Research Article

Photosensitive epilepsy is associated with abnormal excitability of alpha rhythm generating networks

Authors:
Anna Elisabetta Vaudano[1,2*], Andrea Ruggieri[1,2*], Pietro Avanzini[3], Giuliana Gessaroli[2], Gaetano Cantalupo[4], Antonietta Coppola[5], Sanjay Sisodiya[6], and Stefano Meletti[1,2]
* Equally contribution
[1]Department of Biomedical, Metabolic, and Neural Science, University of Modena and Reggio Emilia, NOCSE Hospital, Modena, Italy
[2] Neurology Unit, NOCSAE Hospital, AUSL Modena, Italy
[3] Department of Neuroscience, University of Parma, Parma, Italy
[4]Department of Life and Reproduction Sciences, University of Verona, Verona, Italy
[5]Epilepsy Centre, Department of Neuroscience, Odontostomatology and Reproductive Sciences, Federico II University, Naples, Italy.
[6]Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, National Hospital for Neurology and Neurosurgery, LondonWC1N 3BG, United Kingdom; Epilepsy Society Chalfont-St-Peter Bucks SL9 0RJ, United Kingdom.

Corresponding author: Dr. Stefano Meletti
Corresponding author's address: Department of Biomedical, Metabolic, and Neural Science, University of Modena and Reggio Emilia, NOCSE Hospital, via Giardini 1355, 41126 Modena, Italy.
Corresponding author's phone and fax: Tel: +39-0593961676; Fax: +39-0593961336.
Corresponding author's e-mail address: stefano.meletti@unimore.it
Running title: BOLD correlates of alpha rhythm in epilepsy
Number of words in abstract:
Number of words in main text:
Number of figures: 6
Number of Tables: 2
Supplementary files: 3 figures, 3 tables.

ABBREVIATIONS

BA: Brodmann Area
BOLD: Blood Oxygen Level Dependent
CRT: Controls
EEG-fMRI: Simultaneous Recording EEG and Functional MRI
EMA: Eyelid Myoclonia with Absences
EPI: Echo-Planar Imaging
GGE: Genetic Generalized Epilepsies
FE: Focal Epilepsy
FWHM: Full width at half maximum
FWE: Family Wise Error
FDR: False Discovery Rate
FFT: Fast Fourier Transform
fMRI : functional MRI
GLM: General Linear Model
HRF: Hemodynamic Response Function
ICA: Independent Component Analysis
IED: Interictal Epileptiform Discharges
IPS: Intermittent Photic Stimulation

JAE: Juvanile Absence Epilepsy
JME: Juvanile Myoclonic Epilepsy
MNI: Montreal Neurological Institute
MRI: Magnetic Resonance Imaging
ROI: Region of Interest
PPR: Photoparoxysmal Response
PS: Photosensitivity
SPM: Statistical Parametric Mapping
GSW: Generalized Spike and Wave Discharges
TE: Echo Time
TR: Repetition Time

ABSTRACT

Photosensitivity is a condition in which lights induce epileptiform activities. This abnormal EEG response has been associated with hyperexcitability of the visuo-motor system. In the present work, we evaluate if an intrinsic dysfunction of this network is present during brain activity at rest, independently of any stimulus and of any EEG paroxysmal activity. To address this issue, we investigated the hemodynamic correlates of the spontaneous alpha rhythm, which is considered the hallmark of the brain resting state, in photosensitive patients and in people without photosensitivity. Secondly, we evaluated the whole-brain functional connectivity of the visual thalamic nuclei in the various populations of subjects under investigation.

Forty-four patients with epilepsy and 16 healthy controls underwent an electroencephalography correlated functional magnetic resonance imaging study, during an eyes-closed condition. The following patients' groups were included: (a) Genetic Generalized Epilepsy (GGE) with photosensitivity (*GGE PS+*); 16 subjects (mean age 25±10 years); (b) GGE without photosensitivity (*GGE PS-*), 13 patients (mean age 25±11 years); (c) Focal Epilepsy, 15 patients (mean age 25±9 years). For each subject, the posterior alpha power variations were convolved with the standard hemodynamic response function and used as a regressor in a general linear model. Within and between groups second level analyses were performed. Whole brain functional connectivity was evaluated for two thalamic regions of interest based on the BOLD findings that included the posterior thalamus (pulvinar) and the medio-dorsal thalamic nuclei.

GGE PS+ demonstrated a significant greater mean alpha-power with respect controls and other epilepsy groups. In photosensitive epilepsy, alpha-related BOLD signal changes demonstrated lower BOLD deactivation relative to all other groups at the occipital, sensory-motor, anterior cingulate and supplementary motor cortex. Coherently, the same brain regions demonstrated an abnormal connectivity with the visual thalamus only in *GGE PS+*.

As predicted, our findings indicate that the cortical-subcortical network generating the alpha oscillation at rest is different in people with epilepsy and visual sensitivity. Such difference consists of a decreased alpha-related inhibition of the visual cortex and sensory-motor networks at rest. These findings represent the substrate of the clinical manifestations (i.e. myoclonus) of the photoparoxysmal response. Moreover, our results provide the first evidence on the existence of a functional link between the circuits that trigger the visual sensitivity phenomenon and the posterior alpha rhythm generation.

Keywords: photosensitivity; epilepsy; alpha rhythm; BOLD; functional connectivity.

INTRODUCTION

Photosensitivity refers to a condition in which epileptiform activity is induced by flickering lights, such as flashes on television or produced by video games on computer screens. As an EEG trait, it is characterised by the occurrence of a photoparoxysmal response during

intermittent photic stimulation (Kasteleijn-Nolst Trenitè et al, 2001) and it is reported in about 10% of patients with epilepsy compared with less than 0.5% in otherwise healthy individuals (Gregory et al, 1993). Photosensitivity is the distinctive hallmark of photosensitive occipital lobe epilepsy, which is a focal reflex epilepsy syndrome (Guerrini et al, 1995), and Jeavons syndrome (Jeavons, 1977), which is characterised by eyelid myoclonia with or without impaired consciousness after eye closure. Visual sensitivity is also frequently reported as a reflex trait in patients with genetic generalised epilepsies (formerly known as idiopathic generalised epilepsies), particularly juvenile myoclonic epilepsy, for which the incidence of photosensitivity ranges from 30% (Wolf and Goosses, 1986) to 90% (Appleton et al, 2000) of patients. The high heritability of photosensitivity is widely recognised. Recent evidence points to *CHD2* as a novel gene implicated in photosensitive epilepsy, with patients exhibiting a higher prevalence of unique *CHD2* variants than a control cohort representative of the general population (Galizia et al, 2015).

Precipitation of seizures in photosensitive patients inevitably depends on the activation of a critical neuronal population in the occipital cortex (Wilkins et al, 2004). The distinguishing feature of photosensitive individuals seems to lie in intrinsic hyperexcitability of the visual cortex, which can predispose to large-scale neuronal synchronization. Studies of visual evoked potentials and transcranial magnetic stimulation in patients with photosensitive genetic generalised epilepsy (Strigaro et al, 2012, 2015; Brigo et al, 2013) identified an abnormal excitability profile of the visual cortex, which coexisted with defective contrast gain control mechanisms (Porciatti et al, 2000). However, although hyperexcitability of the visual cortex explains some ictal and EEG findings, it does not entirely elucidate the range of photosensitivity-associated EEG and clinical correlates. Indeed, photoparoxysmal responses are often generalised, and photic stimulation ultimately can elicit seizures with motor components, which are a particularly frequent reflex trait in patients with genetic generalised epilepsies.

Previous EEG-correlated functional MRI (EEG-fMRI) studies have detected photoparoxysmal-related activations in parietal and premotor cortices (Moeller et al., 2009; Bertolini et al., 2014). We recently elucidated the BOLD (blood oxygen level dependent) response to eye-closure in patients with Jeavons Syndrome, discovering the involvement of substantially the same brain circuits (Vaudano et al, 2014). Interestingly, both the study of Vaudano et al, (2014) and the study of Moeller et al, (2009) reveals the presence of an abnormal increase of BOLD signal *before* the appearance of spikes and waves discharges, thus underscoring that signal changes might be linked to an intrinsic dysfunction of this network.

Taken together, current data indicate that photosensitivity is the expression of a visual system alteration, not only limited to the occipital cortex, but expressed as an extended and functional system. For this reason, we believe that a fundamental question, still substantially not investigated, concerns the neural correlates of the alpha rhythm in photosensitive patients. The alpha rhythm, first described by Berger in the 1929 (Berger, 1929), represents the posterior rhythm of the brain. Historically, alpha oscillations have been thought as an idling rhythm, indicating inactivity of brain regions (Pfurtscheller et al, 1996). More recently, the view has changed toward a functional role of alpha oscillation in inhibiting neural regions not relevant in the task-related contest (Klimesch et al, 2007; Mazaheri and Jensen, 2010). Therefore, alpha oscillations in the primary visual areas may represent a mechanism to modulate incoming information. The ‘gating function’ theory, along with the classical alpha desynchronization, predicts greater alpha activity (i.e. greater alpha power) in inhibited cortical areas and lower alpha activity (i.e. lower alpha power) in areas engaged in information processing (Volkmann, 1986; Toscani et al, 2010).

Recently, concurrently recorded EEG-fMRI has been used to search the entire brain for metabolic and/or hemodynamic correlates of the posterior alpha rhythm (Goldman et al, 2002;

Laufs et al, 2003, 2006; Moosmann et al, 2003; Feige et al, 2005; de Munck et al, 2007; Tyvaert et al, 2008; Sadaghiani et al, 2010). The cortical correlation between the posterior alpha modulation and the BOLD signal was negative in every study. This means that the higher the power of the alpha rhythm, the lower was the BOLD signal. The negative alpha-related BOLD signal is evident and maximal over the posterior visual areas, but extends to parietal and prefrontal cortical regions in the majority of studies.

On the basis of this knowledge, we tested the hypothesis that the hemodynamic correlates of the alpha rhythm in photosensitive patients are different with respect to normal subjects and people with epilepsy without photosensitivity. We used EEG-fMRI to find out if the fluctuations in the alpha rhythm correlate with changes in the BOLD signal in cortical and subcortical regions. Specifically, we predicted that alpha-related BOLD signal decreases in the visual system (and beyond, as demonstrated in previous studies) are reduced in photosensitive patients. Then, we evaluated the whole-brain functional connectivity of the visual thalamus (pulvinar), a region implicated in the genesis of the alpha rhythm in animals and humans (Moruzzi and Magoun 1949; Lopez da Silva et al, 1973, 1980; Chatila et al, 1993; Hughes and Crunelli 2005; Liu et al, 2012) in the various sub-populations under investigation.

METHODS

STUDY POPULATIONS AND SETTING

We retrospectively reviewed the entire cohort of patients with epilepsy who underwent an EEG-fMRI study for different purposes, between September 2008 and September 2015 at our Department (total of 260 patients). For the purpose of this study, only patients who had a good quality 10 minutes resting-state fMRI recording with eyes-closed and that fulfilled the following inclusion criteria were considered: (*a*) older than 16 years of age; (*b*) normal structural brain MRI on conventional diagnostic protocol at 3 Tesla; (*c*) absence of sleep EEG figures and absence of interictal events during scanning, or with fewer than 2 spikes/min. Patients with epileptic encephalopathies were further excluded from this study.

We therefore focused on a pool of 44 patients affected by the following epileptic syndromes (according to the definitions of the Commission on Classification and Terminology of the International League Against Epilepsy, Berg et al, 2010):

(a) Genetic Generalized Epilepsies with photosensitivity (GGE *PS*+). This group of patients consisted of 16 subjects (mean age 25 ± 10 years), 11 female and 5 males. For all these patients a 32-channel EEG recording was available within the three months before the fMRI study with an intermittent photic stimulation (IPS) protocol according to international guidelines (Kasteleijn-Nolst Trenite et al, 1999). Photosensitivity was diagnosed if subjects had a photoparoxysmal response (PPR) to IPS (Kasteleijn-Nolst Trenité et al., 2001). In all patients, PPR consisted of generalized spike and wave discharges (type III and IV PPR) (Waltz et al, 1992).

Patients were classified as affected by Juvenile Myoclonic Epilepsy (JME) or Jeavons Syndrome (Eyelid Myoclonia with Absences, EMA)(Jeavons, 1977). The specific inclusion criteria for diagnosis of EMA, beyond the presence of photosensitivity, were the followings (Appleton et al, 1993; Giannakodimos and Panayiotopoulus 1996; Striano et al., 2002): (*i*) age of onset between 2 and 14 years; (*ii*) eyelid myoclonus with or without absences; (*iii*) related generalized paroxysmal activity; (*iv*) eye-closure-induced seizures, EEG paroxysms or both (within 0.5-4 seconds after eye-closure). At the time of the study, self-induction was not reported by any patients. All patients had a photoparoxysmal response with eyelid flickering.

(b) Genetic Generalized Epilepsies without photosensitivity (GGE *PS*-). This group of patients consisted of 13 subjects (mean age 25 ± 11 years), 9 female and 4 males. Patients were classified as affected Juvenile Absence Epilepsy (JAE) or GGE with tonic-clonic seizures only.

(c) Focal non-lesional epilepsy group (FE). This group of patients consisted of 15 subjects (mean age 25 ± 9 years), 10 female and 5 males. As inclusion criteria, these patients were affected by cryptogenic focal epilepsy (normal structural MRI). All had clear focal interictal discharges and seizures with or without consciousness impairment.
None of the patients belonging either to GGE *PS*- and FE groups ever showed PPR to IPS or abnormal sensitivity to the closure of eyes in previous EEG recordings.
Table 1 reports the demographic and electroclinical variables of the three epilepsy groups. Per protocol, all patients were administered a general intelligence evaluation within 1 to 6 months prior to fMRI recordings [Wechsler Adult Intelligence Scale – Fourth Edition (WAIS-IV)]. Full-scale intelligence quotient (IQ) was within the normal range in all recruited subjects.
(d) Controls (CRT). Sixteen healthy volunteers (10 female and 6 male subjects, mean age 25 ± 5 years) with no history of neurologic or psychiatric disorders participated in the study.
The four study groups had the same age and gender distribution. Neither patients nor controls had been taking neuroactive drugs (alcohol and caffeine included) for 72 h prior to the study, except for the patients' antiepileptic treatment. As far as valproic acid (VPA) treatment, a drug that in previous studies has been demonstrated to lower brain rhythms power (Larsson et al., 2005, 2012; Clemens et al., 2007), it was assumed orally by nine out of 16 GGE-PS+ patients, by eight out of 13 GGE-PS- patients and by 4 out of 15 focal epilepsy patients. The mean VPA dose in each group was 750 ± 200 mg/day (GGE-PS+), 800 ± 300 mg/day (GGE-PS-), and 800 ± 200 mg/day (FE), resulting in no significant difference (one-way ANOVA, $p > 0.1$).
Subjects' neurologic, and ophthalmologic examinations were normal. All subjects were right-handed based on the Edinburgh Handedness Inventory.
The human ethics committee of the University of Modena and Reggio Emilia approved this study. Written informed consent was obtained from all the 60 subjects, and by their parents if underage.

EEG RECORDINGS

Scalp EEG was recorded by means of a 32-channel MRI-compatible EEG recording system (Micromed, Mogliano Veneto, Italy). Electrodes were placed according to conventional 10–20 locations. FCz was the reference. ECG was recorded from 2 chest electrodes. Electrode impedance was kept below 10 kOhms. Prior to in-magnet EEG recording, 10 min of out-of magnet EEG data were collected in a room adjacent to the scanner. Foam pads were used to help secure the EEG leads, minimize motion, and improve patient comfort. Data were transmitted via an optic fiber cable from the high-input impedance amplifier (5 kHz sampling rate) to a computer located outside the scanner room. To avoid saturation, the EEG amplifier had a resolution of 22 bits with a range of ±25.6 mV. An anti-aliasing hardware band-pass filter was applied with a bandwidth between 0.15–269.5 Hz.
Subjects' behavior was constantly observed and recorded by means of a small camcorder positioned on the head coil inside the scanner pointing to the patient's face to obtain split-screen video-EEG documentation during the fMRI recording (Ruggieri et al, 2015).
The subjects were asked to rest with eyes closed, to not sleep, and to keep still during fMRI acquisitions. All recordings were performer in the early afternoon in a dimly-illuminated room with a constant luminance (=ca 25 Lux). Sedation was never used.

fMRI DATA ACQUISITION

Functional data were acquired using a Philips Intera system at 3T and a gradient-echo echo-planar sequence from 30 axial contiguous slices (TR=3000 ms; in-plane matrix= 64x64; voxel size: 4x4x4 mm) over one 10-min session per patient (200 images). A high-resolution T1-weighted anatomical image was acquired for each patient to allow anatomical localization. The

volume consisted of 170 sagittal slices (TR= 9.9 ms; TE= 4.6 ms; in plane matrix= 256x256; voxel size=1x1x1 mm).

EEG ARTIFACTS CORRECTION AND PROCESSING

The correction of the gradient artifact was performed offline by means of the Brain Quick System Plus software (Micromed, Mogliano Veneto, Italy) (Allen et al, 2000). The EEG data were then exported in the .edf format and reviewed and analyzed by means of the BrainVision Analyzer 2.0 software (Brain Products, Munich, Germany). After down-sampling to 250 Hz, a band-pass filter between 1 and 70 Hz was applied to the continuous recording and channels showing high impedance or electrode displacement artifacts were interpolated through a cubic spline. Pulse related artifacts were removed offline from the EEG trace recorded during scanning using the EEG processing package of Brain Analyzer (Brain Products, Munich, Germany) (Allen et al, 1998).
The pre-processed EEG data were then submitted to an Independent Component Analysis (ICA) as previously described (Ruggieri et al, 2015; Avanzini et al, 2014).
For each participant, the 30 EEG channels signal was decomposed into 30 components (between F0 to F29). Each component resulting from ICA separation is characterized by a time course, describing the morphology of the component over time, and by a specific topography (i.e. an array containing the weights the specific component has on each channel). In patient populations, two expert epileptologists (A.E.V., A.R.) reviewed both the standard EEG recordings and the relative individual components of the ICA-processed recordings to detect interictal epileptic discharges (IED). The independent components that on visual inspection showed IEDs were marked as epi-IC and discarded from subsequent alpha frequency and power estimations. Moreover, to avoid interaction of interictal events or remaining small motion artifacts with the alpha power variation analysis, we replaced the EEG during these events with a signal obtained by interpolating the EEG before and after the event, as previously applied (Tyvaert et al, 2008). For each subject no more than 5% of the EEG was subjected to interpolation.

ESTIMATION OF THE ALPHA POWER AND VALIDATION

The **Supplementary Figure 1** illustrates the main steps of individual alpha band calculation. The independent components that on visual inspection showed rhythmic activity in the 8 – 12 Hz band were marked as alpha-related IC (αIC). For all αIC in each subject, artifact-free time course was divided into 3s epochs (= to repetition time) using a Hanning time window (epochs were overlapped by 50%) and submitted to Fast Fourier Transform (FFT). The relative power was computed between 7.5 and 12.5 Hz with a frequency resolution equal to 0.1 Hz. The FFT revealed a number of αIC ranging from a minimum of 2 to a maximum of 20 components for each subject. Then, one posterior alpha component originating from the early visual cortex was selected based on the following criteria: (1) a peak in the alpha range as revealed by FFT and (2) a medial-posterior topography of the mixing weights, which expresses the relative strength at which each component time course is expressed at each electrode. This component is reliably observed when ICA is applied to EEG data (Makeig et al, 2004a, 2004b). As a next step, for each subject the mean individual alpha frequency (Hz) and its respective power (uV^2/Hz) were calculated for the selected component. We followed this ICA procedure, as in previous EEG-fMRI studies (Feige et al., 2005; Sheeringa et al., 2010, 2012) to ensure that the EEG-based regressors described an EEG activity as much as possible related to the occipital generators of the alpha rhythm, making them free from volume-conduction and without a-priori choices that could have weakened/blurred their time course.
Finally, after the ICA component selections, the spectral power of the alpha component of the given subject was integrated across each epoch and used as “alpha” regressor for the same

subject's fMRI time series. The regressor therefore quantifies the alpha-band power of the medial-posterior alpha component within each scan interval.
To ensure the validity of the EEG data and analysis obtained during the functional image acquisition, the same EEG pre-processing and analysis was performed for the 10 minutes of resting-state acquired prior to the fMRI protocol in each subject. As a next step, we compared the mean alpha frequency and the mean alpha power of the EEG outside the scanner with those obtained during the scan time.

fMRI ANALYSIS: ALPHA-BOLD CORRELATION

Matlab 7.1 and SPM8 (Welcome Department of Imaging Neuroscience, London, United Kingdom) software were used for fMRI data analysis. All functional volumes were slice-time corrected, realigned to the first volume acquired, spatially normalized into standard space and smoothed with 8 X 8 X 8 mm full-width half maximum (FWHM) Gaussian Kernel.
First-level analysis: The alpha power variations were convolved with the standard hemodynamic response function (HRF), down-sampled to the MRI frequency and used as a regressor in a single general linear model (GLM). Movement artifacts identified by analysis of Video-EEG recordings (blinking, lip smacking, swallowing, head movements) were considered as confounds in the model (Ruggieri et al, 2015). In addition, 24 realignment parameters [six scan realignments parameters from image pre-processing and a Volterra expansion of these (Friston, et al, 1996)] were included in the model as confounds. A t-contrast was specified, testing for the column "alpha regressor".
Second-level group analysis: the statistical images resulting from single-subject contrasts were submitted to a second level (group) random-effect analysis to look for effect on the BOLD signal at the population level. A full factorial design, as implemented in SPM8, was used to test for between groups' effects (one-way ANOVA; four groups): GGE *PS+*; GGE *PS-*; FE and healthy controls. Subjects' age and sex were included in the model as covariates.
The statistical inferences for first- and second-level analyses were set to the threshold of $p<0.05$, corrected for family wise errors (FWE) in order to show significant BOLD changes at whole-brain. If there were no cluster surviving at this level, the threshold was changed to $p<0.05$ corrected for False Discovery Rate (FDR). Controlling the FDR with the criterion FDR=0.05 increases the number of false positives relative to FWE rates techniques, but also increases the ability to detect meaningful signal (Bennett et al., 2009; Genovese et al., 2002; Nichols and Hayasaka, 2003).
The resulting statistical maps have been warped to the PALS-B12 atlas in Caret for visualization purposes (Caret, http://brainvis.wustl.edu/wiki/index.php/Caret:About) (van Essen, 2005).

fMRI ANALYSIS: RESTING-STATE FUNCTIONAL CONNECTIVITY

Considering previous studies (Liu et al, 2012) and the group-level results of the current study (see below), two thalamic regions-of-interest (ROIs) were defined for further functional connectivity analysis. The BOLD signal time course was extracted from each ROI (5 mm-sphere) for each subject by means of marsbar software (http://marsbar.sourceforge.net/). The first ROI consisted of the bilateral posterior clusters showing negative correlation with the posterior alpha (i.e. the pulvinar, PUL); the second ROI was the bilateral medial dorsal region showing significantly positive correlation with the posterior alpha modulation (i.e. medial dorsal nuclei, MDN). Both ROIs served separately as the seed from which the functional connectivity to the rest of the brain was evaluated for all the subjects considered together and for each group independently. The nuisance variables consisting of 24 realignment parameters and three compartment signals (modeling the average signal in the grey matter, white matter,

cerebrospinal fluid) were included in the model. Then, functional connectivity maps of controls and epilepsy subpopulations were evaluated and compared.

RESULTS

ESTIMATION OF THE ALPHA POWER

All the subjects demonstrated a component with a medial posterior topography and a single peak in the alpha frequency range, each within 8-12 Hz, the typical limit for this age group as reported in the literature (Nunez et al, 2001) (**Fig. 1, Panel A**). The individual alpha frequency and its respective power were averaged across each sub-population and compared (ANOVA). Across all the investigated subjects, the individual peak alpha frequency showed a normal distribution within the alpha band, both outside and inside the scanner (**Fig. 1, Panel B**). No group differences were evident when comparing the mean alpha frequency between the different populations (**Fig.1, Panel C**). On the contrary, the mean alpha power was higher in the *GGE PS+* population compared to controls and other epilepsies ($p<0.01$ for all comparison) (**Fig. 1, Panel D**). The EEG inside and outside the scanner appeared similar on visual inspection in all the subjects. Nevertheless, the alpha power recorded during fMRI was reduced in all the investigated populations with an average power reduction of 30%. The mean power reduction was slightly different across the four investigated cohorts, but there was no effect of group on power reduction. This finding is consistent with previous published data (Laufs et al, 2003) and is probably linked to the EEG preprocessing, especially the gradient artifact suppression algorithm. No difference for the mean alpha frequency peak was observed comparing the EEG inside versus outside the scanner.

ALPHA-BOLD CORRELATION: WHOLE POPULATION RESULTS

When considering all the subjects as a unique population (N=60), the alpha power time series correlated positively with brain activity in the bilateral dorsal cingulate cortex and medial thalamic nuclei and negatively with activity in broad areas of cerebral cortex including the superior and inferior parietal lobule, the occipital cortex (middle occipital gyrus and lingual gyrus), the precuneus, the premotor and motor regions (supplementary motor area, frontal operculum, precentral cortex), the middle and superior temporal gyrus. At subcortical level, the bilateral posterior thalamus, the basal ganglia and brainstem were negatively correlated with the posterior alpha power (**Fig. 2, Panel A**). These findings are concordant with those of previous reports (Goldman et al, 2002; Moosman et al, 2003; Goncalves et al, 2006; DiFrancesco et al, 2008; Tyvaert et al, 2008; Liu et al, 2012; Omata et al, 2013). The **Supplementary Table 1** shows the details of the positively and negatively correlated areas.

In addition, we used the digital version of the Morel Atlas of human thalamus (Morel et al, 1997) based on the MNI template (Krauth et al, 2010) to further relate EEG-fMRI findings to specific thalamic nuclei (**Fig. 2, Panel B**). The subcortical thalamic negative correlate of the alpha rhythm was clearly localized at the pulvinar level without involving the lateral geniculate nucleus.

ALPHA-BOLD CORRELATION: SUBPOPULATION FINDINGS AND COMPARISONS

The brain regions that correlated with alpha power in each single sub-population are illustrated in the **Fig. 3** and summarized in the **Supplementary Table 2**.

GGE PS+ group shown positive BOLD correlation at the bilateral dorsal cingulate cortex and caudate nucleus. A negative correlation between alpha predictor and BOLD signal was found predominantly in the parietal and frontal (motor-premotor) cortical areas. Conversely, the posterior occipital regions [Brodmann Area (BA) 18-19] demonstrated a decrease alpha power-related BOLD signal in all the other epileptic populations (*GGE PS-* and *FE*) as well as in the

healthy controls. Parietal, temporal and frontal (motor-premotor) cortical areas were negatively correlated to the alpha in all these three subgroups with substantial uniformity between them.
The comparisons of alpha-power BOLD maps between the patients' groups and between patients and controls revealed significant differences only in the *GGE PS+* population. Specifically, with respect to all other comparisons, *GGE PS+* showed a relative increased BOLD signal in a broad and symmetrical network that includes the pre-post central gyrus, the supplementary motor area, the insula, the precuneus and the temporal (BA 20-22) and occipital cortex (BA 18-19) (**Fig. 4**). This result is the consequence of a decreased anti-correlation between BOLD signal and the alpha power in patients with photosensitivity. No other differences were observed at the considered statistical threshold either in terms of increased or reduced BOLD correlation to the alpha power. **Table 2** illustrates in details the results of the between-groups comparisons.

FUNCTIONAL CONNECTIVITY MAPS

At the population level (N=60) the maps for the functional connectivity with the seeds in the *MDN* and *PUL* are depicted in the **Supplementary Figure 2**.
Statistical comparison of the *MDN*-related functional connectivity maps between controls and epilepsy sub-populations revealed an increased correlation between resting state BOLD activity in the MDN and bilateral orbitofrontal cortex (OFC) in GGE PS+ compared to both healthy controls and focal epilepsy patients (**Fig. 5**). No further differences in functional connectivity maps were detected.
The formal comparison of the *PUL*-related functional connectivity maps between the epilepsy populations and between patients and controls, revealed again significant differences only for the *GGE PS+* subgroup. *GGE PS+* demonstrated consistent increased correlation from the *PUL* to the basal ganglia (putamen and caudate), the anterior cingulate cortex, the dorsolateral prefrontal and the parietal cortex with respect to all the other subgroups (*GGE PS-*, *FE* and *CRT*). A significant decreased connectivity between the *PUL* and the somatosensory and visual cortex was observed in the *GGE PS+* versus healthy subjects at the considered threshold (**Fig. 6**).

DISCUSSION

This is the first EEG-fMRI investigation of the resting-state alpha rhythm in patients with non-lesional focal and genetic generalized epilepsy, with and without photosensitivity. As predicted, our findings indicate that the cortical-subcortical network generating the alpha oscillation at rest is different in people with epilepsy and visual sensitivity. Such difference is independent from the occurrence of overt epileptic activity on scalp EEG and is characterized by: (1) an abnormal hemodynamic coupling between the EEG alpha power fluctuation and the BOLD signal; and (2) an altered functional connectivity between the pulvinar, which is highly implicated in the alpha rhythm generation, and the rest of the brain. The main finding of the study indicates a reduced alpha-related inhibition in photosensitive epilepsy (i.e. lower BOLD deactivation when alpha power increases) that involves not only the occipital cortex but rather extending also to the supplementary motor area, the sensorimotor and the premotor cortex. Coherently, the same brain regions demonstrated an abnormal connectivity with the visual thalamus only in *GGE PS+*.

THE ALPHA RHYTHM IN PHOTOSENSITIVE EPILEPSY

The EEG analysis of the alpha rhythm features showed a significant higher alpha power in *GGE PS+* versus other epilepsy groups and controls, hence confirming a peculiar "alpha phenotype" of this epileptic trait, at least for the investigated group of photosensitive patients.

Changes of the alpha rhythm features have been associated with epilepsy in several neurophysiological studies (Stoller 1949; Larsson et al., 2005, 2012; Clemens et al., 2007, 2008; Pyrzowski et al., 2015). The majority of the studies reported a peak alpha frequency reduction in a heterogeneous population of mixed epilepsy phenotypes, rather than changes in alpha power (Miyauchi et al., 1991; Larsson et al., 2005; Clemens et al., 2007; Visani et al., 2010). However, very few studies investigated the alpha rhythm features in selected patients with photosensitive epilepsy and compared it with other epilepsy syndromes without photosensitivity (Visani et al., 2010). It should also be underscored that our EEG analysis is based on an Independent Component Analysis of the EEG signal and that the 'alpha regressor' and all alpha calculations were based on the selection of the 'posterior alpha component' on ICA EEG decomposition. This means that the reported alpha features index the oscillatory activity of EEG signal generated from the posterior occipital (calcarine/pericalcarine) cortex. To our knowledge no other study has used this approach to investigate alpha rhythm fluctuation in epilepsy. Considering that this methodology is quite simple and reproducible, and that it could be applied also to routine EEG recordings, it should be relatively straightforward for future studies to test our findings on a larger cohort of photosensitive patients. This also implies that at present it is difficult to directly compare our results with previous studies.
Remarkably, the majority of the investigated patients were under antiepileptic drug (AED) treatment at the time of the study. In previous studies, changes in the intrinsic brain rhythms power have been attributed to AED, especially to valproate (Larsson et al., 2005, 2012; Clemens et al., 2007). However, in the current research, an AED effect on alpha power estimation appears unlikely, as valproate and levetiracetam, the most common drugs used in our patients with genetic generalized epilepsy, were homogenously distributed across *PS+* and *PS-* patients. In particular, the average oral dose of valproic acid across the different patients' groups was similar.

REDUCED INHIBITION IN THE SENSORY-MOTOR SYSTEM IN PHOTOSENSITIVITY

BOLD correlates of the posterior alpha activity under rest indicated a reduced anti-correlation between the sensory-motor system and the alpha power in patients with photosensitivity (Fig. 4). Classically, BOLD-fMRI studies reported predominantly negative hemodynamic responses to the alpha oscillations in the occipital lobe (primary and secondary visual cortex) and fronto-parietal cortex (Laufs et al., 2003; Goncalves et al., 2006; DiFrancesco et al., 2008). Overall, negative correlation between the alpha power and the BOLD signal coincides with a decrease of cortical neuronal activity, i.e. a state of inhibition. Indeed, during large-scale synchrony as in alpha rhythm, activity in just a small fraction of neurons within a cortical column may be sufficient to give rise to a strong EEG signal, while the inactive majority maintains overall metabolism low and thus the BOLD effect small (Laufs et al., 2003). In this light, the higher hemodynamic signal in the sensory-motor system in *GGE PS+* during spontaneous alpha, reflects an increased neuronal activity and consequently, we suggest, a lack of inhibition of these regions. This view support previous observations in photosensitivity by means of neurophysiological and neuroimaging studies, as well as the clinical correlates of PPR that often consist of motor phenomena (i.e. myoclonus). Studies using transcranial magnetic stimulation documented increased visuo-motor hyperexcitability during photic stimulation in GGE patients (Strigaro et al, 2015, 2013; Groppa et al, 2008) and even in healthy individuals with PPR (Siniatchkin et al, 2007). Advanced EEG connectivity analyses show an abnormal pattern, with increased coupling between posterior and anterior frontal areas both at rest and during PPR in patients with *GGE PS+* (Varotto et al, 2012; Moeller et al, 2013). Finally, functional EEG-fMRI studies have detected increased neuronal activity of parietal and premotor cortices during paroxysmal activity evoked by visual stimuli (Moeller et al, 2009; Bartolini et al, 2014).

The brain nodes that demonstrated abnormal behaviour in *GGE PS+* under spontaneous alpha oscillations included, beyond the sensory-motor cortex in strictu sensu, the premotor regions (supplementary motor area, middle and inferior frontal gyrus) and also non-motor areas in the temporal, parietal and occipital lobes. Notably, the same premotor areas were involved in the abnormal response to eye-closure in patients with EMA (Vaudano et al, 2014), even in the absence of paroxysmal activity on EEG. Overall, our findings confirm and complete the hypothesis of an intrinsic "visuo-motor hyperexcitability" in *GGE PS+*. The fact that this up-regulation is present under resting (unstimulated) conditions and even during the alpha rhythm, which is considered a hallmark of the brain resting state (Goncalves et al, 2006), indicates probably a genetic predisposition to generate synchronous paroxysmal activity.

THALAMIC CONTRIBUTION TO ALPHA RHYTHM GENERATION

The correlation between the posterior alpha modulation and the BOLD signal in the thalamus has been reported to be positive (de Munk et al., 2007; Fiege et al., 2005; Goldman et al., 2002; Moosmann et al., 2003; Goncalves et al., 2006; Omata et al., 2013), negative (Lindgren et al., 1999; Moosmann et al., 2003), or even near zero (Laufs et al., 2003). Recently, Liu and colleagues (2012) described both positive and negative correlations between the thalamic nuclei and spontaneous modulation of posterior alpha. Our study is the first that replicates and thus confirms that different thalamic nuclei have a different behaviour during alpha rhythm fluctuations. In particular, the negative correlation covers the posterior visual thalamus while positive hemodynamic changes were constrained to the medial dorsal nuclei of the thalamus. The exact contribution of thalamic activity to the cortical oscillations that determine the EEG alpha rhythm is still under investigation. A plausible scheme includes a complex interplay between the primary visual cortex and the visual part (pulvinar and lateral geniculate nucleus, LGN) and reticular nuclei of the thalamus (Lopes da Silva et al., 1973, 1980; Fuentealba and Steriande 2005; Lorincz et al., 2009). Within the posterior thalamic nuclei, the pulvinar, rather than lateral geniculate nucleus, is more likely associated with the spontaneous modulation of posterior alpha rhythm. Animals showing alpha equivalent activity, such as dogs and cats, all have a pulvinar in their visual system, whereas the pulvinar does not seems to exist in brains, such as those of rodents, whose visual system also shows little alpha activity (Highes and Crunelli, 2005; Pessoa and Adolph, 2010). In addition the extensive connections of the pulvinar with the entire cortex (see **supplementary Figure 2**, and Kaas and Lyon, 2007), make this thalamic nucleus well suitable to behave as the subcortical control hub for the widespread cortical alpha activity. Consistently, we also found the involvement of the posterior thalamus with preference for the pulvinar over the lateral geniculate nucleus. Furthermore, the cortical regions that were functionally connected to the seed in the pulvinar largely coincided with the cortical regions that were negatively correlated with the posterior alpha rhythm (**Figure 2**). As far as the medial dorsal nuclei, the increased neuronal activity during increased alpha power probably is not directly linked to alpha waves generation, but rather reflects activity related to the arousal level, which is indirectly linked to cortical alpha rhythm fluctuations (Liu et al., 2012; Omata et al., 2013). Indeed, these thalamic nuclei are considered to be part of the ascending reticular activating system, and in particular its dorsal pathways (Moruzzi and Magoun, 1949; Brown et al, 2012).

THALAMO-CORTICAL FUNCTIONAL CONNECTIVITY IN PHOTOSENSITIVE EPILEPSY

Given the above reported assumptions in normal subjects and moving to the main objective of our work, the question is if the thalamic contribution to the alpha power is different in *GGE PS+*. The whole-brain functional connectivity of the posterior and medial thalamic nuclei was different in photosensitive patients with respect to controls and other epilepsy groups. Notably, it has been recently demonstrated in healthy subjects (Sheeringa et al., 2012)

that the increased local alpha synchronization originating from the early visual cortex is associated with decreased fMRI resting-state connectivity within the visual system. The fact that alpha-band neuronal synchronization is inversely related to connectivity between the primary visual cortex and closely connected regions in both the dorsal and ventral visual stream regions suggests that local alpha-band synchronization in healthy people serves to *reduce* the communication between closely connected regions.

Conversely, *GGE PS+* presented an increased resting connectivity of the medial dorsal thalamus-orbitofrontal cortex circuit (**Fig. 5**). The orbitofrontal cortex (OFC) is a functionally complex structure, which is implicated in high-level cognition processes as well as mediating aspects of emotions and behaviour. Different functions have been linked to OFC activity, including executive control, decision-making, and top-down modulation of bottom-up processes (for review see Elliott et al., 2000; Damasio, 1995). This brain region, and particular the lateral OFC portion (BA11, BA47), has strong connection with the amygdala and insula, structures that are involved in emotional experience and expression (Augustine, 1996; Fink et al, 1996). The fact that this thalamic-prefrontal network is hyper-coupled during rest in patients with photosensitivity might reflect a disruption of cognitive and emotional processes linked to these physiological functions. Notably, JME, in which photosensitivity is commonly observed, has been correlated with psychiatric, behavioral and cognitive deficits, including those of decision-making and executive control (for review see Wolf et al, 2015). This conclusion remains speculative and further data linking the functional connectivity pattern and neuropsychological and psychiatric profile *in GGE PS+* needs to be obtained.

When the seed was placed in the pulvinar, *GGE PS+* patients demonstrated again a different pattern of cortical resting-state connectivity compared to the other populations. Increased connectivity was constrained to the prefrontal cortex (anterior cingulate, dorsolateral prefrontal cortex) and basal ganglia. This visual thalamus-basal ganglia hyper-connectivity could be a promoter of motor cortex hyper-excitability representing a biomarker of increased visuo-motor outflow, which could facilitate myoclonus triggered by visual stimuli. On the contrary, the visual thalamus showed a reduced functional connectivity with the sensory and visual cortex.

The disrupted connectivity between the pulvinar and the visuo-parietal/motor-premotor system further supports the view that photosensitivity in epilepsy is due to abnormal activity of the occipito-frontal circuits, that persists even during rest and independently of visual stimulation and/or pathological paroxysmal activity. Notably, we recently demonstrated by voxel-based morphometry in patients with EMA that the pulvinar has also an abnormal grey matter concentration in these patients, suggesting that microstructural alterations can act over the same pulvinar-fronto-occipital circuit (Vaudano et al, 2014).

METHODOLOGICAL CONSIDERATION

In the present work, the alpha-related fMRI data analyses have been performed by means of a parametric approach within the SPM package, using a statistical threshold of $p < 0.05$ corrected for FWE or FDR. Recent reports have reported a high risk of inflated false positive rate in fMRI group analyses using parametric methods with common fMRI software tools (i.e. SPM, FSL), even when applying a conservative FWE statistical threshold control (Eklund et al., 2012, 2016). These arguments speak to the need of using nonparametric permutation approaches to controls properly the rate of false positive BOLD changes (Eklund et al., 2016).

As far as our findings, the reproducibility of the fMRI results across different analyses strengthens our main conclusion about alpha generators in GGE-PS+ population. Nevertheless, given the high concerns risen by these recent studies, we have re-analyzed the second-level alpha-related fMRI data and the groups' comparisons by means of non-parametric permutation method for multiple-comparisons correction (AFNI, 3dClustSim

function; http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html) to achieve a family-wise error rate (FWER) at p=0.05 at the cluster level. We applied the updated version (May, 2015) of the 3dClustSim function (Cox et al., 2016). This program takes into account the smoothness of the residual dataset and whole-brain mask as inputs, and creates Monte-Carlo simulations (10,000) of noise-datasets with the specified smoothness. It then creates a frequency distribution of noise-cluster sizes and advises the *p* value and cluster extent required to control the FWER at a chosen level. Group-level analyses were thresholded at the voxel-level at $p< 0.001$ and cluster size of 210, which provides $p < 0.05$ (corrected).
Second-level whole-population analysis (N=60), as well as sub-population groups findings did not demonstrated any difference between the applied statistical methods (parametric versus non-parametric). In particular, we did not found clusters mismatches between the two approaches. The groups' comparison analyses results shown instead a few differences in term of total number of clusters identified and relative Z-score. Nevertheless, although a few clusters disappeared, the main BOLD changes survived. In particular, the comparison between GGE-PS+ and the other cohorts confirmed, in the former, an increased excitability under spontaneous alpha oscillations over the premotor-motor and parieto-occipital cortices (**Supplementary Table 3** and **Supplementary Figure 3**). Thus, this additional statistical analysis do not change the principal findings of our parametric SPM approach and hence the main conclusions of the present work, i.e. that people with epilepsy and visual sensitivity, compared to other epileptic syndromes and healthy controls, present a decreased alpha-related inhibition of the sensory-motor and visual networks at rest.

STUDY LIMITATION

As a limit of the present work, we did not explore the hemodynamic correlates of other endogenous EEG frequency bands (i.e. delta, gamma or theta). This additional analyses would be interesting, especially in relation to gamma band activity for the well-known influence of this frequency band in photosensitivity. However, because the data presented here are the first to explore the relationships between spontaneous brain rhythmicity and epilepsy in term of generating networks, we decide to focus to the alpha rhythm, as first step. A cautionary note concerns the generalization of the observed alpha rhythm findings. Indeed, it should be noted that about 70% of the *GGE PS+* was constituted by subjects affected by Jeavons syndrome (EMA). This means that our EEG (and BOLD) findings could be strongly driven by this subpopulation of photosensitive patients. This is relevant also in consideration of the recent genetic findings of a high incidence of *CHD2* mutations in EMA [Galizia et al., 2015]. The influence of sub-syndromes' effects (i.e. JME versus EMA), as well as the effects of specific genetic defects should be tested in future studies. Finally, although we clearly demonstrated that there is a correlation between alpha rhythm and the BOLD signal (and that this relationship is different in different epilepsy populations), our results do not imply direct causality in either direction.

CONCLUSIONS

The findings of our works provide for the first time, the existence of a functional link between the circuits demonstrated to trigger the PPR phenomenon and the ones implicated in the generation of the posterior alpha rhythm. As a general conclusion, we suggest that the "enduring propensity" to generate seizures and/or epileptic activity in patients with photosensitivity is due to the intrinsic susceptibility of a complex thalamo-cortical system, which is indexed by the resting EEG alpha oscillations. One important point of strength of the present work is that a multimodal approach of investigating the characteristics of alpha power and the intrinsic networks generating it, results in a concordant picture. Our results represent

an additional important piece of evidence regarding photosensitivity and contribute to expand the "system epilepsy" concept (Avanzini et al., 2012) for this epileptic trait.

Acknowledgements

We thank Francesca Benuzzi for helping with EEG-fMRI preparation and scanning.

Funding

S. Meletti received Research grant support from the Italian Ministry of Health (MOH), from the non-profit organization CarisMO Foundation; A.E. Vaudano received Research Grant support from the non-profit "Fondazione Epilessia LICE Onlus" and Italian Ministry of Health (MOH), Emilia-Romagna Region (N. PRUA1GR-2013-00000120). A. Ruggieri received a PhD bursary from the University of Modena and Reggio Emilia

Supplementary materials

Supplementary Figure 1: Single-subject main steps of individual alpha band calculation. (A) Representative image of EEG trace recorded inside the scanner after the correction of the gradient and cardiac artifact. The EEG trace is shown in double banana montage. (B) ICA analysis decomposition related to the same EEG page shown in (A). The 30 EEG channels (the ECG and EMG channels are not considered) are decomposed in 30 components (between F0 to F29). Within them, the components F07, F09, F11 and F13 (underlined in blue), were visually identified as the alpha activity of the subject and marked as alpha-related IC (αIC). (C) FFT analyses of all the 30 components. The red dotted lines identify the power spectrum with a clear peak in the alpha band range that in this case corresponds to the IC F07, F09, F13. (D) 3D brain, spatial topography of the posterior central component selected for subsequent fMRI analysis (in this case F13).

Supplementary Figure 2: Functional connectivity maps with seed regions at medio-dorsal thalamic nuclei (MDN) (A) and Pulvinar (PUL) (B) ($p < 0.05$ corrected for FWE). The functional maps are overlaid onto the canonical T1 image as implemented in SPM, axial slices. Correlations with the MDN seed are observed at the anterior and posterior cingulate cortex, the brainstem, the cerebellum, the supplementary motor area, and the limbic mesial structures (insula, hippocampus and parahippocampal gyrus). The cortical regions that were functionally connected to the PUL seed included the brainstem, the striate and peristriate cortex, the regions belonging to the sensory-motor and default-mode networks, the anterior cingulate cortex, the hippocampus, the amygdala and parahippocampal structures, and the cerebellum.

Supplementary Figure 3. Comparisons of alpha-power BOLD maps between the patients' groups and between patients and controls obtained using non-parametric permutation method (AFNI's 3dClustSim function). Only regions survived to statistical threshold of $\alpha < 0.05$ (voxel-wise $p < 0.001$ and cluster size ≥ 210 voxels) are showed. The functional maps are shown on the normalized SPM-glass brain (left images) and warped to the PALS-B12 atlas in caret (mesial and dorsal view) for right (R) and left (L) hemisphere (right images). The red arrow on the SPM-glass brain indicates the global maximum. The white lines on the PALS-B12 atlas show the surface landmarks as implemented in Caret: the Central Sulcus, the medial wall dorsal segment, and the medial wall ventral segment, the sylvian fissure and calcarine sulcus. See text for details.

REFERENCES

Allen PJ, Polizzi G, Krakow K, Fish DR, Lemieux L. Identification of EEG events in the MR scanner: the problem of pulse artifact and a method for its subtraction. Neuroimage. 1998;8:229-39.
Allen PJ, Josephs O, Turner R. A method for removing imaging artifact from continuous EEG recorded during functional MRI. Neuroimage. 2000;12:230-9.
Appleton R, Beirne M, Acomb B. Photosensitivity in juvenile myoclonic epilepsy. Seizure. 2000;9:108–11.
Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans.
Brain Res Brain Res Rev. 1996;22(3):229-44.
Avanzini P, Vaudano AE, Vignoli A, Ruggieri A, Benuzzi F, Darra F, *et al*. Low frequency mu-like activity characterizes cortical rhythms in epilepsy due to ring chromosome 20. Clin Neurophysiol. 2014;125:239-49.
Avanzini G, Manganotti P, Meletti S, Moshé SL, Panzica F, Wolf P, *et al*. The system epilepsies: a pathophysiological hypothesis. Epilepsia. 2012;53(5):771-8.
Bartolini E, Pesaresi I, Fabbri S, Cecchi P, Giorgi FS, Sartucci F, *et al*. Abnormal response to photic stimulation in juvenile myoclonic epilepsy: an EEG-fMRI study. Epilepsia. 2014;55(7):1038-47.
Bennett CM, Wolford GL, Miller MB. The principled control of false positives in neuroimaging Soc Cogn Affect Neurosci. 2009;4(4):417-22.
Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, *et al*. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia. 2010;51:676-85.
Berger, H. U¨ ber das Elektrenkephalogramm des Menschen. Arch. Psychiatr. 1929;87:527–70.
Brigo F, Bongiovanni LG, Nardone R, Trinka E, Tezzon F, Fiaschi A, *et al*. Visual cortex hyperexcitability in idiopathic generalized epilepsies with photosensitivity: a TMS pilot study. Epilepsy Behav. 2013;27(2):301-6.
Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW. Control of sleep and wakefulness. Physiol Rev. 2012;92(3):1087-187.
Chatila M, Milleret C, Rougeul A, Buser P. Alpha rhythm in the cat thalamus. C. R. Acad. Sci. III 1993;316:51-8.
Clemens B, Bessenyei M, Piros P, Tóth M, Seress L, Kondákor I. Characteristic distribution of interictal brain electrical activity in idiopathic generalized epilepsy. Epilepsia. 2007;48(5):941-9.
Clemens B. Valproate decreases EEG synchronization in a use-dependent manner in idiopathic generalized epilepsy. Seizure. 2008;17(3):224-33.
Cox RW, Reynolds RC, Taylor PA. AFNI and Clustering: False Positive Rates Redux BioRxiv. 216;26:1-15.
Damasio AR. On some functions of the human prefrontal cortex. Ann N Y Acad Sci. 1995;769:241-51.
de Munck JC, Goncalves SI, Huijboom L, Kuijer JP, Pouwels PJ, Heethaar RM, *et al*. The hemodynamic response of the alpha rhythm: an EEG/fMRI study. NeuroImage. 2007;35:1142–51.
Difrancesco MW, Holland SK, Szaflarski JP. Simultaneous EEG/functional magnetic resonance imaging at 4 Tesla: correlates of brain activity to spontaneous alpha rhythm during relaxation. J Clin Neurophysiol. 2008;25(5):255-64.
Elliott R, Dolan RJ, Frith CD. Dissociable functions in the medial and lateral orbitofrontal cortex: evidence from human neuroimaging studies. Cereb Cortex. 2000;10(3):308-17.

Eklund A, Andersson M, Josephson C, Johannesson M, Knutsson H. Does parametric fMRI analysis with SPM yield valid results? An empirical study of 1484 rest datasets. Neuroimage. 2012;61(3):565-78.
Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A. 2016;113(28):7900-5.
Feige B, Scheffler K, Esposito F, Di Salle F, Hennig J, Seifritz E. Cortical and subcortical correlates of electroencephalographic alpha rhythm modulation. J. Neurophysiol. 2005;93:2864-72.
Fink GR, Markowitsch HJ, Reinkemeier M, Bruckbauer T, Kessler J, Heiss WD. Cerebral representation of one's own past: neural networks involved in autobiographical memory. J Neurosci. 1996;16(13):4275-82.
Friston KJ, Williams S, Howard R, Frackowiak RS, Turner R. Movement-related effects in fMRI time-series. Magn Reson Med. 1996;35:346-55.
Fuentealba P, Steriade M. The reticular nucleus revisited: intrinsic and network properties of a thalamic pacemaker. Prog Neurobiol. 2005;75(2):125-41.
Galizia EC, Myers CT, Leu C, de Kovel CG, Afrikanova T, Cordero-Maldonado ML, *et al*. CHD2 variants are a risk factor for photosensitivity in epilepsy. Brain. 2015;138:1198-207.
Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 2002;15(4):870-8.
Goldman RI, Stern JM, Engel J Jr, Cohen MS. Simultaneous EEG and fMRI of the alpha rhythm. Neuroreport. 2002;13:2487-92.
Gonçalves SI, de Munck JC, Pouwels PJ, Schoonhoven R, Kuijer JP, Maurits NM, *et al*. Correlating the alpha rhythm to BOLD using simultaneous EEG/fMRI: inter-subject variability. Neuroimage. 2006;30(1):203-13.
Gregory RP, Oates T, Merry RT. Electroencephalogram epileptiform abnormalities in candidates for aircrew training. Electroencephalogr Clin Neurophysiol. 1993;86:75-7.
Groppa S, Siebner HR, Kurth C, Stephani U, Siniatchkin M. Abnormal response of motor cortex to photic stimulation in idiopathic generalized epilepsy. Epilepsia. 2008;49(12):2022-9.
Guerrini R, Dravet C, Genton P, Bureau M, Bonanni P, Ferrari AR, *et al*. Idiopathic photosensitive occipital lobe epilepsy. Epilepsia.1995;36(9):883-91.
Henssen A, Zilles K, Palomero-Gallagher N, Schleicher A, Mohlberg H, Gerboga F, *et al*. Cytoarchitecture and probability maps of the human medial orbitofrontal cortex. Cortex. 2016;75:87-112.
Hughes SW, Crunelli V. Thalamic mechanisms of EEG alpha rhythms and their pathological implications. Neuroscientist. 2005;11,357-72.
Kaas JH, Lyon DC. Pulvinar contributions to the dorsal and ventral streams of visual processing in primates. Brain Res Rev. 2007;55:285-96.
Kasteleijn-Nolst Trenité DG, Binnie CD, Harding GF, Wilkins A. Photic stimulation: standardization of screening methods. Epilepsia. 1999;40:75-9.
Kasteleijn-Nolst Trenité DG, Guerrini R, Binnie CD, Genton P. Visual sensitivity and epilepsy: a proposed terminology and classification for clinical and EEG phenomenology. Epilepsia. 2001;42:692-701.
Klimesch W, Sauseng P, Hanslmayr S. EEG alpha oscillations: the inhibition-timing hypothesis. Brain Res Rev. 2007;53(1):63-88.
Krauth A, Blanc R, Poveda A, Jeanmonod D, Morel A, Székely G. A mean three-dimensional atlas of the human thalamus: Generation from multiple histological data. Neuroimage. 2010;49:2053-62.
Jeavons PM. Nosological problems of myoclonic epilepsies in childhood and adolescence. Dev Med Child Neurol. 1977;19:3–8.

Larsson PG, Kostov H. Lower frequency variability in the alpha activity in EEG among patients with epilepsy. Clin Neurophysiol. 2005;116(11):2701-6.
Larsson PG, Eeg-Olofsson O, Lantz G. Alpha frequency estimation in patients with epilepsy. Clin EEG Neurosci. 2012;43(2):97-104.
Laufs H, Kleinschmidt A, Beyerle A, Eger E, Salek-Haddadi A, Preibisch C, *et al*. EEG-correlated fMRI of human alpha activity. Neuroimage 2003;19:1463-1476.
Laufs H, Lengler U, Hamandi K, Kleinschmidt A, Krakow K. Linking generalized spike-and-wave discharges and resting state brain activity by using EEG/fMRI in a patient with absence seizures. Epilepsia. 2006;47:444-448.
Lindgren KA, Larson CL, Schaefer SM, Abercrombie HC, Ward RT, Oakes TR, *et al*. Thalamic metabolic rate predicts EEG alpha power in healthy control subjects but not in depressed patients. Biol Psychiatry. 1999;45(8):943-52.
Liu Z, de Zwart JA, Yao B, van Gelderen P, Kuo LW, Duyn JH. Finding thalamic BOLD correlates to posterior alpha EEG. Neuroimage. 2012;63(3):1060-9
Lopes da Silva FH, van Lierop TH, Schrijer CF, van Leeuwen WS. Organization of thalamic and cortical alpha rhythms: spectra and coherences. Electroencephalogr Clin Neurophysiol. 1973;35:627-39.
Lopes da Silva FH, Vos JE, Mooibroek J, Van Rotterdam A. Relative contributions of intracortical and thalamo-cortical processes in the generation of alpha rhythms, revealed by partial coherence analysis. Electroencephalogr. Clin. Neurophysiol. 1980;50:449-56.
Lorincz ML, Kekesi KA, Juhasz G, Crunelli V, Hughes SW. Temporal framing of thalamic relay-mode firing by phasic inhibition during the alpha rhythm. Neuron.2009; 63:683-96.
Makeig S, Debener S, Onton J, Delorme A. Mining event-related brain dynamics. Trends Cogn Sci. 2004a;8:204-10.
Makeig S, Delorme A, Westerfield M, Jung TP, Townsend J, Courchesne E. Electroencephalographic brain dynamics following manually responded visual targets. PLoS Biol. 2004b;2:e176.
Mazaheri A, Jensen O. Rhythmic pulsing: linking ongoing brain activity with evoked responses. Front Hum Neurosci. 2010;4:177.
Miyauchi T, Endo K, Yamaguchi T, Hagimoto H. Computerized analysis of EEG background activity in epileptic patients. Epilepsia. 1991;32(6):870-81
Moeller F, Siebner HR, Ahlgrimm N, Wolff S, Muhle H, Granert O, *et al*. fMRI activation during spike and wave discharges evoked by photic stimulation. Neuroimage 2009;48:682-95.
Moeller F, Muthuraman M, Stephani U, Deuschl G, Raethjen J, Siniatchkin M. Representation and propagation of epileptic activity in absences and generalized photoparoxysmal responses. Hum Brain Mapp. 2013;34(8):1896-909.
Morel A, Magnin M, Jeanmonod D. Multiarchitectonic and stereotactic atlas of the human thalamus. J Comp Neurol. 1997;387(4):588-630.
Moosmann M, Ritter P, Krastel I, Brink A, Thees S, Blankenburg F, *et al.* Correlates of alpha rhythm in functional magnetic resonance imaging and near infrared spectroscopy. Neuroimage. 2003;20:145-58.
Moruzzi G, Magoun HW. Brain stem reticular formation and activation of the EEG. Electroencephalogr. Clin. Neurophysiol. 1949;1:455-73.
Nichols T, Hayasaka S. Controlling the familywise error rate in functional neuroimaging: a comparative review. Stat Methods Med Res. 2003;12(5):419-46.
Nunez PL, Wingeier BM, Silberstein RB. Spatial-temporal structures of human alpha rhythms: theory, microcurrent sources, multiscale measurements, and global binding of local networks. Hum Brain Mapp. 2001;13(3):125-64.

Omata K, Hanakawa T, Morimoto M, Honda M. Spontaneous Slow Fluctuation of EEG Alpha Rhythm Reflects Activity in Deep-Brain Structures: A Simultaneous EEG-fMRI Study. PLoS One. 2013;8(6):e66869.
Pessoa L, Adolphs R. Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. Nat Rev Neurosci. 2010;11:773-83.
Pyrzowski J, Siemiński M, Sarnowska A, Jedrzejczak J, Nyka WM. Interval analysis of interictal EEG: pathology of the alpha rhythm in focal epilepsy. Sci Rep. 2015;5:16230.
Porciatti V, Bonanni P, Fiorentini A, Guerrini R. Lack of cortical contrast gain control in human photosensitive epilepsy. Nat Neurosci. 2000;3:259–63.
Pfurtscheller G, Stancák A Jr, Neuper C. Post-movement beta synchronization. A correlate of an idling motor area? Electroencephalogr Clin Neurophysiol. 1996;98(4):281-93.
Ruggieri A, Vaudano AE, Benuzzi F, Serafini M, Gessaroli G, Farinelli V, *et al*. Mapping (and modeling) physiological movements during EEG-fMRI recordings: the added value of the video acquired simultaneously. J Neurosci Methods. 2015;239:223-37.
Sadaghiani S, Scheeringa R, Lehongre K, Morillon B, Giraud AL, Kleinschmidt A. Intrinsic connectivity networks, alpha oscillations, and tonic alertness: a simultaneous electroencephalography/functional magnetic resonance imaging study. J Neurosci. 201;30:10243-50.
Scheeringa R, Fries P, Petersson KM, Oostenveld R, Grothe I, Norris DG *et al*. Neuronal Dynamics Underlying High- and Low-Frequency EEG Oscillations Contribute Independently to the Human BOLD Signal. Neuron. 2011; 69: 572–583.
Scheeringa R, Petersson KM, Kleinschmidt A, Jensen O, Bastiaansen MC. EEG α power modulation of fMRI resting-state connectivity. Brain Connect. 2012;2(5):254-64.
Siniatchkin M, Moeller F, Shepherd A, Siebner H, Stephani U. Altered cortical visual processing in individuals with a spreading photoparoxysmal EEG response. Eur J Neurosci. 2007;26:529–536.
Strigaro G, Prandi P, Varrasi C, Monaco F, Cantello R. Defective visual inhibition in photosensitive idiopathic generalized epilepsy. Epilepsia. 2012;53:695-704.
Strigaro G, Falletta L, Varrasi C, Rothwell JC, Cantello R. Overactive visuomotor connections underlie the photoparoxysmal response. A TMS study. Epilepsia. 2015;56(11):1828-35.
Stoller A. Slowing of the alpha-rhythm of the electroencephalogram and its association with mental deterioration and epilepsy. J Ment Sci. 1949;95(401):972-84.
Tyvaert L, Levan P, Grova C, Dubeau F, Gotman J. Effects of fluctuating physiological rhythms during prolonged EEG-fMRI studies. Clin Neurophysiol. 2008;119(12):2762-74.
Toscani M, Marzi T, Righi S, Viggiano MP, Baldassi S. Alpha waves: a neural signature of visual suppression. Exp Brain Res. 2010;207(3-4):213-9.
Van Essen DC. A population-average, landmark- and surface-based (PALS) atlas of human cerebral cortex. NeuroImage 2005;28:635-62.
Varotto G, Visani E, Canafoglia L, Franceschetti S, Avanzini G, Panzica F. Enhanced frontocentral EEG connectivity in photosensitive generalized epilepsies: a partial directed coherence study. Epilepsia. 2012;53(2):359-67.
Vaudano AE, Ruggieri A, Tondelli M, Avanzini P, Benuzzi F, Gessaroli G, *et al*. The visual system in eyelid myoclonia with absences. Ann Neurol. 2014 Sep;76(3):412-27.
Visani E, Varotto G, Binelli S, Fratello L, Franceschetti S, Avanzini G, *et al*. Photosensitive epilepsy: spectral and coherence analyses of EEG using 14Hz intermittent photic stimulation. Clin Neurophysiol. 2010;121(3):318-24.
Volkmann FC. Human visual suppression. Vision Res. 1986;26(9):1401-16.
Waltz S, Christen HJ, Doose H. The different patterns of the photoparoxysmal response--a genetic study. Electroencephalogr Clin Neurophysiol. 1992;83(2):138-45.

Wilkins AJ, Bonanni P, Porciatti V, Guerrini R. Physiology of human photosensitivity. Epilepsia. 2004;45:7-13.
Wolf P, Goosses R. Relation of photosensitivity to epileptic syndromes. J Neurol Neurosurg Psychiatry. 1986;49:1386–91.
Wolf P, Yacubian EM, Avanzini G, Sander T, Schmitz B, Wandschneider B, *et al*. Juvenile myoclonic epilepsy: A system disorder of the brain. Epilepsy Res. 2015;114:2-12.

Figures Legend

Figure 1: Alpha power estimation and analysis. Panel A: individual alpha band identification and calculation in a representative example. After visual identification of the αIC (top image), the posterior alpha component is selected based on the power spectrum (left bottom image) and average topography (right bottom image). Panel B to D represents histograms of alpha parameters recorded outside (grey) and inside the scanner (black). Panel B: histograms show the Gaussian distribution of individual peak alpha frequency within alpha band. Panel C: histograms illustrate the mean individual alpha frequency in the different subgroups of patients and in controls. Panel D: histograms display the mean alpha power in the different subgroups of patients and in controls. The *GGE PS+* shown significantly higher alpha power compared to others both during scanning and outside. The mean alpha power was significantly lower inside then outside the scanner in all the studied population. The bars represent the Standard Error. ***p<0.01*.

Figure 2: Whole-population BOLD findings. Panel A: Alpha-BOLD correlation map related to all the subjects considered together (p<0.05 corrected for FWE). The functional maps have been warped to the PALS- B12 atlas in caret (dorsal view) for right (R) and left (L) hemisphere (left side images) and overlaid onto representative canonical slices, axial, coronal and sagittal view (right side images) for visualization purposes. The yellow colour indentifies the positive correlations to alpha power, the light-blue colour the negative correlations. See text for details.
Panel B and C: Relationship between BOLD thalamic changes and thalamic nuclei as provided by digital version of the Morel's atlas of human thalamus (Morel et al, 1997; Krauth et al, 2010). Thalamic positive (Panel B) (p< 0.05 corrected for FDR) and negative (Panel C) (p< 0.05 corrected for FWE) BOLD correlations are overlaid onto the MNI template (1mm) (axial slices) as provided by FSL (FMRIB Software Library) after linear transformation using FMRIB Linear Image Registration Tool [FLIRT]. For each panel, the localization of the BOLD change is compared with the thalamic atlas. The thalamic atlas is displayed onto the canonical T1-1mm image as implemented in FSL (FMRIB Software Library). Canonical T1 slices have been zoomed for visualization purposes. The thalamic nuclei have been indicated by different colors for visualization purposes and grouped according to Morel, 1997. LGn, green color: lateral group of thalamic nuclei which includes the ventroposterior complex, ventral lateral posterior/anterior, and ventral anterior and ventral medial nuclei. AGn, red color: anterior group of thalamic nuclei which includes anteroventral, anteromedial, anterodorsal, and lateral dorsal nuclei. PGn, blue color: the posterior group of thalamic nuclei which includes medial and lateral geniculate nuclei, posterior, suprageniculate/limitans, lateral pulvinar nucleus (PuL), Medial pulvinar nucleus (PuM) and Anterior Pulvinar nucleus (PuA). Pink color shows the mesial group of thalamic nuclei: MDmc: Mediodorsal nucleus, magnocellular division; MDpc: Mediodorsal nucleus, parvocellular division; CL: Central lateral nucleus. Scale Bar of 10 mm. R= right; L= left.

Figure 3: Alpha-BOLD correlation maps related to each single population of subjects (*p*<0.05, FDR corrected). The functional maps have been warped to the PALS-B12 atlas in caret (mesial

and dorsal view) for right (R) and left (L) hemisphere. The yellow-red colour indentifies the positive correlations to alpha power, the light-blue colour the negative correlations. See text for details.

Figure 4: Comparisons of alpha-power BOLD maps between the patients' groups and between patients and controls (p<0.05, FDR corrected). The functional maps are shown on the normalized SPM-glass brain (left images) and warped to the PALS-B12 atlas in caret (mesial and dorsal view) for right (R) and left (L) hemisphere (right images). The red arrow on the SPM-glass brain indicates the global maximum. The white lines on the PALS-B12 atlas show the surface landmarks as implemented in Caret: the Central Sulcus, the medial wall dorsal segment, and the medial wall ventral segment, the sylvian fissure and calcarine sulcus. See text for details.

Figure 5: BOLD changes (derived from statistical comparison p<0.05 corrected for FDR) between functional connectivity with seed region at MDN in GGE PS+ versus CRT. The resulted map has been warped to the PALS-B12 atlas in caret (dorsal and ventral view) for right (R) and left (L) hemisphere. For localization purposes, functional results on the right hemisphere were plotted and compared against six Brodmann Areas indicated by the white numbers. The displayed Brodmann Areas cover part of the pre-central regions and the orbitofrontal cortex subdivisions (Henssen et al., 2016). Only increases of functional connectivity in GGE PS+ population were detected. See text for details.

Figure 6: BOLD changes derived from statistical comparison (p< 0.05 corrected for FDR) between functional connectivity with seed regions at Pulvinar in GGE PS+ versus CRT and other epileptic populations. Panel A: differences in functional connectivity between *GGE PS+* and controls. The resulted map has been warped to the PALS-B12 atlas, dorsal view for right (R) and left (L) hemisphere (left image) and to flat template for the right hemisphere (right image). For localization purposes, functional results on the right hemisphere (RH) were plotted and compared against Brodmann Areas indicated by the white numbers. Increases in functional connectivity are shown in yellow-red, decreases in blue. See text for details. Panel B: Decreases in functional connectivity in *GGE PS+* versus all other population of subjects (p< 0.05, uncorrected). Each subjects' subgroup in indicated by a specific colour. The regions coloured in pink represent the overlapping clusters from all the comparisons. R= right.