a significant decrease of CFFF ($p < 0.0001$) while CFFF was significantly increased during LFN (Figure 3).

d2 Test

Table 2 shows the results for the d2 test. Mean values of GZ, KL and F% are given during each experimental condition the. Numerically the total number of letters marked (GZ) and the number of correct letters marked (KL) were the lowest in the HFN condition, while the percentage of errors was the highest, but this difference was not statistically significant.

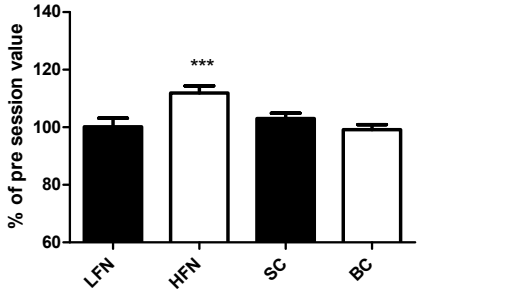

Figure 2 Mean PPVT reaction time change during the experimental conditions expressed as a percentage of the baseline value (* = p < 0.001) (mean+/-SEM).**

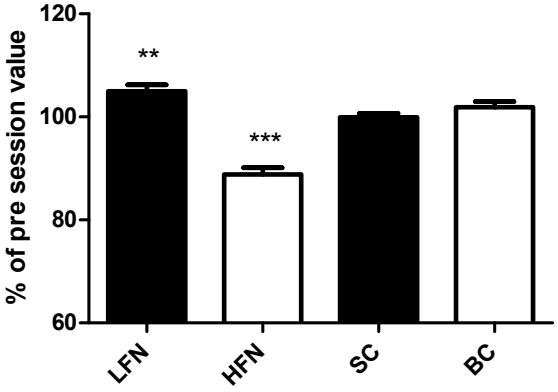

Figure 3 Mean CFFF change during the experimental conditions expressed as a percentage of the baseline value (= p < 0.01; *** = p < 0.001) (mean+/-SEM).**

Fatigue Visual Numeric Scale

The FVNS fatigue score tended to increase during all four conditions. However, the statistical analysis for the averaged individual changes showed that the increase was significant only during HFN (Figure 4).

Discussion

Taken together our results suggest that supraorbital neurostimulation using the Cefaly® device decreases arousal and induces fatigue. This cannot be considered at this stage as a hypnotic effect in the sense of inducing sleep and decreasing sleep latency but rather as a sedative effect in terms of a reduction of alertness and vigilance. Interestingly, this is only the case with high (120 Hz-HFN) and not with low frequency (2.5 Hz-LFN) stimulation. LFN even has an opposite effect in one psychophysical test, the critical flicker fusion frequency. Below we will examine these results in more detail and speculate on possible mechanisms.

The Psychomotor Vigilance Task measures the reaction time (RT) and is considered as the gold standard for measuring sleepiness [21]. That it is readily reproducible is demonstrated by the fact that during the blank condition (BC) the change compared to baseline was less than 1.5%. Sham (SC) and LFN induced non significant increases in RT of respectively 8.9 ms and 8.6 ms. By contrast, HFN increased RT by an average of 36.7 ms, i.e. by more than 10%. Critical flicker fusion frequency is known to decrease with fatigue. While unchanged during SC and minimally increased during BC (+ 0.9 Hz), it increased during LFN (+ 1.9 Hz) possibly suggesting a mild increase in vigilance. Again HFN contrasted with all other conditions by a marked decrease (-4.6 Hz) in CFFF, indicating a decrease in arousal. This result is concordant with that of the subjective fatigue rating on the Fatigue

Table 2 d2 results

N = 30	LFN	HFN	SC	BC
Mean value of GZ	560 ± 77	544 ± 80	587 ± 57	562 ± 70
Mean value of KL	215 ± 40	214 ± 50	229 ± 42	217 ± 43
Mean value of F%	6.95% ± 6.81	8.37% ± 8.38	6.02% ± 5.98	6.72% ± 6.16

Visual Numerical Scale (FVNS). The subjects rated their fatigue higher during all experimental conditions than at baseline, which was not significant and might be related to the mental strain due to the recordings or to a learning effect in using the numerical scale. However, the increase of the FVNS score during HFN was three times greater (+ 72.1%). The d2 test for attention and concentration was in our study the only one for which the HFN condition induced no significant effect. Nevertheless the numerical changes during HFN are in line with the other results as they show a lower number of total letters marked and of correct letters marked as well as a higher number of errors. The lack of significance could have at least two explanations. First, the d2 test was administered at an earlier time point (between 10 and 15 minutes) during the experimental condition compared to the other tests (from 15 minutes onwards). The duration of HFN might thus not have been long enough to produce significant d2 test changes. Second, this test was performed only once to avoid a learning effect and the pre- and per-condition comparison had therefore to be replaced by a comparison between conditions, hence weakening the sensitivity of the test to detect a change.

To the best of our knowledge, this is the first time that the effect of transcutaneous neurostimulation on arousal and fatigue was studied in humans and there are no similar studies available in animals. The neurobiological mechanisms through which HFN induces sedation remain therefore speculative. Some insight can nonetheless be gained from the studies of transcutaneous neurostimulation in Alzheimer's patients and from those in experimental animals of the central nervous system consequences of electroacupuncture. A Dutch group reported in a series of publications that transcutaneous electrostimulation was able to improve memory, alertness [26,27] and rest-activity rhythm [28] in Alzheimer's disease. This effect was attributed to activation of the hippocampus and the suprachiasmatic nucleus both by direct spinal cord afferents [29] and via the dorsal raphe nucleus and locus coeruleus [30,31]. Although vigilance was not specifically measured in these studies, the observed cognitive and behavioural effects would suggest increased arousal and vigilance rather than sedation like in our study. This opposite effects can probably be explained by the different stimulation protocols. First, Alzheimer patients received transcutaneous neurostimulation over paravertebral back muscles daily during 6 [26] or 3 hours [27,28] for 6 weeks while we used a single 20-minute session of supraorbital neurostimulation. In a more recent randomized sham-controlled pilot trial of right median nerve stimulation, Scherder et al [32] found no significant effect on memory in Alzheimer's disease and the same group reported that cranial electrostimulation had no effect on rest-activity rhythm neither at low frequency [33] nor at high frequency [34]. More interestingly, we found a hypnotic effect with high frequency (120 Hz) stimulation, whereas the beneficial effects in Alzheimer's disease were obtained with burst of stimuli (9 pulses at 160 Hz) delivered at a low frequency of 2 Hz, a frequency that in our study concordantly increased critical flicker fusion frequency. One may assume that high and low frequency stimulations can have different effects on central nervous system structures and thus on arousal, but this remains to be proven in an adequate study.

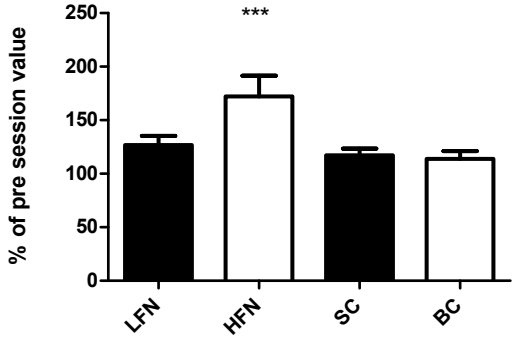

Figure 4 Mean change in FNVS score during the experimental conditions expressed as a percentage of the baseline value (* = p < 0.001) (mean+/-SEM).**

Transcranial direct current stimulation (tDCS) is able to modulate cortical activity under certain conditions and in certain brain areas. It is extremely unlikely, however, that the supraorbital TNS used in this study influences directly the underlying brain structures, i.e. the frontal lobes, for at least two reasons. First, The small electrode surface (7 cm^2) and distance between the two electrodes (5 mm) restrict the skin surface affected by the current as well as current penetration into deeper structures. Second, the TNS applied current is composed of biphasic rectangular impulses with an electrical mean equal to zero, while tDCS uses a direct current. The current characteristics

and the mechanisms of action are thus different between trigeminal TNS and tDCS. Moreover, in a recent study [35], weak transcranial electrical DC or AC currents over the prefrontal cortex had no effect on mood or EEG in healthy subjects. Interestingly, sleepiness was reported rarely both in the active (0.11%) and sham stimulation groups (0.08%).

Experimental studies on the mode of action of electroacupuncture in pain are relevant to this discussion because many of the central nervous system structures activated by electroacupuncture like the monoaminergic brain stem nuclei, the hypothalamic arcuate nucleus or the periaqueductal gray matter also play a role in vigilance states (36,37,38,39). A simple straightforward explanation for the sedative effects found in our study would be an effect of the transcutaneous stimulation on monoaminergic brain stem nuclei such as locus coeruleus that receives direct spinal input [40]. The locus coeruleus is also thought to mediate the anti-epileptic effect of high frequency transcutaneous stimulation of the ophthalmic nerve [41]. However, in animals high frequency electroacupuncture was found to increase neuronal activity in brain stem nuclei [36], in particular in dorsal raphe nuclei [37]. Increased activity of these nuclei that belong to the ascending activating reticular system would be associated with increased rather than decreased arousal and vigilance. Electroacupuncture over peripheral nerves also activates the hypothalamic arcuate nucleus in animals [39]. The arcuate nucleus plays a pivotal role in electroacupuncture-induced cardiovascular inhibition [39], but also in vigilance states via its reciprocal connections with orexin-containing lateral hypothalamic neurons and the ventrolateral periaqueductal gray matter (38,42). A change in activity levels of the orexin-arcuate-periaqueductal gray matter circuit could occur during supraorbital neurostimulation and might explain the decrease in vigilance. Future studies of supraorbital neurostimulation coupled to functional cerebral imaging studies could verify this hypothesis. Further studies are also needed to verify whether the sedative effects of HFN as evidenced here by psychophysical tests have electroencephalographic correlates and if they are associated with hypnotic effects such as sleep latency reduction.

Conclusion

To sum up, we have shown in healthy volunteers that supraorbital high frequency neurostimulation applied with the Cefaly® device modifies concordantly several psychophysical tests in a way that is compatible with decreased vigilance and arousal, while sham stimulation has no effect and low frequency neurostimulation, if anything, tends to increase arousal. The precise mechanisms of action of HFN on the CNS arousal systems are not known and warrant further studies. Meanwhile supraorbital HFN with the Cefaly® device opens interesting perspectives for an adverse effect-free treatment of hyperarousal states, and possibly sleep disorders.

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Authors' contributions

MP has participated in the design of the study, performed the experiments and provided a draft of the results. CB has participated in the design of the study and in the statistical analysis of the results. SLS has made the literature search and JS has interpreted the results in the light of the available literature data and drafted the final manuscript. All authors read and approved the final manuscript.

Competing interests

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RESEARCH ARTICLE Open Access

Abnormal cortical responses to somatosensory stimulation in medication-overuse headache

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Abstract

Background: Medication-overuse headache (MOH) is a frequent, disabling disorder. Despite a controversial pathophysiology convincing evidence attributes a pivotal role to central sensitization. Most patients with MOH initially have episodic migraine without aura (MOA) characterized interictally by an absent amplitude decrease in cortical evoked potentials to repetitive stimuli (habituation deficit), despite a normal initial amplitude (lack of sensitization). Whether central sensitization alters this electrophysiological profile is unknown. We therefore sought differences in somatosensory evoked potential (SEP) sensitization and habituation in patients with MOH and episodic MOA.

Methods: We recorded median-nerve SEPs (3 blocks of 100 sweeps) in 29 patients with MOH, 64 with MOA and 42 controls. Episodic migraineurs were studied during and between attacks. We measured N20-P25 amplitudes from 3 blocks of 100 sweeps, and assessed sensitization from block 1 amplitude, and habituation from amplitude changes between the 3 sequential blocks.

Results: In episodic migraineurs, interictal SEP amplitudes were normal in block 1, but thereafter failed to habituate. Ictal SEP amplitudes increased in block 1, then habituated normally. Patients with MOH had larger-amplitude block 1 SEPs than controls, and also lacked SEP habituation. SEP amplitudes were smaller in triptan overusers than in patients overusing nonsteroidal anti-inflammatory drugs (NSAIDs) or both medications combined, lowest in patients with the longest migraine history, and highest in those with the longest-lasting headache chronification.

Conclusions: In patients with MOH, especially those overusing NSAIDs, the somatosensory cortex becomes increasingly sensitized. Sensory sensitization might add to the behavioral sensitization that favors compulsive drug intake, and may reflect drug-induced changes in central serotoninergic transmission.

Background

Medication-overuse headache (MOH) is a complication of episodic headaches characterized by more than 15 headache days per month and arising from an excessive intake of analgesics or specific anti-migraine drugs, or both [1]. MOH is a disabling health problem that affects 2-4% of the general population and causes considerable long-term morbidity and disability [2]. Most patients attending headache clinics for chronic daily headache have MOH [1,3]. Although MOH evolves from primary as well as secondary headaches the most prevalent initial headache type is episodic migraine without aura and most patients return to the episodic pattern after drug withdrawal [1].

How and why medication overuse leads to chronic episodic headache is unknown. Possible culprits for pain chronification include central sensitization and defective central pain control systems [4]. The addictive behavior and high relapse rates after withdrawal may depend on orbitofrontal cortex hypofunction [5]. The observation that MOH develops predominantly in migraineurs without aura suggests that this headache type possesses pathophysiological peculiarities that could favour drug-induced chronification.

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During the pain-free interval in episodic migraine without aura repeated sensory stimuli delivered using various modalities elicit abnormal cortical responses characterized by deficient habituation contrasting with a normal-amplitude initial evoked potential elicited by a small number of stimuli [6]. Current hypotheses attribute this neurophysiological abnormality to cortical hyper-excitability probably arising from deficient intracortical inhibition [7], or to low sensory cortical preactivation levels ultimately due to abnormal functioning of monoaminergic projections from the brainstem [6,8]. Habituation is considered a protective mechanism intended to prevent neuronal stress and excessive accumulation of metabolites such as lactate and protons that are likely to induce cortical spreading depression or trigeminovascular activation, or both. Evidence suggesting that lack of habituation can promote migraine attacks comes from the observation that it culminates just before the onset of an attack, in the pre-ictal phase [9-11]. During the attack, habituation normalizes, thus transiently activating the protective mechanisms thought to prevent attack recurrence [10-14].

A neurophysiological technique ideally suited to investigate how sensory cortices respond to repetitive stimulation consists of testing somatosensory evoked potentials (SEPs). SEPs are obtained by weak sensory stimuli ideal for disclosing sensitization (reflected by an increased response amplitude to low numbers of stimuli) and habituation (reflected by a decrease in response amplitude after high numbers of stimuli) [15,16], and proved highly sensitive in disclosing abnormal habituation in migraineurs studied interictally, i.e. a clear-cut lack of habituation from the 2nd block of averaged responses onwards [17]. To the best of our knowledge no study has investigated SEP sensitization and habituation in patients with MOH. Having this information may shed light on the mechanisms underlying headache chronification during acute medication overuse.

We used therefore SEPs to investigate whether medication overuse sensitizes the sensory cortices, whether sensitization varies according to the drug overused, and whether the cortical response patterns, sensitization and habituation, differ between patients with episodic migraine without aura recorded in ictal and interictal phases and those with MOH. We also sought possible correlations between the electrophysiological patterns and clinical features including duration of migraine history, duration of headache chronification and class of drugs overused.

Methods

Subjects-Among consecutive patients attending our headache clinic, 93 patients gave informed consent to participate in the study (Table 1), which was approved by the local ethics committee.

According to the revised ICHD-II criteria [1], 29 patients (35 ± 11 years; 23 women) were diagnosed as having MOH during their first visit, a diagnosis that was confirmed 2 months after withdrawal treatment. These patients were stratified according to the class of drug overused: triptans (n = 9), nonsteroidal antiinflammatory drugs (NSAIDs) (n = 10) or a combination of both (n = 10). Before progressing to MOH, all patients had a clear-cut history of episodic migraine without aura (ICHD-II code 1.1). With the exception of 2 patients who had a mild headache, all MOH patients (n = 27) underwent the SEP recordings in a pain-free state. The 2 patients who had a headache had no associated migrainous features. Because MOH patients tend to take acute medications compulsively and frequently during the day, it was impossible to prevent them from taking a medication on the day of recordings. We managed, however, to perform the recordings at least 3 hours after last medication intake. The 64 patients who had episodic migraine without aura (ICHD-II code 1.1) were assigned to two subgroups: 41 patients (34 ± 9 years; 23 women) were recorded during the interictal period, i.e. at least three days before and after an attack, and 23 patients (33 ± 12 years; 20 women) during the ictal period, i.e. from 12 hours before to 12 hours after an attack. The latter were not allowed to take any acute medication before the end of recordings.

For comparison we recorded SEPs in 42 healthy volunteers of comparable age and sex distribution (mean age: 33 ± 13; 26 women); they had no personal or familial history (1st or 2nd degree relatives) of migraine and no detectable medical condition.

To avoid variability due to hormonal changes, women were recorded outside their pre-menstrual or menstrual periods.

Data acquisition

SEPs were elicited by electrical stimulation applied to the right median nerve at the wrist using a constant current square wave pulse (0.1 ms width, cathode proximal), a stimulus intensity set at 1.5 times the motor threshold, and a repetition rate of 4.4 Hz. The active electrodes were placed over the contralateral parietal area (C3', 2 cm posterior to C3 in the International 10-20 system) and on the fifth cervical spinous process (Cv5), both referenced to Fz; the ground electrode was on the right arm [18]. SEP signals were amplified with a Digitimer™ D360 pre-amplifier (Digitimer Ltd, UK) (band-pass 0.05-2500 Hz, Gain 1000) and recorded with a CED™ power1401 device (Cambridge Electronic Design Ltd, Cambridge, UK).

Table 1 Demographics data of study participants and headache profiles of patients

	HV (n = 42)	MOii n = 41)	MOi (n = 23)	MOH (n = 29)	Triptans (n = 9)	NSAIDs (n = 10)	Both (n = 10)
Women (n)	26	23	20	23	7	8	8
Age (years)	32 ± 13	34 ± 9	33 ± 12	35 ± 11	32 ± 8	35 ± 9	34 ± 12
Duration of history of migraine (years)		18.0 ± 12.7	16.7 ± 10.9	18.4 ± 11.0	18.3 ± 9.6	22.4 ± 9.2	13.0 ± 13.7
Days with headache/month (n)		2.1 ± 1.9	3.5 ± 2.3	25.9 ± 6.1	22.1 ± 6.2	25.0 ± 7.4	29.4 ± 1.6
Severity of headache attacks (0-10)		6.8 ± 0.8	7.2 ± 1.2	7.2 ± 0.8	7.4 ± 1.1	7.2 ± 0.5	7.1 ± 0.8
Nausea/vomiting (n)		25	16	24	8	9	7
Photophobia (n)		37	21	27	8	10	9
Phonophobia (n)		31	20	27	7	10	10
Pulsating (n)		38	21	26	9	9	8
Duration of the chronic headache (years)				3.0 ± 3.2	1.9 ± 1.8	3.5 ± 3.1	3.3 ± 3.9
Tablet intake/month (n)				74.2 ± 80.8	28.7 ± 16.3	50.5 ± 38.5	127.3 ± 106.5
Motor threshold (mA)	8.4 ± 1.3	8.6 ± 1.3	8.5 ± 1.5	8.7 ± 1.2	8.8 ± 1.3	9.1 ± 1.1	8.3 ± 1.1

Data expressed as mean ± SD. HV healthy volunteers; MOii episodic migraneurs without aura studied interictally; MOi episodic migraneurs without aura studied ictally; N number of subjects.

Subjects sat relaxed in a comfortable chair in a well-lit room with eyes open. They were asked to fix attention on the stimulus-induced thumb movement. During continuous median-nerve stimulation at the wrist, we collected 300 sweeps of 50 ms, sampled at 5000 Hz. All recordings were averaged off-line using the Signal™ software package version 3.10 (CED Ltd).

Three hundred artefact-free evoked responses recorded in each subject were averaged ("grand average"). After digital filtering of the signal between 0-450 Hz, the various SEP components (N13, N20, P25 and N33) were identified according to their respective latencies. We measured peak-to-peak amplitudes of the cervical N13 component (recorded under the active Cv5 electrode), and the cortical N20-P25 and P25-N33 components (recorded under the active C3' scalp electrode).

Thereafter, the 300 evoked responses were partitioned in 3 sequential blocks of 100 responses (Figure 1). Each block was averaged off-line ("block averages") and analyzed for N20-P25 amplitudes. Sensitization was defined as an increased N20-P25 amplitude recorded during block 1 (after a low number of 100 stimuli), whereas habituation was expressed as the change in N20-P25 amplitude in blocks 2 and 3 compared to block 1 (over a high number of 300 repetitive stimuli).

Statistical Methods

We used the Statistical Package for the Social Sciences (SPSS) for Windows, version 15.0 for all analyses. For grand average SEPs, component amplitudes were tested in a one-way analysis of variance (ANOVA) with group factor "subjects" (MOH patients, episodic migraineurs without aura studied ictally or interictally, and healthy subjects). To assess changes in SEP amplitude between blocks 1, 2 and 3 SEP N20-P25 amplitudes were tested first with a repeated-measure ANOVA with group factor "subjects" and repeated measures factor "block" then using as group factor "MOH subgroups" (MOH-triptans, MOH-NSAIDs, MOH-combination, and normal subjects). Tukey's test was used for post hoc analyses. Pearson's correlation coefficient was calculated to test correlations between SEP amplitudes or habituation and clinical data (disease duration, days with headache, number of tablets taken per month, duration of chronic headache). P values less than 0.05 were considered to indicate statistical significance.

Results

Assessable SEP recordings were obtained from all patients and controls participating in the study (Figure 1). On grand average SEP recordings after electrical median nerve stimulation latencies of N13, N20, P25 and N33 components were not different between groups (for each measure $F(3,131)$, $p > 0.05$) whereas their amplitudes significantly differed between groups ($F(3,131) = 2.75$, $p = 0.045$). Post hoc analysis showed a higher N20-P25 amplitude in patients with MOH and migraineurs without aura studied ictally than in the subgroup studied interictally and controls (Figure 2).

ANOVA testing SEP amplitude block averages disclosed a main effect for factors group ($F(3,131) = 3.83$, $p = 0.01$) and block ($F(2,262) = 4.13$, $p = 0.017$), and a significant interaction of group by block ($F(6,262) = 2.42$, $p = 0.027$). Post hoc analysis showed in each block a higher N20-P25 amplitude in patients with MOH and migraineurs without aura studied ictally than in the subgroup studied interictally and controls (Figure 3). In controls and migraineurs without aura studied ictally, N20-P25 amplitude decreased from block 1 to block 3, i.e. habituated, while in

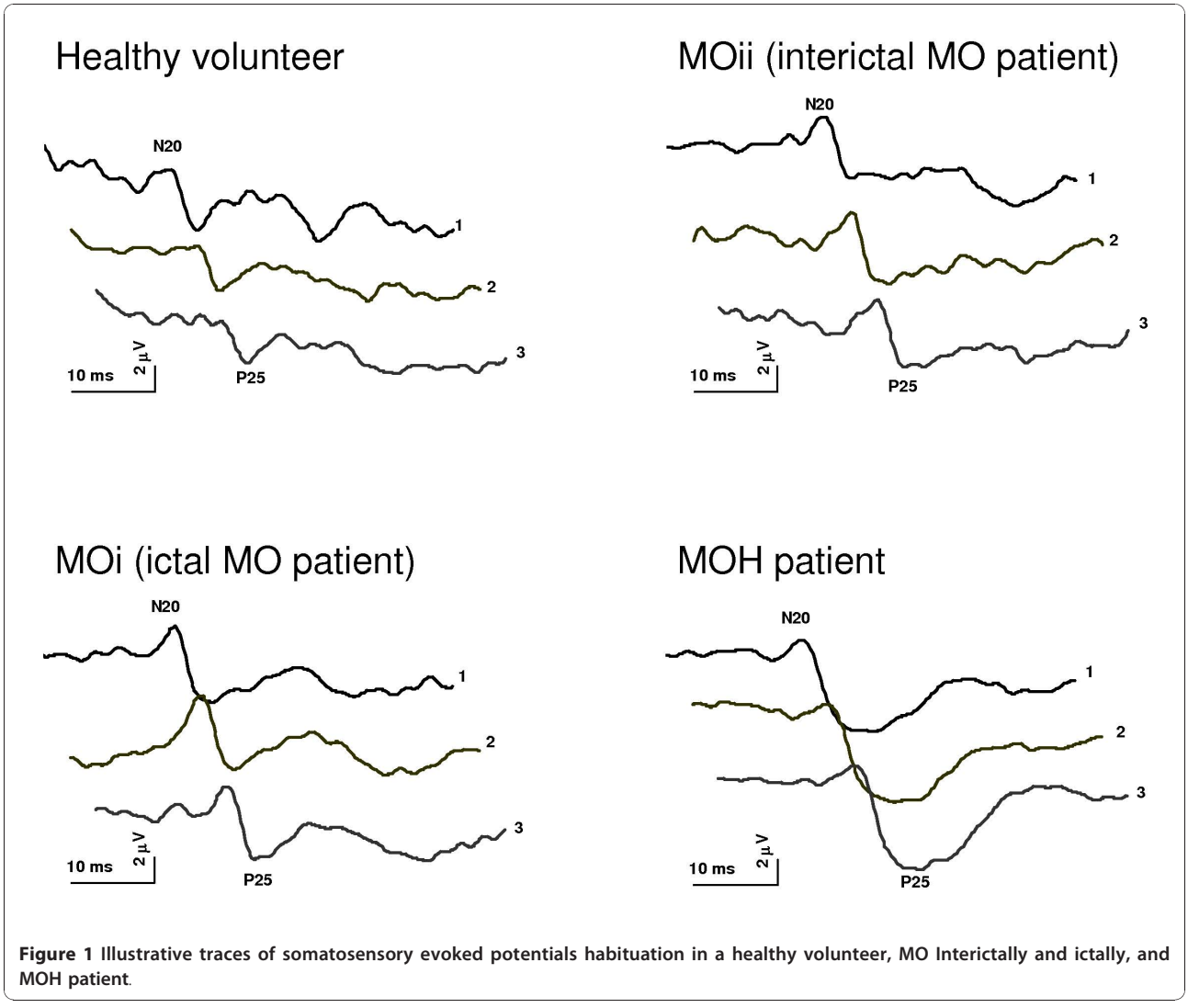

Figure 1 Illustrative traces of somatosensory evoked potentials habituation in a healthy volunteer, MO Interictally and ictally, and MOH patient.

patients with MOH and migraineurs with aura studied interictally it remained unchanged from block 2 onwards, i.e. did not habituate.

Conversely, ANOVA testing block 1 SEP amplitudes showed a main effect only for factor group ($F(3,131) = 2.73$, $p = 0.046$) (Figure 3). Post hoc analysis showed that N20-P25 amplitudes were higher in patients with MOH and migraineurs without aura studied ictally than in the subgroup studied interictally and controls.

When we stratified the data for patients with MOH according to the class of drugs overused, triptans, NSAIDs or both combined, ANOVA for SEP amplitudes in the various blocks, showed a main effect for factor "drug" ($F(2,26) = 3.57$, $p = 0.042$). Post hoc analysis disclosed smaller N20-P25 amplitudes in patients overusing triptans than in those overusing NSAIDs or both medications combined. In addition, group analysis between triptan overusers and controls showed that the N20-P25 amplitude in block 1 was normal in patients ($F(1,49) = 1.08$, $p = 0.3$) (Figure 4).

Pearson's test disclosed various correlations between SEP amplitude and clinical variables. In patients with MOH, N20-P25 amplitude on SEP grand average correlated negatively with disease duration (i.e. combined duration of episodic and chronic headache phases, $r = -0.411$, $p = 0.046$). Conversely, grand average N20-P25 amplitude ($r = 0.477$, $p = 0.016$) as well as block 1 N20-P25 amplitude ($r = 0.454$, $p = 0.023$) correlated positively with duration of the chronic headache phase.

Discussion

The distinct changes we found in cortical responses to low and high numbers of sensory stimuli in patients with MOH suggest that the underlying brain mechanisms are altered and differ from those acting in patients with episodic migraine without aura. Low numbers of

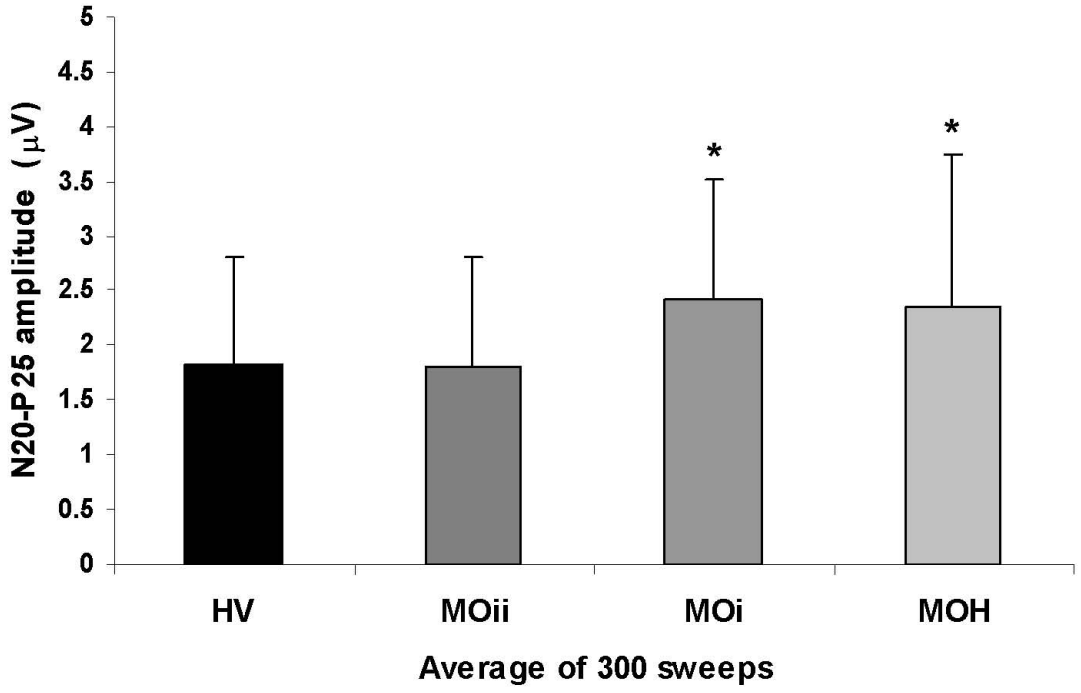

Figure 2 Somatosensory evoked potential (SEP) amplitude grand average in each study group (HV, healthy volunteers; MOii, migraine without aura interictally; MOi, migraine without aura Ictally; MOH, medication overuse headache; data expressed as mean ± SEM).

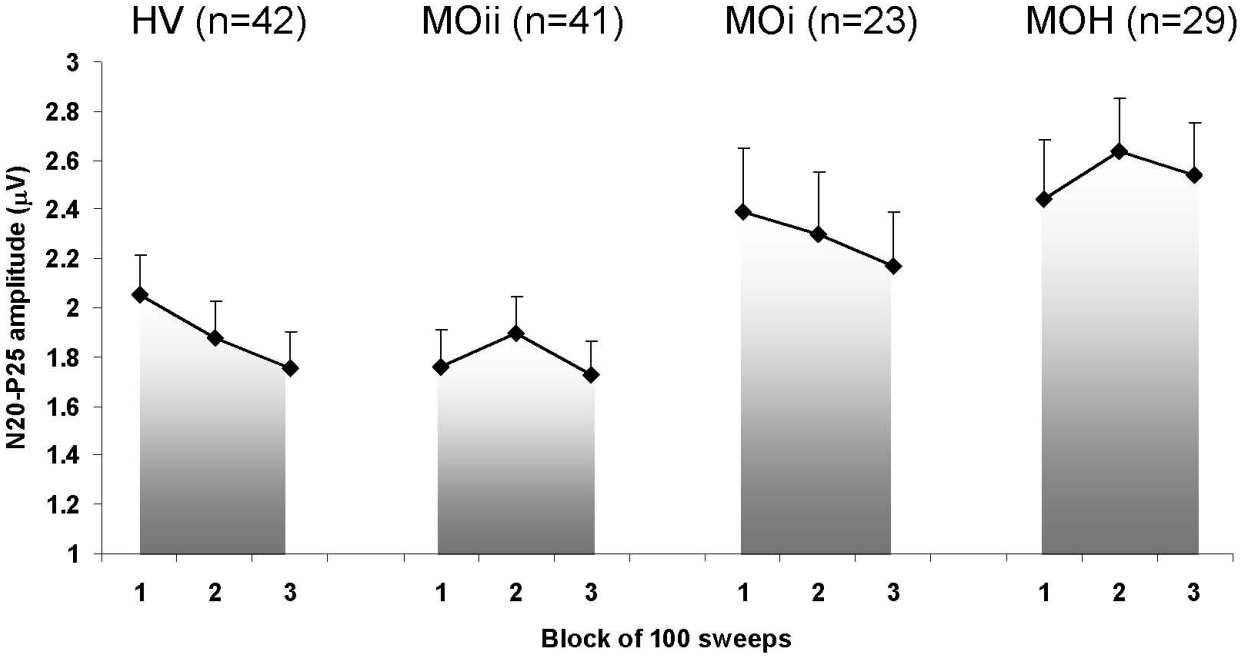

Figure 3 Somatosensory evoked potential (SEP) amplitude block averages in each study group (HV, healthy volunteers; MOii, migraine without aura interictally; MOi, migraine without aura Ictally; MOH, medication overuse headache; data expressed as mean ± SEM).

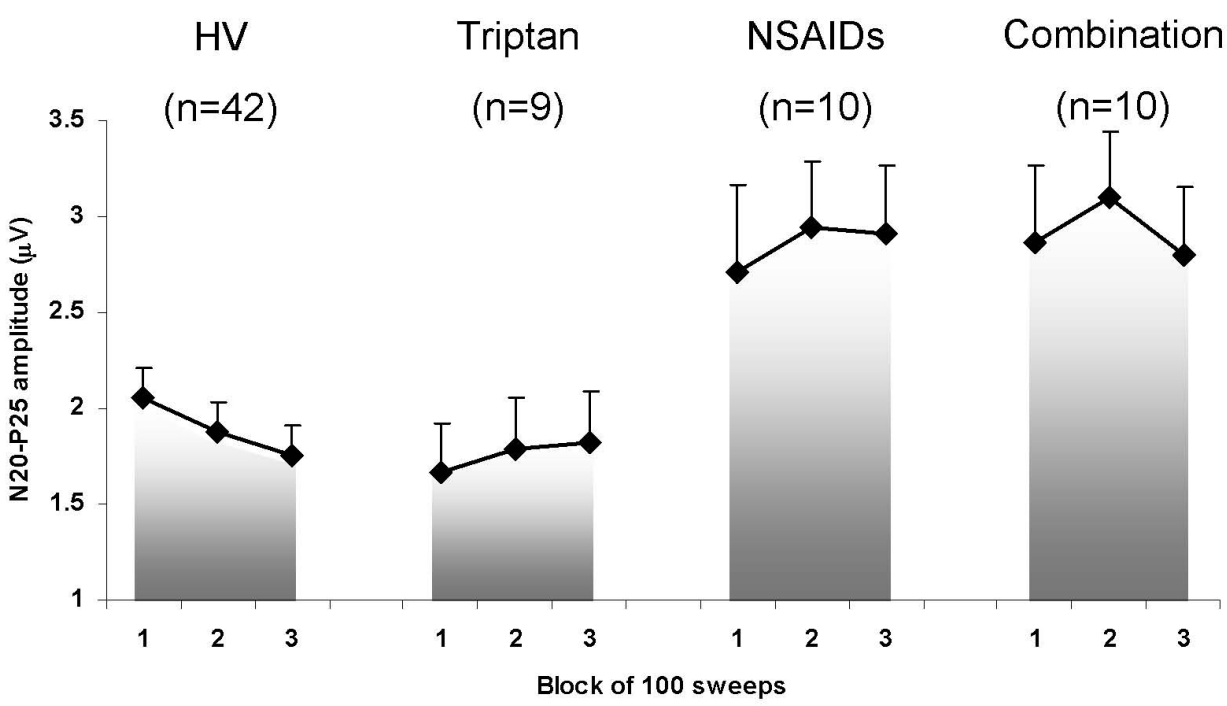

Figure 4 Somatosensory evoked potential (SEP) amplitude block averages in patients with medication overuse headache (MOH) subgroups and a healthy volunteer (HV) (data expressed as mean ± SEM).

median nerve electrical stimuli (block 1) disclosed sensory cortex sensitization in patients with MOH and in episodic migraineurs recorded ictally, whereas amplitude changes over sequential block averagings were consistent with habituation in healthy volunteers and episodic migraineurs recorded ictally, but not in MOH patients and episodic migraineurs recorded interictally. In MOH patients, SEP amplitude was lowest in those with the longest history of migraine, whereas it was highest in those with the longest period of headache chronification, suggesting that the electrophysiologic changes reflect chronification. Patients who overused triptans had lower SEP amplitudes than those who overused NSAIDs or both anti-migraine medications combined, indicating that sensitization varies according to the drug overused.

The combination of an initial SEP amplitude increase (sensitization) along with the subsequent lack of habituation suggests that the electrophysiological pattern underlying MOH differs from that underlying episodic migraine. In episodic migraine, SEP recordings show two characteristic changes: a lack of habituation on interictal recordings, and sensitization during the attack. The habituation deficit normalizes during attacks, whereas sensitization disappears between attacks, but in the immediate pre-ictal phase both sensitization and absent habituation may co-exist [9-11]. The electrophysiological pattern we found in MOH may therefore suggest that the sensory cortex is locked in a pre-ictal state associating both hyper-sensitivity (due to sensitization) and hyper-responsiveness (due to deficient habituation), which contrasts with episodic migraine where these cortical states alternate. It is likely that the disclosure of this peculiar electrophysiological pattern was made possible by the fact that we avoided to record MOH patients during a full-blown migraine attack. The SEP pattern associating sensitization and lack of habituation that we compared with a "persistent pre-ictal state", closely resembles the response patterns generated by central sensitized neuronal circuits. Sensitization refers to a facilitatory process that competes with its opposite, habituation to determine the final behavioural outcome after stimulus repetition. This has been called the "dual process" theory [15,16]. Illustrative of central sensitization are the plastic changes in neural structures belonging to the "pain matrix" [19] that result in decreased nociceptive thresholds and increased responsiveness to noxious and innocuous peripheral stimuli [20]. Studies in animals [21] and humans [22] show that SEP amplitudes increase when transient intense activation of nociceptive afferents induces central sensitization, as

happens in clinical pain conditions including chronic headache. Our study shows that sensitization, as reflected by increased initial SEP amplitudes, is common to MOH and migraine attacks, although we did not record MOH patients during an attack. A clinical consequence of central sensitization is cutaneous allodynia. It was shown to be prevalent during episodic migraine attacks at cephalic and extracephalic sites [23,24], but even more so in chronic migraine [25]. It is associated with increased nociceptive reflexes [26,27], but, interestingly, in MOH trigeminal evoked potentials were increased, whereas nociceptive blink reflexes remained unchanged, suggesting as in our study that sensitization takes place at supraspinal levels [28].

Our finding that the SEP amplitude increase in MOH is proportional to the duration of headache chronification suggests that medication overuse and increased headache frequency promote or reinforce central sensitization, but leaves open the question of the culprit. Conversely, since total duration of the migraine disorder correlates inversely with SEP amplitudes, the SEP amplitude increase is likely related to factors other than migraine duration and simply repetition of attacks. In keeping with this interpretation, patients who overused triptans alone had no initial SEP amplitude increase indicating that the major culprit for central sensitization in MOH could be NSAIDs. The neurobiological underpinning for this difference remains to be determined. An observation that might favour of NSAIDs consumption as a factor promoting sensitization is that NSAIDs increase spinal expression of inducible cyclo-oxygenase-2 [29], an enzyme that contributes to sensitization in a rat model of inflammatory pain [30].

Another possible link between central sensitization, migraine and anti-migraine drugs is monoaminergic transmission in the central nervous system (CNS). Although both triptan and NSAID overuse lead to headache chronification, only the latter is accompanied by SEP sensitization. We hypothesize that this difference is due to a more profound decrease of 5-HT transmission after NSAID overuse. Between attacks, migraine patients have low blood 5-HT levels whereas the reverse is true ictally [31]. Serotonin synthesis in the brain increases during attacks, and this increase is partly counteracted by acute triptan treatment [32]. Chronic administration of triptans in rats, however, increases 5-HT synthesis in several cortical projection areas of the dorsal raphe nucleus [33] possibly reflecting down-regulation or desensitization of 5-HT1 receptors. By contrast, in rats chronically treated with analgesics, 5-HT2A receptors are down-regulated [34] and the 5-HT transporter is up-regulated in the cortex [34] and in platelets [35]. Upregulated platelet 5-HT transporters [35] and decreased whole blood 5-HT levels [36] tend to normalize after drug withdrawal. Collectively, these experimental data suggest that anti-migraine drug overuse can disrupt central 5-HT transmission. In chronic triptan overuse both pre- and postsynaptic 5-HT1 receptors may become desensitised with the ensuing net effect that serotonergic transmission may be only mildly impaired. During analgesic and NSAID overuse, however, the combination of receptor desensitisation and transporter upregulation may lead to serotonergic hypoactivity. Together with noradrenaline and dopamine, serotonin is crucial for tuning cortical excitability including sensitization and habituation processes and its effect in animals varies with concentration and duration of application [37]. A more severe hypofunction of 5-HT transmission after NSAID overuse may thus explain the SEP sensitisation observed in this subgroup of MOH patients. Whether the difference between the drug classes with regard to central sensitisation is related to the clinical observation that withdrawal headache is much shorter after triptan than after analgesic overuse [38] remains to be determined in a properly designed prospective study comparing clinical outcome and electrophysiological patterns.

The association of electrophysiological sensitisation, i. e. increased 1^{st} block SEP amplitude, and lack of habituation.in MOH patients overusing NSAIDs is intriguing. It is at odds with the electrophysiological pattern associating high amplitude in 1^{st} block and normal habituation found during migraine attacks [10-14], but, as mentioned before, it has been described in the pre-ictal phase [9-11]. One possible explanation for the lack of habituation in episodic migraineurs between attacks is the "ceiling theory" [39] postulating that there is a low preactivation level of sensory cortices, also responsible for the low 1^{st} block amplitudes, would allow a larger range of activation before habituation occurs [6,8]. The habituation deficit in NSAIDs overusers cannot be explained by the "ceiling theory" since their high 1^{st} block amplitude indicates rather that the somatosensory cortex is sensitised. There is at present no straight forward explanation for this pattern. It is likely, however, that other neurobiological mechanisms that participate in the production of habituation are impaired. For instance, inhibitory interneurons could be hypofunctioning because of the reduction in serotonergic transmission induced by the prolonged NSAID overconsumption. This hypothesis can be tested experimentally by searching if habituation normalizes during full-blown attacks in MOH patients like in episodic migraine and by exploring inhibitory cortical interneurons with dedicated neurophysiological studies such as that of cortical silent periods using transcranial magnetic stimulation. Given the similar neural mechanisms underlying sensory and behavioural sensitization [40], the interesting question arises

whether the sensory sensitization in patients with MOH parallels behavioural sensitization. Behavioural sensitization is paradigmatic of how the serotonergic, dopaminergic, and noradrenergic systems interact and contribute to central sensitization [41]. Brain circuits involved in addictive behaviour include ventral and dorsal striatum, amygdala and orbitofrontal cortex and are heavily modulated by dopaminergic projections from the ventral tegmental area of the midbrain, serotonergic projections from the median and dorsal raphe nuclei, and noradrenergic projections from the locus coeruleus [4,42]. According to DSM-IV criteria, many MOH patients manifest a dependence behaviour [43]. The latter has been associated with orbito-frontal cortex hypoactivity [44], an abnormality also found in subgroups of MOH patients [5]. The orbito-frontal cortex is thought to modulate habituation mechanisms [45] and orbito-frontal lesions induce SEP sensitization and lack of habituation [46], precisely the two sensory abnormalities we found in patients with MOH. Our findings along with current knowledge on the neurobiology of drug overuse therefore suggest that future studies seeking correlations between electrophysiological and metabolic measures should focus on the orbito-frontal cortex. In our study we did not control for associated depression and anxiety. Despite the evidence that cortical pain-related evoked potentials in MOH do not differ between subgroups of patients with or without depressive symptoms [28], it may still be appropriate to control for psychiatric comorbidity in future studies.

Conclusions

Cortical responses to repetitive sensory stimuli are abnormal in patients with MOH. Increased response amplitudes after low numbers of stimuli indicate sensory sensitization and lack of amplitude decrease during subsequent stimulations reflects a habituation deficit. This cortical response pattern is similar the one found in the immediate pre-ictal phase in episodic migraine, but different from the interictal and ictal patterns. It suggests that the somatosensory cortex has become persistently sensitized and that the migraine generating mechanisms in the central nervous system are not shut off. The sensitization is obvious in patients overusing NSAIDs and almost non-existent or masked in those who overuse only triptans. The different electrophysiological pattern between drug classes may be related to the clinical observation that withdrawal headache is shorter lasting in triptan overusers than in NSAID overusers. We postulate that the abnormal sensory processing in MOH patients reflects a drug-induced impairment of central serotonin neurotransmission, that the decrease of serotonergic activity is more profound after chronic NSAID overconsumption and that the cortical sensory sensitization parallels the behavioural sensitization that accompanies drug overuse and is crucially modulated by the medial orbitofrontal cortex.

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Authors' contributions

GC made substantial contributions to acquisition of data, analysis and interpretation of data as well as in drafting the manuscript. AC, VP, JS and FP were implied in the interpretation of data as well as in drafting the manuscript; gave critical revision of the manuscript for important intellectual content. CDL, MG and SLS were implied in recording data. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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ORIGINAL

Changes in visual-evoked potential habituation induced by hyperventilation in migraine

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Abstract Hyperventilation is often associated with stress, an established trigger factor for migraine. Between attacks, migraine is associated with a deficit in habituation to visual-evoked potentials (VEP) that worsens just before the attack. Hyperventilation slows electroencephalographic (EEG) activity and decreases the functional response in the occipital cortex during visual stimulation. The neural mechanisms underlying deficient-evoked potential habituation in migraineurs remain unclear. To find out whether hyperventilation alters VEP habituation, we recorded VEPs before and after experimentally induced hyperventilation lasting 3 min in 18 healthy subjects and 18 migraine patients between attacks. We measured VEP P100 amplitudes in six sequential blocks of 100 sweeps and habituation as the change in amplitude over the six blocks. In healthy subjects, hyperventilation decreased VEP amplitude in block 1 and abolished the normal VEP habituation. In migraine patients, hyperventilation further decreased the already low block 1 amplitude and worsened the interictal habituation deficit. Hyperventilation worsens the habituation deficit in migraineurs possibly by increasing dysrhythmia in the brainstem-thalamo-cortical network.

Keywords Migraine · Hyperventilation · Visual-evoked potentials · Habituation · Brainstem · Thalamo-cortical activity

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Introduction

Stress is a well known trigger factor for migraine [1]. Stress is often associated with hyperventilation (HV). HV induces several physiological changes in the human central nervous system and does so by altering eucarbia, local cerebral blood flow, brain tissue oxygenation, pH and lactate [2–5]. For instance, HV slows the electroencephalogram (EEG) by increasing delta-power and decreasing alpha-power [6, 7]. It also changes somatosensory-evoked potential latency [8], reduces the long-latency somatosensory-evoked magnetic fields [9], shortens the cortical silent period [10], and reduces the phospene threshold [11] elicited by transcranial magnetic stimulation (TMS). On functional neuroimaging studies, HV decreases or even abolishes the occipital cortex response to visual stimulation [12, 13]. The physiological changes induced in the brain culminate just after experimentally induced HV begins [14, 15].

A widely accepted and standardized test to assess excitability in the occipital cortex is the visual-evoked

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potential (VEP). As happens for other sensory modalities, during repeated stimulation the VEP habituates or adapts, i.e. progressively decreases in amplitude. Besides intervening in learning processes [16], habituation serves as a protective mechanism against excessive neuronal stress and accumulation of metabolites such as lactate and protons [17]. In healthy subjects, various stimulation procedures modulate VEP habituation. For example, low-frequency repetitive TMS [18] and tonic pain induced in the hand during a cold pressor test [19] abolish, whereas psychoactive drugs such as fluoxetine improve habituation [20].

In migraine patients, VEP habituation is reduced or abolished between attacks [21, 22]. Low-frequency (inhibitory) rTMS worsens the habituation deficits whereas high-frequency (facilitatory) repetitive TMS reverses it [18]. By contrast, tonic pain induced by the cold pressor test leaves the habituation deficit unchanged [23]. In migraineurs, evoked responses recorded from the visual cortex therefore display an abnormal VEP habituation pattern and the visual cortex responds inadequately to specific external or internal factors, for example HV. The neural mechanisms underlying deficient-evoked potential habituation in migraineurs remain unclear. Nor is it clear whether HV induces similar changes in habituation in healthy subjects and patients with migraine. A better neurobiological insight into habituation mechanisms would help understand the interictal pathophysiology of migraine.

In this study, to investigate the potential role of HV in modulating the interictal abnormal information processing in migraine, we studied whether and how HV influences visual (occipital) cortical responses. In healthy subjects and migraine patients without aura studied between attacks before and after deep-breathing-induced HV, we recorded VEPs to checkerboard stimulation, measured N1–P1 and P1–N2 amplitudes to a low number of stimuli and assessed VEP habituation over subsequent amplitude blocks. Moreover, we search for correlations among the VEP amplitude changes and clinical variables.

Methods

Subjects

We enrolled a group of 18 consecutive migraine patients without aura (MO, ICHD-II code 1.1) (11 women and 7 men, mean age 30.5 years) who underwent VEP recordings during the interictal period, i.e. attack-free for at least 3 days before and after the recording sessions, and a group of 18 age-matched healthy subjects (12 women and 6 men, mean age 27.1 years) recruited from among medical school students and healthcare professionals. Inclusion criteria were absence of any overt medical condition, and no personal or family history of migraine or epilepsy. Women participants were always recorded at mid-cycle.

All participants received a complete description of the study and granted informed consent. The project was approved by the ethical review board "Sapienza" University of Rome, Polo Pontino. Participants taking regular medications and subjects who failed to reach a best corrected visual acuity of >8/10 were excluded.

Visual-evoked potentials

Subjects were sitting in a semi-dark, acoustically isolated room in front of a TV monitor surrounded by a uniform luminance field of 5 cd/m^2. To obtain a stable pupillary diameter, each subject adapted to the ambient room light for 10 min before VEP recording. VEPs were elicited by monocular right eye stimulation. Visual stimuli consisted of full-field checkerboard patterns (contrast 80%, mean luminance 250 cd/m^2) generated on a TV monitor and reversed in contrast at a rate of 3.1 s^{-1}. At the viewing distance of 114 cm, the single check edges subtended a visual angle of 15 min. Subjects were instructed to fixate with their right eye a red dot in the middle of the screen with the contralateral eye covered by a patch to maintain stable fixation. VEPs were recorded from the scalp through silver cup electrodes positioned at Oz (active electrode) and at Fz (reference electrode 10/20 system). A ground electrode was placed on the right forearm. Signals were amplified by Digitimer^{TM} D360 pre-amplifiers (band-pass 0.05–2,000 Hz, gain 1,000) and recorded by a CED^{TM} power 1401 device (Cambridge Electronic Design Ltd, Cambridge, UK). A total of 600 consecutive sweeps each lasting 200 ms were collected and sampled at 4,000 Hz.

After applying off-line a 35 Hz low-pass digital filter, cortical responses were partitioned in six sequential blocks of 100, consisting of at least 95 artifact-free sweeps. Responses in each block were averaged off-line ("block averages") using the Signal^{TM} software package version 3.10 (CED Ltd).

VEP components were identified according to their latencies: N1 was defined as the most negative peak between 60 and 90 ms, P1 as the most positive peak following N1 between 80 and 120 ms, and N2 as the most negative peak following P1 at between 125 and 150 ms (Fig. 1). We measured the peak-to-peak amplitude of both the N1–P1 and P1–N2 complex. Habituation was defined both as the change in amplitude of N1–P1 and P1–N2 recorded during the six blocks and the slope of the linear regression line for the six blocks. VEP habituation was evaluated before and immediately after HV. All recordings were collected in the morning (between 09.00 and 11.00 a.m.) by the same investigator.

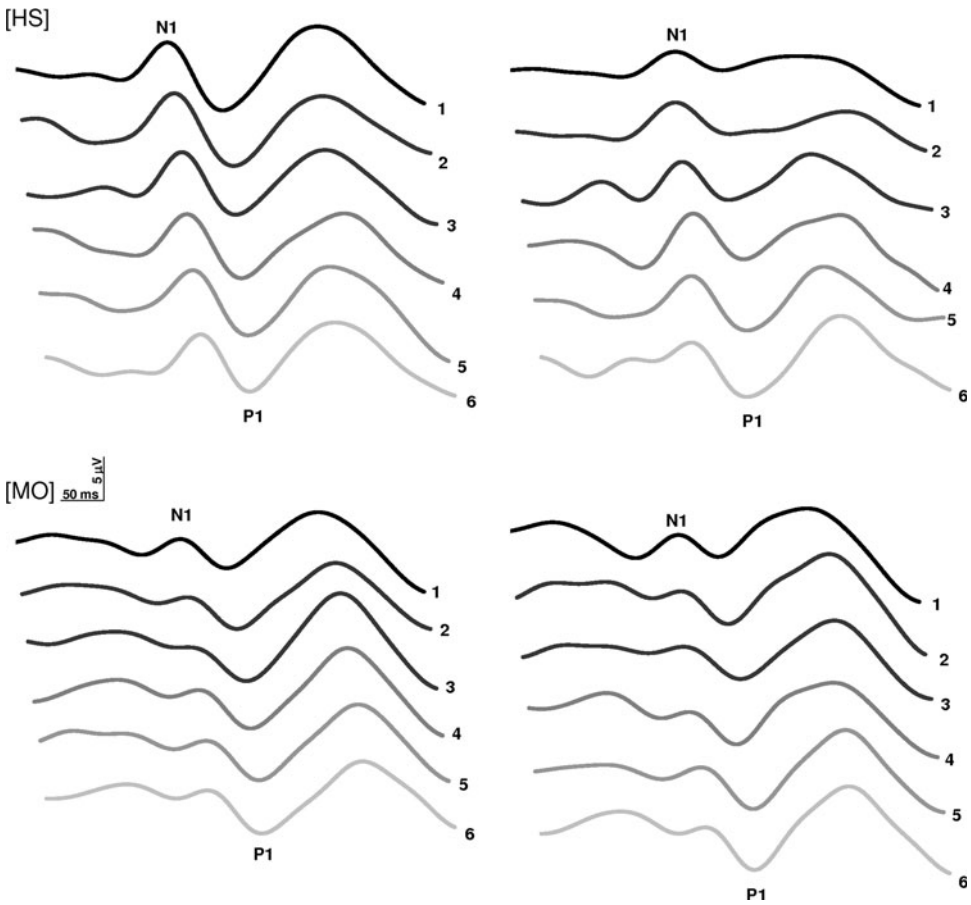

Fig. 1 Representative recordings of visual-evoked potential (VEP) habituation at baseline (*left*) and after 3-min hyperventilation (*right*) in a healthy subject [*HS*] and a migraine patient without aura [*MO*]

Hyperventilation

The subjects were instructed to practice voluntary HV for 3 min by breathing deeply at a constant rate paced by a metronome at 40 times per minute. The post-HV VEP was recorded immediately after HV.

Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS) for Windows, version 15.0 for all analyses. We constructed a multivariate analysis of variance (ANOVA) taking as a within-subject factor "block" and as between-subject factors "Group" (HS, MO) and "time" (before and after HV). A regression analysis was used to disclose linear trends in VEP amplitude across blocks in each condition and group (slope). Student's paired-sample *t* test was used to compare block 1 VEP amplitude before and after HV in both groups. Fisher's least significant difference (LSD) test was used for post hoc analysis. Pearson's correlation test was used to search for correlations among the VEP amplitude slopes and clinical variables. *P* values less than 0.05 were considered to indicate statistical significance.

Results

VEP recordings from all participants yielded analyzable data (Table 1).

During the third minute of HV most subjects complained of light headedness and a sensation of cold. One subject had a mild right carpal spasm that resolved rapidly with normal breathing. The mean breathing rate per minute was similar in healthy subjects and patients ($P > 0.05$).

ANOVA testing amplitude in averaged N1–P1 VEP amplitude blocks disclosed a main effect for factor block [$F_{(5,340)} = 6.76$, $P < 0.001$], a significant two-way interaction of group by block [$F_{(5,340)} = 2.73$, $P = 0.019$], and session by block [$F_{(5,340)} = 4.45$, $P = 0.001$], but not a three-way interaction of block by session and group [$F_{(5,340)} = 0.58$, $P = 0.708$]. Linear regression analysis showed that VEP amplitudes recorded in all blocks differed between sessions in both groups [in healthy subjects $F_{(1,34)} = 9.02$, $P = 0.005$ and in patients $F_{(1,34)} = 5.50$, $P = 0.025$]. Post hoc analysis showed that before induced hyperventilation in healthy subjects the linear trend in VEP amplitudes decreased from blocks 1 to 6 (−0.12), whereas in patients it increased [+0.03; $F_{(1,34)} = 9.49$, $P = 0.004$]. Conversely, after hyperventilation, the linear trend in

Table 1 Clinical and demographic characteristics of healthy subjects (HS) and migraine patients without aura (MO)

Characteristics	HS ($n = 18$)	MO ($n = 18$)
Women (n)	12	11
Age (years)	27.1 ± 7.7	30.5 ± 9.5
Duration of migraine history (years)		18.0 ± 3.1
Attack frequency/month (n)		2.0 ± 1.4
Attack duration (hours)		20.2 ± 18.3
First minute of hyperventilation (rate/min)	44.1 ± 5.1	44.7 ± 4.8
Second minute of hyperventilation (rate/min)	48.4 ± 5.2	44.8 ± 4.7
Third minute of hyperventilation (rate/min)	49.8 ± 4.3	47.7 ± 3.8

Data are expressed as mean ± SD

VEP amplitudes increased from blocks 1 to 6 in both groups [in healthy subjects +0.04, in patients +0.19; $F_{(1,34)} = 4.19$, $P = 0.04$] (Fig. 2). Paired t test showed that the baseline block 1 VEP amplitude decreased significantly after hyperventilation in both groups [healthy subjects, $t_{(1,17)} = 3.18$, $P = 0.005$, and patients $t_{(1,17)} = 3.12$, $P = 0.006$].

ANOVA testing amplitude in averaged P1–N2 VEP amplitude blocks disclosed a main effect for factor block [$F_{(5,340)} = 2.47$, $P = 0.032$], a significant interaction of session by block [$F_{(5,340)} = 3.34$, $P = 0.006$], but not of block by group [$F_{(5,340)} = 0.32$, $P = 0.89$] and of block by session and group [$F_{(5,340)} = 1.09$, $P = 0.365$]. Linear regression analysis showed that VEP amplitudes recorded in all blocks differed between sessions in the healthy subjects group only [in healthy subjects $F_{(1,34)} = 15.51$, $P < 0.001$ and in patients $F_{(1,34)} = 0.78$, $P = 0.383$]. Post hoc analysis showed that before induced hyperventilation in healthy subjects the linear trend in VEP amplitudes decreased from blocks 1 to 6 (−0.11), whereas in patients it increased [+0.05, $F_{(1,34)} = 9{,}66$, $P = 0.003$ vs. controls]. Conversely, after hyperventilation, the linear trend in VEP amplitudes increased from blocks 1 to 6 in both groups [in healthy subjects +0.11, in patients +0.17, $F_{(1,34)} = 0.42$, $P = 0.517$] (Fig. 2). Paired t test showed that the baseline block 1 VEP amplitude was unchanged after hyperventilation in both groups [healthy subjects, $t_{(1,17)} = 0.29$, $P = 0.588$, and patients $t_{(1,17)} = 0.17$, $P = 0.677$].

Pearson's test disclosed no significant correlation between clinical characteristics and both VEP amplitude slopes in migraine patients.

Discussion

In the healthy subjects and patients with migraine without aura recorded between attacks, we studied 3-min deep-breathing-induced HV significantly changed the VEPs to

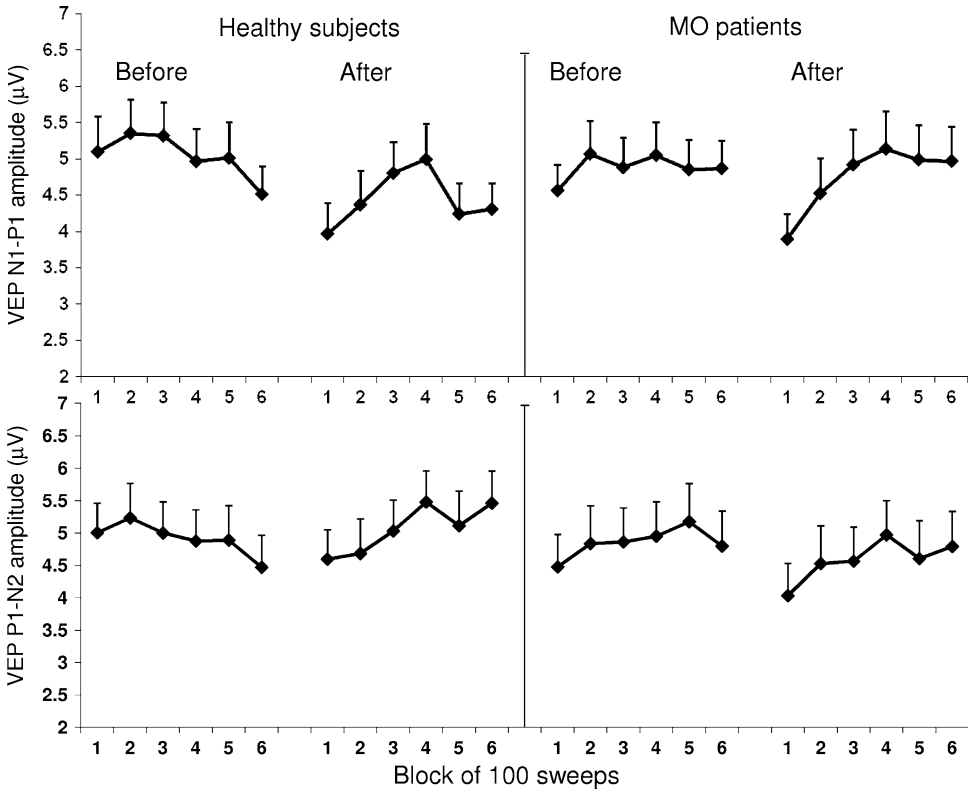

Fig. 2 Visual-evoked potential N1–P1 block amplitudes (mean + SEM) before and after hyperventilation lasting 3 min in healthy subjects and migraine patients without aura

checkerboard stimulation. Another finding was that HV induced similar changes in VEP patterns in healthy subjects and migraine patients. After HV, VEP amplitudes to a low number of stimuli, i.e. in the first block of 100 averaged responses, decreased and the normal VEP amplitude habituation between the first and sixth block of averaged responses disappeared. These changes in cortical responsiveness confirm that deep-breathing-induced HV induces transient physiologic changes in brain functions, and does so by influencing the visual (occipital) cortex activation. As expected, subjects reported experiencing classic HV-induced symptoms including light headedness and sensations of cold, suggesting that they hyperventilated effectively. Two participants, a healthy woman and a male migraineur, also manifested symptoms of mild spasmophilia.

The reduced amplitudes in the N1–P1 first amplitude block after HV in healthy subjects and patients receive support from current neurobiological knowledge on HV-induced changes in hemodynamic status and EEG activity. In healthy subjects, HV reduces cerebral blood flow and causes marked EEG slowing [4]. The EEG changes may reflect causes other than reduced blood flow, given that cerebral vasoconstriction without concomitant alkalosis and a low partial pressure of carbon dioxide (pCO_2) leave the EEG unchanged [24–26]. After HV, EEG delta power increases and alpha power decreases [6, 7, 24]. Observations that are especially relevant to the reduced amplitudes we found in the first N1–P1 amplitude block are that EEG alpha activity predominates in occipito-parietal areas and studies using simultaneous EEG and near-infrared spectroscopy show that low alpha amplitudes in healthy subjects are related to small oxygenation responses and low VEP amplitude [27]. For sake of completeness, we report that some early studies showed no significant HV-induced effects on VEP amplitude [28, 29]. However, relevant technical and methodological considerations render unlikely a direct comparison of findings from these studies with those hereby presented (different stimulus parameters, frequency of pattern reversal, spatial features of checks, sweep recording times, and experimental procedure for VEP acquisition during or after HV).

Several observations help explain which brain areas mediate the HV-induced EEG changes in VEP amplitudes. Because cortical rhythmic activity arises from an interplay between thalamic relay cells with cells in the reticular nuclei and cortico-cortical reverberant loops [30, 31], the VEP changes we recorded after deep-breathing-induced HV could plausibly depend on thalamic neuronal hyperpolarization. This neural mechanism accords perfectly with early evidence that lesions involving the anterior pole of the thalamus (nucleus centralis lateralis) abolish the cortical response to HV [32, 33]. Another major brain nervous structure involved in HV-induced EEG changes is the reticular formation. The mesencephalic reticular formation is as sensitive to CO_2 as the classic respiratory centres, and hypocapnia may disinhibit the normally inhibited neurons in the mesencephalic reticular formation that synchronize cortical activity thereby resulting in slow-wave EEG [34, 35]. Hypercapnia can produce cortical arousal and hypocapnia cortical depression by acting directly on mesencephalic structures [35]. Stimuli activating the mesencephalic reticular formation also facilitate oscillatory activity in the gamma-frequency range and enhance the stimulus-specific synchronization of neuronal spike responses in the visual cortex of cats [36]. The HV-induced reduction in N1–P1 VEP first block amplitude we found in healthy subjects and migraineurs might reflect a transient thalamic dysfunction possibly arising when hypocapnia related to HV interferes with neural activity in brainstem respiratory centres [26, 37, 38].

The second distinctive finding in our study is that in healthy subjects (both N1–P1 and P1–N2 amplitudes) and patients with migraine (N1–P1 amplitude only), experimentally induced-HV dampened VEP amplitude habituation. A possible explanation calls into question the temporal relationship between VEP recordings and the duration of HV-induced EEG changes. Alpha power recovers rapidly soon after HV ends [15], and the recovery time course matches that of the blood-oxygen-level-dependent (BOLD)-contrast functional MRI signal [39]. Our VEP recordings started immediately after induced HV began and lasted ±200 s. We might therefore have recorded the last six blocks of 100 responses during the alpha activity recovery phase that leads to an increase in VEP amplitude after the first block, and thus lack of habituation measured over successive blocks. Although this explanation receives apparent support from our finding that VEP amplitude tends to habituate between the fourth and sixth block (−18.8%) (Fig. 2), HV induced no 4–6 block habituation in migraine patients (−3.2%, $P = 0.07$ vs. controls), possibly because other external and internal factors intervened.

Our new findings also expand our previous report describing deficient VEP habituation at rest in migraine patients between attacks [21, 22] now showing that HV worsens this deficit mainly by decreasing further the already abnormally low amplitude in the first VEP block. In patients, unlike healthy subjects, habituation failed to return between the fourth and sixth blocks suggesting that in migraineurs the brain mechanisms responsible for habituation are malfunctioning. In an earlier study we showed that the late evoked component of VEP gamma band oscillations, which reflects visual stimulus processing by cortical neurons, does not habituate normally in migraineurs [40]. We attributed this finding to a functional

thalamus disconnection due to hypofunctioning serotonergic projections from the brainstem [40, 41] and thus proposed including migraine in the so-called thalamo-cortical dysrhythmia syndromes [42, 43]. The so-called chemically addressed state-setting brain stem nuclei modulate thalamo-cortical activity and cortical excitability [44]. These state-setting systems in the brain stem contain noradrenergic, serotoninergic and histaminergic neurons that are chemosensitive to CO_2 tensions [45–47]. Evidence that serotonergic raphe neurons, for instance, increase their firing rate during hypercapnia explains their role in respiratory control but also in arousal. If their firing rate decreases during HV then decreased firing might further aggravate their hypofunction in migraineurs and worsen the thalamocortical dysrhythmia. Here, whether the known persistent increase in lactate levels induced by HV [2, 4, 48] plays a role in the VEP changes remains to be determined. Collectively the above-mentioned findings seem to support our earlier hypothesis that the interictal habituation deficit in migraine reflects reduced thalamocortical drive and hence a low preactivation level of sensory cortices [21, 22].

In our study, VEP changes could in theory also derive from reduced efficiency of inhibitory circuits. But comparison between the time course of HV-induced modifications in inhibitory mechanisms as revealed by TMS studies (5–10 min after HV ends [10, 11]) and in our sensorial responses following repetitive stimulation (immediately after HV ends) renders unlikely that our HV-induced VEP amplitude changes derive from transient inhibitory cortical dysfunction.

Finally, certain limitations of the present study should be acknowledged. First, investigators were not blinded for subjects' diagnosis, thing that typically happens in this kind of study. Second, although all participants to the study reported classic HV-induced symptoms or manifested mild spasmophilia, the investigators performed no measure of end-tidal pCO_2 to ensure adequate hyperventilation level. Such a missing data should not be considered detrimental since a study comparing EEG modifications induced by standardized (i.e. with end tidal pCO_2 measures) and non-standardized hyperventilation (i.e. without end tidal pCO_2 measures) showed that both procedures changed the spectral power density of EEG in all frequency bands [6].

In conclusion, experimentally induced-HV lasting 3 min decreases VEP amplitudes to a low number of stimuli (first block) and abolishes normal VEP habituation during subsequent visual stimulation. The VEP changes in healthy subjects and migraine patients suggest that hypocapnia induces changes in chemosensitive aminergic nuclei in the brain stem. In migraine patients the HV-induced changes worsen patients' pre-existing thalamo-cortical dysrhythmia. These findings raise the intriguing question of how hyperventilation changes VEPs in migraine patients recorded during the attack, when VEP habituation becomes normal [49].

Conflict of interest None.

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