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Visual cues of threat elicit greater steady-state electroencephalographic responses than visual reminders of death

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Highlights

- We tested whether visual reminders of death have a greater impact on the magnitude of steady-state EEG visual responses compared to unspecific threat-related content.
- In two separate experiments, we found greater frequency-tagged EEG spectral power associated with threat-related images.
- Bayesian statistics support the notion of strong evidence for this alternative hypothesis.

Abstract

Terror management theory (TMT) suggests that reminders of death activate an exclusive anxiety mechanism different from the one activated by other types of symbolic threats. This notion is supported by evidence showing how experimental participants verbally reflecting on their own death are then influenced in their opinions and behaviours. A previous study showed that magnitude of electroencephalography (EEG) activity is greater when images depicting death-related content are coupled with painful thermal stimuli compared to threat-related content. Here we expand on previous research by testing whether similar effects may be brought about by passive observation of generic visual reminders of death. More precisely, we hypothesised that fast periodic presentation of death-related vs. more generic threat-related images determine a preferential modulation of brain activity measured by means of EEG. In two experiments, we found that images depicting death content elicit lower frequency-tagged EEG response compared to more generic threat images. Visual evoked potentials revealed that a brief change of the scene from neutral to threat content elicits greater amplitude at the late latencies (compatible with a P300 potential), particularly at the parieto-occipital sites. Altogether, our findings suggest that, in a context where no reflection on death cues is allowed and no threatening stimuli in other modality occur, visual death cues trigger lower neural synchronisation than that elicited by similarly negative and arousing cues with divergent threatening meaning.

Keywords: affective pictures, death, electroencephalography, terror management theory, threat.

Introduction

Emotionally intense cues gain preferential access to attentional resources due to their intrinsic motivational relevance compared to neutral cues (Margaret M. Bradley et al., 2003; Vuilleumier, 2005). According to the dimensional circumplex model of emotion (Russell, 1980), visual stimuli with positive or negative valence draw attention and tax cognitive resources more than neutral stimuli (Margaret M. Bradley et al., 2003; Keil et al., 2003). Similarly, visual stimuli that possess arousing content, and that leads to changes in the emotional/affective states of the onlooker, can gain preferential access to cognitive resources (Bernat, Patrick, Benning, & Tellegen, 2006; M M Bradley, Codispoti, Cuthbert, & Lang, 2001)

A great deal of experimental work has studied the effect of standardised affective pictures (often extracted from the International Affective Picture System, IAPS; Lang et al., 2008) on subjective and cortical correlates of emotional states in healthy laboratory volunteers (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Hajcak, MacNamara, Foti, Ferri, & Keil, 2013). Studies investigating the brain correlates of affective pictures often rely on electrophysiological responses obtained through the recording of electroencephalography (EEG). Experimenters measure event-related potentials (ERPs) during the presentation of images at a slow pace but also steady-state evoked potentials and spectral magnitude of EEG activity during the fast presentation of visual stimuli (Keil et al., 2003; Kemp, Gray, Eide, Silberstein, & Nathan, 2002).

Here we built on previous research on affective pictures to address a question that has only recently attracted the attention of neuroscientists: do cues of death have a specific effect on brain activity? The recent interest in studying the effect of death–related brain processing is motivated by findings in the field of experimental and social psychology supporting the notion that human’s awareness of death has a unique and pervasive effect on individuals’ behaviour, opinions and attitudes. According to the terror management theory (TMT), a dominant theory in the past 30 years (see Pyszczynski, Solomon, & Greenberg, 2015 for a critical review), the basic psychological conflict between wanting to live and having the self-awareness that death is inevitable generates an unfortunate by-product in humans, namely existential anxiety. Cultural and personality factors may act as mediators of anxiogenic effects caused by the awareness of death. Believing that we are a valuable member of a group/community that shares a set of values and beliefs provides a sense of meaningful continuation after our biological death (Pyszczynski, Greenberg, Solomon, Arndt, & Schimel, 2004).

Effects of death reminders on behaviour and subjective experience have been replicated in approximately 20 different countries with more than 5000 studies since the 80s (Hayes, Schimel, Arndt, & Faucher, 2010; Rosenblatt, Greenberg, Solomon, Pyszczynski, & Lyon, 1989 and Burke, Martens, & Faucher, 2010 for a meta-analytic assessment). For instance, reminders of mortality are associated with generating negative reactions against individuals who threaten the participant's personal beliefs, and increased support for ideas that are consistent with one's worldview (Greenberg et al., 1990; Greenberg, Porteus, Simon, Pyszczynski, & Solomon, 1995; Schimel et al., 1999). Research has shown that these effects are best observed if distraction from death content and a time lapse occurs. TMT theorists proposed a dual-process model to account for different cognitive defences individuals put forth to manage the existential dread associated with death reminders. According to the model, 'proximal' defences (i.e. explicit, voluntary, conscious) imply denying or rationalising death. In contrast, 'distal' defences (i.e. implicit, automatic, unconscious) imply development of self-esteem and cultural beliefs as to buffer existential anxiety (Greenberg, Arndt, Simon, Pyszczynski, & Solomon, 2000). This way of defending against thoughts of death does not necessarily elicit a conscious experience of anxiety or distress (Winkielman & Berridge, 2004 for a critical discussion). The classical experimental manipulation entails having individuals pondering their own mortality (i.e. mortality salience) and then diverting their focus of attention from the idea of death, thus ensuring unconscious distal effects would arise and exert implicit modulation of the dependent variable (Burke et al., 2010).

Despite a wealth of research investigating both proximal and distal effects on psychological variables, few studies have investigated the neural evidence in support of TMT and the dual defence model. For instance, Henry et al. (2010) found a modulation of ERPs amplitude when participants were viewing emotional facial expressions of in-group and outgroup members following mortality salience induction. This suggested that mortality threat may have an influence on the neural processes associated with person perception and categorisation. However, the authors made no mention of how these findings would be interpreted according to the dual process hypothesis. Conversely, Klackl et al. (2013) found an increase in late ERPs while participants were processing death-related compared to generally unpleasant words. They interpreted such increase as a neurophysiological correlate of proximal defences.

At variance with this interpretation we recently proposed that brain responses may reflect activation both proximal and distal defence mechanisms occurring during elaboration of death-related cues (see Valentini, Koch, Nicolardi, & Aglioti, 2015 for a critical discussion). Regardless of what mechanism may explain the neural effects observed so far, studies reported specific effects of death-related content using either the traditional “mortality salience” mind-set induction (Henry et al., 2010; Valentini, Koch, & Aglioti, 2014; Valentini, Koch, Nicolardi, et al., 2015) or linguistic cues without requiring participants to reflect on their own death or a distraction procedure (Han, Qin, & Ma, 2010; Klackl et al., 2013; Quirin et al., 2012). In a very recent experiment (Valentini, Nicolardi, & Aglioti, 2017), we coupled affective pictures to painful stimuli of different intensities in the context of a conditioning paradigm, and found that visual reminders of death were associated with greater alpha desynchronisation over parieto-occipital electrodes when compared to threat-related pictures. These findings suggest the presence of a contextual crossmodal modulation acting on visual EEG responses. Such modulation may reveal competitive cortical mechanisms during perception of visual reminders of death compared with similarly arousing negative valence images.

Building on previous evidence, here we tested the hypothesis that passive observation of visual death-related content would trigger greater synchronised brain activity recorded from parietal and occipital regions of the scalp when compared to threat-related content. To this aim we adopted Fast Periodic Visual Stimulation (FPVS), without co-occurring stimuli in other sensory modalities (Rossion, 2014). FPVS brings about neuronal synchronisation locked to the periodic rate of a flickering stimulus, thus producing a periodic EEG response that can be studied in both the frequency and in the time domain (see Norcia, Gregory Appelbaum, Ales, Cottereau, & Rossion, 2015 for a review; Regan, 1977). Most recent applications showed that high-order periodic changes (e.g. face vs. object) of the visual features do produce consistent detectable responses (Rossion & Boremanse, 2011).

We used an oddball design (Liu-Shuang, Norcia, & Rossion, 2014) with neutral images constituting the standard familiar events and the death or threat scenes being the oddball deviant event. Images were delivered with a stimulus onset asynchrony of 200 ms, thus resulting in a base rate presentation of 5 Hz (*F*). We presented the oddball/deviant each five images per second, thus resulting in a deviant rate of 1 Hz (*F/5*). The activity elicited by the base rate presentation can be considered as a general measure of visual processing with mixed contribution of low and high order elaboration (e.g. Dzhelyova, Jacques, & Rossion, 2017) whereas the activity elicited by the oddball frequency can be conceived as reflecting the pure contrast between the two experimental conditions. Besides being successfully implemented in the study of high-level visual processes, the frequency-tagging approach has already proved to be sensitive and specific also in the study of auditory and somatosensory processes (Colon, Legrain, & Mouraux, 2012; Nozaradan, Peretz, Missal, & Mouraux, 2011). Crucially, this design allowed us to obtain objectively detectable responses (i.e. at predefined frequencies) through which contrast the magnitude of visual-related brain responses linked to death or threat scenes. According to the previous literature, we expected greater power of synchronised EEG activity during the presentation of deviant (i.e. 1 Hz) death-related content whereas no significant difference was expected at the base rate presentation (i.e. 5 Hz). These hypotheses were tested using both frequentist and Bayesian hypothesis testing approach. The rationale of using Bayesian analyses is twofold. First, they allow us to quantify the evidence supporting both H0 and H1 (Wagenmakers, 2007). Therefore, we provide evidence not only on whether a difference takes place but also on the direction of this difference (i.e., not only if H0≠H1 but also if- H0<H1 or H0>H1). Second, they allowed us to test the robustness of the findings by assessing the role of different probability distributions (i.e. priors).

Materials and Methods

Participants

Eighteen right-handed healthy individuals (13 females) participated in *experiment 1* (mean±SD age, 28.9±9.2). Twenty-three right-handed healthy individuals (15 females) participated in *experiment 2.* However, one female participant was excluded because of her extremely poor performance in the behavioural task. Thus, data analyses were performed on the remaining 22 individuals in *experiment 2* (mean±SD age, 23.7±4.3). All participants had normal or corrected-to-normal vision and were naïve as to the purpose of the experiment. None had a history of neurological or psychiatric disease. All gave written informed consent and were debriefed at the end of the experiment. The experimental procedures were approved by the University of Essex ethics committee and were in accordance with the standards of the Declaration of Helsinki.

Stimuli

On the basis of the methodological approach used in a previous study (Valentini et al., 2017), 66 images from the IAPS database (Lang, Bradley, & Cuthbert, 2005) were extracted (22 with neutral valence, 22 with death-related content and 22 with threat-related content). A smaller dataset (n=27) of 9 images per each affective category (9 neutral, N; 9 death-related, D; 9 threat-related, T) were selected for being further submitted to an online survey, in which 45 respondents rated the images according to their ability to evoke a sense of threat, brevity of life, fear of death, disgust, and surprise on a 1 to 5 scale ("Not at all", "Slightly", "Moderately", "Very", "Extremely"; see Appendix 1). The current study capitalised on a variable range of image types. For example, death-related images depicted a dead individual in a coffin sorrounded by grieving individuals (#2799), a graveside scene with two individuals standing by a tombstone (#9220) and a bunch of skulls (#9440). Conversely, threat-related images showed scenes of social violence (#2691) and distress (#9429), food contaminated with insects (#7380) and a snake in attack posture (#1090). From the results of the survey, 8 images from each category (n=24) were selected for the experiment (displayed in Appendix 2). All images were grey-scaled and presented at a 320x240 pixels resolution in an upright position The E-Prime© software (Schneider, Eschman, & Zuccolotto, 2002) was used to control the onset/offset of visual stimuli, displayed on a 15x11.4 inches CRT monitor, with a refresh rate of 85Hz and resolution of 800x600.

EEG recording

Sixty-two Ag/AgCl electrodes (Easycap, BrainProducts GmbH, Gilching, Germany) were used to obtain EEG recordings (Synamps RT, Neuroscan, Compumedics). The electrodes were placed according to the positions of the 10-20 International System. The impedance of the electrodes was kept below 10 kΩ, and the EEG signal was amplified and digitised at 1000 Hz. The reference was at the left earlobe and the ground at AFz.

Experimental design and procedure

Experiment 1

Participants were seated in a comfortable chair in a silent, temperature-controlled room at a distance of approximately 70 cm from the screen (mean±SD, 73.2±4.7 cm) with a visual angle of ~3.1° in width and ~2.2° in height away from fixation. After EEG cap montage and quality check of the signal, four recording blocks were performed. The experimental design contrasted the observation of images with death- or threat-related content during either periodic or aperiodic presentation rate (Fig. 1, top and central panels), thus leading to four experimental conditions: Periodic Death (PD), Periodic Threat (PT), Aperiodic Death (AD), Aperiodic Threat (AT).

All the images (N, D, T) were delivered at a fixed rate of 200 ms per image (±2 ms average delay), thus building the base rate presentation pattern (F = 5 Hz). Images were presented in a square wave mode according to the screen refresh rate (i.e. there was blunt change from one image to the other). Deviant events in the periodic condition were delivered at a frequency of 1 Hz, that is once each four repetitions of four different neutral images (F/5 = 1 Hz) (see Rossion, Torfs, Jacques, & Liu-Shuang, 2015 as reference). The rationale of using an aperiodic or non-periodic presentation relies on controlling for the effect of presentation pattern in interpreting differences between visual content in the deviant frequency. Fast periodic visual stimulation allowed us to elicit a fast periodic response in the EEG that can be measured in the time and frequency domain (Regan, 1966; 1968, 1977).

Each stimulus list consisted of 40 stimuli and was repeated 14 times for a total of 560 events per block. Each repeated list contained eight aversive images with death- or threat-related content (112 in total) and thirty-two images with neutral content (448 in total), and lasted for 112 s. The order of blocks was balanced across participants. As mentioned above, the periodic condition presented an equal number of neutral images between each aversive deviant image. On the contrary, the position of the images in the aperiodic blocks was pseudorandomised to ensure that there was no stable recurrent pattern of images across participants. No aversive image was repeated in a single list run of both periodic and aperiodic presentation. Moreover, we prevented the very same neutral image from being repeated as this would have caused a significant change in the perceived duration of the event (400 ms rather than 200 ms). In addition, at least two neutral images were interleaved between two consecutive aversive images.

A blue fixation cross was superimposed on the images and pseudorandomly turned to yellow throughout the experimental testing. Participants were asked to verbally recall the number of times the cross changed colour from blue to yellow at the end of each block. The aim of the fixation task was to ensure participants remained focussed on the images throughout the experiment and at the same time did not focus their overt attention on the content of the images, thus fostering a covert attentional effect of the image content of brain activity. The colour change of the fixation cross was randomly arranged during the aperiodic blocks. The fixation cross did never change colour immediately before or after a deviant image, and never on a deviant image. This caveat prevented a direct interference of the fixation task on the putative effects associated with the passive observation of the deviant images.

Prior to starting the experiment participants were familiarised with the speed of the presentation of the background images and practiced the fixation cross task. In between experimental blocks participants were given a short pause of 2 minutes in order to avert boredom or tiredness caused by the task and to help participants maintain focus on the fixation task. At the end of the visual presentation participants were fully debriefed and were asked a few questions. Participants were asked whether they could tell apart different types of images and whether they felt emotions during the experimental session (see Appendix 6 for a template of the debriefing questions). The experiment took approximately 60 minutes from EEG set-up to debriefing.

Experiment 2

Participants sat at a distance of approximately 60 cm from the screen (mean±SD, 59.9±2.0) with a visual angle of ~3.91° in width and ~2.94°in height away from fixation. The experimental design and procedure of *experiment 2* was identical to *experiment 1* except for what follows. Each of the 4 blocks contained a single list of 560 images and lasted for 112 s. We changed the delivery pattern as we noticed that in *experiment 1* the EEG captured the refresh rate of stimulation lists administered by the stimulation software. The spectral response can be appreciated in Fig. 2 (panel A) and is centred on 0.125 Hz (and corresponding to 8000 ms resulting from the list cycle of 200 ms * 40 images). As this response could virtually affect the identification of the 1 Hz deviant response we changed the list cycle to eliminate this technical flaw.

Experiment 2 implemented a different control condition. We replaced the aperiodic presentation with periodic presentation of scrambled images as to be able to further generalise the purported difference between periodic presentation of death and threat-related pictures and exclude the contribution of low-order effects. We contrasted the observation of either intact or scrambled images with death- or threat-related content during periodic presentation rate (Fig. 1, bottom panel), thus leading to four experimental conditions: Intact Death (ID), Intact Threat (IT), Scramble Death (SD), Scramble Threat (ST). Scrambled versions of the stimuli were made by using the functions pixelate and mosaic (cell size 15) in Photoshop (Adobe).

Data analysis

EEG data pre-processing

EEG data were pre-processed and analyzed using EEGLAB (Delorme & Makeig, 2004) and Letswave 5 (http://nocions.webnode.com/letswave). Individual participants' data were first re-sampled at 500 Hz. Power line-related sinusoidal artifacts (50-100 Hz) were removed using Cleanline (http://www.nitrc.org/projects/cleanline). Data were then band-pass filtered from 0.1 to 100 Hz (filter order 2) and further corrected using the Artifact Subspace Reconstruction (ASR) plugin (Mullen et al., 2013). We used only the "burst" and "window" criteria with conservative values of 15 and 0.5 respectively. These criteria allowed us to keep all the stimulus events within each recording block while correcting the signal from significant artifacts. Data were then re-referenced to the average of the earlobes and further processed using independent component analysis (ICA, Makeig, Bell, Jung, & Sejnowski, 1996) to subtract eye and muscle-related artifacts, aided by the multiple artifact rejection algorithm (MARA, Winkler, Haufe, & Tangermann, 2011). Data resulting from the ICA were re-

referenced to the average of all electrodes and cropped in equally sized data chunks (113.19 s and 113.17 for *experiment 1* and *2* respectively).

Frequency spectrum analysis

We applied a discrete Fourier transform and output the normalised power (μV^2) spectra (N/2) for the frequency range 0-500 Hz for each participant. This operation yielded spectra with high frequency resolution of 0.009 Hz for both experiments, thus ensuring an optimal signal-to-noise ratio and precise identification of the response at the frequencies of interest (i.e., 5 Hz, 1 Hz) with the exclusion of the deviant and base rate harmonics.

We took into account the variations of noise across the EEG spectrum by computing two types of noise-subtracted spectra. We subtracted, at each bin of the frequency spectra, the average power measured at 20 surrounding frequency bins (10 on each side, excluding the immediately adjacent two bins). We also computed the signal-to-noise ratio (SNR) which was calculated as the ratio between the amplitude at each frequency and the average amplitude of the 20 surrounding frequency bins (excluding the immediately adjacent two bins; e.g., Liu-Shuang et al., 2014). As statistical findings did not differ between the two types of noise-subtracted spectra we used SNR spectra for graphical purposes and also reported SNR spectral values to assist the reader in further assessing the magnitude of the two main responses, particularly the response at the deviant frequency.

To estimate differences between conditions in the deviant (1 Hz) and base rate (5 Hz) frequencies on the noise-subtracted data we averaged spectral power over a pool of electrodes. First, we averaged power across the whole scalp by pooling all the electrodes, thus providing a preliminary assessment of the experimental effects without assumptions on their topographical distribution. Second, we analysed differences on three a priori regions of interest (ROI) established at the level of the scalp occipital and bilateral parieto-occipital regions. The electrodes chosen for the occipital and parieto-occipital ROIs were selected based on the profile of the largest power detected across the electrodes in the target regions that importantly confirmed the pattern of peak activity observed in the previous literature (Rossion et al., 2015; Norcia et al., 2015). Similar to the findings in the context of fast presentation of faces (e.g., Dzhelyova et al., 2017), we observed two different topographical profiles for the deviant and base rate responses (see Fig. 2 and 4). Therefore, we built different regions of interest for the deviant and base rate response. The left parieto-occipital ROI (LPO) contained electrodes PO7, P5, P7 for 1 Hz response and electrodes O1, PO3, PO5, PO7, P3, P5, and P7 for 5 Hz response. The right parieto-occipital ROI (RPO) contained electrodes PO8, P6, P8 for 1 Hz response and electrodes O2, PO4, PO6, PO8, P4, P6, and P8 for 5 Hz response. The central occipital ROI (OCC) contained electrodes O1, O2, Oz, POz for both 1 Hz and 5 Hz responses.

Time domain analysis

Pre-processed averaged-referenced data were band-pass filtered with a Butterworth filter from 1 to 30 Hz with filter order 4. We then applied a fast Fourier transform (FFT) notch filter with 0.1 Hz width to selectively remove the dominating base frequency (5 Hz) and the following 4 harmonics (Dzhelyova & Rossion, 2014). Continuous data were then segmented in 1200 ms epochs (6 cycles of the base rate) for each experimental condition and time-locked to the onset of the deviant event. Epochs were then baseline corrected using the 200 ms pre-stimulus activity (1 cycle of the base rate). These baseline-corrected epochs were then grand-averaged for each of the four conditions separately. Overall, there were 2016 epochs in *experiment 1* and 2464 in *experiment 2* (no epoch was excluded in the pre-processing stage).

Statistical data handling

We computed Wilcoxon signed-ranks test to assess differences between ratings provided in the preliminary survey. We computed ANOVA to analyse behavioural accuracy in the fixation cross task. We first computed the proportion of correct reports by dividing the reported changes by the expected correct amount of changes in each block, thus resulting in a reference accuracy index of 1.

In the analysis of EEG data, we first extracted the mean power within 0.98 and 1.02 Hz for the deviant rate (1 Hz) and from 4.9 and 5.1 Hz for the base rate (5 Hz) as to account for small temporal jitters at the two experimental frequencies. We then performed t-tests for each baseline-subtracted spectral response and ROI to assess the different expected pairwise differences. On the basis of previous research we expected to find no difference between death and threat scenes during aperiodic (*experiment 1*, AD vs. AT) and periodic scramble presentation (*experiment 2*, SD vs. ST). At the same time, we expected the magnitude of spectral activity to be greater for each level of *content* during periodic than during aperiodic presentation (*experiment 1*, AD vs. PD and AT vs. PT) or periodic scramble presentation (*experiment 2*, SD vs. ID and ST vs. IT). Finally, the most relevant hypothesis posited that death pictures during periodic presentation should have shown greater spectral magnitude than threat pictures in both experiments (PD vs. PT and ID vs. IT). We report Cohen's *d* (using pooled SD of death and threat conditions) as measure of effect size.

To estimate the time course of the effects observed in the frequency domain, we performed an analysis of the temporal envelope time-locked to the deviant stimulus. Therefore, time domain analysis was performed only on the ROIs that revealed a difference in spectral power between death- and threat-related scenes during periodic presentation in both experiments. To this aim we computed whole-waveform t-tests with correction for multiple comparisons, by means of the cluster-level randomisation (Maris & Oostenveld, 2007) to identify differences between amplitudes across the experimental conditions. Cluster-level randomisation allowed us to control the Type-1 error rate involving multiple comparisons in the temporal dimension (1000 permutations; Z>2 threshold; P<0.05, two tailed).

As additional informative analysis, we also performed Bayesian t-tests to further assess the main directional hypothesis that death pictures during periodic presentation should have shown greater spectral magnitude than threat pictures in both experiments (PD vs. PT and ID vs. IT). The advantage of using Bayesian statistics (Jarosz & Wiley, 2014 for a discussion) rests on the ability to quantify the evidence in support of both H0 and H1. Here, we aimed to ponder evidence supporting i) no significant difference in EEG power between death and threat, or ii) greater power for death vs. threat, or iii) lower EEG power for death vs. threat.

We extracted Bayes factors (BF) using JASP (JASP Team, 2018) for the three competing models only for the 1 Hz deviant frequency and only on the ROIs that best accounted for associative visual processing (namely, RPO and LPO) in each experiment. As we had no clear estimate of the expected effect size of the difference between death and threat EEG power, we sat the Cauchy prior distribution width to the JASP default value of r = 0.707. However, we also computed robustness and sensitivity analyses to look into the role of priors and sample size in estimating the BF (see Appendix 6).

Results

Preliminary survey on visual stimuli

Median ratings (±IQR) for each of the 8 selected IAPS pictures per category are reported in Appendix 3. We first median-centered each type of rating by dividing each median per its group median and then divided this value by the group median rating attributed to the neutral images for that type of psychological construct (e.g. rating of valence). Table 1 shows the median ratio for the selected D and T pictures.

We then computed the Wilcoxon matched pairs test to assess differences between death and threat-related ratings. The lack of difference between death and threat-related content in valence and arousal confirmed the effectiveness of the selection procedure (valence: Z=1.61, P=0.11; arousal: Z=1.63, P=0.10). There was no difference between death and threat-related content also in perceived disgust (Z=0.03, P=0.98), sense of threat (Z=0.89, P=0.37), or surprise (Z=0.21, P=0.83). As expected, the respondents assigned greater ratings of fear of death for death-related content (2±2 vs. 1±1; Z=2.81, P=0.005), and greater sense of brevity of life (2±1.5 vs. 1±0.5; Z=4.12, P<0.001).

Behavioural task

Experiment 1

ANOVA revealed no difference in accuracy across the different types of *presentation* (F_{17}=0.18, P=0.68) or *content* (F_{17}=0.24, P=0.63). No interaction between these two factors was detected (F_{17}=1.78, P=0.20).

Experiment 2

ANOVA revealed no difference in accuracy across the different types of *presentation* (F_{21}=0.08, P=0.77) or *content* (F_{21}=0.85, P=0.37). No interaction between these two factors was detected (F_{21}=0.11, P=0.75).

In sum, both experiments showed that participants remained engaged throughout the experiment and were focussed on the fixation task, thus being largely accurate in detecting fixation changes regardless of the experimental condition. This also ensures that the deviant-related visual activity measured through EEG is purportedly independent from voluntary overt attention.

Frequency analysis

Table 2 provides an overall summary of this analysis.

Experiment 1

Deviant frequency (1 Hz)

Fig. 2 (panel A) shows the grand-averaged SNR spectral response to deviant pictures averaged across the whole scalp electrodes, as well as the peak topographical activity located at bilateral parieto-occipital regions. T-tests on whole scalp baseline-subtracted spectra revealed no difference in spectral power during aperiodic presentation (AD vs. AT: t_{17}=-1.06, 95% CI [-.01, .004], P=0.30; d=0.26). As expected, the response was greater for periodic than aperiodic presentation for both types of pictures, thus confirming the effective tagging of the deviant event in the periodic presentation (AD vs. PD: t_{17} =-3.60, 95% CI [-.03, -.009], P=0.002, d=1.01; AT vs. PT: t_{17}=-2.77, 95% CI [-.05, -.007], P=0.01, d=0.90). However, we found no difference in spectral power between death- and threat-related scenes during periodic presentation (PD vs. PT: t_{17}=-1.55, 95% CI [-.03, -.004], P=0.14; d=0.35).

The analysis of RPO ROI confirmed the same pattern (Fig. 3, left). That is, no difference between content during aperiodic presentation (AD vs. AT: t_{17}=-1.12, 95% CI [-.05, .05], P=0.90; d=0.04) and greater power during periodic than aperiodic presentation (AD vs. PD: t_{17}=-3.10, 95% CI [-.17, -.03], P=0.007, d=1.07; AT vs. PT: t_{17}=-3.94, 95% CI [-.24, -.07], P=0.01, d=1.22). Again, we found no difference in scalp spectral power between death-and threat-related scenes during periodic presentation (PD vs. PT: t_{17}=-1.50, 95% CI [-.14, .02], *P*=0.15; d=0.40).

The analysis of LPO ROI revealed differences in spectral power across all the planned comparisons (Fig. 3, centre). There was a general a general greater activation during presentation of threat scenes regardless of the presentation modality (AD vs. AT: t_{17}=-3.73, 95% CI [-.07, -.02], *P*=0.002; d=0.95;; AT vs. PT: t_{17}=-3.52, 95% CI [-.18, -.04], *P*=0.003, d=0.94; PD vs. PT: t_{17}=-2.49, 95% CI [-.12, -.01], *P*=0.02; d=0.52),, but also greater spectral power during observation of deviant death scenes during periodic than aperiodic presentation (AD vs. PD: t_{17}=-4.77, 95% CI [-.13, -.05], *P*<0.001, d=1.46). On the contrary, the analysis of OCC ROI revealed no difference in spectral power (Fig. 3, right) during aperiodic presentation (AD vs. AT: t_{17}=0.48, 95% CI [-.01, .02], *P*=0.64; d=0.14), as well as during periodic presentation (PD vs. PT: t_{17}=-1.62, 95% CI [-.06, .01], *P*=0.12; d=0.44) and within the same content when contrasting the two presentation modalities (AD vs. PD: t_{17}=-0.23, 95% CI [-.02, .02], *P*=0.82, d=0.06; AT vs. PT: t_{17}=-1.83, 95% CI [-.07, .005], *P*=0.08, d=0.58))

Base frequency (5 Hz)

Fig. 4 (panel A) shows the grand-averaged SNR spectral response to each picture averaged across the whole scalp electrodes, as well as the peak topographical activity located at bilateral parieto-occipital regions. T-tests revealed no difference in the whole scalp spectral power during aperiodic presentation (AD vs. AT: t_{17}=-0.25, 95% CI [-.003, .002], *P*=0.80; d=0.01). Similarly, no difference was found for each type of content between the two types of presentation pattern (AD vs. PD: t_{17}=1.66, 95% CI [-.001, .01], *P*=0.11, d=0.14; AT vs. PT: t_{17}=-0.82, 95% CI [-.01, .003], *P*=0.42, d=0.07). We found no difference in spectral power between death- and threat-related scenes during periodic presentation (PD vs. PT: t_{17}=-1.66, 95% CI [-.01, .001], *P*=0.11; d=0.22).

The same pattern resulted from the analysis of the RPO ROI (Fig. 5, left). There was no difference in power during aperiodic presentation (AD vs. AT: t_{17}=0.17, 95% CI [-.01, .01], P=0.86; d=0.01) as well as during periodic presentation (PD vs. PT: t_{17}=-1.56, 95% CI [-.03, .004], P=0.14; d=0.18) and within the same content when contrasting the two presentation modalities (AT vs. PT: t_{17}=-0.90, 95% CI [-.01, .01], P=0.38, d=0.05;AD vs. PD: t_{17}=1.50, 95% CI [-.004, .02], P=0.15, d=0.14)).

Similarly, the analysis of LPO ROI (Fig. 5, centre) revealed no differences in spectral power across the planned comparisons (AD vs. AT: t_{17}=-0.37, 95% CI [-.01, .004], P=0.72; d=0.02; AT vs. PT: t_{17}=-0.84, 95% CI [-.01, .01], P=0.41, d=0.11; PD vs. PT: t_{17}=-1.70, 95% CI [-.03, .003], P=0.11; d=0.30) but a significant greater spectral magnitude during aperiodic than periodic presentation of death scenes (AD vs. PD: t_{17}=2.21, 95% CI [.0003, .01], P=0.04, d=0.20).

The analysis of OCC ROI revealed no difference in spectral power across all the planned comparisons (Fig. 5, right; AD vs. AT: t_{17}=-0.26, 95% CI [-.01, .01], P=0.80; d=0.02; AD vs. PD: t_{17}=0.53, 95% CI [-.01, .01], P=0.60, d=0.04; AT vs. PT: t_{17}=-0.48, 95% CI [-.01, .01], P=0.64, d=0.04; PD vs. PT: t_{17}=-0.95, 95% CI [-.02, .01], P=0.35; d=0.10).

Bayesian assessment of parieto-occipital activity during deviant frequency (1 Hz)

Bayesian t-test on RPO ROI revealed only anecdotal evidence for the null hypothesis (non-directional PD ≠ PT model), with a BF of 1.59 and a 95% credible interval range from -1.03 to 0.17, thus suggesting the presence of a likely difference between the two conditions. The test of the alternative models led to supporting evidence for lower EEG power during PD than PT. We obtained moderate/strong evidence for H0 when adopting a directional PD > PT model, with a BF of 9.08 and a 95% credible interval range from 0.004 to 0.43. And yet we gathered only anecdotal evidence for H- in the model assuming PD < PT, with a BF of 1.14 and a 95% credible interval range from -1.06 to -0.04.

A similar pattern was observed for the left hemisphere ROI. Bayesian t-test on LPO ROI revealed anecdotal evidence for the null hypothesis (non-directional PD ≠ PT model), with a BF of 0.38 and a 95% credible interval range from -1.37 to -0.05. We obtained strong/moderate evidence for H0 when adopting a directional PD > PT model, with a BF of 11.98 and a 95% credible interval range from 0.01 to 0.29. Similarly, we gathered a moderate evidence for H- in the model assuming PD < PT, with a BF of 5.18 and a 95% credible interval range from -1.37 to -0.12. See Appendix 6 for sensitivity analyses and robustness checks.

Experiment 2

Deviant frequency (1 Hz)

Fig. 2 (panel B) shows the spectral response to deviant pictures averaged across the whole scalp electrodes. T-tests revealed no difference in the whole scalp spectral power between the two scramble control conditions (SD vs. ST: t_{21}=-0.001, 95% CI [-.004, .004], *P*=1.00; d=0.80). The response was greater for intact than scramble pictures, thus confirming the effective tagging of the deviant event when images could be properly discriminated (SD vs. ID: t_{21}=-3.99, 95% CI [-.05, -.01], *P*<0.001, d=0.72; ST vs. IT: t_{21}=-4.63, 95% CI [-.003, -.03], *P*<0.001, d=1.31). Importantly, we found a difference in spectral power between intact death- and threat-related scenes, an effect that was explained by greater spectral power during observation of deviant threat than death scenes (ID vs. IT: t_{21}=-3.37, 95% CI [-.05, .01], *P*=0.003; d=0.59).

The analysis of RPO ROI and LPO ROI (Fig. 6, left and centre) confirmed the same pattern observed for the whole scalp. That is, no content-related difference in spectral power during scramble presentation (SD vs. ST, RPO ROI: t_{21}=0.04, 95% CI [-.01, .01], *P*=0.97; d=0.01; LPO ROI: t_{21}=-0.80, 95% CI [-.02, .01], *P*=0.43; d=0.26) and greater power for intact than scramble presentation (SD vs. ID, RPO ROI: t_{21}=-3.81, 95% CI [-.14, -.04], *P*=0.001, d=1.07; LPO ROI: t_{21}=-3.02, 95% CI [-1.59, -.03], *P*=0.006, d=0.92; ST vs. IT, RPO ROI: t_{17}=-4.86, 95% CI [-.29, -.12], *P*<0.001, d=1.40; LPO ROI: t_{21}=-4.39, 95% CI [-.31, -.11], *P*<0.001, d=1.33). Crucially, we found significant greater spectral power during periodic presentation of intact threat- than death-related scenes (ID vs. IT, RPO ROI: t_{21}=-3.52, 95% CI [-.18, -.05], *P*=0.002; d=0.68; LPO ROI: t_{21}=-3.80, 95% CI [-.18, -.05], *P*=0.001; d=0.64).

Conversely, the analysis of OCC ROI (Fig. 6, right) revealed difference in spectral power only between scramble and intact presentation per each type of content, in that intact images had greater power than scramble ones (SD vs. ID: t_{21}=-3.12, 95% CI [-.12, -.02], *P*=0.005, d=0.82; ST vs. IT: t_{17}=-3.08, 95% CI [-.14, -.03], *P*=0.006, d=0.86) whereas no difference between content was observed within each type of presentation modality (SD vs. ST: t_{21}=-0.30, 95% CI [-.01, .01], *P*=0.77; d=0.08; ID vs. IT: t_{21}=-0.41, 95% CI [-.07, .05], *P*=0.68; d=0.09).

Bayesian assessment of parieto-occipital activity during deviant frequency (1 Hz)

Bayesian t-test on RPO ROI revealed only anecdotal evidence for the null hypothesis (non-directional ID ≠ IT model), with a BF of 0.05 and a 95% credible interval range from -1.59 to -0.30, thus suggesting the presence of a likely difference between the two conditions. The test of the alternative models led to supporting evidence for lower EEG power during ID than IT. We obtained strong evidence for H0 when adopting a directional ID > IT model, with a BF of 16.26 and a 95% credible interval range from 0.01 to 0.17. In a complementary manner, we gathered a strong/very strong evidence for H- in the model assuming ID < IT, with a BF of 38.88 and a 95% credible interval range from -1.58 to -0.30.

The left hemisphere ROI presented a similar pattern. Bayesian t-test on LPO ROI revealed anecdotal evidence for the null hypothesis (non-directional ID ≠ IT model), with a BF of 0.03 and a 95% credible interval range from 0.35 to 1.66. Notably, we obtained moderate/strong evidence for H0 when adopting a directional ID > IT model, with a BF of 11.98 and a 95% credible interval range from 0.01 to 0.29. Similarly, we gathered a strong evidence for H- in the model assuming ID < IT, with a BF of 16.88 and a 95% credible interval range from -0.32 to -0.002. See Appendix 6 for sensitivity analyses and robustness checks.

Base frequency (5 Hz)

Fig. 4 (panel B) shows the spectral response to each picture averaged across the whole scalp electrodes. T-tests revealed no difference in whole scalp spectral power during scramble (SD vs. ST: t_{21}= 1.15, 95% CI [-.001, .003], *P*=0.26; d=0.09) and intact (ID vs. IT: t_{21}=-0.04, 95% CI [-.003, .003], *P*=0.97; d=0.00) presentation. Yet, there was a clear difference between the two types of presentation pattern (SD vs. ID: t_{21}=-3.70, 95% CI [-.01, -.003], *P*=0.001, d=0.44; ST vs. IT: t_{21}=-3.86, 95% CI [-.01, -.004], *P*=0.001, d=0.59) which was explained by greater spectral power during presentation of intact scenes.

The differences observed at whole scalp level was confirmed by the analysis of the parieto-occipital ROIs. Essentially, there was no effect of content in either the scramble (SD vs. ST, RPO: t_{21}=1.86, 95% CI [-.001, .01], *P*=0.08; d=0.13; LPO: t_{21}=0.47, 95% CI [-.005, .01], *P*=0.64; d=0.04; OCC: t_{21}=0.71, 95% CI [-.01, .01], *P*=0.49; d=0.08) or the intact (ID vs. IT, RPO: t_{21}=-0.07, 95% CI [-.01, .01], *P*=0.94; d=0.01; LPO: t_{21}=0.33, 95% CI [-.005, .01], *P*=0.74; d=0.03; OCC: t_{21}=0.67, 95% CI [-.01, .01], *P*=0.51; d=0.09) presentation. However, there was a robust difference between the two types of presentation pattern across the three ROIs. This was explained by greater spectral power during observation of intact than scramble scenes in the RPO (SD vs. ID, t_{21}=-3.94, 95% CI [-.03, -.001], *P*=0.001; d=0.47; ST vs. IT, t_{21}=-3.81, 95% CI [-.04, -.01], *P*=0.001; d=0.64) and LPO (SD vs. ID, t_{21}=-2.27, 95% CI [-.02, -.001], *P*=0.03; d=0.37; ST vs. IT, t_{21}=-2.34, 95% CI [-.02, -.001], *P*=0.03; d=0.45) ROIs. Conversely, the difference was explained by greater power during presentation of scramble scenes in the OCC ROI (SD vs. ID, t_{21}=2.17, 95% CI [.001, .05], *P*=0.04; d=0.31 and ST vs. IT, t_{21}=2.06, 95% CI [-.0001, .04], *P*=0.05; d=0.33).

Time course of the difference between death and threat during periodic presentation

Following up on the frequency domain results, we detected a significant difference between periodic death and threat scenes only in the LPO ROI in *experiment 1*. Time domain analysis of deviant-locked potentials revealed an increase in amplitude of the positive potential during PT than PD (t_{17}=6.64, CI [-1.00, -.58], P<0.001) at 380–476 ms post-stimulus (Fig. 8, A), with a maximum at PO7 (1.91 μv, 380 ms).

In *experiment 2* the analysis of LPO ROI revealed a positive potential that significantly differed in amplitude between ID and IT waveforms at 230–476 ms post-stimulus (Fig. 8, B, top). The difference was explained by greater amplitude for intact threat- than death-related pictures (t_{21}=8.91, CI [-1.31, -.75], P<0.001) with a peak on P7 (3.26 μv, 354 ms). The analysis of RPO ROI revealed a positive potential that significantly differed in amplitude between ID and IT waveforms at 422–513 ms post-stimulus (Fig. 8, B, bottom). The difference was explained by greater amplitude for intact threat- than death related pictures (t_{21}=5.92, CI [-1.24, -.59], P<0.001) with a peak on P8 (2.96 μv, 422 ms).

Discussion

In two different experiments, we tested whether passive observation of quickly presented images (200 ms each) depicting either death- or threat-related scenes differentially affects robust (i.e. high SNR) electrophysiological spectral responses (Fig. 1). We obtained an implicit discrimination between the two categories using a concomitant orthogonal task (i.e. detection of fixation cross changes) that ensured deployment of covert attention (e.g. Rossion et al., 2015) to the affective scenes. As the two image categories were found to be matched in terms of negative valence and arousal in a preliminary survey (Table 1), we assumed that differences in spectral EEG magnitude were due to the specific different meaning of the two set of affective pictures. More specifically, according to the main tenet of the TMT, we expected death-related content to be associated with greater synchronised EEG activity than threat-related content.

Our results do not support this hypothesis and rather indicate that the detection of brief changes of scene content (i.e. from neutral to death or threat) at the specific deviant frequency rate (i.e. 1 Hz) is associated with greater EEG magnitude when the pictures entailed general unspecific threat content (Figs. 2, 3, 6; Table 2). Whilst the first experiment indicated this difference being spatially distributed only at the left parieto-occipital region (Fig. 2, panel A; Fig. 3), the second experiment (methodologically improved and with a larger sample size) revealed a more pervasive difference distributed over the entire scalp and not only limited to the left parieto-occipital region (Fig. 2, panel B; Fig. 6). Interestingly though, the analysis of the occipital ROI did not show a difference in spectral power between the two categories, thus suggesting that the difference observed at the deviant rate may be entirely accounted for by associative and higher order cortices while being unaffected by different bottom-up salience of the stimulus material. Importantly, these differences were not explained by variation in age and gender in both experiments (see Appendix 4 in supplementary material).

Crucially, these results were confirmed and complemented by Bayesian analyses (Appendix 5) that showed how, even with wide priors, data collected in both experiments do not support the null hypothesis of no difference in EEG magnitude during deviance detection. However, the hypothesis that death images elicited greater magnitude than threat was not supported either, and in fact we found moderate to very strong evidence in favour of greater parieto-occipital EEG power (regardless of the hemisphere) during threat than death deviant images, particularly so in experiment 2.

The time course of the difference between death and threat pictures revealed a late prolonged modulation (ranging from 230 to 513 ms) that was predominant at the level of the left parieto-occipital region of the scalp (Fig. 8). Research suggests right hemisphere dominance for emotional processing, particularly of emotional facial expressions (Calvo, Rodríguez-Chinea, & Fernández-Martín, 2014; Demaree, Everhart, Youngstrom, & Harrison, 2005; Gainotti, 2012). However, the right hemisphere dominance finds support mainly in studies where visual stimuli appear with a visual angle beyond the parafoveal boundary (~5° away from fixation). This was not the case in the current study. In fact, the visual angle of affective scenes in either experiments was not beyond parafoveal boundary (i.e. <4°). Crucially, evidence of right lateralisation becomes strong only if levels of valence and arousal are experimentally manipulated (reviewed in Olofsson, Nordin, Sequeira, & Polich, 2008). In fact, as valence and arousal were allegedly perceptually and emotionally matched in the current study, the left hemisphere sensitivity we found may be explained by brain processes associated with object recognition and visual semantic processing rather than only affective processing per se (Gainotti, 2011; Rossion et al., 2000).

The more general spectral response at the base rate frequency (5 Hz) is believed to reflect a mixture of low- and high-level processes (Dzhelyova et al., 2017) and it may be considered as a marker of short term adaptation to visual stimuli. The analysis of the base rate spectral power revealed a partly counterintuitive pattern. Although in both experiments either control conditions (i.e. aperiodic and scramble) were generally eliciting lower magnitude (Fig. 4), we found a smaller magnitude for responses obtained during periodic rather than aperiodic presentation of death-related scenes at the left parieto-occipital ROI in *experiment 1* (Fig. 5). Similarly, we found a greater spectral power during presentation of scrambled images at the OCC ROI in *experiment 2* (Fig. 7). Despite the small size of these effects, they be may be interpreted as reflecting a reduced neural adaptation to more constantly changing visual patterns (i.e. the aperiodic and scramble presentation modalities).

It is noteworthy that the identification of differences between conditions is secured through a robust objective neural marker (Norcia et al., 2015) occurring at a specific pre-determined EEG frequency (i.e. 1 Hz and 5 Hz) and grounded on a priori knowledge. Such methodological and analytical approach allowed us to assess whether a subtle difference in the threat meaning of the images would be associated with a distinct magnitude of the EEG objective marker. Other authors used a similar approach to study early processing of affective pictures and provided substantial evidence that frequency-tagged EEG responses can be enhanced by differences in emotional valence of the visual stimulus (Keil et al., 2003; Keil, Moratti, Sabatinelli, Bradley, & Lang, 2005) even at very fast presentation rates (12 Hz – 83 ms; Peyk, Schupp, Keil, Elbert, & Junghöfer, 2009). Interestingly, more recent evidence seems to support the notion that lower presentation rates (as the ones used in the present study) result in better cortical discrimination of the emotional cue (Bekhtereva & Müller, 2015). The fact that threatening content elicits greater EEG amplitude of frequency-tagged responses than neutral and even pleasant pictures agrees with previous research (Keil et al., 2003). This finding may reflect the engagement of attentional resources by emotional stimuli. However, most of the published studies did not attempt to equate the emotional arousal and therefore the reported differences are most often explained by differences in the arousal dimension alone (Olofsson et al., 2008; Rozenkrants, Olofsson, & Polich, 2008). Here, we expand on previous research by showing that even subtle differences in the meaning/content of affective pictures belonging to the same valence (namely, unpleasant negative valence pictures) can be captured by robust objective EEG markers.

As Pyszczynski et al. (2015) acknowledged in their comprehensive review of TMT research, the goal of disentangling the specificity of death-related cognition from other psychological/conceptual threats is crucial to the generalisability of the TMT. As TMT states that death is a unique paramount motivator of human behaviour, it follows that neural activity associated with the elaboration of this concept should display some level of specificity when compared to other conceptual threats. Although the current bulk of evidence seems to support this conclusion, it does not necessarily preclude less specific interpretations. In fact, other researchers have sought to argue that all threats are functionally equivalent (Jonas et al., 2014; McGregor, Nash, Mann, & Phills, 2010) and may rely on a common overarching anxiety biological system (McGregor, 2006; Tritt, Inzlicht, & Harmon-Jones, 2012).

The current findings seem consistent with our most recent observations on the effect of visual reminders of death on visual EEG responses. When coupled to painful stimuli visual reminders of death induced greater alpha desynchronisation but no greater amplitude in visual evoked potentials, which are expression of synchronised brain activity (see Fig. 5 in Valentini et al., 2017). The current study targeted synchronised activity with more sensitivity and precision than in the previous study. In addition, we studied visual responses in isolation, without co-occurring threatening stimuli in another sensory modality. Lastly, here we tested the difference between death and threat content using two different control conditions in two separated samples.

Although our findings seem not to support a neural dominance of death-related cortical processing they do not necessarily falsify the main tenet of the TMT. In fact, the impersonal connotation of the visual stimuli may trigger proximal (i.e. conscious) and distal (i.e. unconscious) defences processes of lower magnitude than the one triggered during reflective/contemplative situations as in the classical mortality salience procedure (Rosenblatt, Greenberg, Solomon, Pyszczynski, & Lyon, 1989). However, the very same founders of the TMT also showed that the purported effects of reminding people of their death can be achieved by simply showing the word "*death*" at a subliminal level (Arndt, Allen, & Greenberg, 2001; Arndt, Greenberg, Pyszczynski, & Solomon, 1997). Other studies, primed words at awareness level and showed again similar differences between death-related content vs. a negative control content (e.g. Shihui Han, Qin, & Ma, 2010; Johannes Klackl, Jonas, & Kronbichler, 2013). Therefore, we should conclude that regardless of whether reminders of death are delivered in a self-relevant contemplative rather than self-irrelevant passive context, we would still be able to observe the effects associated with the intrinsic motivational relevance of death-related content.

In this respect it is important to note that longer delays between reminders of death and the measurement of the dependent variable are crucial in increasing the effect-size of the distal effects on behavioural and subjective variables (Martens, Burke, Schimel, & Faucher, 2011; Steinman & Updegraff, 2015). This is the reason why other authors interpreted the death-related effects on EEG measures as an index of proximal defences (Klackl et al., 2013). And yet, as EEG measures can reflect both conscious and unconscious processes, we recently contended this interpretation by showing that EEG measures co-vary with subjective measures of anxiety and affect as well as with individual differences in self-esteem (Valentini, Koch, Nicolardi, et al., 2015), thus being sensitive measures of both manifest and latent psychological processes. In the current study, at variance with studies using subliminal priming, participants were implicitly aware of the content of the visual stimulus as demonstrated by the responses collected in the debriefing phase of each experiment (Appendix 6). Therefore, we cannot exclude that participants would have activated proximal defences even though they were not explicitly made aware of the death-related content before or during the experimental testing.

Notwithstanding whether proximal or distal defences were at stake in our paradigm, we found no preferential impact of visual cues of death content on brain responses using a very precise and sensitive EEG approach. Another possible reason why threatening pictures elicited a greater EEG magnitude may concern the variability in the content eventually expressed by this category of images compared to the death-related category (see Appendix 1). Indeed, although we controlled for an important physical feature such as colour by delivering images in greyscale representation, it is still possible that the diversity of content of the threat category (e.g., violence, danger, disgusting material) required a greater amount of neural associative resources as to accomplish the scene identification irrespective of the emotional impact of the specific image.

Another potential modulating factor is the participants' age. Here we verified that no influence of age was at stake (appendix 4) as previous studies investigated the hypothesis that older individuals would show a different behavioural response to reminders of death than young individuals. (Maxfield et al., 2007) reported greater MS effects (i.e. harsher evaluations of moral transgression) for younger than older adults. This effect seemed to be moderated by greater executive functioning in older adults (Maxfield, Pyszczynski, Greenberg, Pepin, & Davis, 2012). However, more recent studies seem to suggest that either the effect of age emerges when MS is heightened (Maxfield et al., 2014) or that no difference between younger and older adults can be appreciated (Bevan, Maxfield, & Bultmann, 2014; Maxfield, Pyszczynski, Greenberg, & Bultmann, 2017). To the best of our knowledge, the only study investigating the difference associated with reminders of death in brain responses of younger and older adults (Bluntschli, Maxfield, Grasso, & Kisley, 2018) reported greater event related electroencephalography amplitudes in response to death-related than negative words, whereas older adults showed the opposite pattern. If we were to compare our study to Bluntschli et al.'s we would have expected even greater brain reactivity to threat-than death-related visual cues in older adults. While this whole evidence points to a potential difference following reminders of death across different population ages it is unclear yet what is the behavioural pattern associated with it, and even more so the brain correlates.

Altogether, our findings add to the ongoing debate by showing that implicit processing of images representing death content is associated with lower magnitude of associative visual activity (indexed by synchronised parieto-occipital EEG activity) when compared to images depicting threatening scenes that do not directly involve death-related content.

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Figure captions

Fig. 1. We recorded EEG during fast periodic visual stimulation (FPVS) using an oddball design with the neutral images constituting the standard familiar events and death or threat images the deviant event. All the images were delivered at 200 ms each thus ensuing in a 5Hz stimulation base rate (black). Deviant events in the periodic condition were delivered at a frequency of 1 each five events, thus resulting in a 1 Hz deviant rate (red), whereas no deviant rate was meant to be established in the aperiodic control condition (orange). We compared the experimental periodic presentation with two control presentations. In *experiment 1* the control presentation occurred in aperiodic manner, that is without a precise pattern, thus giving rise to four experimental conditions: Periodic Death (PD, top), Periodic Threat (PT), Aperiodic Death (AD, centre), Aperiodic Threat (AT). *Experiment 2* consisted of the very same procedure with the exception that a periodic presentation of scrambled death or threat images (which then became unrecognizable) replaced the aperiodic presentation. Therefore, similarly to *experiment 1*, the design entailed four experimental conditions: Periodic Death (PD, top), Periodic Threat (PT), Scramble Death (SD, bottom), Scramble Threat (ST).

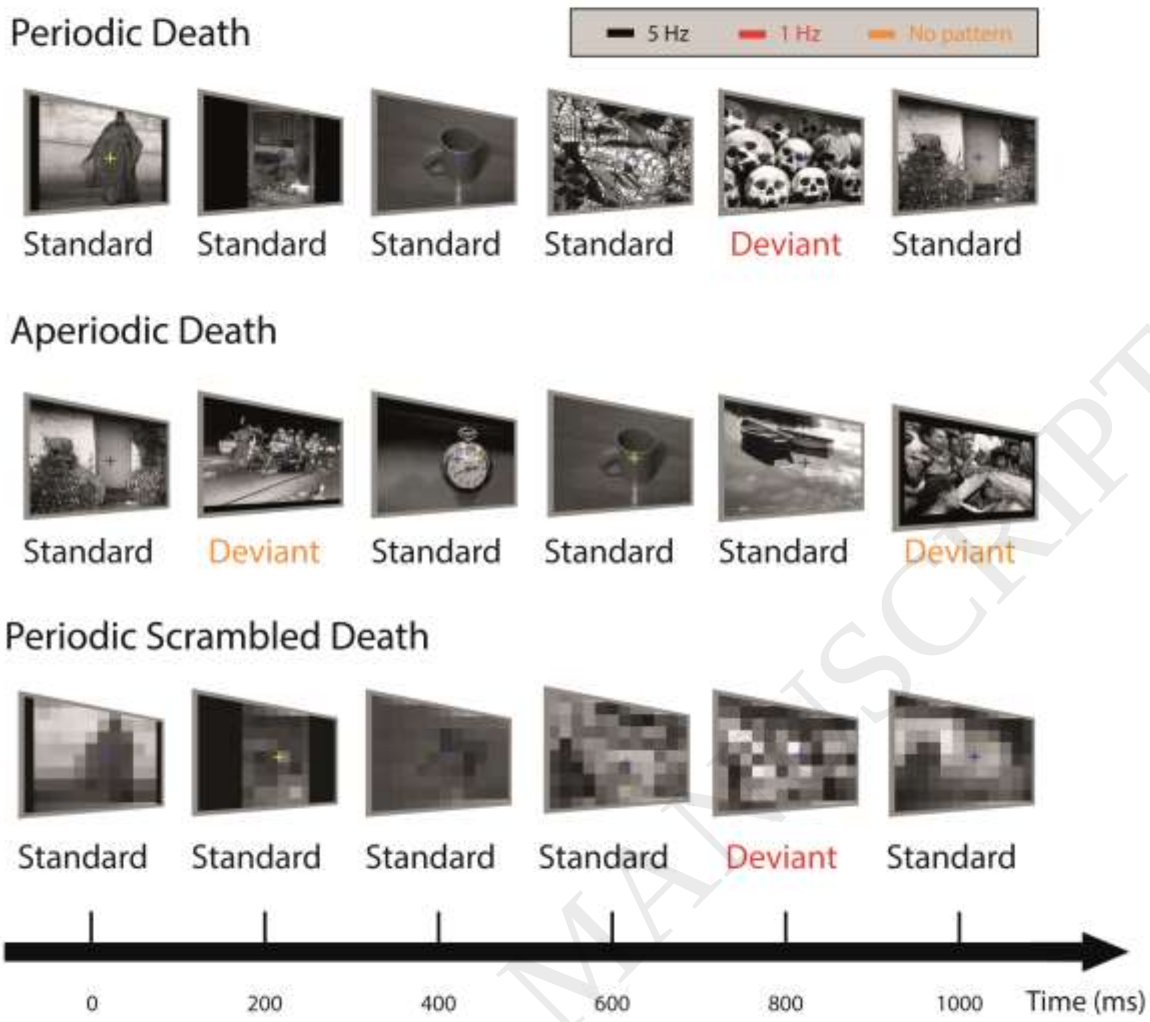

Fig. 2. Deviant rate response. SNR baseline-corrected power spectra grand-averaged across the entire EEG electrodes set-up in the four different conditions recorded in *experiment 1* (A) and *experiment 2* (B). Death-related signal is depicted in red while threat-related signal in blue. Control conditions are mattified. Peak topographical activity at 1 Hz was distributed at bilateral parieto-occipital scalp regions in both experiments. Greater power was observed in periodic conditions, particularly in *experiment 2* where scrambled control images did not elicit any deviant-related response.

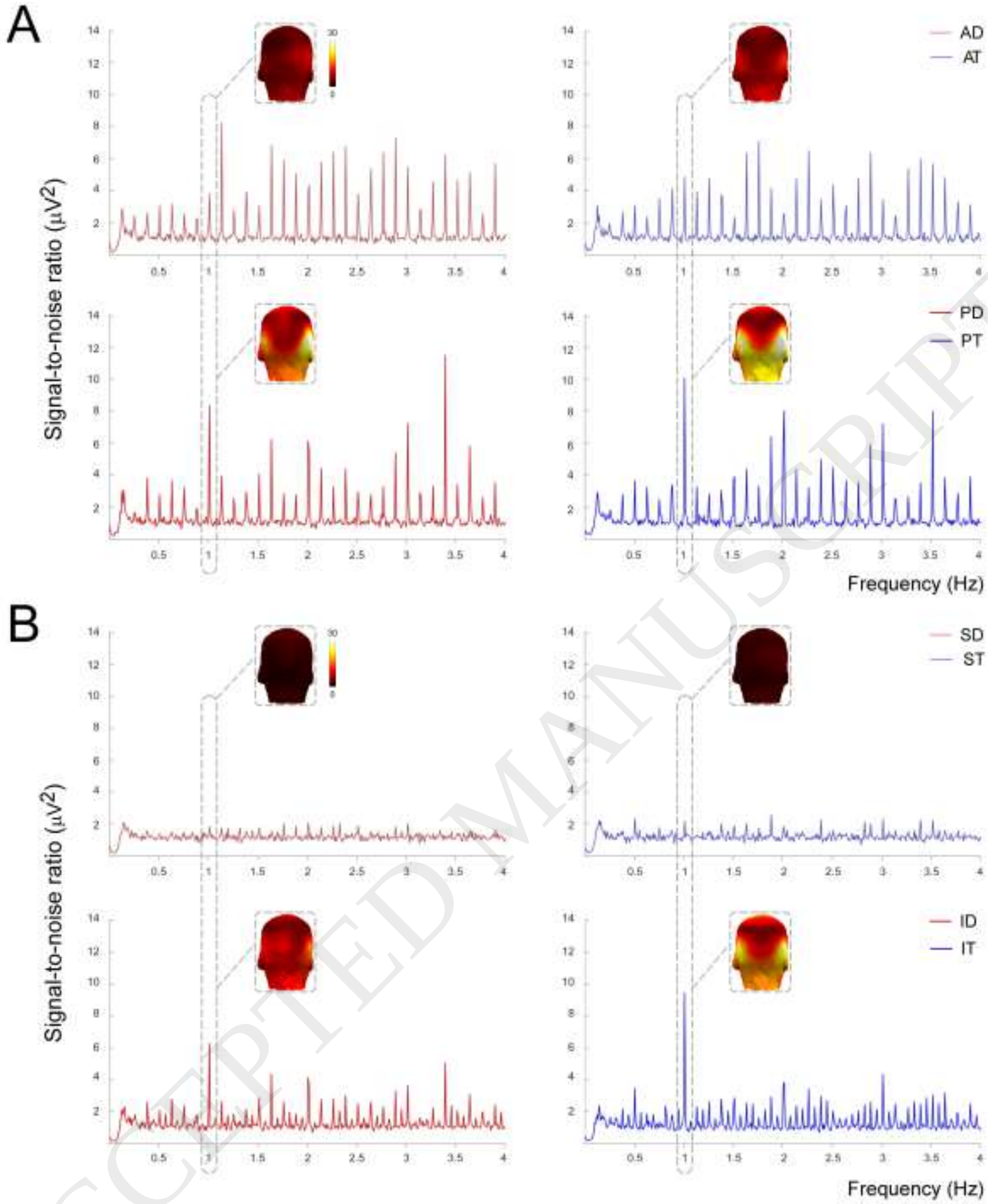

Fig. 3. Deviant rate response in *experiment 1*. Box plots representing the grand-median of baseline-subtracted mean spectral power extracted between 0.98 and 1.02 Hz (y axis) in the four different conditions (x axis). The boxes represent the 25th and 75th percentiles whilst whiskers represent extreme values. Death-related signal is depicted in red while threat-related signal in blue. Control conditions are mattified. Asterisks represent statistical two-

tailed significance (***P≤0.001, **P≤0.01, *P≤0.05). Note only the left parieto-occipital ROI revealed a greater spectral magnitude during periodic presentation of threat than death scenes.

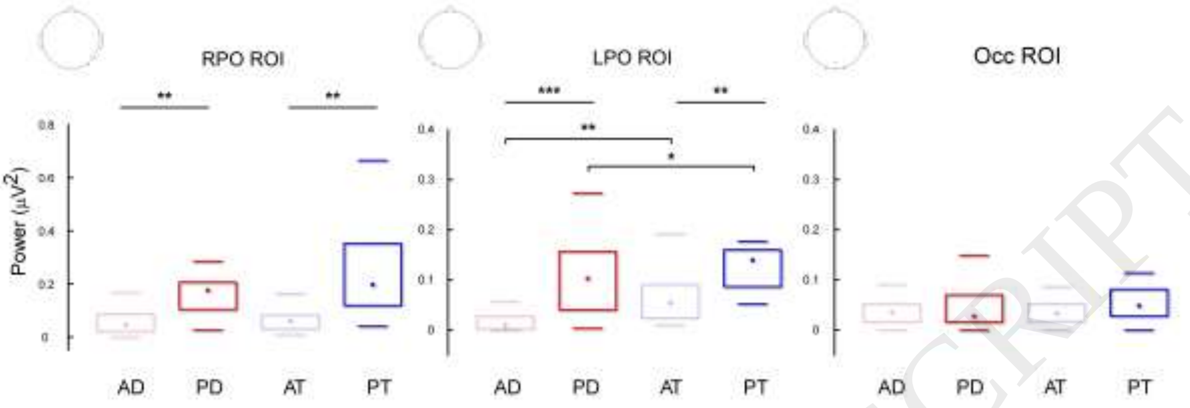

Fig. 4. Base rate response. SNR baseline-corrected power spectra grand-averaged across the entire EEG electrodes set-up in the four different conditions recorded in *experiment 1* (A) and *experiment 2* (B). Base rate harmonics are visible at 10 and 15 Hz. Death-related signal is depicted in red while threat-related signal in blue. Control conditions are mattified. Peak topographical activity at 5 Hz was distributed at bilateral parieto-occipital scalp regions in both experiments.

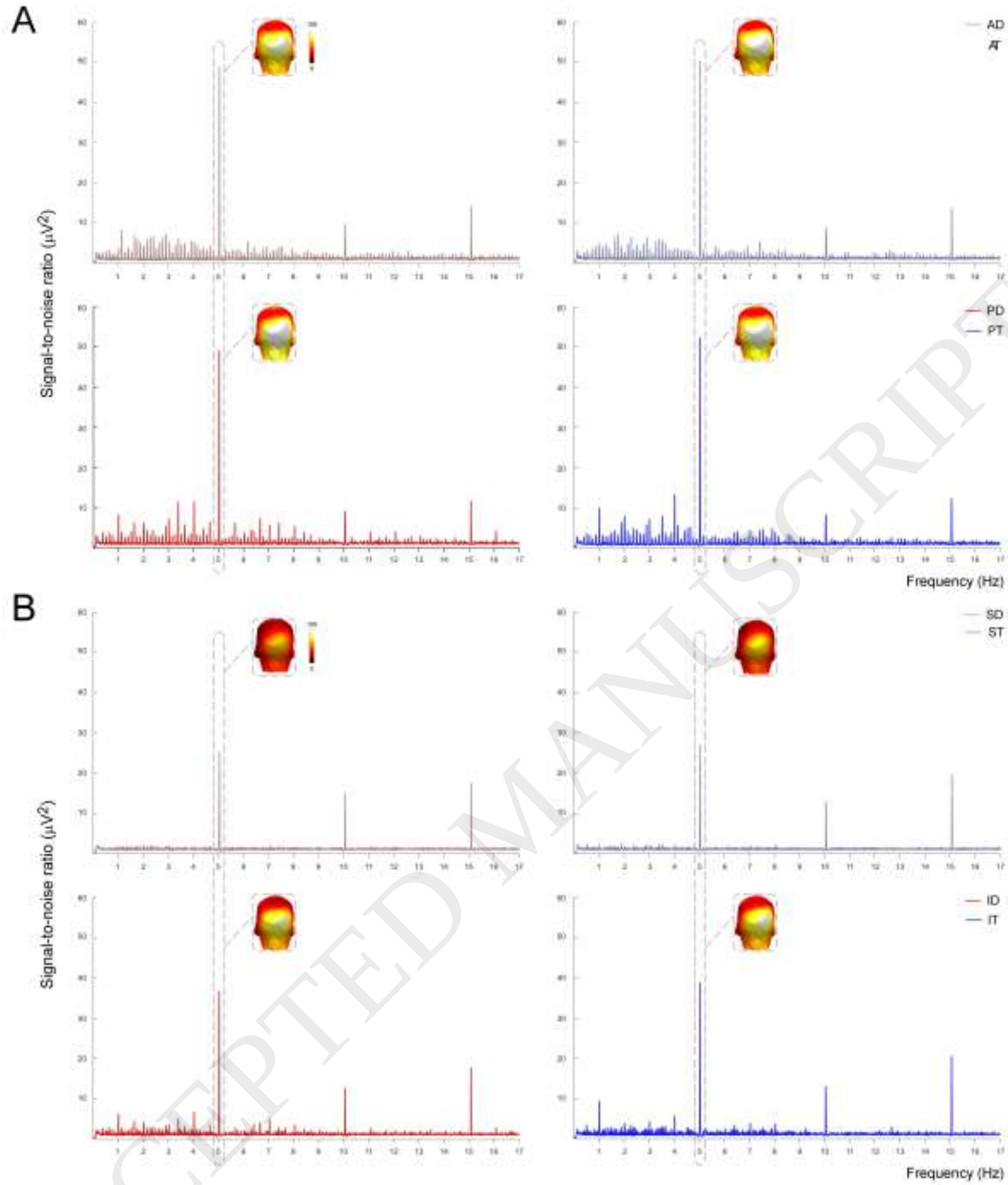

Fig. 5. Base rate response in *experiment 1*. Box plots representing the grand-median of baseline-subtracted mean spectral power extracted between 4.9 and 5.1 Hz (y axis) in the four different conditions (x axis). The boxes represent the 25th and 75th percentiles whilst whiskers represent extreme values. Death-related signal is depicted in red while threat-related signal in blue. Control conditions are mattified. Asterisks represent statistical two-tailed significance (***P≤0.001, **P≤0.01, *P≤0.05). Note only the left parieto-occipital ROI

revealed a greater spectral magnitude for death scenes during aperiodic than periodic presentation.

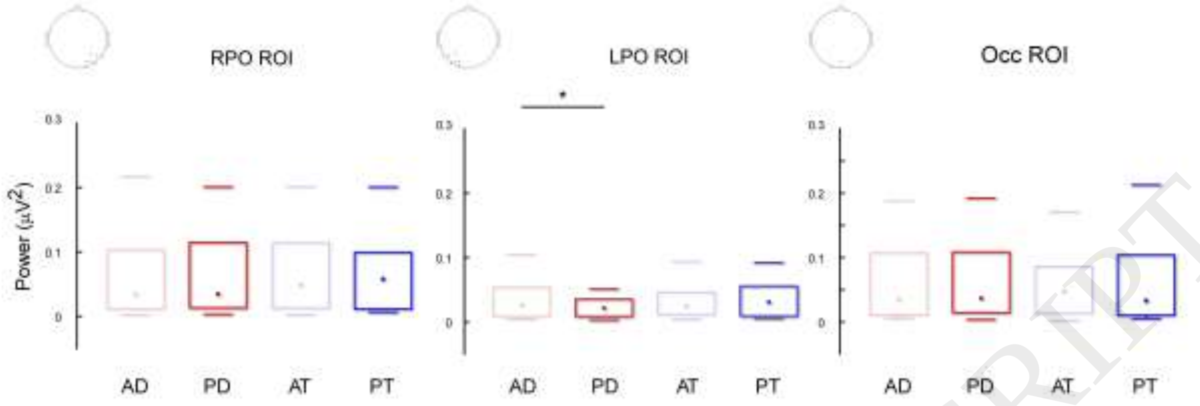

Fig. 6. Deviant rate response in *experiment 2*. Box plots representing the grand-median of baseline-subtracted mean spectral power extracted between 0.98 and 1.02 Hz (y axis) in the four different conditions (x axis). The boxes represent the 25th and 75th percentiles whilst whiskers represent extreme values. Death-related signal is depicted in red while threat-related signal in blue. Control conditions are mattified. Asterisks represent statistical two-tailed significance (***P≤0.001, **P≤0.01, *P≤0.05). Note the parieto-occipital ROIs revealed a greater spectral magnitude during periodic presentation of threat than death scenes.

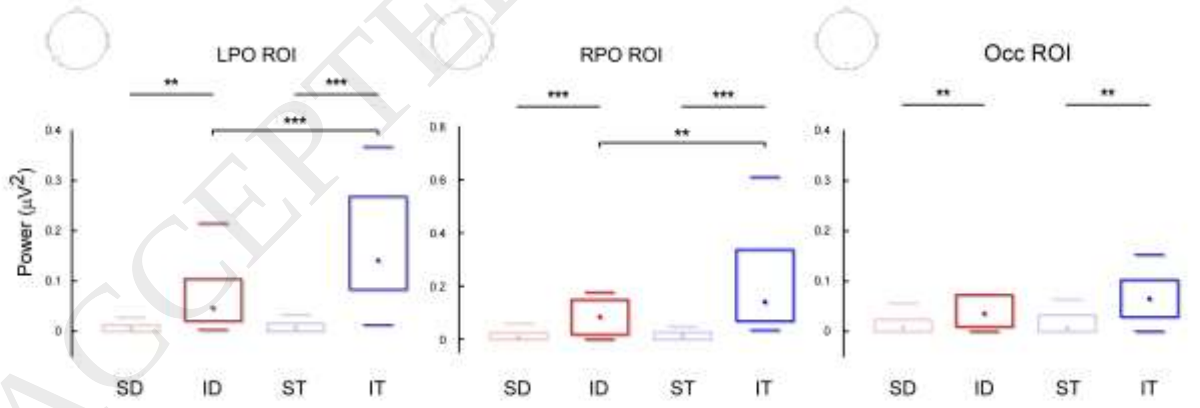

Fig. 7. Base rate response in *experiment 2*. Box plots representing the grand-median of baseline-subtracted mean spectral power extracted between 4.9 and 5.1 Hz (y axis) in the four different conditions (x axis). The boxes represent the 25th and 75th percentiles whilst whiskers represent extreme values. Death-related signal is depicted in red while threat-

related signal in blue. Control conditions are mattified. Asterisks represent statistical two-tailed significance (***P≤0.001, **P≤0.01, *P≤0.05). Note both the parieto-occipital ROIs revealed a greater spectral magnitude for death scenes during periodic than aperiodic presentation. Conversely, this pattern was reversed at the occipital ROI.

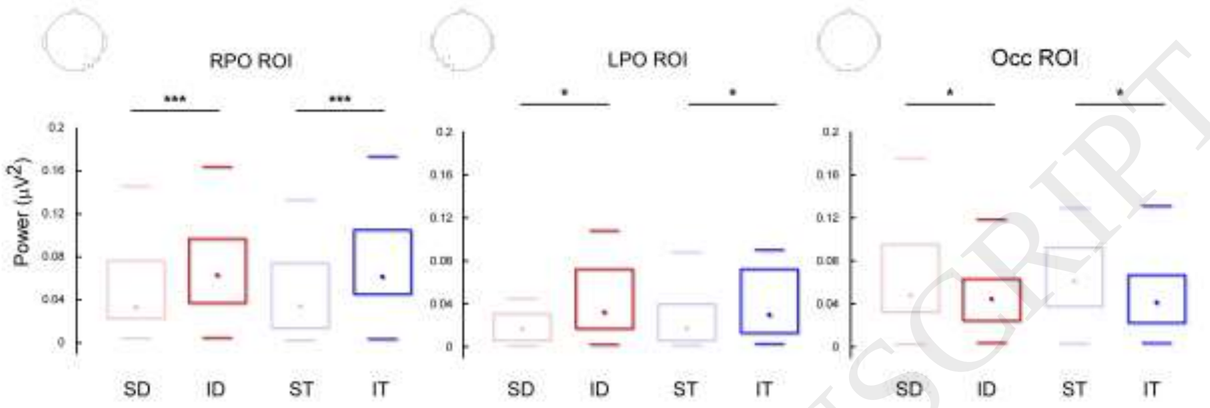

Fig. 8. Grand average evoked potentials recorded during *experiment 1* (A) and *experiment 2* (B), showing the time-course of the periodic deviant effect. Scalp topographies represent the maximal activity in the two main experimental conditions and the scalp distribution of the difference. A; T-test on the LPO ROI waveform revealed greater positive amplitude at 380–476 ms post-stimulus during the periodic threat (PT, blue) than death (PD, red) condition. B; T-test revealed the same pattern in *experiment 2*. The modulation of the positive potential was earlier at the LPO (230–476 ms) than RPO (422–513 ms) ROI.

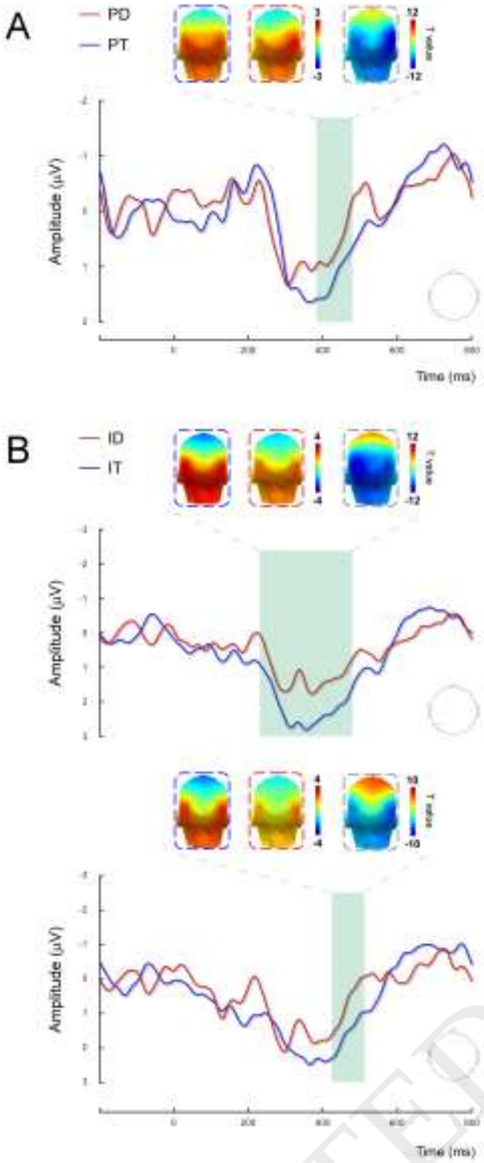
A
PD
PT
3
-3
12
-12
T value
Amplitude (μV)
-2
-1
0
1
2
0
200
400
600
800
Time (ms)
B
ID
IT
4
-4
12
-12
T value
Amplitude (μV)
4
-4
10
-10
T value
Amplitude (μV)
0
200
400
600
800
Time (ms)

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Table 1. Median-centred (±IQR) ratio (divided by the median rating attributed to neutral pictures) for the final sample of 8 death- and threat-related pictures selected from the IAPS database, according to their ability to evoke disgust, brevity of life, fear of death, sense of threat, surprise, valence and arousal.

	AROUSAL	BREVITY OF LIFE	DISGUST	FEAR OF DEATH	SENSE OF THREAT	SURPRISE	VALENCE
D	1.40 (0.40)	2.00 (1.50)	2.00 (1.5)	2.00 (1.50)	1.00 (0.66)	1.83 (0.64)	0.50 (0.33)
T	1.80 (0.60)	1.00 (0.50)	1.50 (2)	1.00 (0.50)	1.00 (0.60)	1.97 (0.75)	0.50 (0.16)

Table 2. Mean (±SEM) power for both deviant and base rate across the four experimental conditions in experiment 1 (top) and 2 (bottom). Summary values are display according to the pooled activity across the scalp, the right parietal-occipital (RPO) region of interest (ROI), the left parietal-occipital (LPO) ROI, and the occipital (OCC) ROI.

Experiment 1	**Deviant frequency (1 Hz)**			
	Aperiodic Death	**Periodic Death**	**Aperiodic Threat**	**Periodic Threat**
Whole scalp	0.02 (± 0.004)	0.04 (± 0.01)	0.02 (± 0.004)	0.04 (± 0.01)
RPO ROI	0.07 (± 0.02)	0.18 (± 0.03)	0.08 (± 0.02)	0.23 (± 0.04)
LPO ROI	0.02 (± 0.01)	0.11 (± 0.02)	0.07 (± 0.01)	0.18 (± 0.04)
OCC ROI	0.04 (± 0.01)	0.04 (± 0.01)	0.04 (± 0.01)	0.07 (± 0.02)
	Base rate frequency (5 Hz)			
Whole scalp	0.02 (± 0.005)	0.02 (± 0.004)	0.02 (± 0.005)	0.03 (± 0.01)
RPO ROI	0.07 (± 0.02)	0.06 (± 0.01)	0.07 (± 0.02)	0.08 (± 0.02)
LPO ROI	0.04 (± 0.01)	0.03 (± 0.01)	0.04 (± 0.01)	0.04 (± 0.01)
OCC ROI	0.06 (± 0.02)	0.06 (± 0.01)	0.07 (± 0.02)	0.07 (± 0.02)
Experiment 2	**Deviant frequency (1 Hz)**			
	Scrambled Death	**Intact Death**	**Scrambled Threat**	**Intact Threat**
Whole scalp	0.02 (± 0.003)	0.04 (± 0.01)	0.01 (± 0.002)	0.07 (± 0.01)
RPO ROI	0.02 (± 0.01)	0.11 (± 0.03)	0.02 (± 0.005)	0.22 (± 0.04)
LPO ROI	0.01 (± 0.004)	0.1 (± 0.004)	0.01 (± 0.03)	0.22 (± 0.05)
OCC ROI	0.01 (± 0.005)	0.09 (± 0.03)	0.02 (± 0.01)	0.1 (± 0.03)
	Base rate frequency (5 Hz)			
Whole scalp	0.02 (± 0.003)	0.02 (± 0.003)	0.02 (± 0.003)	0.02 (± 0.003)
RPO ROI	0.05 (± 0.01)	0.07 (± 0.01)	0.05 (± 0.01)	0.07 (± 0.01)
LPO ROI	0.03 (± 0.01)	0.04 (± 0.01)	0.03 (± 0.01)	0.04 (± 0.01)
OCC ROI	0.09 (± 0.02)	0.06 (± 0.01)	0.08 (± 0.02)	0.06 (± 0.01)

Appendix 1.

Examples of questions from the online preliminary survey

“Please explain how much the image evoked”:

	Not at all	**Slightly**	**Moderately**	**Very**	**Extremely**
Fear of death					
Sense of brevity of life					
Sense of threat					
Surprise					

“Did you experience the image as”:

Negative **Mildly negative** **Neutral** **Mildly positive** **Positive**

“Did you experience the image as”:

Calming **Mildly calming** **Neither calming nor arousing** **Mildly arousing** **Arousing**

Appendix 2.

Detail of stimulus material

Visual stimuli used in the two experiments.

Appendix 3.

Table reporting median (and interquartile range) rating for each image belonging to the three different affective picture categories.

		Arousal	Brevity of Life	Disgust	Fear of Death	Sense of threat	Surprise	Valence
Death	2799.jpg	4 (3;5)	2 (1;4)	3 (1.5;4)	3 (1;4)	3 (2;4)	1 (1;3)	1 (1;1)
	6020.jpg	3 (3;4)	2 (1;3)	1 (1;1)	2 (1;3)	1 (1;2)	1 (1;2)	2 (1;3)
	9000.jpg	3 (3;3)	2 (1;3.5)	1 (1;1)	1 (1;2)	1 (1;1)	1 (1;1)	2 (2;3)
	9220.jpg	4 (3;5)	2 (2;3)	4 (2;5)	3 (1;4)	3 (1;3)	2 (1;3.5)	1 (1;1)
	9400.jpg	3 (3;4)	2 (1;3)	2 (1;3)	2 (1;3)	1 (1;3)	1 (1;2)	2 (1;2.5)
	9440.jpg	3 (3;4)	2 (1;3.5)	2 (1;3.5)	2 (1;3.5)	1 (1;3)	1 (1;2)	1 (1;2)
	9480.jpg	4 (3;4)	3 (1.5;4)	2 (1;3)	3 (2;4)	2 (1;3)	1 (1;2)	1 (1;2)
	9910.jpg	4 (3;4)	1 (1;2)	1 (1;2)	2 (1;4)	2 (1.5;4)	1 (1;2.5)	2 (2;3)
Threat	1090.jpg	3 (3;4)	1 (1;2)	2 (1;3)	1 (1;2)	3 (2;3.5)	1 (1;2)	1 (1;2)
	2691.jpg	3 (3;4)	1 (1;1)	1 (1;2)	1 (1;1)	2 (1;2)	1 (1;2)	2 (1;2)
	6561.jpg	4 (3;5)	2 (1;4)	2 (1;4)	2 (1;4)	4 (2.5;5)	2 (1;2)	1 (1;1)
	6571.jpg	4 (3;5)	1 (1;3)	3 (1;4)	1 (1;3)	3 (2;4)	2 (1;2.5)	1 (1;1.5)
	6838.jpg	4 (3;5)	1 (1;1)	4 (4;5)	1 (1;1)	2 (1;3)	3 (1.5;4)	1 (1;2)
	7380.jpg	4 (3;4.5)	1 (1;3)	1 (1;2.5)	1 (1;3)	2 (2;3)	1 (1;2)	1 (1;2)
	9429.jpg	3 (3;5)	1 (1;3)	2 (1;3)	2 (1;3)	3 (1.5;4)	1 (1;2)	1 (1;2)
	9495.jpg	3 (3;3)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	3 (3;3)
Neutral	2514.jpg	1 (1;2)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	4 (3;5)
	5390.jpg	2 (1;3)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	4 (3;5)
	2620.jpg	2 (2;3)	1 (1;2)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	3 (3;4)
	2518.jpg	2 (1;3)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	4 (3;5)
	5731.jpg	3 (2;3)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;2)	3 (3;4)
	7009.jpg	3 (3;3)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;2)	3 (3;3)
	7160.jpg	3 (2;3)	1 (1;2)	1 (1;1)	1 (1;1)	1 (1;1)	1 (1;1)	3 (3;3)
	7190.jpg	2.78 (0.7)	1.56 (0.98)	1.04 (0.21)	1.13 (0.5)	1.07 (0.25)	1.31 (0.75)	3.22 (0.73)

Appendix 4.

Effect of gender and age

We analysed the effect of gender and age on the significant differences in spectral power elicited by periodic deviant (1 Hz rate) death- and threat-related scenes in both experiments (PD, PT in experiment 1 and ID, IT in experiment 2). Gender and age were used as categorical or continuous covariate in separate general linear models to test both their main effect and their interaction with the effect of content (death and threat) in the two main regions of interest (LPO, RPO). Findings are summarised in the table below. Neither main effects no interactions were detected, thus suggesting that our main findings would not be explained by a significant variation in the age and gender of our participants.

ROI	**Age**		**Gender**	
	Main effect	Interaction	Main effect	Interaction
LPO (exp. 1)	F = 1.10; p = 0.30	F = 0.22; p = 0.64	F = 0.0001; p = 0.99	F = 0.11; p = 0.74
LPO (exp. 2)	F = 1.06; p = 0.31	F = 0.04; p = 0.84	F = 0.49 p = 0.49	F = 0.24; p = 0.62
RPO (exp. 2)	F = 1.90; p = 0.17	F = 0.18; p = 0.67	F = 0.009; p = 0.92	F = 0.50; p = 0.48

Appendix 5.

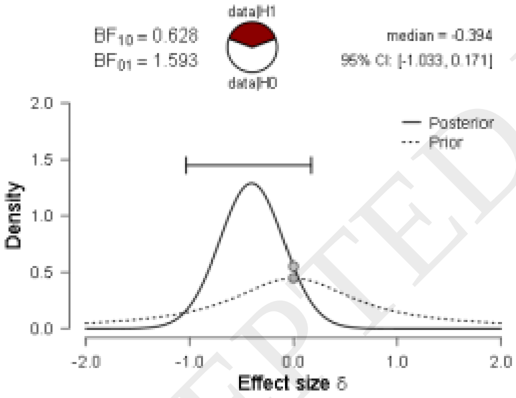
Bayesian paired t-test of parietal-occipital differences in power during deviant 1 Hz frequency presentation

Experiment 1. RPO ROI

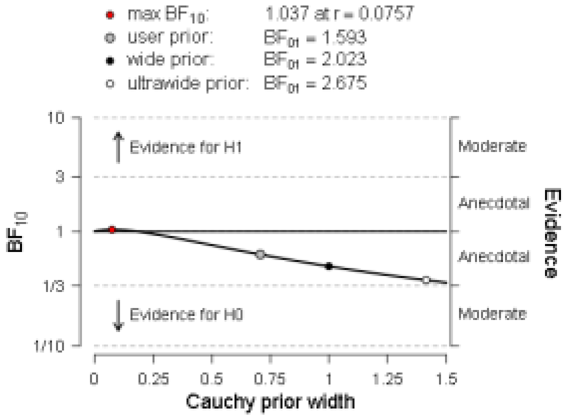
Non-directional hypothesis (PD ≠ PT)

	BF_{01}	**error %**
PD ≠ PT	1.59	0.001

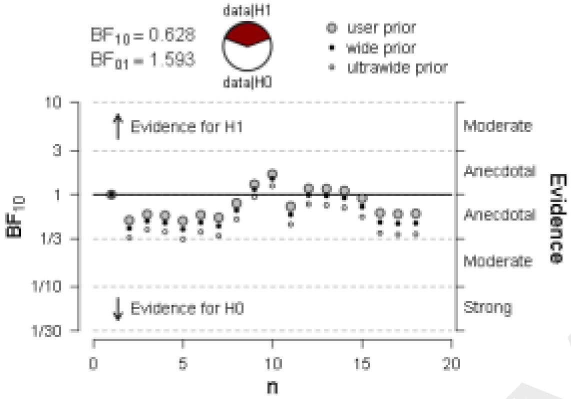
Prior and Posterior inferential plot



Bayes Factor Robustness Check



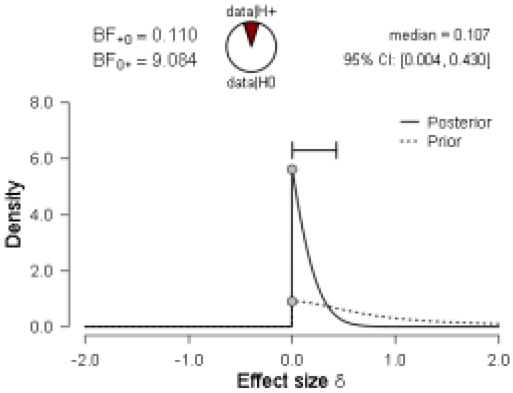
Sequential Analysis



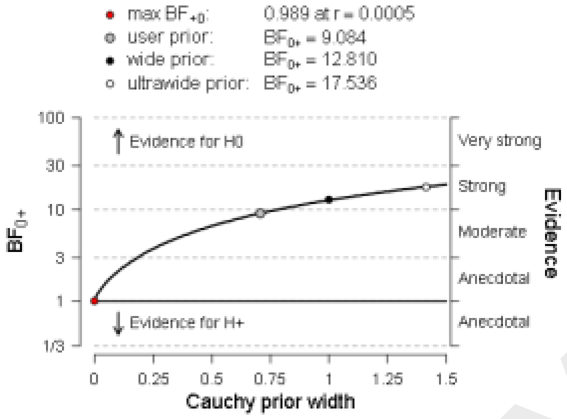
Directional hypothesis (PD > PT)

	BF_{01}	error %
PD > PT	9.08	~ 4.470e -4

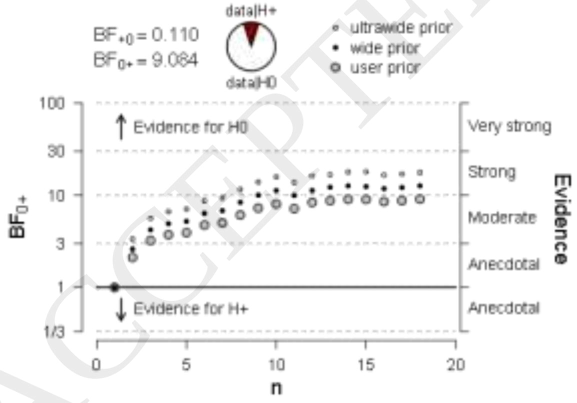
Prior and Posterior inferential plot



Bayes Factor Robustness Check



Sequential Analysis

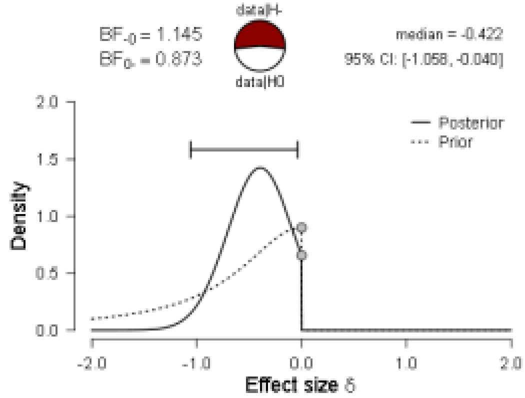


Directional hypothesis (PD < PT)

	BF_{01}	error %

	BF_{01}	error %
PD < PT	0.87	~ 0.007

Prior and Posterior inferential plot



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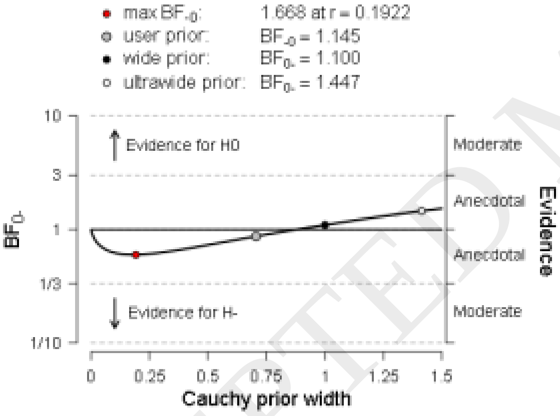
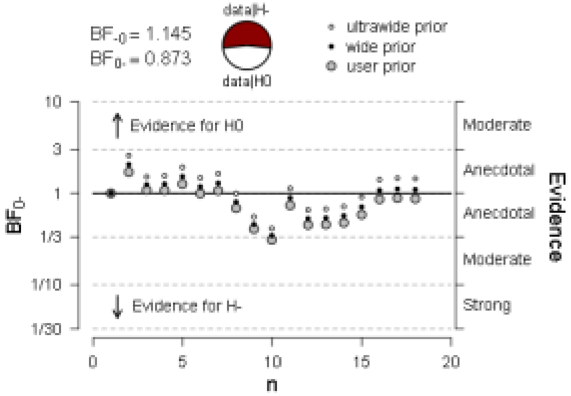


Sequential Analysis

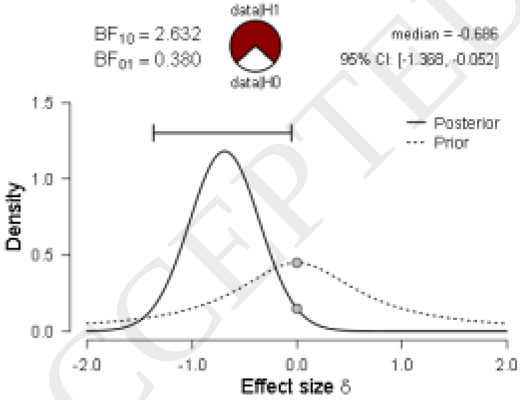


Experiment 1. LPO ROI

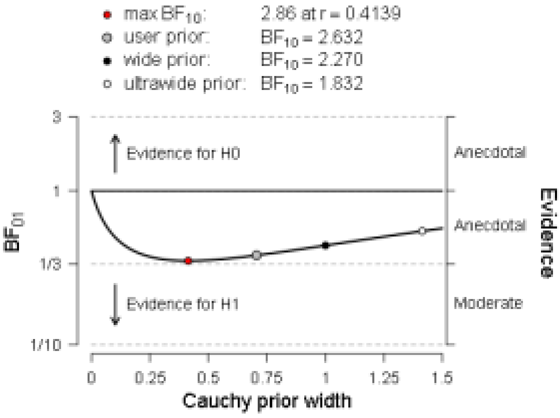
Non-directional hypothesis (PD ≠ PT)

	BF_{01}	error %
PD ≠ PT	0.38	0.004

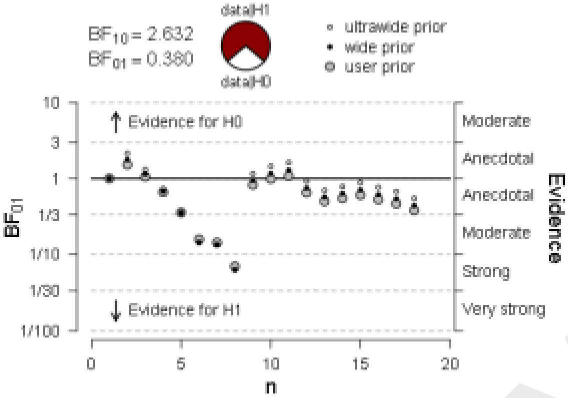
Prior and Posterior inferential plot



Bayes Factor Robustness Check



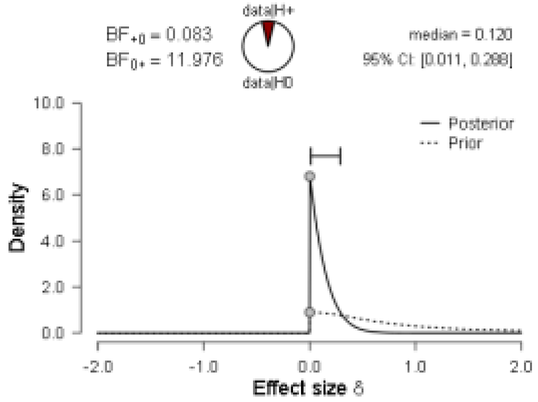
Sequential Analysis



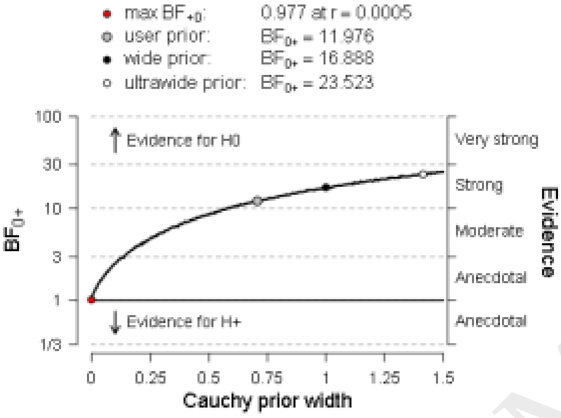
Directional hypothesis (PD > PT)

	BF_{01}	error %
PD > PT	11.98	~ 1.151e -5

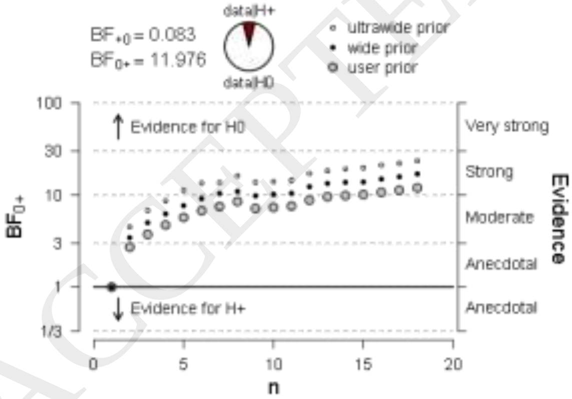
Prior and Posterior inferential plot



Bayes Factor Robustness Check



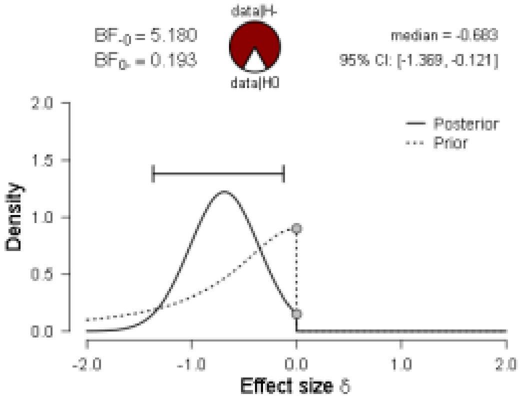
Sequential Analysis



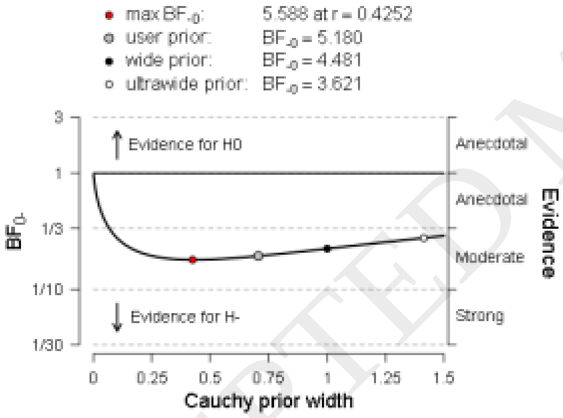
Directional hypothesis (PD < PT)

	BF_{01}	error %
PD < PT	0.19	~ 0.002

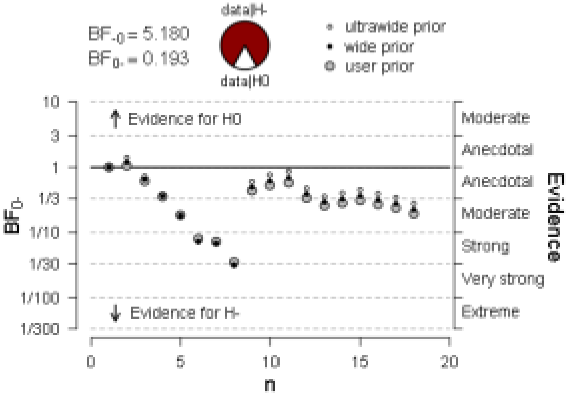
Prior and Posterior inferential plot



Bayes Factor Robustness Check



Sequential Analysis

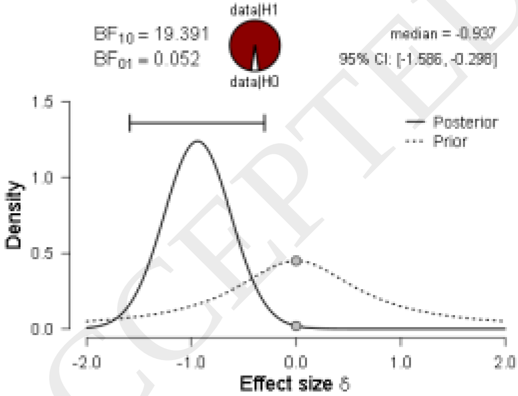


Experiment 2. RPO ROI

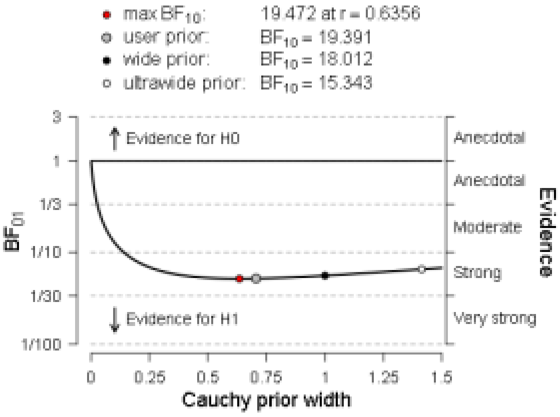
Non-directional hypothesis (ID ≠ IT)

	BF_{01}	error %
ID ≠ IT	0.05	3.566e -4

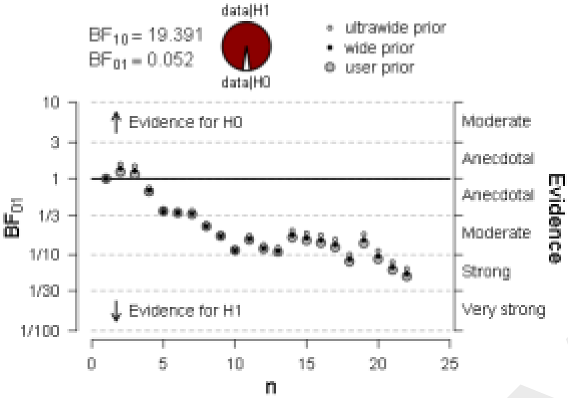
Prior and Posterior inferential plot



Bayes Factor Robustness Check



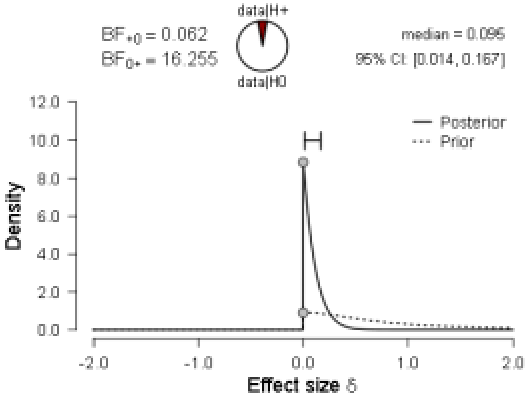
Sequential Analysis



Directional hypothesis (ID > IT)

	BF_{01}	**error %**
ID > IT	16.26	~ 0.008

Prior and Posterior inferential plot



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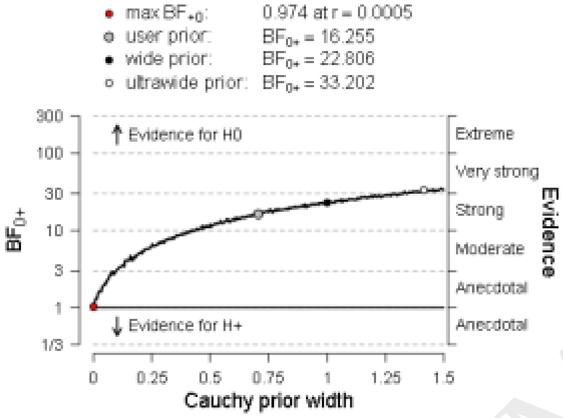
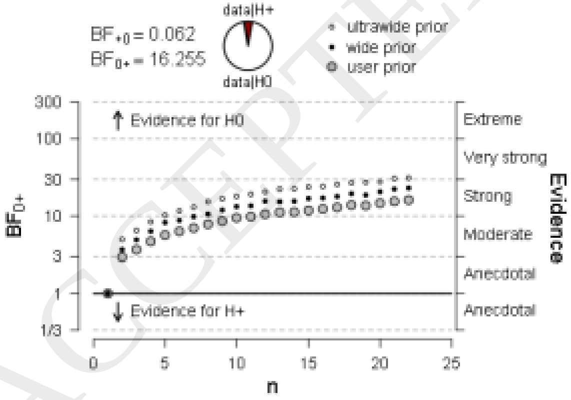


Sequential Analysis

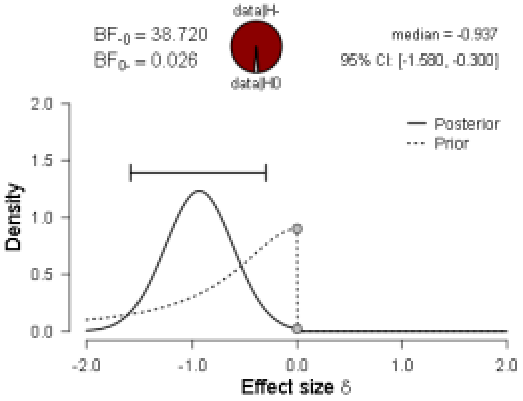


Directional hypothesis (ID < IT)

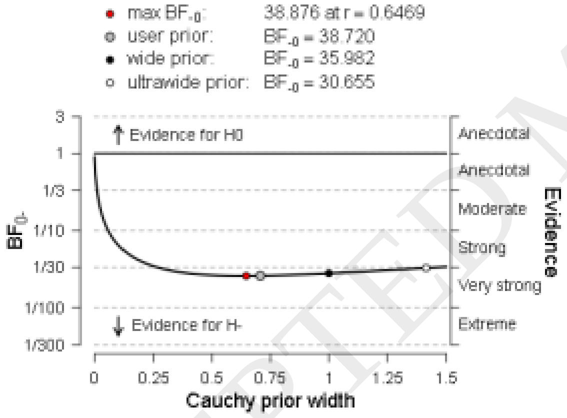
	BF_{01}	error %

	BF_{01}	**error %**
ID < IT	0.03	~ 2.126e -4

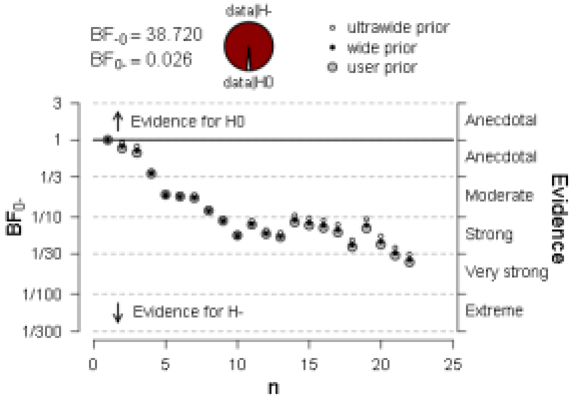
Prior and Posterior inferential plot



Bayes Factor Robustness Check



Sequential Analysis

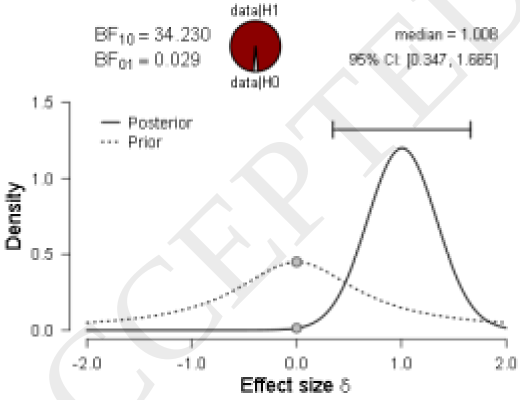


Experiment 2. LPO ROI

Non-directional hypothesis (ID ≠ IT)

	BF_{01}	error %
ID ≠ IT	0.029	1.249e -4

Prior and Posterior inferential plot



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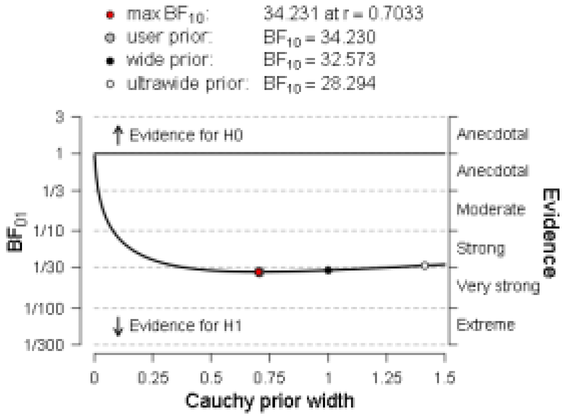
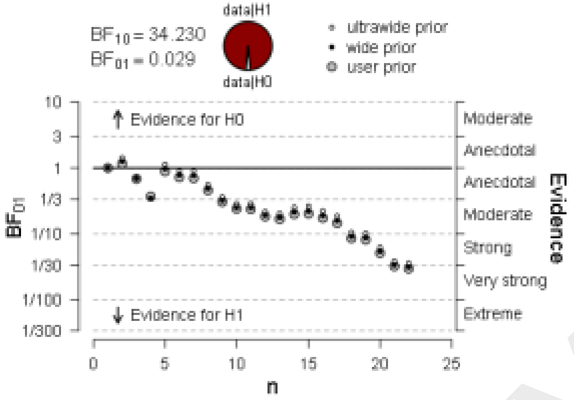


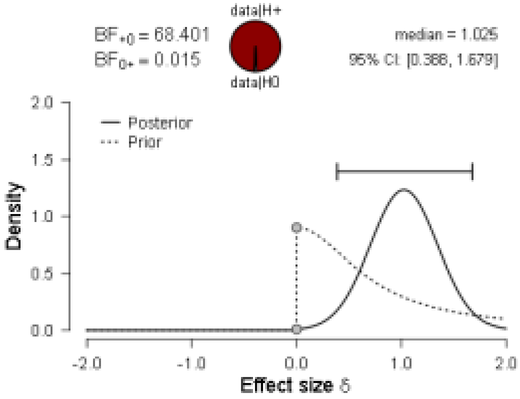
Sequential Analysis



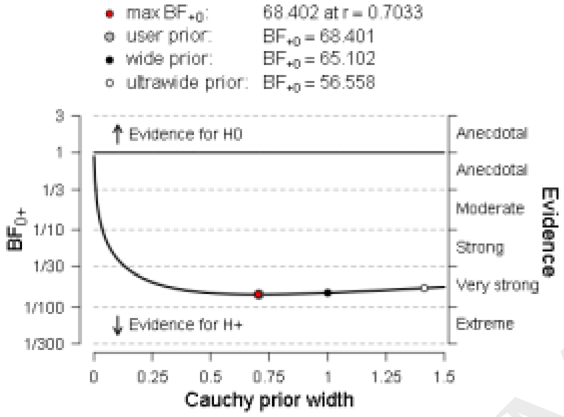
Directional hypothesis (ID > IT)

	BF_{01}	error %
ID > IT	0.015	~ 8.975e -5

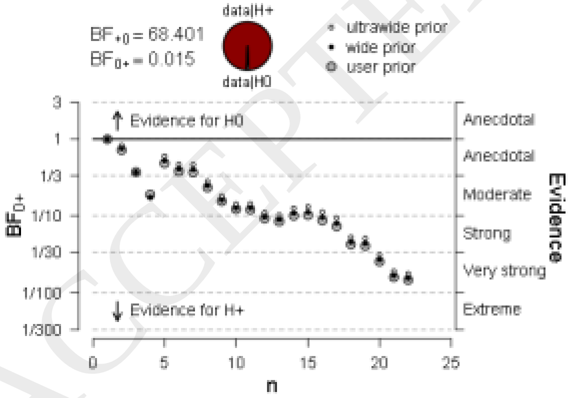
Prior and Posterior inferential plot



Bayes Factor Robustness Check



Sequential Analysis

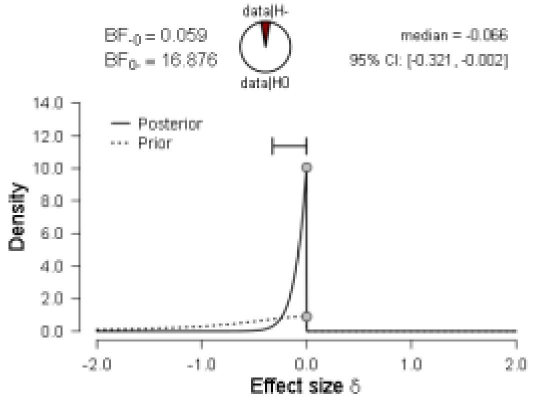


Directional hypothesis (ID < IT)

	BF$_{01}$	**error %**

	BF_{01}	error %
ID < IT	16.88	~ 0.001

Prior and Posterior inferential plot



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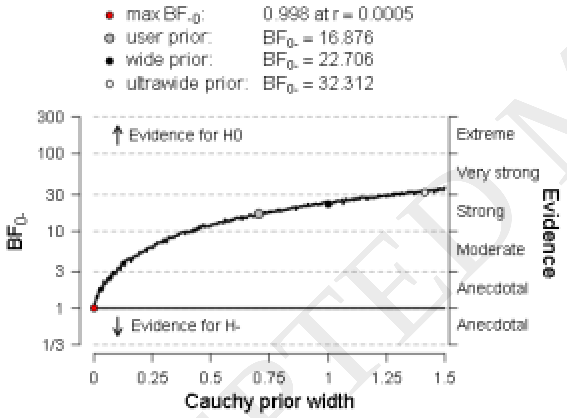
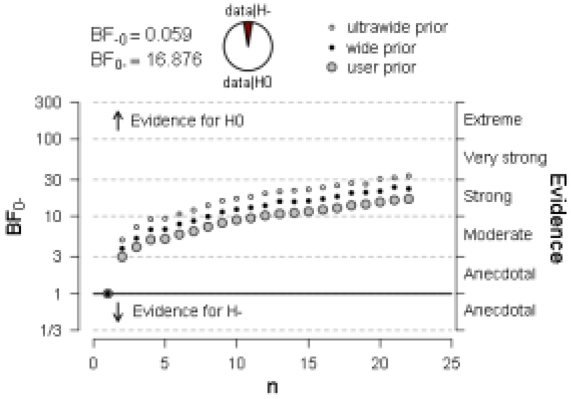


Sequential Analysis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BF0- = 16.876
data|H-
data|H0
ultrawide prior
wide prior
user prior
300
100
30
10
3
1
1/3
BF0-
Evidence for H0
Evidence for H-
Extreme
Very strong
Strong
Moderate
Anecdotal
Anecdotal
Evidence
0
5
10
15
20
25
n

Appendix 6.

Debriefing questions

"Can you tell apart the different content or meaning represented in the images?"

"Did you feel (or still feeling) emotions during the experiment? If so, which type of emotion?"

Written reports in *experiment 1*:

- Participants recalled had seen 3 to 11 different images overall. The mean (±SD) amount of different images seen was 6.67 (±2.95)
- 44.4% of all participants used the word "violence" while other 44.4% of all participants used the word "religious" to describe some of the images they saw.
- 33.3% of all participants reported feeling some emotion during the experiment. They described their emotional state as being "anxious", "frightened" and "discomfort". The participants attributed these feelings to the recalled images.

Written reports in *experiment 2*:

- Participants recalled from 0 to 9 overall. The mean (±SD) amount of different images seen was 3.56 (±2.14).
- 30.43% of all participants remembered a mug/cup. 30.43% of all participants recalled skulls. 26% of all participants recalled images similar to a boat or a lake. Only one participant recalled the image of a snake.

- 36.36% of all participants experienced feeling anxiety, a “dark” emotion, depressive state, unpleasant mood, as well as feeling frightened. 27.27% of all participants did not report any experienced emotion during the experiment.