Exploring cortical excitability and photosensitivity in neurological disorders using pupillary response to sparse multifocal stimuli

A thesis

submitted for the degree of Doctor of Philosophy of The Australian National University

by

Eman Nassim Ali

(May 2016)

I declare that the research material in this thesis has not been submitted or accepted for the award of any other degree or diploma at any university. The material presented in this thesis is my own original work. Experimental data Collection and analysis was carried out by myself. This thesis, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made in the text. The experimental chapters are presented as papers for publication.

Eman Nassim Ali

2016

The studies in this paper have been present as platform and poster presentations at National and International conferences

ALI, E. N., LUECK, C. J., CARLE, C. F., KOLIC, M. & MADDESS, T. Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective perimetry. International Pupil Colloquium, 2015 Oxford, UK.

ALI, E. N., LUECK, C. J., MARTIN, K., BORBELJ, A. & MADDESS, T. Characterization of the pupillary response in epilepsy patients using multifocal objective pupillographic perimetry. International Pupil Colloquium, 2015 Oxford, UK.

ALI, E. N., MADDESS, T., CARLE, C. F. & LUECK, C. J. Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective pupillometry. J Neurology, 2014 Joint Congress of European Neurology, Istanbul, TURKEY S110-S111.

A further seven presentations of the work in my thesis have been given by my collaborators at the NANOS, AAN, and ARVO conferences between 2012 and 2016. I also published the following paper during the course of my PhD which arose from my preceeding MSc in Neuroscience at the ANU.

ALI, E. N., MADDESS, T., JAMES, A. C., VOICU, C. & LUECK, C. J. 2014. Pupillary response to sparse multifocal stimuli in multiple sclerosis patients. *Multiple Sclerosis Journal,* 20**,** 854-861.

Acknowledgments

In the process of writing this thesis I found my way, I found my voice, I found my true aim.

I would like to thank all the participants in my research; it was humbling to see them give their time in support of science in spite of their illnesses.

I would like to thank the people whose encouragement made me go on, starting with Professor Ted Maddess who gave me the chance to work in his group. He was always fully supportive, kind, and always went the extra mile to teach me, not only about science but also about life. He backed me up even during the times I doubted myself the most.

I would also like to extend my thanks to Professor Christian Lueck who gave me the chance to conduct my research in The Canberra Hospital and allowed me to use the facilities of the neurology department. I thank him for his continuous feedback regarding my work and for adding that extra touch of elegant English to my writing since it is not my mother tongue.

I thank Dr. Corrine Carle for being that friend that teaches you all the inside-and-outs of any new group you join, for supplying me with valuable references and scientific materials, and for teaching me how to operate my research-related device and the software needed for analysis.

I thank Maria Kolic for being a role model in clinical research organisation, recruitment, lab manners and data filing.

My thanks also go to Kate Martin, Angela Borbelj, and the rest of the staff of the neurophysiology lab at the Canberra Hospital for welcoming me into their lab despite their hectic schedules.

I thank the rest of the group: Professor Andrew James, the computer wizard, Dr Andrew Bell the eternal optimist, Dr Marconi Barbosa, Dr Faran Sabeti, Ms Bryony Curran, and Mr Adnan Muhammad for being a second family to me in Canberra.

I am grateful for the financial support I received from the Saudi Arabian Ministry of Higher Education, The Australian National University, and the the Australian Research Council through the ARC Centre of Excellence in Vision Science.

Also, I Thank Dr Anna Cowan who accepted me into the ANU neuroscience Masters program which led me to continue into the PhD program.

I also thank my real family: my mother, without whose prayers I would not have reached where I am today; my late father, who taught me that we should take advantage of every opportunity for learning; my brother, Fahad, who continued to encourage me in the same manner as if my father was still alive; and my sisters, Hala and Fatemah, who always backed me up.

I thank my two angels, Aysha and Ayah: they deserve an award for putting up with me all this time and, above all, my dear husband, Adel, whose encouragement and understanding were truly amazing throughout.

I hope to take the tools I have learned through my research back to my country, Saudi Arabia, which backed me financially during this once-in-a-lifetime journey.

Abstract

Epilepsy and migraine are two distinct paroxysmal neurological disorders yet both disorders overlap in multiple aspects. The objective of this thesis was to study two of the phenomena shared by these disorders, namely photosensitivity and cortical hyper-excitability. For this purpose, I chose the *multifocal objective pupillographic perimetry* device (mfPOP) to study the largely subcortical part of the visual pathway responsible for the pupillary response. The main objectives were first, to examine whether the objective perimetry results differed from the general population in these two disorders, second to explore whether there were differences between sub-types of the disorders and, third, to estimate the safety and tolerability of the mfPOP protocols that involve light stimulation. We also examined the effects of treatments that might affect brain excitability. I then went further and analysed electroencephalograms (EEG) recorded from epilepsy subjects looking at changes in the alpha rhythm entrainment in response to photic stimulation as an added indicator of cortical excitability. These effects were correlated with the mfPOP results.

Regarding photosensitivity, I found that the mfPOP device appeared to be safe when used in migraine subjects, even when a method designed specifically designed to stimulate the melanopsin containing retinal ganglion cells was used: these cells have been implicated in the mechanism of exacerbation of migraine headaches by light. Safety was also supported clinically and objectively for epilepsy patients by using EEGs and finding them to be clear of epileptiform activities during mfPOP testing.

Regarding cortical excitability, I found that this phenomena did indeed affect the pupil derived perimetry responses, with the responses being increased post-ictally and inter-ictally in epilepsy patients while being normal in the inter-ictal period in migraineurs and decreased after migraine attacks. These changes were reversed by two distinct classes of medication, anti-epileptics in epilepsy and triptans in migraine patients. Some disease-specific differences in the location of visual field defects were observed, especially for response sensitivity. Disease dependent changes in mfPOP response delays were observed, which were more generalised across the visual fields more than the sensitivity changes.

These findings were further supported by demonstrating that the alpha entrainment was more pronounced in epilepsy patients than the normal population and that it was also affected by medication use. These findings were consistent with the fact that both disorders have been shown to demonstrate an increase in cortical excitability in between attacks, this increase being more pronounced in epilepsy subjects. Medication consumption has a dampening effect on this excitability. During a migraine attack, this excitability is believed to transition to cortical spreading depression rather than to the hyper-synchronous activity that characterizes epilepsy.

These results, combined with our earlier study of Multiple Sclerosis, demonstrate that mfPOP is a valuable tool in studying neurological disorders especially in populations with motor deficits. With its safety now relatively well established it can help shed a light on the pathophysiology of these disorders and aid in their diagnosis.

Table of contents

Chapter 1.

Introduction

1.1. Overview

Epilepsy and migraine are among the most prevalent neurological conditions, and although they are two distinct neurological disorders characterized by transient paroxysmal episodes of altered brain function resulting in recurrent attacks of nervous system dysfunction with a return to baseline between attacks (Haut et al., 2006), both disorders converge in multiple aspects. It was as far back as 1906 that the coexistence of the two disorders in some subjects was highlighted by Gowers (Gowers, 1906). He went further to describe the occurrence of the visual, sensory and motor prodromes, and the associated symptoms of vertigo, pain, somnolence and delirium in both disorders. Since then, these similarities have been further highlighted in many studies that found that the presence of one disorder increases the probability of the other occurring in the same subject (Ottman and Lipton, 1996). Furthermore, the phenomenon of photosensitivity is shared by both disorders, and the underlying pathophysiology is attributed to altered ion channels and ion transporters (Ryan and Ptacek, 2010). These changes lead to a state of cortical hyper-excitability which is critical for generating epileptic seizures, and is also demonstrated between migraine attacks using visual evoked responses (Ambrosini et al., 2003), trans-cranial magnetic stimulation (Aurora et al., 1999) and electroencephalogram (Nyrke et al., 1990). However, hyper-excitability during a migraine attack is believed to transition to cortical spreading depression (CSD) rather than to the hyper-synchronous activity that characterises epilepsy (Parisi et al., 2008). Cortical hyper-excitability is believed to be the reason why treatments involving antiepileptic drugs are useful in both conditions (Silberstein

et al., 2012, Bianchin et al., 2010, Ziemann et al., 1996). Whether this excitability extends to involve subcortical pathways is not yet known.

We chose to explore changes in the pupillary response pathway in migraine and epilepsy because it serves as a subcortical pathway which is subject to cortical influence and it is part of the visual pathway which is primarily responsible for photosensitivity. We used the multifocal pupillary objective perimetry (mfPOP) device by itself or in conjunction with electroencephalography (EEG) to reach our goals.

In this introduction I will first highlight further the similarities between epilepsy and migraine; second, I will explore in depth the concept of cortical excitability and, third, I will discuss photosensitivity, its triggers and its postulated mechanisms. Then I will identify how the photic drive response seen in EEGs relates to both photosensitivity and hyper-excitability and, finally, I will discuss multifocal methods and why I chose the mfPOP device to explore these phenomena and how it may increase our understanding of the pathophysiology of migraine and epilepsy.

1.2. Epilepsy and migraine

1.2.1. Definitions

Migraine is a recurrent headache disorder that manifests itself in attacks lasting 4–72 h. Typical characteristics are unilateral location, pulsating quality, moderate or severe intensity, aggravation by routine activity and association with nausea and/or photophobia and phonophobia. It is divided into two major subtypes: migraine with

aura and migraine without aura according to the International Classification of Headache Disorders third edition (CHD-3) (2004, 2013).

Epilepsy is defined according to the International League Against Epilepsy (ILAE) as a disease of the brain with the possible features: (1) at least two unprovoked (or reflex) seizures occurring more than 24 hours apart; (2) one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; (3) diagnosis of an epilepsy syndrome (Fisher et al., 2014). Epilepsy can be broadly classified into two main types: generalized, which mainly includes idiopathic generalised epilepsy (IGE), and focal. IGE, is believed to have a strong underlying genetic basis, while focal epilepsies are mostly considered to be due to underlying focal pathology such as hippocampal sclerosis or an area of cortical dysgenesis (Fisher et al., 2014).

The term migraine-triggered seizures or "migralepsy" was introduced by Lennox and Lennox in 1960 and describes a condition in which ophthalmic migraine is followed by symptoms characteristic of epilepsy (Lennox and Lennox, 1960). Although this term has been the object of debate, it was included in the ICHD-3 (2013). In addition, two other disorders that highlight the coexistence of headache and epilepsy were also included in the ICHD-3: they are hemicrania epileptica, and postictal headaches.

1.2.2. Clinical aspects

Both epilepsy and migraine are chronic diseases with episodic attacks. Interictally, both disorders exhibit an increased predisposition to future attacks. During attacks, both are clinically manifested by repeated episodes of paroxysmal events, often preceded by prodromes and/or auras. **(Table 1)** summarises the clinical aspects that are common to

the two disorders (Bianchin et al., 2010). In addition, occasional attacks of both disorders may fail to stop resulting in status epilepticus or status migrainosus.

Epilepsy and migraine can precede or succeed each other, or even occur simultaneously. With respect to epilepsy the temporal relationship of headache and migraine occurrence is shown in **(Figure 1)** (Bianchin et al., 2010). 5% to 15% of patients with epilepsy report headaches pre-ictally. Ictal headaches are reported by less than 5% of patients and may reflect a true epileptic phenomenon, impairment of conciseness provoked by seizure leading to failure of subjects to report headaches may be attributed to this low prevalence. Postictal headaches occur in 10% to 50% of patients with epilepsy, often resembling migraines and responding to migraine treatments such as ergotamine derivatives or to triptans (Bianchin et al., 2010).

Some epileptic syndromes show a strong association with migraine by history, like benign occipital epilepsy of childhood (Caraballo et al., 2008). Others share genetic risk factors involving various components of ionic channels like those of benign rolandic epilepsy (Clarke et al., 2009), familial occipito-temporal lobe epilepsy (Deprez et al., 2007), and familial hemiplegic migraines (Barrett et al., 2008).

A further similarity between the two disorders that further supports the notion of a sometimes shared underlying mechanism is that some antiepileptic medications are useful in treating both conditions, specifically sodium valproate and topiramate which are used as preventive medications (Silberstein et al., 2012).

I will next discuss in details two major areas of similarity between migraine and epilepsy, namely cortical hyper-excitability and photo-sensitivity.

Table 1. Symptoms common to both migraine and epilepsy. Adapted with permission from (Bianchin et al., 2010)

Symptom	Migraine	Epilepsy
Systemic		
Vomiting	+	±
Nausea	+	±
Diarrhea	±	−
Headache	+	±
Visual disturbances		
Coloured circles	−	+
Black and white lines	+	−
Blindness	±	±
Blurred vision	+	+
Visual triggering factors	+	+
Other neurologic		
Olfactory	±	+
Vertigo	+	±
Confusion	±	+
Loss of consciousness	±**	+
Impaired consciousness	±	+
Loss of memory	±	+
Post event lethargy	+	+
Depersonalization	±	+
Paresthesias	+	+
Hemiparesis	±**	+
Hemi-sensory loss	±**	+
Aphasia	±**	+

+ denotes presence; − denotes absence; ± denotes rarely reported
**Present in hemiplegic migraine.

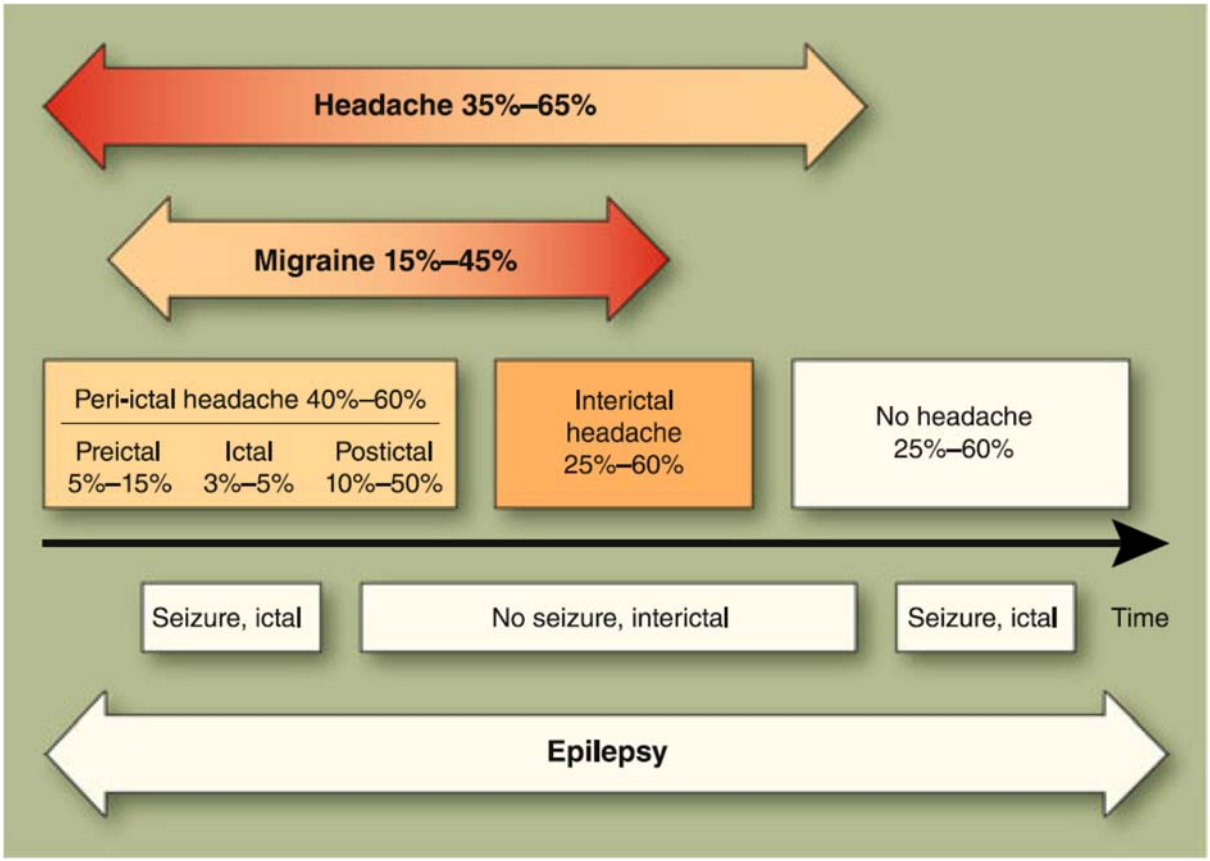


Figure 1. Time distribution of headache and migraine in epilepsy. Migraine and epilepsy are comorbid. They are bi-directional and one can precede or succeed the other or even occur simultaneously. Numbers represent approximate percentage range of headache occurring in respect of an epilepsy attack. According with seizures, headache can be classified as interictal, preictal, ictal, or postictal. Adapted with permission from (Bianchin et al., 2010).

1.3. Cortical hyper-excitability

The nervous system represents a complex arrangement of highly specialised neural circuits which are critically dependent on healthy excitatory and inhibitory systems.

Excitability of cell membranes appears to be a fundamental factor in the brain's susceptibility to various disorders. Excitation is mainly facilitated by the action of glutamate on N-methyl-d-aspartate (NMDA), and non-NMDA receptors, while inhibition is mainly mediated by the action of gamma-aminobutyric acid (GABA) on GABAA and GABAB receptors. Abnormal reorganization of brain circuits can result in various neurological disorders including epilepsy and migraine (Badawy et al., 2012).

In animal studies cortical excitability has been studied using direct current stimulation (DCS) where it was found that cortical neuronal depolarization is caused by anodal DCS at a subthreshold level, while they are hyperpolarized by cathodal DCS (Purpura and McMurtry, 1965). Alteration of membrane potential causes in turn alteration of spontaneous neuronal discharge (Purpura and McMurtry, 1965) and if the current is applied for a sufficient long period (10-30 minutes) after effects lasting for hours can be achieved (Bindman et al., 1962). changes in cyclic AMP generation are suggested to form the neurochemical basis of changes induced by anodal polarization (Hattori et al., 1990) . The after-effects of DCS are suggested to be due to NMDA receptor dependent (Liebetanz et al., 2002). Furthermore cyclic AMP accumulation is proposed as the basis of changes seen in induced chronic epilepsy animal models (Hattori et al., 1992) (Hattori et al., 1993) (Hattori, 1990). Also Agonists of all three major ionotropic glutamate receptors, quisqualate, kainate, and NMDA, were effective in inducing cortical spreading depression in turtles a key step in the generation of migraine aura (see below) (Lauritzen et al., 1988).

1.3.1. Migraine and cortical excitability

Mechanisms involved in the generation of a migraine headache are complex and not fully understood. It is believed that neurotransmitter disturbances, especially calcitonin

gene-related peptide and serotonin (Ogilvie et al., 1998), channelopathies (like the P/Q-type Ca2+ channel defects in familial hemiplegic migraine) (Ophoff et al., 1998), and cortical spreading depression with subsequent release of inflammatory mediators (Pietrobon, 2005) all play a role in migraine headache generation. The proposed cascade of events that leads to a migraine attack are summarized in **(Figure 2)** (Lance, 1996). At the start of a migraine attack, visual auras may appear. They consist of a scotoma with a scintillating border that usually begins near the centre of vision as a twinkling star and then develops into an expanding half-circle that slowly expands across the visual field toward the periphery. Looking at the underlying mechanism of these scotomas, Lashley postulated in 1941 that the scotoma results from a region of depressed neural activity in the visual cerebral cortex and that the scintillations result from a bordering region of intense cortical excitation (Tfelt-Hansen, 2010). This depression was later found to be associated with a reduction regional cerebral blood flow starting at the parieto-occipital region and spreading forward at a rate of 2-3 mm/min, corresponding to the speed of both the fortification spectra movement over the field of vision and the cortical spreading depression (CSD) observed by Leão in rabbits' EEGs. Leão was attempting to develop a model of experimental epilepsy by electrically stimulating the cortical surface; instead, he found that a weak electrical stimulation elicited a decrease in the spontaneous activity on EEG that spread out from the stimulated region in all directions at a rate of 3-5 mm/min and that spontaneous activity recovery occurred over 5 to 10 min (Leao, 1986). Thus, CSD is a wave of profound depression in neural activity preceded by neuronal activation, and so it is believed to underlie migraine aura and to be a trigger for the headache pain in migraine (Grafstein, 1956).

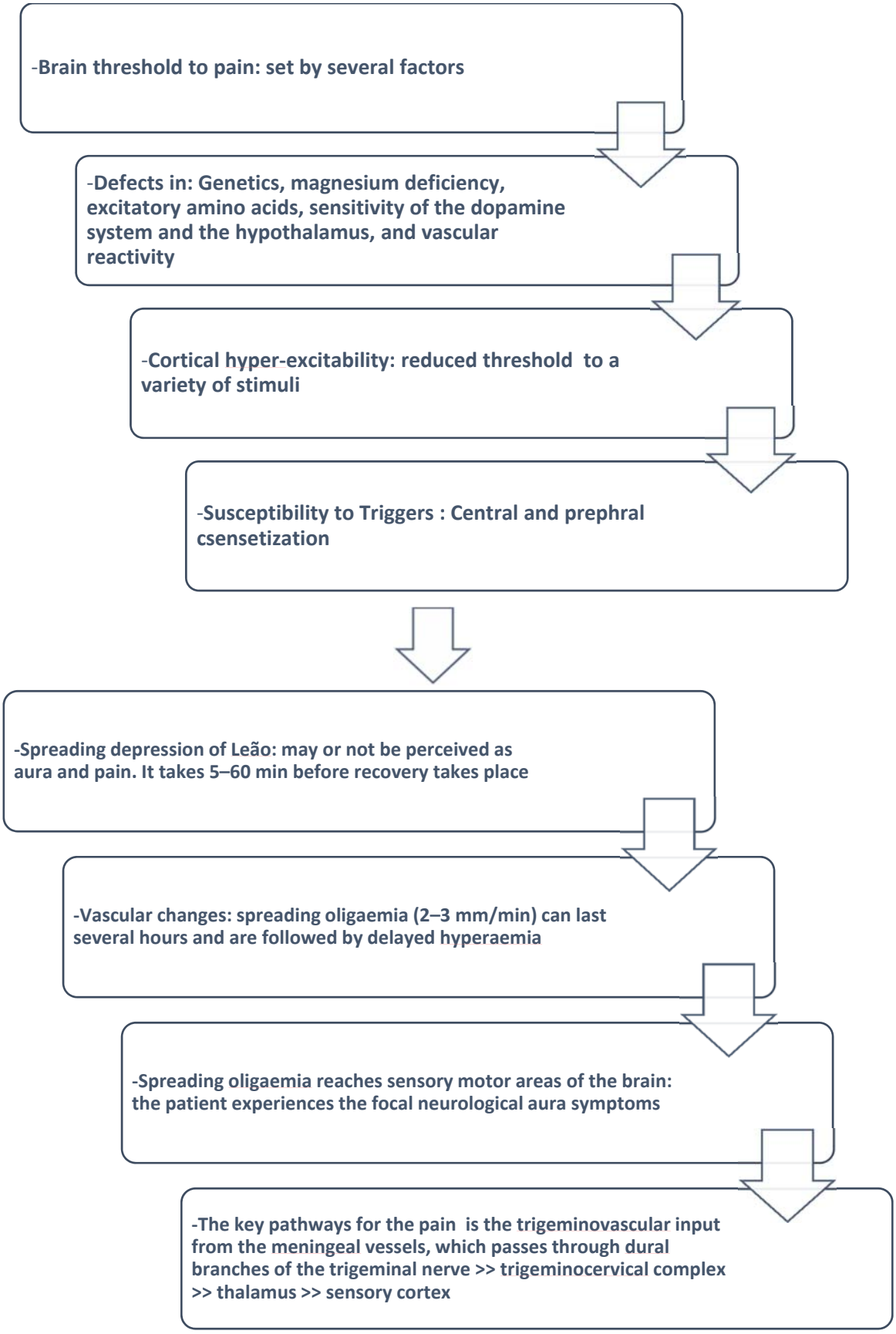


Figure 2. Mechanism of migraine attack. A flow chart showing the cascades of events underlying the pathophysiology of migraine headaches.

The existence of cortical hyper-excitability in migraine was confirmed using visual evoked potentials (Lehtonen, 1974, Connolly et al., 1982) which demonstrated evidence of increased P100 amplitude, as well as on transcranial magnetic stimulation (TMS). For both motor and visual occipital cortex TMS has been used to show increased cortical excitability between migraine attacks. Visual hyper-excitability occurred when TMS stimulation was delivered over the occipital cortex, leading to the perception of 'phosphenes' (flashes of light) which were reported by the subjects (Aurora et al., 1999, Bohotin et al., 2003, Gunaydin et al., 2006). Highly excitable neurons need to be stimulated less than depressed neurons in order to elicit either visual or motor responses, thus, TMS can be used to measure cortical excitability *in vivo*. Occipital cortical TMS studies reported evidence of hyper-excitability due to decreased inhibition, particularly in migraine with aura (Gunaydin et al., 2006, Aurora et al., 1999). Motor hyper-excitability has been demonstrated by recording a muscle response in the form of a twitch using electromyography (EMG) when TMS stimuli are delivered over the primary motor cortex. The results of such techniques showed a pattern of changes in migraine similar to those seen in epilepsy, although of much smaller magnitude. This provides more evidence supporting an overlap between the two paroxysmal disorders (Badawy and Jackson, 2012). Moreover, cortical excitability changes were found to change dynamically with respect to migraine attack timing (Bohotin et al., 2003, Judit et al., 2000).

1.3.2. Epilepsy and cortical excitability

Disturbance in the neuronal excitatory/inhibitory balance leading to the formation of hyper-excitable seizure networks is an important proposed mechanism underlying the pathophysiology of epilepsy (McCormick and Contreras, 2001). Alterations in cortical

excitability have been observed for 24, and even up to 48, hours before and after seizures. In addition, several factors have been found to alter this excitability including menstrual cycle, time of day, sleep and sleep deprivation, possibly explaining why these factors are considered to be epilepsy triggers in themselves. Epilepsy treatments including: antiepileptic medications irrespective of the underlying mechanism and target receptor, successful epilepsy surgery, vagal nerve stimulation or thalamic deep brain stimulation have all shown reduction of the baseline hyper-excitability to normal or near normal values in patients who have become seizure-free (Badawy et al., 2012). The mechanism, as proved by transcranial magnetic stimulation, understood to be through changes in either intracortical excitability caused by GABA-controlled interneuronal circuits in the motor cortex or changes in motor thresholds dependent on ion channel conductivity (Ziemann et al., 1996).

1.3.3. A shared mechanism for epilepsy and migraine

Ottoman and Lipton proposed that the comorbidity of migraine and epilepsy cannot be explained by genetic mechanisms alone, instead, they proposed that a state of brain hyper-excitability that results from genetic as well as environmental risk factors increases the risk of both conditions, thus leading to their comorbid association (Ottman and Lipton, 1996).

An example of shared genetic mechanisms between the two disorders is familial hemiplegic migraine (FHM) that predisposes to both migraine and epilepsy as a result of alteration in ion channels responsible for cell membrane homeostasis. FHM is an autosomal dominant syndrome characterized by severe migraine. It arises as a result of mutations in genes coding for the membrane ion transport proteins *CACNA1A* (P/Q-

type voltage-gated calcium channel), *ATP1A2* (Na^+-K^+ ATPase), or *SCN1A* (voltage-gated sodium channel). Mutations in all these genes can also cause *generalized*, and in some cases *focal* epilepsy, and are associated with the co-occurrence of FHM and seizures in the same family members (Barrett et al., 2008).

In general, many neurological episodic disorders – which include migraine and many types of epilepsies – are due to defects in ion channels and/or ion transport proteins that result in a reduced safety margin to regular stressors which in turn act as trigger factors that overcome homeostatic mechanisms that prevent the development of a pathological state (Ryan and Ptacek, 2010). **(Figure 3)** summarises the cellular events leading to the generation of an epileptic or a migraine event. A trigger is required as an initiator of an attack in both disorders causing glutamate release via voltage-gated Na^+ channels. This leads to hyper-excitability resulting in the development of hypersynchronous neuronal discharges through the AMPA receptors leading to the generation and spread of epilepsy, while in migraine NMDA receptors mediate the transition of hyper-excitability into CSD. This may explain why some antiepileptic medications do prevent migraine attacks whereas others, notably those acting on voltage-gated Na^+ channels or which act via GABAergic mechanisms (e.g. phenytoin , carbamazepine, vigabatrin and clonazepam), do not prevent migraine attacks (Rogawski, 2012). The broad range of propagation rates that happens in epilepsy as opposed to the relatively quick propagation in migraine is due to the fact that in epilepsy the glutamate release is entirely a synaptic process, while in migraine a wave of neuronal and glial depolarization that has a well-defined rate of propagation of about 3 mm/min occurs via intercellular gap junctions leading to CSD (Rogawski, 2012). As CSD propagates glutamic acid, released during CSD, convert into c- aminobutyric acid (GABA). This conversion has been suggested as the possible mechanism of hyperpolarizing type of

inhibition at the late phase of unit activity blockade after CSD (Mesgari et al., 2015). Applying a low concentration of a $GABA_A$ blocker has been shown to generate spiking activity during the late excitability state of CSD in epileptic human brain tissues and in non-epileptic rat brain (Dreier et al., 2012, Mesgari et al., 2015). These Changes during the late hyper-excitability phase of CSD have been suggested to contribute not only to the pathophysiology of migraine but to epilepsy as well (Dreier et al., 2012). In addition, brain pathologies associated with CSD include concussion, hypoxia and ischemia (Somjen, 2001) all of which are associated with epilepsy. Add to that neocortical CSD propagate to hippocampal structures- an area involved in the most common type of focal epilepsy temporal lobe epilepsy- affecting the hippocampal function (Wernsmann et al., 2006). As addressed above, migraine and epilepsy share many similarities in regards to cortical excitability and thus underlying pathophysiological mechanisms.

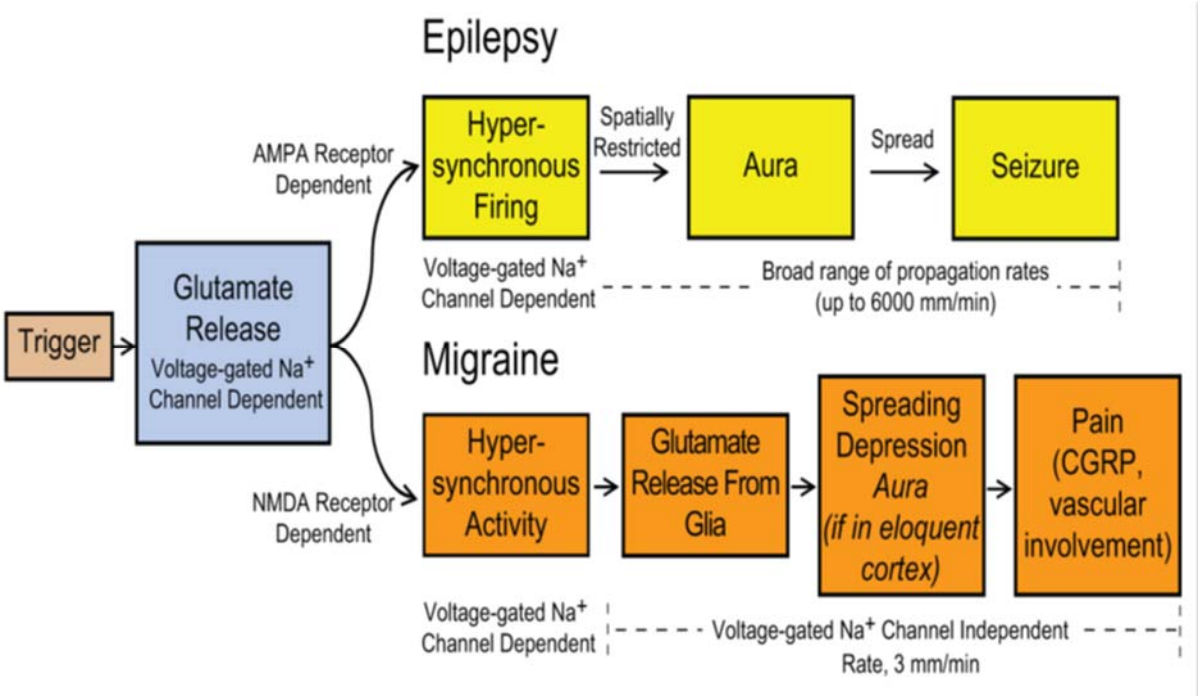


Figure 3: cellular events in the evolution of an epileptic seizure and migraine attack. Highlighting the similarities and differences. In epilepsy, synaptic glutamate release acting through AMPA receptors is a trigger factor, and synaptic activity is required for propagation. In migraine, synaptic glutamate acting through NMDA receptors is a trigger factor. Once established, synaptic activity may no longer be necessary and glutamate release from glia is the predominant factor that drives the advancing front of spreading depression. The spreading depression wave triggers the release of mediators that activate the trigeminovascular system, resulting in headache pain. Voltage-gated Na+ channel dependence (tetrodotoxin-sensitivity) implies the involvement of synaptic mechanisms. CGRP, calcitonin gene-related peptide. Adapted with permission from (Rogawski, 2012)**.**

1.4. Photosensitivity

Another important association between migraine and epilepsy is photosensitivity. I will outline several studies that have investigated the triggering role of photosensitivity in both headache and epilepsy.

1.4.1. Migraine and photosensitivity

Factors that induce attacks in susceptible migraine individuals, alone or in combination, are known as migraine triggers (Zagami and Bahra, 2006). They usually precede attacks by less than 48 hours (Zagami and Bahra, 2006). Identifying such trigger factors is important in management because treatment programs emphasise awareness and avoidance (Friedman and De ver Dye, 2009). In a literature review of migraine triggers, visual disturbances – defined as flicker, glare, and eyestrain – were identified as a trigger in 27 – 75% of cases (Martin, 2010). In general, light characteristics that can potentially cause discomfort are summarized as follows:

1) **Spatial pattern.** Patterns containing periodic elongated parallel lines of alternate light and dark stripes have been perceived as uncomfortable for migraine subjects (migraineurs) (82%) compared to non-migraine headache sufferers (18%) (Marcus and Soso, 1989). The pattern should stimulate a large portion of the visual cortex, for example by covering a substantial portion of the central visual field. Smooth-edged patterns are much less effective as visual triggers.

2) **Temporal pattern**. Periodic flicker at about 3 Hz for several seconds was perceived as a visual stressor in 80.6% of classic migraine subjects in comparison to only 14.9% of normal controls (Hay et al., 1994).

3) **Light intensity**. Between attacks, migraine sufferers reported decreased light discomfort threshold to whole-field flashes of around 95 Lux compared to controls at 200 Lux ($p < 0.00005$) (Main et al., 1997).

4) **Light wavelength.** In one study, red colour was reported as uncomfortable when migraineurs were asked to manipulate the colour of light falling on a passage of high contrast text (Chronicle and Wilkins, 1991). However, in another study results indicated that the migraine group had significantly lower discomfort thresholds at both low (blue) and high (red) wavelengths compared with tension-type headache and control groups (Main et al., 2000).

New insights into how light could modulate photosensitivity in migraine subjects was the subject of a study conducted by Noseda *et al* (Noseda et al., 2010) in which they examined the exacerbation of migraine headache by light in blind people. Six subjects had no light perception, either due to enucleation of the eye or to damage to the optic nerve (i.e. lacking input from all retinal layers) and when exposed to light, the intensity of these patients' headaches was unaffected, suggesting that migraine photophobia depends on signals relayed from the retina to the brain via the optic nerve. On the other hand, 14 legally blind subjects with preserved non-image-forming pathways (i.e. capable of detecting light in the face of markedly deficient image-forming perception, <20/200 vision) with histological evidence of total loss of the outer photoreceptor layer and preservation of the inner layers, had increase in both migraine headache intensity and photophobia with increased ambient light despite degeneration of both rods and cones. These findings led the authors to speculate that the exacerbation must be related to a non-image forming visual pathway mediated via the melanopsin-containing (intrinsically photosensitive) retinal ganglion cells (Hattar et al., 2002). Major differences between the image forming and non-forming pathways are summarized in **(Table 2).**

Table 2. Differential features between the classical and the melanopsin photoreceptive pathways. Adapted from (Benarroch, 2011)

Pathway	**Classical (image-forming)**	**Melanopsin (non-imaging-forming)**
Photoreceptor cell	Rods and cones	ipRGCs
Photo-pigment	Rhodopsin cone opsins	Melanopsin
Light sensitivity	All visible wavelengths	Most sensitive to blue wavelengths
Response to light	Hyperpolarization	Depolarization
Receptive fields	Very small	Very large
Properties	Fine spatial resolution	Temporal integration of ambient light (irradiance)
Main target of ganglion cells	Lateral geniculate nucleus Superior colliculus Olivary pretectal nucleus	Suprachiasmatic nucleus Subparaventricular zone Ventrolateral preoptic area Intrageniculate leaflet of the lateral geniculate nucleus Olivary pretectal nucleus
Function	Image formation Pupillary light reflex (early and transient response)	Entrainment of circadian clock Light-induced sleep regulation and inhibition of melanotonin secretion Pupillary light reflex (sustained response)
Involvement in disease	*Rod – cone dystrophies. *Mitochondrial optic neuropathy by loss of RGCs and optic nerve atrophy e.g. Leber hereditary optic neuropathy (LHON) and autosomal-dominant optic atrophy	Seasonal affective disorder Glaucoma

Unlike the image-forming pathway, which is generated by photo-activation of the opsin-based photo pigments in both the rods and cones and subsequent activation of the retinal ganglion cells (RGCs) whose axons form the optic nerve that travel to the lateral geniculate nucleus, the superior colliculus, and terminate in the visual cortex, the non-image forming pathway is mediated by a specialized pathway originating from intrinsically photosensitive RGCs (ipRGCs). These ipRGCs are not only activated by input from rods and cones but also activated directly by light acting intrinsically via the unique photo-pigment melanopsin. These retinal ganglion cells are unique in their ability to transduce light into electrical energy **(Figure 4)**. The axons of these Melanopsin-containing ipRGCs project via the optic nerve to several targets in the diencephalon and midbrain. These cells provide a major contribution to the afferent limb of the pupillary light reflex by sending a direct projection to the the suprachiasmatic nucleus, inter-geniculate leaflet, and, subsequently, the olivary pretectal nucleus of the midbrain; this nucleus projects to the Edinger-Westphal nucleu, which sends efferents to the ciliary ganglion.

Noseda's group went further to investigate whether the central trigemino-vascular pathway, which is responsible for carrying the pain signal from the dura mater to the brain, is also regulated by the non-image forming signals from the eyes and thus the melanopsin-containing ipRGCs. Using single-unit recording and neural tract tracing in the rat they found that the axon projections of the ipRGCs and dura-sensitive neurons, i.e. activity modulated by light, converged in the posterior thalamus and from there they projected extensively to the somatosensory cortex, visual, and associative cortices, leading them to hypothesize that migraine pain is modulated at the level of the thalamus by retinal activation of the ipRGCs **(Figure 5)**.

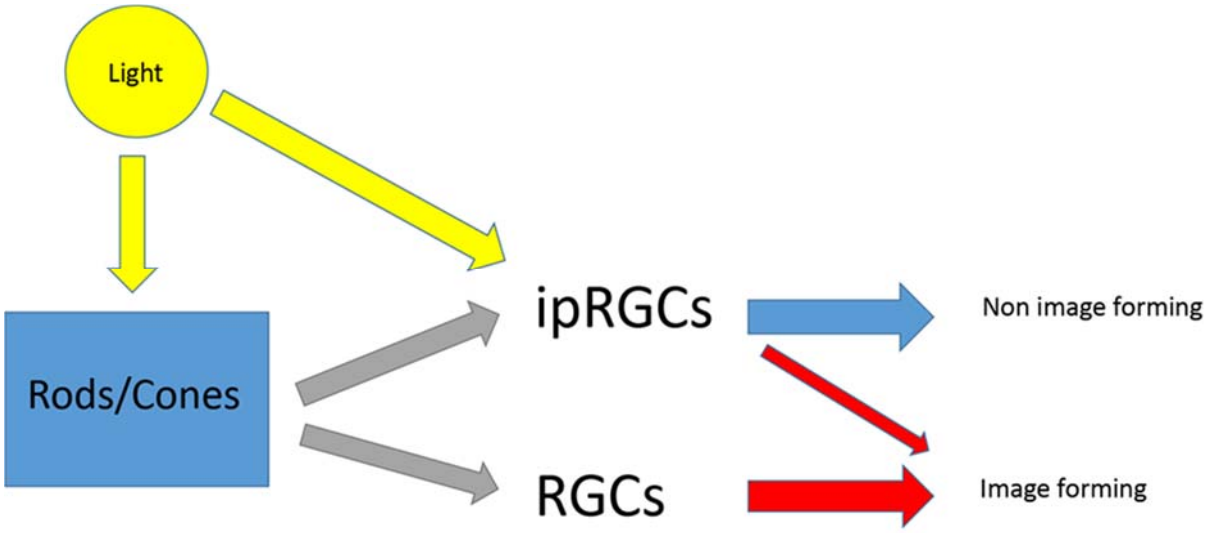


Figure 4. Visual pathways. *The image forming pathway:* light activates cones and rods in the retina which intern activate the RGCs and information is carried by the optic nerve (large red arrow). The non-image forming pathway is mediated by the ipRGCs, which in addition to being activated by rods and cones, they are intrinsically activated by light via the photo-active pigment melanopsin (large blue arrow). In addition ipRGCs also provide input to the image forming pathway (small red arrow) (Matynia, 2013)

ipRGCs = intrinsically photosensitive retinal ganglion cells, RGCs= retinal ganglion cells.

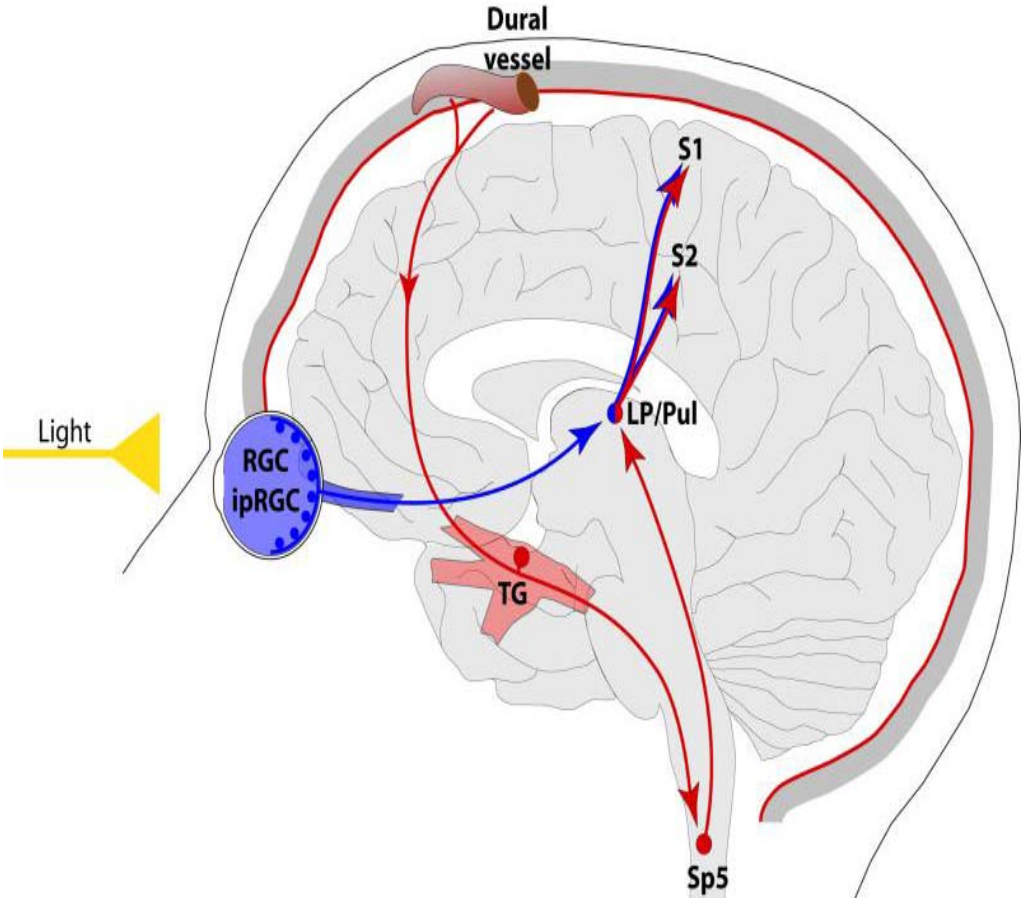


Figure 5. Proposed mechanism for exacerbation of migraine headache by light through the convergence of the photic signals from the retina and nociceptive signals from the meninges onto the same thalamic neurons that project to the somatosensory cortices. Red depicts the trigeminovascular pathway. Blue depicts visual pathway from the retina to the posterior thalamus. Abbreviations: RGC, retinal ganglion cells; ipRGC, intrinsically-photosensitive retinal ganglion cells; TG, trigeminal ganglion; Sp5, spinal trigeminal nucleus; LP, lateral posterior nucleus; Pul, pulvinar; S1, primary somatosensory cortex; S2 secondary somatosensory cortex. Adapted with permission from (Noseda and Burstein, 2011)

In addition to being responsible for adaptation of static pupillary size to light (Kawasaki and Kardon, 2007), ipRGCs have also been implicated in the entrainment of the biological clock to the dark-light cycle and suppression of melatonin release by light (Gooley et al., 2003).

Since 1998 when Provencio *et al* first identified a novel opsin from the dermal melanophore cells of the frog and called it *melanopsin* (Provencio et al., 1998). Since its subsequent discovery in humans (Provencio et al., 2000), several studies have highlighted the specific characteristics of these ipRGCs. Located in the ganglion cell layer of the retina, these cells have a giant somata and long, branching dendritic processes. The M1 subtype of ipRGCs extend their processes to the outer sublayer of the inner plexiform layer (where an "off" response is triggered upon the interaction with blue – short wavelength – S cones), and the M2 subtype project to the inner sublayer (where the rods and red –long wavelength – L cones, and green – medium wavelength – M cones provide an "on" response) (Hattar et al., 2002, Baver et al., 2008).

The retinal area with the highest ipRGC density was found to be located around the fovea with peak density of 20-25 cells/mm^2 (Dacey et al., 2005). Melanopsin has a peak spectral sensitivity at ~ 480 nm, i.e. blue/cyan light (Dacey et al., 2005). The probability of absorbing a photon by ipRGCs is >1 million times lower than in rods or cones for a given area of photostimulation (Do et al., 2009). Therefore, in order to ensure optimal melanopsin-driven sustained pupil responses, a bright light stimulus presented at greater than about 1 per second is needed (Park et al., 2011).

New modalities of migraine treatments have emerged based on our knowledge of the melanopsin-containing ipRGCs, such as pharmacological manipulation of melanopsin via the a small molecule (opsinamide) that antagonizes melanopsin-mediated phototransduction (Jones et al., 2013). Even before the discovery of the ipRGCs, blocking blue wavelengths

using tinted lenses in children with migraine was found to reduce frequency, duration and intensity of migraine attacks (Good et al., 1991).

Therefore, the study of the pupillary response to understand the effects of ipRGC stimulation on migraine pathophysiology may have a role in helping to decrease the burden of this disorder.

1.4.2. Epilepsy and photosensitivity

1.4.2.1. Definitions

In order to address photosensitivity in epilepsy patients I will start with some definitions.

- *Photic-induced seizures* (PIS) constitute part of a larger subtype of epilepsies known as reflex epilepsies. They are defined as seizures provoked by visual stimulation. The usual stimulus is a flashing light, but it can be patterns of lines, gratings, checkerboards, or other configurations (Fisher et al., 2005).
- *Photosensitivity* is an abnormal visual sensitivity of the brain in response to flickering or intermittent light sources or patterns; it is expressed in the electroencephalogram (EEG) as a generalized spike-and-wave discharge (photoparoxysmal response, PPR) (Covanis, 2005, Fisher et al., 2005, Harding, 1994).

1.4.2.2. Epidemiology

The estimated prevalence of seizures from light stimuli is approximately 1 per 4,000 individuals aged 5-24 years. 2% of epileptic patients have PIS but its prevalence increases up to 10% when patients aged 7–19 years are studied (Fisher et al., 2005). It is more common in

females and it is believed that there may be an important genetic component, probably autosomal dominant inheritance with reduced penetrance, although no major photosensitivity gene has yet been identified. The most common form of PIS is a generalized tonic-clonic convulsion (79%), followed by absence seizures (occurring in 10% of patients) and myoclonic seizures (occurring in 6% of patients), with focal seizures occurring in 5% (Fisher et al., 2005). The pathophysiology of photosensitivity is believed to be related to increased excitability of the occipital cortex as demonstrated by functional MRI and transcranial magnetic stimulation studies (Chiappa et al., 1999, Siniatchkin et al., 2007).

1.4.2.3. Light characteristics

Seizures can be provoked by certain TV shows, movie screen images, video games, natural stimuli (e.g. sun on water), public displays, and many other sources. Identifying light characteristics that provoke PIS is of great importance. Several studies have attempted to do so (Covanis, 2005, Harding et al., 2005, Parra et al., 2005, Wilkins, 1995). These characteristics are summarised as follows:-

1. **Stripe patterns:** with sharp edges, high contrast and a 50:50 duty-cycle.
2. **Flicker**: at > 3 Hz, the risk is increased when the stimulus contains 5 or more parings of black/red or blue/red alternation (Parra et al. 2005).
3. **Checkerboard patterns** are about 5 times less likely to generate PIS than patches of stripes that have aspect ratios of 20 or more. Sine-wave gratings are quite ineffective.
4. **Intensities** of 0.2-1.5 million candlepower are in the range to trigger seizures.
5. **Frequencies** of 15-25 Hz are most provocative (range is 1-65 Hz).
6. **Stimuli subtending more than a quarter of the central 10 degrees of the visual field;** much of the visual cortex is dedicated to that part of visual space, and so concurrent stimulation of a large part of the cortex can be achieved by stimulating just

that area of visual space. Stimulating greater than 10% of the cortex is required to generate a PIS.

7. **Synchronous activation of both eyes** increases the probability of epileptiform EEG by about 4-fold over a broad range of luminance.

Combination of both of spatial and temporal stimulus aspects are required to have a 60% chance of inducing PIS.

1.4.2.4. Light-emitting devices and photic induced seizures

Recommendations on reducing the risk of seizures have been developed by agencies in the United Kingdom, Japan, and the International Telecommunications Union, affiliated with the United Nations. Guidelines were developed following several incidents. In 1993, three people in the United Kingdom reported seizures while watching a television commercial for *Golden Wonder Pot Noodle* due to rapidly flashing contrast changes. This led the British government to respond by investigating what could be done to prevent future similar occurrences. The television regulatory agency introduced broadcast guidelines and has subsequently refined and updated them (Fisher et al., 2005). Another incident occurred in 1997 in Japan in which an episode of the *Pokemon* cartoon caused 685 children to visit hospitals with 560 cases shown to have had proven seizures triggered by a four-second sequence of alternating saturated red and blue light used in the program. Only 24% of those who had a seizure during the cartoon had previously experienced a seizure. Japan subsequently adopted formal guidelines on flashing and regular patterns based on the United Kingdom guidelines (Fisher et al., 2005). To facilitate broadcasting screening, the United Kingdom introduced an automatic screening device, “The Harding Test” (Cambridge Research Systems, Rochester, U.K.), which is an automated test for PIS-provocative image sequences in television content. The test screens for luminance flash activity, red flash

activity, extended flash warnings, number of luminance flashes, and number of red flashes (Harding and Takahashi, 2004).

All these guidelines that evaluate the safety of artificial light-emitting sources in patients with PIS rely on comparison against stimulus parameters which are known to provoke seizures. However, such guidelines lack objective proof of safety. Moreover, no guidelines exist for light-emitting medical devices. One approach is to assess their safety using electroencephalography (EEG) while testing subjects under controlled conditions; such an approach has been used to assess pattern-induced seizures (Wilkins et al., 1979). For better understanding of how to use EEGs in such circumstances I will briefly review EEG changes during photic stimulation.

1.4.2.5. Photosensitivity and electroencephalography

During routine EEGs, intermittent photic stimulation (IPS) delivered via a stroboscopic light source is used as a provoking method to detect additional abnormalities. IPS effects on the human EEG were first studied by Adrian and Matthews in the 1930s (Schomer and Lopes da Silva, 2005). Walter et al were the first to report paroxysmal discharges elicited by IPS using strobe light (Walter et al., 1946). The standard screening methods for assessing photosensitivity involve the presentation of IPS of varying frequencies (depending on the EEG laboratory) in trains of about 5 seconds duration, first with eyes closed and then with eyes open in a room with reduced illumination (Bickford et al., 1952). The three main EEG changes induced by IPS are: 1) photoparoxysmal response (PPR); 2) photomyoclonic response, and 3) photo-entrainment or photic drive response (PDR) (Schomer and Lopes da Silva 2005).

1. **Photoparoxysmal response (PPR).** This occurs in 2.8% of patients referred for an EEG examination (Jeavons and Harding, 1975). It is characterized by spike-and-wave

or polyspike-and wave complexes which are bilaterally synchronous, symmetrical, and generalized. The response may outlast the stimulus by a few seconds. PPR can be elicited by a broad range of IPS frequencies (1 – 65 Hz), most commonly 15-18 Hz (15 Hz when eyes are closed and 20 Hz when they are open). Frequencies of 15 and 20 Hz were reported to be the most commonly provoking frequencies in up to 96% of patients (Harding 1994), although this is subject to variability and may range from one flash per second to up to 84 flashes per second.

2. **Photo-myoclonic responses (PMR).** These are characterized by forehead and muscle twitching in response to light flashes and disappear with eye opening (Fisher et al. 2005). The signal on EEG is electromyographic in origin, arising in the orbicularis oculi and frontalis muscles in particular, though it can be associated with eyelid flutter (Fisher et al. 2005). The PMR is time-locked to the stimulus (Kasteleijn-Nolst Trenite et al., 2001). It is triggered by IPS frequencies ranging from 12 to 18 Hz. It occurs in in 0.3% of normal subjects, and 3% of patients with epilepsy (Schomer and Lopes da Silva 2005). Other causes that may contribute to the occurrence of PMR include brain stem lesions, psychiatric disorders such as anxiety, alcohol withdrawal in chronic alcoholics, barbiturate withdrawal, and severe hypocalcaemia (Schomer and Lopes da Silva 2005).
3. **Photo-entrainment of alpha rhythm - Photic drive response- (PDR).** This is a physiologic response consisting of rhythmic RRG activity time-locked to the stimulus at a frequency identical to, or harmonically-related to, that of the stimulus. It is elicited over the posterior region of the head by IPS frequencies of about 5 to 30 Hz (Schomer and Lopes da Silva 2005). It is more likely to occur around a baseline background activity of 2-4 Hz (Blum and Rutkove, 2007). The PDR is considered abnormal in the following circumstances: 1) an amplitude asymmetry greater than

50% at all frequencies of stimulation usually associated with structural brain disease, 2) an asymmetry in the development of the photic driving response associated with focal lesions of varying locations (60%) or associated with generalized cortical atrophy and/or ventricular enlargement (40%), or 3) the presence of unusual high-amplitude single spikes evoked by individual light flashes seen in seen in patients with diffuse encehalopathies (Coull and Pedley, 1978).

The source of the PDR is not fully understood. It was thought to be similar to visual evoked potentials due to the facts that the background rhythm is linked to the timing of the photic stimulator, the first response appearing shortly after the start of the stimulator (<100ms), and stopping when the stimulator stops (Blum and Rutkove, 2007). However, this does not explain why it happens in some people more than in others, nor why the response is not only identical to the IPS frequency but to its harmonics as well.

Unlike the PPR, changes in the PDR in epilepsy patients are less explored. Our interest in the PDR in epilepsy and our attempt to understand it better by assessing the pupillary response derive from several facts: first, that the PDR has been linked to cortical excitability (Simon et al., 1982); second, anatomically the thalamus acts as a convergence point for three important pathways, the visual pathway (which relays to the lateral geniculate nucleus); the thalamo-cortical pathway which plays a role in spike wave generation in epilepsy (involving the thalamic reticular nucleus) (Huguenard, 1999); and third, the alpha rhythm generator- which is entrained during PDR- and involves a complex interaction between cortical and thalamic oscillators particularly the lateral geniculate nucleus (Hughes and Crunelli, 2005). For these reasons I speculated that the PDR would differ in epilepsy patients from the normal population.

1.5. Multifocal methods and pupillary response assessment in neurological disorders

Visual evoked potentials (VEPs) have been used for many years in clinical neurology to demonstrate conduction delay in an optic nerve affected by inflammatory lesions, e.g. optic neuritis in multiple sclerosis (Polman et al., 2005). Changes in evoked potentials have also been seen in conditions leading to anatomical changes such as ischemic damage (Stoerig et al., 2002), neurotransmitter abnormalities such as Parkinson's disease (Muthane et al., 1993), phenylketonuria (Schafer and McKean, 1975), and in paroxysmal disorders such as migraine (Kennard et al., 1978) and epilepsy (Geller et al., 2005). However, these full-field pattern reversal stimuli involve presenting a single large stimulus to a relatively large proportion of the visual field, and measure a single aggregate VEP (the P100) using scalp electrodes placed over the occipital cortex. This technique has the potential to miss lesions which affect only a small portion of the visual field, since the VEP will pool responses from healthy and affected parts of the visual field. Due to these limitations the *multifocal VEP* (mfVEP) was developed. In mfVEPs many regions of the visual field can be tested independently and concurrently (Baseler et al., 1994), and this version of VEP has been reported to be better at detecting small lesions affecting the optic nerve (Davie et al., 1995). A more refined method has been developed by our laboratory in the Australian National University (James, 2009, Ruseckaite et al., 2005, James et al., 2005, James, 2003, Maddess et al., 2005) using temporally-sparse dichoptic stimuli. This method greatly enhances the signal-to-noise ratio permitting shorter test duration which is of more clinically-acceptable length.

When considering multiple sclerosis (MS) as an example of a neurological disease and comparing different evoked potentials used to evaluate visual involvement, VEPs to full-field

pattern reversal stimuli can demonstrate increased latency in up to 90% of cases of clinically-definite MS (Losseff et al., 1996). However, a more recent assessment of the sensitivity suggests that it is lower than previously reported, somewhere between 25% to 83% (Maddess et al., 2005). A conventional mfVEP stimuli yields a sensitivity of 92% but misdiagnoses more than 20% of the normal population. Sparse mfVEPs demonstrate comparative sensitivity of 92% but at a false-positive rate of 0% (Ruseckaite et al., 2005).

In the pursuit of further refinements *multifocal pupillographic perimetry* (mfPOP) was attempted **(Figure 6)** (Sutter, 1996, Tan et al., 2001, Wilhelm et al., 2000a). This technique allows objective perimetry based on the pupillary response pathway; thus it does not require the use of electrodes and involves little setup time. Like mfVEPs, mfPOP is capable of testing many parts of the visual fields of both eyes simultaneously. By testing both eyes with independent stimuli and recording from both pupils, the device can distinguish localized afferent and efferent defects (Bell et al., 2010, Carle et al., 2011b). Up to 176 pupillary responses can be obtained and analysed for both amplitude and delay (time-to-peak) **(Figure 7)**.

MfPOP has been evolving over the last 10 years. Improvements have included slightly overlapping of bigger stimuli, and the introduction of sparse stimuli (Bell et al., 2010), luminance balanced stimuli, and clustered volleys stimuli (Sabeti et al., 2014). In the latter instead of appearing randomly across the field, the stimuli are presented in volleys within the hemifields but are randomized within each hemi-field. All these modifications have led to successive increases in signal-to-noise Ratio (SNR), permitting increasingly more stimuli per visual field, reducing test duration, and providing improved sensitivity and specificity in several diseases.

To date, mfPOP has been successfully evaluated in several conditions, including diabetic retinopathy (Bell et al., 2010, Sabeti et al., Sabeti et al., 2015), macular degeneration (Sabeti et al., 2012, Sabeti et al., 2014), and glaucoma (Carle et al., 2011a, Carle et al., 2015). When used in MS patients, the predictive power of mfPOP to diagnose MS expressed as the percentage area under the curve (AUC) of the receiver operating characteristic (ROC) was found to be 69.8% for time-to-peak in the relapsing remitting MS group and increased to 85.5% in the progressive MS group; diagnostic power followed disability (Ali et al., 2014). Re-analysis of the published data using a method that examines asymmetries in response between the left and right eyes raises the corresponding %AUC values to 79.6 ± 3.03 and 92.3 ± 5.23 (mean ± SE). The method employed in that MS study did not use the newer mfPOP methods with their increased signal quality.

Accurate fast and objective measurements of visual fields were the goals set when designing the mfPOP device based on techniques originally developed for evoked potentials (James, 2003, James et al., 2005, Ruseckaite et al., 2005). Although mfPOP has never been used to evaluate neither migraine nor epilepsy due to the fact that the two conditions were considered relative contraindications due to the fears that light emitted by the device during examination may exacerbate both conditions. Mfpop protocols do not fulfil light characteristics that are known to exacerbate epilepsy (described above). Overall, the mfPOP stimuli are unlikely to generate PSE on a number of counts. Firstly stimulation between the eyes is never co-synchronous for any particular stimulus region. Each region stimulates much less than 10% of the cortex – this is perhaps the most important factor. The stimuli do not contain 5 to 8 stripes. The stimuli do not have sharp edges and, in fact, their edges are roughly sine-wave gratings. The stimulus delivery rate at any one location is certainly less than 3 Hz. It is the case that our flicked stimuli alternate for several cycles at 15 Hz. However, their small

projected cortical excitation area, monocular presentation, blurred edges and lack of any stripes should render them quite ineffective at generating PSE.

The same can be said for migraine, yet to further reinforce its safety we designed two studies to indicate that commonly used mfPOP protocols are safe in the two conditions. Furthermore, protocols specifically designed and proven to stimulate melanospin containing RGCs in glaucoma subjects (Carle et al., 2015) were used in migraine patients. As mentioned above these ipRGS were found to play a role in exacerbation of headache by light (Noseda et al., 2010). Carle *et al.* (2015) found that several characteristics of the responses obtained to blue stimulation through mfPOP were indeed melanopsin-influenced. And although cone photoreceptors have participated in those responses to the blue protocol as sources of both excitatory and inhibitory input, pupil constriction amplitudes in the blue protocol were also substantially influenced by melanopsin.

In addition to determining safety, our mfPOP studies will shed the light the pathophysiology of cortical excitability changes in migraine and epilepsy. Although the pupillary response is believed to be generated through a subcortical pathway it is important to note that the primary nucleus driving this response, the pretectal olivary nucleus (PON) receives significant cortical, ventral thalamic, midbrain and retinal inputs **(Figure 8)**. Studies have identified well-defined ipsilateral projections from both striate and extra-striate visual areas to the PON such as area 17/V1 (Benevento et al., 1977), area 19 (Distler and Hoffmann, 1989, Lui et al., 1995), the pupilloconstrictor region of area 20a (Distler and Hoffmann, 1989), V4 (Dineen and Hendrickson, 1983), the frontal and supplementary eye fields (Huerta et al., 1986, Leichnetz, 1982, Shook et al., 1990), dorsal prelunate, preoccipital cortex (Asanuma et al., 1985) , inferior temporal cortex (Steele and Weller, 1993) and lateral intraparietal region (Asanuma et al., 1985). Based on the number and variety of cortical regions projecting to

PON, a wide range of visual and oculomotor signals must have an impact on this nucleus and thus on the pupillary light reflex (Gamlin, 2006).

The PON has reciprocal connections to the ventral thalamus, namely the ventral Lateral geniculate nucleus (LGNv), pre-geniculate nucleus (PGN) (Conley and Friederich-Ecsy, 1993, Edwards et al., 1974, Graybiel, 1974, Ribak and Peters, 1975, Steele and Weller, 1993) and inter-geniculate leaflet (IGL) (Moore et al., 2000, Morin and Blanchard, 1998). This is an added reason why we feel that the pupillary response may serve as an important pathway to study migraine and epilepsy since the thalamus play a role in the pathogenesis of both disorders. In migraine the trigeminal ganglion signals are relayed to the spinal trigeminal nucleus, which projects to the lateral posterior nucleus and the pulvinars – both parts of the thalamus - before relaying the input into the primary somatosensory cortex **(Figure 5)** and in epilepsy, the thalamo-cortical pathway plays an important role in spike wave generation in epilepsy (involving the thalamic reticular nucleus) (Huguenard, 1999).

Putting in mind that mfPOP is a subtype of evoked response generated by the pupillary pathway, with indirect cortical influence rather than being a direct cortically driven evoked response such as the VEP, we feel it will be ideal to study cortical excitability changes in migraine and epilepsy.

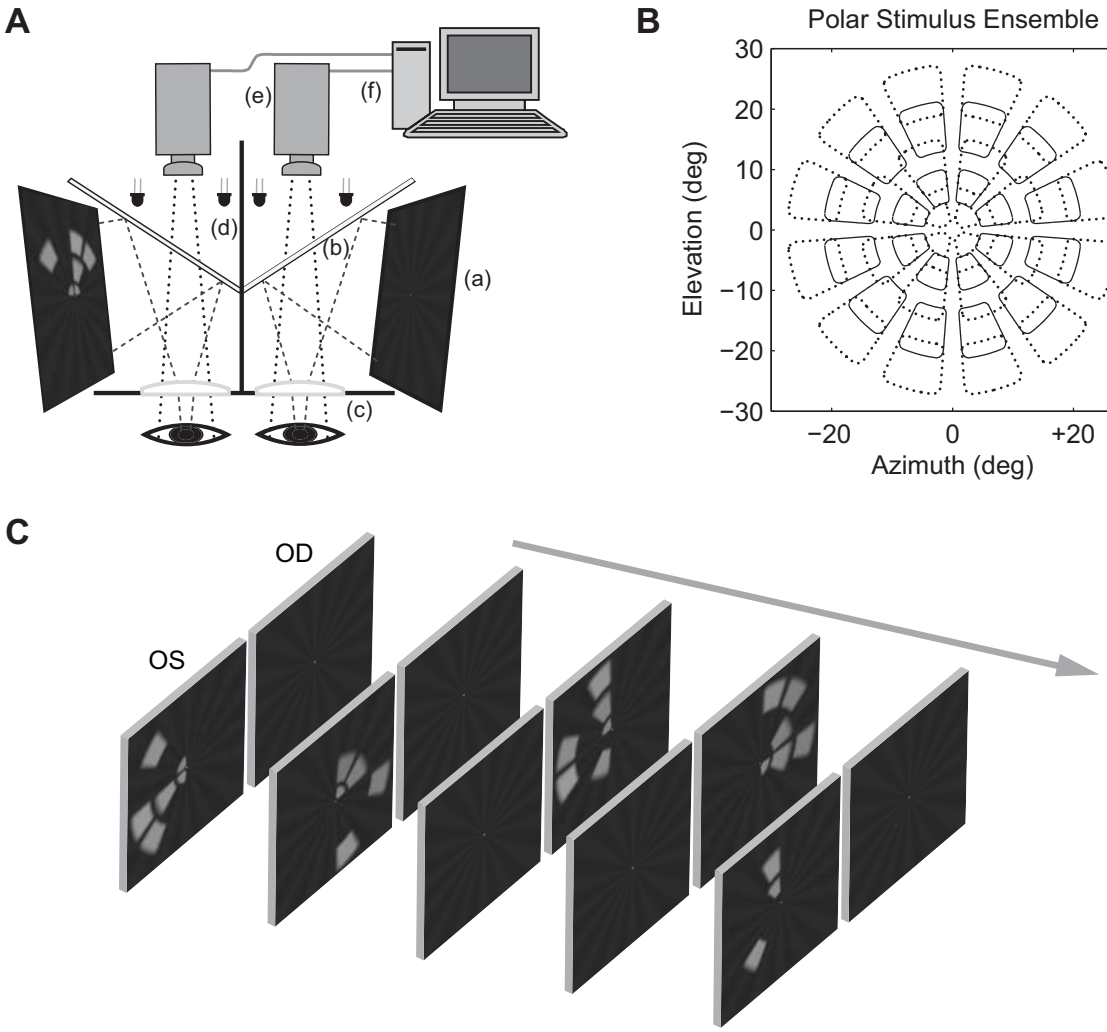
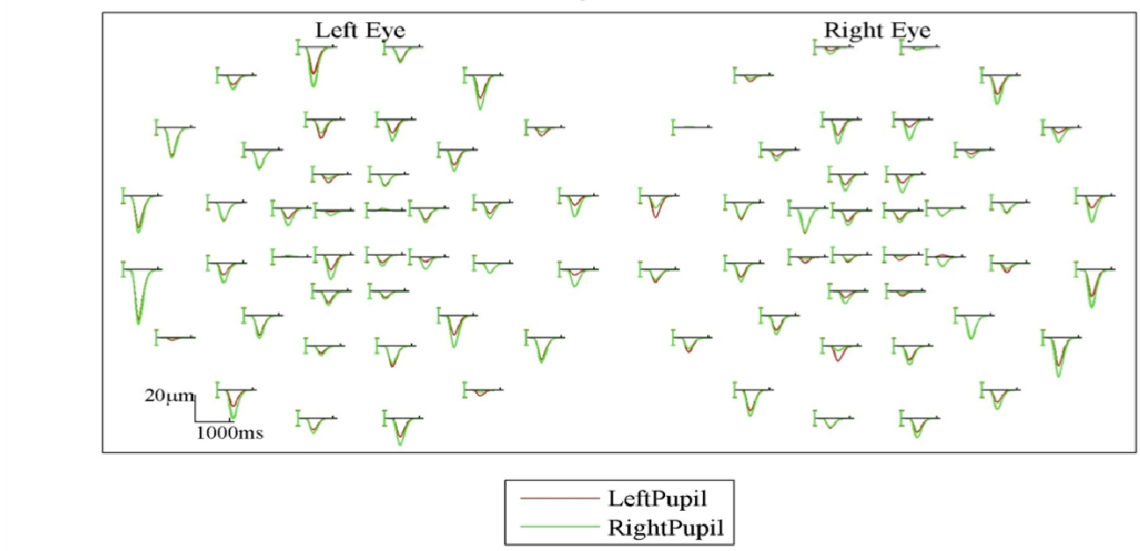


Figure 6. Measurement of pupillary responses. A) Schematic of the nuCoria Field Analyser®. Stimuli are presented independently on two LCD monitors (a). These images are reflected by two dichroic mirrors (b) allowing infrared light to pass while reflecting shorter wavelengths. Viewing distance is increased to optical infinity by plano-convex lenses (c). Each eye views only one monitor, the images being fused by the subject into a cyclopean view. Infrared illumination of the eyes is provided by infrared light-emitting diodes (d) facilitating the monitoring of each pupil by separate infrared video cameras (e). Pupil diameters are then extracted in real-time and recorded by a computer (f). B) The 44 stimulus regions per eye were arranged in a dartboard-like polar layout extending to 30° from fixation. C) Showing the independent stimuli (dichoptic) from a series of video frames of the test sequence. Stimuli were pseudo-randomly presented to each hemifield of each eye in a consecutive series. The faint background starburst pattern assists the subjects to fuse the images.



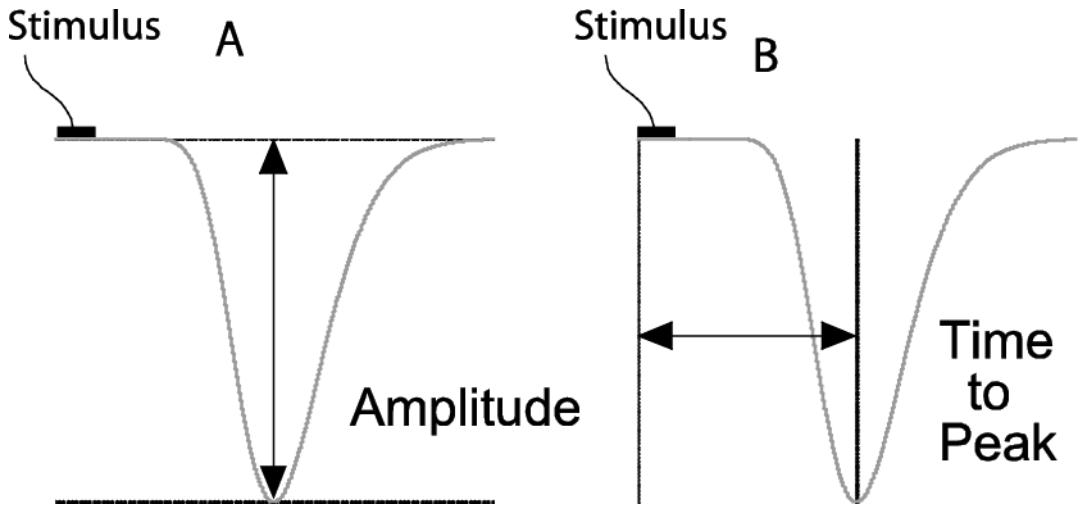


Figure 7. Example mfPOP response waveforms from an individual subject (Above). The mean pupil responses to stimuli present to each of the 44 test regions were obtained from both eyes concurrently from 6 minutes of stimulation. Downward deflection indicates contraction. The red and green traces of the upper plot are the responses of the left and right pupils. (Below) Pupil responses are analysed according to amplitude and delay (time-to-peak).

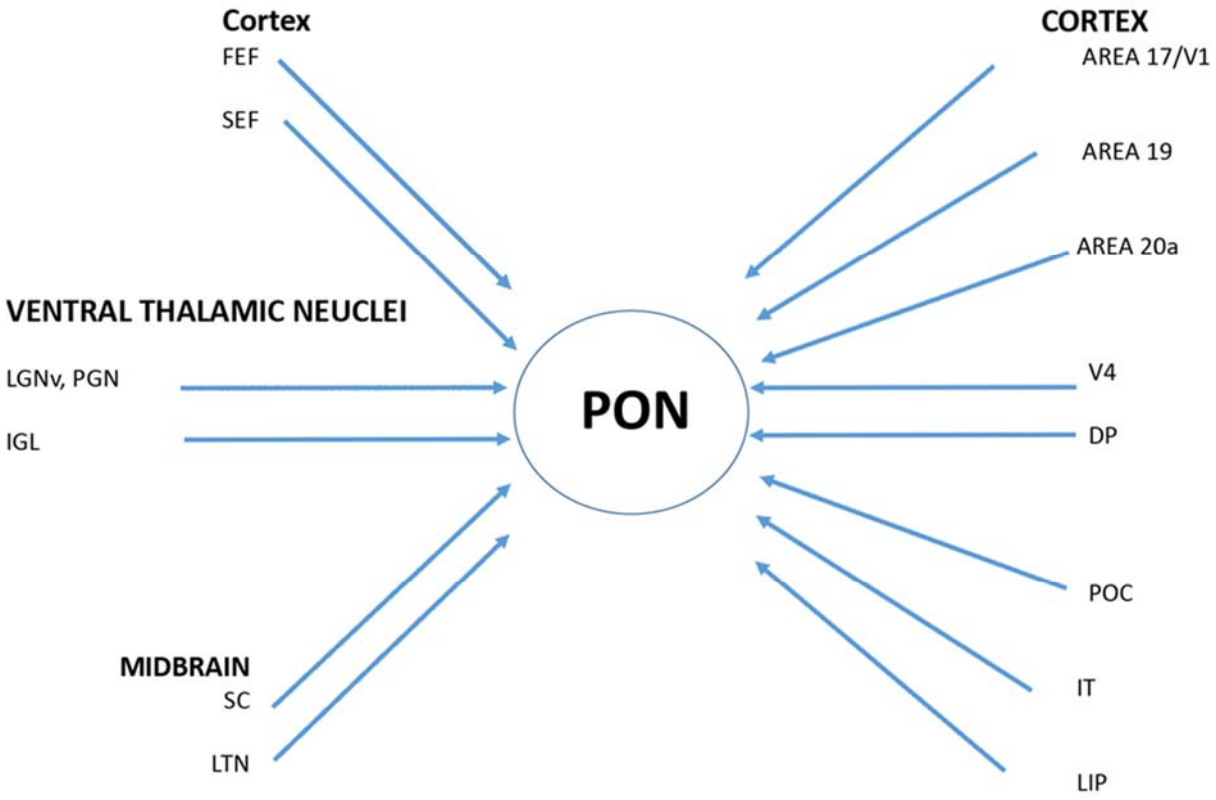


Figure 8. Non-retinal afferent connections of the pretectal olivary nucleus.

Adopted from (Gamlin, 2006).

FEF=frontal eye fields; SEF= supplementary eye fields; LGNv= ventral lateral geniculate nucleus; PGN= pregeniculate nucleus; IGL= intergeniculate leaflet; SC= superior colliculus; LTN= lateral terminal nucleus; DP= dorsal prelunate; POC= preoccipital cortex; IT= inferior temporal cortex; LIP= lateral intraparietal region.

References

2004. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia,* 24 Suppl 1**,** 9-160.

2013. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia,* 33**,** 629-808.

ALI, E., MADDESS, T., JAMES, A., VOICU, C. & LUECK, C. 2014. Pupillary response to sparse multifocal stimuli in multiple sclerosis patients. *Multiple Sclerosis Journal,* 20**,** 854-861.

AMBROSINI, A., DE NOORDHOUT, A. M., SANDOR, P. S. & SCHOENEN, J. 2003. Electrophysiological studies in migraine: a comprehensive review of their interest and limitations. *Cephalalgia,* 23 Suppl 1**,** 13-31.

ASANUMA, C., ANDERSEN, R. A. & COWAN, W. M. 1985. The thalamic relations of the caudal inferior parietal lobule and the lateral prefrontal cortex in monkeys: divergent cortical projections from cell clusters in the medial pulvinar nucleus. *J Comp Neurol,* 241**,** 357-81.

AURORA, S. K., CAO, Y., BOWYER, S. M. & WELCH, K. M. 1999. The occipital cortex is hyperexcitable in migraine: experimental evidence. *Headache,* 39**,** 469-76.

BADAWY, R. A. & JACKSON, G. D. 2012. Cortical excitability in migraine and epilepsy: a common feature? *J Clin Neurophysiol,* 29**,** 244-9.

BADAWY, R. A., LOETSCHER, T., MACDONELL, R. A. & BRODTMANN, A. 2012. Cortical excitability and neurology: insights into the pathophysiology. *Funct Neurol,* 27**,** 131-45.

BARRETT, C. F., VAN DEN MAAGDENBERG, A. M., FRANTS, R. R. & FERRARI, M. D. 2008. Familial hemiplegic migraine. *Adv Genet,* 63**,** 57-83.

BASELER, H. A., SUTTER, E. E., KLEIN, S. A. & CARNEY, T. 1994. The topography of visual evoked response properties across the visual field. *Electroencephalogr Clin Neurophysiol,* 90**,** 65-81.

BAVER, S. B., PICKARD, G. E., SOLLARS, P. J. & PICKARD, G. E. 2008. Two types of melanopsin retinal ganglion cell differentially innervate the hypothalamic suprachiasmatic nucleus and the olivary pretectal nucleus. *Eur J Neurosci,* 27**,** 1763-70.

BELL, A., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Dichoptic multifocal pupillography reveals afferent visual field defects in early type 2 diabetes. *Invest Ophthalmol Vis Sci,* 51**,** 602-8.

BENARROCH, E. E. 2011. The melanopsin system: Phototransduction, projections, functions, and clinical implications. *Neurology,* 76**,** 1422-7.

BENEVENTO, L. A., REZAK, M. & SANTOS, A. 1977. An autoradiographic study of the projections of the pretectum in the rhesus monkey (Macaca mulatta): evidence for sensorimotor links to the thalamus and oculomotor nuclei. *Brain Res,* 127**,** 197-218.

BIANCHIN, M. M., LONDERO, R. G., LIMA, J. E. & BIGAL, M. E. 2010. Migraine and epilepsy: a focus on overlapping clinical, pathophysiological, molecular, and therapeutic aspects. *Curr Pain Headache Rep,* 14**,** 276-83.

BICKFORD, R. G., SEM-JACOBSEN, C. W., WHITE, P. T. & DALY, D. 1952. Some observations on the mechanism of photic and photometrazol activation. *Electroencephalogr Clin Neurophysiol,* 4**,** 275-82.

BINDMAN, L. J., LIPPOLD, O. C. & REDFEARN, J. W. 1962. Long-lasting changes in the level of the electrical activity of the cerebral cortex produced bypolarizing currents. *Nature,* 196**,** 584-5.

BLUM, A. S. & RUTKOVE, S. B. 2007. *The Clinical Neurophysiology Primer,* Totowa, New Jersey, USA., Humana press.

BOHOTIN, V., FUMAL, A., VANDENHEEDE, M., BOHOTIN, C. & SCHOENEN, J. 2003. Excitability of visual V1-V2 and motor cortices to single transcranial magnetic stimuli in migraine: a reappraisal using a figure-of-eight coil. *Cephalalgia,* 23**,** 264-70.

CARABALLO, R. H., CERSOSIMO, R. O. & FEJERMAN, N. 2008. Childhood occipital epilepsy of Gastaut: a study of 33 patients. *Epilepsia,* 49**,** 288-97.

CARLE, C. F., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2015. Blue Multifocal Pupillographic Objective Perimetry in Glaucoma. *Invest Ophthalmol Vis Sci,* 56**,** 6394-403.

CARLE, C. F., JAMES, A. C., KOLIC, M., LOH, Y. W. & MADDESS, T. 2011a. High-resolution multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci,* 52**,** 604-10.

CARLE, C. F., MADDESS, T. & JAMES, A. C. 2011b. Contraction anisocoria: segregation, summation, and saturation in the pupillary pathway. *Invest Ophthalmol Vis Sci,* 52**,** 2365-71.

CHIAPPA, K. H., HILL, R. A., HUANG-HELLINGER, F. & JENKINS, B. G. 1999. Photosensitive epilepsy studied by functional magnetic resonance imaging and magnetic resonance spectroscopy. *Epilepsia,* 40 Suppl 4**,** 3-7.

CHRONICLE, E. P. & WILKINS, A. J. 1991. Colour and visual discomfort in migraineurs. *Lancet,* 338**,** 890.

CLARKE, T., BASKURT, Z., STRUG, L. J. & PAL, D. K. 2009. Evidence of shared genetic risk factors for migraine and rolandic epilepsy. *Epilepsia,* 50**,** 2428-33.

CONLEY, M. & FRIEDERICH-ECSY, B. 1993. Functional organization of the ventral lateral geniculate complex of the tree shrew (Tupaia belangeri): II. Connections with the cortex, thalamus, and brainstem. *J Comp Neurol,* 328**,** 21-42.

CONNOLLY, J. F., GAWEL, M. & ROSE, F. C. 1982. Migraine patients exhibit abnormalities in the visual evoked potential. *J Neurol Neurosurg Psychiatry,* 45**,** 464-7.

COULL, B. M. & PEDLEY, T. A. 1978. Intermittent photic stimulation. Clinical usefulness of non-convulsive responses. *Electroencephalogr Clin Neurophysiol,* 44**,** 353-63.

COVANIS, A. 2005. Photosensitivity in idiopathic generalized epilepsies. *Epilepsia,* 46 Suppl 9**,** 67-72.

DACEY, D. M., LIAO, H. W., PETERSON, B. B., ROBINSON, F. R., SMITH, V. C., POKORNY, J., YAU, K. W. & GAMLIN, P. D. 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature,* 433**,** 749-54.

DAVIE, C. A., BARKER, G. J., WEBB, S., TOFTS, P. S., THOMPSON, A. J., HARDING, A. E., MCDONALD, W. I. & MILLER, D. H. 1995. Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axon loss. *Brain,* 118 (Pt 6)**,** 1583-92.

DEPREZ, L., PEETERS, K., VAN PAESSCHEN, W., CLAEYS, K. G., CLAES, L. R., SULS, A., AUDENAERT, D., VAN DYCK, T., GOOSSENS, D., DEL-FAVERO, J. & DE JONGHE, P. 2007. Familial occipitotemporal lobe epilepsy and migraine with visual aura: linkage to chromosome 9q. *Neurology,* 68**,** 1995-2002.

DINEEN, J. T. & HENDRICKSON, A. 1983. Overlap of retinal and prestriate cortical pathways in the primate pretectum. *Brain Res,* 278**,** 250-4.

DISTLER, C. & HOFFMANN, K. P. 1989. The pupillary light reflex in normal and innate microstrabismic cats, II: Retinal and cortical input to the nucleus praetectalis olivaris. *Vis Neurosci,* 3**,** 139-53.

DO, M. T., KANG, S. H., XUE, T., ZHONG, H., LIAO, H. W., BERGLES, D. E. & YAU, K. W. 2009. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature,* 457**,** 281-7.

DREIER, J. P., MAJOR, S., PANNEK, H. W., WOITZIK, J., SCHEEL, M., WIESENTHAL, D., MARTUS, P., WINKLER, M. K., HARTINGS, J. A., FABRICIUS, M., SPECKMANN, E. J. & GORJI, A.

2012. Spreading convulsions, spreading depolarization and epileptogenesis in human cerebral cortex. *Brain,* 135**,** 259-75.
EDWARDS, S. B., ROSENQUIST, A. C. & PALMER, L. A. 1974. An autoradiographic study of ventral lateral geniculate projections in the cat. *Brain Res,* 72**,** 282-7.
FISHER, R. S., ACEVEDO, C., ARZIMANOGLOU, A., BOGACZ, A., CROSS, J. H., ELGER, C. E., ENGEL, J., JR., FORSGREN, L., FRENCH, J. A., GLYNN, M., HESDORFFER, D. C., LEE, B. I., MATHERN, G. W., MOSHE, S. L., PERUCCA, E., SCHEFFER, I. E., TOMSON, T., WATANABE, M. & WIEBE, S. 2014. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia,* 55**,** 475-82.
FISHER, R. S., HARDING, G., ERBA, G., BARKLEY, G. L. & WILKINS, A. 2005. Photic- and pattern-induced seizures: a review for the Epilepsy Foundation of America Working Group. *Epilepsia,* 46**,** 1426-41.
FRIEDMAN, D. I. & DE VER DYE, T. 2009. Migraine and the environment. *Headache,* 49**,** 941-52.
GAMLIN, P. D. 2006. The pretectum: connections and oculomotor-related roles. *Prog Brain Res,* 151**,** 379-405.
GELLER, A. M., HUDNELL, H. K., VAUGHN, B. V., MESSENHEIMER, J. A. & BOYES, W. K. 2005. Epilepsy and medication effects on the pattern visual evoked potential. *Doc Ophthalmol,* 110**,** 121-31.
GOOD, P. A., TAYLOR, R. H. & MORTIMER, M. J. 1991. The use of tinted glasses in childhood migraine. *Headache,* 31**,** 533-6.
GOOLEY, J. J., LU, J., FISCHER, D. & SAPER, C. B. 2003. A broad role for melanopsin in nonvisual photoreception. *J Neurosci,* 23**,** 7093-106.
GOWERS, W. R. 1906. Clinical Lectures ON THE BORDERLAND OF EPILEPSY. III.-MIGRAINE. *Br Med J,* 2**,** 1617-22.
GRAFSTEIN, B. 1956. Mechanism of spreading cortical depression. *J Neurophysiol,* 19**,** 154-71.
GRAYBIEL, A. M. 1974. Visuo-cerebellar and cerebello-visual connections involving the ventral lateral geniculate nucleus. *Exp Brain Res,* 20**,** 303-6.
GUNAYDIN, S., SOYSAL, A., ATAY, T. & ARPACI, B. 2006. Motor and occipital cortex excitability in migraine patients. *Can J Neurol Sci,* 33**,** 63-7.
HARDING, G. 1994. Photosensitivity: a vestigial echo? The first Grey Walter Lecture. *Int J Psychophysiol,* 16**,** 273-9.
HARDING, G., WILKINS, A. J., ERBA, G., BARKLEY, G. L. & FISHER, R. S. 2005. Photic- and pattern-induced seizures: expert consensus of the Epilepsy Foundation of America Working Group. *Epilepsia,* 46**,** 1423-5.
HARDING, G. F. A. & TAKAHASHI, T. 2004. Regulations: What Next? *Epilepsia,* 45**,** 46-47.
HATTAR, S., LIAO, H. W., TAKAO, M., BERSON, D. M. & YAU, K. W. 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science,* 295**,** 1065-70.
HATTORI, Y. 1990. Regional difference in responsiveness of adenosine-sensitive cyclic AMP-generating systems in chronic epileptic cerebral cortex of the rat. *J Neurochem,* 55**,** 1294-303.
HATTORI, Y., MORIWAKI, A., HAYASHI, Y., ISLAM, N. & HORI, Y. 1993. Characterization of adenosine receptor-mediated generation of cyclic AMP in slices of rat cerebral cortex with chronic epileptic activity. *Neurochem Res,* 18**,** 1009-14.
HATTORI, Y., MORIWAKI, A., HAYASHI, Y., LU, Y. F. & HORI, Y. 1992. Increased responses to adenosine and 2-chloroadenosine of cyclic AMP-generating systems in the primary cortical region of cobalt-induced epilepsy in the rat. *Jpn J Physiol,* 42**,** 151-7.
HATTORI, Y., MORIWAKI, A. & HORI, Y. 1990. Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex. *Neurosci Lett,* 116**,** 320-4.

HAUT, S. R., BIGAL, M. E. & LIPTON, R. B. 2006. Chronic disorders with episodic manifestations: focus on epilepsy and migraine. *Lancet Neurol,* 5**,** 148-57.
HAY, K. M., MORTIMER, M. J., BARKER, D. C., DEBNEY, L. M. & GOOD, P. A. 1994. 1044 women with migraine: the effect of environmental stimuli. *Headache,* 34**,** 166-8.
HUERTA, M. F., KRUBITZER, L. A. & KAAS, J. H. 1986. Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys: I. Subcortical connections. *J Comp Neurol,* 253**,** 415-39.
HUGHES, S. W. & CRUNELLI, V. 2005. Thalamic mechanisms of EEG alpha rhythms and their pathological implications. *Neuroscientist,* 11**,** 357-72.
HUGUENARD, J. R. 1999. Neuronal circuitry of thalamocortical epilepsy and mechanisms of antiabsence drug action. *Adv Neurol,* 79**,** 991-9.
JAMES, A. C. 2003. The pattern-pulse multifocal visual evoked potential. *Invest Ophthalmol Vis Sci,* 44**,** 879-90.
JAMES, A. C., RUSECKAITE, R. & MADDESS, T. 2005. Effect of temporal sparseness and dichoptic presentation on multifocal visual evoked potentials. *Vis Neurosci,* 22**,** 45-54.
JAMES, G., KOLIC, ESSEX, MADDESS Objective Pupillographic Multifocal Perimetry for Glaucoma ARVO Meeting April 11, 2009 2009.
JEAVONS, P. M. & HARDING, G. F. A. 1975. *Photosensitive Epilepsy,* London, William Heinemann.
JONES, K. A., HATORI, M., MURE, L. S., BRAMLEY, J. R., ARTYMYSHYN, R., HONG, S. P., MARZABADI, M., ZHONG, H., SPROUSE, J., ZHU, Q., HARTWICK, A. T., SOLLARS, P. J., PICKARD, G. E. & PANDA, S. 2013. Small-molecule antagonists of melanopsin-mediated phototransduction. *Nat Chem Biol,* 9**,** 630-5.
JUDIT, A., SANDOR, P. S. & SCHOENEN, J. 2000. Habituation of visual and intensity dependence of auditory evoked cortical potentials tends to normalize just before and during the migraine attack. *Cephalalgia,* 20**,** 714-9.
KASTELEIJN-NOLST TRENITE, D. G., GUERRINI, R., BINNIE, C. D. & GENTON, P. 2001. Visual sensitivity and epilepsy: a proposed terminology and classification for clinical and EEG phenomenology. *Epilepsia,* 42**,** 692-701.
KAWASAKI, A. & KARDON, R. H. 2007. Intrinsically photosensitive retinal ganglion cells. *J Neuroophthalmol,* 27**,** 195-204.
KENNARD, C., GAWEL, M., RUDOLPH NDE, M. & ROSE, F. C. 1978. Visual evoked potentials in migraine subjects. *Res Clin Stud Headache,* 6**,** 73-80.
LANCE, J. W. 1996. Migraine: then and now. *Med J Aust,* 164**,** 519-20.
LAURITZEN, M., RICE, M. E., OKADA, Y. & NICHOLSON, C. 1988. Quisqualate, kainate and NMDA can initiate spreading depression in the turtle cerebellum. *Brain Res,* 475**,** 317-27.
LEAO, A. A. 1986. Spreading depression. *Funct Neurol,* 1**,** 363-6.
LEHTONEN, J. B. 1974. Visual evoked cortical potentials for single flashes and flickering light in migraine. *Headache,* 14**,** 1-12.
LEICHNETZ, G. R. 1982. Connections between the frontal eye field and pretectum in the monkey: an anterograde/retrograde study using HRP gel and TMB neurohistochemistry. *J Comp Neurol,* 207**,** 394-404.
LENNOX, W. G. & LENNOX, M. A. 1960. *Epilepsy and related disorders,* Boston, Little, Brown and Company.
LIEBETANZ, D., NITSCHE, M. A., TERGAU, F. & PAULUS, W. 2002. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain,* 125**,** 2238-47.
LOSSEFF, N. A., WEBB, S. L., O'RIORDAN, J. I., PAGE, R., WANG, L., BARKER, G. J., TOFTS, P. S., MCDONALD, W. I., MILLER, D. H. & THOMPSON, A. J. 1996. Spinal cord atrophy and

disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain,* 119 (Pt 3)**,** 701-8.
LUI, F., GREGORY, K. M., BLANKS, R. H. & GIOLLI, R. A. 1995. Projections from visual areas of the cerebral cortex to pretectal nuclear complex, terminal accessory optic nuclei, and superior colliculus in macaque monkey. *J Comp Neurol,* 363**,** 439-60.
MADDESS, T., JAMES, A. C. & BOWMAN, E. A. 2005. Contrast response of temporally sparse dichoptic multifocal visual evoked potentials. *Vis Neurosci,* 22**,** 153-62.
MAIN, A., DOWSON, A. & GROSS, M. 1997. Photophobia and phonophobia in migraineurs between attacks. *Headache,* 37**,** 492-5.
MAIN, A., VLACHONIKOLIS, I. & DOWSON, A. 2000. The wavelength of light causing photophobia in migraine and tension-type headache between attacks. *Headache,* 40**,** 194-9.
MARCUS, D. A. & SOSO, M. J. 1989. Migraine and stripe-induced visual discomfort. *Arch Neurol,* 46**,** 1129-32.
MARTIN, P. R. 2010. Behavioral management of migraine headache triggers: learning to cope with triggers. *Curr Pain Headache Rep,* 14**,** 221-7.
MATYNIA, A. 2013. Blurring the boundaries of vision: novel functions of intrinsically photosensitive retinal ganglion cells. *J Exp Neurosci,* 7**,** 43-50.
MCCORMICK, D. A. & CONTRERAS, D. 2001. On the cellular and network bases of epileptic seizures. *Annu Rev Physiol,* 63**,** 815-46.
MESGARI, M., GHAFFARIAN, N., KHALEGHI GHADIRI, M., SADEGHIAN, H., SPECKMANN, E. J., STUMMER, W. & GORJI, A. 2015. Altered inhibition in the hippocampal neural networks after spreading depression. *Neuroscience,* 304**,** 190-7.
MOORE, R. Y., WEIS, R. & MOGA, M. M. 2000. Efferent projections of the intergeniculate leaflet and the ventral lateral geniculate nucleus in the rat. *J Comp Neurol,* 420**,** 398-418.
MORIN, L. P. & BLANCHARD, J. H. 1998. Interconnections among nuclei of the subcortical visual shell: the intergeniculate leaflet is a major constituent of the hamster subcortical visual system. *J Comp Neurol,* 396**,** 288-309.
MUTHANE, U. B., SATISHCHANDRA, P. & SUBHASH, M. N. 1993. Visual and auditory evoked potentials in early onset Parkinson's disease and their relationship to cerebrospinal fluid monoamine metabolites. *Mov Disord,* 8**,** 344-8.
NOSEDA, R. & BURSTEIN, R. 2011. Advances in understanding the mechanisms of migraine-type photophobia. *Curr Opin Neurol,* 24**,** 197-202.
NOSEDA, R., KAINZ, V., JAKUBOWSKI, M., GOOLEY, J. J., SAPER, C. B., DIGRE, K. & BURSTEIN, R. 2010. A neural mechanism for exacerbation of headache by light. *Nat Neurosci,* 13**,** 239-45.
NYRKE, T., KANGASNIEMI, P. & LANG, H. 1990. Alpha rhythm in classical migraine (migraine with aura): abnormalities in the headache-free interval. *Cephalalgia,* 10**,** 177-81.
OGILVIE, A. D., RUSSELL, M. B., DHALL, P., BATTERSBY, S., ULRICH, V., SMITH, C. A., GOODWIN, G. M., HARMAR, A. J. & OLESEN, J. 1998. Altered allelic distributions of the serotonin transporter gene in migraine without aura and migraine with aura. *Cephalalgia,* 18**,** 23-6.
OPHOFF, R. A., TERWINDT, G. M., FRANTS, R. R. & FERRARI, M. D. 1998. P/Q-type Ca2+ channel defects in migraine, ataxia and epilepsy. *Trends Pharmacol Sci,* 19**,** 121-7.
OTTMAN, R. & LIPTON, R. B. 1996. Is the comorbidity of epilepsy and migraine due to a shared genetic susceptibility? *Neurology,* 47**,** 918-24.
PARISI, P., PICCIOLI, M., VILLA, M. P., BUTTINELLI, C. & KASTELEIJN-NOLST TRENITE, D. G. 2008. Hypothesis on neurophysiopathological mechanisms linking epilepsy and headache. *Med Hypotheses,* 70**,** 1150-4.

PARK, J. C., MOURA, A. L., RAZA, A. S., RHEE, D. W., KARDON, R. H. & HOOD, D. C. 2011. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci,* 52**,** 6624-35.

PARRA, J., KALITZIN, S. N. & LOPES DA SILVA, F. H. 2005. Photosensitivity and visually induced seizures. *Curr Opin Neurol,* 18**,** 155-9.

PIETROBON, D. 2005. Migraine: new molecular mechanisms. *Neuroscientist,* 11**,** 373-86.

POLMAN, C. H., REINGOLD, S. C., EDAN, G., FILIPPI, M., HARTUNG, H. P., KAPPOS, L., LUBLIN, F. D., METZ, L. M., MCFARLAND, H. F., O'CONNOR, P. W., SANDBERG-WOLLHEIM, M., THOMPSON, A. J., WEINSHENKER, B. G. & WOLINSKY, J. S. 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol,* 58**,** 840-6.

PROVENCIO, I., JIANG, G., DE GRIP, W. J., HAYES, W. P. & ROLLAG, M. D. 1998. Melanopsin: An opsin in melanophores, brain, and eye. *Proc Natl Acad Sci U S A,* 95**,** 340-5.

PROVENCIO, I., RODRIGUEZ, I. R., JIANG, G., HAYES, W. P., MOREIRA, E. F. & ROLLAG, M. D. 2000. A novel human opsin in the inner retina. *J Neurosci,* 20**,** 600-5.

PURPURA, D. P. & MCMURTRY, J. G. 1965. INTRACELLULAR ACTIVITIES AND EVOKED POTENTIAL CHANGES DURING POLARIZATION OF MOTOR CORTEX. *J Neurophysiol,* 28**,** 166-85.

RIBAK, C. E. & PETERS, A. 1975. An autoradiographic study of the projections from the lateral geniculate body of the rat. *Brain Res,* 92**,** 341-68.

ROGAWSKI, M. A. 2012. Migraine and Epilepsy-Shared Mechanisms within the Family of Episodic Disorders.

RUSECKAITE, R., MADDESS, T., DANTA, G., LUECK, C. J. & JAMES, A. C. 2005. Sparse multifocal stimuli for the detection of multiple sclerosis. *Ann Neurol,* 57**,** 904-13.

RYAN, D. P. & PTACEK, L. J. 2010. Episodic neurological channelopathies. *Neuron,* 68**,** 282-92.

SABETI, F., MADDESS, T., ESSEX, R. W. & JAMES, A. C. 2012. Multifocal pupillography identifies ranibizumab-induced changes in retinal function for exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci,* 53**,** 253-60.

SABETI, F., MADDESS, T., ESSEX, R. W., SAIKAL, A., JAMES, A. C. & CARLE, C. F. 2014. Multifocal pupillography in early age-related macular degeneration. *Optom Vis Sci,* 91**,** 904-15.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T. 2015. Multifocal Pupillography Identifies Changes in Visual Sensitivity According to Severity of Diabetic Retinopathy in Type 2 Diabetes. *Invest Ophthalmol Vis Sci,* 56**,** 4504-13.

SCHAFER, E. W. & MCKEAN, C. M. 1975. Evidence that monoamines influence human evoked potentials. *Brain Res,* 99**,** 49-58.

SCHOMER, D. L. & LOPES DA SILVA, F. H. 2005. *Niedermeyer's Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*

Philadelphia, PA, USA, Lippincott Williams & Wilkins.

SHOOK, B. L., SCHLAG-REY, M. & SCHLAG, J. 1990. Primate supplementary eye field: I. Comparative aspects of mesencephalic and pontine connections. *J Comp Neurol,* 301**,** 618-42.

SILBERSTEIN, S. D., HOLLAND, S., FREITAG, F., DODICK, D. W., ARGOFF, C. & ASHMAN, E. 2012. Evidence-based guideline update: pharmacologic treatment for episodic migraine prevention in adults: report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society. *Neurology,* 78**,** 1337-45.

SIMON, R. H., ZIMMERMAN, A. W., TASMAN, A. & HALE, M. S. 1982. Spectral analysis of photic stimulation in migraine. *Electroencephalogr Clin Neurophysiol,* 53**,** 270-6.

SINIATCHKIN, M., GROPPA, S., JEROSCH, B., MUHLE, H., KURTH, C., SHEPHERD, A. J., SIEBNER, H. & STEPHANI, U. 2007. Spreading photoparoxysmal EEG response is associated with an abnormal cortical excitability pattern. *Brain,* 130**,** 78-87.

SOMJEN, G. G. 2001. Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol Rev,* 81**,** 1065-96.

STEELE, G. E. & WELLER, R. E. 1993. Subcortical connections of subdivisions of inferior temporal cortex in squirrel monkeys. *Vis Neurosci,* 10**,** 563-83.

STOERIG, P., ZONTANOU, A. & COWEY, A. 2002. Aware or unaware: assessment of cortical blindness in four men and a monkey. *Cereb Cortex,* 12**,** 565-74.

SUTTER, E. E. 1996. the field topography of the pupillary response: rod and cone inputs. *Invest Ophthalmol Vis Sci,* 3157.

TAN, L., KONDO, M., SATO, M., KONDO, N. & MIYAKE, Y. 2001. Multifocal pupillary light response fields in normal subjects and patients with visual field defects. *Vision Res,* 41**,** 1073-84.

TFELT-HANSEN, P. C. 2010. History of migraine with aura and cortical spreading depression from 1941 and onwards. *Cephalalgia,* 30**,** 780-92.

WALTER, W. G., DOVEY, V. J. & SHIPTON, H. 1946. Analysis of the electrical response of the human cortex to photic stimulation. *Nature,* 158**,** 540.

WERNSMANN, B., PAPE, H. C., SPECKMANN, E. J. & GORJI, A. 2006. Effect of cortical spreading depression on synaptic transmission of rat hippocampal tissues. *Eur J Neurosci,* 23**,** 1103-10.

WILHELM, H., NEITZEL, J., WILHELM, B., BEUEL, S., LUDTKE, H., KRETSCHMANN, U. & ZRENNER, E. 2000. Pupil perimetry using M-sequence stimulation technique. *Invest Ophthalmol Vis Sci,* 41**,** 1229-38.

WILKINS, A. J. 1995. *Visual Stress,* New York, Oxford Press.

WILKINS, A. J., DARBY, C. E., BINNIE, C. D., STEFANSSON, S. B., JEAVONS, P. M. & HARDING, G. F. 1979. Television epilepsy--the role of pattern. *Electroencephalogr Clin Neurophysiol,* 47**,** 163-71.

ZAGAMI, A. & BAHRA, A. 2006. *Symptomatology of migraines without aura.In The Headaches,* Philadelphia, Lippincott Williams & Wilkins.

ZIEMANN, U., LONNECKER, S., STEINHOFF, B. J. & PAULUS, W. 1996. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann Neurol,* 40**,** 367-78.

Aims and summary

The purpose of this thesis was to evaluate the phenomena of photosensitivity and cortical hyper-excitability in two distinct but overlapping disorders, namely epilepsy and migraine using the multifocal objective pupillographic perimetry device, which allows assessment of the cortical and subcortical parts of the visual pathway responsible for the pupillary response. Based on the above knowledge, our hypotheses are as follows:

1- The mfPOP protocols that involve light are safe when used to examine epilepsy and migraine subjects.
2- Cortical excitability changes in migraine and epilepsy disorders extend to involve subcortical pathways namely the pupillary response.
3- Stimulating melanospin containing retinal ganglion cells will lead to exacerbation of migraine headaches.

The objectives of the research in this thesis were done to first evaluate the multifocal objective pupillographic perimetry responses in normal, migraineurs, and patients with epilepsy, and to assess if there are subtypes of epilepsy and migraine which can be identified by mfPOP.

Second, to provide evidence that mfPOP protocols, that involve light stimulation, are safe and tolerable when used in migraine and epilepsy patients. This is of great importance before using this device on a wide scale in these groups of subjects. To further reinforce this point protocols designed specifically to stimulate melanopsin-containing retinal

ganglion cells in migraine subjects were used to examine whether stimulating this pathway will lead to exacerbation of migraine headaches.

The third Objective was to evaluate the factors that might influence these responses such as attack timing and medication use. Finally EEGs recorded from epilepsy subjects looking at changes in the alpha rhythm entrainment in response to photic stimulation were analysed serving as an added indicator of cortical excitability.

Chapter 2.

Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective pupillometry:

a randomized controlled cross over study

Abstract

Hypothesis: Stimulating melanospin containing retinal ganglion cells may lead to exacerbation of migraine headaches**. Aim:** To establish the effects of stimulating intrinsically-photosensitive retinal ganglion cells (ipRGCs) on migraine severity and pupillary response characteristics, and to determine if there are differences in the pupillary response characteristics between migraineurs and controls**. Design/Methods:** A randomized, open labelled, crossover study tested migraineurs and normal controls using multifocal pupillographic objective perimetry (mfPOP) with 44 test-regions/eye. A blue protocol (BP) stimulated ipRGCs, and a yellow protocol (YP) stimulated cone photoreceptors. Migraine diaries assessed migraine severity. Responses were analyzed according to response time-to-peak and standardized amplitude (AmpStd). **Results:** 36 migraineurs (42.0 ± 16.5 years, 23 females) and 24 normal controls (39.2 ± 14.8 years, 14 females) were tested. Only one patient had difficulty completing the tests and reported the occurrence of an aura. The percentage of subjects developing a migraine attack did not differ after either protocol, either during the 1st day (odds ratio 1.0; 95% confidence interval 0.2-4.4, p = 0.48) or during the first three days after testing (odds ratio 0.8; 95% confidence interval 0.3-2.1, p = 0.68). Migraine days/week did not increase following testing with either protocol in comparison to the baseline week (1.4 ± 1.6 pre-testing (mean ± SD), 1.3 ± 1.4 post-BP, and 1.3 ± 1.2 post-YP; p = 0.96), neither did other measures of severity. Pupillary response characteristics including AmpStd and time-to-peak did not differ between migraineurs and controls. Looking at effects of headache characteristics on the pupillary response in migraineurs we found that a migraine attack occurring prior to testing had a significant independent effect in lowering AmpStd while a history of triptan use increased AmpStd. **Conclusions:** Stimulating ipRGCs did not affect migraine occurrence or severity. Pupillary response

characteristics were influenced by the occurrence of a recent migraine attack and a history of triptan use; these changes might be the result of the cortical spreading depression which is associated with migraine attacks.

2.1. Introduction

Migraine headaches are associated with cortical spreading depression (CSD) which consists of a wave of profound depression in neural activity preceded by neuronal activation. CSD is believed to underlie migraine aura and to be a trigger for the headache pain in migraine (Grafstein, 1956). Light is a well-recognized trigger of migraine attacks (Martin, 2010). How the pathways involved in light perception might be affected in migraine subjects is still not fully understood. Noseda *et al* (2010) have recently described a retino-thalamic pathway which arises from a subset of retinal ganglion cells (RGCs) called intrinsically-photosensitive RGCs (ipRGCs). This pathway may be responsible for headache exacerbation. ipRGCs contain the photosensitive pigment, melanopsin, and relay their responses to cells in the posterior thalamus, among other targets. These same cells in the thalamus also receive input from the trigemino-vascular pathway which is believed to carry the pain signal arising from the dura mater during migraine attacks (Noseda et al., 2010). The output of these cells is fed to the somatosensory cortex. Melanopsin, and thus direct ipRGC activation, is most sensitive to blue light, its sensitivity peaking at 479 nm (Kawasaki and Kardon, 2007). Also, intense stimuli of long duration are required for optimal activation of the ipRGCs. Whether such stimuli have the ability to stimulate this retino-thalamic pathway, and so be capable of exacerbating a migraine attack, remains to be explored. A more recent study showed that melanopsin is expressed in both human and mouse trigeminal ganglion neurons- classic pain sensory neurons- and these isolated neurons

respond to blue light stimuli with a delayed onset and sustained firing, similar to the melanopsin-dependent intrinsic photosensitivity observed in ipRGCs. They have also been shown to be responsible for light detection in the Central nervous system via a non-optic nerve pathway (Matynia et al., 2016). A previous study conducted by Main *et al.* (2000) observed that migraine patients found both short (blue) and long (red) wavelengths of light significantly more uncomfortable between attacks in comparison to normal controls and subjects with tension-type headache. However, these results were only based on subjective measures.

Multifocal Pupillographic Objective Perimetry (mfPOP) is a diagnostic technique that objectively assesses visual function using the pupillary response. The device tests 44 locations in the visual fields of both eyes concurrently using pupil responses. By testing both eyes with independent stimuli and recording the response of both pupils the device can distinguish localized afferent and efferent defects (Bell et al., 2010, Carle et al., 2011b). Alterations in pupillary response have been described in migraine patients clinically: prolonged mydriasis has been reported during migraine attacks, sometimes persisting for up to three months after an attack, suggesting a dysfunction of the ipsilateral ganglionic system(Cambron et al., 2014, Barriga et al., 2011). However, conventional pupillometry has so far failed to confirm these findings (Cambron et al., 2014). In addition, visual field defects have been documented in relation to migraine attacks (McKendrick and Badcock, 2004b, McKendrick and Badcock, 2004a). To date, the diagnostic power of mfPOP in detecting visual field defects has been successfully evaluated in several conditions including multiple sclerosis (Ali et al., 2014), diabetic retinopathy (Bell et al., 2010, Sabeti et al., Sabeti et al., 2015), macular degeneration (Sabeti et al., 2012, Sabeti et al., 2014), and glaucoma (Carle et al., 2011a, Carle et al., 2015). Consequently, in view of the confusion in the literature addressing changes in

the pupillary response in migraine this study was designed to use mfPOP to characterize the pupillary responses and document visual field defects in patients with migraine. The stimuli used in previous studies with mfPOP were transiently-presented yellow stimuli designed to drive red and green cones. We have used long-duration blue mfPOP stimuli targeting ipRGCs in a study of 19 glaucoma patients and 24 control subjects (Carle et al., 2010, Carle et al., 2015) without obvious side-effects, though migraineurs were not specifically examined.

This study aimed first to determine whether testing migraine patients with mfPOP would exacerbate their symptoms if a long-duration blue stimulus designed to stimulate the ipRGCs was used. This stimulus was compared to the standard mfPOP transient yellow stimulus designed to drive cone photoreceptor input to ipRGCs rather than stimulate their intrinsic response. A second aim was to estimate the power of mfPOP to quantify effects of migraine. The final aim was to determine the pupillary response characteristics and pattern of visual field defects of migraine subjects, along with any factors that might influence them.

2.2. Methods

2.2.1. Study Design and Subjects

A randomized, controlled, open labeled crossover single-site study was undertaken over a one year period between 2012 and 2013 **(Figure 1).**

Subjects with migraine were recruited from staff and students at The Australian National University and via local neurologists at The Canberra Hospital in Canberra, Australia. Recruitment occurred via poster advertisement, letters, and email circulation. Informed, written consent was obtained from all subjects. The study conformed to the Declaration of Helsinki guidelines and was approved by both the Human Research

Ethics Committee of the Australian National University (2012/278) and the ACT Health Human Research Ethics Committee (ETH.3.12.064).

Inclusion criteria comprised: 1) age above 18 years, 2) a clear diagnosis of migraine with or without aura according to International Headache Society criteria (2013) 3) corrected visual acuity in both eyes better than 6/12. Exclusion criteria included: 1) a history of other visual or neurological disturbance that might affect visual assessment, 2) a history of epilepsy, 3) colour blindness, 4) pregnancy, 5) medication that could affect pupillary responses, 6) migraine headache occurring within the 24 hours prior to testing. The control group consisted of age- and sex-matched normal healthy controls.

Sample size calculation suggested that a total of 22 migraine subjects and 22 controls would be needed. In order to detect an effect size of 40% increase in migraine headache or aura occurrence after testing with the mfPOP device. The power was set at 80% using a two-sided t-test at the level of $p = 0.05$. This calculation was done using the sample size formula for proportions (Lawrence M. Friedman, 2010).

A screening session to establish eligibility was performed for each participant during which a complete history to establish the diagnosis by the investigator along with background information regarding age at migraine onset, typical triggers, pattern, frequency and duration of migraine, presence of photophobia, other headaches, time since the most recent headache attack, and medication use (therapeutic or preventative) was obtained. Medications were divided according to class into triptans, opioids or over-the-counter (OTC) medications (NSAIDs, aspirin and paracetamol). A complete neurological examination to exclude other neurological disorders was conducted including confrontation visual field testing.

Subjects and controls were asked to complete a baseline headache diary for one week. This was felt to be an appropriate duration granted the short mfPOP testing time. Participants were then randomized using Research Randomizer software (Urbaniak and Plous, 2013) to undergo one of the two mfPOP testing protocols **(Figure 1)**. The randomized crossover design minimized the influence of confounding covariates because each patient acted as their own control. After each test a second diary was completed for a week followed by a washout period of a week. Subjects then underwent the other mfPOP protocol (see below) after which they completed a third headache diary. For ethical reasons, the use of subjects' usual pain-relieving medications was permitted. This enabled evaluation of trends in medication use before and after testing and was felt to be likely to increase adherence to the study. All participants were advised not to smoke, drink caffeinated beverages, or consume alcohol during the six hours prior to testing.

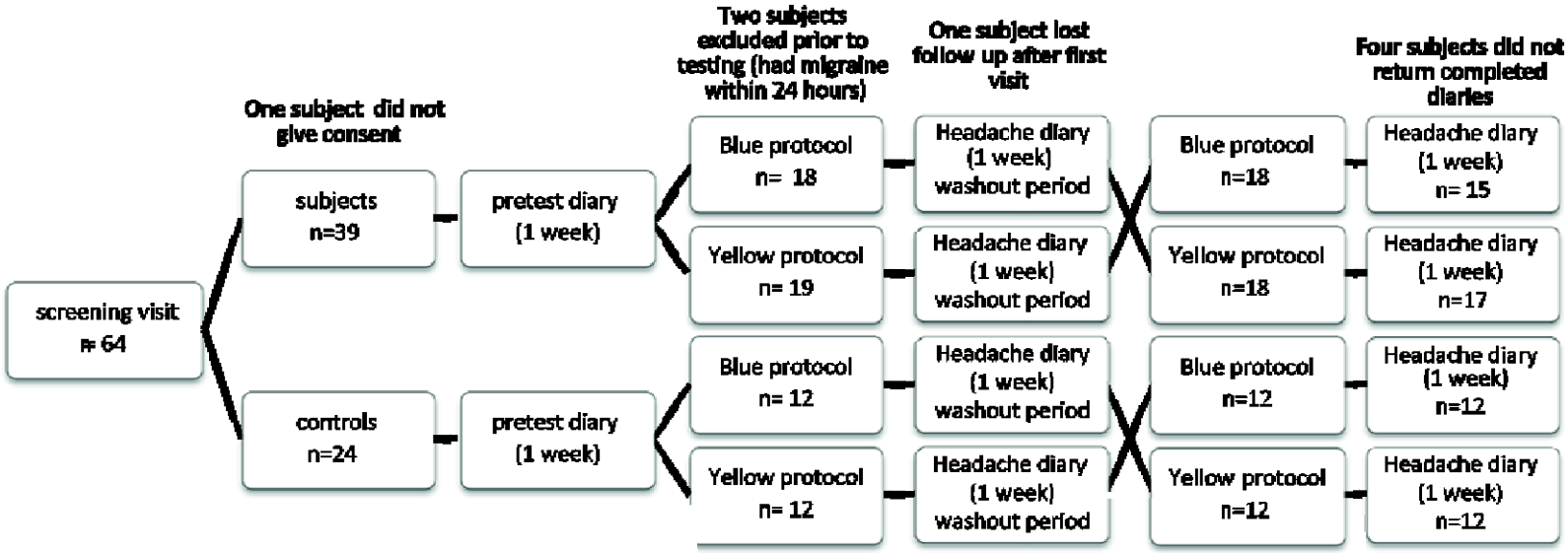


Figure 1. Schema: Cross over study design.

2.2.2. Migraine diaries

A validated migraine headache diary devised by The Diagnostic Headache Diary Study Group (Jensen et al., 2011) was used on the three occasions described above **(Figure 2)**.

Parameters recorded included whether the subject experienced a migraine headache (yes/no), severity on a scale of 1-3 (1= not bad, 2= quite bad, 3= very bad), duration (estimated from the time the first symptoms were noticed until the time the headache finally subsided), characteristics (throbbing or compressing /unilateral or bilateral), associated factors (presence of aura, photophobia, phonophobia, nausea vomiting), precipitating and relieving factors, as well as medication consumption (including type, dosage and frequency).

2.2.3. MfPOP assessment

All subjects underwent mfPOP assessment using the nuCoria Field Analyser® prototype (nuCoria Pty Ltd, Canberra, Australia) **(Figure 3).** The components of the FDA-cleared device are described in **(Figure 3A)**. Corrective lenses compensate for any refractive error. Stimuli were presented by a pair of liquid crystal displays (LCD) and reflected by cold dichroic mirrors to the two eyes simultaneously, these types of mirrors were used to allow infra-red light through to permit illumination and videoing of the irises. The cameras operated a 60 frames/s and a resolution of 512x768 pixels. The forty-four pseudo-randomly presented individual stimuli/eye were arranged in a dart-board like pattern extending to ±30°eccentricity of visual field **(Figure 3B, C)**. To reduce the effects of possible light scatter from adjacent regions a background illumination of 10 cd/m^2 was used to adapt rod photoreceptor responses (note that commercial perimeters provide this light level to light adapt the rods to reduce

responses to weak scattered light). To minimise any accommodative response the display included a small central (binocular) red cross to assist the subject to fixate at optical infinity, in addition, computer monitoring of fixation was used. Stimuli were presented dichoptically to each eye, and both direct and consensual responses were obtained from each tested visual field region.

Relative (rather than absolute) pupil diameter was recorded by video cameras using infrared illumination with settings unified for both protocols. Only the lower 75% portion of the pupil was recorded to avoid potential problems generated by ptosis. Stimuli were presented in 9 segments. If more than 15% of a segment was lost, that particular recording segment was repeated.

Start of registration period _____ Stop of registration Period _____

Please read carefully the instructions. Complete one column **every** evening by ticking the applicable boxes.

		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1. Day and date of the month		___	___	___	___	___	___	___	___	___	___	___	___	___	___
2. Did you have a headache today? (if no, go directly to question 15)	No Yes	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
3. If yes, when did you first notice it?	(hr:min)														
4. When did it finally go?	(hr:min)														
5. In the hour *before* it started, did you notice eyesight interference such as flashing lights, zigzag lines or blind spots?	No Yes	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
6. Was the headache on one side of the head or both ?	On one side On both sides	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
7. What was the headache like?	Throbbing Pressing	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
8. Did physical activity (such as walking upstairs) make the headache worse?	No Yes	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
9. How bad was your headache overall? (please see instructions)	Not bad Quite bad Very bad	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
10. Were you nauseated ? (did you feel you were going to be sick)?	No A little More than a little	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
11. Did you throw up?	No Yes	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
12. Were you bothered by the light?	No Yes	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
13. Were you bothered by noise?	No Yes	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐	☐ ☐
14. Did you do anything, or did anything happen, that may have caused the attack?	If yes, please specify:														
15. Did you take any medication(s) today for headache or for any other pain? **For each medication, please enter:** **a) the name** **b) the number you took** **c) the time(s) you took it (hr:min)**															

Figure 2. Sample migraine diary (Jensen et al., 2011)

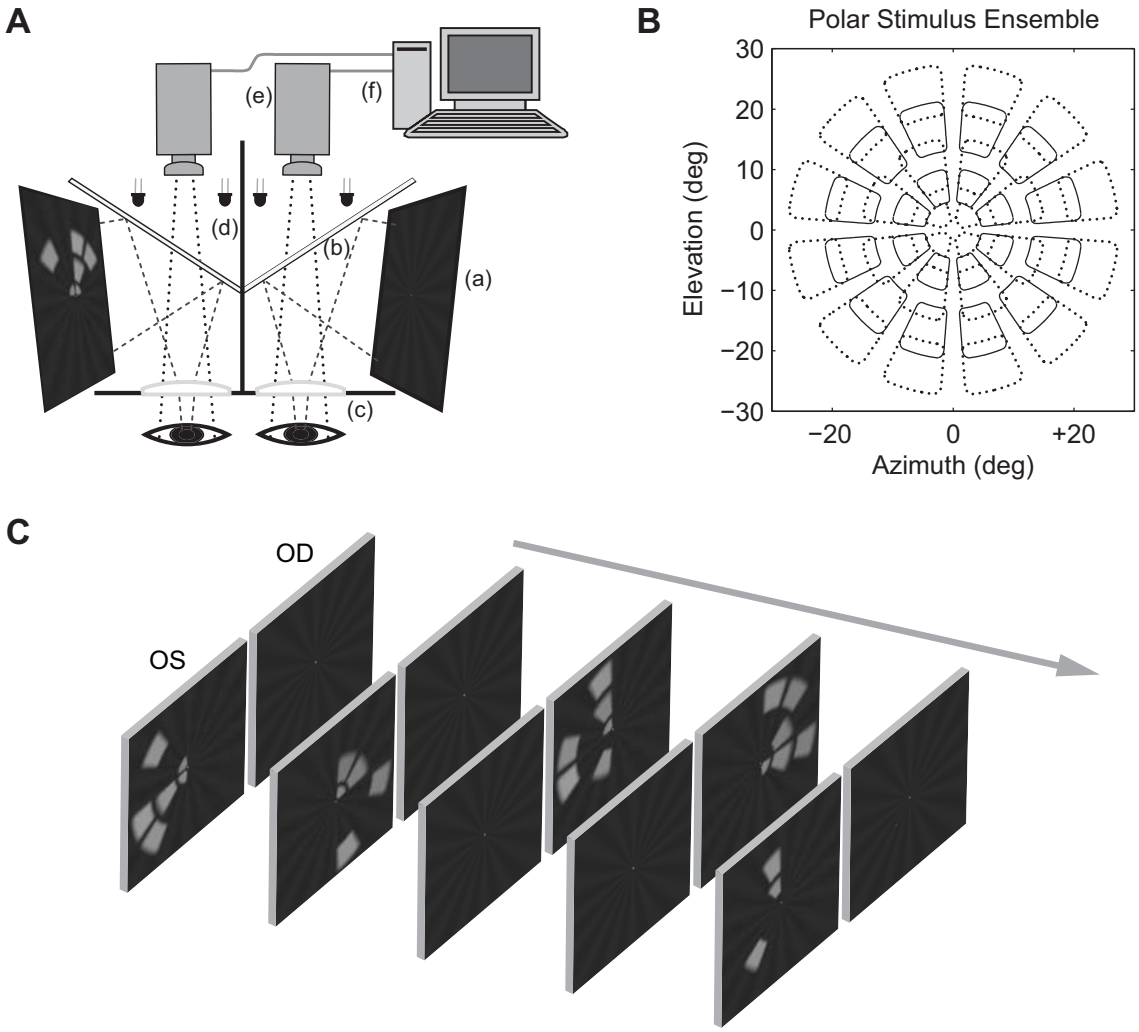


Figure 3. Measurement of pupillary responses. **A)** Schematic of the nuCoria Field Analyser®. Stimuli are presented independently on two LCD monitors (a). These images are reflected by two dichroic mirrors (b) allowing infrared light to pass while reflecting shorter wavelengths. Viewing distance is increased to optical infinity by plano-convex lenses (c). Each eye views only one monitor, the images being fused by the subject into a cyclopean view. Infrared illumination of the eyes is provided by infrared light-emitting diodes (d) facilitating the monitoring of each pupil by separate infrared video cameras (e). Pupil diameters are then extracted in real-time and recorded by a computer, this also allowed the investigator to have a view of the pupil images during real-time recording (f). **B)** The 44 stimulus regions per eye were arranged in a dartboard-like polar layout extending to 30° from fixation. **C)** Showing the independent stimuli (dichoptic) from a series of video frames of the test sequence. Stimuli were pseudo-randomly presented to each hemifield of each eye in a consecutive series. The faint background starburst pattern assists the subjects to fuse the images.

2.2.4. Stimulus characteristics

There were two stimulus methods (protocols) used and the characteristics of each protocol are summarized in **(Table 1)**.

The "yellow" protocol (YP) – the standard mfPOP testing protocol – was designed to stimulate cone photoreceptors. It involved stimuli with a maximum luminance of 150 cd/m^2 and individual stimulus duration of 33 msec. Test duration was 6 minutes divided into 9 segments, each of 40 s duration, with additional rest periods of several seconds between segments.

The "blue" protocol (BP) was designed to stimulate the intrinsic response of ipRGCs (Carle et al., 2015), and involved presentation of blue stimuli corresponding to 479 nm with a maximum luminance of 75 cd/m^2 **(Figure 4)**. This wavelength was chosen based on the response of a primate melanopsin-expressing ganglion cell to a 470 nm light pulse where those cells continued to fire action potentials for 30 seconds after the end of the light stimulus (Lucas et al., 2001, Kawasaki and Kardon, 2007). Stimulus chromaticities are given in Table 1, and the relative cone excitations are given in **(Figure 4)**. Evidence that the BP protocol does simulate the ipRGCs has been published elsewhere (Carle et al., 2015).

For both protocols the array of 44 stimuli extended to ±30° eccentricity to cover the area of retina with the highest ipRGC density (greatest toward the fovea with peak density of 20-25 cells/mm^2) (Dacey et al., 2005). The probability of absorbing a photon by an ipRGC is >1 million times lower than in rods or cones for a given area of photic stimulation (Do et al., 2009). Hence, the BP stimulus duration was increased from the standard 33msec to 1 sec to ensure optimal melanopsin-driven sustained pupil

responses (Park et al., 2011). Also ipRGCs integrate the melanopsin signal over a second or more (Dacey et al. 2005). As with YP the test duration was six minutes. That test duration is the standard mfPOP testing duration used in many studies (excluding breaks) and was felt to be sufficient based on previous work by Cao who found that a mean time of 6.88 minutes was needed to trigger a migraine in 61.5% of subjects shown a provoking visual stimulus (Cao et al., 1999).

Table 1. Stimulus characteristics

Protocol	Stimulus colour C.I.E. *x,y* coordinates	Stimulus maximum luminance	Background luminance	Stimulus duration	Recording duration
Blue Protocol:	[0.145, 0.113]	75cd/m²	10 cd/m²	1 sec	6 minutes
Yellow Protocol:	[0.377, 0.464]	150cd/m²	10 cd/m²	33 msec	6 minutes

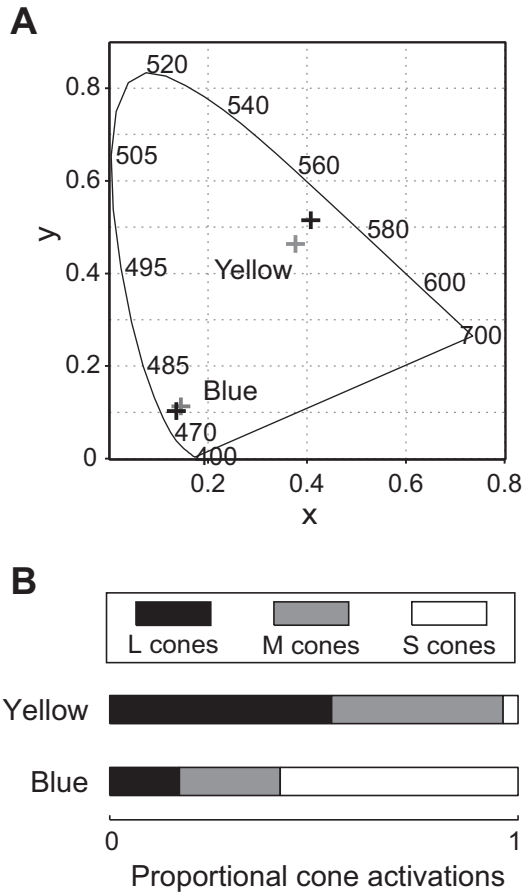


Figure 4. Stimulus characteristics. (A) C.I.E. coordinates for the colors used in the study. The crosses in grey represent the background, and the black crosses are the stimulus. **(B)** Proportional cone activations for the yellow and blue protocols.

2.2.5. Data Analysis

Analysis was conducted using MATLAB software (MathWorks, Natick, MA). Response waveforms – for both direct and consensual responses – from each region of the visual field were obtained and fitted to a log-normal function as follows:

$$v(t)= A \exp (-[\ln(t/t_p)]^2 \ / \ 2\sigma^2)$$

Where *v(t)* is the response waveform, A is the peak amplitude, *t* is the time at which each estimation is made, t_p is the time to peak, and σ is the width of the response (Bell et al., 2010, Carle et al., 2011a, Carle et al., 2013).

This allowed the characterization of the responses according to standardized amplitude (AmpStd) and time-to-peak **(Figure 5)**. AmpStd assessed any change in pupil size corrected to the mean diameter of the population rather than using absolute pupil size and was expressed in decibels (dB). It was derived from contraction amplitude as follows:

AmpStd = contraction amplitude (μm) x 3500/c

Where *c* is the mean pupil diameter based on the value of a line fitted to the entire 240 seconds of pupil diameter data recorded during each test, and 3500 μm is the nominal population mean.

AmpStd was used to overcome inter-subject variation in mean pupil diameter and also improved tolerance to non-circular pupils. The higher the AmpStd, the larger the magnitude of pupillary constriction.

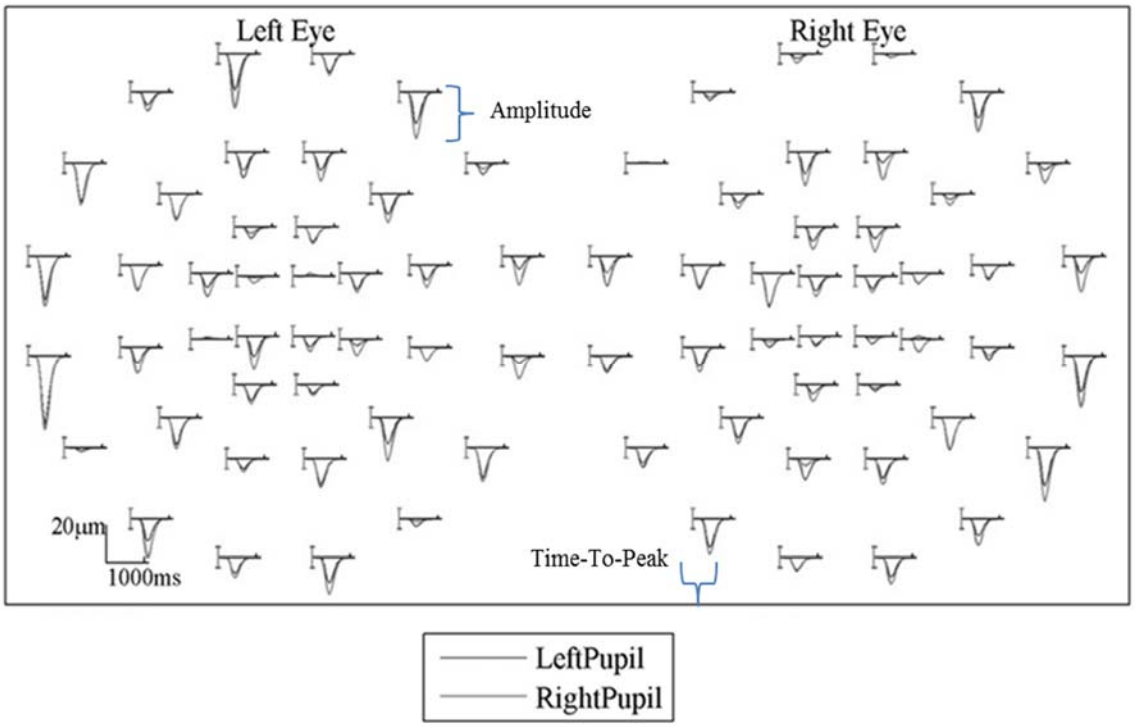


Figure 5. Example mfPOP response waveforms from an individual subject. The mean pupil responses to stimuli present to each of the 44 test regions were obtained from both eyes concurrently from 6 minutes of stimulation. Downward deflection indicates contraction. The black and grey traces are the mean responses of the left and right pupils.

Student's t-tests and Fisher's exact tests were used to compare baseline characteristics. Odds ratios, McNemar's and Cochran's q tests, and one-way between-subjects ANOVAs were conducted to compare the number of subjects developing migraine after each protocol – the primary outcome – and the effect of mfPOP testing on other migraine parameters. Multivariate linear models were used to assess the independent effects of migraine parameters on the pupillary response.

The percentage area under the curve (AUC) of the receiver operating characteristic (ROC) plot was used as a measure of the power of mfPOP to predict migraine diagnosis i.e**.** it *quantified the overall ability of the mfPOP to discriminate between individuals with and without migraine***.** ROC plots were constructed for both AmpStd and time-to-peak in both protocols, using either the single worst region in each field (i.e. the one most deviating from normal) or the mean of the five worst regions, looking at either single eyes or at the asymmetry between anatomically equivalent regions of the two eyes (Bell et al., 2010).

2.3. Results

Forty migraine patients **(Table 2)** were screened and 39 enrolled **(Figure 1)**. Thirty-eight subjects completed testing with both mfPOP protocols. Two subjects were excluded because they developed a migraine within the 24 hours prior to testing, one patient withdrew after the first test, and four subjects did not return completed diaries. In all, thirty-two sets of completed migraine diaries were returned and analyzed. In addition, 24 age- and gender-matched controls were studied.

Table 2. Subject characteristics

	Subjects (n=36)	***Controls (n=24)***
Age (mean ± SD)	42.0 ± 16.5	39.2 ± 15.2
Male: Female	1 : 1.8	1: 1.5
Migraine type		-
• **With aura**	26 (72%)	
• **Without aura**	15 (42%)	
Mean age of onset (±SD)	17.8 ± 9.11	-
Mean disease duration (±SD)	24.3 ± 16.7	-
Treatment		-
• **Preventative**	8 (22%)	
• **During attacks**		
Over the counter	25 (69%)	
Triptans	14 (39%)	
Opioids	13 (36%)	
Ergot	2 (6%)	
Mean attacks per month (±SD)	2.62 ± 5.17	-
Mean headache duration (hours) (±SD)	11.75 ± 16.5	-
Trigger		-
• **Light**	15 (41%)	
• **Other**	32 (88%)	
Photosensitivity	34 (94%)	-

2.3.1. Effects of mfPOP stimulation on migraine headache severity parameters

Only one patient had difficulty completing the BP, reporting the occurrence of an aura at the end of the test. Otherwise, all patients reported no discomfort during testing apart from mild tearing due to lack of blinking. The effects of testing on other migraine parameters are summarized in **(Table 3)** which shows the same number of patients – four subjects (12.5%) – developing a migraine attack in the first day after testing with either BP or YP. The difference was not significant (odds ratio 1.0; 95% confidence interval 0.2-4.4, p = 0.48). The results remained non-significant testing both protocols over the first 72 hours, with 11 subjects (34.4%) developing migraine post-BP and 13 subjects (41%) post-YP (odds ratio 0.8; 95% confidence interval 0.3-2.1, p = 0.68). This period of 72 hours was examined based on evidence that it may take up to 48 hours from a trigger for a migraine to occur (Zagami and Bahra, 2006). In comparison to pre-testing, migraine days / week were not significantly increased: 1.4 ± 1.6 pre-testing (mean ± SD), 1.3 ± 1.4 post-BP, and 1.3 ± 1.2 post-YP (both p = 0.96). Other migraine parameters including attack severity, attack duration, and percentage of patients taking medication before and after each test were also not significantly different.

Table 3. Effects of mfPOP stimulation on migraine headache severity parameters

Parameter	*Pre-testing*	*Post-BP*	*Post-YP*	*P value*
Patients experiencing migraine in the 1st day post testing (number, %)	-	4 (12.5%)	4 (12.5%)	0.48[a]
Patients experiencing migraine within 3 days post testing (no, %)	-	11(34.4%)	13(41%)	0.68[a]
Migraine days/week(mean ± SD)	1.4 ± 1.6	1.3 ± 1.4	1.3 ± 1.2	0.96[b]
% of patients experiencing quite bad to very bad migraine	34%	25%	43%	0.10[c]
Mean attack duration (hours)	1.41	1.09	1.07	0.71[b]
% of patients taking medication	50%	50%	53%	0.93[c]

[a]McNemar's test
[b] One way ANOVA
[c] Cochran's Q test

2.3.2. Changes in the mfPOP pupillary response characteristics

Results for mean AmpStd and time-to-peak are summarized in **(Table 4)** No significant differences were found between patients and controls during either BP or YP. However, there was a shorter time-to-peak during YP (493.5 ± 25.7 ms in controls and 494.9 ± 22.3 ms in migraineurs) compared to BP (594.9 ± 55.2 ms in controls and 604.3 ± 48.8 in migraineurs), probably related to the large difference in the duration of the stimuli and the very slow response time of ipRGCs.

2.3.3. Effects of headache characteristics on the pupillary response in migraineurs

The closer a migraine attack occurred prior to the time of testing, the more negative the effect upon AmpStd, i.e. less pupillary constriction in both protocols **(Table 5)**. The greatest reduction was seen if the attack occurred within one week prior to testing, followed by two weeks and, lastly, two months prior to testing. There was no "predictive" effect of mfPOP associated with a migraine that was about to occur in the week following testing, i.e. no changes in the pupillary response were seen before a migraine attack was about to happen (results not shown). Looking at the effect of medications, the use of triptans was associated with a significant increase in AmpStd in both YP and BP (0.45 ± 0 .09 dB, and 0.48 ± 0.07 dB, respectively, $p < 0.001$). This was not seen for other therapeutic medications (OTC medications, opioids, etc.) or preventative therapies. Although the presence of other types of headaches (mainly tension headaches) was associated with a significant change in amplitude, the number of patients was too small to allow further comment.

Table 4. mfPOP results for the Blue and Yellow protocols.

		Control (mean ± SE)	**Migraine (mean ± SE)**
Blue protocol	**AmpStd (dB)**	9.48 ± 10.4	9.05 ± 11.5
	Time-to-peak (ms)	594.9 ± 55.2	604.3 ± 48.8
Yellow protocol	**AmpStd (dB)**	11.4 ± 5.23	10.8 ± 6.25
	Time-to-peak (ms)	493.5 ± 25.7	494.9 ± 22.3

Table 5. Independent Effects of headache Parameters on AmpStd in migraine subjects.

	Blue protocol		Yellow protocol	
	dB ± SE	**P value**	**dB ± SE**	**P value**
(Reference) *	-0.96 ± 0.11	-	-0.70 ± 0.09	-
Migraine within last 60 days (n=26)	-0.45 ± 0.16	0.004	-0.08 ± 0.11	0.501
Migraine within last 2 weeks (n=24)	-0.64 ± 0.14	<0.001	-0.65 ± 0.11	<0.001
Migraine within last week (n=19)	-0.80 ± 0.10	<0.001	-0.57 ± 0.08	<0.001
Female gender (n=22)	0.07 ± 0.10	0.448	0.42 ± 0.07	<0.001
Triptan (n=13)	0.45 ± 0.09	<0.001	0.48 ± 0.07	<0.001
Other headaches (n=2)	2.40 ± 0.25	<0.001	-1.17 ± 0.14	<0.001

* Male patients aged 40 without migraine in the last 60 days and no triptan use. AmpStd values used were the mean for each subject of the 22 weakest contracting regions relative to normal in decibels. Other factors included in the multivariate linear models include age and consensual pupillary responses.

2.3.4. Visual field defects detected by the mfPOP

(Figure 6) Shows examples of visual field abnormalities seen in migraine subjects demonstrating deviations in AmpStd from normal (images taken in response to the YP). In general, these defects were seen with both the YP and BP. They were mainly concentrically located, affecting the peripheral visual fields and were asymmetric between the two eyes: in some cases they were monocular but a few were homonymous in nature. These defects were seen less in subjects with migraine without aura, and when measurement were further in time from the last migraine attack. Grey scale plots **(Figure 7)** representing the mean deviations in AmpStd taken from all migraine subjects in response to both the YP and BP from normal controls, again abnormalities were on average more pronounced in the periphery of the visual fields. Although averages around rings are sometimes used in multi-focal methods there appears to be no obvious ring-structure.

2.3.5. The power of mfPOP to predict the diagnosis of migraine

(Table 6) shows that the ROC %AUC ranged between 65% and 77% for AmpStd, with BP performing marginally better than YP. Better performance was achieved using smaller subsets of regions (the single most deviating test region in visual field or the mean of the worst 5 regions), rather than the mean pupil response across regions indicating significant localised scotomas. When asymmetry between the eyes was analysed, the %AUC increased to 88% for BP and 81% for YP. The asymmetry values is calculated as absolute value of the difference between visual field locations that are analytical equivalent, i.e. temporal and temporal field locations, nasal and nasal locations. Thus there are 44 asymmetry measures per patient (Bell et al., 2010, Sabeti et al. 2014, Sabeti et al., 2015).

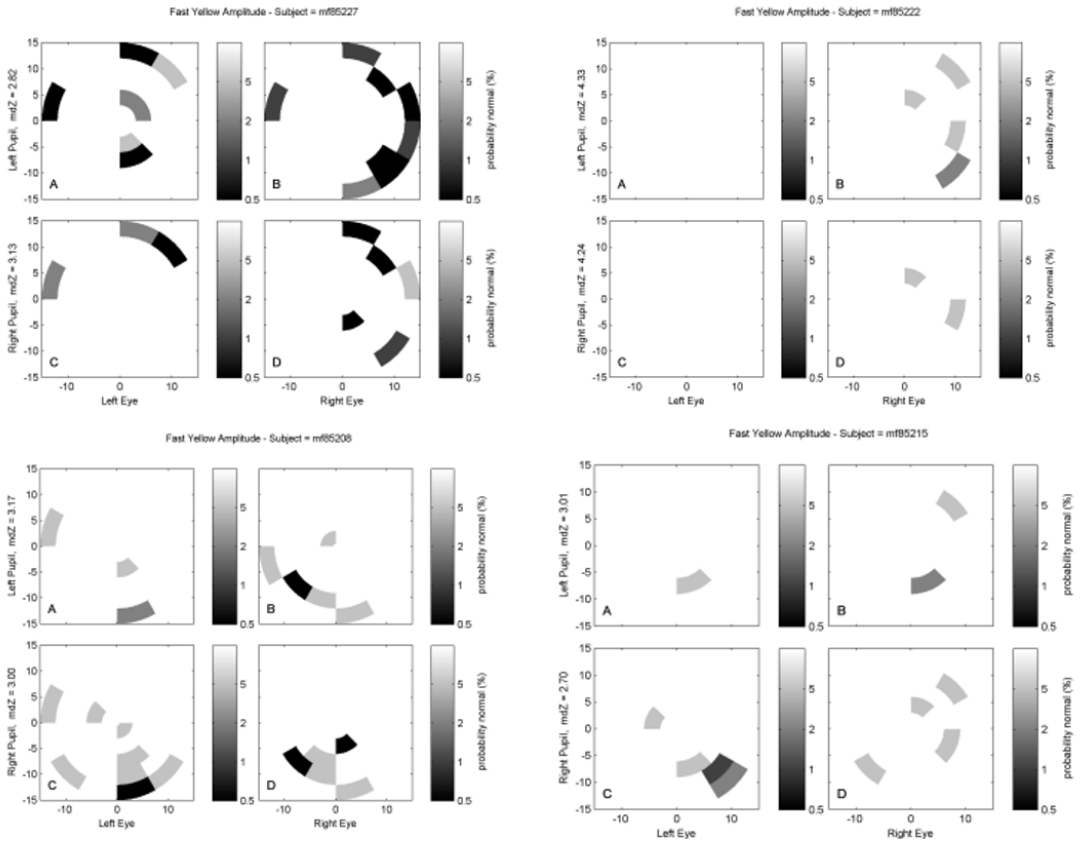


Figure 6. Examples of visual field defects from the YP detected by the mfPOP device showing deviation in AmpStd from normal in four migraine subjects, results from each subject being in a 2 x 2 block of plots labelled A to D. The darker the region the more it deviates from normal. The top row of each set of four represents responses recorded by the left pupil, and the bottom row the right pupil. Direct responses are thus labelled A and D, and consensual responses B and C. Afferent defects will be in agreement for an eye and across pupils (across a vertical pair), effect defects would be consistent for a given pupil (across a horizontal pair) and not in agreement across eyes. Here the defects mainly appear to be afferent.

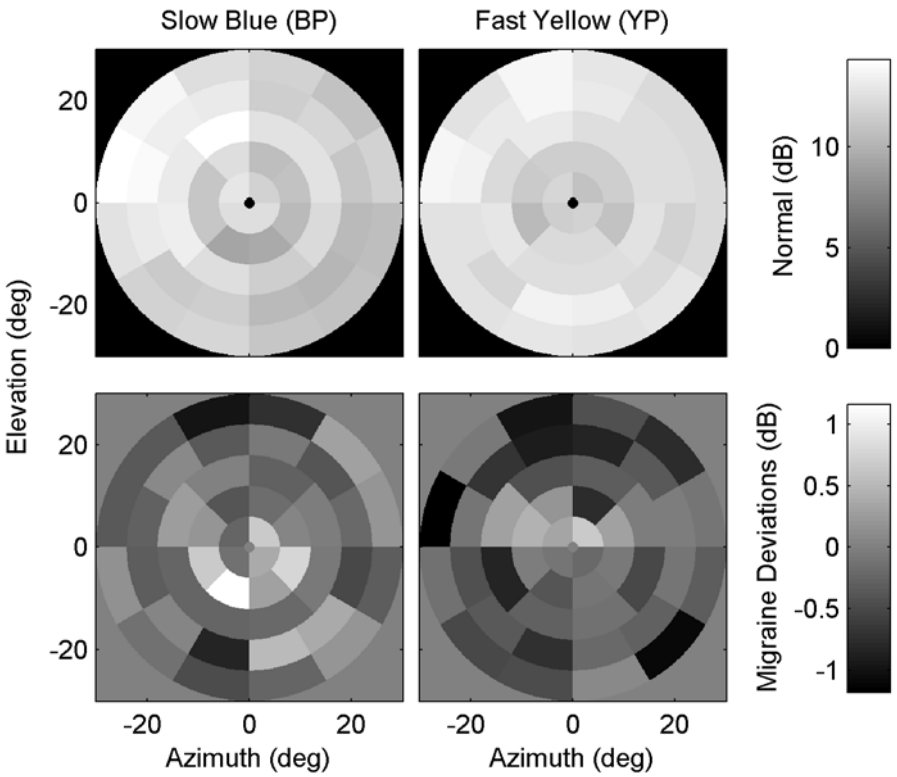


Figure 7. Gray scale plots showing mean deviations of AmpStd in migraine patients from normal controls in response to both the YP (right column) and BP (left column) (top row) represents normal controls; (bottom row) represents migraine effects (difference from normal base on linear models). Patients show reductions in sensitivity (darker tones) that are perhaps greatest in the periphery.

	Blue protocol (%)	Yellow protocol (%)
AmpStd each eye:		
. Worst point / 44	76.7 ± 4.40	72.0 ± 4.65
. Mean of worst 5 pts	65.2 ± 5.06	68.4 ± 4.88
Asymmetry between eyes:		
. Worst point / 44	88.3 ± 4.06	80.6 ± 4.24
. Mean of worst 5 pts	73.8 ± 4.52	75.0 ± 4.88

Table 6. %AUCs for blue and yellow protocols.

2.4. Discussion

The results show that the mfPOP device is relatively safe and well tolerated when used in migraine patients. There was no increase in the incidence or severity of migraine headaches, even when using the blue stimulus which was specifically designed to stimulate the melanopsin-containing ipRGCs. We have shown similar results in epilepsy patients (Chapter 3), and the lab has recorded over 14,000 mfPOP tests on over 3800 subjects without a complaint of a migraine or seizure. The mean pupillary response characteristics determined by mfPOP in migraine patients did not differ from those of controls. However, there was a significant reduction in pupillary constriction associated with a migraine attack occurring prior to testing. Being a user of triptans was associated with the opposite effect i.e. pupillary constriction was increased. mfPOP was moderately able to distinguish patients from controls with BP performing better than YP detecting visual fields defects consistent with the scintillating scotomas reported by migraine patients.

Based on the work of Noseda *et al.* that pointed to a role of the melanopsin containing ipRGCs in the exacerbation of migraine headaches (Noseda et al., 2010) new modalities to treat migraine have emerged such as pharmacological manipulation of melanopsin (Jones et al., 2013) or blocking of blue wavelengths using tinted lenses (Good et al., 1991). Therefore, it is important to understand the effects of ipRGC stimulation on migraine pathophysiology. This study is the first to use a stimulus specifically designed to target the melanopsin-containing ipRGCs in order to look for

an effect on migraine occurrence. In addition to being responsible for the Post-illumination Pupil Response (PIPR), results by Gamlin *et al* also suggested that melanopsin has a substantial influence on early pupillary response constriction in macaques. This was proved during retinal illumination at 493 nm and pharmacological blockade of rod and cone inputs, and finding the pupillary response to be present despite being delayed by approximately 1 s and more sluggish than normal. This consistent with the idea that the ipRGCs controls the acute phase of the pupillary response (Gamlin et al., 2007).

Furthermore Carle *et al* found that the stimulus response functions - namely the pupil constriction amplitudes - for the blue stimuli obtained through mfPOP matches that of the melanopsin containing RGCs (Carle et al., 2015). The stimuli were specifically designed to eliminate light characteristics that could potentially cause discomfort in migraine subjects; stimuli were small and brief (33 to 1000 ms) and were delivered randomly to any location in the visual field (i.e. stimulation of the two eyes was never co-synchronous in any part of the visual field). Thus, each stimulus activated much less than 10% of the visual cortex at any given time (Hay et al., 1994). The stimuli did not contain stripes or checks (Marcus and Soso, 1989), had smooth edges like sine-wave gratings, and contained no high spatial frequencies above 2.0 cyc/deg. Thus, color and duration were the main characteristic that could have contributed to any effect that the stimulus might have had on triggering a migraine attack.

When looking at the mean pupillary responses obtained by mfPOP, the results are in agreement with Cambron *et al.* (Cambron et al., 2014) who studied autonomic function in migraine patients during both ictal and interictal phases using pupillometry and found no significant difference between migraine sufferers and controls in either phase in terms of latency, amplitude of constriction, minimum diameter, constriction and re-

dilatation speeds. However it is important to point out that, when looking at changes in responses to some parts of the visual field as opposed to the overall mean across the field, it was possible to detect differences when testing was conducted shortly after an attack or after triptan use. These changes were unlikely to be due to a generalized autonomic dysfunction accompanying migraine attacks as such changes would generate equal defects in all regions. One speculation is that the abnormalities represent small visual field defects (or migraine scotomas) resulting from cortical spreading depression (CSD) (Grafstein, 1956). Looking further at these defects they were found to be asymmetric between the two eyes, concentrically arranged, and localized to the peripheral fields (Figs. 6 and 7). In some subjects they were monocular and a very small number had homonymous defects. With increasing time after an attack these defects were observed to be smaller in magnitude. The results are consistent with previous work by the MacKendrick group who found similar defects using static and temporal modulation perimetry (McKendrick et al., 2000). None of the visual defects they detected were consistent with a cortical locus (i.e. bilateral homonymous deficits), which made them speculate that they were due to a pre-cortical visual dysfunction. They suggested that defects in the magnocellular visual pathways which also contribute to the pupillary response (Alpern and Campbell, 1962{Tsujimura, 2003 #475) may be responsible for theses visual disturbances based on selective loss for targets temporally modulated by either motion or flicker (McKendrick et al., 2001). They excluded the retina to be the origin of theses magnocellular defects when they simultaneously recorded retinal and cortical visually evoked electrophysiological responses in-between migraine attacks using pattern-reversal electro-retinograms (PERGs) and pattern visual evoked responses (PVERs), and found PERG to be normal while defects were detected on PVERs (Nguyen et al., 2012, Nguyen et al., 2014). mfPOP responses are generated

mainly through a subcortical pathway with some cortical influence – including an enhanced inhibitory effect – at the level of the Edinger-Westphal nucleus (Barbur, 1995). If the observed effect of decreased pupillary constriction was due to a direct influence from the cortex during cortical spreading depression (CSD), an increased, rather than a decreased, contraction would have been observed due to the loss of the inhibitory effect on the pupillary pathway leading to pupillary constriction. This was the opposite of what occurred. This allows speculation that these changes may arise independently from the cortex and that the spreading depression might extend and involve a subcortical pathway, namely the pupillary pathway leading to pupillary dilatation.

Although the use of pain-relieving medications in this study may have altered the course of migraine and set the migraine brain excitability to a different threshold, the fact that patients were allowed to use their own medication permitted assessment of the effect of triptan use; interestingly, this was associated with increased pupillary constriction. This finding is the opposite of what might have been expected because the serotonergic effects of triptan overdose are well known to cause mydriasis. The mfPOP system does not use absolute pupil size, but rather changes in pupil size relative to the mean and, interestingly, the triptan effect was restricted to some parts of the visual field. If the effect of triptans was on the iris as a whole, this should have resulted in a global visual field change. The fact that it did not suggests that the triptans could have had an effect on cortical hyperexcitability (Coppola and Schoenen, 2012).

Our study did have some limitations, the subjects included had infrequent migraine attacks (mean attacks were 2.6 per month); and we had a short baseline period (only two weeks).

Conclusions

This study has demonstrated that stimulation of melanospin-containing ipRGCs did not alter migraine severity parameters, and that overall pupillary responses did not differ in migraine patients from normal controls. Abnormalities were detected only if testing was carried out shortly after an attack or if subjects were taking triptans. mfPOP proved to be an important tool in studying visual pathophysiology in migraine subjects and was able to map visual field defects and pupil response changes.

References

2013. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia,* 33**,** 629-808.

ALI, E., MADDESS, T., JAMES, A., VOICU, C. & LUECK, C. 2014. Pupillary response to sparse multifocal stimuli in multiple sclerosis patients. *Multiple Sclerosis Journal,* 20**,** 854-861.

ALPERN, M. & CAMPBELL, F. W. 1962. The spectral sensitivity of the consensual light reflex. *J Physiol,* 164**,** 478-507.

BARBUR, J. L. 1995. A study of pupil response components in human vision. *In:* ROBBINS, J. G., DJAMGOZ, M. B. A. & TAYLOR, A. (eds.) *Basic and clinical prespectives in vision research* New York: Plenum Press.

BARRIGA, F. J., LOPEZ DE SILANES, C., GILI, P. & PAREJA, J. A. 2011. Ciliary ganglioplegic migraine: migraine-related prolonged mydriasis. *Cephalalgia,* 31**,** 291-5.

BELL, A., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Dichoptic multifocal pupillography reveals afferent visual field defects in early type 2 diabetes. *Invest Ophthalmol Vis Sci,* 51**,** 602-8.

CAMBRON, M., MAERTENS, H., PAEMELEIRE, K. & CREVITS, L. 2014. Autonomic function in migraine patients: ictal and interictal pupillometry. *Headache,* 54**,** 655-62.

CAO, Y., WELCH, K. M., AURORA, S. & VIKINGSTAD, E. M. 1999. Functional MRI-BOLD of visually triggered headache in patients with migraine. *Arch Neurol,* 56**,** 548-54.

CARLE, C., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Multifocal Pupillographic Objective Perimetry in Glaucoma using Blue Stimuli. *International Perimetry Society 19th Annual Meeting, ,*

. Puerto de la Cruz, Tenerife.

CARLE, C. F., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2015. Blue Multifocal Pupillographic Objective Perimetry in Glaucoma. *Invest Ophthalmol Vis Sci,* 56**,** 6394-403.

CARLE, C. F., JAMES, A. C., KOLIC, M., LOH, Y. W. & MADDESS, T. 2011a. High-resolution multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci,* 52**,** 604-10.

CARLE, C. F., JAMES, A. C. & MADDESS, T. 2013. The pupillary response to color and luminance variant multifocal stimuli. *Invest Ophthalmol Vis Sci,* 54**,** 467-75.

CARLE, C. F., MADDESS, T. & JAMES, A. C. 2011b. Contraction anisocoria: segregation, summation, and saturation in the pupillary pathway. *Invest Ophthalmol Vis Sci,* 52**,** 2365-71.
COPPOLA, G. & SCHOENEN, J. 2012. Cortical excitability in chronic migraine. *Curr Pain Headache Rep,* 16**,** 93-100.
DACEY, D. M., LIAO, H. W., PETERSON, B. B., ROBINSON, F. R., SMITH, V. C., POKORNY, J., YAU, K. W. & GAMLIN, P. D. 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature,* 433**,** 749-54.
DO, M. T., KANG, S. H., XUE, T., ZHONG, H., LIAO, H. W., BERGLES, D. E. & YAU, K. W. 2009. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature,* 457**,** 281-7.
GAMLIN, P. D., MCDOUGAL, D. H., POKORNY, J., SMITH, V. C., YAU, K. W. & DACEY, D. M. 2007. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Res,* 47**,** 946-54.
GOOD, P. A., TAYLOR, R. H. & MORTIMER, M. J. 1991. The use of tinted glasses in childhood migraine. *Headache,* 31**,** 533-6.
GRAFSTEIN, B. 1956. Mechanism of spreading cortical depression. *J Neurophysiol,* 19**,** 154-71.
HAY, K. M., MORTIMER, M. J., BARKER, D. C., DEBNEY, L. M. & GOOD, P. A. 1994. 1044 women with migraine: the effect of environmental stimuli. *Headache,* 34**,** 166-8.
JENSEN, R., TASSORELLI, C., ROSSI, P., ALLENA, M., OSIPOVA, V., STEINER, T., SANDRINI, G., OLESEN, J. & NAPPI, G. 2011. A basic diagnostic headache diary (BDHD) is well accepted and useful in the diagnosis of headache. a multicentre European and Latin American study. *Cephalalgia,* 31**,** 1549-60.
JONES, K. A., HATORI, M., MURE, L. S., BRAMLEY, J. R., ARTYMYSHYN, R., HONG, S. P., MARZABADI, M., ZHONG, H., SPROUSE, J., ZHU, Q., HARTWICK, A. T., SOLLARS, P. J., PICKARD, G. E. & PANDA, S. 2013. Small-molecule antagonists of melanopsin-mediated phototransduction. *Nat Chem Biol,* 9**,** 630-5.
KAWASAKI, A. & KARDON, R. H. 2007. Intrinsically photosensitive retinal ganglion cells. *J Neuroophthalmol,* 27**,** 195-204.
LAWRENCE M. FRIEDMAN, C. D. F., DAVID L. DEMETS 2010. *Fundamentals of Clinical Trials*, Springer

LUCAS, R. J., DOUGLAS, R. H. & FOSTER, R. G. 2001. Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat Neurosci,* 4**,** 621-6.
MAIN, A., VLACHONIKOLIS, I. & DOWSON, A. 2000. The wavelength of light causing photophobia in migraine and tension-type headache between attacks. *Headache,* 40**,** 194-9.
MARCUS, D. A. & SOSO, M. J. 1989. Migraine and stripe-induced visual discomfort. *Arch Neurol,* 46**,** 1129-32.
MARTIN, P. R. 2010. Behavioral management of migraine headache triggers: learning to cope with triggers. *Curr Pain Headache Rep,* 14**,** 221-7.
MATYNIA, A., NGUYEN, E., SUN, X., BLIXT, F. W., PARIKH, S., KESSLER, J., PEREZ DE SEVILLA MULLER, L., HABIB, S., KIM, P., WANG, Z. Z., RODRIGUEZ, A., CHARLES, A., NUSINOWITZ, S., EDVINSSON, L., BARNES, S., BRECHA, N. C. & GORIN, M. B. 2016. Peripheral Sensory Neurons Expressing Melanopsin Respond to Light. *Front Neural Circuits,* 10**,** 60.
MCKENDRICK, A. M. & BADCOCK, D. R. 2004a. An analysis of the factors associated with visual field deficits measured with flickering stimuli in-between migraine. *Cephalalgia,* 24**,** 389-97.
MCKENDRICK, A. M. & BADCOCK, D. R. 2004b. Decreased visual field sensitivity measured 1 day, then 1 week, after migraine. *Invest Ophthalmol Vis Sci,* 45**,** 1061-70.

MCKENDRICK, A. M., VINGRYS, A. J., BADCOCK, D. R. & HEYWOOD, J. T. 2000. Visual field losses in subjects with migraine headaches. *Invest Ophthalmol Vis Sci,* 41**,** 1239-47.

MCKENDRICK, A. M., VINGRYS, A. J., BADCOCK, D. R. & HEYWOOD, J. T. 2001. Visual dysfunction between migraine events. *Invest Ophthalmol Vis Sci,* 42**,** 626-33.

NGUYEN, B. N., MCKENDRICK, A. M. & VINGRYS, A. J. 2012. Simultaneous retinal and cortical visually evoked electrophysiological responses in between migraine attacks. *Cephalalgia,* 32**,** 896-907.

NGUYEN, B. N., VINGRYS, A. J. & MCKENDRICK, A. M. 2014. The effect of duration post-migraine on visual electrophysiology and visual field performance in people with migraine. *Cephalalgia,* 34**,** 42-57.

NOSEDA, R., KAINZ, V., JAKUBOWSKI, M., GOOLEY, J. J., SAPER, C. B., DIGRE, K. & BURSTEIN, R. 2010. A neural mechanism for exacerbation of headache by light. *Nat Neurosci,* 13**,** 239-45.

PARK, J. C., MOURA, A. L., RAZA, A. S., RHEE, D. W., KARDON, R. H. & HOOD, D. C. 2011. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci,* 52**,** 6624-35.

SABETI, F., MADDESS, T., ESSEX, R. W. & JAMES, A. C. 2012. Multifocal pupillography identifies ranibizumab-induced changes in retinal function for exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci,* 53**,** 253-60.

SABETI, F., MADDESS, T., ESSEX, R. W., SAIKAL, A., JAMES, A. C. & CARLE, C. F. 2014. Multifocal pupillography in early age-related macular degeneration. *Optom Vis Sci,* 91**,** 904-15.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T. 2015. Multifocal Pupillography Identifies Changes in Visual Sensitivity According to Severity of Diabetic Retinopathy in Type 2 Diabetes. *Invest Ophthalmol Vis Sci,* 56**,** 4504-13.

URBANIAK, G. C. & PLOUS, S. 2013. Research Randomizer 4.0 ed. http://www.randomizer.org/

ZAGAMI, A. & BAHRA, A. 2006. *Symptomatology of migraines without aura.In The Headaches,* Philadelphia, Lippincott Williams & Wilkins.

Chapter 3.

Response characteristics of multifocal objective pupillographic perimetry in epilepsy patients

Abstract

Aim: To investigate the pattern of pupillary abnormalities in patients with epilepsy using Multifocal Objective Pupil Perimetry (mfPOP), and to conduct a preliminary investigation of its safety. While the stimuli provided during mfPOP fall well outside the usual parameters for epileptogensis, a preliminary investigation is warranted.

Design/Methods: A cross-sectional, open-labelled, study of 15 consecutive patients with epilepsy and 15 controls who underwent mfPOP during routine EEG testing. mfPOP responses were obtained from 44 regions of both visual fields simultaneously. Each region was analysed according to response time-to-peak and standardised amplitude. The proportion of patients developing a seizure, a photo-paroxysmal response (PPR), or increased epileptiform activity on their EEG during mfPOP was used as the outcome measure of safety. **Results:** All subjects tolerated mfPOP testing well. No patient developed an epileptic aura or clinical seizure during (or shortly after) testing. There was no evidence of a PPR or increased epileptic activity in any subject. Pupillary responses were larger in patients with generalised epilepsy than in controls by a mean of 3.8 dB ± 1.43 dB (mean ± SE), and these changes where perhaps biased to the superior field. The use of antiepileptic medication reduced pupillary responses by a mean of 4 dB ± 1.74 dB. A mean delay of 24.9 ± 10.2 ms in the time-to-peak of the pupillary response was seen in patients with focal epilepsy. Changes in response delay seemed to be expressed uniformly across the visual field.

Conclusions: Performing EEG during testing has provided preliminary evidence of the safety of mfPOP in patients with epilepsy. The high level of inter-connectivity of the pupillary system with many brain areas means that mfPOP may represent a useful tool in the study of epilepsy.

3.1. Introduction

Visual stimuli provoking photic-induced seizures seen on electroencephalography (EEG) as a photo-paroxysmal response (PPR) are believed to act through increasing occipital cortex excitability. This has been demonstrated using functional MRI (Chiappa et al., 1999) and trans-cranial magnetic stimulation (Siniatchkin et al., 2007). The pretectal olivary nuclei are highly interconnected with many brain areas including the cerebral cortex (Gamlin, 2006). These interconnections influence the Edinger Westphal nucleus leading to alterations in the pupillary response. How changes in cortical excitability might influence the pupillary response in epilepsy patients has not been yet explored.

Multifocal Pupillographic Objective Perimetry (mfPOP) was introduced about a decade ago (Tan et al., 2001, Wilhelm et al., 2000b). More recently, a version with FDA clearance has been developed by our group, the nuCoria Visual Field Analyser (nCFA). It objectively assesses visual function using the pupillary response and has been successfully evaluated in several conditions including diabetic retinopathy (Bell et al., 2010, Sabeti et al., Sabeti et al., 2015), macular degeneration (Sabeti et al., 2012, Sabeti et al., 2014), glaucoma (Carle et al., 2011a, Carle et al., 2015), and multiple sclerosis (Ali et al., 2014). mfPOP is potentially useful in the study of epilepsy by better characterizing the pupillary response and we will investigate whether it is influenced by cortical excitability or factors that alter that excitability such as anti-epileptic medications.

Our previous study of mfPOP in migraine (Chapter 2) suggests that mfPOP may quantify changes in brain excitability due to either treatment effects or the time since a migraine attack (Lueck et al., 2014). Therefore it is reasonable to examine possible effects of antiepileptic medication, and also any differential effects of focal versus generalised epilepsy in comparison to normal population.

Since the mfPOP device delivers fixed-intensity supra-threshold visual stimuli presented randomly to various locations in the visual field at a rate of 22 stimuli per second, its safety in patients with epilepsy requires investigation. That being said, its stimulus parameters fall well outside those expected to be epileptogenic (Harding et al., 2005). Over the past 10 years the group has done over 14,000 mfPOP tests on over 3800 adults without a report of an epileptic seizure. Over 900 subjects have had 6 or more mfPOP tests, where 3 or more tests were done in a single sitting for every subject.

Current guidelines evaluating the safety of artificial light-emitting sources in patients with photosensitive epilepsy (PSE) rely on comparison against stimulus parameters which are known to provoke seizures (Harding et al., 2005). However, these guidelines lack objective proof of the safety of the individual sources. We were interested to see if mfPOP produced signs of epileptogenesis in actual epilepsy patients using electroencephalography (EEG) and investigate its tolerability during perimetric testing.

Our study was carried to investigate the pattern of pupillary abnormalities in patients with epilepsy using mfPOP, and to conduct a preliminary investigation of its safety in the same population of patients

3.2. Methods

3.2.1. Study Design

This cross-sectional, open-labelled study was conducted at The Canberra Hospital, Canberra, Australia. The primary endpoint was the proportion of patients developing any of the following: aura/seizure during (or shortly after) testing, a photo-paroxysmal response (PPR), or increased epileptiform activity on the EEG during mfPOP testing. Secondary endpoints included establishing the pattern of mfPOP abnormalities in patients with epilepsy.

The study conformed to the Declaration of Helsinki guidelines and approval from both the human research ethics committee of the Australian National University (protocol 2012/303) and the ACT Health Human Research Ethics Committee (ETH 4.12.080) overseeing The Canberra Hospital was obtained. Informed written consent was obtained from all subjects.

3.2.2. Subjects

Successive patients undergoing routine clinical EEG were enrolled. Participants were 18 years of age or older divided into: 1) subjects, if they had a clear diagnosis of epilepsy by history and medical records; or 2) controls, if they were being evaluated by EEG for other reasons such as syncope or psychiatric illness. Exclusion criteria included pregnancy or breast feeding, a seizure occurring within the previous 24 hours, a history of other visual or neurological disturbance that might affect visual assessment, and consumption of medication that could interfere with pupillary responses.

3.2.3. Study settings and procedures

All participants underwent routine EEG testing with activity recorded from 19 scalp electrodes according to the international 10-20 system (1958). The EEG protocol included standard provocation techniques of hyperventilation and intermittent photic stimulation (IPS). After completion of the standard EEG (30 min) subjects were placed in a sitting position and were asked to look into the mfPOP device. The electrodes remained attached and EEG recording continued during mfPOP testing.

3.2.4. mfPOP stimulation

mfPOP responses were obtained using a nuCoria Field Analyser® (nCFA) prototype (nuCoria Pty Ltd, Canberra, Australia) as described in Chapter 1 **(Figure 1)** . In summary, subjects viewed liquid crystal displays (LCDs) provided independent stimuli to each eye separately out to 30° eccentricity. Corrective lenses were supplied as necessary. Forty-four regions per visual field were tested with yellow stimuli exhibiting peak luminance ranging from 138.2–290.0 cd/m^2, the luminances being selected to generate approximately equally reliable responses from each visual field location. The background illumination was 10 cd/m^2. Pseudo-randomly sequenced stimuli were presented independently to each eye (i.e. dichoptic stimulation) resulting in 44 independent direct and consensual pupillary responses/eye. Each of the pulsed stimuli lasted for 33 ms and stimuli were separated by an average inter-stimulus interval of 4 seconds, thereby providing a mean presentation rate of 22 stimuli/s. Each measured response was thus the average for 90 stimulus presentations. The test period was divided into nine segments of 40 seconds (6 min total). Infrared video cameras measured pupil diameter.

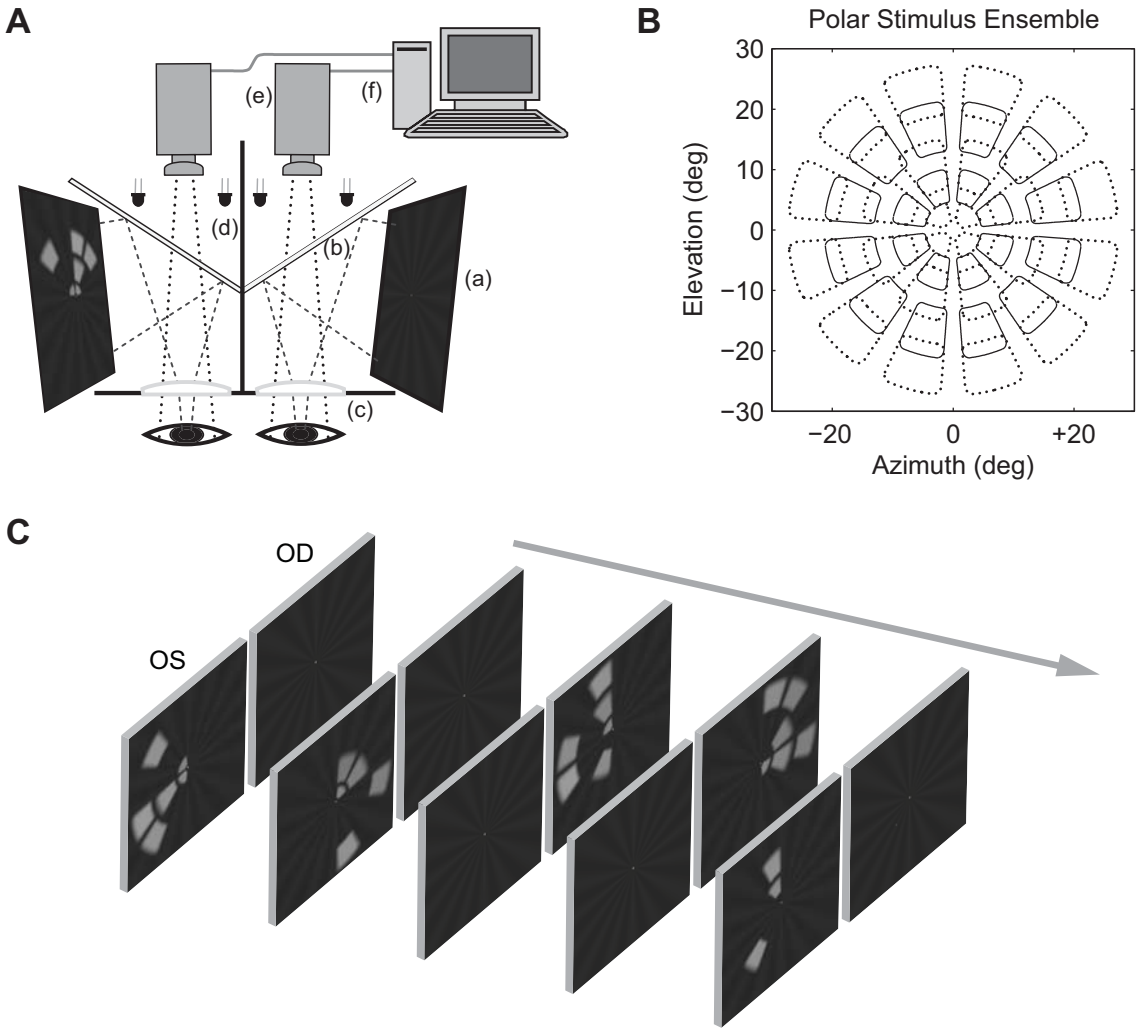


Figure 1. Measurement of pupillary responses. **A)** Schematic of the nuCoria Field Analyser®. Stimuli are presented independently on two LCD monitors (a). These images are reflected by two dichroic mirrors (b) allowing infrared light to pass while reflecting shorter wavelengths. Viewing distance is increased to optical infinity by plano-convex lenses (c). Each eye views only one monitor, the images being fused by the subject into a cyclopean view. Infrared illumination of the eyes is provided by infrared light-emitting diodes (d) facilitating the monitoring of each pupil by separate infrared video cameras (e). Pupil diameters are then extracted in real-time and recorded by a computer (f). **B)** The 44 stimulus regions per eye were arranged in a dartboard-like polar layout extending to 30° from fixation. **C)** Showing the independent stimuli (dichoptic) from a series of video frames of the test sequence. Stimuli were pseudo-randomly presented to each hemifield of each eye in a consecutive series.

3.2.5. Data analysis

EEG interpretation was undertaken independently by experienced neurologists. mfPOP analysis was conducted using MATLAB software (MathWorks, Natick, MA, USA). Baseline characteristics (age, gender) were compared between patients and controls using Student's *t*-test and chi-square, respectively. Fisher's exact test was used to compare the proportion of subjects developing any of the primary outcome measures before and after mfPOP testing.

For the secondary outcome measures, a total of 176 response waveforms per subject for both direct and consensual responses were obtained and these were then fitted to a log-normal function as described elsewhere (Bell et al., 2010, Carle et al., 2011a). Each regional response was analysed for deviation from normal according to response time-to-peak (TTP), expressed in milliseconds (ms), and standardized amplitude (AmpStd), expressed in decibels (dB) **(Figure 2)**. AmpStd was used to overcome inter-subject variation in mean pupil diameter: it assessed changes in pupil size after correcting to the mean diameter of the population rather than using absolute pupil size.

The effects of multiple epilepsy parameters on pupillary characteristics were examined using multivariate linear models. These parameters included seizure type (generalized vs. focal), family history of epilepsy, light already identified as trigger for epilepsy, gender, age, and consumption of antiepileptic medications.

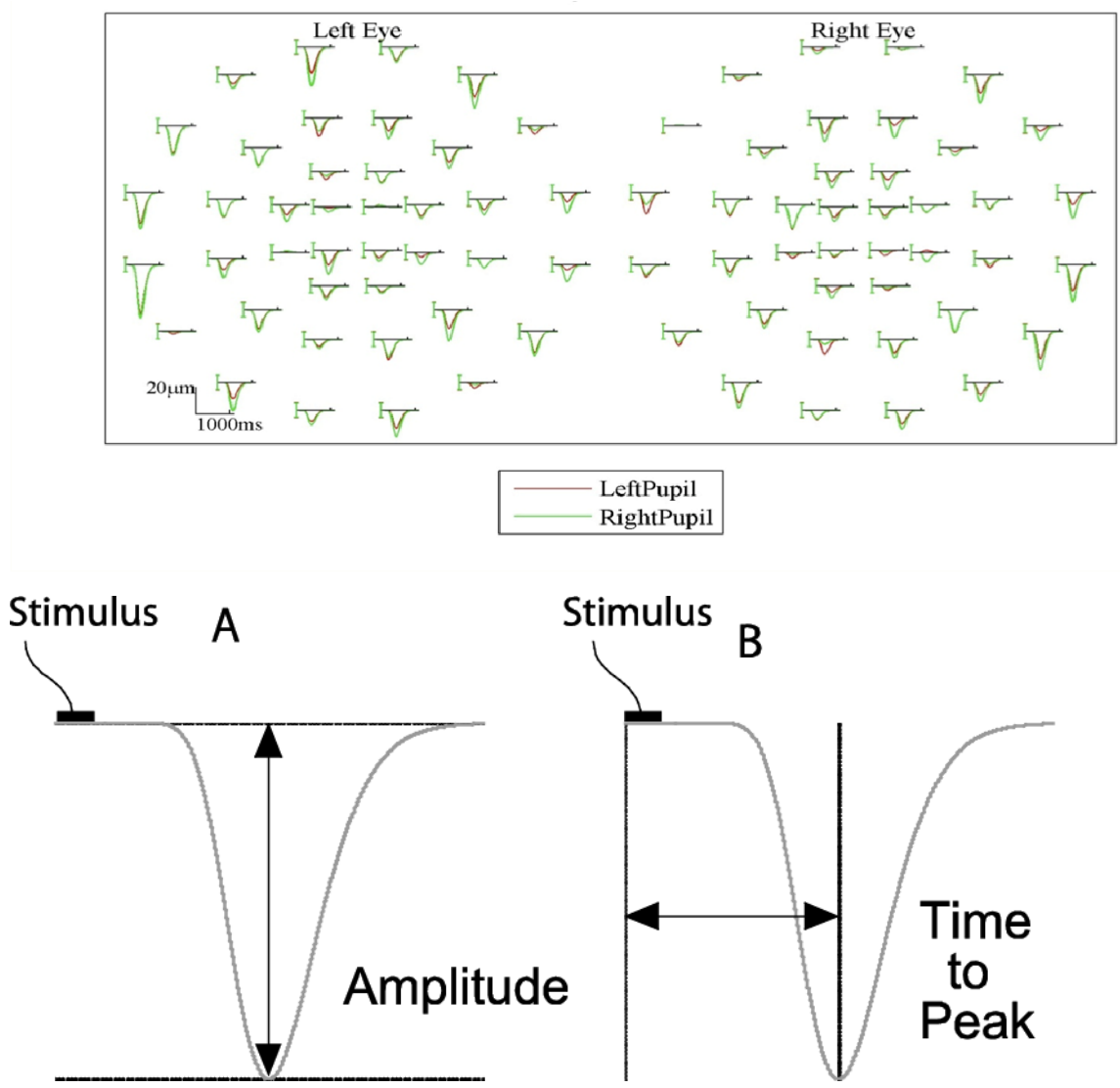


Figure 2. Example mfPOP response waveforms from an individual subject (above). The mean pupil responses to stimuli present to each of the 44 test regions were obtained from both eyes concurrently from 6 minutes of stimulation. Downward deflection indicates contraction. The red and green traces are the responses of the left and right pupils. (Below) Pupil responses are analysed according to amplitude and delay (time to peak).

3.3. Results

3.3.1. Demographics

A total of 15 subjects (8 males; mean age ± SD 47.3 ± 4.6 years), including three with known photic induced seizures, and 15 controls (9 males; mean age 52.7 ± 4.6 years) were studied. There were no significant differences between the two groups regarding either age or gender. In the subject group, 11 had generalized epilepsy and seven had focal epilepsy according to the ILAE classification (Berg et al., 2010), three patients had both types**.** Eleven subjects were taking antiepileptic medications. In the control group, three individuals were taking antiepileptic medications, two as a mood stabilizer and one in case a diagnosis of epilepsy was not confirmed. Antiepileptic medications included carbamazepine, oxcarbazepine, valproic acid, phenytoin, lamotrigine, levetiracetam, gabapentin and pregabalin. There were no differences in the median baseline absolute pupil sizes for controls and epileptics which were 3.59 ± 0.623, and 3.56 ± 0.326 mm (median ± SD).

3.3.2. Safety of the mfPOP device

Both subjects and controls tolerated mfPOP testing well. None developed clinical seizures, aura, or PPR on EEG. Prior to mfPOP testing, four subjects from the epilepsy group showed evidence of epileptiform activity on EEG with one subject developing electrophysiological subclinical seizure activity starting before mfPOP testing. This activity continued during and after mfPOP testing, but there was no sign of exacerbation by the perimetry. Otherwise, there was no evidence of abnormality on EEG during the mfPOP testing segment in any of the subjects or controls.

3.3.3. Abnormalities detected by mfPOP

3.3.3.1. AmpStd:

The mean AmpStd across test regions **(Table 1A)** for the control group was 19.8 ± 1.1 dB (mean ± SE). For patients with generalized epilepsy, a significant increase of 3.8 dB ± 1.43 dB in AmpStd ($p = 0.01$) was seen, i.e. a 2.40-fold increase (95% CI: 1.26 - 3.31) compared to controls. The use of antiepileptic medication reduced AmpStd by 4.0 dB ± 1.74 dB ($p = 0.02$), i.e. a 2.53-fold reduction (95% CI: 1.14 - 5.65). There was no effect of focal epilepsy, family history of epilepsy, light previously identified as trigger for epilepsy, gender, or age. The gray-scale plots **(Figure 3A, 3B)** demonstrated enhanced sensitivity (increased AmpStd) above the equator relative to controls in the generalized epilepsy group ($p < 0.05$) with an inferior-to-superior gradient. The focal epilepsy group only showed mild suppression of sensitivity, especially inferiorly.

3.3.3.2. Time-to-peak:

The mean TTP across regions **(Table 1B)** for controls was 477.5 ± 6.19 ms (mean ± SE). A mean increase in TTP of 24.9 ± 10.2 ms was seen in patients with focal epilepsy ($p = 0.02$) but there was no significant increase in patients with generalized epilepsy. As previously described in other mfPOP studies (Ali et al., 2014) female gender was associated with significantly shorter TTP by about 22 ms. As subjects aged their responses slowed by the rate of 9.79 ms per 10 years (also as reported in previous mfPOP studies) (Ali et al., 2014).

The gray-scale plots **(Figure 3C, 3D)** showed that the focal epilepsy group **(Figure 3D)** had more regions showing significantly increased TTP compared to the generalized group ($p < 0.05$) **(Figure 3C)**.

Table 1. Independent effects of patient parameters on AmpStd and Time-to Peak

A) Independent Effects of Patient Parameters on AmpStd			
	Mean dB ± SE	**T stat**	**P value (Two sided)**
Constant*	19.8 ± 1.07	18.5	-
Generalized epilepsy	3.80 ± 1.43	2.72	0.01
Focal epilepsy	1.43 ± 1.76	0.81	0.42
Anti-epileptics	-4.04 ± 1.74	2.32	0.02

B) Independent Effects of patient parameters on time-to-peak			
	Mean ms ± SE	**T stat**	**P value (Two sided)**
Constant**	477.5 ± 6.19	77.1	-
Generalized epilepsy	-8.87 ± 8.10	1.09	0.28
Focal epilepsy	24.9 ± 10.2	2.44	0.02
female	-22.1 ± 8.5	2.61	0.01
Age effect relative to 47 y (ms/decade)	9.79 ± 2.44	4.11	0.001<

* Independent effects on AmpStd estimated by a multivariate linear model, the factors were fitted as contrasts to a reference value termed a constant which is the mean response in dB for the control group. The values of the other factors thus represent the differences from the constant (strictly the global mean for male control subjects aged 47 years) and the significances of those differences, and their t- and p-values. For the

constant (reference value) the t-static indicates its significance relative to a response of 0.

** A similar model was used and the Constant for time-to-peak constituted the mean response time-to peak in milliseconds (ms) from all regions of both eyes and both direct and consensual responses of the control group. When compared to the constant positive values will indicate a slower time-to-peak, i.e. additional delay.

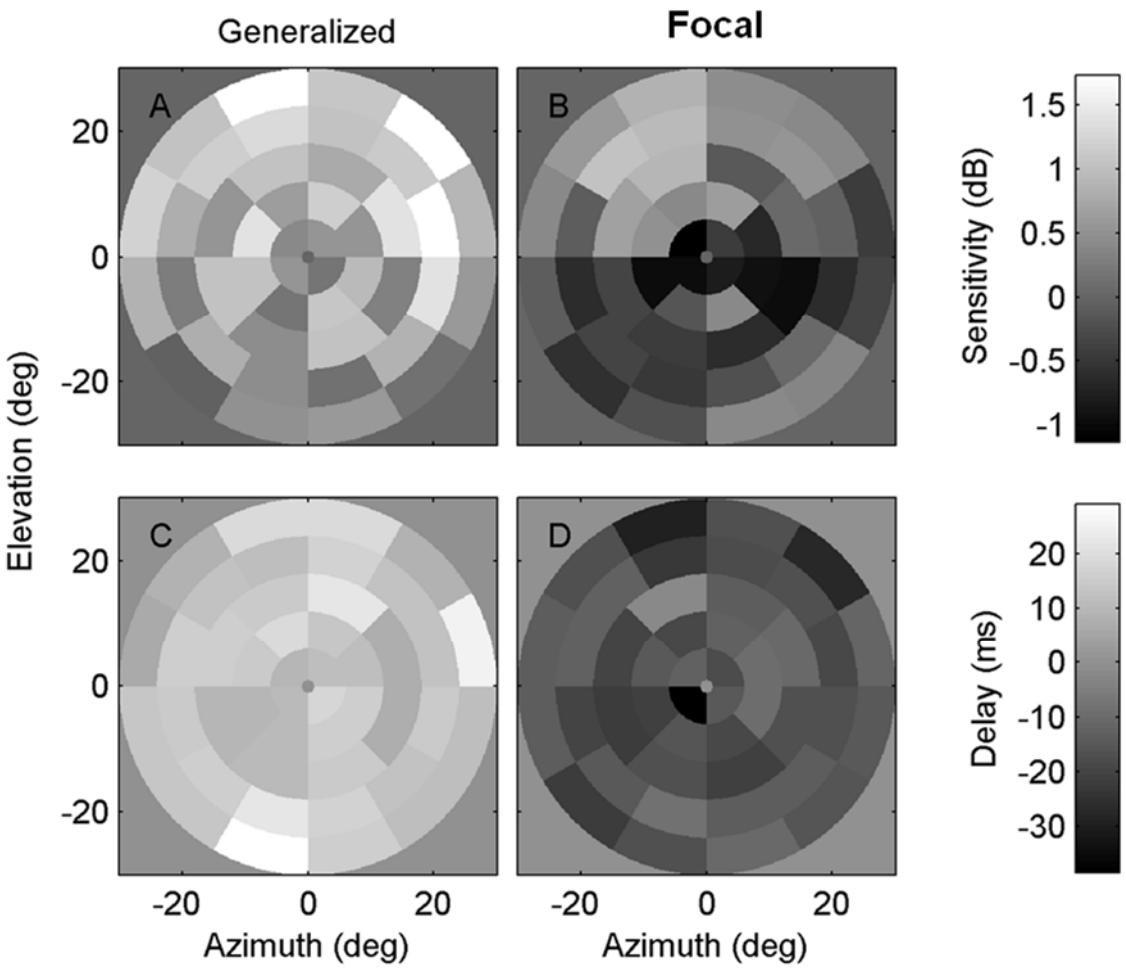


Figure 3. Gray scale plots. **The top row are the controls and the bottom plots are the differences from the controls for the cases described and all the numbers come from a single multivariate model.** The top row (A, B) represented the mean region-by-region contraction AmpStd deviations from controls in the generalized epilepsy group (A) and focal epilepsy group (B); n.b. lighter regions represent increased dB sensitivity compared to control values, darker regions reduced sensitivity.

The bottom row (C, D) shows mean contraction time-to-peak deviations from controls in the generalized epilepsy group (C) and focal epilepsy group (D). The darker regions represent faster TTP than control values, brighter regions indicate longer TTP.
For all panels the background grey represents 0 change compared to controls.

3.4. Discussion

This study has demonstrated an enhancement of the pupillary contractions in the form of increased pupillary response standardized amplitudes in patients with generalized epilepsy, an effect which appears to be reversed by antiepileptic medication. In addition, the time-to-peak of the pupillary response was increased in patients with focal epilepsy. This study has also added to data on the safety of the mfPOP device in patients with epilepsy, determined both clinically and electro-physiologically.

This is believed to be the first study to examine and characterise the pupillary response in patients with epilepsy during the interictal phase. The enhanced pupillary constriction observed in the generalized epilepsy group could be due to altered autonomic function, something which has been reported to cause either pupillary mydriasis or miosis during the ictal phase as part of general autonomic nervous system inhibition or activation. Alternatively, the overactive pupillary responses could be part of a more generalized cortical excitability which is associated with generalized epilepsies (Badawy et al., 2014). Disturbance in the neuronal excitatory/inhibitory balance leading to the formation of hyper-excitable seizure networks is an important proposed mechanism underlying the pathophysiology of epilepsy (McCormick and Contreras, 2001). Alterations in cortical excitability have been observed for 24, and even up to 48, hours before and after seizures. In addition, several factors have been found to alter this excitability including menstrual cycle, time of day, sleep and sleep deprivation, possibly explaining why these factors are considered to be epilepsy

triggers in themselves (Badawy et al., 2012). About half of the input to the Edinger-Westphal nucleus arises from the extrastriate cortex (Gamlin, 2006).This cortical influence is believed to be inhibitory in nature based on studies showing that when transcranial magnetic stimulation was placed over the occipital cortex in humans it inhibited the pupil constriction elicited by a light stimulus given to the retina, although not causing pupillary dilation (Kardon, 2005). In the light that generalised epilepsy subjects do have increased cortical excitability this should have led to inhibition of the parasympathetic pupillary response, thus leading to pupillary dilatation. This is to the contrary to what we found, making us speculate that pupillary response exhibit a subcortical excitability independent from cotical influence. Whether this overactivity also contributes to photosensitivity in patients with generalized epilepsy needs further exploration (Wolf and Goosses, 1986). Comparing these results to our results in migraine patients - which is another disorder believed to exhibit cortical hyperexcitability- we found that shortly after an attack there was a reduction in pupillary contraction, which was believed to be due cortical spreading depression occurring during and shortly after migraine attacks. If we also considered a decrease in cortical influence over the pupillary response in that situation we would speculate a loss of inhibition and thus increased pupillary constriction like what is observed in fatigue and drowsiness in humans and in animals (Kardon, 2005) ,yet we saw the opposite effect further supporting the notion that the changes in the pupillary response excitability is independent from cortical influence.

The use of antiepileptic medication led to a reduction of the pupillary response. Many antiepileptic medications have anticholinergic activity which would be expected to lead to pupillary dilation and/or reduced constriction. On the other hand, cortical hyper-excitability has been shown to be decreased in patients taking antiepileptic medications

(Badawy et al., 2010) and this may account for the observed reduction in pupillary response. Future research is needed to clarify this issue and there may be implications for the selection of medication used for treating patients with photosensitive epilepsy. Furthermore in the coming chapter (Ali, 2016) we will study the interaction between the alpha rhythms on the EEG and the photic stimulation and how they affect mfPOP pupillary parameters, this will further reinforce the role of mfPOP characterise changes of cortical excitability.

The explanation for the increase in time-to-peak of the pupillary response in patients with focal epilepsy is not clear. One possibility is that structural abnormalities, which are known to be more common in focal seizures, have altered structures involved in the pupillary pathway.

A consensus view of stimuli likely to provoke visually-evoked seizures has been proposed by the Epilepsy Foundation of America (Harding et al., 2005). Although guidelines exist for commercial television broadcasters in some countries (Harding and Takahashi, 2004), there is no definite objective evidence that implementing these guidelines actually prevents clinical or subclinical events. Similarly, there is no standardised protocol for testing light-emitting medical devices. A logical step would be to assess safety of all medical light-emitting devices using EEG testing in the way that this study did to demonstrate the safety of the mfPOP device in epilepsy; however, the small sample size and the fact that no patient demonstrated a clear PPR on EEG during routine IPS are limitations to our study. The demonstration of a PPR during IPS but not during the mfPOP would have provided stronger evidence of safety. This would have been difficult to achieve since the chance a person referred to an EEG lab from the population develop PPR is only 2.8 /100 regardless of the diagnosis (Jeavons and Harding, 1975).

Our study had limitations in the form of small sample size and the control group not consisting of healthy volunteers instead they were subjects with disorders other than epilepsy referred to the neurophysiology lab for EEGs, but who had been shown to not have epilepsy.

In conclusion, standardization of testing light emitting medical devices is of importance when assessing their safety in patients with photosensitive epilepsy. More studies looking at changes in the pupillary response in the interictal phase are needed.

References

1958. Report of the committee on methods of clinical examination in electroencephalography. *Electroencephalography and Clinical Neurophysiology,* 10**,** 370-375.

ALI, E. 2016. *Exploring cortical excitability and photosensitivity in neurological disorders using pupillary response to sparse multifocal stimuli* The Australian National University

ALI, E., MADDESS, T., JAMES, A., VOICU, C. & LUECK, C. 2014. Pupillary response to sparse multifocal stimuli in multiple sclerosis patients. *Multiple Sclerosis Journal,* 20**,** 854-861.

BADAWY, R. A., LOETSCHER, T., MACDONELL, R. A. & BRODTMANN, A. 2012. Cortical excitability and neurology: insights into the pathophysiology. *Funct Neurol,* 27**,** 131-45.

BADAWY, R. A., MACDONELL, R. A., BERKOVIC, S. F., NEWTON, M. R. & JACKSON, G. D. 2010. Predicting seizure control: cortical excitability and antiepileptic medication. *Ann Neurol,* 67**,** 64-73.

BADAWY, R. A., VOGRIN, S. J., LAI, A. & COOK, M. J. 2014. Does the region of epileptogenicity influence the pattern of change in cortical excitability? *Clin Neurophysiol*.

BELL, A., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Dichoptic multifocal pupillography reveals afferent visual field defects in early type 2 diabetes. *Invest Ophthalmol Vis Sci,* 51**,** 602-8.

BERG, A. T., BERKOVIC, S. F., BRODIE, M. J., BUCHHALTER, J., CROSS, J. H., VAN EMDE BOAS, W., ENGEL, J., FRENCH, J., GLAUSER, T. A., MATHERN, G. W., MOSHE, S. L., NORDLI, D., PLOUIN, P. & SCHEFFER, I. E. 2010. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia,* 51**,** 676-85.

CARLE, C. F., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2015. Blue Multifocal Pupillographic Objective Perimetry in Glaucoma. *Invest Ophthalmol Vis Sci,* 56**,** 6394-403.

CARLE, C. F., JAMES, A. C., KOLIC, M., LOH, Y. W. & MADDESS, T. 2011. High-resolution multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci,* 52**,** 604-10.

CHIAPPA, K. H., HILL, R. A., HUANG-HELLINGER, F. & JENKINS, B. G. 1999. Photosensitive epilepsy studied by functional magnetic resonance imaging and magnetic resonance spectroscopy. *Epilepsia,* 40 Suppl 4**,** 3-7.

GAMLIN, P. D. 2006. The pretectum: connections and oculomotor-related roles. *Prog Brain Res,* 151**,** 379-405.

HARDING, G., WILKINS, A. J., ERBA, G., BARKLEY, G. L. & FISHER, R. S. 2005. Photic- and pattern-induced seizures: expert consensus of the Epilepsy Foundation of America Working Group. *Epilepsia,* 46**,** 1423-5.

HARDING, G. F. A. & TAKAHASHI, T. 2004. Regulations: What Next? *Epilepsia,* 45**,** 46-47.

JEAVONS, P. M. & HARDING, G. F. A. 1975. *Photosensitive Epilepsy,* London, William Heinemann.

KARDON, R. 2005 Anatomy and Physiology of the Autonomic Nervous System. *Walsh and Hoyt's Clinical Neuro-Ophthalmology* Spencer S. Eccles Health Sciences Library

LUECK, C., ALI, E., CARLE, C. & MADDESS, T. 2014. 47. : Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective pupillometry. *Journal of Clinical Neuroscience,* 21**,** 2048.

MCCORMICK, D. A. & CONTRERAS, D. 2001. On the cellular and network bases of epileptic seizures. *Annu Rev Physiol,* 63**,** 815-46.

SABETI, F., MADDESS, T., ESSEX, R. W. & JAMES, A. C. 2012. Multifocal pupillography identifies ranibizumab-induced changes in retinal function for exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci,* 53**,** 253-60.

SABETI, F., MADDESS, T., ESSEX, R. W., SAIKAL, A., JAMES, A. C. & CARLE, C. F. 2014. Multifocal pupillography in early age-related macular degeneration. *Optom Vis Sci,* 91**,** 904-15.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T. 2015. Multifocal Pupillography Identifies Changes in Visual Sensitivity According to Severity of Diabetic Retinopathy in Type 2 Diabetes. *Invest Ophthalmol Vis Sci,* 56**,** 4504-13.

SINIATCHKIN, M., GROPPA, S., JEROSCH, B., MUHLE, H., KURTH, C., SHEPHERD, A. J., SIEBNER, H. & STEPHANI, U. 2007. Spreading photoparoxysmal EEG response is associated with an abnormal cortical excitability pattern. *Brain,* 130**,** 78-87.

TAN, L., KONDO, M., SATO, M., KONDO, N. & MIYAKE, Y. 2001. Multifocal pupillary light response fields in normal subjects and patients with visual field defects. *Vision Res,* 41**,** 1073-84.

WILHELM, H., NEITZEL, J., WILHELM, B., BEUEL, S., LUDTKE, H., KRETSCHMANN, U. & ZRENNER, E. 2000. Pupil perimetry using M-sequence stimulation technique. *Invest Ophthalmol Vis Sci,* 41**,** 1229-38.

WOLF, P. & GOOSSES, R. 1986. Relation of photosensitivity to epileptic syndromes. *J Neurol Neurosurg Psychiatry,* 49**,** 1386-91.

Chapter 4.

Photic drive response in epilepsy patients

Abstract

Purpose: Photic drive responses (PDRs) have been used to explore cortical hyper-excitability in neurological disorders. We quantified changes PDR in epilepsy patients and looked for interactions with responses obtained from multifocal objective pupillographic perimetry (mfPOP).

Methods: This was a cross-sectional study of 15 consecutive epilepsy patients (mean age ± SD 47.3 ± 4.6 years; 8 males), and 15 controls (mean age 52.7 ± 4.6 years; 9 males) undergoing routine EEG with standard intermittent photic stimulation (IPS), and testing with the mfPOP device. EEG spectral amplitudes during IPS were obtained using the discrete Fourier transform. N-fold changes in PDR (expressed in dB) when IPS and alpha bands overlapped: the *alpha-band gain*, were examined and also their interaction with mfPOP responses. Alpha-band gain was determined by comparing eyes-open and eyes–closed conditions, with and without IPS. mfPOP responses were obtained from 44 regions/visual field. Response time-to-peak and standardized amplitude was recorded for each test region.

Results: A linear model indicated that an epileptic attack within 1 month increased the alpha-band gain by 1.33 dB (p=0.01). Generalised epilepsy (i.e. no focal epilepsy) decreased the alpha-band gain by -1.03 dB (p=0.03). For each decade increase in age the gain increased by 0.36 dB (p=0.007). For every 1 dB increase in alpha-band gain

pupil responses were reduced by 0.21 ± 0.09 dB on average across the field (p=0.024).

Conclusions: Investigating alpha-band gain offers another way to quantify cortical hyper-excitability in epilepsy patients. Responses to mfPOP may provide less invasive means to quantify hyper-excitability.

4.1. Introduction

Intermittent Photic stimulation (IPS) is the most commonly used method of cerebral activation for routine electroencephalogram (EEG) examinations. Its effects on the human EEG are reported to have been first noticed by Adrian and Matthews (Schomer and Lopes da Silva, 2005). Since then this technique has been validated as a useful activation method for eliciting paroxysmal EEG activity (Walter et al., 1946). The three main EEG changes induced by IPS are: 1) Photo-entrainment or Photic drive response (PDR); 2) the Photoparoxysmal response (PPR); 3) and the Photomyoclonic response (Schomer and Lopes da Silva 2005). The latter two have been associated with epilepsy whereas changes in the PDR are less explored in epilepsy patients.

PDR is a physiologic response consisting of rhythmic activity time-locked to the stimulus at a frequency identical or harmonically related to that of the stimulus (Noachtar et al., 1999). The sources of the PDR are not fully understood. It was believed to occur as a result of a flash visual evoked response. This was supported by the fact that the background rhythm becomes synchronised to the timing of the photic stimulator with the first response appearing shortly after the start of the stimulation (<100ms), and stopping when the stimulator stops (Blum and Rutkove, 2007). However, this view does not explain why IPS induces not only responses at the fundamental frequency, but also other harmonic responses in higher frequency bands

(Kikuchi et al., 2002). A simple possibility is just that the stimulus response function of the evoked potential is nonlinear, resulting in harmonic responses. Another view has been suggested: that the origin of the alpha rhythm is the output of a non-linear oscillator that can be entrained by forced stimuli at nearby frequencies. This could only happen if the system generating the alpha rhythm was non-linear rather than a narrow-band transmission system acting as a linear filter (Wiener, 1961). This idea was further supported by Gebber *et al* using analysis in the time and frequency domains showing that the alpha rhythm can be entrained to the second or third harmonic of low frequency light flashes (3 to12 Hz) (Gebber et al., 1999). Vogel et al. suggested the addition of a central adrenergic effect contributing to the generation of PDR when it was found that monoamine oxidase inhibitors inhibited the PDR (Vogel et al., 1974).

The PDR has been studied in migraine patients using the "H response", which refers to an increased tendency of EEG rhythms to synchronize to external repetitive stimuli with stimulation frequencies around 20 Hz (Golla and Winter, 1959). This response was suggested to reflect a state of cortical hyper-excitability (Simon et al., 1982) and was able to distinguish migraine from normal subjects and other headache types with reasonable sensitivity and specificity (Fogang et al., 2015, Chorlton and Kane, 2000). Other disorders in which PDR has been studied include schizophrenia (Jin et al., 1995, Jin et al., 1990) and Alzheimer's disease (Kikuchi et al., 2002), both of which were found to produce PDRs that were distinguishable from normal controls. Changes in PDR in epileptic patients have only been evaluated in a few reports, specifically in combination with transcranial Doppler sonography to assess posterior circulation blood flow in epilepsy patients (Diehl et al., 1998), and in evaluating alcohol-induced seizures (Sand et al., 2010).

Our basic aims were to investigate the changes in PDR in epilepsy patients using spectral analysis and to search for clinical correlates of abnormal photic driving such as type of epilepsy, medication consumption, and recent attacks. We also wanted to see whether increased sensitivity of the pupillary response may contribute to a larger magnitude of entrainment so we studied whether pupillary response parameters obtained from the multifocal objective pupillographic perimetry (mfPOP) was correlated with the PDR.

4.2. Methods

4.2.1. Subjects and settings

Successive patients undergoing routine clinical EEG at the Canberra hospital, Canberra, Australia, were enrolled. Participants were 18 years of age or older and were divided into: 1) study subjects, if they had a clear diagnosis of epilepsy by history and medical records, or 2) controls, if they were being evaluated by EEG for other reasons such as syncope or psychiatric illness. Exclusion criteria included pregnancy or breast feeding, a seizure occurring within the previous 24 hours, a history of other visual or neurological disturbance that might affect visual assessment, and consumption of medication that could impair pupillary responses. The study conformed to the Declaration of Helsinki guidelines and approval from both the Human Research Ethics Committee of the Australian National University (protocol 2012/303) and the ACT Health Human Research Ethics Committee (ETH 4.12.080) was obtained. Informed written consent was obtained from all subjects

4.2.2. mfPOP stimulation and recording

All subjects underwent mfPOP assessment using the nuCoria Field Analyser® (nCFA) prototype (nuCoria Pty Ltd, Canberra, Australia), which has FDA 510k clearance. The components of the device are summarised in **(Figure 1A).** Corrective lenses compensated for refractive errors. Stimuli were produced by a pair of LCD displays and reflected by cold dichroic mirrors. Forty-four individual stimuli were arranged in a dart-board like pattern extending to ± 30°eccentricity of visual field **(Figure 1 B, C)**. To reduce the effects of possible light scatter from adjacent regions a background illumination of 10 cd/m^2 was used to adapt rod photoreceptor responses. Stimuli were presented dichoptically (independently) to both eyes concurrently, and the dynamic diameter of each pupil was recorded by infrared video cameras at 30 / s under infrared illumination. The appearance of individual stimuli was governed by pseudo-random sequences that allowed the average response at each test region to be estimated by multiple regression (Carle et al., 2013). The stimulus and recording arrangement resulted in direct and consensual responses from each tested visual field region. Relative (rather than absolute) pupil diameter was recorded and transformed to standardised amplitudes of a 3.5 mm pupil (Bell et al., 2010). Only the lower 75% portion of the pupil was recorded to avoid problems generated by ptosis. Up to 15% data loss from blinks and fixation loss was permitted. If more than 15% was lost, that particular recording segment was repeated. The standard mfPOP testing protocol involved stimuli with a maximum luminance of 150 cd/m^2 and stimulus duration of 33 msec. Test duration for both eyes was 6 minutes.

For the pupillary response analysis, a total of 176 response waveforms per subject (2 eyes × 2 pupils × 44 regions/eye) for both direct and consensual responses were obtained and these were then fitted to a log-normal function as described elsewhere **(Figure 2)** (Carle et al., 2011a, Carle et al., 2013). Each regional response was then

analysed for the amount of deviation from normal according to response time-to-peak (TTP), expressed in milliseconds (ms), and standardized amplitude (AmpStd), expressed in decibels (dB).

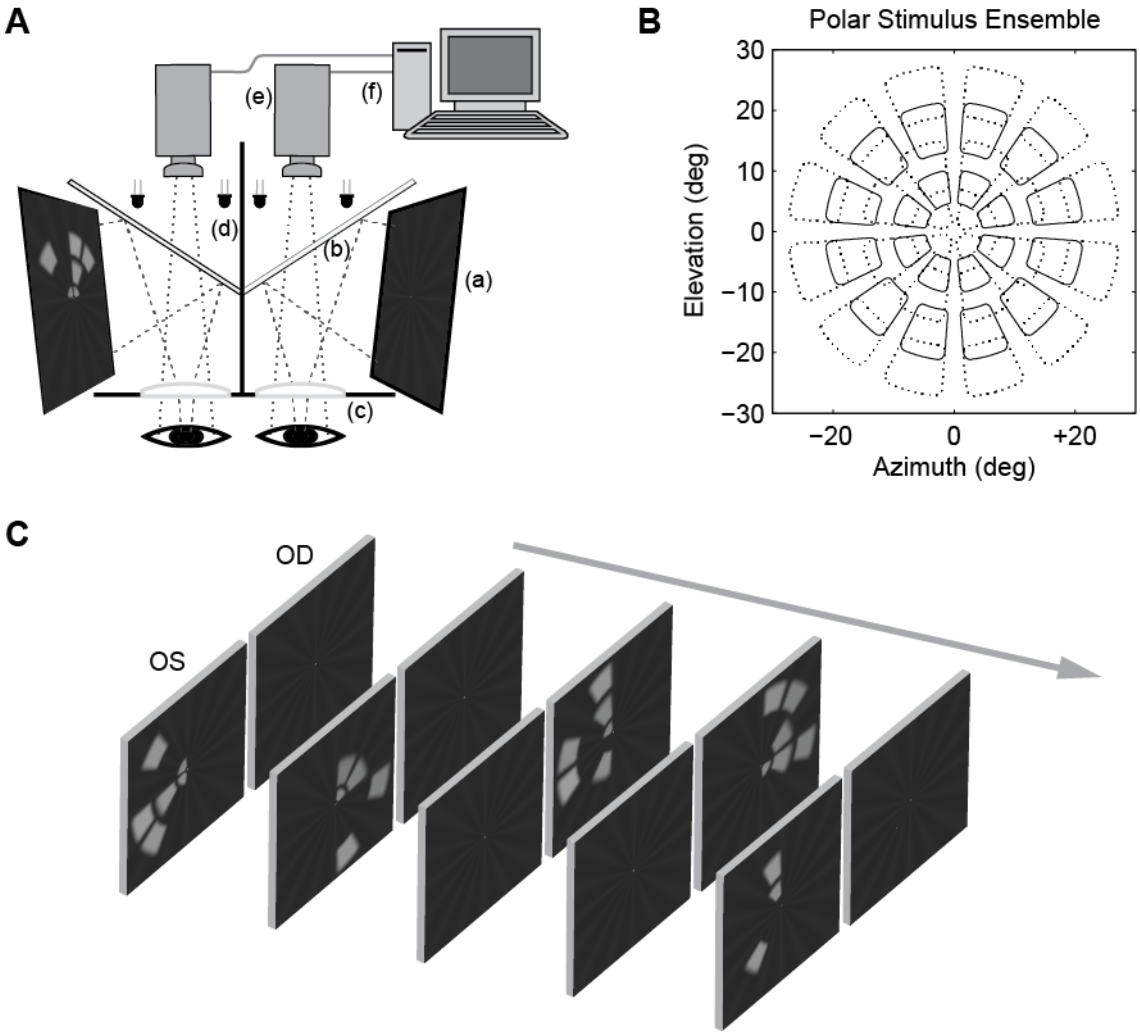


Figure 1. Measurement of pupillary responses. **A**) Schematic of the nuCoria Field Analyser®. Stimuli are presented independently on two LCD monitors (a). These images are reflected by two dichroic mirrors (b) allowing infrared light to pass while reflecting shorter wavelengths. Viewing distance is set to optical infinity by plano-convex lenses (c). Each eye views only one monitor, the images being fused by the subject into a cyclopean view. Infrared illumination of the eyes is provided by infrared light-emitting diodes (d) facilitating the monitoring of each pupil by separate infrared video cameras (e). Pupil diameters are then extracted in real-time at 30 / s and recorded

by a computer (f). **B**) The 44 stimulus regions per eye were arranged in a dartboard-like polar layout extending to 30° from fixation. **C**) Showing the independent stimuli (dichoptic) from a series of video frames of the test sequence. Stimuli were pseudo-randomly presented to each hemi-field of each eye in a consecutive series. A faint background starburst pattern assists the subjects to fuse the images.

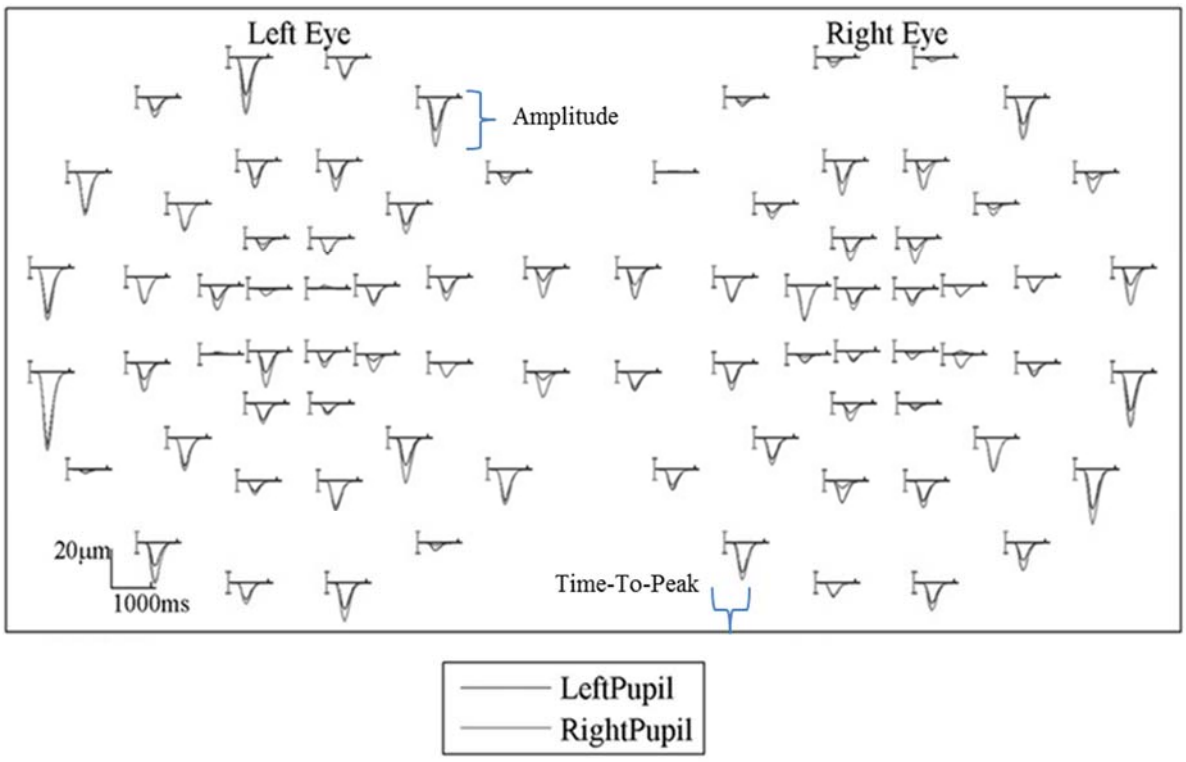


Figure 2. Example mfPOP response waveforms from an individual subject. The mean pupil responses to stimuli present to each of the 44 test regions were obtained from both eyes concurrently from 6 minutes of stimulation. Downward deflection indicates contraction. The black and grey traces are the responses of the left and right pupils.

4.2.3. EEG Intermittent photic stimulation protocol

Each subject underwent a total of 20 minutes of EEG recording. A total of 26 electrodes were placed according to the 10-20 system and included channels for recording horizontal and vertical eye movements and electrocardiograph. The EEG was recorded digitally using ProFusion EEG software version 4.3 (Compumedics, Abbotsford, VIC, Australia). The sampling rate was 250/s. The band pass filter setting was 0.5-70 Hz with a notch filter at 50 Hz. In a dimly lit room, Intermittent Photic Stimulation (IPS) was delivered toward the end of the recording with subjects lying in a semi-supine position 30 cm away from the photo-stimulator stroboscope (Compumedics Neuroscan Model 7097). The stimulus frequencies were 2, 4, 6, 8, 9, 10, 11, 12, 13, 14, 15, 18, 20, 25, 50 flashes/s. EEG recordings were exported using the European Data Format (EDF) and analysed using MATLAB software (MathWorks, Natick, MA, USA). Most analysis presented here was restricted to the occipital responses (O1 and O2).

Each frequency was delivered as a 10 seconds (s) train divided into 5 s with the eyes closed followed by the eyes being opened for the further 5 s. This was followed by a 10 s inter-train interval break. The eyes remained closed for the first 5 s of the break period and then open for the final 5 s and EEG recording continued throughout. The nomenclature for these stimulus conditions is given in **(Table 1A)**, and their sequence

is given in **(Table 1B)**. After the 20 min of IPS testing subjects were placed in the sitting position and were asked to look into the mfPOP device for testing.

Table1A. Epoch definitions

Epoch name	Definition
$EO_{with\ IPS}$	eyes open (EO) with Intermittent Photic Stimulation (IPS), which includes a *visual evoked potential* (VEP)
$EC_{with\ IPS}$	eyes closed (EC) with IPS, which includes the *photic drive response* (PDR)
$EC_{without\ IPS}$	eyes closed (EC) without IPS, which includes the baseline *alpha rhythms*
$EO_{without\ IPS}$	eyes open (EO) without IPS or alpha rhythms

Table1B. Repeated epoch sequence, each of epoch 1 to 4 was 5 seconds in duration

	Epoch 1	**Epoch 2**	**Epoch 3**	**Epoch 4**
Epoch name	$EO_{with\ IPS}$	$EC_{with\ IPS}$	$EC_{without\ IPS}$	$EO_{without\ IPS}$
Eyes	Open	Closed	Closed	Open
alpha rhythm		√	√	
IPS	√	√		

To quantify any interactions between the VEP and the alpha band we calculated the *alpha-band gain*, which is the N-fold change in the VEP when it occurs with the alpha band for each stimulus frequency F, and also for its harmonics (F/2, 2F, 3F, 4F) as follows:-

1) Alpha band gain = $EC_{with\ IPS} - EC_{without\ IPS}$ / $EO_{with\ IPS} - EO_{without\ IPS}$

Where $EC_{\text{with IPS}} - EC_{\text{without IPS}}$ represents: PDR – baseline alpha band;

and $EO_{\text{with IPS}} - EO_{\text{without IPS}}$ represents: VEP – background noise. Hence equation 1 can be written:-

2) Alpha Band Gain = (PDR – baseline alpha band) / (VEP – background noise)

Of course the "baseline alpha amplitude" includes noise. Thus, the alpha-band gain characterises the N-fold change in the PDR relative to the VEP independent of noise and alpha-band strength. The alpha-band gain was calculated for all subjects: controls and epileptic patients. Additionally, groups selected for sub-analyses included all subjects on anti-epileptic medications, controls on anti-epileptics, epileptic subjects on anti-epileptics, subjects with focal epilepsy, and subjects with generalised epilepsy. We also examined the gain for all subjects not on anti-epileptics, controls not on anti-epileptics, epileptics not on anti-epileptics, and subjects with an epileptic attack in the last 2 months or less prior to recording.

4.2.4. Statistical analysis

Analysis of the mfPOP data was conducted using MATLAB software (MathWorks, Natick, MA, USA). Baseline characteristics (age, gender) were compared between patients and controls using Student's *t*-test and chi-square tests, respectively.

Other analysis involved two multivariate linear models. The first examined the alpha-band gain as a function of explanatory variables that included type of epilepsy, recent epileptic attack and age. Here the alpha-band gain was selected to be the mean of the gain across the IPS frequencies 9, 10, 11 Hz and was expressed in decibels (dB) in order to fit additive models and stabilise the variance. The second model examined the effects of explanatory variables on mean pupillary response including alpha-band gain, type of epilepsy, and recent attacks of epilepsy.

Our basic aims were to explore interactions between the alpha band and the responses to IPS, and whether there was any correlation with responses obtained from the pupillography.

4.3. Results

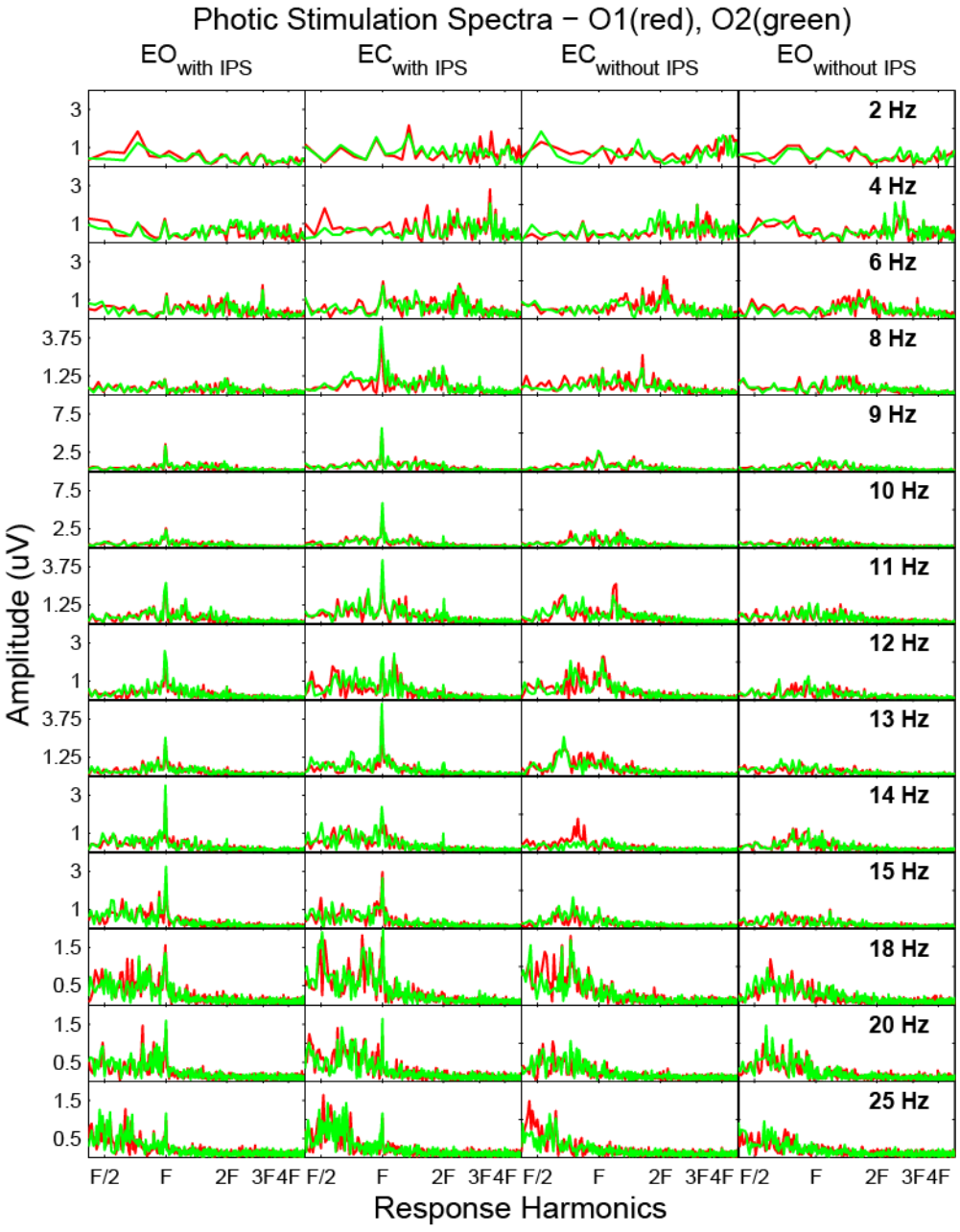
4.3.1. Demographics

A total of 15 subjects (mean age ± SD: 47.3 ± 4.6 years; 8 males), including three with known photosensitivity, and 15 controls (mean age 52.7 ± 4.6 years; 9 males) were studied. There was no significant difference between the two groups regarding either age or gender **(Table 2)**. At the time of the photic stimulation, four patients had focal EEG slowing and three had epileptiform discharges. One control subject had generalised slowing. None of the epileptic patients or controls had a photoparoxysmal response.

Table 2 Demographics

	Subjects (n=15)	**Controls (n=15)**
Age (mean ± SD)	47.3 ± 4.6	52.7 ± 4.6
Female: male	1 : 0.9	1 : 1.5
Type of epilepsy		-
Generalized	11	
Focal	7	
Photosensitivity	3	-
Anti-epileptic medications	11	3
Carbamazepine	1	-
Oxacarbazepine	1	-
Valoproic acid	2	1
Phenytoin	2	-
Lamotrogine	1	-
Levetiracetam	4	2
Gabapentin	1	-
Pregabalin	1	-

3 patients were described as having both generalized and complex partial epilepsy
3 patients were on antiepileptics although they were controls either taking it for other causes (mood stabilizer such as valoproic acid for bipolar disorder, or suspension till diagnosis confirmed to be epilepsy or not
2 patients only were on 2 antiepileptics
.



3.2

Figure 3. Example of the amplitude spectra during intermittent photic stimulation (IPS) of an epilepsy patient. The figure also illustrates the eyes-open (EO) and eyes-closed (EC) epochs and therefore the basis of the alpha-band gain calculations. The red and green lines represent the data from O1 and O2 respectively. The abscissa on each panel is a log scale where octaves of the frequencies are in equal sized steps. Each test is divided into four epochs of 5 seconds duration represented by the four columns. As indicated by the column titles the epoch correspond to: eyes open with intermittent photic stimulation ($EO_{with\ IPS}$), eyes closed with IPS ($EC_{with\ IPS}$), eyes closed without IPS ($EC_{without\ IPS}$)and eyes open without IPS ($EO_{without\ IPS}$), see Table 1A and 1B.
F is the photic stimulation frequency which corresponds to the frequency indicated at the end of each row, and its harmonics are indicated as F/2, 2F, 3F, and 4F. The ordinate represents the amplitude of the spectra measured in μV.

4.3.2. Alpha band gain

Each epoch was analysed using a discrete Fourier transformation yielding amplitude per Hertz (μV/Hz) spectra. (**Figure 3)** shows exemplary results from one subject, the columns of spectra corresponding to the Epochs of Table 1B. In each panel of Figure 3 the spectrum is presented on a logarithmic frequency scale on which octaves of the stimulus frequencies are equal-sized steps. Thus, F is the photic stimulation frequency, the rate of which is indicated by the label at the right end of each row of spectra (e.g. 2 Hz, 4Hz, etc.). The sub-harmonic F/2, and 2^{nd} to 4^{th} harmonics (2F, 3F, and 4F) indicate nonlinear responses to the photic stimuli. During eyes closed (EC, middle two columns of **Figure 3**) the band of alpha frequencies appears to slide from right to left as photic stimulus frequency increases down the rows of the figure. Thus, F is initially below the alpha band (e.g. 2 Hz) and, by the bottom of (**Figure 3)** is above it (e.g. 25 Hz). The red and green lines represent the data from O1 and O2 respectively. Clear peaks at the stimulus frequency (F), and some second harmonics (2F), are seen in many of the spectra during the two IPS phases (EO and EC).

Column 1 of **Figure 3** shows VEP response spectra during the first epoch for the $EO_{with\ IPS}$ condition. Clear VEP peaks are seen at the IPS frequency from about 6 to 25 Hz (50 Hz not shown), reaching a maximum of 3.3 μV at around 14 Hz. Column 3 shows the basic alpha band response spectrum during the third $EC_{without\ IPS}$ epoch, the amplitudes of which also did not exceed 3 μV.

Epoch 2, $EC_{with\ IPS}$, appears to show that when the alpha band and IPS frequencies overlap (8-13 Hz) that the PDR response may be larger than the simple sum of the alpha and VEP amplitudes. We examined this across the subjects by examining the so

called *alpha band gain*: basically the VEP response less noise divided by the PDR less the background alpha amplitude (Equations 1 and 2).

This was repeated for each IPS frequency and each of its harmonics and the results are summarised in **(Figure 4)**. Each bar in **(Figure 4A, B)** is the mean of responses across all subjects and across O1 and O2. The alpha band gain was found to exhibit around a three times N-fold change **(Figure 4C, *)**, indicating that this was a synergistic (super-linear) interaction between the VEP and the alpha generator, and not just an additive effect. This gain was not apparent for pairs of electrodes away from the occipital pole like F7 and F8 (not shown). Interestingly there was also a spike in gain for the 2F frequencies for lower IPS input frequencies **(Figure 4, $^{+}$)**, i.e. when the second harmonic of the VEP overlapped with the alpha band. This would indicate that the actual interaction is between the output response from IPS and the alpha activity, rather than the input IPS frequency and the alpha band. These effects were not observed for electrodes F7 and F8.

When looking at specific subject groups **(Figure 5)** it appeared that the alpha band gain was lower in subjects consuming anti-epileptic medications (**Figure 5**, AntiE), whereas not being on this class of medications (**Figure 5**, Epi_{noAnti}), or having a recent attack of epilepsy within the month preceding testing (**Figure 5**, Recent) both increased alpha band gain.

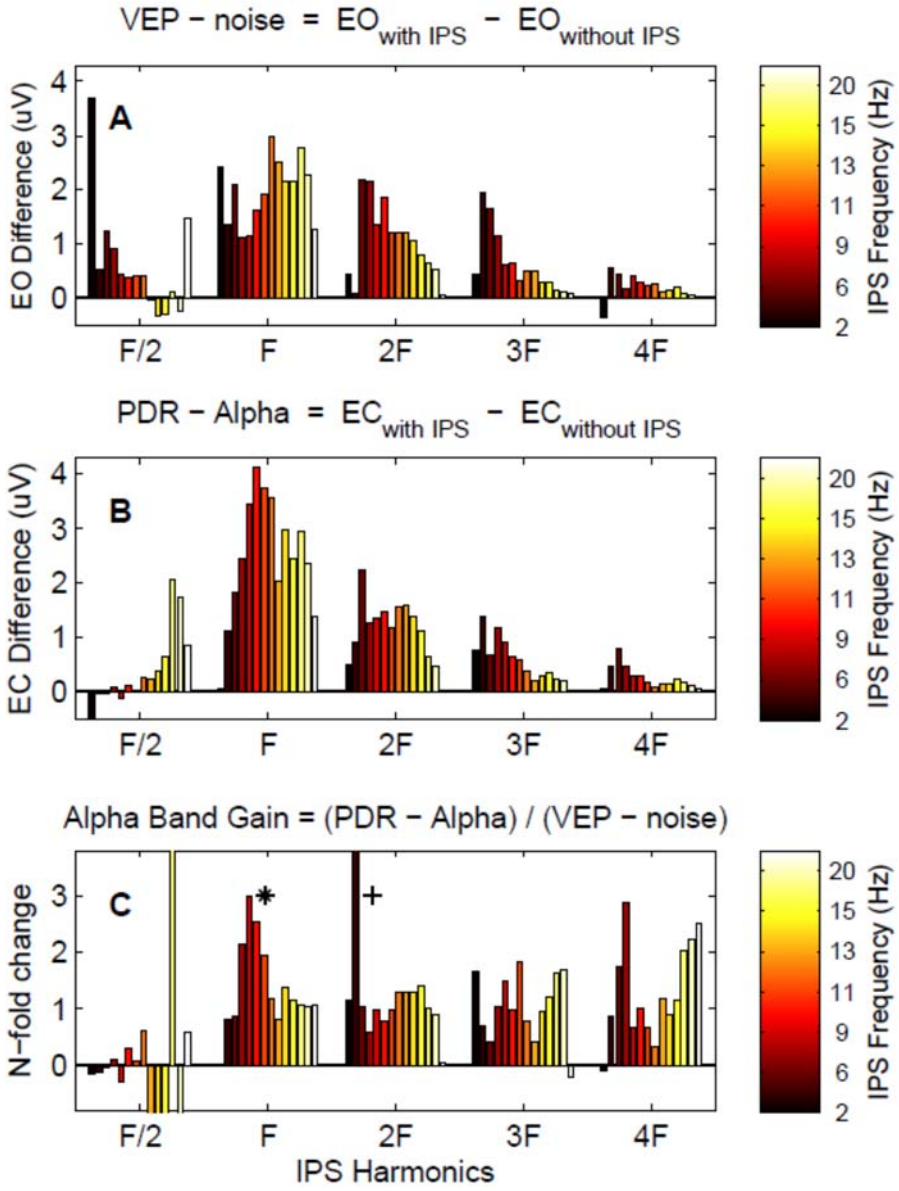


Figure 4. All alpha band gains and their derivation. Each vertical bar is a mean across all subjects for a particular IPS frequency and harmonic. **A**) Represents the mean EO $_{\text{with IPS}}$ – EO $_{\text{without IPS}}$ (= VEP- background noise) for each photic frequency F (coloured bars) and its harmonics. **B)** Represents EC $_{\text{with IPS}}$ – EC $_{\text{without IPS}}$ (= PDR – background alpha). **C**) Shows the alpha band gains, which are each the ratio of EO $_{\text{with IPS}}$ – EO $_{\text{without IPS}}$ / EC $_{\text{with IPS}}$ – EC $_{\text{without IPS}}$.

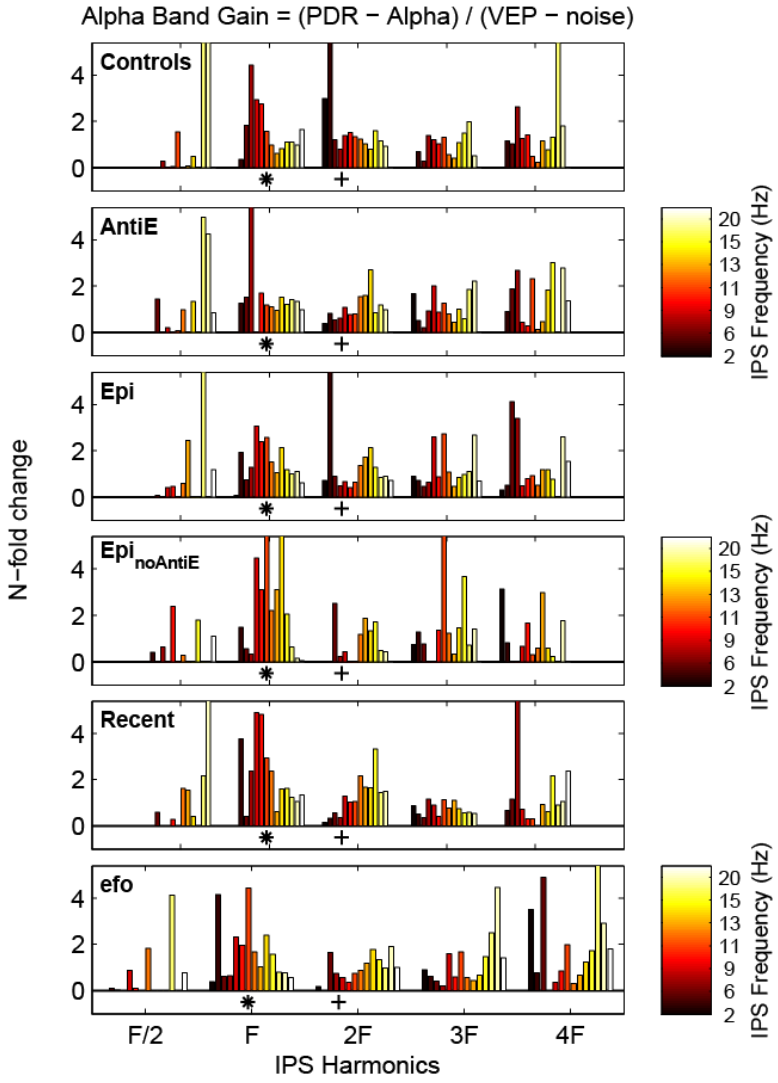


Figure 5. Mean alpha band gain for computed for different subgroups. Each row was derived as for Figure 4C. The subgroups included were from the top down: control subjects (Controls), all subjects on anti-epileptics (AntiE), all epileptics (Epi), epileptic subjects not on anti-epileptics ($Epi_{noAntiE}$), subjects with an epileptic attack in the month prior to recording (Recent), and patients with focal epilepsy (efo). The * indicates responses at the driving frequency (F), and a + indicates an IPS 2nd harmonic that overlaps with the alpha band.

The effect of a recent attack is illustrated more clearly in **(Figure 6)** where a histogram of the gains for individuals was broken-down by the nine subjects who had a recent attack of epilepsy less than a month before testing all nine subjects with recent epilepsy had an alpha band gain larger than the reference alpha band. This is further seen in **(Table 3)** where the multivariate linear model that assessed the factors affecting the alpha band gain showed that recent epileptic attack increased the alpha band gain by 1.33 dB (p=0.01). Generalised epilepsy decreased the gain by 1.03 dB (p=0.03), and for each decade increase in age from controls the gain increased by 0.36 dB (p=0.007).

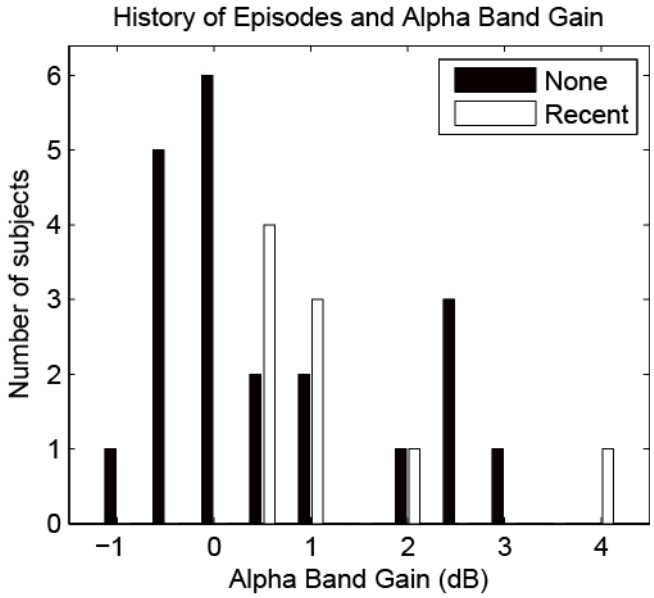


Figure 6. Histogram of the alpha band gains for individuals was broken-down by the nine subjects who had a recent attack of epilepsy less than a month before testing (open bars) and those who had had none (dark bars).

Table 3. Alpha band Gain as a function of explanatory variables

	dB ±SE	t-stat	p
Controls	0.784 ± 0.27	2.878	0.008
Generalized epilepsy	-1.027 ± 0.44	2.325	0.029
Focal epilepsy	-0.155 ± 0.46	0.341	0.736
Recent attack (<1 month)	1.33 ± 0.48	2.76	0.011
Age dB/decade *	0.356 ± 0.12	2.911	0.007

*Age is relative to the mean of 47 years. The dB/decade indicates that the gain increases by 0.356 dB for each decade increase in age.

Table 4. Effect of explanatory variables on mean pupil response AmpStd

	(dB) ± SE	t-stat	p
Controls	12.3 ± 0.24	51.006	0.0000
Generalized epilepsy	0.80 ± 0.2	3.997	0.0001
Focal epilepsy	0.24 ±0.23	1.038	0.301
Recent Attack (<1 month)	0.99 ± 0.24	4.091	0.0001
Alpha Gain dB/dB*	-0.21 ± 0.09	-2.294	0.024

* dB/dB means that for every 1 dB increase in alpha gain meant pupil responses are reduced by 0.21 dB

4.3.3. MfPOP responses

The increase in alpha band gain reduced the mean pupillary response AmpStd, i.e. produced less pupillary constriction **(Table 4)**. For each 1 dB increase in the alpha band gain the pupillary response was found to drop by -0.21 dB (p=0.024). Generalised epilepsy and recent attacks of epilepsy both significantly increased the AmpStd by 0.8 and 0.99 dB respectively.

The grey-scale plots of the average effects on the visual field position for both amplitude of the pupillary response (**Figure 7)** demonstrated enhanced sensitivity relative to controls in the recent attack of epilepsy group. For epilepsy types (both generalised and focal epilepsy) there was suppression of sensitivity especially inferiorly with a possible inferior-to-superior gradient. The data for the colour maps were derived from a linear model and so the results for each group are the estimated independent effects of general or focal epilepsy and recent attacks.

The grey-scale plots for the delays in the pupillary response **(Figure 8)** showed that the focal epilepsy group had more regions showing significantly increased time-to-peak, and recent attack of epilepsy had an earlier time to peak compared to the controls.

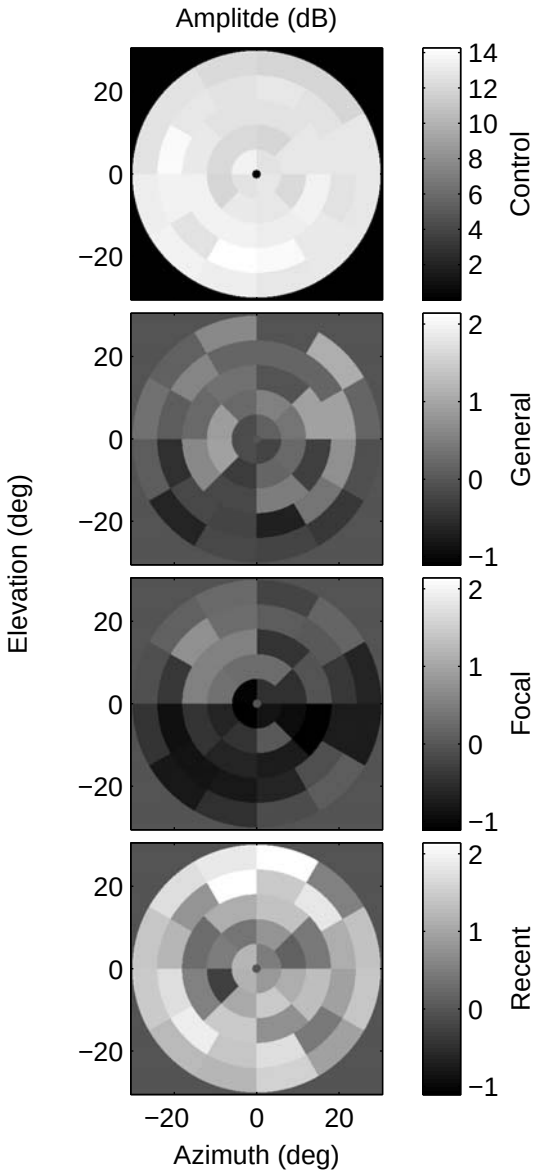


Figure 7. Grey scale plots for mean region by region contraction AmpStd deviations from controls (top) in the generalized epilepsy, focal epilepsy groups, and subjects with recent attack (<1 month). The lighter the region the more increased pupillary response amplitude deviations from control values. Note that for the bottom 3 panels the background grey represents 0 change relative to normal.

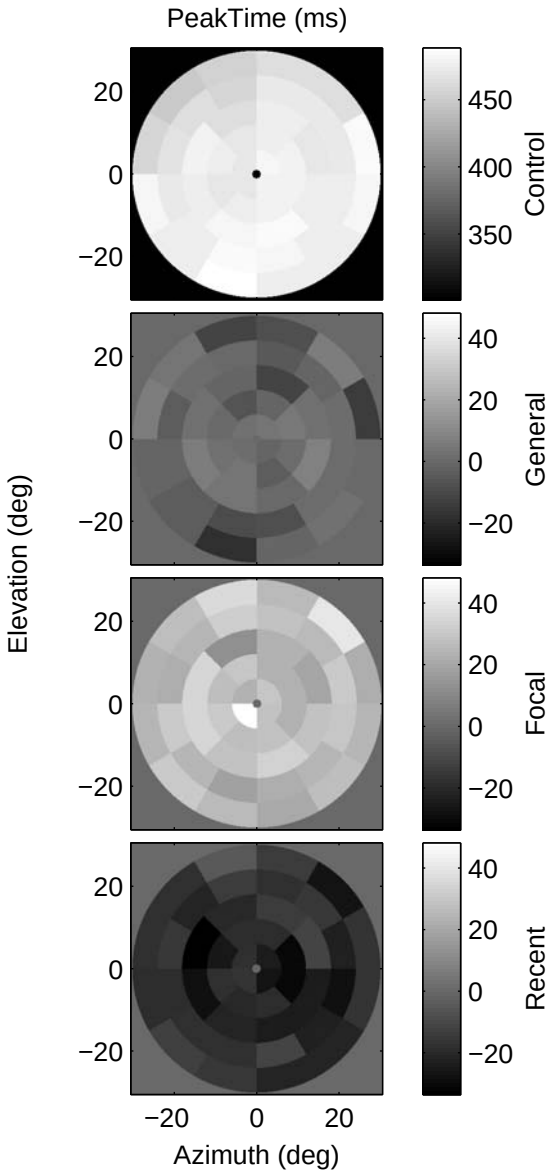


Figure 8. Grey scale plots for mean contraction time-to-peak deviations from controls in the generalized epilepsy, focal epilepsy groups and subjects with recent attack. The lighter regions represent larger delays deviations from controls.

4.4. Discussion

Frequency analyses of EEGs from epilepsy patients and normal controls provided evidence of a synergistic interaction existing between responses to the IPS at F and 2F and the alpha frequencies when the two coincided. This lead to augmented PDRs, which exceeded the simple summation of the VEP response and alpha signal at the interaction frequencies. Further augmentation was seen in epilepsy subjects especially with a history of a recent attack **(Figure 5 and 7, Table 3)**. A decrease in this interaction was observed in subjects consuming anti-epileptic medications and subjects suffering from generalised forms of epilepsy. The pronounced IPS and alpha frequency interaction had a negative effect on pupillary constriction at 0.21 dB of pupil response per dB change in alpha band gain **(Table 4)**.

We observed an increase in PDR of epilepsy patients that is similar to what has been reported in migraine patients (Golla and Winter, 1959, Bjork et al., 2011, Fogang et al., 2015). Both disorders are believed to share a common interictal cortical hyper-excitability state. We have also recorded mfPOP responses in migraineurs, reporting an overall suppression of response in patients relative to controls, and further suppression in patients who had a migraine within the 2 months before testing (Ali et al., 2015). However, our present results on epilepsy may superficially appear to be contrary to a previous study of patients with seizures either due to alcohol or epilepsy showing a decrease in the "H response" rather than an increase as seen in migraine patients in comparison to control groups (Sand et al., 2010). Importantly those authors stimulated at 24 Hz. This held true for recent attack of seizure (regardless of the cause), which was also associated with a decreased 24 Hz driving response in their regression models. The discrepancy with respect to our results of increased PDR are attributed to methodological differences between the two studies where we have investigated

entrainment of the alpha rhythm to the entire IPS frequencies instead of concentrating on 24Hz. In their paper they were also speculated an increase rather than a decrease in the reactivity to the 24 Hz photic driving, which would have been more consistent with a state of cortical hyperexcitability that is known to accompany epileptic attacks (Badawy et al., 2013b). In our case, responses to 25 Hz were quite small **(Figure 3 and 4)**, and did not generate large gain changes **(Figure 5)**.

Another study by Diehl *et al.* looked at the photic driving EEG response and photo reactive cerebral blood flow in the posterior cerebral artery in controls and in patients with epilepsy. (Diehl et al., 1998). PDRs were visually inspected on EEGs and classified as a good driving response if a well-discernible, harmonic or subharmonic EEG synchronization was seen over more than 80% of the stimulus interval. The epilepsy group did not show a higher percentage of good PDR (63.3% in comparison to 81% in the normal control group). It was expected that the good driving response would be accompanied by a higher increase in cerebral blood flow velocities (CBFV) in the posterior cerebral artery (PCA). This was expected given that brain activity, metabolism and blood flow are coupled, yet the increase in CBFV of the PCA in normal controls was found to be higher than in patients with focal epilepsy which may indicate that epileptic patients have a reduced coupling between neuronal activation and blood flow. Again that study used visual inspection rather than computer based spectral analysis. Those findings may explain why in our study the large alpha band gain had a negative effect on pupillary constriction, it may be due to decreased blood flow to the brain stem - a major part of the pupillary pathway response - which is supplied by posterior circulation were the PCA is a branch. Another possibility is pupillary

constriction may represent a protective mechanism by the brain to decrease light entry through the pupils in cases of exaggerated cortical excitability.

Looking at results of steady state visual evoked potentials (SSVEP) we observe similarities to our results, Tsai et al. used SSVEP to contrast reversing gratings and found that amplitudes of visual responses did not saturate at high stimulus contrast in generalised epilepsy patients, as it did in the control subjects (Tsai et al., 2011). They attributed their findings to abnormalities in neuronal gain control. They defined gain control as the machinery by which a system dynamically adjusts its sensitivity to the input allowing for a wide input range and keeping the output in an optimal regime. They went further to use parametric modelling to show that the abnormality lay in reduced inhibition from neighbouring neurons rather than increased excitatory response to the stimulus. (Geller et al., 2005).

Both the use of anti-epileptic medications and seizure frequency have been reported to affect cortical hyper-excitability (Badawy et al., 2013a) and thus as expected they negatively influenced the amplitude of the alpha band gain. The findings here of the generalised epilepsy group having a low alpha band gain was not expected and may be attributed to the consumption of anti- epileptic medications. Again our results were similar to the SSVEP results, when spectral amplitudes were compared between controls, focal, and generalized epilepsy groups (Geller et al., 2005). The maximum amplitude of the fundamental (F1) component of the VEP was shifted to lower frequencies in the generalised epilepsy group relative to the other two groups. Again the authors attributed this to reduced intracortical inhibition in the subjects with generalized epilepsy.

We also found that increase in age led to an increase in the alpha gain which is difficult to explain since with age the response to photic stimulation would be expected to

decrease rather than to increase (Ross et al., 1997). This may have been a function of our particular subjects.

mfPOP gave us an advantage over full-field pupil responses when studying PDR and alpha rhythm changes, A better exposition of this point that while **(Table 4.)** shows no effect upon the mean response amplitude in Focal epilepsy subjects, but there was clearly a gradient of amplitude changes in that group as shown in **(Figure 7).** The gradient goes from positive in the superior field to negative in the inferior field, so on average there was no effect. Clearly measuring a pupil response to a single large stimulus would be akin to assessing the mean response as in **(Table 4.)** Hence the regional analysis afforded by mfPOP was obviously the better approach.

Limitations of our study include the heterogeneity of our control group with some of them having psychiatric disorders such as schizophrenia, which has been shown to lower PDR, particularly in the high alpha frequency band (Jin et al., 1990, Jin et al., 1995), and some being on anti-epileptic medications. Another limitation is that the EEG channels used were according to the 10-20 system, inclusion of an electrode at Oz, which generally has the largest VEP response, would have been useful. Yet in a previous study (Sabeti, 2010) the same apparatus was used to record multi focal visual evoked potentials (mfVEPs), but with a denser 10-10 lay-out showing no real advantage could be cited. The timing of the mfPOP was after the 20 minutes of EEG, this might have led to inter-testing fatigue between subjects and controls i.e. patients are prone to more fatigue than controls.

We have provided evidence that epilepsy patients show augmented photic drive response and that anti-epileptic medication reverses or decreases it. Investigation of the PDR offers yet another way to explore cortical hyper-excitability in epilepsy patients.

mfPOP responses were inversely related to the alpha band gain results and so may provide supplementary data on epilepsy status.

References

ALI, E. N., LUECK, C. J., CARLE, C. F., KOLIC, M. & MADDESS, T. Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective. International Pupil Colloquium, 2015 Oxford, UK.

BADAWY, R. A., VOGRIN, S. J., LAI, A. & COOK, M. J. 2013a. The cortical excitability profile of temporal lobe epilepsy. *Epilepsia,* 54**,** 1942-9.

BADAWY, R. A., VOGRIN, S. J., LAI, A. & COOK, M. J. 2013b. Patterns of cortical hyperexcitability in adolescent/adult-onset generalized epilepsies. *Epilepsia,* 54**,** 871-8.

BELL, A., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Dichoptic multifocal pupillography reveals afferent visual field defects in early type 2 diabetes. *Invest Ophthalmol Vis Sci,* 51**,** 602-8.

BJORK, M., HAGEN, K., STOVNER, L. & SAND, T. 2011. Photic EEG-driving responses related to ictal phases and trigger sensitivity in migraine: a longitudinal, controlled study. *Cephalalgia,* 31**,** 444-55.

BLUM, A. S. & RUTKOVE, S. B. 2007. *The Clinical Neurophysiology Primer,* Totowa, New Jersey, USA., Humana press.

CARLE, C. F., JAMES, A. C., KOLIC, M., LOH, Y. W. & MADDESS, T. 2011. High-resolution multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci,* 52**,** 604-10.

CARLE, C. F., JAMES, A. C. & MADDESS, T. 2013. The pupillary response to color and luminance variant multifocal stimuli. *Invest Ophthalmol Vis Sci,* 54**,** 467-75.

CHORLTON, P. & KANE, N. 2000. Investigation of the cerebral response to flicker stimulation in patients with headache. *Clin Electroencephalogr,* 31**,** 83-7.

DIEHL, B., STODIECK, S. R., DIEHL, R. R. & RINGELSTEIN, E. B. 1998. The photic driving EEG response and photoreactive cerebral blood flow in the posterior cerebral artery in controls and in patients with epilepsy. *Electroencephalogr Clin Neurophysiol,* 107**,** 8-12.

FOGANG, Y., GERARD, P., DE PASQUA, V., PEPIN, J. L., NDIAYE, M., MAGIS, D. & SCHOENEN, J. 2015. Analysis and clinical correlates of 20 Hz photic driving on routine EEG in migraine. *Acta Neurol Belg,* 115**,** 39-45.

GEBBER, G. L., ZHONG, S., LEWIS, C. & BARMAN, S. M. 1999. Human brain alpha rhythm: nonlinear oscillation or filtered noise? *Brain Res,* 818**,** 556-60.

GELLER, A. M., HUDNELL, H. K., VAUGHN, B. V., MESSENHEIMER, J. A. & BOYES, W. K. 2005. Epilepsy and medication effects on the pattern visual evoked potential. *Doc Ophthalmol,* 110**,** 121-31.

GOLLA, F. L. & WINTER, A. L. 1959. Analysis of cerebral responses to flicker in patients complaining of episodic headache. *Electroencephalogr Clin Neurophysiol,* 11**,** 539-49.

JIN, Y., POTKIN, S. G., RICE, D., SRAMEK, J., COSTA, J., ISENHART, R., HEH, C. & SANDMAN, C. A. 1990. Abnormal EEG responses to photic stimulation in schizophrenic patients. *Schizophr Bull,* 16**,** 627-34.

JIN, Y., SANDMAN, C. A., WU, J. C., BERNAT, J. & POTKIN, S. G. 1995. Topographic analysis of EEG photic driving in normal and schizophrenic subjects. *Clin Electroencephalogr,* 26**,** 102-7.

KIKUCHI, M., WADA, Y., TAKEDA, T., OE, H., HASHIMOTO, T. & KOSHINO, Y. 2002. EEG harmonic responses to photic stimulation in normal aging and Alzheimer's disease: differences in interhemispheric coherence. *Clin Neurophysiol,* 113**,** 1045-51.

NOACHTAR, S., BINNIE, C., EBERSOLE, J., MAUGUIERE, F., SAKAMOTO, A. & WESTMORELAND, B. 1999. A glossary of terms most commonly used by clinical electroencephalographers and proposal for the report form for the EEG findings. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl,* 52**,** 21-41.

ROSS, M. H., YURGELUN-TODD, D. A., RENSHAW, P. F., MAAS, L. C., MENDELSON, J. H., MELLO, N. K., COHEN, B. M. & LEVIN, J. M. 1997. Age-related reduction in functional MRI response to photic stimulation. *Neurology,* 48**,** 173-6.
SABETI, M., JAMES, . Dichoptic Multifocal Pupillography Identifies Retinal Dysfunction in Early Age-Related Macular Degeneration. ARVO Meeting, April 11, 2010 2010.
SAND, T., BJORK, M., BRATHEN, G., BRODTKORB, E., MICHLER, R. P. & BOVIM, G. 2010. The EEG response to photic stimulation is not increased in alcohol-related seizures. *Clin Neurophysiol,* 121**,** 1810-5.
SCHOMER, D. L. & LOPES DA SILVA, F. H. 2005. *Niedermeyer's Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*

Philadelphia, PA, USA, Lippincott Williams & Wilkins.
SIMON, R. H., ZIMMERMAN, A. W., TASMAN, A. & HALE, M. S. 1982. Spectral analysis of photic stimulation in migraine. *Electroencephalogr Clin Neurophysiol,* 53**,** 270-6.
TSAI, J. J., NORCIA, A. M., ALES, J. M. & WADE, A. R. 2011. Contrast gain control abnormalities in idiopathic generalized epilepsy. *Ann Neurol,* 70**,** 574-82.
VOGEL, W., BROVERMAN, D. M., KLAIBER, E. L. & KOBAYASHI, Y. 1974. EEG driving responses as a function of monoamine oxidase. *Electroencephalogr Clin Neurophysiol,* 36**,** 205-7.
WALTER, W. G., DOVEY, V. J. & SHIPTON, H. 1946. Analysis of the electrical response of the human cortex to photic stimulation. *Nature,* 158**,** 540.
WIENER, N. 1961. Cybernetics: or control and communication in the animal and the machine. . Cambridge: MIT Press; .

Chapter 5.
Conclusion

We undertook the research in this thesis to establish the use of multifocal pupillographic objective perimetry (mfPOP) in two neurological disorders with many similarities epilepsy and migraine, we also explored the common phenomena of photosensitivity and cortical excitability that both disorders share. We accomplished our aim through three experimental chapters: the first examined migraine patients using protocols designed to stimulate melanopsin containing retinal ganglion cells, which are believed to play a role in exacerbating migraine headaches through light (Noseda et al., 2010). In the second chapter we shed light on pupillary changes in epilepsy patients and we incorporated electroencephalography (EEG) to examine the safety of mfPOP clinically and sub-clinically in epilepsy subjects. In the third chapter again we used EEGs in epilepsy patients to explore a phenomena related to light stimulation, which is the alpha rhythm entrainment also known as the photic drive response. The conclusions of these three chapters can be summarised into three main categories, which are 1) photosensitivity and safety of the mfPOP device in neurological disorders; 2) cortical excitability in migraine and epilepsy and how they can be further explored using mfPOP; and 3) importance of mfPOP in neurological disorders.

5.1. Photosensitivity

As we expect the mfPOP device will be widely available in the near future to test variety of patients, so its safety when used in disorders known to be associated with photosensitivity is of great importance. We have provided evidence that in both epilepsy and migraine patients the use of mfPOP was well tolerated and safe. We went beyond subjective safety measures in migraine patients and made the study a randomised and crossed over design, gave extended headache diaries, and used non-

standard mfPOP protocols, in particular -the blue protocol- to specially target cells known to play a major role in the pathophysiology of that disorder and subsequently insure optimal tolerability and safety. Likewise in epilepsy patients we tested subjects with the mfPOP device concomitantly with ongoing EEG recording as an extra measure of subclinical safety. Rarely do we see such steps are taken in the process of approving a medical device before its wide use. This stemmed from the gap we felt during counselling these subjects before testing and our inability of guaranteeing their safety since scientific data were lacking. The literature would suggest that the small, asynchronous, stimuli of mfPOP would not induce visual distress (Wilkins, 1995), however this was untested. Standardised safety testing of photosensitive subjects whether suffering from headache or epilepsy should be implemented for all light emitting device as part of their routine approval.

5.2. Cortical excitability

One of the many aspects that Migraine and epilepsy share is the phenomena of cortical hyper-excitability, which is critical for generating epileptic seizures, and has been demonstrated between migraine attacks. Hyper-excitability during a migraine attack is believed to transition to cortical spreading depression (CSD) rather than to the hyper-synchronous activity that characterizes epilepsy. Our results have shown that this phenomena spread further to involve the pupillary response pathway which is mainly a subcortical pathway, these results are summarised in **(Figure 1)**. In case of migraine patients we found decreased pupillary constriction to occur after a migraine attack and this effect slowly tapered off with time. Thus, we believe we were detecting a recovery from the cortical spreading depression seen during a migraine attack.

On the other hand patients classified as having generalised epilepsy showed exaggerated pupillary constriction interictally, when testing is carried out long after an attack this increase tended to normalise. Both these phenomena were reversed by using two different classes of medications known to alter cortical hyper-excitability namely triptans - which are selective serotonin 5 HT1 receptor agonists - in migraine subjects, and antiepileptic medications - predominantly voltage dependent Na channel inhibitors - in epilepsy patients. These results lead us to speculate that our findings were mostly a reversal of a central effect of the primary disease rather than an effect of the medications on their own.

In the view of changes in cerebral blood flow in migraine – as evident on apparent water diffusion co-efficient (ADC) on diffusion-weighted MRI during cortical spreading depression (CSD) (Smith et al., 2006, Umesh Rudrapatna et al., 2015)- and epilepsy (Diehl et al., 1998), - we strongly feel that coupling such a modality with mfPOP testing could yield a lot of information that will further elucidate whether these blood flow changes are also contributing to the pupillary response changes.

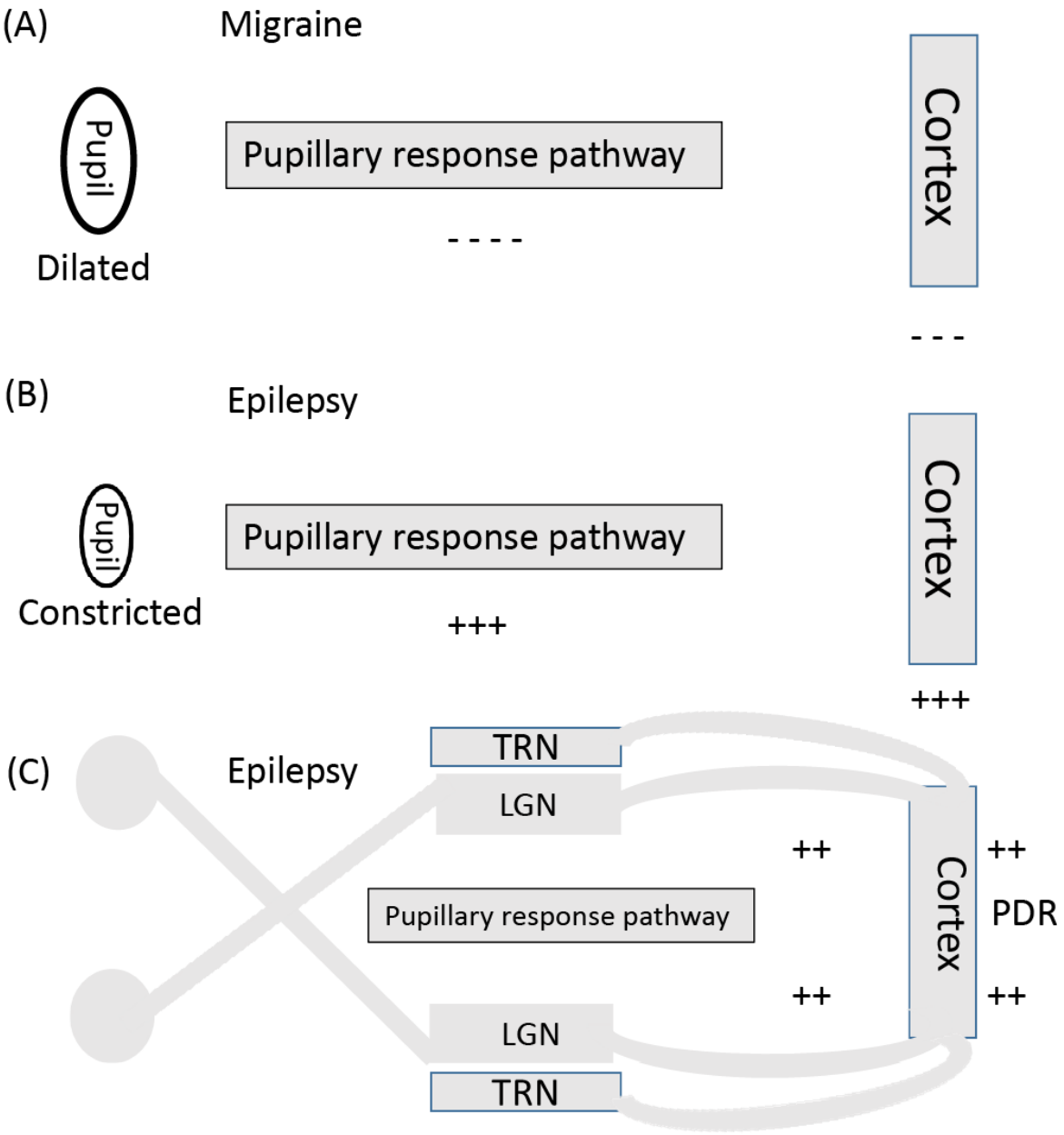


Figure 1. Summary of cortical excitability changes and how they correlated with pupillary response changes. (A) **Chapter 1:** Post migraine attacks, similar to the persistence of the cortical spreading depression a persistence subcortical depression was seen that may have affected the pupillary response pathway leading to more pupillary dilatation, as time progressed following the attack this dilatation normalises. (B) **Chapter 2:** inter-ictally in epilepsy patients and mainly post attacks, cortical hyper-excitability was correlated with a subcortical hyper excitability of the pupillary response leading to exaggerated pupillary constriction, more so for generalised epilepsy than focal. (C) **Chapter 3:** Photic drive response (PDR) was exaggerated in epilepsy patients, and was associated with a suppressive effect on pupil responses at -0.21 dB of pupil response per 1 dB of PDR enhancement. PDR= photic drive response, LGN= lateral geniculate nucleus, TRN= thalamic reticular nucleus.

Based on our knowledge of the common involvement of the thalamus in the visual pathway (mainly the lateral geniculate nucleus), the generation of the alpha rhythm, and the generation of spike wave activity in epilepsy patients via the thalamocortical circuits (mainly through the thalamic reticular nucleus (TRN)), we went further to study the photic drive response (PDR) - which again was used as a marker of cortical excitability - in epilepsy patients where we defined an alpha-band gain. We measured it in both subjects and controls and found the alpha-band gain amplitude was more pronounced in epilepsy patients than normal controls, and again these changes were attenuated by the use of anti-epileptic medications. Interestingly the increase in the alpha-band gain amplitude had an inverse relation to the pupillary constriction amplitude. This may be a protective mechanism by the brain to decrease light entry through the pupils in cases of exaggerated cortical excitability.

All these results are an indication that along with transcranial magnetic stimulation, electroencephalography, and functional MRI, mfPOP is a useful device to study cortical hyper excitability and how it dynamically changes in disorders like epilepsy and migraine.

5.3. Importance of mfPOP as a perimetry device in neurology patients

In addition to shedding the light on the underlying pathophysiology of cortical excitability and how the pupillary response is modulated (as seen above) when the mfPOP device was used in migraine patients it was capable of mapping visual field scotomas. They were found peripherally and concentrically located, changing with time, decreasing with time following attacks, and decreasing with the use of medication namely triptans. Not only were these results consistent with results obtained with other

methods of perimetry, they also contributed to our understanding of the source generator of these scotomas. The source of migraine scotomas has been long debated in the literature. Our results here suggested that both cortical and subcortical visual processing anomalies occur in migraine. MfPOP visual field results are obtained through the pupillary response with less influence from the cortex – particularly with the long blue stimuli used (Carle et al., 2015) - thus giving evidence that subcortical structures do contribute to the source of these scotomas.

So far we have shown results of the application of mfPOP in two neurological disorders namely migraine headaches and epilepsy. We have also investigated its use in previous work on multiple sclerosis (Ali et al., 2014). Ongoing work is exploring its use in detecting abnormalities in stroke and pituitary tumour patients.

Neurology patients are known to have multiple comorbidities in the cognitive and motor domains, these limitations make standard perimetry testing difficult in terms of cooperativity and tolerability. In addition the subjective results of other perimetry methods may be affected by cognitive issues affecting these patients. Therefore the development of a test that is rapid, objective, and does not require motor cooperation in the form of pushing buttons, is of great importance to these patients. These factors were taken into account during the development of the mfPOP device. We hope in the near future to establish the mfPOP device as the standard for perimetry in neurology patients.

References

1958. Report of the committee on methods of clinical examination in electroencephalography. *Electroencephalography and Clinical Neurophysiology,* 10**,** 370-375.

2004. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia,* 24 Suppl 1**,** 9-160.

2013. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia,* 33**,** 629-808.

ALI, E. 2016. *Exploring cortical excitability and photosensitivity in neurological disorders using pupillary response to sparse multifocal stimuli* The Australian National University

ALI, E., MADDESS, T., JAMES, A., VOICU, C. & LUECK, C. 2014. Pupillary response to sparse multifocal stimuli in multiple sclerosis patients. *Multiple Sclerosis Journal,* 20**,** 854-861.

ALI, E. N., LUECK, C. J., CARLE, C. F., KOLIC, M. & MADDESS, T. Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective. International Pupil Colloquium, 2015 Oxford, UK.

ALPERN, M. & CAMPBELL, F. W. 1962. The spectral sensitivity of the consensual light reflex. *J Physiol,* 164**,** 478-507.

AMBROSINI, A., DE NOORDHOUT, A. M., SANDOR, P. S. & SCHOENEN, J. 2003. Electrophysiological studies in migraine: a comprehensive review of their interest and limitations. *Cephalalgia,* 23 Suppl 1**,** 13-31.

ASANUMA, C., ANDERSEN, R. A. & COWAN, W. M. 1985. The thalamic relations of the caudal inferior parietal lobule and the lateral prefrontal cortex in monkeys: divergent cortical projections from cell clusters in the medial pulvinar nucleus. *J Comp Neurol,* 241**,** 357-81.

AURORA, S. K., CAO, Y., BOWYER, S. M. & WELCH, K. M. 1999. The occipital cortex is hyperexcitable in migraine: experimental evidence. *Headache,* 39**,** 469-76.

BADAWY, R. A. & JACKSON, G. D. 2012. Cortical excitability in migraine and epilepsy: a common feature? *J Clin Neurophysiol,* 29**,** 244-9.

BADAWY, R. A., LOETSCHER, T., MACDONELL, R. A. & BRODTMANN, A. 2012. Cortical excitability and neurology: insights into the pathophysiology. *Funct Neurol,* 27**,** 131-45.

BADAWY, R. A., MACDONELL, R. A., BERKOVIC, S. F., NEWTON, M. R. & JACKSON, G. D. 2010. Predicting seizure control: cortical excitability and antiepileptic medication. *Ann Neurol,* 67**,** 64-73.

BADAWY, R. A., VOGRIN, S. J., LAI, A. & COOK, M. J. 2013a. The cortical excitability profile of temporal lobe epilepsy. *Epilepsia,* 54**,** 1942-9.

BADAWY, R. A., VOGRIN, S. J., LAI, A. & COOK, M. J. 2013b. Patterns of cortical hyperexcitability in adolescent/adult-onset generalized epilepsies. *Epilepsia,* 54**,** 871-8.

BADAWY, R. A., VOGRIN, S. J., LAI, A. & COOK, M. J. 2014. Does the region of epileptogenicity influence the pattern of change in cortical excitability? *Clin Neurophysiol*.

BARBUR, J. L. 1995. A study of pupil response components in human vision. *In:* ROBBINS, J. G., DJAMGOZ, M. B. A. & TAYLOR, A. (eds.) *Basic and clinical prespectives in vision research* New York: Plenum Press.
BARRETT, C. F., VAN DEN MAAGDENBERG, A. M., FRANTS, R. R. & FERRARI, M. D. 2008. Familial hemiplegic migraine. *Adv Genet,* 63**,** 57-83.
BARRIGA, F. J., LOPEZ DE SILANES, C., GILI, P. & PAREJA, J. A. 2011. Ciliary ganglioplegic migraine: migraine-related prolonged mydriasis. *Cephalalgia,* 31**,** 291-5.
BASELER, H. A., SUTTER, E. E., KLEIN, S. A. & CARNEY, T. 1994. The topography of visual evoked response properties across the visual field. *Electroencephalogr Clin Neurophysiol,* 90**,** 65-81.
BAVER, S. B., PICKARD, G. E., SOLLARS, P. J. & PICKARD, G. E. 2008. Two types of melanopsin retinal ganglion cell differentially innervate the hypothalamic suprachiasmatic nucleus and the olivary pretectal nucleus. *Eur J Neurosci,* 27**,** 1763-70.
BELL, A., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Dichoptic multifocal pupillography reveals afferent visual field defects in early type 2 diabetes. *Invest Ophthalmol Vis Sci,* 51**,** 602-8.
BENARROCH, E. E. 2011. The melanopsin system: Phototransduction, projections, functions, and clinical implications. *Neurology,* 76**,** 1422-7.
BENEVENTO, L. A., REZAK, M. & SANTOS, A. 1977. An autoradiographic study of the projections of the pretectum in the rhesus monkey (Macaca mulatta): evidence for sensorimotor links to the thalamus and oculomotor nuclei. *Brain Res,* 127**,** 197-218.
BERG, A. T., BERKOVIC, S. F., BRODIE, M. J., BUCHHALTER, J., CROSS, J. H., VAN EMDE BOAS, W., ENGEL, J., FRENCH, J., GLAUSER, T. A., MATHERN, G. W., MOSHE, S. L., NORDLI, D., PLOUIN, P. & SCHEFFER, I. E. 2010. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia,* 51**,** 676-85.
BIANCHIN, M. M., LONDERO, R. G., LIMA, J. E. & BIGAL, M. E. 2010. Migraine and epilepsy: a focus on overlapping clinical, pathophysiological, molecular, and therapeutic aspects. *Curr Pain Headache Rep,* 14**,** 276-83.
BICKFORD, R. G., SEM-JACOBSEN, C. W., WHITE, P. T. & DALY, D. 1952. Some observations on the mechanism of photic and photometrazol activation. *Electroencephalogr Clin Neurophysiol,* 4**,** 275-82.
BINDMAN, L. J., LIPPOLD, O. C. & REDFEARN, J. W. 1962. Long-lasting changes in the level of the electrical activity of the cerebral cortex produced bypolarizing currents. *Nature,* 196**,** 584-5.
BJORK, M., HAGEN, K., STOVNER, L. & SAND, T. 2011. Photic EEG-driving responses related to ictal phases and trigger sensitivity in migraine: a longitudinal, controlled study. *Cephalalgia,* 31**,** 444-55.
BLUM, A. S. & RUTKOVE, S. B. 2007. *The Clinical Neurophysiology Primer,* Totowa, New Jersey, USA., Humana press.
BOHOTIN, V., FUMAL, A., VANDENHEEDE, M., BOHOTIN, C. & SCHOENEN, J. 2003. Excitability of visual V1-V2 and motor cortices to single transcranial magnetic stimuli in migraine: a reappraisal using a figure-of-eight coil. *Cephalalgia,* 23**,** 264-70.

CAMBRON, M., MAERTENS, H., PAEMELEIRE, K. & CREVITS, L. 2014. Autonomic function in migraine patients: ictal and interictal pupillometry. *Headache,* 54**,** 655-62.
CAO, Y., WELCH, K. M., AURORA, S. & VIKINGSTAD, E. M. 1999. Functional MRI-BOLD of visually triggered headache in patients with migraine. *Arch Neurol,* 56**,** 548-54.
CARABALLO, R. H., CERSOSIMO, R. O. & FEJERMAN, N. 2008. Childhood occipital epilepsy of Gastaut: a study of 33 patients. *Epilepsia,* 49**,** 288-97.
CARLE, C., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2010. Multifocal Pupillographic Objective Perimetry in Glaucoma using Blue Stimuli. *International Perimetry Society 19th Annual Meeting, ,*

. Puerto de la Cruz, Tenerife.
CARLE, C. F., JAMES, A. C., KOLIC, M., ESSEX, R. W. & MADDESS, T. 2015. Blue Multifocal Pupillographic Objective Perimetry in Glaucoma. *Invest Ophthalmol Vis Sci,* 56**,** 6394-403.
CARLE, C. F., JAMES, A. C., KOLIC, M., LOH, Y. W. & MADDESS, T. 2011a. High-resolution multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci,* 52**,** 604-10.
CARLE, C. F., JAMES, A. C. & MADDESS, T. 2013. The pupillary response to color and luminance variant multifocal stimuli. *Invest Ophthalmol Vis Sci,* 54**,** 467-75.
CARLE, C. F., MADDESS, T. & JAMES, A. C. 2011b. Contraction anisocoria: segregation, summation, and saturation in the pupillary pathway. *Invest Ophthalmol Vis Sci,* 52**,** 2365-71.
CHIAPPA, K. H., HILL, R. A., HUANG-HELLINGER, F. & JENKINS, B. G. 1999. Photosensitive epilepsy studied by functional magnetic resonance imaging and magnetic resonance spectroscopy. *Epilepsia,* 40 Suppl 4**,** 3-7.
CHORLTON, P. & KANE, N. 2000. Investigation of the cerebral response to flicker stimulation in patients with headache. *Clin Electroencephalogr,* 31**,** 83-7.
CHRONICLE, E. P. & WILKINS, A. J. 1991. Colour and visual discomfort in migraineurs. *Lancet,* 338**,** 890.
CLARKE, T., BASKURT, Z., STRUG, L. J. & PAL, D. K. 2009. Evidence of shared genetic risk factors for migraine and rolandic epilepsy. *Epilepsia,* 50**,** 2428-33.
CONLEY, M. & FRIEDERICH-ECSY, B. 1993. Functional organization of the ventral lateral geniculate complex of the tree shrew (Tupaia belangeri): II. Connections with the cortex, thalamus, and brainstem. *J Comp Neurol,* 328**,** 21-42.
CONNOLLY, J. F., GAWEL, M. & ROSE, F. C. 1982. Migraine patients exhibit abnormalities in the visual evoked potential. *J Neurol Neurosurg Psychiatry,* 45**,** 464-7.
COPPOLA, G. & SCHOENEN, J. 2012. Cortical excitability in chronic migraine. *Curr Pain Headache Rep,* 16**,** 93-100.
COULL, B. M. & PEDLEY, T. A. 1978. Intermittent photic stimulation. Clinical usefulness of non-convulsive responses. *Electroencephalogr Clin Neurophysiol,* 44**,** 353-63.
COVANIS, A. 2005. Photosensitivity in idiopathic generalized epilepsies. *Epilepsia,* 46 Suppl 9**,** 67-72.
DACEY, D. M., LIAO, H. W., PETERSON, B. B., ROBINSON, F. R., SMITH, V. C., POKORNY, J., YAU, K. W. & GAMLIN, P. D. 2005. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature,* 433**,** 749-54.

DAVIE, C. A., BARKER, G. J., WEBB, S., TOFTS, P. S., THOMPSON, A. J., HARDING, A. E., MCDONALD, W. I. & MILLER, D. H. 1995. Persistent functional deficit in multiple sclerosis and autosomal dominant cerebellar ataxia is associated with axon loss. *Brain,* 118 (Pt 6)**,** 1583-92.

DEPREZ, L., PEETERS, K., VAN PAESSCHEN, W., CLAEYS, K. G., CLAES, L. R., SULS, A., AUDENAERT, D., VAN DYCK, T., GOOSSENS, D., DEL-FAVERO, J. & DE JONGHE, P. 2007. Familial occipitotemporal lobe epilepsy and migraine with visual aura: linkage to chromosome 9q. *Neurology,* 68**,** 1995-2002.

DIEHL, B., STODIECK, S. R., DIEHL, R. R. & RINGELSTEIN, E. B. 1998. The photic driving EEG response and photoreactive cerebral blood flow in the posterior cerebral artery in controls and in patients with epilepsy. *Electroencephalogr Clin Neurophysiol,* 107**,** 8-12.

DINEEN, J. T. & HENDRICKSON, A. 1983. Overlap of retinal and prestriate cortical pathways in the primate pretectum. *Brain Res,* 278**,** 250-4.

DISTLER, C. & HOFFMANN, K. P. 1989. The pupillary light reflex in normal and innate microstrabismic cats, II: Retinal and cortical input to the nucleus praetectalis olivaris. *Vis Neurosci,* 3**,** 139-53.

DO, M. T., KANG, S. H., XUE, T., ZHONG, H., LIAO, H. W., BERGLES, D. E. & YAU, K. W. 2009. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature,* 457**,** 281-7.

DREIER, J. P., MAJOR, S., PANNEK, H. W., WOITZIK, J., SCHEEL, M., WIESENTHAL, D., MARTUS, P., WINKLER, M. K., HARTINGS, J. A., FABRICIUS, M., SPECKMANN, E. J. & GORJI, A. 2012. Spreading convulsions, spreading depolarization and epileptogenesis in human cerebral cortex. *Brain,* 135**,** 259-75.

EDWARDS, S. B., ROSENQUIST, A. C. & PALMER, L. A. 1974. An autoradiographic study of ventral lateral geniculate projections in the cat. *Brain Res,* 72**,** 282-7.

FISHER, R. S., ACEVEDO, C., ARZIMANOGLOU, A., BOGACZ, A., CROSS, J. H., ELGER, C. E., ENGEL, J., JR., FORSGREN, L., FRENCH, J. A., GLYNN, M., HESDORFFER, D. C., LEE, B. I., MATHERN, G. W., MOSHE, S. L., PERUCCA, E., SCHEFFER, I. E., TOMSON, T., WATANABE, M. & WIEBE, S. 2014. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia,* 55**,** 475-82.

FISHER, R. S., HARDING, G., ERBA, G., BARKLEY, G. L. & WILKINS, A. 2005. Photic- and pattern-induced seizures: a review for the Epilepsy Foundation of America Working Group. *Epilepsia,* 46**,** 1426-41.

FOGANG, Y., GERARD, P., DE PASQUA, V., PEPIN, J. L., NDIAYE, M., MAGIS, D. & SCHOENEN, J. 2015. Analysis and clinical correlates of 20 Hz photic driving on routine EEG in migraine. *Acta Neurol Belg,* 115**,** 39-45.

FRIEDMAN, D. I. & DE VER DYE, T. 2009. Migraine and the environment. *Headache,* 49**,** 941-52.

GAMLIN, P. D. 2006. The pretectum: connections and oculomotor-related roles. *Prog Brain Res,* 151**,** 379-405.

GAMLIN, P. D., MCDOUGAL, D. H., POKORNY, J., SMITH, V. C., YAU, K. W. & DACEY, D. M. 2007. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Res,* 47**,** 946-54.

GEBBER, G. L., ZHONG, S., LEWIS, C. & BARMAN, S. M. 1999. Human brain alpha rhythm: nonlinear oscillation or filtered noise? *Brain Res,* 818**,** 556-60.

GELLER, A. M., HUDNELL, H. K., VAUGHN, B. V., MESSENHEIMER, J. A. & BOYES, W. K. 2005. Epilepsy and medication effects on the pattern visual evoked potential. *Doc Ophthalmol,* 110**,** 121-31.
GOLLA, F. L. & WINTER, A. L. 1959. Analysis of cerebral responses to flicker in patients complaining of episodic headache. *Electroencephalogr Clin Neurophysiol,* 11**,** 539-49.
GOOD, P. A., TAYLOR, R. H. & MORTIMER, M. J. 1991. The use of tinted glasses in childhood migraine. *Headache,* 31**,** 533-6.
GOOLEY, J. J., LU, J., FISCHER, D. & SAPER, C. B. 2003. A broad role for melanopsin in nonvisual photoreception. *J Neurosci,* 23**,** 7093-106.
GOWERS, W. R. 1906. Clinical Lectures ON THE BORDERLAND OF EPILEPSY. III.-MIGRAINE. *Br Med J,* 2**,** 1617-22.
GRAFSTEIN, B. 1956. Mechanism of spreading cortical depression. *J Neurophysiol,* 19**,** 154-71.
GRAYBIEL, A. M. 1974. Visuo-cerebellar and cerebello-visual connections involving the ventral lateral geniculate nucleus. *Exp Brain Res,* 20**,** 303-6.
GUNAYDIN, S., SOYSAL, A., ATAY, T. & ARPACI, B. 2006. Motor and occipital cortex excitability in migraine patients. *Can J Neurol Sci,* 33**,** 63-7.
HARDING, G. 1994. Photosensitivity: a vestigial echo? The first Grey Walter Lecture. *Int J Psychophysiol,* 16**,** 273-9.
HARDING, G., WILKINS, A. J., ERBA, G., BARKLEY, G. L. & FISHER, R. S. 2005. Photic- and pattern-induced seizures: expert consensus of the Epilepsy Foundation of America Working Group. *Epilepsia,* 46**,** 1423-5.
HARDING, G. F. A. & TAKAHASHI, T. 2004. Regulations: What Next? *Epilepsia,* 45**,** 46-47.
HATTAR, S., LIAO, H. W., TAKAO, M., BERSON, D. M. & YAU, K. W. 2002. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science,* 295**,** 1065-70.
HATTORI, Y. 1990. Regional difference in responsiveness of adenosine-sensitive cyclic AMP-generating systems in chronic epileptic cerebral cortex of the rat. *J Neurochem,* 55**,** 1294-303.
HATTORI, Y., MORIWAKI, A., HAYASHI, Y., ISLAM, N. & HORI, Y. 1993. Characterization of adenosine receptor-mediated generation of cyclic AMP in slices of rat cerebral cortex with chronic epileptic activity. *Neurochem Res,* 18**,** 1009-14.
HATTORI, Y., MORIWAKI, A., HAYASHI, Y., LU, Y. F. & HORI, Y. 1992. Increased responses to adenosine and 2-chloroadenosine of cyclic AMP-generating systems in the primary cortical region of cobalt-induced epilepsy in the rat. *Jpn J Physiol,* 42**,** 151-7.
HATTORI, Y., MORIWAKI, A. & HORI, Y. 1990. Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex. *Neurosci Lett,* 116**,** 320-4.
HAUT, S. R., BIGAL, M. E. & LIPTON, R. B. 2006. Chronic disorders with episodic manifestations: focus on epilepsy and migraine. *Lancet Neurol,* 5**,** 148-57.
HAY, K. M., MORTIMER, M. J., BARKER, D. C., DEBNEY, L. M. & GOOD, P. A. 1994. 1044 women with migraine: the effect of environmental stimuli. *Headache,* 34**,** 166-8.
HUERTA, M. F., KRUBITZER, L. A. & KAAS, J. H. 1986. Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys: I. Subcortical connections. *J Comp Neurol,* 253**,** 415-39.

HUGHES, S. W. & CRUNELLI, V. 2005. Thalamic mechanisms of EEG alpha rhythms and their pathological implications. *Neuroscientist,* 11**,** 357-72.
HUGUENARD, J. R. 1999. Neuronal circuitry of thalamocortical epilepsy and mechanisms of antiabsence drug action. *Adv Neurol,* 79**,** 991-9.
JAMES, A. C. 2003. The pattern-pulse multifocal visual evoked potential. *Invest Ophthalmol Vis Sci,* 44**,** 879-90.
JAMES, A. C., RUSECKAITE, R. & MADDESS, T. 2005. Effect of temporal sparseness and dichoptic presentation on multifocal visual evoked potentials. *Vis Neurosci,* 22**,** 45-54.
JAMES, G., KOLIC, ESSEX, MADDESS Objective Pupillographic Multifocal Perimetry for Glaucoma ARVO Meeting April 11, 2009 2009.
JEAVONS, P. M. & HARDING, G. F. A. 1975. *Photosensitive Epilepsy,* London, William Heinemann.
JENSEN, R., TASSORELLI, C., ROSSI, P., ALLENA, M., OSIPOVA, V., STEINER, T., SANDRINI, G., OLESEN, J. & NAPPI, G. 2011. A basic diagnostic headache diary (BDHD) is well accepted and useful in the diagnosis of headache. a multicentre European and Latin American study. *Cephalalgia,* 31**,** 1549-60.
JIN, Y., POTKIN, S. G., RICE, D., SRAMEK, J., COSTA, J., ISENHART, R., HEH, C. & SANDMAN, C. A. 1990. Abnormal EEG responses to photic stimulation in schizophrenic patients. *Schizophr Bull,* 16**,** 627-34.
JIN, Y., SANDMAN, C. A., WU, J. C., BERNAT, J. & POTKIN, S. G. 1995. Topographic analysis of EEG photic driving in normal and schizophrenic subjects. *Clin Electroencephalogr,* 26**,** 102-7.
JONES, K. A., HATORI, M., MURE, L. S., BRAMLEY, J. R., ARTYMYSHYN, R., HONG, S. P., MARZABADI, M., ZHONG, H., SPROUSE, J., ZHU, Q., HARTWICK, A. T., SOLLARS, P. J., PICKARD, G. E. & PANDA, S. 2013. Small-molecule antagonists of melanopsin-mediated phototransduction. *Nat Chem Biol,* 9**,** 630-5.
JUDIT, A., SANDOR, P. S. & SCHOENEN, J. 2000. Habituation of visual and intensity dependence of auditory evoked cortical potentials tends to normalize just before and during the migraine attack. *Cephalalgia,* 20**,** 714-9.
KARDON, R. 2005 Anatomy and Physiology of the Autonomic Nervous System. *Walsh and Hoyt's Clinical Neuro-Ophthalmology* Spencer S. Eccles Health Sciences Library
KASTELEIJN-NOLST TRENITE, D. G., GUERRINI, R., BINNIE, C. D. & GENTON, P. 2001. Visual sensitivity and epilepsy: a proposed terminology and classification for clinical and EEG phenomenology. *Epilepsia,* 42**,** 692-701.
KAWASAKI, A. & KARDON, R. H. 2007. Intrinsically photosensitive retinal ganglion cells. *J Neuroophthalmol,* 27**,** 195-204.
KENNARD, C., GAWEL, M., RUDOLPH NDE, M. & ROSE, F. C. 1978. Visual evoked potentials in migraine subjects. *Res Clin Stud Headache,* 6**,** 73-80.
KIKUCHI, M., WADA, Y., TAKEDA, T., OE, H., HASHIMOTO, T. & KOSHINO, Y. 2002. EEG harmonic responses to photic stimulation in normal aging and Alzheimer's disease: differences in interhemispheric coherence. *Clin Neurophysiol,* 113**,** 1045-51.
LANCE, J. W. 1996. Migraine: then and now. *Med J Aust,* 164**,** 519-20.
LAURITZEN, M., RICE, M. E., OKADA, Y. & NICHOLSON, C. 1988. Quisqualate, kainate and NMDA can initiate spreading depression in the turtle cerebellum. *Brain Res,* 475**,** 317-27.

LAWRENCE M. FRIEDMAN, C. D. F., DAVID L. DEMETS 2010. *Fundamentals of Clinical Trials*, Springer

LEAO, A. A. 1986. Spreading depression. *Funct Neurol,* 1**,** 363-6.
LEHTONEN, J. B. 1974. Visual evoked cortical potentials for single flashes and flickering light in migraine. *Headache,* 14**,** 1-12.
LEICHNETZ, G. R. 1982. Connections between the frontal eye field and pretectum in the monkey: an anterograde/retrograde study using HRP gel and TMB neurohistochemistry. *J Comp Neurol,* 207**,** 394-404.
LENNOX, W. G. & LENNOX, M. A. 1960. *Epilepsy and related disorders,* Boston, Little, Brown and Company.
LIEBETANZ, D., NITSCHE, M. A., TERGAU, F. & PAULUS, W. 2002. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain,* 125**,** 2238-47.
LOSSEFF, N. A., WEBB, S. L., O'RIORDAN, J. I., PAGE, R., WANG, L., BARKER, G. J., TOFTS, P. S., MCDONALD, W. I., MILLER, D. H. & THOMPSON, A. J. 1996. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain,* 119 (Pt 3)**,** 701-8.
LUCAS, R. J., DOUGLAS, R. H. & FOSTER, R. G. 2001. Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat Neurosci,* 4**,** 621-6.
LUECK, C., ALI, E., CARLE, C. & MADDESS, T. 2014. 47. : Effects of stimulating melanopsin-containing retinal ganglion cells in migraine patients using multifocal objective pupillometry. *Journal of Clinical Neuroscience,* 21**,** 2048.
LUI, F., GREGORY, K. M., BLANKS, R. H. & GIOLLI, R. A. 1995. Projections from visual areas of the cerebral cortex to pretectal nuclear complex, terminal accessory optic nuclei, and superior colliculus in macaque monkey. *J Comp Neurol,* 363**,** 439-60.
MADDESS, T., JAMES, A. C. & BOWMAN, E. A. 2005. Contrast response of temporally sparse dichoptic multifocal visual evoked potentials. *Vis Neurosci,* 22**,** 153-62.
MAIN, A., DOWSON, A. & GROSS, M. 1997. Photophobia and phonophobia in migraineurs between attacks. *Headache,* 37**,** 492-5.
MAIN, A., VLACHONIKOLIS, I. & DOWSON, A. 2000. The wavelength of light causing photophobia in migraine and tension-type headache between attacks. *Headache,* 40**,** 194-9.
MARCUS, D. A. & SOSO, M. J. 1989. Migraine and stripe-induced visual discomfort. *Arch Neurol,* 46**,** 1129-32.
MARTIN, P. R. 2010. Behavioral management of migraine headache triggers: learning to cope with triggers. *Curr Pain Headache Rep,* 14**,** 221-7.
MATYNIA, A. 2013. Blurring the boundaries of vision: novel functions of intrinsically photosensitive retinal ganglion cells. *J Exp Neurosci,* 7**,** 43-50.
MATYNIA, A., NGUYEN, E., SUN, X., BLIXT, F. W., PARIKH, S., KESSLER, J., PEREZ DE SEVILLA MULLER, L., HABIB, S., KIM, P., WANG, Z. Z., RODRIGUEZ, A., CHARLES, A., NUSINOWITZ, S., EDVINSSON, L., BARNES, S., BRECHA, N. C. & GORIN, M. B. 2016. Peripheral Sensory Neurons Expressing Melanopsin Respond to Light. *Front Neural Circuits,* 10**,** 60.

MCCORMICK, D. A. & CONTRERAS, D. 2001. On the cellular and network bases of epileptic seizures. *Annu Rev Physiol,* 63**,** 815-46.
MCKENDRICK, A. M. & BADCOCK, D. R. 2004a. An analysis of the factors associated with visual field deficits measured with flickering stimuli in-between migraine. *Cephalalgia,* 24**,** 389-97.
MCKENDRICK, A. M. & BADCOCK, D. R. 2004b. Decreased visual field sensitivity measured 1 day, then 1 week, after migraine. *Invest Ophthalmol Vis Sci,* 45**,** 1061-70.
MCKENDRICK, A. M., VINGRYS, A. J., BADCOCK, D. R. & HEYWOOD, J. T. 2000. Visual field losses in subjects with migraine headaches. *Invest Ophthalmol Vis Sci,* 41**,** 1239-47.
MCKENDRICK, A. M., VINGRYS, A. J., BADCOCK, D. R. & HEYWOOD, J. T. 2001. Visual dysfunction between migraine events. *Invest Ophthalmol Vis Sci,* 42**,** 626-33.
MESGARI, M., GHAFFARIAN, N., KHALEGHI GHADIRI, M., SADEGHIAN, H., SPECKMANN, E. J., STUMMER, W. & GORJI, A. 2015. Altered inhibition in the hippocampal neural networks after spreading depression. *Neuroscience,* 304**,** 190-7.
MOORE, R. Y., WEIS, R. & MOGA, M. M. 2000. Efferent projections of the intergeniculate leaflet and the ventral lateral geniculate nucleus in the rat. *J Comp Neurol,* 420**,** 398-418.
MORIN, L. P. & BLANCHARD, J. H. 1998. Interconnections among nuclei of the subcortical visual shell: the intergeniculate leaflet is a major constituent of the hamster subcortical visual system. *J Comp Neurol,* 396**,** 288-309.
MUTHANE, U. B., SATISHCHANDRA, P. & SUBHASH, M. N. 1993. Visual and auditory evoked potentials in early onset Parkinson's disease and their relationship to cerebrospinal fluid monoamine metabolites. *Mov Disord,* 8**,** 344-8.
NGUYEN, B. N., MCKENDRICK, A. M. & VINGRYS, A. J. 2012. Simultaneous retinal and cortical visually evoked electrophysiological responses in between migraine attacks. *Cephalalgia,* 32**,** 896-907.
NGUYEN, B. N., VINGRYS, A. J. & MCKENDRICK, A. M. 2014. The effect of duration post-migraine on visual electrophysiology and visual field performance in people with migraine. *Cephalalgia,* 34**,** 42-57.
NOACHTAR, S., BINNIE, C., EBERSOLE, J., MAUGUIERE, F., SAKAMOTO, A. & WESTMORELAND, B. 1999. A glossary of terms most commonly used by clinical electroencephalographers and proposal for the report form for the EEG findings. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl,* 52**,** 21-41.
NOSEDA, R. & BURSTEIN, R. 2011. Advances in understanding the mechanisms of migraine-type photophobia. *Curr Opin Neurol,* 24**,** 197-202.
NOSEDA, R., KAINZ, V., JAKUBOWSKI, M., GOOLEY, J. J., SAPER, C. B., DIGRE, K. & BURSTEIN, R. 2010. A neural mechanism for exacerbation of headache by light. *Nat Neurosci,* 13**,** 239-45.
NYRKE, T., KANGASNIEMI, P. & LANG, H. 1990. Alpha rhythm in classical migraine (migraine with aura): abnormalities in the headache-free interval. *Cephalalgia,* 10**,** 177-81.
OGILVIE, A. D., RUSSELL, M. B., DHALL, P., BATTERSBY, S., ULRICH, V., SMITH, C. A., GOODWIN, G. M., HARMAR, A. J. & OLESEN, J. 1998.

Altered allelic distributions of the serotonin transporter gene in migraine without aura and migraine with aura. *Cephalalgia,* 18**,** 23-6.
OPHOFF, R. A., TERWINDT, G. M., FRANTS, R. R. & FERRARI, M. D. 1998. P/Q-type Ca2+ channel defects in migraine, ataxia and epilepsy. *Trends Pharmacol Sci,* 19**,** 121-7.
OTTMAN, R. & LIPTON, R. B. 1996. Is the comorbidity of epilepsy and migraine due to a shared genetic susceptibility? *Neurology,* 47**,** 918-24.
PARISI, P., PICCIOLI, M., VILLA, M. P., BUTTINELLI, C. & KASTELEIJN-NOLST TRENITE, D. G. 2008. Hypothesis on neurophysiopathological mechanisms linking epilepsy and headache. *Med Hypotheses,* 70**,** 1150-4.
PARK, J. C., MOURA, A. L., RAZA, A. S., RHEE, D. W., KARDON, R. H. & HOOD, D. C. 2011. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci,* 52**,** 6624-35.
PARRA, J., KALITZIN, S. N. & LOPES DA SILVA, F. H. 2005. Photosensitivity and visually induced seizures. *Curr Opin Neurol,* 18**,** 155-9.
PIETROBON, D. 2005. Migraine: new molecular mechanisms. *Neuroscientist,* 11**,** 373-86.
POLMAN, C. H., REINGOLD, S. C., EDAN, G., FILIPPI, M., HARTUNG, H. P., KAPPOS, L., LUBLIN, F. D., METZ, L. M., MCFARLAND, H. F., O'CONNOR, P. W., SANDBERG-WOLLHEIM, M., THOMPSON, A. J., WEINSHENKER, B. G. & WOLINSKY, J. S. 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol,* 58**,** 840-6.
PROVENCIO, I., JIANG, G., DE GRIP, W. J., HAYES, W. P. & ROLLAG, M. D. 1998. Melanopsin: An opsin in melanophores, brain, and eye. *Proc Natl Acad Sci U S A,* 95**,** 340-5.
PROVENCIO, I., RODRIGUEZ, I. R., JIANG, G., HAYES, W. P., MOREIRA, E. F. & ROLLAG, M. D. 2000. A novel human opsin in the inner retina. *J Neurosci,* 20**,** 600-5.
PURPURA, D. P. & MCMURTRY, J. G. 1965. INTRACELLULAR ACTIVITIES AND EVOKED POTENTIAL CHANGES DURING POLARIZATION OF MOTOR CORTEX. *J Neurophysiol,* 28**,** 166-85.
RIBAK, C. E. & PETERS, A. 1975. An autoradiographic study of the projections from the lateral geniculate body of the rat. *Brain Res,* 92**,** 341-68.
ROGAWSKI, M. A. 2012. Migraine and Epilepsy-Shared Mechanisms within the Family of Episodic Disorders.
ROSS, M. H., YURGELUN-TODD, D. A., RENSHAW, P. F., MAAS, L. C., MENDELSON, J. H., MELLO, N. K., COHEN, B. M. & LEVIN, J. M. 1997. Age-related reduction in functional MRI response to photic stimulation. *Neurology,* 48**,** 173-6.
RUSECKAITE, R., MADDESS, T., DANTA, G., LUECK, C. J. & JAMES, A. C. 2005. Sparse multifocal stimuli for the detection of multiple sclerosis. *Ann Neurol,* 57**,** 904-13.
RYAN, D. P. & PTACEK, L. J. 2010. Episodic neurological channelopathies. *Neuron,* 68**,** 282-92.
SABETI, F., MADDESS, T., ESSEX, R. W. & JAMES, A. C. 2012. Multifocal pupillography identifies ranibizumab-induced changes in retinal function for exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci,* 53**,** 253-60.

SABETI, F., MADDESS, T., ESSEX, R. W., SAIKAL, A., JAMES, A. C. & CARLE, C. F. 2014. Multifocal pupillography in early age-related macular degeneration. *Optom Vis Sci,* 91**,** 904-15.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T.

SABETI, F., NOLAN, C. J., JAMES, A. C., JENKINS, A. & MADDESS, T. 2015. Multifocal Pupillography Identifies Changes in Visual Sensitivity According to Severity of Diabetic Retinopathy in Type 2 Diabetes. *Invest Ophthalmol Vis Sci,* 56**,** 4504-13.

SABETI, M., JAMES, . Dichoptic Multifocal Pupillography Identifies Retinal Dysfunction in Early Age-Related Macular Degeneration. ARVO Meeting, April 11, 2010 2010.

SAND, T., BJORK, M., BRATHEN, G., BRODTKORB, E., MICHLER, R. P. & BOVIM, G. 2010. The EEG response to photic stimulation is not increased in alcohol-related seizures. *Clin Neurophysiol,* 121**,** 1810-5.

SCHAFER, E. W. & MCKEAN, C. M. 1975. Evidence that monoamines influence human evoked potentials. *Brain Res,* 99**,** 49-58.

SCHOMER, D. L. & LOPES DA SILVA, F. H. 2005. *Niedermeyer's Electroencephalography: Basic Principles, Clinical Applications, and Related Fields*

Philadelphia, PA, USA, Lippincott Williams & Wilkins.

SHOOK, B. L., SCHLAG-REY, M. & SCHLAG, J. 1990. Primate supplementary eye field: I. Comparative aspects of mesencephalic and pontine connections. *J Comp Neurol,* 301**,** 618-42.

SILBERSTEIN, S. D., HOLLAND, S., FREITAG, F., DODICK, D. W., ARGOFF, C. & ASHMAN, E. 2012. Evidence-based guideline update: pharmacologic treatment for episodic migraine prevention in adults: report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society. *Neurology,* 78**,** 1337-45.

SIMON, R. H., ZIMMERMAN, A. W., TASMAN, A. & HALE, M. S. 1982. Spectral analysis of photic stimulation in migraine. *Electroencephalogr Clin Neurophysiol,* 53**,** 270-6.

SINIATCHKIN, M., GROPPA, S., JEROSCH, B., MUHLE, H., KURTH, C., SHEPHERD, A. J., SIEBNER, H. & STEPHANI, U. 2007. Spreading photoparoxysmal EEG response is associated with an abnormal cortical excitability pattern. *Brain,* 130**,** 78-87.

SMITH, J. M., BRADLEY, D. P., JAMES, M. F. & HUANG, C. L. 2006. Physiological studies of cortical spreading depression. *Biol Rev Camb Philos Soc,* 81**,** 457-81.

SOMJEN, G. G. 2001. Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol Rev,* 81**,** 1065-96.

STEELE, G. E. & WELLER, R. E. 1993. Subcortical connections of subdivisions of inferior temporal cortex in squirrel monkeys. *Vis Neurosci,* 10**,** 563-83.

STOERIG, P., ZONTANOU, A. & COWEY, A. 2002. Aware or unaware: assessment of cortical blindness in four men and a monkey. *Cereb Cortex,* 12**,** 565-74.

SUTTER, E. E. 1996. the field topography of the pupillary response: rod and cone inputs. *Invest Ophthalmol Vis Sci,* 3157.

TAN, L., KONDO, M., SATO, M., KONDO, N. & MIYAKE, Y. 2001. Multifocal pupillary light response fields in normal subjects and patients with visual field defects. *Vision Res,* 41**,** 1073-84.

TFELT-HANSEN, P. C. 2010. History of migraine with aura and cortical spreading depression from 1941 and onwards. *Cephalalgia,* 30**,** 780-92.
TSAI, J. J., NORCIA, A. M., ALES, J. M. & WADE, A. R. 2011. Contrast gain control abnormalities in idiopathic generalized epilepsy. *Ann Neurol,* 70**,** 574-82.
UMESH RUDRAPATNA, S., HAMMING, A. M., WERMER, M. J., VAN DER TOORN, A. & DIJKHUIZEN, R. M. 2015. Measurement of distinctive features of cortical spreading depolarizations with different MRI contrasts. *NMR Biomed,* 28**,** 591-600.
URBANIAK, G. C. & PLOUS, S. 2013. Research Randomizer 4.0 ed. http://www.randomizer.org/
VOGEL, W., BROVERMAN, D. M., KLAIBER, E. L. & KOBAYASHI, Y. 1974. EEG driving responses as a function of monoamine oxidase. *Electroencephalogr Clin Neurophysiol,* 36**,** 205-7.
WALTER, W. G., DOVEY, V. J. & SHIPTON, H. 1946. Analysis of the electrical response of the human cortex to photic stimulation. *Nature,* 158**,** 540.
WERNSMANN, B., PAPE, H. C., SPECKMANN, E. J. & GORJI, A. 2006. Effect of cortical spreading depression on synaptic transmission of rat hippocampal tissues. *Eur J Neurosci,* 23**,** 1103-10.
WIENER, N. 1961. Cybernetics: or control and communication in the animal and the machine. . Cambridge: MIT Press; .
WILHELM, H., NEITZEL, J., WILHELM, B., BEUEL, S., LUDTKE, H., KRETSCHMANN, U. & ZRENNER, E. 2000a. Pupil perimetry using M-sequence stimulation technique. *Invest Ophthalmol Vis Sci,* 41**,** 1229-38.
WILHELM, H., NEITZEL, J., WILHELM, B., BEUEL, S., LUDTKE, H., KRETSCHMANN, U. & ZRENNER, E. 2000b. Pupil perimetry using M-sequence stimulation technique. *Invest Ophthalmol Vis Sci,* 41**,** 1229-38.
WILKINS, A. J. 1995. *Visual Stress,* New York, Oxford Press.
WILKINS, A. J., DARBY, C. E., BINNIE, C. D., STEFANSSON, S. B., JEAVONS, P. M. & HARDING, G. F. 1979. Television epilepsy--the role of pattern. *Electroencephalogr Clin Neurophysiol,* 47**,** 163-71.
WOLF, P. & GOOSSES, R. 1986. Relation of photosensitivity to epileptic syndromes. *J Neurol Neurosurg Psychiatry,* 49**,** 1386-91.
ZAGAMI, A. & BAHRA, A. 2006. *Symptomatology of migraines without aura.In The Headaches,* Philadelphia, Lippincott Williams & Wilkins.
ZIEMANN, U., LONNECKER, S., STEINHOFF, B. J. & PAULUS, W. 1996. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Ann Neurol,* 40**,** 367-78.