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ELECTROPHYSIOLOGICAL CORRELATES OF VISUAL AWARENESS AND ITS DIFFERENT PROPERTIES

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Electrophysiological correlates of visual awareness and its different properties
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SOMMARIO

Due componenti ERP, una precoce deflessione negativa (Visual Awareness Negativity; VAN), ed una più tardiva deflessione positiva (Late Positivity; LP), si suggerisce riflettano proprietà differenti della consapevolezza: rispettivamente, il contenuto fenomenico di una percezione e l'accesso allo stesso.

Il primo esperimento ha indagato la natura graduale o dicotomica della consapevolezza. Lo scopo è stato quello di ricercare i correlati neurali di gradi differenti di consapevolezza visiva analizzando gli ERP in risposta a stimoli a basso contrasto, la cui chiarezza è stata valutata su una scala a quattro punti, la Perceptual Awareness Scale (PAS). I risultati hanno identificato una deflessione negativa sulle aree centro-parietali sinistre (VAN; picco attorno a 280-300ms), seguita da una deflessione positiva bilaterale (LP; ~510-550ms) sulla quasi totalità degli elettrodi. Interessante notare che l'ampiezza di entrambe le deflessioni aumentava in maniera graduale alla consapevolezza visiva, e che i generatori intracranici del contenuto fenomenico (VAN) erano localizzati nel lobo temporale sinistro. I dati suggeriscono quindi che la consapevolezza visiva sia caratterizzata da un aumento graduale della chiarezza percepita a livello comportamentale e neurale, e che il contenuto percettivo emerga da una percoce attivazione locale nelle aree temporali.

Lo scopo del secondo studio è stato quello di utilizzare un approccio integrato quale strumento diagnostico per discriminare tra blindsight e visione degradata conapevole. Il blindsight è l'abilità di alcuni pazienti con deficit di campo visivo (emianopia) di esibire un comportamento apparentemente guidato dalla vista anche nel loro campo cieco, nonostante non riportino consapevolezza degli stimoli. Pazienti con visione degradata consapevole, a differenza dei pazienti con blindsight, dovrebbero quindi mostrare le stesse componenti ERP (VAN e LP) e la stessa modulazione da parte della consapevolezza visiva, come osservato nei soggetti sani. A tal fine, ad una paziente emianopica sono stati presentati stimoli nel campo cieco, le è stato richiesto di discriminarli e poi di valutarli sulla PAS. A livello comportamentale, la sua accuratezza nella discriminazione dipendeva dal livello di consapevolezza, suggerendo quindi visione degradata consapevole. I dati elettrofisiologici hanno mostrato la presenza precocemente della VAN (picco attorno ai 200ms) e della più tardiva LP (da circa 300ms), e, fondamentale, l'ampiezza di entrambe le componenti risultava modulata dal livello di consapevolezza. Le componenti elettrofisiologiche

possono quindi considerarsi uno strumento diagnostico più fine nella valutazione dei pazienti emianopici.

L’ultimo esperimento mirava a meglio caratterizzare i processi cognitivi riflessi nella LP. Non solo è stata infatti associata all’accesso consapevole di un contenuto percettivo, ma anche all’accumulazione di evidenza sensoriale che porta alla presa di decisione. Per distinguere tra le due interpretazioni, sono stati utilizzati stimoli a livelli di contrasto differenti, chiedendo ai partecipanti di discriminarli e poi valutare la qualità della loro percezione sulla PAS. I risultati hanno mostrato che la LP era modulata solo dalle valutazioni soggettive sulla consapevolezza, e non dai livelli differenti di stimolazione sensoriale. I dati suggeriscono che la componente può essere considerata uno stadio intermedio tra il puro input sensoriale e la decisione, riflettendo il livello di accesso alle rappresentazioni interne, indipendentemente, in parte, dall’informazione fisica. Ciò che quindi sembra essere accumulata non è solo l’evidenza sensoriale, ma anche il rumore neurale che è indipendente dallo stimolo e prodotto all’interno del cervello stesso.

In generale, si è confermato che consapevolezza fenomenica e di accesso sono rispettivamente riflesse nella VAN e nella LP. Inoltre, dal momento che la percezione visiva consapevole avviene al di fuori della corteccia visiva primaria, V1 sembra non essere necessaria per l’emergere della consapevolezza.

ABSTRACT

Two ERP components, an early negative deflection (Visual Awareness Negativity; VAN), and a later positive deflection (Late Positivity; LP) are thought to reflect different properties of consciousness: the phenomenal content of a perception and access to it, respectively.

The first experiment investigated the graded vs. dichotomous nature of consciousness. The aim was to search for the neural correlates of different grades of visual awareness analyzing the ERPs to reduced contrast stimuli, whose clarity was rated on the 4-point Perceptual Awareness Scale (PAS). Results revealed a left centro-parietal negative deflection (VAN; peak at ~280-320ms), followed by a bilateral positive deflection (LP; ~510-550ms) over almost all electrodes. Interestingly, the amplitude of both deflections gradually increased along with visual awareness and the intracranial generators of the phenomenal content (VAN) were located in the left temporal lobe. Data thus suggest that visual awareness is characterized by a gradual increase of perceived clarity at behavioral and neural level, and that the perceptual content emerges from early local activation in temporal areas.

The aim of the second experiment was to use an integrative approach as a diagnostic tool to discriminate between blindsight or degraded conscious vision. Blindsight is the ability of some patients with a visual field defect (hemianopia) to exhibit visually guided behavior also in their blind field, despite reporting no awareness of stimuli. Patients with degraded conscious vision, differently from blindsight patients, should thus show the same ERP components (VAN and LP) and the same visual awareness modulation observed in healthy subjects. To this end, a hemianopic patient was presented with stimuli in her blind visual field, asked to discriminate and then rate them on the PAS. At behavioral level, her discrimination accuracy depended on the level of awareness, thus suggesting degraded conscious vision. Electrophysiological data revealed the presence of the early VAN (peak at ~200ms) and the late LP (from ~300ms), and, crucially, the amplitude of both components was modulated by the level of awareness. Electrophysiological signatures can thus be a fine-grained diagnostic tool when assessing hemianopic patients.

The last experiment aimed at better characterizing the cognitive processes reflected in the LP. Not only it has been associated with conscious access to a perceptual content, but also with accumulation of sensory evidence leading to decision-making. To disentangle between the two, stimuli at different contrast levels were presented, asking

participants to perform a discrimination task and then rate the quality of their perception on the PAS. Results showed that the LP was modulated only by the subjective ratings of awareness, and not by the different levels of sensory stimulation. Data suggest that the component can be considered an intermediate stage between merely sensory input and decision, reflecting the level of access to internal representation, partly regardless of the physical information. What thus appears to be accumulated is not only sensory evidence, but also stimulus-independent neural noise produced within the brain itself.

Overall, phenomenal and access consciousness were confirmed to be distinctly reflected in the VAN and LP. Moreover, since conscious visual perception occurs outside the primary visual cortex, V1 appears not to be necessary for the emergence of awareness.

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ABBREVIATIONS

CPP	Centro-Parietal Positivity
EEG	Electroencephalography
ERP	Event Related Potential
LP	Late Positivity
NCC	Neural Correlates of Consciousness
PAS	Perceptual Awareness Scale
RT	Reaction Time
SCD	Scalp Current Density
VAN	Visual Awareness Negativity

GENERAL INTRODUCTION

Consciousness has always been and continues to be a difficult topic in neuroscience research, even if it can be considered the most essential feature that characterizes our mental life. Due to the complexity of the theme, different classifications and definitions of consciousness have been offered throughout the years, each of them focusing on a specific aspect of it. In a recent review (de Graaf et al., 2102), four different distinctions of consciousness have been proposed. The first definition is about *Self-awareness*, referring to all mental aspects that define our experience as being 'someone' different from 'others'. Then there is *Higher-order awareness*, a feature that differentiates humans from other animals and allows us to reflect on things. The comparison between the states of being conscious (e.g. awake) and of not being conscious (e.g. sleeping or comatose) is reflected in the so-called *Medical awareness*. Finally and more interestingly to our research, *Content-Consciousness* is the actual phenomenal quality present in the mind of a subject that is consciously experiencing something.

The main goal of all studies on content-consciousness is to look for its neural correlates (NCCs), defined as those neural processes that are necessary and sufficient to generate a conscious experience (Koch, 2004). The NCCs are usually investigated by comparing identical physical conditions leading or not to a conscious percept (Baars, 1998). Significant achievements in this field of research have been possible also thanks to the investigation of some peculiar neuropsychological syndromes (e.g. blindsight, agnosia or spatial neglect) occurring in brain-damaged patients (Naccache, 2015), where crucial dissociations can be observed. However, different experimental paradigms yielded different results on the brain regions involved in conscious perception: from striate and extrastriate areas (Block, 2005), to higher-level extrastriate regions projecting to the prefrontal cortex (Crick and Koch, 1995, but see also Koch et al., 2016) or a widespread fronto-parietal network (Dehaene and Naccache, 2001). In summary, no satisfactory and unitary conclusion on the NCCs has been reached yet, and the debate is still vividly open.

Another important issue in the study of consciousness regards the measures used to investigate it. Methodologically speaking, in cognitive neuroscience (and every other science that demands to be defined "experimental"), the primary source of evidence should be obtained from objective data, with an almost complete neglect of subjective reports, often considered unreliable. However, the same argument cannot be fully applied to the study of consciousness, a concept that by definition is intrinsically related to a temporally specific and subjective experience. In this case, in fact, objective measures often raises some problems (Schurger and Sher, 2008), mostly resulting in an overestimation of the perceptual experience and an inability to capture some peculiar and more elaborated aspects of perception. For this reason, recently, subjective reports (Ramsøy and Overgaard, 2004; Del Cul et al., 2007) have been preferred, since they allow to assess consciousness immediately after every trial presentation, thus being a more accurate representation of the internal state experienced by the experimental subjects (for a comparison between different report scales, see Overgaard and Sandberg, 2012).

Taken together, these considerations highlight the need of designing experimental studies that combine different measures (objective/behavioral, subjective and also brain-based data) to better investigate and appreciate the composite concept that is consciousness. As regards brain-imaging techniques, recording the electrophysiological brain signals (EEG) and more specifically analyzing the event-related potentials (ERPs; Luck, 2005) proved to be an excellent tool to examine the temporal evolution of brain's activity in response to specific sensory or cognitive events (for a review on conscious perception-related ERPs see Koivisto and Revonsuo, 2010).

The aim of the works presented in the following chapters was thus to use an integrative approach in order to investigate consciousness, specifically visual awareness and its different properties, in both healthy and brain-damaged subjects.

EXPERIMENT 1

This chapter has been published as a scientific paper:

"Tagliabue, C.F., Mazzi, C., Bagattini, C., and Savazzi, S. (2016). Early local activity in temporal areas reflects graded content of visual perception. *Frontiers in Psychology*, 7, 572"

1. Introduction

Consciousness (or awareness) refers to the fact that, when we are awake, we have experiences. Since consciousness gained enough consideration to be investigated in the field of cognitive neuroscience, an intensive search for the neural correlates of consciousness (NCC) has been undertaken. The NCC has been defined by Koch (2004) as "the minimal set of neuronal events and mechanisms jointly sufficient for a specific conscious percept".

Such NCC are usually investigated by contrasting neural responses to physically identical stimuli that are consciously perceived or not, the so-called contrastive analysis (Baars, 1988), used across different experimental paradigms in which visual awareness is manipulated (e.g. masking, change blindness, reduced-contrast stimuli, etc. For a review, see Koivisto and Revonsuo, 2010). fMRI studies have revealed that changes in conscious contents correlate with activation along the ventral visual pathway (e.g. Bar et al., 2001; Pins and ffytche, 2003) with additional involvement of frontal and parietal areas (e.g. Beck et al., 2001; Lumer and Rees, 1999), revealing the key role of dorsal-ventral interactions for visual awareness. The temporal dynamics of such neural processing have been obtained by studying event-related brain potentials (ERPs), the electrical potential changes in response to a given sensory, motor or cognitive event (Luck, 2005). Recent ERP studies have found that conscious perception consistently correlates with an early component called Visual Awareness Negativity (VAN; Koivisto and Revonsuo, 2003), that is a negative amplitude difference wave between aware and unaware trials peaking at about 200 ms after stimulus onset in occipito-temporal sites (Koivisto et al., 2008), but also observed at central, fronto-polar (Wilenius-Emet et al., 2004) and occipital-parietal (Pitts et al., 2014) electrodes. The latency of this component is prolonged (up to 200 ms later) when the contrast of the stimuli is lowered (Ojanen et al., 2003). The VAN is usually followed by a later positive component, called Late Positivity (LP; Del Cul et al., 2007), another difference wave between aware and unaware conditions peaking between 300 and 400 ms after stimulus presentation in parietal and central sites.

Finally, weaker evidence has been found also for an enhancement of P1 amplitude in response to aware trials at around 100-130 ms in the occipital sites (Pins and ffytche, 2003), even if this early positive component might better reflect attention-related processes (Hillyard et al., 1998).

Importantly, it has been proposed (Block, 2005) that a distinction needs to be made between two components of consciousness: phenomenal consciousness, described as the "what-it-is-like" of the experience (the actual content), and access consciousness, that is the ability to report, remember or act on such experiences. Accordingly, different NCC might reflect each one of these components (Block, 1996). Following the classification made by Block, Koivisto and Revonsuo (2010) have proposed that the two components typically found in ERP experiments to correlate with visual awareness (VAN and LP) may represent distinctive NCC of the different properties of consciousness. More specifically, given their latencies and topographies, the VAN, the earlier ERP component, has been interpreted (Koivisto and Revonsuo, 2010) as the neural correlate of phenomenal awareness, whereas the LP, the later ERP component, has been related to access awareness.

Together with the classification of consciousness in the phenomenal and the access components, another important issue that has to be taken into account when investigating visual awareness relates to the way the perceptual experience is reported. Studies on unconscious perception typically require the participants to report whether or not they saw a stimulus, thus measuring their conscious experiences in a dichotomous way (e.g. Baars, 1994). In this perspective, then, consciousness is considered as an all-or-none process. However, it has been argued that conscious perception is a complex phenomenon characterized by different degrees of clarity, thus needing more elaborated report measures to be adopted (Ramsøy and Overgaard, 2004).

In the light of these considerations, in order to obtain more detailed subjective reports Ramsøy and Overgaard (2004) developed a 4-point scale to assess the clarity of perceptual experiences: the Perceptual Awareness Scale (PAS). The four points consistently used by the participants to judge their visual perceptions were: 1) no experience of the stimulus, 2) brief glimpse, 3) almost clear experience and 4) clear experience. The PAS proved to be the most exhaustive measure of visual awareness compared to other graded scales and showed a good correlation between performance and awareness, possibly implying that different cognitive processes actually take place for each level of the scale (Sandberg et al., 2010). The four categories of the PAS thus refer to the quality of the perceptual experience, differently from confidence ratings that mostly involve metacognitive knowledge about the perceptual content (see Sandberg et al., 2010 for a comparison of report methodologies).

Accordingly, an fMRI study (Christensen et al., 2006) investigated the neural correlates of the use of a three-point scale (clear, vague, no perceptual experience) to rate the clarity of visual experiences in response to briefly presented stimuli. Interestingly, the authors revealed that different levels of awareness correlated with different degrees but also with different patterns of brain activation. More specifically, reports of clear experiences activated a network including parietal, temporal, frontal, basal ganglia and thalamic areas, while reports of vague perception resulted in graded activation within the same network but also in specific activations in frontal and insular regions, not seen for reports of clear experiences. Also a recent MEG study (Andersen at al., 2015) found that, during a visual masking task, occipital sources in the VAN time range were more accurate in decoding visual awareness as assessed on the four categories of the PAS, providing further evidence that perceptual awareness may be best described as a graded phenomenon.

So far, just a few papers have used a graded scale to assess visual awareness using EEG. The most evident limitation is that not all the categories of the scale were actually investigated. For example, Melloni and colleagues (2011) studied how previous experience affected conscious perception of stimuli presented at different degradation levels. Participants had to rate target visibility on the PAS, but then the authors decided to recode the scale into a dichotomous scale and found that P200 amplitude was inversely modulated by perceptual awareness. More recently, in another study Koivisto and colleagues (2013) focused on the role of recurrent interactions for categorization of natural scenes and the PAS was used in an object substitution masking experiment. However, due to the small number of ‘no experience’ ratings, behavioral analyses were carried out by pooling together the ratings of the two lowest PAS categories (‘no experience’ and ‘brief glimpse’), showing how reduced perceptual awareness following masking affected categorization performance. Moreover, ERPs were investigated only for masked and unmasked trials, regardless of PAS rating. The situation is more complex when even a more fine-graded continuous scale is used to evaluate subjective visibility by moving a cursor on a horizontal bar where only the extremes are labeled (‘not seen’ and ‘maximal visibility’). Such a scale was employed in an attentional blink (Sergent et al., 2005) and in a masking (Del Cul et al., 2007) experiment; for both tasks the authors found that visibility ratings could be neatly divided into two categories, seen and not seen trials, without intermediate graded ratings, thus showing a sort of nonlinear trend for visual awareness.

It thus seems clear that the difficult part is to get enough trials for each category of the scale, in order to perform analyses on all of them.

The aim of the present study is to explore the possible neural correlates of different grades of visual awareness. To do so, we studied the ERPs in response to reduced contrast visual stimuli at a detection threshold of about 50%. Participants had to judge the brightness of the stimuli and then qualitatively rate their visual experiences on the four-point PAS (Ramsøy and Overgaard, 2004). It was hypothesized that different grades of awareness may be reflected by different amplitudes of the components related to conscious perception (Koivisto and Revonsuo, 2003; Del Cul et al., 2007; Pins and ffytche, 2003). More specifically, if consciousness is indeed a graded phenomenon, then a linear increase of the amplitudes of the components should be observed as a function of visual awareness. Furthermore, from the analysis of the intracranial generators we could draw some conclusions on where visual awareness emerges in the brain.

2. Materials and methods

2.1 Participants

Twenty right-handed participants (13 females, mean age ± standard deviation: 22.5 ± 2.11) were recruited for the study. All reported normal or corrected-to-normal vision and no history of neurological or psychiatric disorders. They all gave their written informed consent to participate in the study. The study was approved by the local Ethics Committee and conducted in accordance with the 2013 Declaration of Helsinki. Data from six participants were excluded because there were not enough trials for analysis (<40 trials per condition) or because they showed an unequal distribution of the two stimulus types (lighter and darker) in one or more conditions. Two participants were not included in the study because of persistent noise in the EEG signal. The final sample was thus composed of twelve participants (11 females, mean age ± standard deviation: 23.08 ± 2.06).

2.2 Stimuli

The stimuli were two-dimensional lighter or darker gray Gaussian patches with a standard deviation of 0.5°, presented for 34 ms on a gray background (8.01 cd/m^2) at an eccentricity of 7° along the vertical meridian and of 12° along the horizontal meridian to the right of the fixation point. Two stimulus luminance values (one lighter and one darker than the background) were determined for each participant by means of a threshold assessment procedure: during this phase five different lighter luminance values (ranging from 6.65 cd/m^2 to 7.60 cd/m^2) and five different darker luminance values (ranging from 8.69 cd/m^2 to 9.77 cd/m^2) were used.

2.3 Threshold Assessment

In a dimly lit testing room participants sat in front of a 17 in. CRT monitor (resolution 1024 x 768, refresh rate of 85 Hz) placed at a viewing distance of 57 cm, with their head laying on a chin rest. The aim was to find two individual luminance values (one lighter and one darker) at which the participants reported to be aware of about 50% of the stimuli. The detection threshold was measured using the method of constant stimuli (Urban, 1910), where the preselected luminance values were presented in a randomized order in the periphery of the right visual field (see “Stimuli” section for details). The procedure included ten blocks: on each block, each luminance value was tested five times, resulting in a total number of 500 trials per participant. On each trial the stimulus appeared after a random interval (300–600 ms) following a brief 1000 Hz warning tone. The participants were asked to keep their eyes on a central fixation cross and press the spacebar whenever they saw a stimulus. At the end of the threshold assessment one lighter luminance value and one darker luminance value related to a 50%-detection threshold were chosen for each participant. These two luminance values were then used in the second phase of the experiment.

2.4 EEG Experiment

Each trial started with a black fixation cross, followed 400 ms later by a 1000 Hz warning tone. After a random interval ranging from 200 to 600 ms a lighter or a darker gray Gaussian patch (whose luminance values were determined in the threshold assessment) was presented for 34 ms in the periphery of the right visual field. A 1000 ms pause was then followed by a response prompt asking the participants to judge the brightness of the stimulus as compared with the gray background, pressing a button for “lighter” and another button for “darker”. The participants were required to answer even if they did not see any stimulus. Then another response prompt asked the participants to rate the quality of their perception on the four-point Perceptual Awareness Scale (PAS; Ramsøy and Overgaard, 2004). The four PAS categories are: 0) no experience of the stimulus, 1) a brief glimpse, meaning that the participant saw something but could not discriminate the brightness of the stimulus, 2) an almost clear experience and 3) a clear experience. Responses were given by pressing four different buttons on the keyboard (Fig. 1A). In order to verify that the participants used the PAS properly, at the end of the experiment we administered an open-ended questionnaire asking them to describe the criteria used for each category of the scale. The experimental session was divided into twenty blocks (66 trials each: 30 lighter, 30 darker and 6 stimulus-absent trials), thus yielding a total of 1320 trials. The order of the trials was fully randomized. Both the threshold assessment and the EEG experiment were programmed and run using E-prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA; https://www.pstnet.com/eprime.cfm).

2.5 EEG recording and event-related brain potential (ERP) analysis

EEG signal was continuously recorded with BrainAmp system (Brain Products GmbH, Munich, Germany – BrainVision Recorder) using a Fast'n Easy cap with 27 Ag/AgCl pellet pin electrodes (EasyCap GmbH, Herrsching, Germany) placed according to the 10–20 International System (O1, O2, P7, P3, Pz, P4, P8, CP5, CP1, CP2, CP6, T7, C3, Cz, C4, T8, FC5, FC1, FC2, FC6, F7, F3, Fz, F4, F8, Fp1, Fp2). Four additional electrodes were used for monitoring blinks and eye movements. Horizontal and vertical eye movements were detected respectively with electrodes placed at the left and right canthi and above and below the right eye. Other two extra electrodes served as ground (AFz) and online reference (right mastoid, RM). All scalp channels were then re-referenced offline to the left mastoid (LM). Electrode impedances were kept below 5 kΩ. The digitization rate was 1000 Hz with a time constant of 10 s as low cut-off and a high cut-off of 250 Hz.

The continuous EEG signal was then processed off-line using Brain Vision Analyzer 2.0. Data were filtered with a high-frequency cutoff of 50 Hz (12 dB/octave) and a low-frequency cutoff of 0.1 Hz (12 dB/octave), and a 50 Hz notch filter was used to remove 50 Hz interference. Channels Fp1 and Fp2 were removed in all participants due to excessive noise. Independent component analysis (ICA) was applied to the whole dataset using the Infomax ICA algorithm (Bell and Sejnowski, 1995) in order to eliminate artefactual ICs. The EEG data were then cut into epochs of 1200 ms starting 200 ms before the onset of the stimulus and segmented trials were baseline corrected on the 200 ms pre-stimulus period. Before averaging, all segments were visually inspected and removed if contaminated by eye movements, blinks, strong muscle activity or excessive noisy EEG. The averaging was carried out for five different conditions: PAS = 0 (correct lighter and darker trials receiving a rating of 0 on the PAS), PAS = 1 (correct lighter and darker trials receiving a rating of 1 on the PAS), PAS = 2 (correct lighter and darker trials receiving a rating of 2 on the PAS), PAS = 3 (correct lighter and darker trials receiving a rating of 3 on the PAS) and Catch (stimulus-absent trials receiving a rating of 0 on the PAS). After pre-processing, the mean number of trials used for the average was 103 for PAS = 0, 75 for PAS = 1, 88.25 for PAS = 2, 65.92 for PAS = 3 and 57.83 for the Catch condition. Finally, for statistical analysis, data were downsampled to 250 Hz.

2.6 Statistical analysis

A repeated-measures analysis of variance (ANOVA) was carried out on the mean percentage of correct responses of each level of the PAS. A non-parametric binomial test was performed on the same measures to determine whether accuracies were significantly different from chance (50%).

Each conscious condition (PAS = 1, 2 and 3) was pairwise compared to the unconscious (PAS = 0) condition with the Mass Univariate ERP Toolbox (Groppe et al., 2011) implemented in Matlab by means of repeated measures, two-tailed t-tests on consecutive mean amplitude time windows of 20 ms, from 0 to 1000 ms at all electrode sites. For the three pairwise comparisons (PAS = 1 vs PAS = 0; PAS = 2 vs PAS = 0; PAS = 3 vs PAS = 0) the classic Benjamini and Hochberg (1995) false discovery rate (FDR) control procedure was applied with an FDR level of 5%.

Other two repeated-measures ANOVAs were then conducted in order to test whether the increment in amplitude of the VAN and the LP as a function of perceived clarity was linear. To do so, we evaluated by means of the trend analysis implemented as part of the analysis performed by the ANOVA in SPSS, whether a linear or nonlinear (quadratic or cubic) function best represented data distribution by using polynomial coefficients. The first ANOVA was performed on the mean amplitude of the significant 20 ms time window (280–300 ms, VAN time range) of each level of the

PAS for electrodes Cp5 and T7 separately (selected on the basis of the previous analysis). The second ANOVA was run on the mean amplitude of a significant 40 ms time window (510–550 ms, LP time range) of each level of the scale for electrode Pz only (selected according to the literature).

The generators contributing to the different levels of awareness as assessed on the PAS were defined using Scalp Current Density (SCD) maps, as implemented in BrainVision Analyzer 2.0. SCD maps are calculated from the Laplacian second derivative of the field potential that is directly proportional to the current density. This technique is independent from the reference electrode and mathematically eliminates the voltage gradients caused by tangential current flows, thus emphasizing the local contributions to the surface maps and providing a better visualization of approximate locations of intracranial generators. SCD topographic maps were computed from the spherical spline interpolation of the surface voltage recording (Perrin et al., 1989) for each conscious-unconscious difference. A fourth-order spherical spline was used with a spline-smoothing coefficient (λ) of 1×10^{-6}. In order to improve the signal-to-noise ratio and to account for inter-individual differences, SCD maps were created on the grand averages of the differences between each conscious condition (PAS = 1, PAS = 2, PAS = 3) and the unconscious condition (PAS = 0). As a result of the three pairwise comparisons, SCD analyses were performed on the VAN (280-300 ms) and LP (510–550 ms) time windows. The display gain of the maps was defined by visually inspecting the baseline period of the SCD maps (from −200 to 0 ms) to better appreciate the contribution of noise to the SCD topographies.

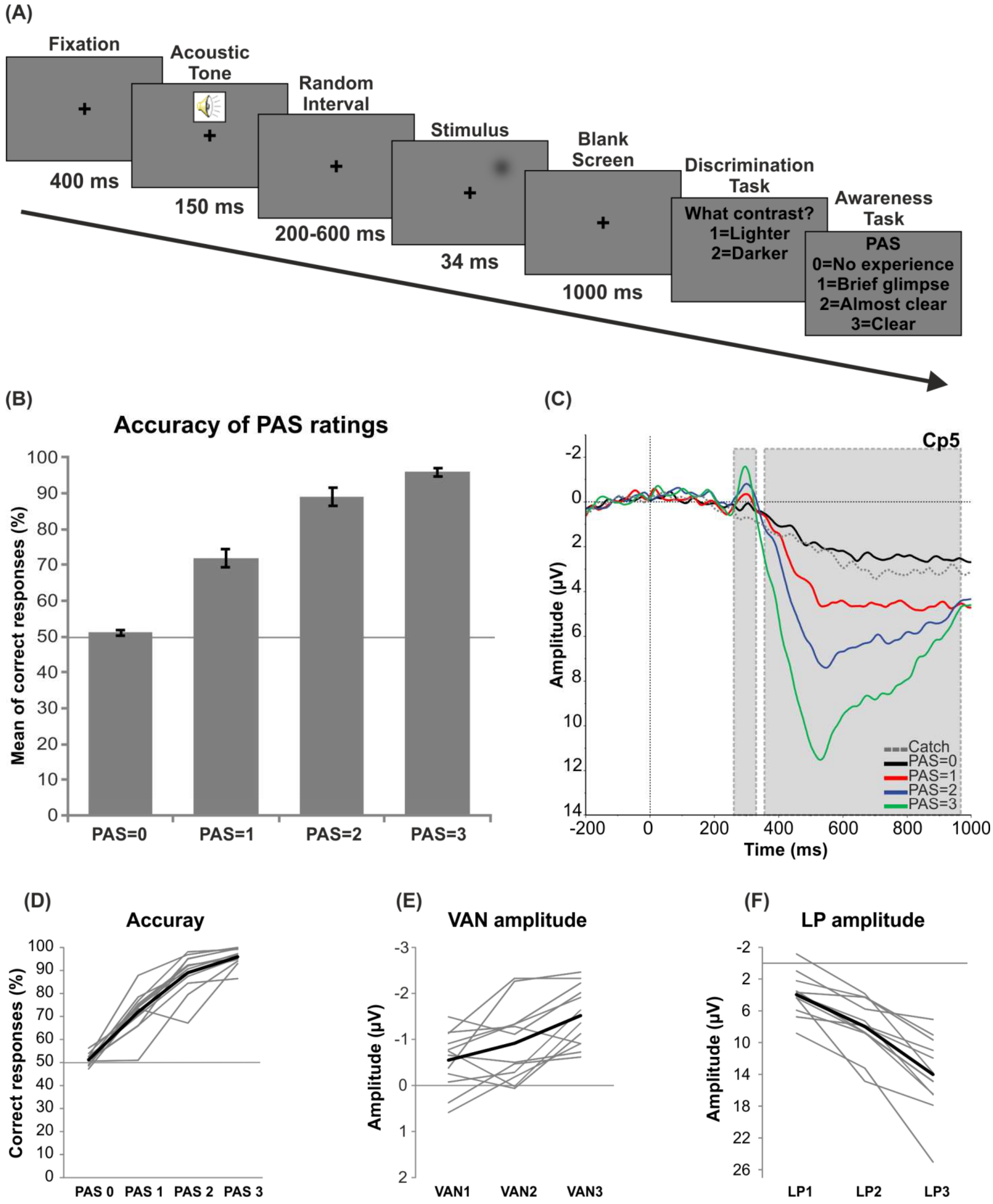


Figure 1. Trial procedure and results: **(A)** Experimental procedure: first, a fixation cross was presented for 400 ms followed by a warning acoustic tone lasting 150 ms. Then, a random interval ranging from 200 to 600 ms preceded the stimulus presentation (34 ms) in the periphery of the right visual field. After a 1000 ms pause participants had to discriminate the brightness of the stimulus (Discrimination task) and then rate the clarity of their perception on

the PAS (Awareness task). **(B)** Behavioral results: mean percentage of correct responses for each level of the PAS. Error bars represent standard errors and the solid line (50%) chance level. **(C)** ERPs: grand average ERPs in response to each category of the PAS and catch trials for electrode Cp5. Gray dotted boxes indicate the components of interest (respectively VAN and LP). **(D)** Single subject behavioral data. The thick black line represents the mean of accuracy. **(E)** Single subject amplitudes of the VAN component as a function of the differences between each conscious condition (PAS = 1, 2 and 3) and the unconscious condition (PAS = 0). The thick black line represents the average of single subject amplitudes. **(F)** Single subject amplitudes of the LP component as a function of the differences between each conscious condition (PAS = 1, 2 and 3) and the unconscious condition (PAS = 0). The thick black line represents the average of single subject amplitudes.

3. Results and Discussion

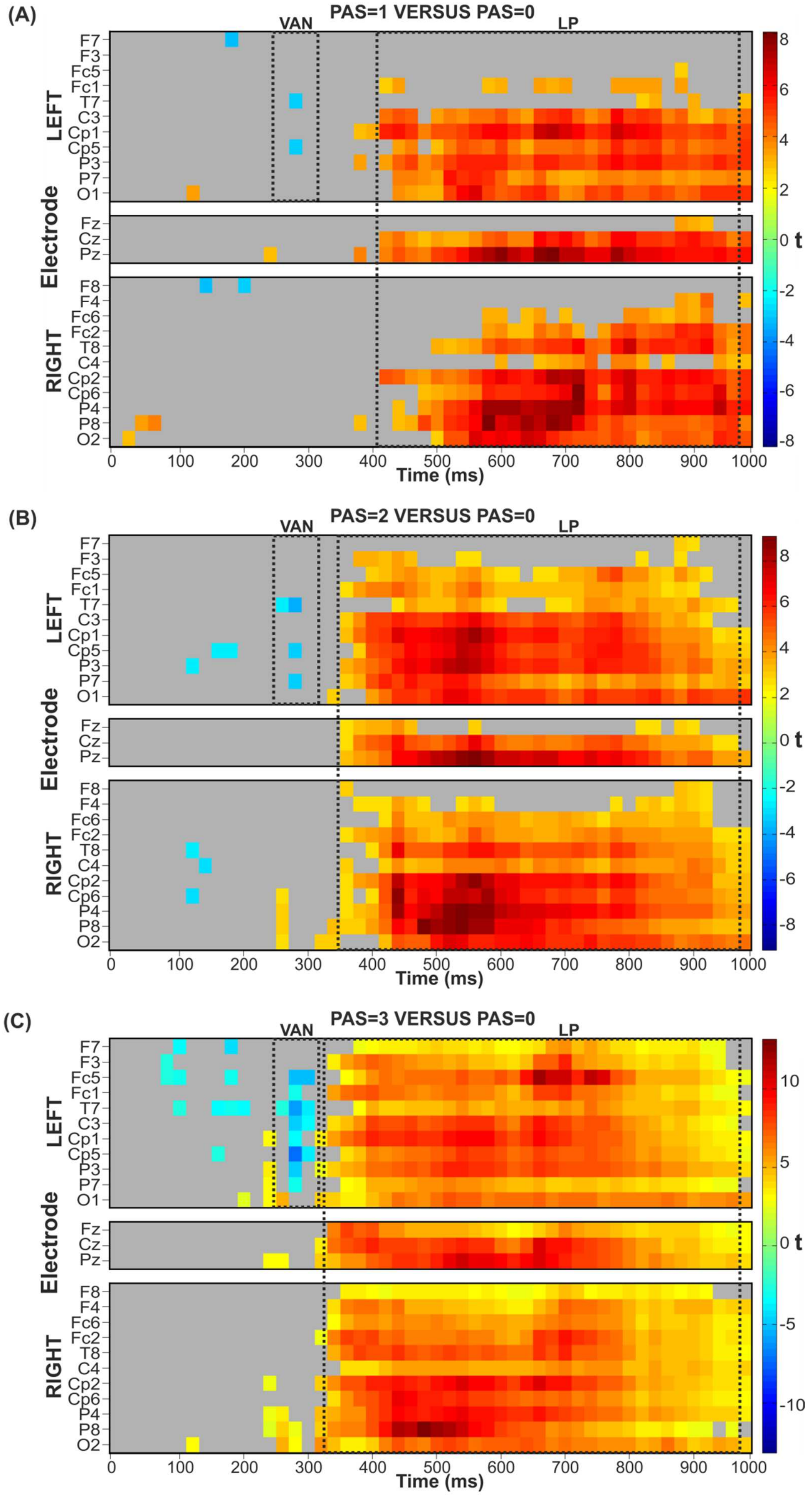
3.1 Behavioral results

After the threshold assessment, the mean luminance value chosen for lighter trials was of 9.23 cd/m^2 and of 7.23 cd/m^2 for darker trials. The mean percentage of catch trials receiving a rating of 0 on the PAS was 92.10% (sd = 5.19), thus revealing the reliability of the participants. For all trials, the mean percentage of PAS = 0 responses given by the participants was 42.66%, for PAS = 1 was 22.66%, for PAS = 2 was 22.88% and for PAS = 3 was 11.78%. A repeated-measures ANOVA conducted on the mean percentage of correct responses for each category of the PAS showed that, as visual awareness increased, also accuracy significantly increased [$F(3,33) = 156.46$, $p < 0.01$; linear trend $F(1,11) = 1279.817$, $p < 0.01$]. The mean percentage of correct responses for PAS = 0 was 51.15%, for PAS = 1 was 72.82%, for PAS = 2 was 85.68% and for PAS = 3 was 95.81% (Fig. 1B). Interestingly, also at the single subject level this linear trend could be observed (Fig. 1D), both for included and excluded (data not shown) participants. Finally, a non-parametric binomial test performed to determine whether the accuracy of each PAS level significantly differed from chance (50%) revealed that the performance when PAS = 1, PAS = 2 and PAS = 3 was significantly above chance level (all ps < 0.01), while for PAS = 0 it was not different from 50% ($p > 0.05$).

3.2 ERP results

Visual inspection of the grand average ERPs of each category of the PAS confirmed the presence of an early negative deflection (VAN) peaking at 300 ms at left channels followed by a later bilateral positive deflection (LP) starting at ~400 ms (Fig. 1C).

To compare each conscious condition (PAS = 1, PAS = 2, PAS = 3) with the unconscious condition (PAS = 0) we analyzed the corresponding mean amplitudes by means of the Mass Univariate analysis (Groppe et al., 2011) in consecutive time windows of 20 ms, starting from 0 to 1000 ms after stimulus onset, at all electrodes. For the PAS = 1 versus PAS = 0 pairwise comparison all significant FDR-corrected p-values were between 0.049056 and 0.001173 (Fig. 2A), for PAS = 2 versus PAS = 0 between 0.049848 and 0.000320 (Fig. 2B) and for PAS = 3 versus PAS = 0 between 0.047111 and 0.000040 (Fig. 2C).


(A)
PAS=1 VERSUS PAS=0
(B)
PAS=2 VERSUS PAS=0
(C)
PAS=3 VERSUS PAS=0
VAN
LP
LEFT
RIGHT
Electrode
F7
F3
Fc5
Fc1
T7
C3
Cp1
Cp5
P3
P7
O1
Fz
Cz
Pz
F8
F4
Fc6
Fc2
T8
C4
Cp2
Cp6
P4
P8
O2
Time (ms)
0
100
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8
6
4
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5
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-10

Figure 2. Raster plots: FDR-controlled t-test from Mass Univariate analyses of the three comparisons between each conscious (PAS = 1, 2 and 3) condition and the unconscious (PAS = 0) condition. T-tests were performed on the mean amplitude of consecutive time windows of 20 ms. **(A)** PAS = 1 versus PAS = 0. **(B)** PAS = 2 versus PAS = 0. **(C)** PAS = 3 versus PAS = 0.

3.2.1 Visual Awareness Negativity (VAN)

In the VAN time range (~280-300 ms) the first conscious-unconscious pairwise comparison performed on PAS = 1 versus PAS = 0 conditions demonstrated that the ERP amplitudes differed significantly at electrodes T7 and Cp5 in the left hemisphere, contralateral to stimulus presentation (Fig. 2A). The two-tailed t-tests of the second pairwise comparison on PAS = 2 versus PAS = 0 conditions revealed a significant difference again at left electrodes T7 and Cp5 but also at electrode P7 (Fig. 2B). Finally, the two-tailed t-tests on PAS = 3 versus PAS = 0 conditions showed similar results to those obtained in the previous comparisons: the VAN effect was broader both in terms of time and number of significant electrodes (P7, P3, Cp5, Cp1, C3, T7, Fc1 and Fc5), spreading to left centro-parietal sites, as depicted in Fig. 2C

To test whether there was a linear increase in the amplitude of the VAN components, two repeated-measures analyses of variance were carried out for electrodes T7 and Cp5 on the mean amplitudes of each level of the PAS in the significant 20 ms time window (280-300 ms). We decided to choose these two channels since both of them resulted significant in all the three conscious-unconscious pairwise comparisons in the VAN time range. The two ANOVAs showed that for both electrodes the amplitude of the VAN increased as a function of visual awareness (T7: $F(3,33) = 16.299$, $p < 0.01$; Cp5: $F(3,33) = 19.435$, $p < 0.01$). Interestingly, the analyses revealed a linear modulation in the increase of both electrode amplitudes (T7: linear trend $F(1,11) = 34.858$, $p < 0.01$; Cp5: linear trend $F(1,11) = 54.263$, $p < 0.01$) and such linear trend was evident in the data of each participant (Fig. 1E).

Conscious conditions thus seemed to elicit more negative responses than the unconscious condition, as revealed by the presence of a reliable negative early component (VAN). The component peaked between 280 and 300 ms and was evident at left lateral posterior channels spreading to more parietal and central sites as visual awareness increased. Moreover, there was a linear graded modulation of the amplitude of the component as a function of the levels of visual awareness.

3.2.2 Late Positivity (LP)

Corresponding pairwise comparisons were performed in the LP time window. The two-tailed t-tests on PAS = 1 versus PAS = 0 conditions showed a widespread LP component starting at ~420 ms post-stimulus and continuing until the end of the epoch (1000 ms), particularly over posterior and central sites, bilaterally (Fig. 2A). The comparison performed on PAS = 2 versus PAS = 0 conditions revealed a widespread LP component that started at ~360 ms and continued up to 1000 ms showing the most consistent effects bilaterally at posterior, central and partly also at prefrontal channels (Fig. 2B). Finally, the two-tailed t-tests on the last conscious-unconscious comparison performed on PAS = 3 versus PAS = 0 found significant differences between the two conditions starting at ~340 ms to 1000 ms over all channels bilaterally (Fig. 2C).

One repeated-measures ANOVA was performed for electrode Pz on the mean amplitudes of each level of the PAS in a 40 ms time window around the peak (510-550 ms) in order to test for a linear increase of the LP component. Given the widespread LP effect, channel Pz was chosen for analysis according to the literature (Del Cul et al., 2007). As for the VAN, the ANOVA revealed that the amplitude of the LP linearly increased with higher ratings of visual awareness ($F(3,33) = 70.277$, $p < 0.01$; linear trend $F(1,11) = 103.177$, $p < 0.01$) and this linear modulation was again found at the individual level, as shown in Fig. 1F.

Responses to perceived trials were thus more positive than to unconscious trials. Such broad LP effect was bilaterally evident at central, posterior and lateral sites spreading also to prefrontal channels as visual awareness increased. Finally, the amplitude of the LP component was linearly modulated by visual awareness.

3.2.3 Scalp Current Density (SCD) Maps

Intracranial generators of the VAN and the LP were defined using SCD maps (Fig. 3). According to the SCD topographies, at the lowest level of visual awareness (PAS = 1), the VAN component was consistent with left temporal generators, contralateral to stimulus presentation. The effect then spread to left posterior parietal areas at the intermediate level of awareness (PAS = 2) and finally activated a complex comprising also fronto-central generators at the highest level of conscious perception (PAS = 3). As regards the LP effect, the SCD topographies were consistent with bilateral posterior, lateral and central generators for the lowest and intermediate levels of visual awareness (PAS = 1 and PAS = 2), while for reports of clear experience (PAS = 3), current density foci were observed over different scalp areas including the prefrontal cortex and seemed larger in the right hemisphere. The intracranial generators of the phenomenal awareness (as assessed by the VAN) were thus found in the left temporal lobe, then the activation spread to posterior, central and prefrontal areas as a function of visual awareness. The LP component, interpreted to reflect access awareness, originated bilaterally in posterior, lateral and central areas extending to prefrontal regions as perceived clarity increased.

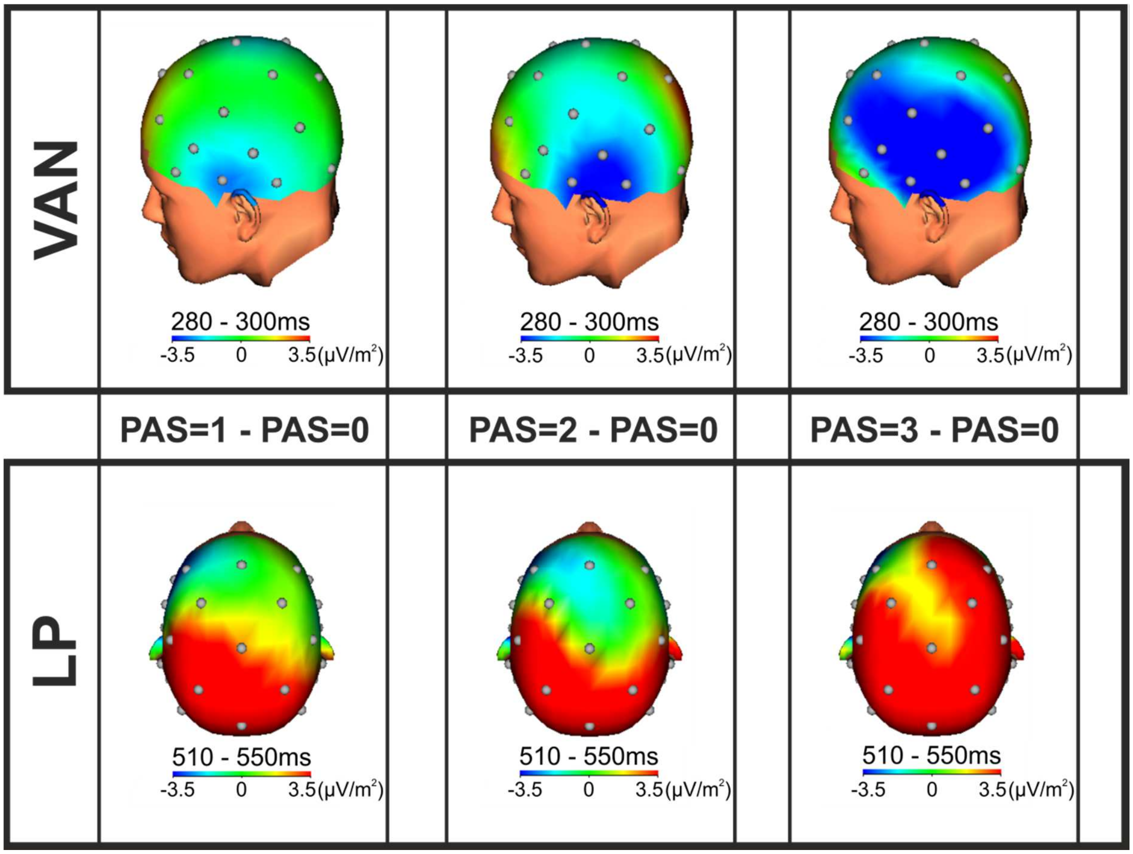

Figure 3. SCD topographic maps: SCD foci for the VAN (upper panel; time window from 280 to 300 ms) and LP (lower panel; time window from 510 to 550 ms) components performed on the grand average of the differences between each conscious condition (PAS = 1, 2 and 3) and the unconscious condition (PAS = 0).

4. General Discussion

In the present study, participants were required to rate the clarity of their perceptual experience of low-contrast stimuli on the four-point Perceptual Awareness Scale (Ramsøy and Overgaard, 2004). We found that their discrimination accuracy increased linearly as visual awareness increased. Moreover, ERP results revealed two electrophysiological components correlating with visual awareness. A negative early deflection, the VAN, peaking around 280-300 ms at lateral, parietal and central sites in the left hemisphere, followed by a later positive component, the LP, starting bilaterally 400 ms after stimulus onset over different scalp regions. As for accuracy, the amplitude of both components was found to increase linearly as a function of visual awareness as assessed on the PAS.

These results provide evidence that visual perceptual experience is characterized by a gradual increase of perceived clarity at both behavioral (accuracies) and neural (amplitudes) level. Such findings seem to be inconsistent with those by Del Cul (2007), in which both accuracies and subjective ratings collected on a continuous scale where only the extremes were labelled exhibited a nonlinear dichotomous distribution and the P300 was the only component whose amplitude varied with a similar sigmoidal trend to subjective ratings. The authors thus concluded that the P300 reflected the final stage of a process that led to an all-or-none reportability of a perceptual experience and they seem to allude to what Block (2005) defined "access consciousness". However, it has been argued that the components in the P300 latency range and, thus, access consciousness might better reflect post-perceptual processes or consequences of consciousness, such as the confidence of the observer (Eimer and Mazza, 2005), different levels of accumulation of sensory evidence (Melloni et al, 2011) or working memory update (Polich, 2007). Given that the PAS is a measure of clarity of the perceptual experience and not a measure of confidence in response accuracy, our data would suggest that the LP might reflect the linear increase of the sensory evidence as the clarity of perceptual experience increases, in line with the interpretation given by Melloni and colleagues (2011).

Importantly, what proved to be the earliest most reliable correlate of phenomenal consciousness (Block, 2005), across different experimental paradigms and attentional manipulations, is the VAN, interpreted (Koivisto and Revonsuo, 2010) as the correlate of the actual content of perception as opposed to later post-perceptual processes (for a review see Railo et al., 2011). In agreement with this interpretation, the quality judgments given by our participants on the PAS were reflected in a concurrent linear modulation of the VAN amplitude, showing that different levels of cortical activity determined different levels of perceptual clarity. Similar results were found by Moutoussis and Zeki (2002) that showed how the difference between perceived and invisible stimuli depended on the strength of brain activation.

Our results are in line with findings obtained with different neuroimaging techniques. In an fMRI study (Christensen et al., 2006), reports of vague perceptual clarity versus clear experiences resulted in graded brain activity but also in unique patterns of cortical activation. As regards MEG, a recent experiment (Andersen et al., 2015) showed that occipital sources at the time window of the VAN could better decode graded levels of perceptual consciousness as assessed on the PAS. Taken together, all these pieces of evidence seem to support the graded nature of visual experience. Moreover, given its early latency, the VAN seems to be the component that better tackles the different degrees of perceived clarity of the phenomenal content.

An important point that deserves some considerations relates to the “where in the brain” perceptual awareness emerges. Our study, together with previous studies (e.g. Koivisto et al., 2013; Sandberg et al., 2013a), seems to indicate that processes correlating with the graded contents of visual experience take place in temporal areas. The presence of early generators (VAN) in such posterior areas might be in line with data on phosphene perception (Bagattini et al., 2015): in this paper, the authors have proved how phosphene perception following occipital TMS stimulation is generated in the temporal cortex, while phosphene perception after parietal stimulation arises from the parietal regions. The fact that different generators have actually been found for the two different stimulation conditions thus confirms that the temporal and parietal cortices themselves are independent generators of conscious visual percepts. Both these and our results seem to be in favor of Zeki’s “micro-consciousness” proposal (Zeki and ffytche, 1998), stating that local early activity in higher-order extrastriate regions plays a key role in generating visual perception. Indeed, it is evident from the analysis of intracranial sources that visual consciousness does not require a later widespread fronto-parietal activation, as proposed by the Global Workspace Theory (GWT; Dehaene, 2014). This, again, is confirmed by phosphene studies (Bagattini et al., 2015), since a patient with a complete lesion of V1 showed differences for phosphene awareness only in an early time window, unlike healthy participants where differences were found also in a later phase in occipital and frontal areas. Likewise, recent MEG findings (Andersen et al., 2015) revealed that frontal sources at the P300 time range could not decode all PAS ratings. All these results seem to strengthen the assumption that such later frontal activity might support those consequences of consciousness (LP or access consciousness; Block, 2005) that are related to the components in the P300 time window (confidence, Eimer and Mazza, 2005;

accumulation of sensory evidence, Melloni et al, 2011; update of working memory, Polich, 2007) and not perceptual awareness itself.

Another interesting aspect is that the PAS (Ramsøy and Overgaard, 2004) proved to be a good report measure to investigate different levels of perceptual clarity. Indeed, in the present study participants could use all the categories of the scale. Besides, we found that different levels of accuracy, and both the access properties (LP) and, more importantly, the actual phenomenal content of consciousness (VAN) differed depending on the levels of the PAS further corroborating the suggestion (Ramsøy and Overgaard, 2004) that each judgment given by the participants actually implies differences in processing. The implications related to such findings are important when considering blindsight patients. Blindsight follows a lesion in the primary visual cortex, resulting in a preserved ability to detect and discriminate visual stimuli presented in the blind field yet reporting no awareness of them: a phenomenon at first described as a case of unconscious vision (Weiskrantz, 1986). The exact mechanisms that are responsible for blindsight are still unknown but some patients with a huge lesion of V1 have been reported to exhibit some residual visual consciousness in their damaged hemifield (Barbur et al., 1993; Zeki and ffytche, 1998). The use of a graded scale, such as the PAS, together with electrophysiological measures, might be helpful in discriminating patients showing a genuine blindsight phenomenon from those having residual conscious vision. In fact, using a dichotomous scale might not be sufficient to detect weaker forms of conscious perception, as already illustrated by Overgaard and colleagues in their seminal paper (2008) and more recently by Mazzi and colleagues (submitted). In these studies, patient GR (Overgaard et al, 2008) and patient SL (Mazzi et al., submitted), both suffering from a damage to the left occipital lobe, exhibited a blindsight behavior when tested with a binary seen/unseen scale, while when using the PAS, visual awareness was predictive of their performance, thus exhibiting conscious, yet degraded, vision. It could, thus, be predicted that patients diagnosed with degraded vision (as assessed on the PAS or another graded scale) would show similar components (VAN and LP) as the healthy participants in the present paper while genuine blindsight patients would not.

To summarize, we found that discrimination performance in a task with low-contrast stimuli increased as a function of visual awareness together with a linear amplitude modulation of the components correlating with the perceptual content (VAN) and post-perceptual processes (LP), suggesting that the nature of visual consciousness might be gradual. We also propose that the conscious phenomenal content of perceptual experiences emerges from the activation in temporal areas, as indicated by the topography of the intracortical generators of the VAN. Finally, the PAS seems to be an exhaustive measure in order to obtain more detailed subjective ratings.

EXPERIMENT 2

1. Introduction

Hemianopia is one of the most common visual field defects that follows a lesion occurring between the optic chiasm and the primary visual cortex (Holmes, 1945). In particular, in homonymous hemianopia the patient has no conscious access to visual information presented in his contralesional hemifield, despite of absence of peripheral damage to the eye.

Interestingly, even if there is no visual awareness for material appearing in the contralesional visual field, some of the patients suffering from hemianopia can show some striking behavior that seems to be visually guided (Weiskrantz, 2009). Such a phenomenon is called 'blindsight' (Weiskrantz et al., 1974). For example, patients can above chance orient their attention to stimuli presented in their blind visual field, without being utterly aware of them (Sanders et al., 1974). Noteworthy, since not all hemianopic patients exhibit blindsight, different neuroanatomical correlates have been related to this particular behavior. Some authors linked blindsight to the preserved functioning of "islands" of neurons within the damaged visual cortex (Fendrich et al., 2001), even if this account has not been completely confirmed. Other studies (Rodman et al., 1989) support the existence of a subcortical pathway projecting from the superior colliculus and the pulvinar towards extrastriate areas in the dorsal stream. Lastly, recent researches (Schmid et al., 2010) identified the lateral geniculate nucleus of the thalamus as a crucial structure in visual functions, necessary for neural activation of extrastriate areas in the absence of V1. No clear consensus on the neural basis of blindsight has been reached yet, also due to the fact that over the last years, alongside the original form of blindsight (later called type 1 blindsight), another variety of such a behavior has been described in a series of experiments, the so-called type 2 blindsight (Weiskrantz, 1998). In type 2 blindsight, patients report a "non-visual" feeling of something happening in the blind field, thus showing a residual awareness that positively correlates with the behavioral outcome in visual tasks (e.g. Stoerig and Barth, 2001; Zeki and ffytche, 1998). However, it has been claimed (Foley, 2014) that patients exhibiting type 2 blindsight might describe their perceptual experiences as non-visual in nature because, as a consequence of the brain damage, they lack the important features that specifically characterize healthy visual perception. So what might happen is that patients misjudge the visual nature of the perceptual experience, since it is extremely different from what it used to be before the lesion. If this is the case, then, type 2 blindsight, differently from type 1 blindsight, can be better described as degraded yet conscious vision.

Another important issue when studying blindsight regards the assessment of residual visual abilities in hemianopic patients. In fact, blindsight has usually been studied by means of dichotomous report scales (e.g. Azzopardi and Cowey, 1997), requiring the patients to say whether or not they perceived something in their blind visual field. In a methodological review on blindsight, Overgaard (2011) stated that conscious experiences are indeed complex, so that binary scales have a too limited capacity to detect weaker forms of consciousness. This is true especially in the case of hemianopic patients where, as said before, the visual experience is completely different from that of neurologically healthy subjects. As a confirm, studies on two different hemianopic patients (GR: Overgaard et al., 2008; SL: Mazzi et al., 2016) found that, when using binary scales, such patients put the threshold for visual awareness higher than when they were required to use graded measures. These different thresholds result in a blindsight behavior when awareness is assessed through dichotomous scales (i.e. above chance performance in the absence of conscious experience), and in degraded conscious sight when using graded scales (i.e. performance positively correlates with visual awareness level). It obviously follows that exhaustive rating scales should be employed to assess residual visual abilities in hemianopic patients, in order not to over or underestimate the visual defect. Particularly, the scale that was used to test the aforementioned patients (GR and SL), and proved to be an exhaustive measure of visual awareness, is the four-point Perceptual Awareness Scale (PAS; Ramsøy and Overgaard, 2004). In addition, electrophysiological data showed that each level of the scale differentially modulated the amplitude of two ERP components (the Visual Awareness Negativity and the Late Positivity) related to conscious perception, and, specifically, the amplitude of both components resulted to be linearly modulated by the level of visual awareness as rated by the participants on the scale (Tagliabue et al., 2016). The combined use of the PAS and electrophysiological measures could thus be a promising approach to discriminate patients exhibiting an authentic blindsight behavior from those suffering from just degraded conscious vision. Patients with degraded vision, in contrast to genuine blindsight patients, might not only have a behavioral performance that positively correlates with visual awareness, but also show similar VAN and LP components (and a similar modulation) as those found in healthy subjects.

The aim of this study is to test a female hemianopic patient (SL), that was previously reported (Mazzi et al, 2016) to suffer from degraded conscious vision assessed at behavioral level. The same assessment procedure was repeated: the patient was presented with stimuli in her blind visual field, asked to first discriminate them and then rate the quality of her perceptual experience on the PAS. In addition, while SL performed the task, the EEG signal was recorded in order to investigate the ERP components and their hypothesized modulation elicited by the material showed in her blind hemifield. The ultimate goal is to evaluate whether electrophysiological signatures can act as an additional and more fine-grained diagnostic tool to confirm the behavioral performance exhibited by patients.

2. Materials and Methods

2.1 Participant

The patient involved in the study is a 49-years old right-handed woman (SL). She suffered from an ischemic stroke with hemorrhagic evolution that completely destroyed her left primary visual cortex (V1), as shown by the MRI (Fig. 1A). A right homonymous hemianopia developed as a consequence of the brain damage and was assessed through a computerized perimetry (Humphrey system; Fig 1B).

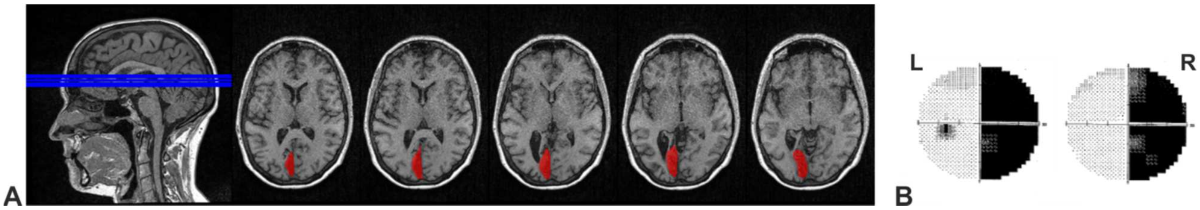

Figure 1. (A) Axial MRI slices showing SL's lesion in the left occipital lobe. **(B)** Visual field plots obtained from computerized Humphrey perimetry that surveys ± 30 degrees for the left (L) and right (R) eye. The black region shows the hemianopic right visual field.

SL was tested about 88 months after the neurological event. The patient gave her written informed consent to participate in the study. The study was approved by the local Ethics Committee and conducted in accordance with the 2013 Declaration of Helsinki.

2.2 Stimuli

The stimuli were two-dimensional lighter or darker gray circles with a diameter of 4°, presented for 72 ms on a gray background (7.47 cd/m^2). The stimuli were presented unilaterally in the blind (right) visual field of the patient, at an eccentricity of 7° along the vertical meridian and of 12° along the horizontal meridian. Two stimulus luminance values (one lighter, 10.46 cd/m^2, and one darker, 0.27 cd/m^2, than the background) were chosen to perform the task.

2.3 Experimental Procedure

In a dimly lit testing room the patient sat in front of a 17-inc CRT monitor (resolution 1024 x 768, refresh rate of 85 Hz) placed at a viewing distance of 57 cm, with her head laying on an adjustable chin rest. Each trial started with a black central fixation cross, followed 400 ms later by a 1000 Hz warning tone lasting 150 ms. A random interval ranging from 200 to 600 ms to avoid expectation preceded stimulus presentation. Lighter or darker gray circles were then presented for 72 ms in the periphery of the blind (right) visual field. A 1000 ms pause was then followed by a response prompt asking the patient to judge the brightness of the stimulus as compared with the gray background, pressing a button for "lighter" and another button for "darker". Stimulus-absent (catch) trials were also included in the experiment and SL was required to guess when no stimulus discrimination was possible. Then another response prompt asked her to rate the quality of her perception on the four-point Perceptual Awareness Scale (PAS; Ramsøy and Overgaard, 2004). The four PAS categories are: 0) no experience of the stimulus, 1) a brief glimpse, meaning that the participant saw something but could not discriminate the brightness of the stimulus, 2) an almost clear experience and 3) a clear experience. Responses were given by pressing four different buttons on the keyboard (Fig. 2).

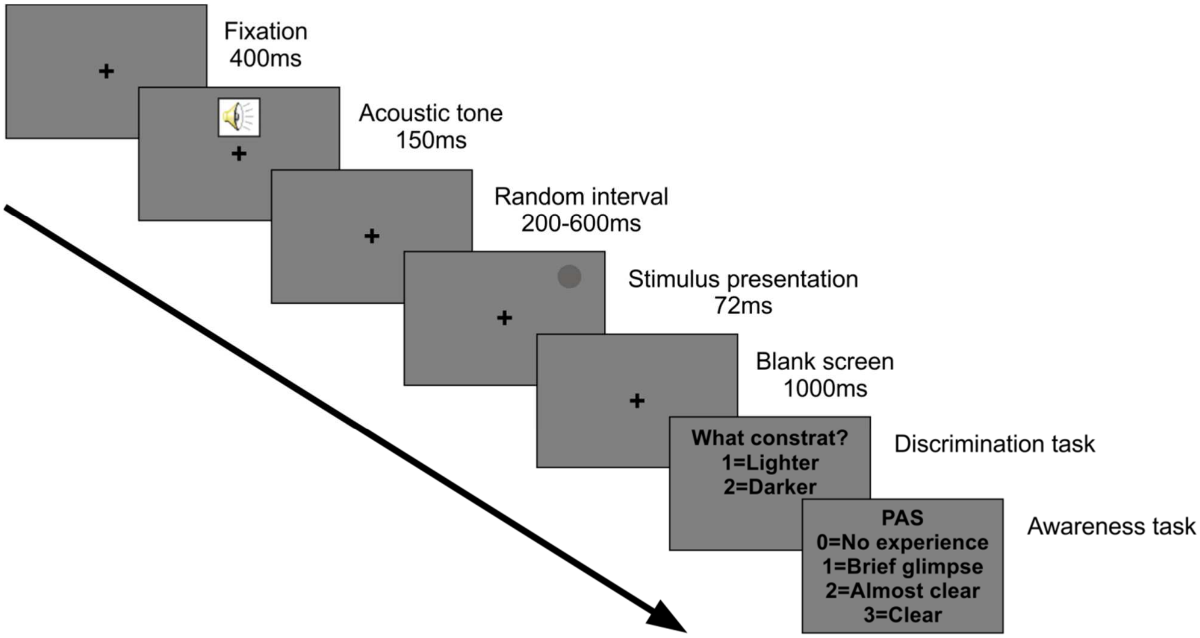

Figure 2. Single trial structure: A fixation cross was presented for 400 ms followed by a warning acoustic tone lasting 150 ms. Then, a random interval preceded the stimulus presentation (72 ms) in the periphery of the (blind) right visual field. After a 1000 ms pause SL had to discriminate the brightness of the stimulus (Discrimination task) and then rate the clarity of her perception on the PAS (Awareness task).

In order to avoid misunderstandings, the use of the PAS was thoroughly discussed with the patient in a training session (Sandberg et al., 2013b). The experimental session was divided into 30 blocks (33 trials each: 15 lighter, 15 darker and 3 stimulus-absent trials), thus yielding a total of 990 trials. The order of the trials was fully randomized. The EEG experiment was programmed and run using E-prime (Psychology Software Tools, Inc., Pittsburgh, PA, USA; https://www.pstnet.com/eprime.cfm). To control SL’s fixation during the presentation of the stimuli, her eye movements were monitored on-line throughout the experiment by means of an infrared camera.

2.4 EEG recording and event-related brain potential (ERP) analysis

EEG signal was continuously recorded with BrainAmp system (Brain Products GmbH, Munich, Germany – BrainVision Recorder) using a Fast'n Easy cap with 59 Ag/AgCl pellet pin electrodes (EasyCap GmbH, Herrsching, Germany) placed according to the 10–05 International System. Four additional electrodes were used for monitoring blinks and eye movements. Horizontal and vertical eye movements were detected respectively with electrodes placed at the left and right canthi and above and below the right eye. Other two extra electrodes served as ground (AFz) and online reference (right mastoid, RM). Electrode impedances were kept below 5 kΩ. The digitization rate was 1000 Hz with a time constant of 10 s as low cut-off and a high cut-off of 250 Hz. The continuous EEG signal was then processed off-line using EEGLAB (Delorme and Makeig, 2004). First of all, data were downsampled to 250 Hz and then filtered with a low-frequency cutoff of 0.1 Hz. A 50 Hz bandpass notch filter (width of 2 Hz) was also applied to remove 50 Hz line noise. All scalp channels were then re-referenced offline to the left mastoid (LM). Independent component analysis (ICA) was applied to the whole dataset using the Infomax ICA algorithm (Bell and Sejnowski, 1995) in order to eliminate artefactual ICs (e.g. eye blinks, saccades, muscle activity). After ICA, a high-frequency filter with a cut-off of 40 Hz was applied. The EEG data were then cut into epochs of 1300 ms starting 300 ms before the onset of the stimulus and segmented trials were baseline corrected on the 300 ms pre-stimulus period. Before averaging, all segments were visually inspected and removed if contaminated by residual eye movements, blinks, strong muscle activity or excessive noisy EEG. Due to the fact that SL never used rating 3 on the PAS, the averaging was carried out for four different conditions only: PAS = 0 (correct lighter and darker trials receiving a rating of 0 on the PAS), PAS = 1 (correct lighter and darker trials receiving a rating of 1 on the PAS), PAS = 2 (correct lighter and darker trials receiving a rating of 2 on the PAS) and Catch (stimulus-absent trials receiving a rating of 0 on the PAS). After pre-processing, the number of trials used for the average was 104 for PAS = 0, 129 for PAS = 1, 102 for PAS = 2 and 59 for the Catch condition.

2.5 Statistical analysis

A non-parametric binomial test was performed on the mean percentage of correct responses of each level of the PAS to determine whether accuracies were significantly different from chance (50%).

For the analysis of the ERPs, each category of the PAS (0, 1 and 2) was compared to the others by means of a non-parametric Monte Carlo percentile two-tailed bootstrap resampling procedure (Efron and Tibshirani, 1993), on each sample (channel x time point) from 0 to 800 ms after stimulus onset. Bootstrap relies on random sampling with replacement, so that each new sample is not identical to the initial one. 10,000 resampled data distributions were created and a 0.05 significance threshold was used. Correction for multiple comparisons was performed through the classic Benjamini and

Hochberg (1995) false discovery rate (FDR) control procedure, with an FDR level of 5%. Before running the analysis, the order of the trials was shuffled within each condition, due to the fact that they had slightly different numbers of trials. Furthermore, the maximum size of each resampled distribution was equated to the size of the less numerous condition.

3. Results and Discussion

3.1 Behavioral Results

The percentage of stimulus-absent trials receiving a rating of 0 on the PAS was 93.3%, thus revealing how SL can most of the times recognize when nothing is presented in her blind field. For all trials collapsed across color, the mean percentage of PAS = 0 responses given by the patient was 29.78%, 46.33% for PAS = 1 and 23.89% for PAS = 2. She never used rating 3. The mean percentage of correct responses when SL used PAS = 0 was 59.33%, 46.76% when trials were rated 1 and 66.98% when she reported an almost clear experience of the stimulus (PAS = 2). A non-parametric binomial test was performed to determine whether the accuracy of each PAS category was significantly different from chance level (50%). The analyses found that the performance when PAS = 0 and PAS = 1 did not differ from 50% (all ps > 0.05), while for PAS = 2 it was significantly above chance level (p < 0.01; Fig. 3).

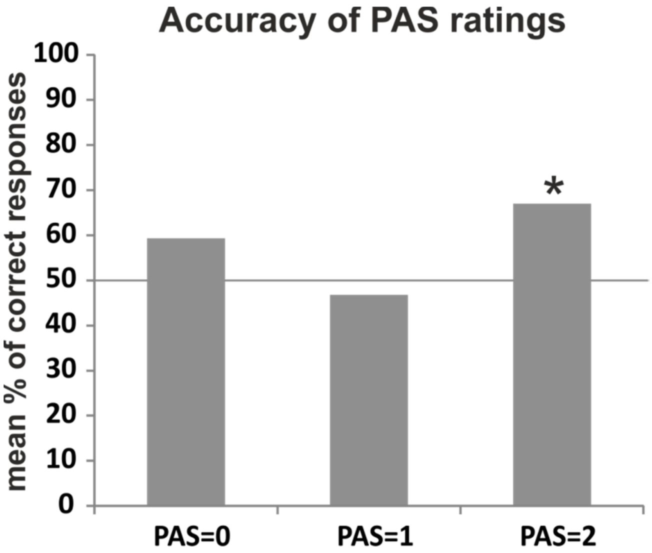

Figure 3. Behavioral results: mean percentage of correct responses for each level of the PAS. The solid line represents (50%) chance level.

The behavioral data thus revealed that, even if SL gave different ratings about her perceptual experience, there is no difference in accuracy between PAS = 0 and PAS = 1 (they are both at chance level), while her performance is above 50% when she reports a clearer visual experience (PAS = 2).

3.2 ERP results

The visual inspection of SL's averaged ERPs (Fig. 4) elicited by each category of the PAS confirmed the presence of an early negative deflection, consistent with the VAN, peaking around 200 ms over left channels. The VAN is followed by a later bilateral positive deflection, consistent with the LP, starting at ~300 ms.

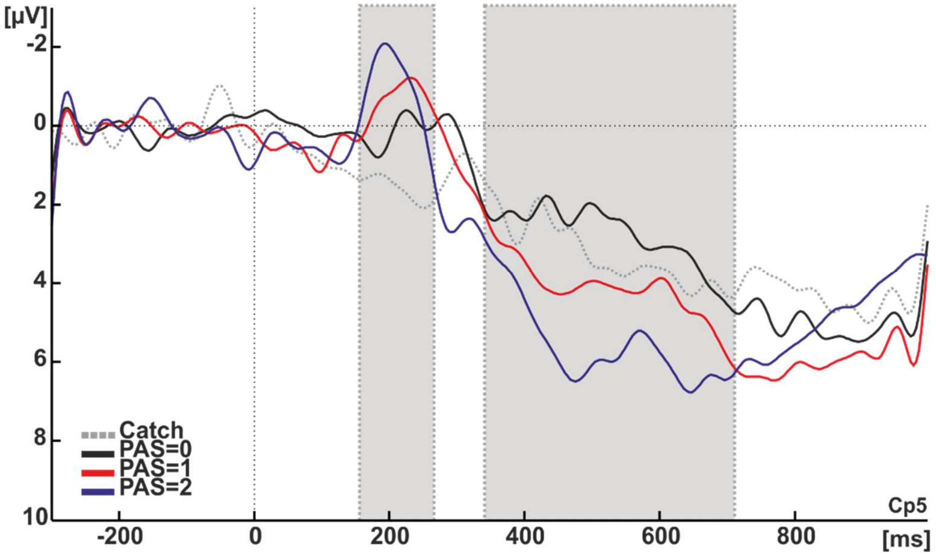

Figure 4. ERPs: grand average ERPs in response to each category of the PAS and catch trials for electrode Cp5. Gray dotted boxes indicate the components of interest (respectively VAN and LP).

Each condition (PAS = 0, PAS = 1, PAS = 2) was compared to the others by means of FDR-corrected boostrap tests on the amplitude of each time point, starting from 0 to 800 ms after stimulus onset, over all electrodes. In the first comparison, PAS = 0 versus PAS = 1, no sustained reliable difference was found between the two PAS conditions, for both VAN and LP (Fig. 5).

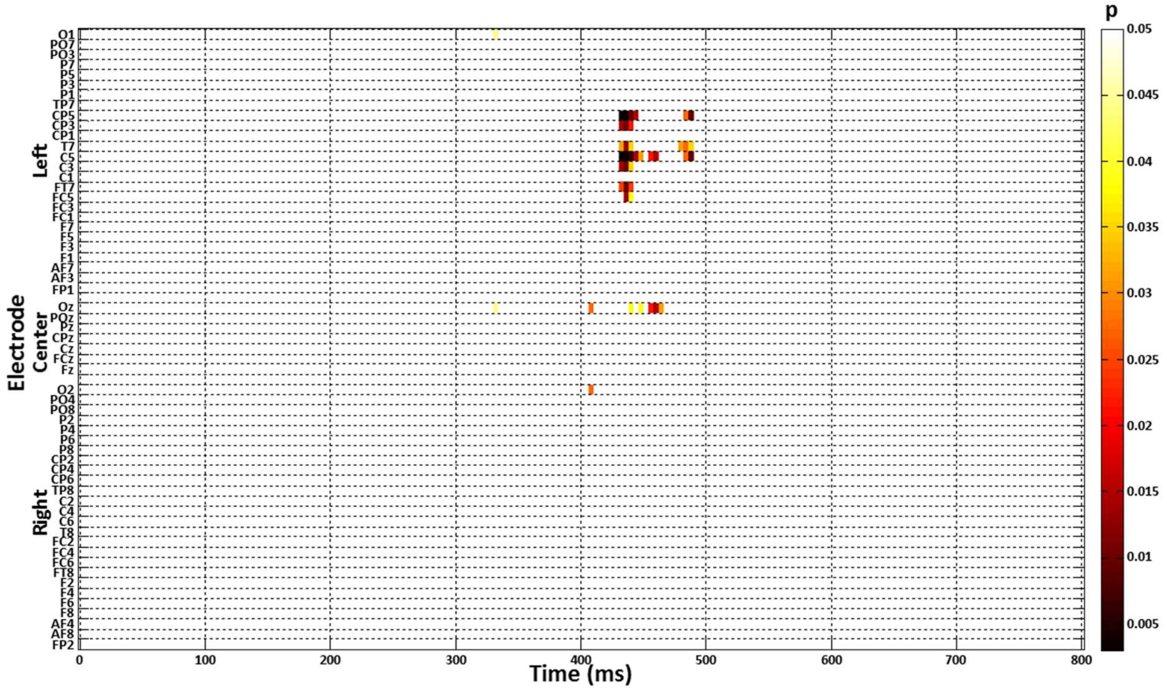

Figure 5. Raster plot of FDR-controlled bootstrap t-tests of PAS = 0 versus PAS = 1 comparison. T-tests were performed on the amplitude of each time point, from 0 to 800 ms after stimulus onset.

The second comparison between PAS = 1 and PAS = 2 (Fig. 6) showed the first significant sustained difference for a negative component consistent with the VAN starting around 150 ms until 210 ms, over left temporal, central and frontal areas (T7, FT7, FC5, FC3, F7, F5). As regards the LP, a significant difference is found from around 250 to 300 ms after stimulus onset over left posterior and centro-parietal channels (O1, PO7, PO3, P7, P5, P3, P1, CP5, CP3, CP1, FC1), while over central and right centro-parietal electrodes the difference is significant from around 270 until 500 ms after stimulation (FCz, Cz, CPz, Pz, POz; P2, P4, P6, CP2, CP4, CP6, TP8, C2, C4, C6, T8, FC2, FC4, FC6, FT8).

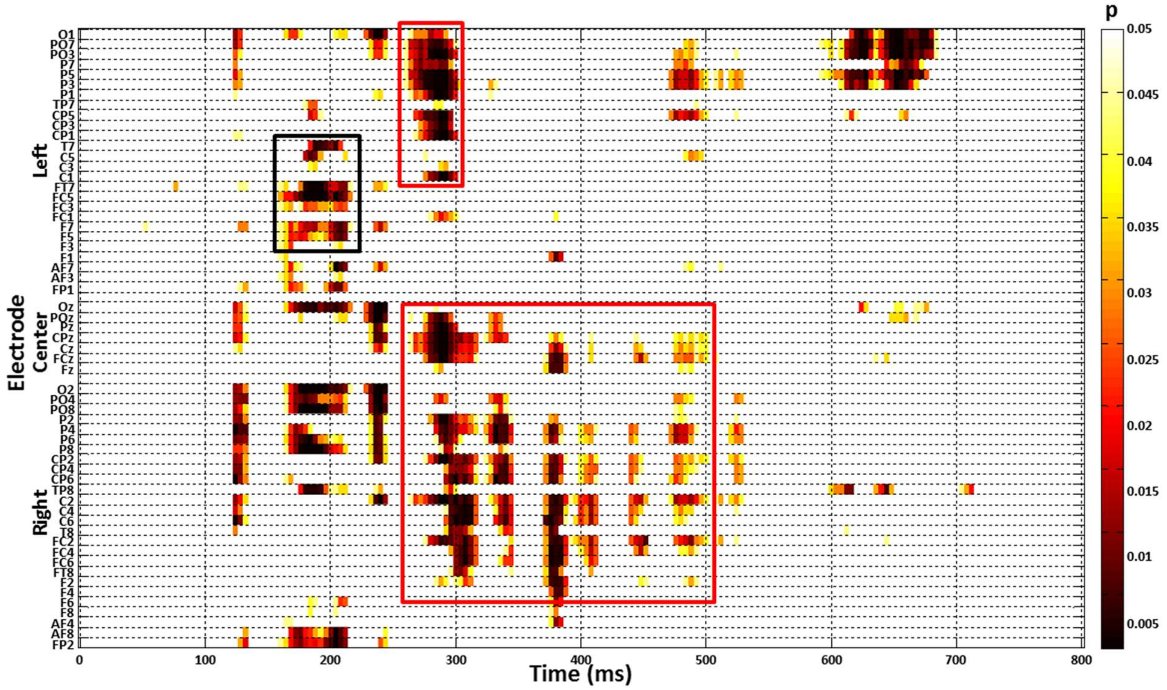

Figure 6. Raster plot of FDR-controlled bootstrap t-tests of PAS = 1 versus PAS = 2 comparison. T-tests were performed on the amplitude of each time point, from 0 to 800 ms after stimulus onset. The black box indicates the electrodes where a difference in the VAN is observed, while the red boxes indicate the electrodes where a difference in the LP is observed.

The last comparison between PAS = 0 and PAS = 2 (Fig. 7) identified a significant difference in the VAN over centro-parietal sites (P7, P5, TP7, CP5, CP3, T7, C5, C3, FT7, FC5, FC3), from around 170 to 230 ms after stimulus onset, while significant differences on the LP were observed bilaterally over the majority of electrodes, starting from 250 until 530 ms.

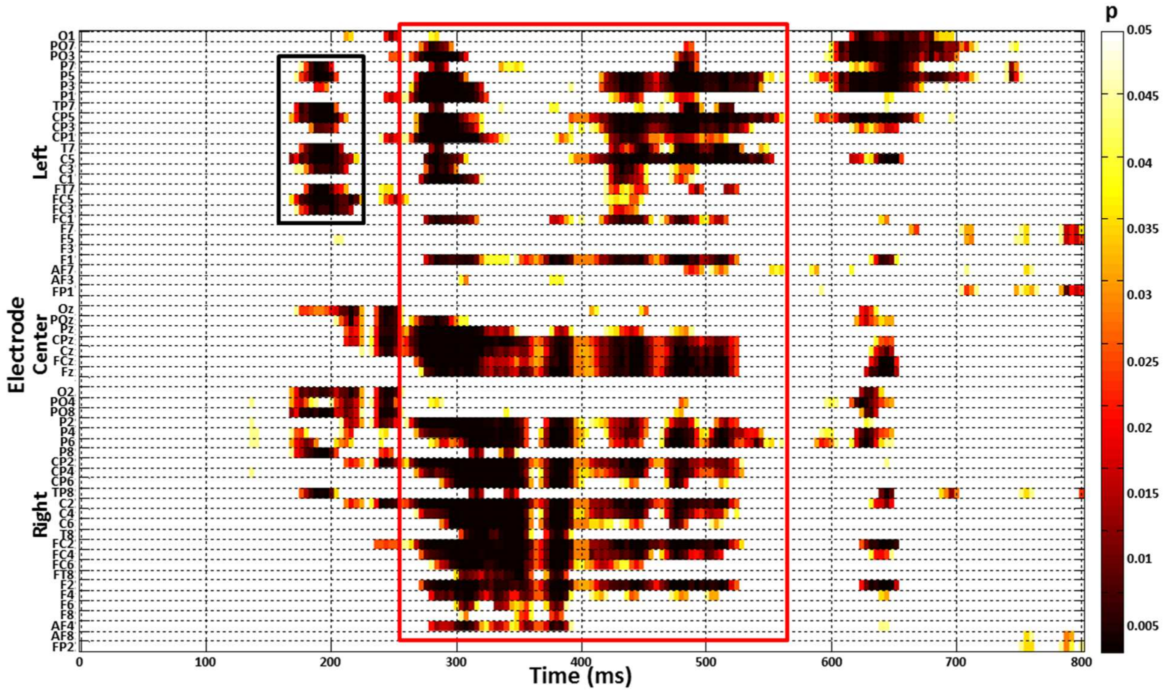

Figure 7. Raster plot of FDR-controlled bootstrap t-tests of PAS = 0 versus PAS = 2 comparison. T-tests were performed on the amplitude of each time point, from 0 to 800 ms after stimulus onset. The black box indicates the electrodes where a difference in the VAN is observed, while the red box indicates the electrodes where a difference in the LP is observed.

The results thus identified no differences in the VAN and LP when comparing the two lowest levels of visual awareness (PAS = 0 vs PAS = 1). Significant differences were instead found in the other comparisons (PAS = 1 vs PAS = 2 and PAS = 0 and PAS = 2), with higher amplitudes of both components related to higher levels of perceptual visual experience.

4. General Discussion

In the present study, we tested SL, a hemianopic patient previously (Mazzi et al., 2016) assessed at behavioral level and showing degraded conscious vision. The patient was furtherly tested in her blind visual field while recording the EEG signal. She was required to discriminate visual stimuli and then rate the clarity of her perceptual experience on the four-point Perceptual Awareness Scale (Ramsøy and Overgaard, 2004). We found that her visual defect was confirmed as degraded conscious vision. Not only she could use 3 out of the 4 possible PAS categories (no experience, brief glimpse and almost clear experience), but, more crucially, her discrimination accuracy depended on the level of awareness. Moreover, ERP results revealed the presence of the VAN, peaking around 200 ms after stimulus onset in the left hemisphere, and the LP, starting bilaterally at 250 ms over different scalp sites. As found in healthy subjects (Tagliabue et al., 2016), the amplitude of both VAN and LP was larger for higher levels of visual awareness, again confirming the conscious nature of her visual perception in the blind field.

These data highlights the usefulness of ERP analysis as an additional tool to graded scales in the assessment of hemianopic patients, in order to disentangle between genuine unconscious vision (blindsight) and degraded yet conscious vision. Despite the absence of significant differences at the neural level between PAS = 0 and PAS = 1, the electrophysiological data are in line with the discrimination accuracy of SL. In fact, for both PAS = 0 and PAS = 1 her discrimination accuracy is not different from chance (50%). The degraded visual input is thus not strong enough to allow SL an above chance performance when rating her perceptual experience as brief glimpse (PAS = 1), contrary to what was seen in healthy subjects (Tagliabue et al., 2016). This again confirms the absolute differences existing between visual perceptions generated by a healthy visual field and by an impaired visual field (Foley, 2014), leading to the necessity to go beyond binary assessing scale that are not able (Overgaard et al., 2008; Mazzi et al., 2016) to catch every aspect of a complex visual experience.

Of great interest, despite the complete destruction of the primary visual cortex confirmed also by fMRI (Celeghin et al., 2015), SL could indeed perceive visual stimuli presented in her blind field. Moreover, these results show a behavior that is different from the classical Riddoch syndrome (Riddoch, 1917), where patients report to be aware of moving stimuli in their blind visual field. In fact, SL reported almost clear experiences for static stimuli. Our present data, together with other findings (see for example Mazzi et al., 2014; Bagattini et al., 2015), are in line with the view that considers primary visual cortex and feedback to it not the only gateway to conscious vision (ffytche and Zeki, 2011). Even more intriguingly, the visual stimuli presented in SL’s blind hemifield could elicit electrophysiological responses in the damaged hemisphere. Activations in the damaged hemisphere has been previously shown (Rossion et al., 2000) while presenting complex stimuli (faces and cars), even if the patient tested in that study (GY) exhibited no awareness of them. In this respect, some studies on patients with visual field defects have reported that extrastriate areas

(Goebel et al., 2001; Bridge et al., 2010), especially area MT, and the lateral intraparietal cortex (Silvanto, 2014) show residual activation even in the absence of a functioning V1, therefore they might be still capable of at least low-level visual functions. Consequently, a residual activation of spared cortical areas might lead to an impaired but conscious visual perception. However, no general agreement has been reached on which is the area or the network of cortical and subcortical areas that, in the absence of V1, subserves such a conscious visual behavior (for a review, see Silvanto, 2015), and the present data cannot be in favor of any of the proposed alternative explanations.

In sum, we found that a hemianopic patient, described (Mazzi et al., 2016) as having degraded conscious vision when tested with visual stimuli presented in her blind hemifield, shows ERP signatures that are consistent with her behavior. In fact, the amplitude of both the VAN and the LP is larger when the patient reports an almost clear visual experience. Even if a larger sample is needed to confirm the present findings, our data speaks in favor of an integrative approach, comprising graded scales of visual awareness and concurrent EEG recording, when assessing residual vision in patients suffering from hemianopia.

EXPERIMENT 3

1. Introduction

Since its discovery (Sutton et al., 1965), the P300, a large centro-parietal positive deflection peaking within a varying time window spanning from 250 to 500 ms after stimulus onset, has been the focus of many debates on its functional significance. Due to the fact that the P300 is modulated by different stimulus manipulations (for a review, see Polich, 2007), a clear general agreement about the cognitive process it represents has not been reached yet. Among the various proposals it has been suggested to reflect context updating (Donchin and Coles, 1988), closure of perceptual events (Desmedt, 1981), allocation of attentional resources (Wickens et al., 1983) or stimulus evaluation (Kutas et al., 1977).

Lately, in the research field on perceptual decisions, a new interesting theory on the cognitive function of the P300 has been put forward. O'Connell and colleagues, in a series of studies (O'Connell et al., 2012; Kelly and O'Connell, 2013; Loughnane et al., 2016), identified a component in the P300 latency range they called Centro-Parietal Positivity (CPP) and suggested it to be the neural correlate of a so-called "decision variable". Such a decision variable is not the correlate of the decision itself, but rather an integration of different signals that must be decoded in order to trigger the actual decision (Shadlen and Kiani, 2013). In line with this view, the CPP build-up rate increases steadily as a function of the incoming sensory evidence strength and peaks at response time (O'Connell et al., 2012). Moreover, within the same level of sensory stimulation, not only the build-up rate of the component is steeper when associated with faster reaction times (RTs), but also pre-target α power, an index of attentional endogenous fluctuations (Thut et al., 2006) inversely correlated with cortical excitability, appears to be larger for slower RTs. The authors thus claimed that the evolution of the CPP, in relation to accumulated sensory evidence and RTs, shows how decision formation is influenced by a combination of exogenous physical factors and fluctuations within the brain itself.

Interestingly, another cognitive process that has been associated with the P300 is the so-called "access awareness" (Block, 2005). According to Block's distinction, access awareness is the ability of the subjects to act, report or remember the phenomenal content of a given perceptual experience and experiments investigating visual awareness (for a review, see Koivisto and Revonsuo, 2010) links access awareness to a component called Late Positivity (LP; Del Cul et al., 2007). The LP is a positive difference wave between aware and unaware trials, peaking between 300 and 400 ms after stimulus onset in centro-parietal sites. The LP not only discriminates between seen and unseen conditions, but its amplitude is also modulated by different levels of visual awareness (Tagliabue et al., 2016), as rated by subjects on the four-point Perceptual Awareness Scale (PAS; Ramsøy and Overgaard, 2004). However, also the actual cognitive underpinnings of the LP as a post-perceptual process still remain unclear (Railo et al., 2011; Rutiku et al., 2015; Salti et al., 2012).

Taken together, these findings highlight the striking resemblance between the CPP and the LP. Both components share the same polarity (positive), latency (peak around 300 – 400 ms after stimulus presentation) and topography (centro-parietal). However, they are suggested to reflect different cognitive processes, thus not solving the confusion about the actual functions represented by such a positive component peaking at 300 ms after stimulation (the P300 component): does it more closely reflect accumulation-to-bound of sensory evidence (as indexed by the CPP in perceptual decision-making studies) or conscious access to the content of the perception (as indexed by the LP in visual awareness experiments)? Crucially, it is unavoidable that awareness and accumulation of sensory evidence are intrinsically linked and confounded, since the more the accumulated evidence, the higher the level of awareness about the presented information. Nevertheless, the main issue is that decision-making experiments are usually designed with stimuli providing different levels of sensory information (such as different percentages of motion coherence or contrast), taking for granted that each level yields a given subjective percept (i.e. targets containing more sensory information are always better processed and perceived than trials with fewer sensory information), without assessing how the participants really perceive the stimuli. On the other hand, visual perception experiments follow the logic of contrastive analysis (Baars, 1988), according to which the neural correlates of consciousness should be investigated by contrasting neural responses to consciously perceived or unperceived physically identical stimuli. As a consequence, by using stimuli with the same amount of sensory information, what visual awareness studies lack is an investigation of the contribution of external stimulation in generating the perceptual experience.

A possible solution to fill the gaps of both literatures is to combine different conditions of visual awareness and sensory stimulation, in order to get a clearer idea 1) about which of the two factors is mainly reflected in the P300 and then 2) about what or if there is a differential contribution from physical stimulation and access to internal representations in perceptual tasks.

In this respect, it might be hypothesized that the P300 is part of a two-stage (Carpenter et al., 2009) decision process. The first stage (detection) detects signals among noise until reaching a predetermined threshold through a random walk, consistent with a diffusion model account (Ratcliff and Rouder, 2000). The output of this first stage is integrated into the second stage (decision), where more higher order factors determine a linear rise to threshold, consistent with the LATER (Linear Approach to Threshold with Ergodic Rate) approach (Carpenter, 1999). As a consequence, this two-stage model explains behavioral variability as resulting not only from the noise accumulated together with sensory stimulation during the first stage, but also from the noise that is generated within the brain itself during the second stage. We might hypothesize that the P300 acts as an intermediate phase between merely sensory input and the decision, thus representing stimulus-independent internal processes. To test this hypothesis and to overcome the limitations of both the sensory-evidence approach (that usually dismisses subjective experience) and the awareness approach (where no manipulation of sensory information is employed), we designed an EEG experimental paradigm where stimuli at different contrast levels were presented, asking participants to perform a discrimination task and then rate the quality of their perception on the four-point PAS (Ramsøy and Overgaard, 2004). Our paradigm, thus, allowed us to investigate the modulation of the P300 for each experimental manipulation alone (sensory evidence and access awareness), while holding constant the orthogonal manipulation (awareness for sensory evidence and sensory evidence for awareness, respectively).

2. Materials and Methods

2.1 Participants

14 participants (7 females, 2 left-handed, mean age ± standard deviation: 23.79 ± 3.17) were recruited for the study. All reported normal or corrected-to-normal vision and no history of neurological or psychiatric disorders. They all gave their written informed consent to participate in the study. The study was approved by the College of Science and Engineering Ethics Committee of the University of Glasgow and conducted in accordance with the 2013 Declaration of Helsinki. Data from three participants were excluded from the analysis because of a low number of trials in one or more conditions.

The final sample was thus composed of 11 participants (6 females, 1 left-handed, mean age ± standard deviation: 23.6 ± 3.32).

2.2 Experimental Procedure

The experiment comprised two sessions performed within two consecutive days. The first session served for threshold assessment (see “Threshold Assessment”) and to familiarize participants with the behavioral task. During the second session, after a threshold re-assessment, participants were prepared for EEG recordings. They then performed a forced choice discrimination task while the EEG was continuously recorded (see “EEG Experiment”).

2.3 Stimuli

The stimuli were two-dimensional light or dark gray Gaussian patches with a standard deviation of 0.65°, presented on a gray background, at an eccentricity of 5° along the vertical meridian and of 10° along the horizontal meridian to the right of the fixation point. Six stimulus luminance values (three lighter and three darker than the background) were determined for each participant by means of a threshold assessment procedure (see next paragraph for further details). The contrast luminance of the stimuli presented varied from 0.025 to 0.116% of the maximal luminance of the brightest (white) and of the darkest (black) colour.

2.4 Thresholds Assessment

In a dimly lit testing room participants sat in front of a CRT monitor (resolution 1280 × 1024, refresh rate of 100 Hz) placed at a viewing distance of 57 cm, with their head laying on a chin rest. The aim of the assessment session was to individually identify six luminance values (three for light and three for dark patches) corresponding to 25%, 50% and 75% of correct detection. The thresholds were measured using the method of constant stimuli (Urban, 1910). At the beginning of the assessment procedure, ten evenly spaced luminance values ranging from 0.025 to 0.116% of the maximal black and maximal white screen luminance were presented in a randomized order, in the periphery of the right visual field (see “Stimuli” for details). This first phase included two blocks: on each block, all luminance values were tested seven times together with 14 stimulus-absent trials (catch trials), resulting in a total number of 308 trials per participant. On each trial the stimulus appeared after a 1000 ms interval following a brief (150 ms) 1000 Hz warning tone. Participants were asked to keep their eyes on a central fixation cross and press the spacebar whenever they saw a stimulus. At the end of the two blocks, data of both light and dark stimulus trials were separately fitted to a sigmoid function and contrast values yielding detection thresholds of 25%, 35%, 50%, 65% and 75% were extracted for each participant. The contrast levels extracted were then tested again in two blocks, including 10 trials for each contrast and stimulus type (light and dark stimuli) and 14 catch trials, resulting in a total number of 228 trials per participant.

On the second day of testing and prior to EEG recording, a short threshold assessment was performed, to verify that participants' performance was comparable to that obtained in the first session. In this case, the contrast values previously identified (5 for light and 5 for dark patches) and contrast levels corresponding to 0% and 100% detection accuracy were each presented seven times together with 14 catch trials, for a total of 182 trials. If luminance values resulting in detection thresholds of about 25%, 50% and 75% were confirmed, they were selected for the behavioural task during the EEG recording. Otherwise, data were once again fitted to a sigmoid function and new contrast levels were extracted and tested with the same procedure. The assessment procedure had to be repeated for 4 subjects.

2.5 EEG Experiment

During EEG, participants performed a two-alternative forced choice discrimination task. Each trial (Fig. 1) started with a black fixation cross, followed 400 ms later by a 1000 Hz warning tone (150 ms). After a 1000 ms interval, a light or a dark gray Gaussian patch (whose luminance values were determined in the threshold assessment) was presented for 30 ms (3 frames) in the periphery of the right visual field. A 1000 ms blank was then followed by a response prompt asking the participants to judge the brightness of the stimulus as compared with the gray background, pressing a button for "lighter" and another button for "darker". The participants were required to answer even if they did not see any stimulus. After the button press, another response prompt asked participants to rate the quality of their perception on the four-point Perceptual Awareness Scale (PAS; Ramsøy and Overgaard, 2004).

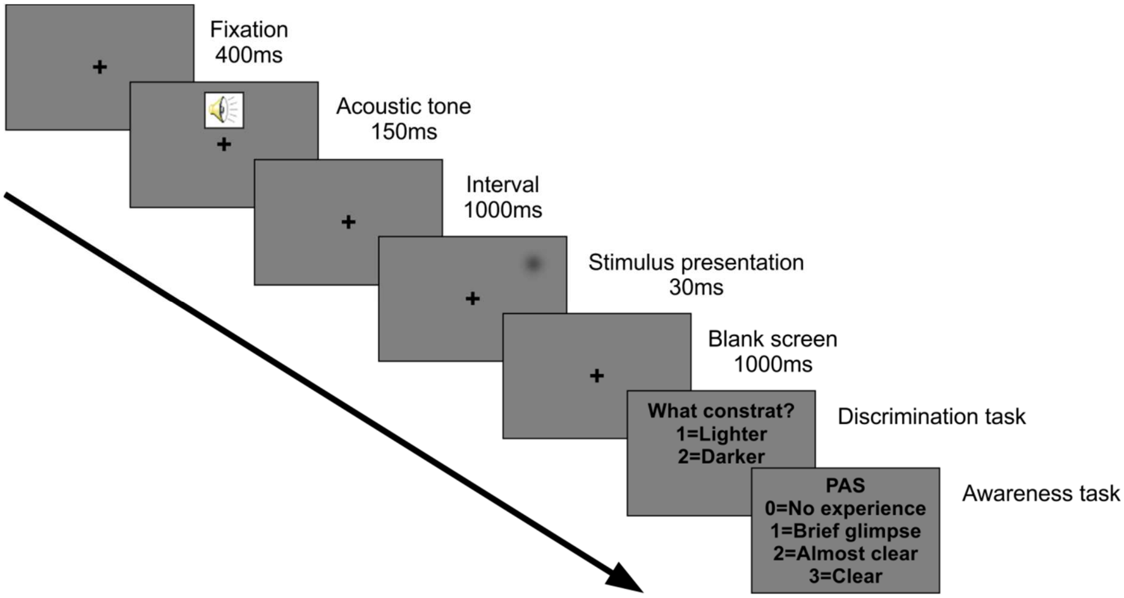

Figure 1. Single trial structure: A fixation cross was presented for 400 ms followed by a warning acoustic tone lasting 150 ms. Then, a 1000 ms interval preceded the stimulus presentation (30 ms) in the periphery of the right visual field. After a 1000 ms pause participants had to discriminate the brightness of the stimulus (Discrimination task) and then rate the clarity of their perception on the PAS (Awareness task).

The four PAS categories are: 0) no experience of the stimulus, 1) a brief glimpse, meaning that the participant saw something but could not discriminate the brightness of the stimulus, 2) an almost clear experience and 3) a clear experience. Responses were given by pressing four different buttons on the keyboard. The experimental session was divided into ten blocks. Each block was composed of 80 trials: 10 trials for each individually adjusted stimulus contrast (25%, 50% and 75% of detection threshold) and stimulus type (light and dark), together with 20 catch trials, thus yielding a total of 800 trials. The order of the trials was fully randomized. Both the threshold assessment and the actual behavioral task were programmed and run in MATLAB (MathWorks Inc.), using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997).

2.6 EEG recording and Event-Related Brain Potential (ERP) Analysis

EEG signal was continuously recorded with BrainAmp system (Brain Products GmbH, Munich, Germany – BrainVision Recorder) using a Fast'n Easy cap with 61 Ag/AgCl pellet pin electrodes (EasyCap GmbH, Herrsching, Germany) placed according to the 10–05 International System. An additional electrode was positioned on the outer canthus of the left eye to record eye movements (after being referenced to Fp1), whereas horizontal eye movements were detected by referencing AF7 to AF8 off-line. Two extra electrodes served as ground (TP9) and on-line reference (AFz). All scalp channels were re-referenced off-line to the average of all electrodes. Electrode impedances were kept below 10 kΩ. The digitization rate was 1000 Hz with a time constant of 10 s as low cut-off and a high cut-off of 100 Hz.

The continuous EEG signal was pre-processed off-line using Brain Vision Analyzer 2.0 (BrainProducts). Data were filtered with a second order high-frequency cutoff of 85 Hz and a second order low-frequency cutoff of 0.1 Hz. A band rejection filter with a bandwidth of 2 Hz was then used to remove 50 Hz interference. Independent component analysis (ICA; Bell and Sejnowski, 1995) was applied to remove eye blinks and muscle artifacts. The EEG data were then cut into epochs of 1300 ms starting 300 ms before the onset of the stimulus and baseline corrected to 300 ms pre-stimulus period. All segments were visually inspected and removed if still contaminated by residual eye movements, blinks, strong muscle activity or excessive noisy EEG. On average, ~5% of the trials were discarded. Finally, for statistical analysis, data were down-sampled to 250 Hz before averaging.

Analysis of the Event-Related brain Potentials (ERPs) was performed using the Fieldtrip toolbox (Oostenveld et al., 2011; see http://www.ru.nl/neuroimaging/fieldtrip). Averaging was carried out separately for each Contrast and Rating condition. To evaluate the unique impact of visual awareness on P300, we randomly selected trials within the same PAS rating so that the average would include an equal number of trials with different contrast stimuli, in order to control for the contrast factor. In a second analysis, we focused on the impact of physical properties of the stimuli, i.e. different contrasts, on P300. In this case, within the same contrast, trials were randomly selected so that the average would include an equal number of trials receiving different perceptual ratings on the PAS, so that to control for the perceptual rating factor.

Because of a low number of trials for the 25% contrast – rating 3 and 75% contrast – rating 0 combinations, comparisons between perceptual ratings 0, 1 and 2 included trials with contrasts corresponding to 25% and 50% detection thresholds; comparisons between perceptual ratings 1, 2 and 3 included trials with contrasts corresponding to 50% and 75% detection thresholds. For the same reason, the comparison between contrasts 25% and 50% only included ratings of 0, 1 and 2 and the comparison between contrasts 50% and 75% only included ratings of 1, 2 and 3 (Fig. 2).

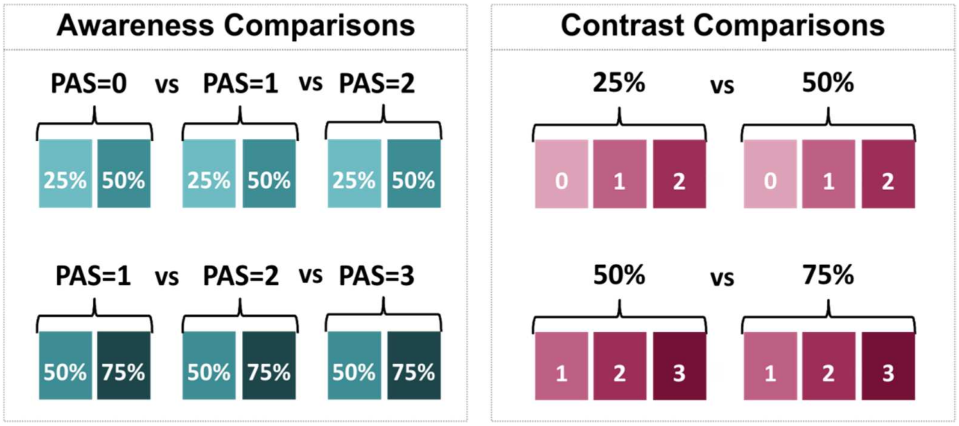

Figure 2. Trial sorting and comparisons. Left panel: comparisons performed to investigate the awareness effect. Different scores at the awareness scale were compared by including an equal number of trials for each contrast level. To compare rating 0 vs rating 1 vs rating 2, contrasts corresponding to 25% and 50% of detection threshold were included (left upper panel), whereas contrasts at 50% and 75% were considered to compare rating 1 vs rating 2 vs rating 3 (left bottom panel). Right panel: comparisons performed to investigate the contrast effect. Contrasts were compared by equating the number of trial for each rating. Contrast levels corresponding to 25% and 50% of detection threshold included equal number of trials rated as 0, 1 and 2 on the PAS (right upper panel), whereas 50% and 75% of detection threshold were compared including an equal number of trials with ratings 1, 2 and 3 (right bottom panel).

The mean number of trials for each condition of each comparison is: 72.82 for 25% vs 50% (contrasts), 66.82 for 50% vs 75% (contrasts), 48.55 for 0 vs 1 vs 2 (ratings) and 44.55 for 1 vs 2 vs 3 (ratings). Finally, for each subject also the average of the catch trials was computed (mean number of trials: 186.18).

2.7 Statistical Analysis

To evaluate the effectiveness of the experimental manipulations, two separate repeated-measures analyses of variance (ANOVA) were carried out on discrimination accuracy for trials sorted according to both perceptual rating (within-subject factor: PAS. 4 levels: PAS=0, PAS=1, PAS=2 and PAS=3) and contrast level (within-subject factor: Contrast. 3 levels: 25%, 50% and 75%). Indeed, if our manipulation was effective, we expect that accuracy increases as both the perceptual ratings and contrast levels increase.

To investigate the effect of different levels of visual awareness (rating 0 vs 1 vs 2; rating 1 vs 2 vs 3) and different contrast conditions (contrast 25% vs 50%; contrast 50% vs 75%) on EEG data, non-parametric cluster-based permutation analyses were used (Maris and Oostenveld, 2007). For every sample (channel x time point), conditions were compared by means of a repeated-measures ANOVA (for rating comparison) or of a paired-samples T-Test (for contrast comparison), on a time window from 0 to 900 ms after stimulus presentation. Those samples whose F- or t-value exceeded a critical value ($p < 0.05$) were selected and clustered according to spatial and temporal adjacency, then, within every cluster, F- or t-values were summed to calculate cluster-level statistics. The maximum cluster was thus used in the test statistics. These cluster-based statistics were evaluated through a non-parametric permutation analysis, which included 500 random sets of permutations. For each permutation, cluster-based statistics were calculated and a reference distribution was built, from which the Monte Carlo p-value was estimated according to the proportion of the randomization null distribution exceeding the maximum cluster statistic. When ANOVAs on the rating comparisons resulted significant, post-hoc analyses were performed through non-parametric cluster-based permutation t-tests between each rating condition. The paired-samples T-Tests were run on the mean amplitude of the significant time window identified by the main ANOVA.

In order to ensure that the random selection of trials performed to equate the number of trials was not biasing the results, the trial sampling was repeated 500 times for each comparison and the statistical analyses were performed for each random draw. The p values obtained after each draw and statistical analysis were averaged together for each comparison, to confirm the significant effects.

To further investigate the contribution of sensory stimulation, we compared EEG responses evoked by a different amount of physical information (different contrasts), but resulting in the same subjective report on the PAS. To this end, ERPs derived from trials corresponding to 25 and 50% detection threshold and rated as 1 on the PAS were compared to ERPs calculated as average signal of trials corresponding to 50 and 75% detection threshold and also rated as 1. The same comparison was repeated for rating 2, comparing the average response at 25 and 50% versus the EEG response evoked by contrasts at 50 and 75% detection threshold (Fig. 2). For both comparisons, 500 paired-samples t-test were run on the mean amplitude of a 350 – 450 ms time window of electrode Pz (the electrode that showed the largest effects).

Lastly, a cluster-based permutation t-test was performed on catch trials to test for a statistical difference from the baseline (-300 to 0 ms before stimulus onset). For every channel x time point sample, the comparison was run on a time window from 0 to 900 ms after stimulus presentation.

3. Results and Discussion

3.1 Behavioural Results

After the threshold assessment, the mean luminance value chosen was 0.0425% for 25%, 0.0488% for 50% and 0.0569% for 75% detection threshold (lighter and darker stimuli collapsed together). For trials at 25% detection threshold, the mean percentage of PAS = 0 responses given by the participants was 55.09%, 25.32% for PAS = 1, 14.45% for PAS = 2 and 5.14% for PAS = 3. For trials at 50% detection threshold, the mean percentage of PAS = 0 responses given by the participants was 36.05%, 26.45% for PAS = 1, 24.86% for PAS = 2 and 12.64% for PAS = 3. Finally, for trials at 75% detection threshold, the mean percentage of PAS = 0 responses given by the participants was 18.18%, 22.09% for PAS = 1, 30.68% for PAS = 2 and 29.05% for PAS = 3. The mean percentage of catch trials receiving a rating of 0 on the PAS was 88.14% (sd = 17.47), thus revealing the reliability of the participants. For trials sorted according to the different visual awareness levels as rated by participants (PAS = 0, PAS = 1, PAS = 2, PAS = 3), the repeated-measures ANOVA conducted on the mean percentage of correct responses revealed that, as visual awareness increased, also accuracy significantly increased [Greenhouse-Geisser adjusted $F(1.688,16.882) = 113.168$, $p < 0.01$; linear trend $F(1,10) = 1000.716$, $p < 0.01$; Fig. 3A]. For trials sorted according to the different sensory stimulation levels (25%, 50%, 75%), the repeated-measures ANOVA on accuracy showed that, as sensory information increased, also accuracy significantly increased [$F(2,20) = 35.53$, $p < 0.01$; linear trend $F(1,10) = 89.89$, $p < 0.01$; Fig. 3B]. These results thus confirm that the experimental manipulations carried out (visual awareness and sensory evidence) were indeed effective.

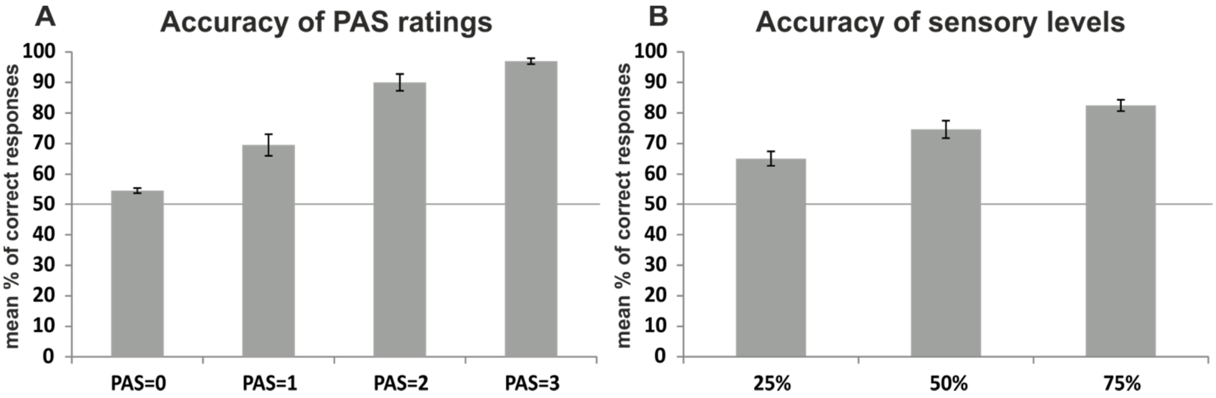

Figure 3. Behavioral results. **(A)** Mean percentage of correct responses as a function of PAS rating. **(B)** Mean accuracy for each contrast level. Error bars represent standard errors and the solid line (50%) chance level.

3.2 ERP Results

To investigate the awareness and the sensory evidence effects, we performed a series of non-parametric cluster-based permutations ANOVAs and t-tests, after controlling for the orthogonal factor by randomly selecting and numerically equating trials within each experimental condition (see “Statistical Analysis” section for details).

3.2.1 Awareness Rating

1) PAS = 0 vs PAS = 1 vs PAS = 2

For this analysis, trials at 25% and 50% detection threshold were used (Fig. 2). Visual inspection of the mean grand average ERPs obtained after 500 random selections of trials confirmed the presence of a positive deflection, compatible with the P300 component, starting around 250 ms after stimulus onset (Fig. 4A). The non-parametric cluster-based permutation ANOVA, performed on a single sampling run, found a significant positive cluster over centro-parietal and frontal electrodes, on an interval from 264 to 848 ms after stimulus onset ($p_{cluster} < 0.01$). Post-hoc comparisons between each awareness level, performed through cluster-based permutation t-tests averaging over the significant time window identified in the main analysis (264 - 848 ms), showed that each condition was significantly different from the others. Specifically, the comparison between PAS = 1 and PAS = 0 identified a significant positive cluster of centro-parietal electrodes and a significant negative cluster on frontal areas (all $ps_{cluster} < 0.01$; Fig. 4B). The comparison between PAS = 2 and PAS = 1 found the same positive and negative clusters at centro-parietal and frontal electrodes, respectively (all $ps_{cluster} < 0.01$; Fig. 4C). Finally, the comparison between PAS = 2 and PAS = 0 found the positive centro-parietal and the negative frontal clusters (all $ps_{cluster} < 0.01$; Fig. 4D). After repeating the random selection and the cluster analyses 500 times, the presence of the two clusters was confirmed: the positive centro-parietal cluster of electrodes remained significant from around 400 to 700 ms after stimulus onset, while the negative cluster resulted to be significant from around 520 to 670 ms, as shown by the topography of the averaged p-values (Fig. 4E) and the number of times out of 500 that centro-parietal electrodes resulted significant (Fig. 4F). Overall, these results show that the P300 is modulated by the level of visual awareness as rated by the participants, with higher amplitudes corresponding to higher quality in the perceptual experience.

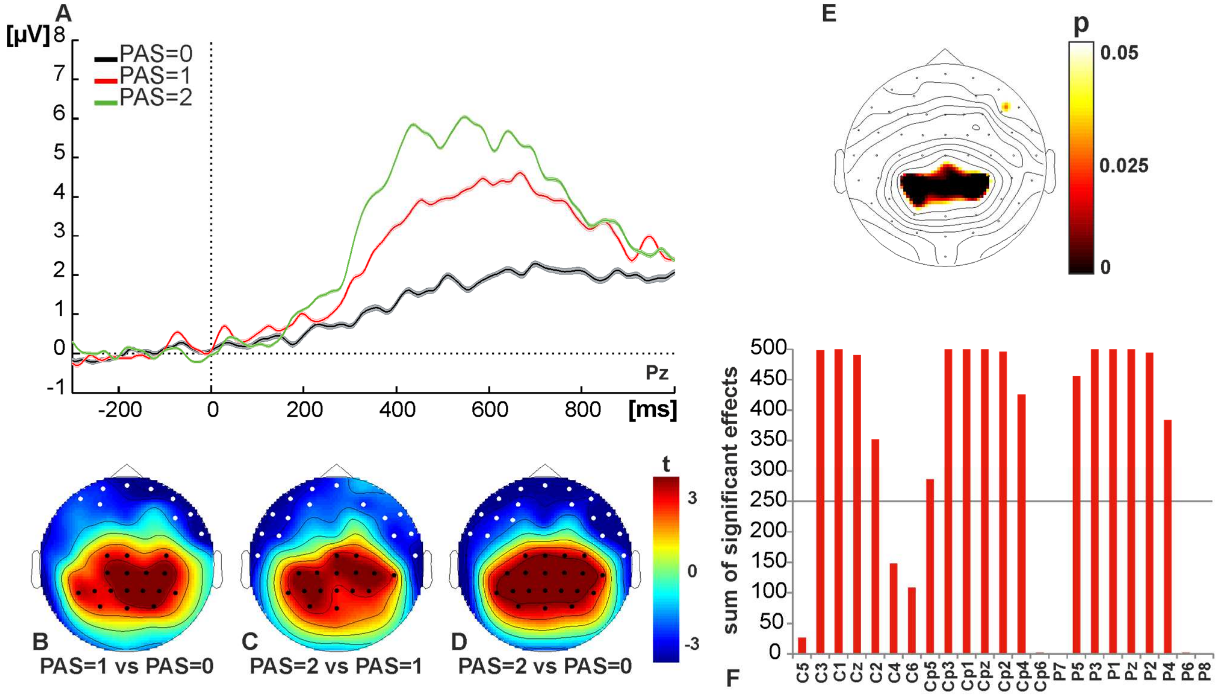

Figure 4. Effect of rating on P300 amplitude (PAS=0 vs PAS=1 vs PAS=2). **(A)** Mean grand average ERP waves over electrode Pz, obtained for each PAS category after 500 random draws. Shaded areas represent standard errors at each time point. **(B)** Post-hoc comparison between PAS=1 and PAS=0. **(C)** Post-hoc comparison between PAS=2 and PAS=1. **(D)** Post-hoc comparison between PAS=2 and PAS=0. Black dots represent a significant positive cluster, whereas white dots represent a significant negative cluster. **(E)** Topography of the averaged p-values over 500 random draws from 400 to 700 ms after stimulus onset, the time window where the most consistent effect was found, i.e. when the majority of electrodes showed a significant difference between conditions 500 times out of 500 cluster-based ANOVAs. **(F)** Sum of the significant effects at centro-parietal electrodes after 500 random

draws at 400 ms after stimulus onset, when the effect (significant difference between rating conditions after 500 random draws) was maximum.

2) PAS = 1 vs PAS = 2 vs PAS = 3

For this analysis, trials at 50% and 75% detection threshold were used (Fig. 2). Again, visual inspection of the mean grand average ERPs obtained after 500 random selections of trials showed the presence of a positive P300 component, starting around 250 ms after stimulus presentation (Fig. 5A). The non-parametric cluster-based permutation ANOVA, performed on a single sampling run, found a significant cluster of centro-parietal electrodes from 228 to 696 ms after stimulation ($p_{cluster} < 0.01$). Post-hoc comparisons between each PAS rating, performed through cluster-based permutation t-tests on the mean amplitude of the significant time window (228 - 696 ms), resulted in significant differences between each awareness condition. The comparison between PAS = 2 and PAS = 1 found a significant difference in a positive centro-parietal cluster of electrodes ($p_{cluster} < 0.01$; Fig. 5B). Also the comparison between PAS = 3 and PAS = 2 identified a significant positive cluster over centro-parietal areas ($p_{cluster} < 0.01$; Fig. 5C). Lastly, the comparison between PAS = 3 and PAS = 1 identified a significant positive centro-parietal cluster ($p_{cluster} < 0.01$) and a significant negative left frontal cluster of electrodes ($p_{cluster} < 0.05$; Fig. 5D). The random sampling performed 500 times and the following ANOVAs confirmed the presence of the positive centro-parietal cluster of electrodes, from around 270 to 470 ms after stimulus onset, as shown by the topography of the averaged p-values (Fig. 5E) and the number of times out of 500 that centro-parietal electrodes resulted significant (Fig. 5F). In line with the previous comparison, the amplitude of the positive deflection increased as a function of visual awareness.

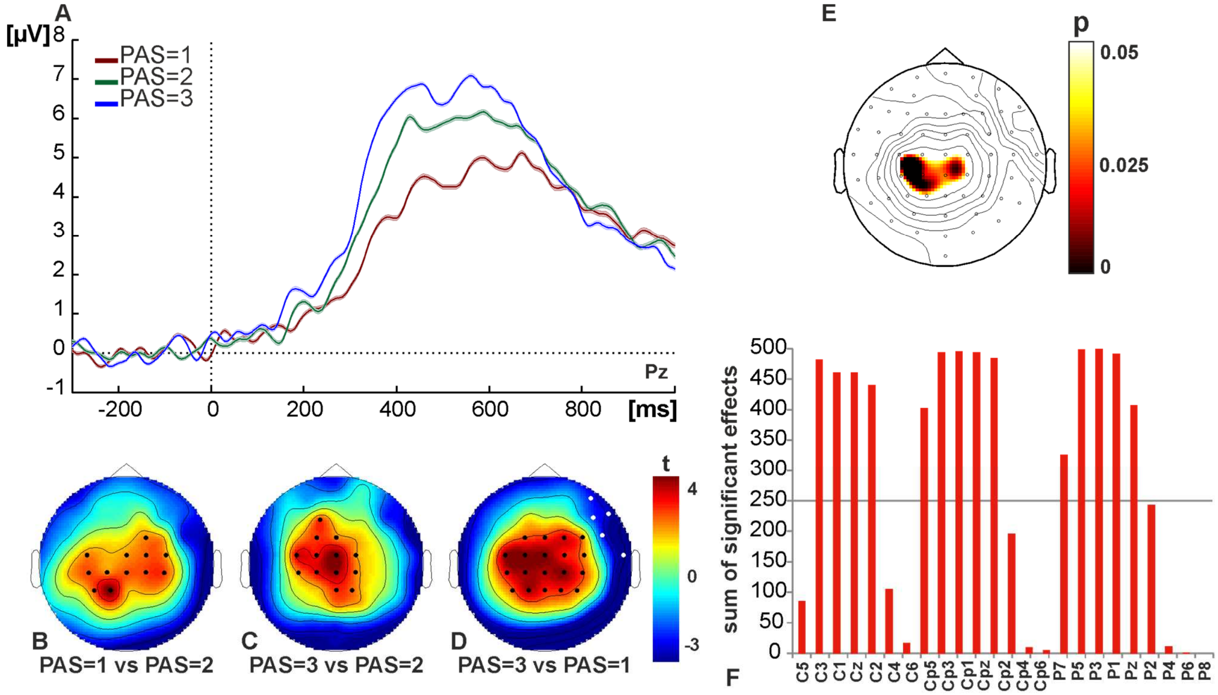

Figure 5. Effect of rating on P300 amplitude (PAS=1 vs PAS=2 vs PAS=3). **(A)** Mean grand average ERP waves over electrode Pz, obtained for each PAS category after 500 random draws. Shaded areas represent standard errors at each time point. **(B)** Post-hoc comparison between PAS=1 and PAS=2. **(C)** Post-hoc comparison between PAS=3 and PAS=2. **(D)** Post-hoc comparison between PAS=3 and PAS=1. Black dots represent a significant positive cluster, whereas white dots represent a significant negative cluster. **(E)** Topography of the averaged p-values over 500 random draws from 320 to 420 ms after stimulus onset, the time window where the most consistent effect was found, i.e. when the majority of electrodes showed a significant difference between conditions 500 times out of 500 cluster-based ANOVAs. **(F)** Sum of the significant effects at centro-parietal electrodes after 500 random draws at 400 ms after stimulus onset, when the effect (significant difference between rating conditions after 500 random draws) was maximum.

3.2.2 Sensory Stimulation

1) 25% Contrast vs 50% Contrast

For this analysis, trials rated as 0, 1 and 2 on the PAS were used (Fig. 2). Visual inspection of the mean grand average ERPs obtained after 500 random selections of trials confirmed the presence of a positive deflection, compatible with the P300 component, starting around 250 ms after stimulation (Fig. 6). The non-parametric cluster-based permutation t-test, performed on a single sampling run, did not find any significant cluster of electrodes (all $ps_{cluster} > 0.05$) when comparing the two contrasts. The absence of any effect was confirmed after running the random sampling and the analyses 500 times. The P300, albeit present, was thus not modulated by the different levels of sensory stimulation provided.

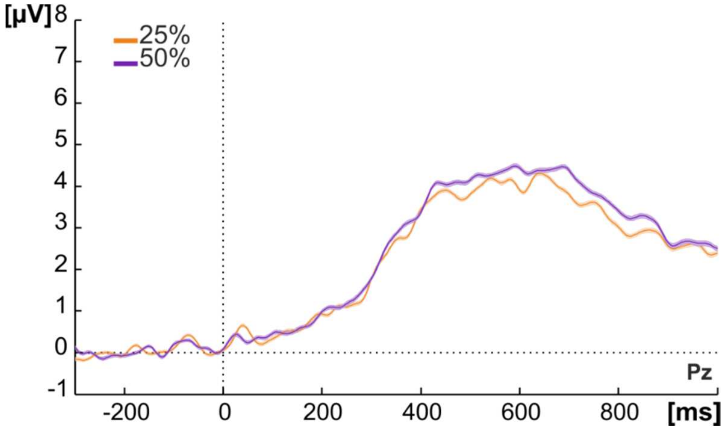

Figure 6. Mean grand average ERP waves over electrode Pz, obtained for each contrast level after 500 random draws. Shaded areas represent standard errors at each time point.

2) 50% Contrast vs 75% Contrast

For this analysis, trials rated as 1, 2 and 3 on the PAS were used (Fig. 2). Again, visual inspection of the mean grand average ERPs obtained after 500 random selections of trials showed the presence of a positive P300-like component, starting around 250 ms after stimulus presentation (Fig. 7). The non-parametric cluster-based permutation t-test, performed on a single sampling run, did not identify any significant cluster of electrodes (all $ps_{cluster} > 0.05$) for the difference between the two contrast conditions. The 500 random samplings and analyses confirmed the absence of the effect, so there is no modulation of the amplitude of the component as a function of increasing sensory stimulation.

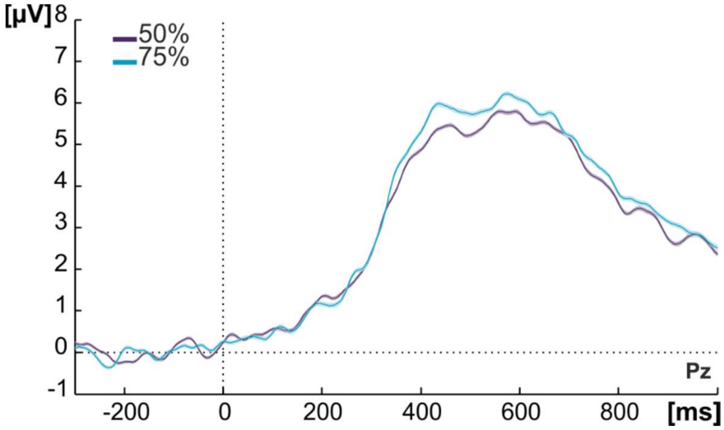

Figure 7. Mean grand average ERP waves over electrode Pz, obtained for each contrast level after 500 random draws. Shaded areas represent standard errors at each time point.

3.2.3 Differences between same Ratings

The contribution of sensory stimulation was further investigated by comparing EEG signal associated with the same subjective report on the PAS, but different amounts of physical information (contrasts).

1) Rating 1

For this analysis, trials rated 1 on the PAS were used (Fig. 2). Trials at 25% and 50% were collapsed and compared to the average of contrast conditions at 50%+75% (Fig. 8). Out of 500 paired-samples t-tests on the mean amplitude of electrode Pz, within a time window from 350 to 450 ms, 61 tests resulted to be significant. Since the number of significant tests did not reach the confidence interval of 95%, the effects found can thus be considered random, meaning that sensory stimulation does not contribute to the modulation of the P300 component.

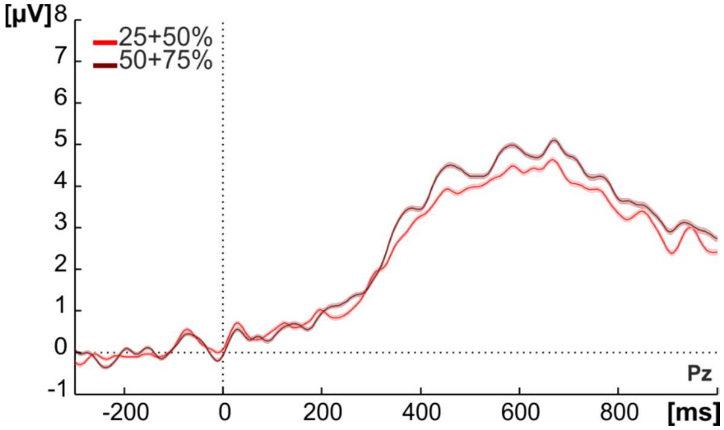

Figure 8. Mean grand average ERP waves over electrode Pz, obtained for each rating 1 (25+50% and 50+75%) after 500 random draws. Shaded areas represent standard errors at each time point.

2) Rating 2

For this analysis, trials rated 2 on the PAS were used (Fig. 2) and the statistical analysis was performed to compare the average signal evoked by trials at 25% and 50% to the average of 50% and 75% sensory level (Fig. 9). The 500 paired-samples t-tests, on the mean amplitude of electrode Pz from 350 to 450 ms, resulted to be significant 36 times. Again, since the number of significant tests did not exceed the 95% confidence interval, the effect can be considered null, confirming the results found for the comparison of the two rating 1.

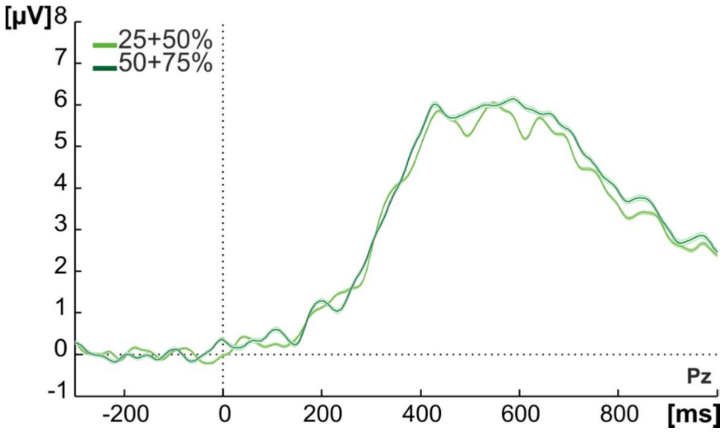

Figure 9. Mean grand average ERP waves over electrode Pz, obtained for each rating 2 (25+50% and 50+75%) after 500 random draws. Shaded areas represent standard errors at each time point.

3.2.4 Catch trials

The cluster-based permutation t-test performed on the whole epoch (0 to 900 ms) between ERP amplitude evoked by catch trials (Fig. 10A) and pre-stimulus (-300 to 0 ms) baseline interval, revealed two significant clusters of electrodes (Fig. 10B). The first cluster was a positive cluster over centro-parietal areas ($p_{cluster} < 0.01$, starting at 248 ms until the end of the epoch), and the second was a negative cluster on frontal channels ($p_{cluster} < 0.05$, from 296 ms until the end of the epoch). These results thus show that even if no sensory stimulation was provided, the centro-parietal component is still present. Since in the case of catch trial no sensory stimulation can be accumulated, this finding may support the hypothesis that instead an accumulation of internal evidence takes place, regardless of the presence of the actual physical stimulus.

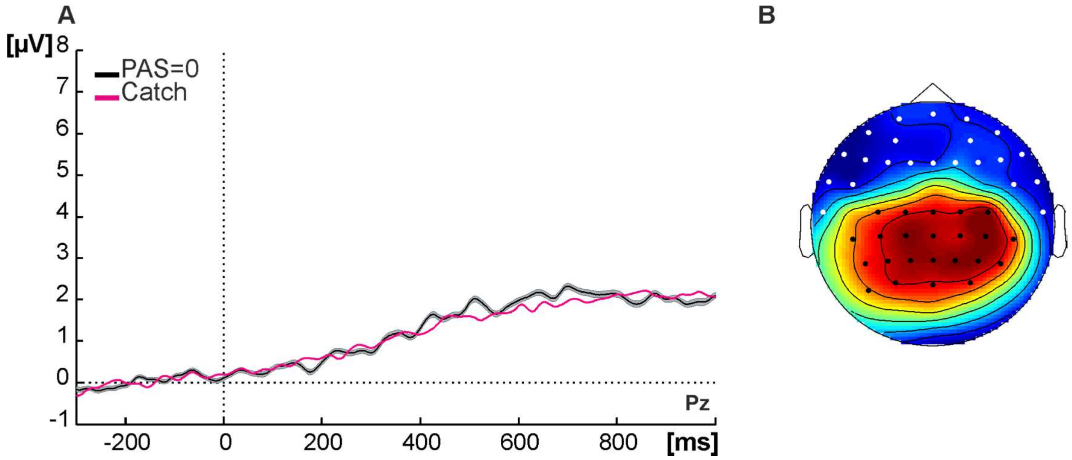

Figure 10. Catch trials. **(A)** Visual comparison between mean grand average ERP waves of PAS=0 (obtained after 500 random draws from 25+50% trials) and grand average ERPs of catch trials over electrode Pz. Shaded areas of PAS=0 ERPs represent standard errors at each time point. **(B)** Comparison between catch trials and pre-stimulus baseline interval. Black dots represent a significant positive cluster, whereas white dots represent a significant negative cluster.

4. General Discussion

The aim of the present study was to evaluate the contribution of both physical stimulation and subjective rating of visual awareness in perceptual decision-making tasks. In standard conditions, sensory evidence and visual awareness proceed together and are thus confounded, with higher levels of visual awareness elicited by stronger stimulation, so that it is difficult to disentangle their unique contribution when coming to a decision. Crucially, our experimental manipulations allowed us to investigate each process (sensory evidence and visual awareness) alone. Participants had to discriminate stimuli at different levels of sensory evidence and rate the quality of their visual experience on the graded Perceptual Awareness Scale (Ramsøy and Overgaard, 2004). The P300, a component that has been related to both accumulation of sensory evidence (O'Connell et al., 2012) and access to the phenomenal content of a given perception (Del Cul et al., 2007), resulted to be modulated only by the level of visual awareness rated by the participants, and did not depend on the actual strength of the stimulation provided, as also confirmed in the case of catch trials.

These results thus provide further evidence in favor of the contribution of endogenous fluctuations in decision formation. In fact, previous studies (Kelly and O'Connell, 2013; Twomey et al., 2015; Loughnane et al., 2016) assessed the impact of internal variations on decision-making by analyzing reaction times (RTs). Sorting trials according to RTs (from slower to faster) within each sensory level revealed how the build-up rate of the P300 increased as a function of speed, i.e. steeper build-up rates of the P300 were associated with faster RTs. Moreover, this endogenous variability was explained by attentional fluctuations in pre-stimulus parieto-occipital α power (Kelly and O'Connell, 2013). The problem with RTs is that they also represent a confound, since they can both reflect the ease of sensory evidence accumulation and the speed of conscious access, thus being an indirect measure of the actual perception of the stimuli. On the contrary, asking the participants to rate the level of visual awareness provides a direct subjective measure of perceptual variability within the same level of physical stimulation.

A previous study (de Lange et al., 2011) tried to investigate the effect of visual awareness on sensory evidence accumulation. In their research, through masking, the authors presented a sequence of high and low visibility stimuli, without asking the participants to judge their perceptual experience. They found that, despite the accumulation of sensory evidence was possible even in the absence of visual awareness, there were qualitative differences in evidence accumulation that instead depended on the higher or lower visibility of the stimuli. At the neural level, these qualitative differences were reflected in an early (~50 ms) frontal top-down biasing effect, present only for highly visible material: in series of high visibility trials evidence is rapidly accumulated to the decision bound, so that there is no need of further accumulation once this bound is reached. A similar top-down effect was previously found by the same group (de Lange et al., 2010) at a later latency (~200-300 ms) over centro-parietal areas, where changes in neural activity were inversely correlated to the amount of accumulated sensory evidence, suggesting that the more the sensory evidence collected, the fewer the resources deployed to process further information. However, in our electrophysiological data, we seem to observe a quantitative more than a qualitative difference in evidence accumulation. In fact, the P300 component is always present with the same centro-parietal topography for trials at the different contrast levels, at the different awareness levels and also in catch trials, with amplitude modulations that are related only to the subjective experience rated by the participants on the PAS (Ramsøy and Overgaard, 2004). The latency of the effect (from ~300 ms) can also suggest that trying to access perceptual information might represent a top-down post-stimulus endogenous process, that is decoupled from exogenous factors (as revealed by the presence of the effect also in catch trials). It is thus a top-down process related to the read-out of the perceptual information (be it present or not), and hence different from the biasing effect found by de Lange and colleagues, where there is a strategic modulation in information acquisition exclusively depending on the strength of the sensory stimulation initially provided (2011) or accumulated across time (2010). This post-stimulus endogenous process might also be different from another post-sensory process identified around 300 ms (Philiastides et al., 2006). The component found by these authors is stronger with higher sensory evidence and its presence is strictly task-related, so it has been proposed to represent the evidence that actually goes through the decision-making process. Anyway, again, the fact that in our study we found a P300 also when processing catch trials does not allow us to interpret the reflected process in the same way as Philiastides and collaborators (2006), but it might be better described as access to the internal evidence. Another confirm that the P300 reflects access to the evidence might be found in a work of Melloni and colleagues (2011). When assessing the modulation of expectations on the correlates of visual awareness, the amplitude of the P300 appeared to be larger for more visible stimuli than for less visible trials, but only when such stimuli were presented in an ascending sequence of six increasing contrast levels, and not when the sequence was descending. The results thus suggest that the effect was observed when perception relied on sensory stimulation, again pointing to a sort of top-down post-stimulus process that is about the search and the read-out of the evidence, not based on prior established expectations.

The P300 found in our experiment is also different from a mere decision variable (Shadlen and Kiani, 2013), since we did not observe the component to reach a common boundary as in the experiments conducted by O’Connell’s group (O’Connell et al., 2012; Kelly and O’Connell, 2013; Twomey et al., 2015; Loughnane et al., 2016). The amplitude of the component continued to be modulated by visual awareness and exhibited larger peaks for higher awareness levels, differently from the decision variable identified by O’Connell and collaborators that reaches at different latencies a fixed amplitude for all levels of sensory stimulation. The same continuous modulation was shown also in other studies (Philiastides et al., 2006; Philiastides et al., 2014) and the authors suggested that the reaching of a fixed amplitude might be due to the complex nature of the task employed by O’Connell’s group, where more cognitive resources are required and mixed together, thus leading to a common amplitude boundary.

Taken together, our results suggest that the P300 better reflects the so-called access consciousness (Block, 2005), a higher order process that allows the later manipulation of the content of a perceptual experience by a wide range of output systems. Access consciousness can thus be thought as a transitional stage between sensory stimulation and decision making, where the evidence and, importantly, also the absence of it, is read out to be the input of further processing. The finding that the amplitude of the P300 is modulated by the subjective ratings of visual awareness, and not by the different amounts of physical stimulation, might support the hypothesis that such a signal is not totally determined by the strength of the sensory evidence (Kelly and O’Connell, 2013). Rather, according to the two-stage decision-making model (Carpenter et al., 2009), this electrophysiological component reflects the quantitative differences in the accumulation of both sensory evidence and, crucially, also stimulus-independent neural noise, that is produced within the brain itself. As a consequence, the accumulation of evidence deriving from both exogenous and endogenous factors then leads to the observed behavioral variability.

GENERAL CONCLUSION

In the debate on the graded vs. dichotomous nature of visual awareness (Ramsøy and Overgaard, 2004), we were able to provide some evidence that electrophysiological signatures related to conscious visual perception, the VAN (Koivisto and Revonsuo, 2003) and the LP (Del Cul et al., 2007), showed an amplitude linear modulation as a function of visual awareness, rated by subjects on a graded scale. In addition, we saw that the phenomenal content of perception (as reflected in the VAN) was generated in an early time window in temporal, and not occipital, cortical areas, suggesting that conscious visual perception takes place outside the primary visual cortex (Zeki and ffytche, 1998).

These results were confirmed when the same paradigm was employed with a hemianopic patient exhibiting degraded conscious vision (Mazzi et al., 2016). The patient could report some degrees of awareness for stimuli presented in her blind hemifield. Moreover, the electrophysiological data (both the VAN and the LP) correlated with her behavioral performance and were modulated by the level of awareness, suggesting that ERP analysis might be a helpful instrument in assessing blindsight versus degraded conscious vision patients. Visual stimuli in the blind visual field could thus elicit brain responses in the damaged hemisphere, suggesting again that residual awareness might occur regardless of V1 effective functionality.

Finally, a further investigation was carried out to better characterize the post-perceptual processes that are reflected in the LP, considered by different lines of research both as access consciousness (Koivisto and Revonsuo, 2010) and as sensory evidence accumulation (O’Connell et al., 2012). Our findings revealed that the amplitude of the component was modulated by the level of awareness, and not by the actual physical stimulation. These results thus highlight the importance of internal representations in the process that leads to decision-making: what takes place is an accumulation of both noise coming with the sensory input and, more importantly, of noise that is generated within the brain itself.

Taken together, our experimental manipulations confirmed the existence at the neural level of two different properties, previously proposed by Block (2005), that characterize visual awareness: phenomenal consciousness and access consciousness. Even if it is not clear yet whether V1 directly or indirectly contributes to visual awareness and there is still an ongoing debate on the anatomical correlates of both phenomenal and access consciousness, converging studies (see Koch et al., 2016 for a recent review) pointed to a restricted localization in a so-called “temporo-parietal-occipital hot zone”, especially for content consciousness. So, specific perceptual experiences might happen without an amplification coming from a fronto-parietal network (Dehaene, 2014), that might be instead responsible of attention allocation and task monitoring and reporting.

REFERENCES

Andersen, L.M., Pedersen, M.N., Sandberg, K., and Overgaard, M. (2015). Occipital MEG activity in the early time range (<300 ms) predicts graded changes in perceptual consciousness. *Cerebral Cortex*, 26, 2677-2688.

Azzopardi, P., and Cowey, A. (1997) Is blindsight like normal, near-threshold vision? *Proceedings of the National Academy of Sciences of the United States of America*, 94, 14190–14194.

Baars, B.J. (1988). *A cognitive theory of consciousness*. Cambridge: Cambridge University Press.

Baars, B.J. (1994). A thoroughly empirical approach to consciousness. *Psyche*. 1, 1-21.

Bagattini, C., Mazzi, C., and Savazzi, S. (2015). Waves of awareness for occipital and parietal phosphenes perception. *Neuropsychologia*, 70, 114-125.

Bar, M., Tootell, R.B.H., Schacter, D.L., Greve, D.N., Fischl, B., Mendola, J.D., Rosen, B.R., and Dale, A.M. (2001). Cortical Mechanisms Specific to Explicit Visual Object Recognition. *Neuron*, 29, 529-535.

Barbur, J.L., Watson, J.D.G., Frackowiak, R.S.J., and Zeki, S. (1993). Conscious visual perception without V1. *Brain*, 116, 1293-1302.

Beck, D.M., Rees, G., Frith, C.D., and Lavie, N. (2001). Neural correlates of change detection and change blindness. *Nature Neuroscience*, 4, 645-650.

Bell, A.J., and Sejnowski, T.J. (1995). An information-maximization approach to blind separation and blind deconvolution. *Neural Computation*, 7, 1129-1159.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society - Series B (Methodological)*, 57, 289–300.

Block, N. (1996). How can we find the neural correlate of consciousness? *Trends in Neurosciences*, 19, 456-459.

Block, N. (2005). Two neural correlates of consciousness. *Trends in Cognitive Sciences*, 9, 46-52.

Brainard, D.H. (1997). The Psychophysics Toolbox. *Spatial Vision*, 10, 437–442.

Bridge, H., Hicks, S.L., Xie, J., Okell, T.W., Mannan, S., Alexander, I., Cowey, A., and Kennard, C. (2010). Visual activation of extra-striate cortex in the absence of V1 activation. *Neuropsychologia*, 48, 4148-4154.

Carpenter, R.H.S. (1999). A neural mechanism that randomizes behaviour. *Journal of Consciousness Studies*, 6, 13-22.

Carpenter, R.H.S., Reddi, B.A.J., and Anderson, A.J. (2009). A simple two-stage model predicts response time distributions. *The Journal of Physiology*, 587, 4051-4062.

Celeghin, A., Savazzi, S., Barabas, M., Bendini, M., and Marzi, C.A. (2015). Blindsight is sensitive to stimulus numerosity and configuration: evidence from the redundant signal effect. *Experimental Brain Research*, 233, 1617-1623.

Christensen, M.S., Ramsøy, T.Z., Lund, T.E., Madsen, K.H., and Rowe, J.B. (2006). An fMRI study of the neural correlates of graded visual perception. *NeuroImage*, 31, 1711-1725.

Crick, F., and Koch, C. (1995). Are we aware of neural activity in primary visual cortex? *Nature*, 375, 121-123.

De Graaf, T.A., Hsieh, P., and Sack, A.T. (2012). The ‘correlates’ in neural correlates of consciousness. *Neuroscience and Biobehavioral Reviews*, 36, 191-197.

De Lange, F.P., Jensen, O., and Dehaene, S. (2010). Accumulation of evidence during sequential decision making: the importance of top-down factors. *The Journal of Neuroscience*, 30, 731-738.

De Lange, F.P., van Gaal, S., Lamme, V.A.F., and Dehaene, S. (2011). How awareness changes the relative weights of evidence during human decision-making. *PLoS Biology*, 9, e1001203.

Dehaene, S. (2014). *Consciousness and the brain: deciphering how the brain codes our thoughts*. New York: Penguin.

Dehaene, S., and Naccache, L. (2001). Towards a cognitive neuroscience of consciousness: basic evidence and a workspace framework. *Cognition*, 79, 1-37.

Del Cul, A., Baillet, S., and Dehaene, S. (2007). Brain Dynamics Underlying the Nonlinear Threshold for Access to Consciousness. *PloS. Biology*, 5, 2408-2423.

Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics. *Journal of Neuroscience Methods*, 134, 9-21.

Desmedt, J. (1981). P300 in serial tasks: An essential post-decision closure mechanisms. *Progress in Brain Research*, 54, 682-686.

Donchin, E., and Coles, M.G. (1988). Is the P300 component a manifestation of context updating? *Behavioral and Brain Sciences*, 11, 357-374.

Efron, B., and Tibshirani, R.J. (1993). *An Introduction to the Bootstrap*. New York: Chapman & Hall.

Eimer, M., and Mazza, V. (2005). Electrophysiological correlates of change detection. *Psychophysiology*, 42, 328-342.

Fendrich, R., Wessinger, C.M., and Gazzaniga, M.S. (2001). Speculations on the neural basis of islands of blindsight. *Progress in Brain Research*, 134, 353-366.

ffytche, D.H., and Zeki, S. (2011). The primary visual cortex, and feedback to it, are not necessary for conscious vision. *Brain*, 134, 247-257.

Foley, R. (2014). The case for characterizing type-2 blindsight as a genuinely visual phenomenon. Consciousness and Cognition, 32, 56-67.

Goebel, R., Muckli, L., Zanella, F.E., Singer, W., and Stoerig, P. (2001). Sustained extrastriate cortical activation without visual awareness revealed by fMRI studies of hemianopic patients. *Vision Research*, 41, 1459-1474.

Groppe, D.M., Urbach, T.M., and Kutas, M. (2011). Mass univariate analysis of event-related brain potentials/fields I: A critical tutorial review. *Psychophysiology*, 48, 1711–1725.

Hillyard, S.A., Vogel, E.K., and Luck, S.J. (1998). Sensory gain control (amplification) as a mechanism of selective attention: electrophysiological and neuroimaging evidence. *Philosofical Transactions of the Royal Society of London. Series B: Biological Sciences*, 353, 1257-1270.

Holmes, G. (1945). The organization of the visual cortex in man. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 132, 348-361.

Kelly, S.P., and O'Connell, R.G. (2013). Internal and external influences on the rate of sensory evidence accumulation in the human brain. *The Journal of Neuroscience*, 33, 19434-19441.

Koch, C. (2004). *The Quest for Consciousness: A Neuroscientific Approach*. Denver: Roberts & Co.

Koch, C., Massimini, M., Boly, M., and Tononi, G. (2016). Neural correlates of consciousness: progress and problems. *Nature Reviews. Neuroscience*, 17, 307-321.

Koivisto, M., Kastrati, G., and Revonsuo, A. (2013). Recurrent processing enhances visual awareness but is not necessary for fast categorization of natural scenes. *Journal of Cognitive Neuroscience*, 26, 223-231.

Koivisto, M., Lähteenmäki, M., Sørensen, T.A., Vangkilde, S., Overgaard, M., and Revonsuo, A. (2008). The earliest electrophysiological correlate of visual awareness? *Brain and Cognition*, 66, 91-103.

Koivisto, M., and Revonsuo, A. (2003). An ERP study of change detection, change blindness, and visual awareness. *Psychophysiology*, 40, 423-429.

Koivisto, M., and Revonsuo, A. (2010). Event-related brain potential correlates of visual awareness. *Neuroscience and Biobehavioral Reviews*, 34, 922-934.

Kutas, M., McCarthy, G., and Donchin, E. (1977). Augmenting mental chronometry: the P300 as a measure of stimulus evaluation. *Science*, 197, 792–795.

Loughnane, G.M., Newman, D.P., Bellgrove, M.A., Lalor, E.C., Kelly, S.P., and O'Connell, R.G. (2016). Target selection signals influence perceptual decisions by modulating the onset and rate of evidence accumulation. *Current Biology*, 26, 496-502.

Luck, S.J. (2005). *An introduction to the event-related potential technique*. Cambridge: MIT Press.

Lumer, E.D., and Rees, G. (1999). Covariation of activity in visual and prefrontal cortex associated with subjective visual perception. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 1669 –1673.

Maris, E., and Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-data. *Journal of Neuroscience Methods*, 164, 177-190.

Mazzi, C., Bagattini, C., and Savazzi, S. (2016). Blind-sight vs. Degraded-Sight: different measures tell a different story. *Frontiers in Psychology*, 7, 901.

Mazzi, C., Mancini, F., and Savazzi, S. (2014). Can IPS reach visual awareness without V1? Evidence from TMS in healthy subjects and hemianopic patients. *Neuropsychologia*, 64, 134-144.

Melloni, L., Schwiedrzik, C.M., Müller, N., Rodriguez, E., and Singer, W. (2011). Expectations change the signatures and timing of electrophysiological correlates of perceptual awareness. *The Journal of Neuroscience*, 31, 1386-1396.

Moutoussis, K., and Zeki, S. (2002). The relationship between cortical activation and perception investigated with invisible stimuli. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 9527-9532.

Naccache, L. (2015). Visual consciousness explained by its impairments. *Current opinion in neurology*, 28, 45-50.

O'Connell, R.G., Dockree, P.M., and Kelly, S.P. (2012). A supramodal accumulation-to-bound signal that determines perceptual decisions in humans. *Nature Neuroscience*, 15, 1729-1735.

Ojanen, V., Revonsuo, A., and Sams, M. (2003). Visual awareness of low-contrast stimuli is reflected in event-related brain potentials. *Psychophysiology*, 40, 192-197.

Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J.M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, 2011, 156869.

Overgaard, M. (2011). Visual experience and blindsight: a methodological review. *Experimental Brain Research*, 209, 473-479.

Overgaard, M., Fehl, K., Mouridsen, K., Bergholt, B., and Cleeremans, A. (2008). Seeing without seeing? Degraded conscious vision in a blindsight patient. *PLoS ONE*, 3, e3028.

Overgaard, M., and Sandberg, K. (2012). Kinds of access: different methods for report reveal different kinds of metacognitive access. *Philosofical Transactions of the Royal Society of London. Series B: Biological Sciences*, 367, 1287-1296.

Pelli, D.G. (1997). The VideoToolbox software for visual psycho-physics. *Spatial Vision*, 10, 437–442.

Perrin, F., Pernier, J., Bertrand, O., and Echallier, J.F. (1989). Spherical splines for scalp and current density mapping. *Electroencephalography and Clinical Neurophysiology*, 72, 184–187.

Philiastides, M.G., Ratcliff, R., and Sajda, P. (2006). Neural representation of task difficulty and decision making during perceptual categorization: a timing diagram. *The Journal of Neuroscience*, 26, 8965-8975.

Philiastides, M.G., Heekeren, H.R., and Sajda, P. (2014). Human scalp potentials reflect a mixture of decision-related signals during perceptual choices. *The Journal of Neuroscience,* 34, 16877-16889.

Pins, D., and ffytche, D. (2003). The neural correlates of conscious vision. *Cerebral Cortex*, 13, 461-474.

Pitts, M.A., Metzler, S., and Hillyard, S.A. (2014). Isolating neural correlates of conscious perception from neural correlates of reporting one's perception. *Frontiers in Psychology,* 5, 1078.

Polich, J. (2007). Updating P300: an integrative theory of P3a and P3b. *Clinical Neurophysiology*, 118, 2128-2148.

Railo, H., Koivisto, M., and Revonsuo, A. (2011). Tracking the processes behind conscious perception: a review of event-related potential correlates of visual consciousness. *Consciousness and Cognition*, 20, 972-983.

Ramsøy, T.Z., and Overgaard, M. (2004). Introspection and subliminal perception. *Phenomenology and the Cognitive Sciences*, 3, 1-23.

Ratcliff, R., and Rouder, J.N. (2000). A diffusion model account of masking in two-choice letter identification. *Journal of Experimental Psychology: Human Perception and Performance*, 26, 127-140.

Rodman, H.R., Gross, C.G., and Albright, T.D. (1989). Afferent basis of visual response properties in area MT of the macaque. I. Effects of striate cortex removal. *The Journal of Neuroscience,* 96, 2033-2050.

Rossion, B., de Gelder, B., Pourtois G., Guérit, J.M., and Weiskrantz, L. (2000). Early extrastriate activity without primary visual cortex in humans. *Neuroscience Letters*, 279, 25-28.

Rutiku, R., Martin, M., Bachmann, T., and Aru, J. (2015). Does the P300 reflect conscious perception or its consequences? *Neuroscience*, 298, 180-189.

Salti, M., Bar-Haim, Y., and Lamy, D. (2012). The P3 component of the ERP reflects conscious perception, not confidence. *Consciousness and Cognition*, 21, 961-968.

Sandberg, K., Bahrami, B., Kanai, R., Barnes, G.R., Overgaard, M., and Rees, G. (2013a). Early visual responses predict conscious face perception within and between subjects during binocular rivalry. *Journal of Cognitive Neuroscience*, 25, 969–985.

Sandberg, K., Bibby, B.M., and Overgaard, M. (2013b). Measuring and testing awareness of emotional face expressions. *Consciousness and Cognition*, 22, 806-809.

Sandberg, K., Timmermans, B., Overgaard, M., and Cleeremans, A. (2010). Measuring consciousness: is one measure better than the other? *Consciousness and Cognition*, 19, 1069-1078.

Sanders, M.D., Warrington, E.K., Marshall, J., and Weiskrantz, L. (1974). “Blindsight”: vision in a field defect. *Lancet*, 1, 707-708.

Schmid, M.C., Mrowka, S.W., Turchi, J., Saunders, R., Wilke, M., Peters, A.J., Ye, F.Q., and Leopold, D.A (2010). Blindsight depends on the lateral geniculate nucleus. *Nature*, 466, 373-377.

Schurger, A., and Sher, S. (2008). Awareness, loss aversion, and post-decision wagering. *Trends in Cognitive Sciences*, 12, 209-210.

Sergent, C., Baillet, S., and Dehaene, S. (2005). Timing of the brain events underlying access to consciousness during the attentional blink. *Nature Neuroscience*, 8, 1391-1400.

Shadlen, M.N., and Kiani, R. (2013). Decision making as a window on cognition. *Neuron*, 80, 791-806.

Silvanto, J. (2014). Is primary visual cortex necessary for visual awareness? *Trends in Neurosciences*, 37, 618-619.

Silvanto, J. (2015). Why is “blindsight” blind? A new perspective on primary visual cortex, recurrent activity and visual awareness. *Consciousness and Cognition*, 32, 15-32.

Stoerig, P., and Barth, E. (2001). Low-level phenomenal vision despite unilateral destruction of primary visual cortex. *Consciousness and Cognition*, 10, 574-587.

Sutton, S., Braren, M., Zubin, J., and John, E. (1965). Evoked potential correlates of stimulus uncertainty. *Science*, 150, 1187-1188.

Tagliabue, C.F., Mazzi, C., Bagattini, C., and Savazzi, S. (2016). Early Local Activity in Temporal Areas Reflects Graded Content of Visual Perception. *Frontiers in Psychology*, 7, 572.

Thut, G., Nietzel, A., Brandt, S.A., and Pascual-Leone, A. (2006). Alpha-band electroencephalographic activity over occipital cortex indexes visuospatial attention bias and predicts visual target detection. *The Journal of Neuroscience*, 26, 9494-9502.

Twomey, D.M., Murphy, P.R., Kelly, S.P., and O’Connell R.G. (2015). The classic P300 encodes a build-to-threshold decision variable. *The European Journal of Neuroscience,* 42, 1636-1643.

Urban, F.M. (1910). The method of constant stimuli and its generalizations. *Psychological Review*, 17, 229-259.

Weiskrantz, L. (1986). *Blindsight – a case study and implications*. Oxford: Oxford University Press.

Weiskrantz, L. (1998). Consciousness and commentaries. In *Towards a Science of Cosnciousness II – The Second Tucson Discussions and Debates*, eds S.R. Hameroff, A.W. Kaszniak, and A.C. Scott. Cambridge: MIT Press, 371-377.

Weiskrantz, L. (2009). *Blindsight*. Oxfor: Oxford University Press.

Weiskrantz, L., Warrington, E.K., Sanders, M.D., and Marshall J. (1974). Visual capacity in the hemianopic field following a restricted occipital ablation. *Brain*, 97, 709-728.

Wickens, C., Kramer, A., Vanasse, L., and Donchin, E. (1983). The performance of concurrent tasks: a psychophysiological analysis of the reciprocity of information processing resources. *Science*, 221, 1080–1082.

Wilenius-Emet, M., Revonsuo, A., and Ojanen, V. (2004). An electrophysiological correlate of human visual awareness. *Neuroscience Letters*, 354, 38-41.

Zeki, S., and ffytche, D.H. (1998). The Riddoch syndrome: insights into the neurobiology of conscious vision. *Brain*, 121, 25-45.

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