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Different responses of spontaneous and stimulus-related alpha activity to ambient luminance changes

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Abstract

Alpha oscillations are particularly important in determining our percepts and have been implicated in fundamental brain functions. Oscillatory activity can be spontaneous or stimulus-related. Furthermore, stimulus-related responses can be phase- or non-phase-locked to the stimulus. Non-phase-locked (induced) activity can be identified as the average amplitude changes in response to a stimulation, while phase-locked activity can be measured via reverse-correlation techniques (echo function). However, the mechanisms and the functional roles of these oscillations are far from clear. Here, we investigated the effect of ambient luminance changes, known to dramatically modulate neural oscillations, on spontaneous and stimulus-related alpha. We investigated the effect of ambient luminance on EEG alpha during spontaneous human brain activity at rest (experiment 1) and during visual stimulation (experiment 2). Results show that spontaneous alpha amplitude increased by decreasing ambient luminance, while alpha frequency remained unaffected. In the second experiment, we found that under low-luminance viewing, the stimulus-related alpha amplitude was lower, and its frequency was slightly faster. These effects were evident in the phase-locked part of the alpha response (echo function), but weaker or absent in the induced (non-phase-locked) alpha responses. Finally, we explored the possible behavioural correlates of these modulations in a monocular critical flicker frequency task (experiment 3), finding that dark adaptation in the left eye decreased the temporal threshold of the right eye. Overall, we found that ambient luminance changes impact differently on spontaneous and stimulus-related alpha expression. We suggest that stimulus-related alpha activity is crucial in determining human temporal segmentation abilities.

Introduction

The human brain can be conceived as a dynamical system where billions of neurons synchronize their activity to generate a coherent and stable representation of the world. Neuronal oscillations play a special role in this synchronization, and in particular, alpha oscillations (8–13 Hz) are known to shape perception (VanRullen & Koch, 2003; VanRullen, 2016). Alpha amplitude and phase are related to stimulus processing and cortical excitability (Nunn & Osselton, 1974; Linkenkaer-Hansen *et al.*, 2004; Hanslmayr *et al.*, 2005; Romei *et al.*, 2008; Busch *et al.*, 2009), as well as cognitive and memory functions (Klimesch *et al.*, 1993; Klimesch, 1999; Bonnefond & Jensen, 2012). Furthermore, alpha rhythm peak frequency is linked to visual temporal resolution (Varela *et al.*, 1981; Cecere *et al.*, 2015; Samaha & Postle, 2015; Milton & Pleydell-Pearce, 2016). These independent functions suggest that the alpha rhythm nests partial independent oscillators which serve different processes. A first attempt to disentangle these differences is to investigate the alpha basic oscillatory mechanisms. A common conception is that cortical oscillatory activity comprises both spontaneous and stimulus-related components, possibly reflecting semi-independent functions (David *et al.*, 2006). The spontaneous activity reflects ongoing oscillatory mechanisms, while the stimulus-related activity reflects oscillatory mechanisms sensitive to stimulation. Furthermore, stimulus-related activity can be decomposed in an 'induced' response and an 'evoked' response. The 'induced' response is characterized by stimulus-related changes in oscillatory amplitude, which are not necessarily phase-locked to the stimulus (i.e. their latency vary trial-by-trial), and these amplitude modulations tend to disappear in the time-domain averaged data. In contrast, the 'evoked' activity reflects

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only the phase-locked EEG activity synchronized with the stimulus onset. Recently, by applying a reverse-correlation technique between a random luminance sequence and the corresponding EEG responses, the electroencephalogram 'impulse response function' (or echo function) of the visual system has been modelled (VanRullen & Macdonald, 2012). Crucially, the echo function reflects the stimulus-related phase-locked activity of the EEG signal, by measuring how much the EEG response correlates with the visual stimulation, over time. The amplitude and the frequency of the echo function are correlated with the spontaneous resting alpha (VanRullen & Macdonald, 2012). At the same time, it has been shown that attentional allocation is inversely correlated with spontaneous alpha amplitude (Sauseng *et al.*, 2005), and it correlates positively with the echo function amplitude (VanRullen & Macdonald, 2012). Together, these findings suggest that the two rhythms reflect partially independent functions; however, the nature of these differences is still unclear.

To clarify the functional differences between spontaneous and stimulus-related brain rhythms, we experimentally manipulated neuronal oscillations by modulating luminance viewing conditions. In fact, luminance viewing conditions are known to influence visual and cognitive abilities (Vandewalle *et al.*, 2009; Barbur & Stockman, 2010). The latency and integration time of visual processing – from retinal to higher processing sites – progressively increases at low-luminance (Kammer *et al.*, 1999), as well as the alpha amplitude (Min *et al.*, 2013; Brodoehl *et al.*, 2015), and the frequency of behavioural visual oscillations (Benedetto *et al.*, 2016). Moreover, brief dark exposure produces adaptive changes in cortical excitability (Huang *et al.*, 2015).

In experiment 1, we investigated the effect of ambient luminance on the spontaneous alpha activity recorded at rest. In experiment 2, we extended this investigation to stimulus-related brain activity, looking at possible luminance modulations over phase-locked (echo) and non-phase-locked EEG activity. Visual temporal segmentation abilities are known to correlate with brain rhythms, and in particular, are reported to be tightly linked with alpha frequency (Samaha & Postle, 2015). Thus, in experiment 3, we explored some functional correlates of the luminance-based modulations of brain oscillations by estimating an index of temporal segmentation abilities (monocular critical flicker frequency, CFF) under different contralateral luminance viewing conditions.

Material and methods

All experiments were conducted in a quiet, dark room (mean ambient luminance < 0.01 cd/m^2). For experiment 1 and 2, electrophysiological activity was continuously recorded at 1024 Hz using a 64 channel ActiveTwo Biosemi system. Horizontal and vertical eye movements were recorded by three additional electrodes: one below the left eye and two at bilateral outer canthi. After giving written informed consent, 16, 12 and 13 subjects took part in experiments 1, 2 and 3, respectively (including two authors). A total of 21 participants were recorded (10 women, mean age and standard deviation: 30 $\pm$ 4). The three groups of participants were practically overlapping: six participants performed both experiments 1 and 2, eight participated in experiments 1 and 3, and five in experiments 2 and 3. All had normal or corrected-to-normal vision. Two subjects from experiment 1 did not show alpha activity and were thus discarded from further analyses. The main goal of this study was to compare the effect of different luminance viewing condition over spontaneous and stimulus-related EEG alpha activities. For this reason, we restricted our analysis to the electrode POz that is known to convey the strongest stimulus-related response (VanRullen & Macdonald, 2012; Chang *et al.*, 2017) as well as showing a strong alpha activity at rest. For experiments 1 and 2, stimuli were generated using the MATLAB Psychophysics toolbox (Brainard, 1997) and displayed at 57 cm on a gamma-corrected CRT monitor (640 $\times$ 480 pixels, 160 Hz). For experiment 3, stimuli were presented using Python (Peirce, 2007) on a gamma-corrected CRT monitor (800 $\times$ 600 pixels, 60 Hz) and a white LED controlled by Arduino Uno serially connected to the PC (1 15 200 baud rate) (Teikari *et al.*, 2012). Response in experiment 3 was recorded via a potentiometer driven by Arduino Uno. Data were analysed with EEGLAB (Delorme & Makeig, 2004), FieldTrip (Oostenveld *et al.*, 2011) and custom Matlab code. The low-luminance viewing condition was achieved by applying a neutral density filter (NDF) in front of the monitor (NDF: 2.5 LU, experiments 1 and 2), or in front of the left eye (NDF: 1.5 LU, experiment 3). All experiments were approved by the Centre National de la Recherche Scientifique ethical committee.

Experiment 1: spontaneous alpha activity

The experimental procedure is shown in Fig. 1A. We recorded blocks of 1 min of EEG activity while participants (N = 14) maintained fixation on a dot presented on a grey screen (resting state, with eyes open). To maintain alertness, after each resting period, participants performed a reaction time (RT) task to a visual target presented above a movie shown in the screen centre (active task, 2 min long). The experiment consisted of three consecutive sessions. In the first and the third session, five resting blocks of 1 min per session were recorded for each participant over 13 min, under high-luminance viewing conditions (mean luminance of 51.8 cd/m^2). The second session was performed under low-luminance viewing condition, achieved by positioning a neutral density filter (NDF, 2.5 LU) in front of the monitor. Fourteen minutes of resting were collected per participant over 40 min.

The EEG was re-referenced to the common average and band-passed filtered (1–256 Hz, 4th order Butterworth IIR filter). Each 1-min recording was split in 5 s epochs (from 5 to 60 s). Artefacts were detected and removed in two steps: first, we visually inspected the epochs and those with gross muscular artefacts were rejected. Subsequently, we applied ICA to remove artefacts (Jung *et al.*, 2000). We standardized the EEG responses according to the global standard deviation of each participant, across conditions. For each participant and condition, we investigated from POz two main indices: the individual alpha amplitude (spontaneous IAA) and the individual alpha frequency (spontaneous IAF). To compute the spontaneous IAA, we first defined the alpha range as the frequency band between 8 and 13 Hz. Next, we computed for each epoch the alpha spectrum using a Fourier transform. The spontaneous IAA was defined for both high- and low-luminance condition, as the mean of the alpha spectra across epochs. To determine the spontaneous IAF, we computed the mean amplitude spectrum of the alpha band (8–13 Hz), using a Fast Fourier transform. We averaged the alpha spectra across all epochs and defined spontaneous IAF as the centre of mass (or spectral centroid) of the mean alpha spectrum (Klimesch *et al.*, 1993).

Experiment 2: stimulus-related alpha activity

White-noise visual luminance sequences were displayed within a disc of 3.5° radius presented in the vertical meridian centred at 7.5° above the fovea on a black background. Each randomly generated luminance sequence (6.25 s) was tailored to have equal amplitude at

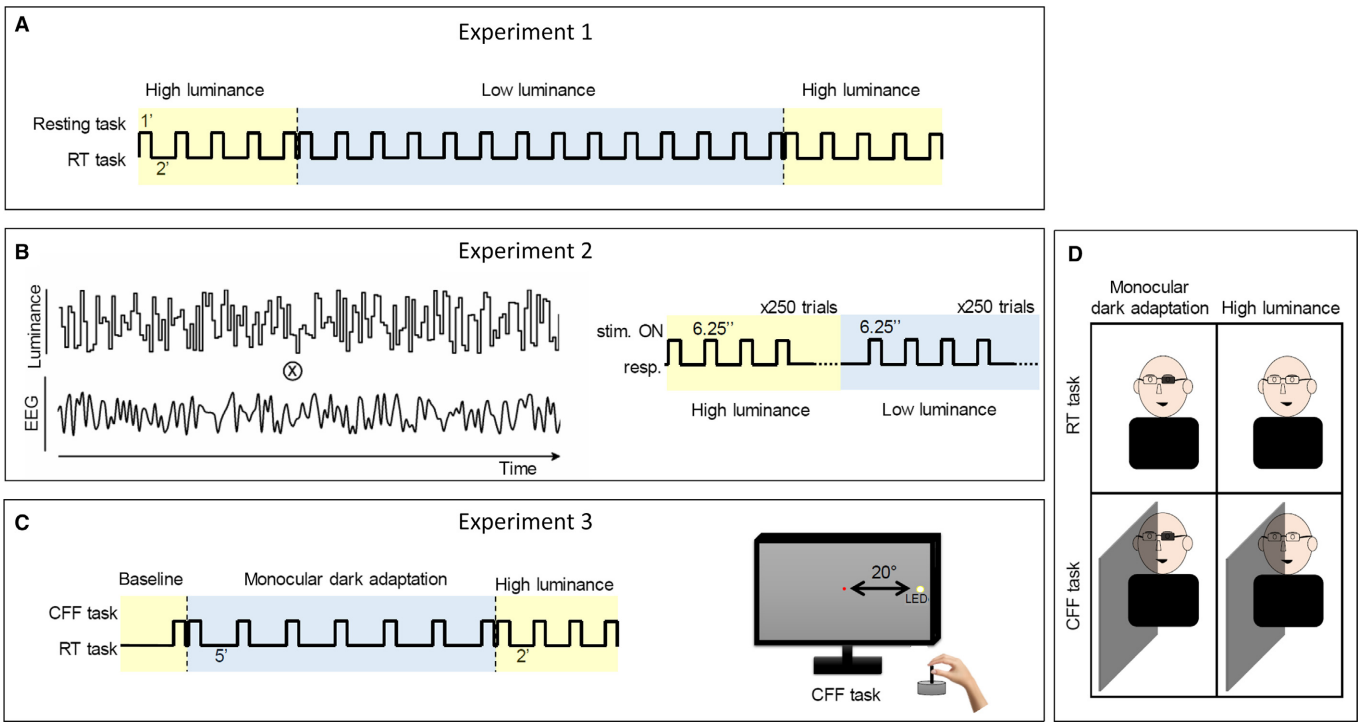

FIG. 1. Schematic of the procedures for experiments 1,2 and 3. (A) Schematic of the procedure of experiment 1. One minute of resting state was followed by 2 min of RT task. Globally, 10 min of resting state was recorded under high-luminance and 14 min under low-luminance viewing condition. (B) Schematic of experiment 2. For each participant, we computed the individual echo by cross-correlating the random luminance sequence of the visual stimulation with the EEG response of the POz electrode. Each stimulation lasted for 6.25 s, and 250 trials were recorded under high- and low-luminance viewing condition. (C) schematic of experiment 3. After a training conducted under high-luminance viewing condition, 10 CFF thresholds were recorded as a baseline, right before starting the monocular dark adaptation. During the monocular dark adaptation, CFF thresholds were acquired followed by 5 min of RT task. CFF threshold was tested seven times over 30 min of global monocular dark adaptation, for a total of 70 CFF threshold values. CFF was then computed 7 times more under high-luminance viewing condition, again after CFF task participants performed 2 min of RT task. The right insert shows an example of the CFF task: the flickering LED was 20° distant from the centre, participants had 6 s to select with a potentiometer the CFF threshold. (D) An example showing the experimental apparatus for the CFF experiment. During monocular dark adaptation, participants wore goggles with a NDF on the left eye. During the CFF task, a board was positioned between the subject's nose and the centre of the screen and the stimulus was presented on the right hemi-field. Only the right (non-adapted) eye was tested. Note that, in this way, the retinal adaptation of the tested eye was identical between the monocular dark-adaptation and the light-adaptation condition.

all frequencies, by normalizing the amplitudes of its Fourier components before applying an inverse Fourier transform. Sequences ranged from black (0.02 cd/m^2) to white (110 cd/m^2). Observers (N = 12) covertly monitored the stimulus to detect a 1 s long target square (3.75 degrees) appearing inside the disc on a random 25% of trials. The target was presented at random times within the sequence, excluding the first and last 0.25 s. The area within the square followed the same sequence of luminance changes as the disc stimulus, but scaled in amplitude using a QUEST procedure so that detection performance was fixed at approximately 82% (Watson & Pelli, 1983). A schematic of the procedure is shown in Fig. 1B. Observers were instructed to press a button at the end of the sequence if they had detected the target. The experiment consisted of 250 trials and each participant performed the task both under high- and low-luminance viewing condition. The order of the conditions was random, and 5 min of dark adaptation preceded the low-luminance condition. Target-present and target-absent trials were included in the cross-correlation analysis, as it was verified elsewhere that the echo function is high consistent in both conditions (VanRullen & Macdonald, 2012).

The EEG was re-referenced to the common average and down-sampled to 160 Hz before cross-correlation analysis. To avoid on/off transient, all stimulus time points except the first 0.5 s and the last 1.5 s of the sequence were entered in the cross-correlation. To obtain the echo function, we averaged the single-trial cross-correlations (Lalor *et al.*, 2006; VanRullen & Macdonald, 2012) between the luminance sequence and the simultaneously acquired EEG time series (VanRullen & Macdonald, 2012). This results in a single correlation value over the whole time series for each time lag. The cross-correlation procedure aims to calculate the 'impulse response function' of the EEG, as follows:

$$\mathrm{IRF}\ (t)\ =\ \sum_{T} \mathrm{stim}(T) \cdot \mathrm{eeg}\ (T\ +\ t)$$

where t denotes the time lag between the two signals and T designates all sampling-points, stim and eeg denote the standardized stimulus sequence and the corresponding standardized EEG response, respectively. To compute the echo function, we calculated all time lags between −0.2 and 1.5 s of the cross-correlation. While more classical methods rely on the EEG dynamics following a single transient signal (e.g. visual evoked response analysis, VEP), our approach has the ecological advantage to evaluate the EEG rhythms during a continuous visual stimulation. Therefore, the 'impulse response function' can be conceived as the superimposition of VEPs to each stimulus frame (weighted by the stimulus luminance on that frame), rather than a standard VEP evoked by the sequence onset (Lalor *et al.*, 2006; VanRullen & Macdonald, 2012; Chang *et al.*, 2017). In practice, the impulse response function tends to show a much stronger and longer-lasting alpha oscillation, the 'perceptual

echo' (VanRullen & Macdonald, 2012), which makes it more suitable than a VEP for studying evoked (phase-locked) alpha oscillations.

The literature on the echo function consistently shows that before 0.25 s, the echo overlaps with the early broadband part of the IRF, similar to a VEP (İlhan & VanRullen, 2012; VanRullen & Macdonald, 2012). In other words, cross-correlation lags before 0.25 s reveal a broadband ERP (0–30 Hz) that is not specific to alpha oscillations. Accordingly, we defined our temporal window of interest with lags between 0.25 and 1 s. Individual alpha amplitude (echo IAA) and individual alpha frequency (echo IAF) were computed at the electrode POz within these lags (VanRullen & Macdonald, 2012; Chang *et al.*, 2017). The echo IAA was defined, for both high- and low-luminance condition, as the mean of the alpha amplitude spectrum computed using the Fourier transform, obtained from averaging all the echoes across trials. To determine the echo IAF, we computed the alpha spectrum of the averaged echoes, via a Fast Fourier transform. The echo IAF was defined as the centre of mass of the mean echo function spectrum. To increase frequency resolution of the echo IAF, the signal was zero-padded (30 s).

In addition, we investigated the phase difference between the two conditions. We selected a time window of 300 ms between 0.25 and 0.55 from stimulus onset, where the alpha amplitude was maximal for both conditions (see Fig. 4B). For each participant, the echo was previously bandpass filtered ± 1 Hz around its IAF (ideal band pass), instantaneous analytic phase was obtained by taking the angle of the Hilbert-transform of echo functions within the window of interest.

Finally, we also investigated the activity induced by the stimulation. To investigate the induced EEG response, we computed the induced IAA and induced IAF for the EEG data over the entire stimulation sequence duration, excluding the first 0.5 s and the last 1.5 s of each stimulus sequence to avoid on/off artefact (e.g. ERPs). Even though the EEG signal is phase-locked to the onset of the luminance sequence, it bears no phase relation to each individual frame, as the frames happen continually and with random luminance. Therefore, analyses of the induced response involved averaging the EEG response in the frequency domain following an FFT (as for the spontaneous responses), and not in the temporal domain (as for the echoes). Induced IAA was computed as spontaneous IAA in experiment 1. The EEG responses were standardized according to the global standard deviation of each participant, across both conditions. We computed for each trial the alpha amplitude spectrum via a Fourier transform. The induced IAA was defined for both high- and low-luminance condition, as the mean of the averaged alpha spectra. Moreover, we averaged the alpha spectra (8–13 Hz) across trials to compute the centre of mass of the mean alpha spectrum (induced IAF). Before computing the Fourier transform, the signal was zero-padded to increase frequency resolution (30 s).

Experiment 3: critical flicker frequency

A schematic of the experiment 3 is shown in Fig. 1C and D. The stimulus was a white LED flickering (square wave from 70 to 20 Hz, 65 cd/m^2) 20° right to the centre, and it was visible only by the right eye thanks to a board positioned between the subject's nose (head fixed on a chin-rest) and the centre of the screen segregating the visual field of the two eyes. We asked subjects ($N = 13$) to adjust the frequency of the flicker with a potentiometer, until reaching the critical flicker frequency (CFF), that is the minimum frequency at which the light is perceived as steady instead of flickering. To investigate the role of luminance viewing in determining CFF, participants performed the task while the contralateral left eye was dark-adapted (dark-adapted condition, DA) or light-adapted (light-adapted condition, LA) with a steady light. In this way, we assured an identical retinal adaptation of the tested eye (right eye), but different cortical excitability states for the two conditions (LA, DA). After a training, the baseline monocular CFF was computed for each individual subject (10 trials). After the baseline recording, the left eye of the participants was patched with a NDF (1.5 LU, DA condition). A monocular CFF session recording was presented every 5 min. About 10 trials were acquired for each testing session; each trial lasted for 6 s during which subjects were required to adjust the flickering frequency, and the starting flickering frequency was fixed at 70 Hz. The filter was removed from the left eye in the subsequent light-adaptation condition (LA), and four sessions of 10 trials each were recorded every 2 min from the patching removal. For this condition, six subjects additionally performed three more sessions (seven sessions). To maintain alertness, after each CFF session, the board was removed, and participants performed a reaction time task to a black blob presented beside a movie shown in the screen centre (2.5 × 2 deg). The RT task interleaved the CFF sessions and lasted for 5 and 2 min under DA and LA condition, respectively. Only data from CFF were analysed. A linear mixed-effect model analysis was conducted on the logarithm of the CFF, with subject variability modelled as a random effect, and condition (baseline, dark adaptation and light adaptation) or session as fixed effects (model: CFF ~ condition/session + (condition/session|subject)). The associated method 'ANOVA' returns the F-statistics and *P*-values for the fixed effect terms (where the degrees of freedom are assumed to be constant and equal to n-k, where n is the number of observations and k is the number of fixed effect). For the analysis on the effect of session, we contrasted each experimental session with the baseline.

Results

Experiment 1: Spontaneous alpha activity

We analysed the effect of ambient luminance on resting EEG alpha for 14 participants at the POz electrode. The amplitude spectrum was computed for both high- and low-luminance recordings (Fig. 2A). Figure 2B and D show the spontaneous IAA for the two conditions. A two-tailed paired *t*-test showed that alpha amplitude was higher at low-luminance compared to high-luminance viewing conditions ($t_{13} = -2.36$, $P = 0.034$). Similarly, we compared the spontaneous IAF for the two luminance conditions (Fig. 2C and E). No differences were found in spontaneous IAF for high- and low-luminance conditions ($t_{13} = 0.17$, $P = 0.8$).

Experiment 2: Stimulus-related alpha activity

For each participant ($N = 12$), we computed the echo function at high- and low-luminance for the POz electrode (two representative subjects are shown in Fig. 4A). Figure 3A shows the mean spectrum ± 1 SEM of the echo EEG, for both viewing conditions. We compared the echo IAA for the two luminance conditions with a two-tailed *t*-test, and we found that alpha amplitude was higher at high-luminance compared with low-luminance ($t_{11} = 4.63$, $P < 0.001$. See Fig. 3B and D). Thus, we investigated the effect of luminance on echo IAF (Fig. 3C and E) and we found a significant shift of about 0.15 Hz towards higher frequencies at low-luminance compared with high-luminance ($t_{11} = -2.41$, $P = 0.034$). Taken together, these results show that the echo function response is

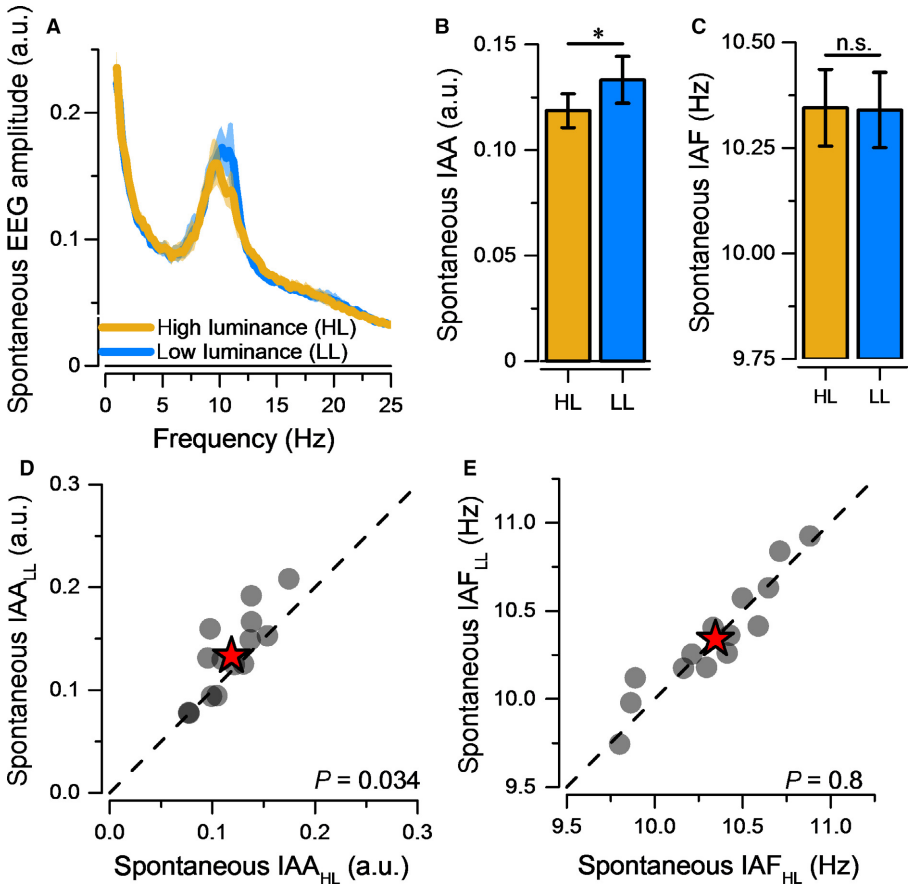

FIG. 2. Experiment 1: spontaneous alpha activity IAA and IAF. Main results from experiment 1. (A) Group-mean spontaneous EEG amplitude (±SEM) at high- and low-luminance (in yellow and blue, respectively) for the electrode POz. (B) IAA group mean and SEM at high- and low-luminance. (C) IAF group mean and SEM at high- and low-luminance. (D) IAA for single subject (dots) and group-mean (star) and for both luminance conditions. Most of the points cluster above the equality line (dashed line) confirming a IAA difference for the two luminance conditions. (E) Scatter plot of the IAF computed at high- and low-luminance for each participant (dots) and group mean (star). The points are distributed around the equality line (dashed line), confirming no differences in IAF for the two conditions. Asterisks (in B and C) mark the statistical significance (n.s. > 0.05 > * > 0.01).

consistently modulated by luminance viewing condition, along both amplitude and frequency dimensions.

Thus, we investigated the luminance viewing condition effect on the phase of the echo function. We found a strong phase opposition between the echoes recorded at high- and low-luminance, maximally expressed at POz (Fig. 4C). To have a meaningful phase estimation, the analysis was restricted to a time window of 300 ms around the time of the maximal stimulus-related alpha activity, from 0.25 to 0.55 s (Fig. 4B). During the maximal amplitude of the echo function, we confirmed a strong phase opposition (3.15 ± 0.26 rad). To verify that the phase difference was not uniformly distributed across all phases, we performed a Rayleigh test on the phase differences confirming the presence of a non-uniform phase distribution centred around π (i.e. phase opposition, $P < 0.001$). Next, we asked whether the phase shift could be driven by a fixed physiological neural delay, known to be caused by luminance differences (Kammer *et al.*, 1999). We investigated the correlation between the echo IAFs and the phase difference under different luminance viewing (Fig. 4D). A constant time delay (i.e. irrespective of the frequency of the echo IAFs) would result in a positive correlation, while a constant phase delay would result in a null correlation. We revealed a strong positive correlation between phase differences and echo IAFs (Pearson's $r = 0.57$; $P = 0.003$). Additionally, we ran a partial correlation analysis to rule out the contribution of the different conditions. This analysis confirmed the presence of a genuine positive correlation (Pearson's $r = 0.59$; $P = 0.003$). Taken together, these results suggest that luminance changes produced a shift in the phase of the echo function, but this shift is mainly driven by a constant neural delay. Note that, it was possible to perform a linear regression on circular phase data, because the measured phase differences were all comprised between π/2 and 3π/2, so there was no 'wraparound' issue around 0 or 2π. The phase lags, when expressed in ms (taking into account the echo IAF for each subject/condition), were clustered around 46 ± 10 ms, a value consistent with the previously reported physiological delay of about 15 ms for each log-unit attenuation of luminance (Julesz & White, 1969; Williams & Lit, 1983), predicting here a neural delay around 40 ms.

Additionally, we also investigated the induced alpha spectrum obtained during the stimulation. Fig. 5A shows the main results of these analyses for high- and low-luminance conditions. We evaluated the correlation between echo IAA and the induced IAA (Fig. 5B). We found a positive correlation between the two indexes (Pearson's $r = 0.54$; $P = 0.005$). The partial correlation analysis, controlling for the viewing conditions, revealed a clear positive trend between the two indexes (Pearson's $r = 0.36$; $P = 0.08$). The same comparison was run for the IAF (Fig. 5C) and revealed a significant positive correlation (Pearson's $r = 0.44$; $P = 0.029$), confirmed also by the partial correlation analysis (Pearson's $r = 0.45$;

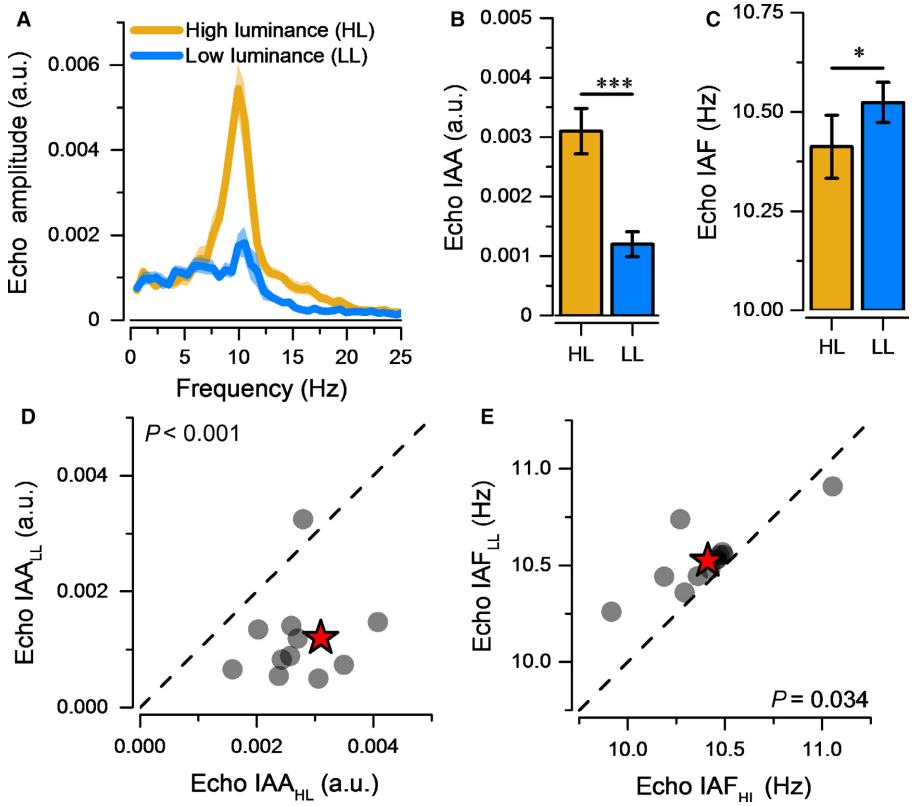

FIG. 3. Experiment 2: echo function IAA and IAF. (A) Group-mean echo function spectrum and SEM at high- and low-luminance (yellow and blue lines, respectively) for the POz electrode. (B) Bar plot of the echo IAA (±1 SEM) at high- and low-luminance. (C) Bar plot of the echo IAF (±1 SEM) at high- and low-luminance. (D) Scatter plot of the echo IAA at high- and low-luminance. The points cluster below the equality line (dashed line), indicating a difference in echo IAA for the two conditions. The star indicates the group-mean IAA. (E) Scatter plot of the echo IAF across single subjects for the two luminance conditions. The cloud of dots scatters above the equality line, indicating that the echo IAF was higher at low-luminance. The star indicates the group-mean IAF. Asterisks (in B and C) mark the statistical significance (0.05 > * > 0.01, 0.001 > ***).

$P = 0.029$). Taken together, these results suggest that echo and induced activity are well correlated (as expected given the intimate connection between these two indexes), but also show some crucial differences in their response to luminance changes. In fact, similarly to what was found for the phase-locked (echo) alpha, results showed a decrease in induced IAA for the low-luminance condition ($t_{11} = 3.97$; $P = 0.002$. Figure 5D). However, this difference was much reduced, compared to the one found for the echo function. Interestingly, no difference was found regarding the induced IAF ($t_{11} = -0.33$; $P = 0.74$. Figure 5E). Note that, we also replicated our analysis of the induced response (as in Fig. 5) after subtracting the trial-averaged ERP from each trial. Consistently with the fact that the induced response has almost no phase-locked components after 500 ms from stimulus onset, this subtraction did not alter the results (control analysis is not reported in the manuscript).

Experiment 3: critical flicker frequency

We next investigated on 13 participants the potential perceptual consequences of the luminance-induced alpha modulations in a monocular CFF task that is suggested to be linked with EEG alpha expression (Chyatte, 1958; Kooi *et al.*, 1958; May *et al.*, 2014). A linear mixed-effect model on the CFF timecourse showed a significant effect of time session (fixed effect 'time session' with 'subject' as random effect. $F_{14,1725} = 4.4208$, $P < 0.001$. See Fig. 6A). Contrasts between session and baseline revealed that the CFF in the first session of the binocular light adaptation was the only threshold significantly different from the baseline ($P = 0.047$). We next investigated the global effect of monocular dark adaptation on CFF. Figure 6B shows the group-mean CFF shifts between the two conditions. The test between monocular dark-adaptation and binocular light-adaptation conditions revealed a significant difference between conditions (fixed effect 'condition', random effect 'subject'. $F_{2,1737} = 14.592$, $P < 0.001$), indicating that CFF was consistently higher during DA compared to LA, by about 2 Hz. No differences were present between DA and baseline or LA and baseline ($P > 0.05$). To verify whether this difference was mainly driven by the first session recorded after DA (i.e. the one showing the strongest effect), we replicated the same analysis after excluding this session. Again, the test revealed a significant difference between conditions (fixed effect 'condition', random effect 'subject'. $F_{2,1607} = 8.414$, $P < 0.001$). Figure 6C shows an analysis run on single subjects (including the first LA session in the analysis), additionally tested with a bootstrap *t*-test (10 000 repetitions with replacement, $n = 40$). About nine subjects showed a statistically significant difference in CFF between monocular dark-adaptation and binocular light-adaptation conditions ($P < 0.01$), while four participants showed a trend in the same direction without reaching significance ($P > 0.05$).

Discussion

We evaluated the influence of ambient luminance changes on the alpha dynamic characteristics. First, we investigated the effect of luminance changes on the spontaneous alpha rhythm recorded during resting with eyes open (experiment 1). In line with the existing literature

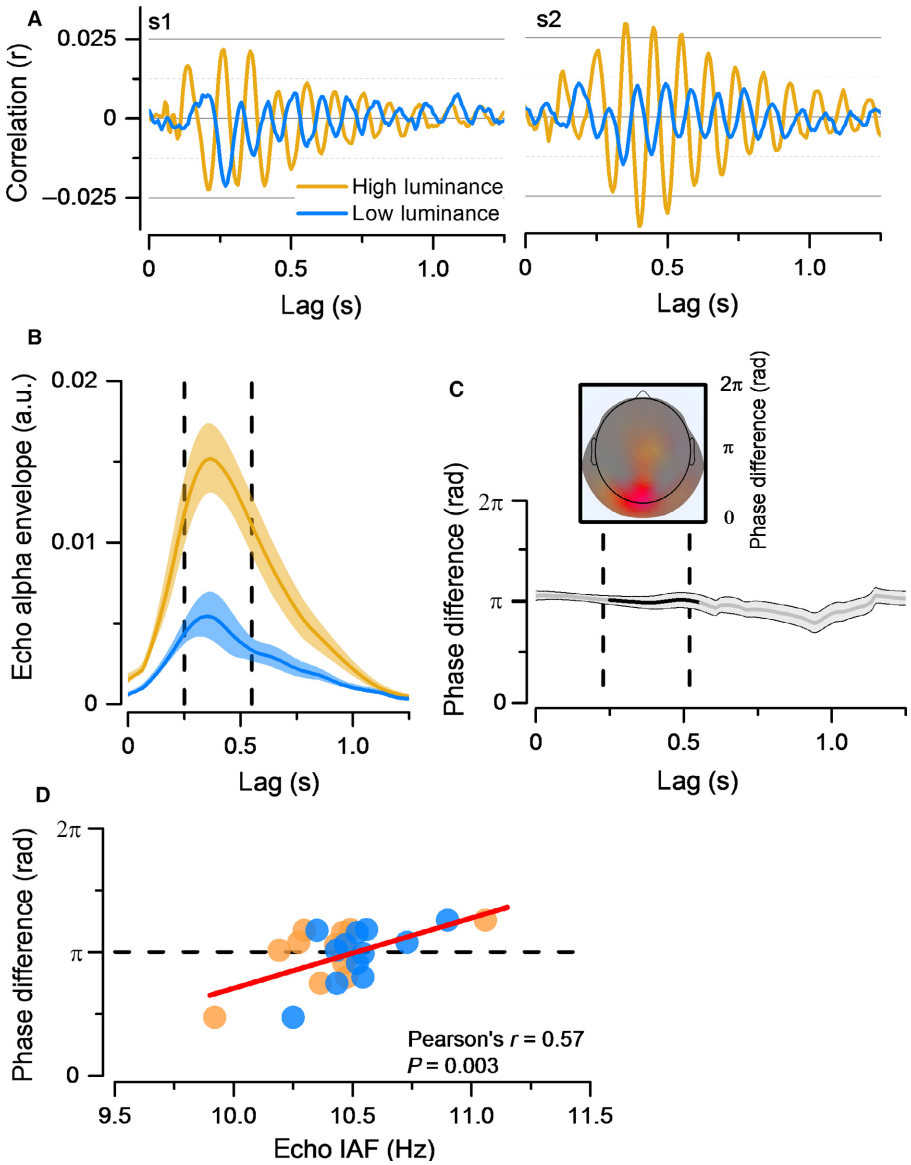

FIG. 4. Experiment 2: echo function phase analysis. Example of echoes for two representative subjects (A) at high- and low-luminance (yellow and blue line, respectively). (B) Alpha amplitude envelope for both high-and low-luminance echoes. Dashed lines mark the temporal window of interest for the phase analysis in panels C, D, (between 0.25 and 0.55 s). (C) Group-mean phase difference (±1 SEM) between the two echo function conditions. Dashed lines mark the temporal window of interest for the phase analysis (shown in B). The top panel shows the grand-mean topographic representation of phase differences averaged over the temporal window of interest. Colour code represents phase differences in radians. Topography was masked (grey transparency) by the averaged amplitude of echo functions. (D) Scatter plot for echo phase difference as a function of echo IAF. The red line reports the linear regression model, showing a positive correlation between the two variables (P = 0.003), indicating the presence of a constant time delay between conditions. Dashed line shows the mean phase difference.

(Min *et al.*, 2013), we found that ambient luminance alters the spectral amplitude in the alpha range during resting. We found a spontaneous IAA enhancement at low-luminance compared to high-luminance, particularly visible in the upper-alpha band. Traditionally, this alpha amplitude enhancement is interpreted as a consequence of the metabolic deactivation of the underlying cortex at low luminance (Moosmann *et al.*, 2003; Brodoehl *et al.*, 2015), reflected in a strong occipital alpha synchronization in the EEG. Interestingly, luminance changes produced no effects on the spontaneous IAF.

Next, we investigated the stimulus-related alpha (experiment 2). Contrary to the spontaneous alpha rhythm at rest, the phase-locked alpha amplitude (echo IAA) was strongly attenuated in the low-luminance viewing condition. Furthermore, we found that the alpha frequency of the phase-locked alpha (echo IAF) shifted towards higher frequencies at low-luminance compared to high-luminance viewing. The alpha amplitude modulation might reflect a reduced capability of the visual system to synchronize its responses to the stimuli, due to a degradation of the signal-to-noise ratio at low luminance. The frequency shift might reveal a basic adaptive strategy to balance the reduced inflow of good quality visual information under low luminance, with an oversampling of the visual inputs. In other words, when the visual inputs are reliable (i.e. under high-luminance viewing) the system facilitates the retention of the sensory representation over time; conversely, when the visual inputs are degraded (i.e. under low-luminance viewing), the system underweights its sensory representations and updates them more quickly, that is at a

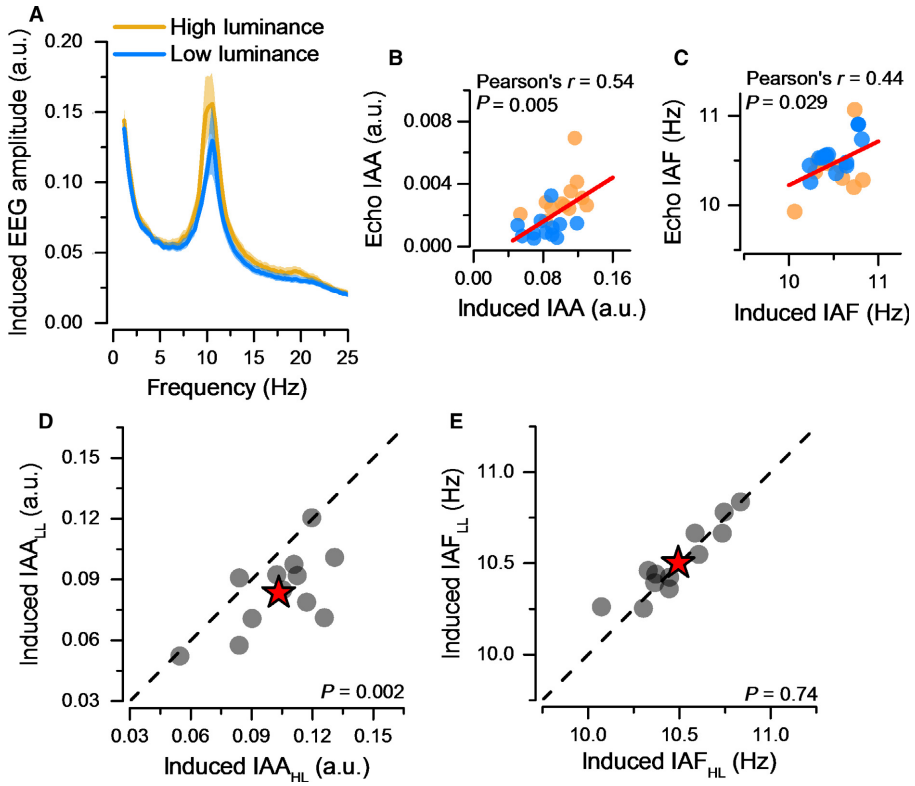

FIG. 5. Experiment 2: induced alpha activity IAA and IAF. (A) Group-mean (±1 SEM) spectrum of the induced EEG recorded at POz, at low-luminance (blue line) and high-luminance (yellow line). (B and C) Correlation between the IAA (B) and IAF (C) computed for the phase-locked (echo) and the induced EEG response. Dots represent the data for single subject at high- and low-luminance (yellow and blue, respectively). Red lines report the best linear model. (D) Scatter plot showing induced IAA for single subjects (dots) and group mean (star) under different luminance viewing conditions. EEG amplitude was higher at high-luminance. Dashed line marks the equality points. (E) Same as D but for induced IAF. No differences were found between induced IAF for the two conditions.

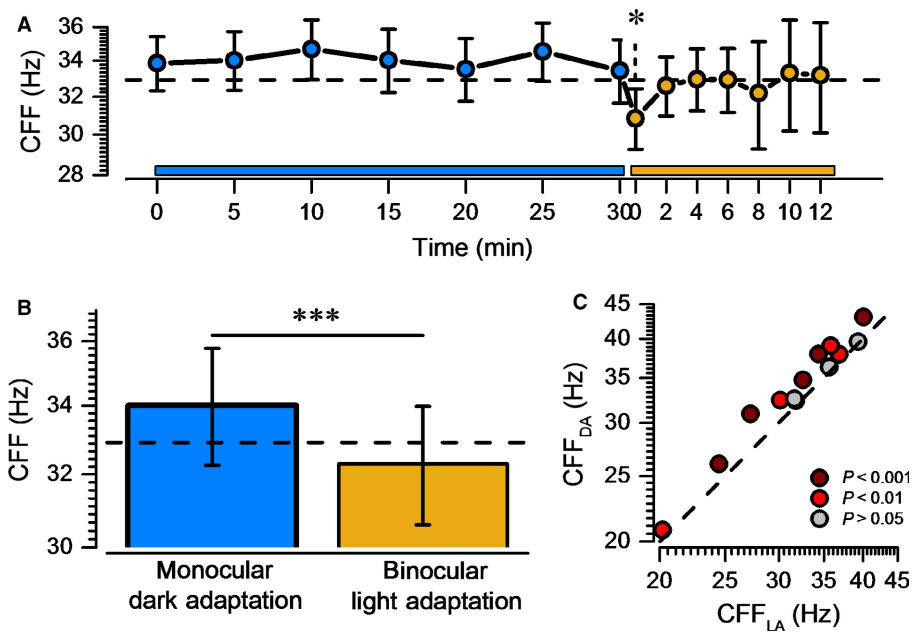

FIG. 6. Experiment 3: CFF. (A) CFF and SEM timecourse as a function of time from monocular dark adaptation (DA, blue dots) and binocular light adaptation (LA, yellow dots). Horizontal dashed line shows the baseline computed before DA. Horizontal blue and yellow lines mark the duration of the DA period and of the LA, respectively. (B) Grand-mean and SEM of the CFF for DA (blue bar) and LA (yellow bar). Horizontal dashed line shows the baseline. **C**, CFF for DA and LA for all subjects. Statistics were computed via a bootstrap procedure. Equality line is shown as dashed line. Asterisks (in A and B) indicate the statistical significance (0.05 > * > 0.01, 0.001 > ***). Colour codes (in C) mark the statistical significance of the difference.

faster frequency. Additionally, we found a consistent phase opposition between the echo functions at high- and low-luminance. It is known that at low-luminance visual processing is slowed down and delayed by about 15 ms for each log-unit attenuation of luminance (Julesz & White, 1969; Williams & Lit, 1983; Kammer *et al.*, 1999), resulting in a constant delay of about 40 ms, in our experiment. A similar constant temporal delay in neural processing would generate a smaller phase difference for slower alpha frequencies and larger for faster alpha frequencies, resulting in a positive correlation between echo IAFs and IAF phase difference. As a matter of fact, we found a positive correlation between the phase difference and the echo IAFs, suggesting that the phase shift reported here is mainly driven by a constant neural delay.

Finally, we investigated the alpha activity induced by visual stimulation in experiment 2 (induced activity, see methods for details). We found that induced and echo IAFs are strongly correlated for both high- and low-luminance conditions. Differently from what shown here from echo IAF, induced IAF did not change across luminance viewing condition, while induced IAA slightly increased at high-luminance viewing. These results contrast with that from the echo function and confirm that the echo and the induced alpha possess peculiar and partially independent properties.

Having found that luminance differences produce a dissociation between spontaneous and stimulus-related alpha activity, we investigated possible behavioural functions linked with these different oscillatory responses. The critical flicker frequency (CFF) is an index of visual temporal segmentation abilities, revealing basic aspects of our visual temporal resolution. It has been suggested that CFF and alpha activities might correlate (Chyatte, 1958; Kooi *et al.*, 1958; May *et al.*, 2014); however, the majority of the reported effects are shown for clinical populations (Karp *et al.*, 1962; May *et al.*, 2014), rely on somatosensory tasks (May *et al.*, 2014), or their results have been questioned (Dondero *et al.*, 1956; Karp *et al.*, 1962). Recent findings have shown that the alpha frequency is crucial in determining our temporal segmentation abilities (Varela *et al.*, 1981; Cecere *et al.*, 2015; Samaha & Postle, 2015; Milton & Pleydell-Pearce, 2016), suggesting a possible link between CFF and alpha oscillations. According to this evidence and our reported findings, we investigated here the effects of luminance viewing condition on CFF, to determine influences of spontaneous and phase-locked alpha activity over our visual temporal resolution. It is known that CFF is modulated by both retinal and central visual processes (Wells *et al.*, 2001) and that binocular light adaptation modulates the critical flicker frequency: it decreases during dark adaptation and increases in the course of light adaptation (Fedorov & Mkrticheva, 1938). Moreover, 3 h of monocular light-deprivation are known to produce a decrease in the CFF for the non-occluded eye (Allen, 1923). Interestingly, it has also been shown that the light adaptation of one eye can modulate the CFF of the other eye in an opposite way (Lipkin, 1962). In his experiment, Lipkin adapted one eye with a steady light and tested the non-adapted eye. He found that an adapting luminance on one eye progressively reduced the CFF on the contralateral eye. Here, we adopted a similar procedure: we dark-adapted the left eye of the subjects for 30 min by applying a NDF patch, while testing the non-deprived right eye (dark adaptation, DA). Next, we removed the patch and continued testing the right eye for 12 min (light adaptation, LA). Note that, in this way, we kept constant the retinal adaptation of the tested eye, while manipulating only the extraretinal light adaptation. In agreement with Lipkin (1962), we showed that only 30 min of monocular dark adaptation induced a fast and transient decrease in contralateral CFF threshold that gradually disappeared after about 12 min from adaptation. Much evidence suggests that this phenomenon could be considered as a plasticity response of the primary visual cortex to luminance changes.

Recently, it has been shown that dark exposure reduces tonic inhibition in visual cortex (Huang *et al.*, 2015) and that monocular deprivation alters early components of visual evoked potentials as well as producing a GABA concentration decrement in the primary visual cortex of adult humans (Lunghi *et al.*, 2015a,b). Interestingly, GABA is suggested to play a key role in generating and maintaining alpha oscillations (Klimesch *et al.*, 2007; Jensen & Mazaheri, 2010), and pharmacological GABA enhancers can reduce spontaneous alpha amplitude (Lozano-Soldevilla *et al.*, 2014). Here, we show that phase-locked alpha – but not spontaneous alpha activity – fits nicely this evidence: echo alpha amplitude decreases at low-luminance, while spontaneous alpha amplitude increases. Moreover, we also found that our visual temporal resolution (once factored out the retinal contribution) matches the dynamics of phase-locked alpha frequency: it is higher under low-luminance viewing condition compared to high-luminance.

To sum up, we show here that the luminance viewing condition strongly impacts over our alpha expression, affecting in a peculiar way spontaneous and induced activity, as well as the EEG visual impulse response function (echo function). Moreover, we speculate that the visual impulse response function changes across different luminance conditions reflect an important plasticity phenomenon impacting brain rhythms: the visual cortex modulates its impulse response function depending on the luminance viewing condition, and these modulations impact on very low-level stages of visual processing, such as flicker perception. However, future experiments would be needed to provide more concrete evidence for this hypothesis.

Conflict of interest

The authors declare no competing financial interests.

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Author contributions

A.B. and R.V. conceived the study and designed the methodology; A.B. and D.L-S. performed the experiment; all authors performed the analyses of the experimental data and wrote the article.

Data accessibility

The article's supporting data and materials will be accessible on 10.6084/m9.figshare.5688796

Abbreviations

CFF, critical flicker frequency; DA, dark adaptation; IAA, individual alpha amplitude; IAF, individual alpha frequency; LA, light adaptation; NDF, neutral density filter.

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