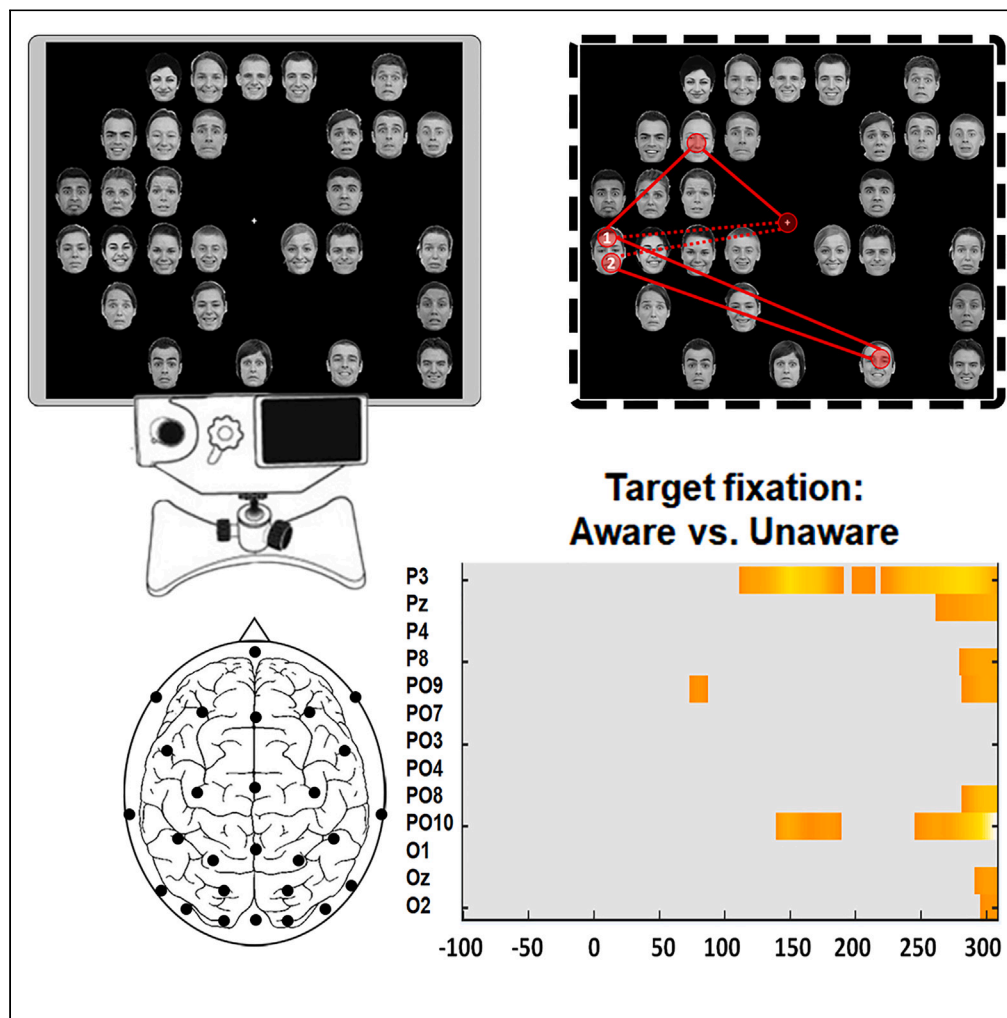


Article

Fixation-related electrical potentials during a free visual search task reveal the timing of visual awareness



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Highlights

Electrophysiological correlates of awareness emerge at 110 ms in free visual search

Conscious processing of fearful faces emerges later in the N170 time range

We did not find evidence for unconscious processing of fearful expressions

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Article

Fixation-related electrical potentials during a free visual search task reveal the timing of visual awareness

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SUMMARY

It has been repeatedly claimed that emotional faces readily capture attention, and that they may be processed without awareness. Yet some observations cast doubt on these assertions. Part of the problem may lie in the experimental paradigms employed. Here, we used a free viewing visual search task during electroencephalographic recordings, where participants searched for either fearful or neutral facial expressions among distractor expressions. Fixation-related potentials were computed for fearful and neutral targets and the response compared for stimuli consciously reported or not. We showed that awareness was associated with an electrophysiological negativity starting at around 110 ms, while emotional expressions were distinguished on the N170 and early posterior negativity only when stimuli were consciously reported. These results suggest that during unconstrained visual search, the earliest electrical correlate of awareness may emerge as early as 110 ms, and fixating at an emotional face without reporting it may not produce any unconscious processing.

INTRODUCTION

Although the visual field of humans spans nearly 180°,^{1,2} effective visual processing occurs mostly at the center, in the so-called functional visual field.³ Consequently, when exploring their visual environment, humans will produce multiple saccades and fixations, orienting their gaze toward different parts of the visual field to process the stimuli that capture their attention or are relevant to their goals.^{4,5} In such visual serial search tasks, it has long been known that exploration time increases as a function of the number of items in the visual field.⁶ Variations in the speed of detection have been found across different stimuli and have been shown to depend on a number of parameters, including the physical characteristics of the stimuli and the features that distinguish the stimuli from irrelevant distractors.⁷

While central fixation is important for conscious processing, it is not sufficient to generate awareness. Indeed, when exploring the environment, stimuli may be fixated without being consciously detected, and decades of behavioral experiments using visual search paradigms have shown that, during serial search, targets may be fixated without being consciously seen.⁸

Human faces, in particular those displaying emotional expressions, constitute a particularly important category of stimuli in that they are crucial to our daily social life. Emotional faces have received much scrutiny following early observations indicating that they can readily capture attention.^{9–11} Behavioral studies examining the speed of visual search and detection have reported that emotional faces are detected faster than other types of stimuli.^{12–14} In line with these observations, studies of patients with spatial attention deficits have shown that emotional faces attract attention more efficiently than non-emotional stimuli,^{11,15} possibly due to their behavioral relevance. In a similar vein, patients with visual deficits have been found to process emotional faces without awareness (a phenomenon termed affective blindsight^{16,17}). This finding has been replicated in healthy controls using visual masking.^{16–20}

However, objections have been voiced suggesting that such attentional effects may be driven essentially by the low-level characteristics of the stimuli,²¹ while others have argued that they occur only if sufficient attentional resources are available, and that increasing the attentional requirements of a concurrent task prevents faces from capturing attention.²² Furthermore, nonconscious or subliminal viewing has been questioned and suggested to be a degraded form of conscious vision.^{23,24}

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Recently, neuroscience has begun to explore in more depth the neural events that reflect perceptual awareness and their temporal dynamics (see the study by Mudrik and Deouell²⁵ for a recent review). Using electrophysiological measures, in particular event-related potentials (ERPs), different paradigms have been created to prevent conscious perception and thus investigate how visual awareness emerges. Such paradigms are designed either to interfere with the normal perception of stimuli, or to divert attention from them.²⁵ The former paradigms include techniques such as stimulus masking²⁶ (where stimuli are presented very briefly and are followed by a mask that blocks conscious detection), stimulus crowding^{27,28} (where a target is presented along with numerous other stimuli, causing it to go undetected), or interocular suppression²⁹ (where different stimuli are presented simultaneously to each eye, allowing only one to be consciously detected). The latter include paradigms where attention is directed toward irrelevant aspects of the visual scene, away from the target information (e.g., inattention blindness or attentional blink paradigms^{30,31}; see the study by Railo et al.³² for a review).

By comparing conscious and unconscious presentations of stimuli, different studies have attempted to identify the electrical brain responses associated with awareness. Two possible ERP markers have been put forward as possible electrical correlates of visual awareness. The first one to be highlighted is a late positive potential situated over centro-parietal electrode sites around 300–600 ms after the stimulus onset. This ERP wave, termed the P300, emerges when participants detect stimuli in tasks manipulating visibility.³³ However, arguments against the P300 being the most reliable correlate of awareness have underlined the fact that other factors, such as task relevance, may be critical for its appearance.^{34,35} Moreover, the P300 has been found to correlate with processes separate from awareness, such as working memory,^{36,37} context updating,³⁸ and post-perceptual processing.³⁹ Some studies have further shown that conscious reporting can take place before the onset of the P300,⁴⁰ shedding more doubt on the correlation between the P300 and awareness.

Subsequently, a number of observations have pointed to an earlier negative deflection over temporo-occipital regions that may in fact index awareness.⁴¹ This earlier component is observed after ~200 ms post-stimulus and presents as a greater negativity for conscious compared to unconscious stimulus presentations, consequently dubbed the visual awareness negativity (VAN). The VAN has been found with different methods of awareness manipulation, even when controlling for the task relevance of the stimuli, objective task performance, and a variety of types of attention (see the study by Förster et al.⁴¹ for a review). This component has been posited to be the earliest correlate of visual awareness in the human brain.^{41–43}

A controversy has emerged regarding which, if any, of these two markers actually reflects awareness, especially as they are loosely linked to two recent influential theories.^{44,45} One of them, the global neuronal workspace theory,⁴⁴ postulates that awareness arises when sensory information, coded by modular cerebral networks, is amplified by attention and subsequently recruits neurons widely distributed in the brain. This process creates a “neuronal workspace” in which information becomes available for different complex processes, such as working memory, perceptual categorization, or memorization, allowing the emergence of awareness.⁴⁴ In this framework, awareness has been hypothesized to be indexed by the P300.

The second theory highlights the role of recurrent processing in enabling awareness.⁴⁵ This theory states that the feedforward sweep projecting information bottom-up through the cortical hierarchy is not sufficient to produce awareness. Rather, it is the subsequent feedback projections from higher to lower-tier areas following the initial feedforward sweep that are required to produce conscious perception.⁴⁵ Such feedback activity is largely localized to early regions receiving sensory (e.g., visual) information and suggests an earlier timescale for the emergence of awareness more akin to the VAN.⁴¹

Both theories have empirical support for their interpretations^{41,46–48} and the question remains open as to which one better accounts for perceptual awareness.

The discrepancies in findings regarding the electrical correlates of awareness and unconscious emotion processing may be due to the way awareness was manipulated in previous paradigms. Indeed, awareness is generally impeded by modifying viewing procedures that are rarely, as noted above, if ever, found under normal viewing conditions.

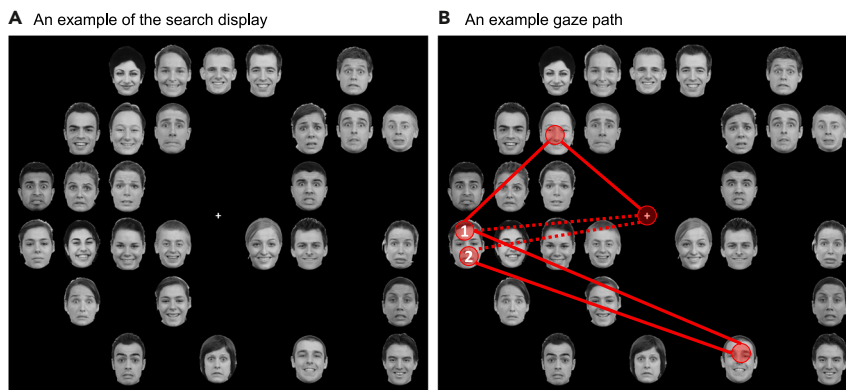


Figure 1. An example of the search display in each trial

(A) Here, the target is a single neutral face among happy and fearful distractor faces.

(B) An example gaze path. Only fixations on the targets are marked and analyzed in this study. The number inside the circle indicates the order of the fixations on the same target in one trial. In this example, one or two fixations can be made on the neutral target face. If the first fixation on the target is followed by a saccade to another face image and is not followed by a report for the target, this fixation would be identified as an unaware fixation. Then, in this trial, the last fixation on the target followed by a saccade to the central fixation point (dashed line) and subsequently a correct target localization would be identified as the aware fixation. If, however, the first fixation on the target is followed by a saccade to the central fixation point and a correct target localization, it would be identified as the aware fixation, and there would be no unaware fixation from this trial. See the [STAR Methods](#) for a detailed description of the procedure.

One way to circumvent this issue would be to allow the participants to explore the visual scene freely during the electroencephalographic recordings. To our knowledge, unconstrained visual search has barely been used in tasks measuring electrophysiological outcomes. Since natural viewing conditions entail saccades and multiple fixations that do not systematically produce awareness, we decided to take advantage of the so-called “normal blindness” or look-but-failed-to-see phenomenon⁸ to investigate the neural correlates of visual awareness and simultaneously to explore unconscious processing of emotional faces.

In the current study, participants were allowed to explore a visual scene freely, in search of a target facial expression. These visual scenes were composed of multiple faces displaying neutral, happy, and fearful expressions. On each trial, participants were asked to localize a specific target facial expression (a single fearful or a neutral expression) among the other emotions (see [Figure 1A](#)). Eye-movements and electroencephalography were recorded simultaneously during the search. The electrical potentials triggered at each fixation on the target face stimulus (the fixation-related potentials or FRPs) were computed. Separate FRPs were obtained when the target was fixated but not reported (unaware condition), and when the target was fixated and subsequently reported (aware condition). These were further separated according to target expression (fearful vs. neutral).

On each given trial, a variable number of fixations could occur for the target without giving rise to awareness. The first such fixation on the target was used to compute the “unaware” FRP. The last fixation on the target comprised the “aware” FRPs (see [Figure 1B](#)). However, to ensure that this target fixation was not the last saccade but was followed by another saccade as for unaware fixations, participants were instructed to gaze at a specific location in the middle of the screen after they detected the target. This would activate the mouse cursor, which allowed them to respond manually by clicking on the target location.

The first aim of the current study was to identify the neural correlate of awareness: we aimed to establish whether awareness arises early (~200 ms) or later (>300 ms) in the stream of visual processing. Moreover, we aimed to identify the neural activity underlying conscious and, if present, unconscious processing of fearful and neutral faces, using free visual search. Specifically, we reasoned that if a fearful expression was processed without awareness, the FRPs would be significantly different between fearful and neutral target expressions, in unaware fixations. However, if the processing of a fearful expression required awareness, we would only find FRP differences in aware fixations.

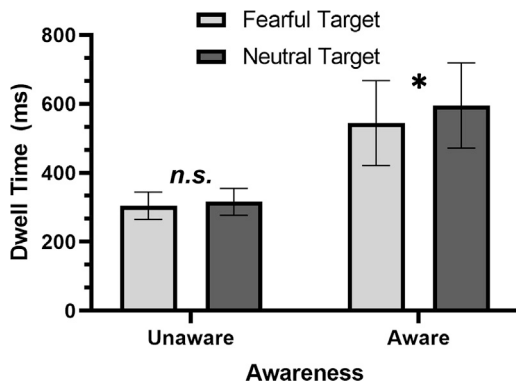


Figure 2. Dwell time data in different awareness conditions for fearful and neutral targets

Data are represented as mean \pm SEM. Note: * indicates $p < 0.001$ in the 2 (awareness: aware, unaware) \times 2 (target emotion: fearful, neutral) repeated-measures ANOVA.

RESULTS

Behavioral results: Accuracy and dwell time

The overall accuracy (proportion of correct responses) was 0.97 ($SD = 0.03$) on the target face localization task, indicating a ceiling effect and suggesting that the task was relatively easy; no further analysis was performed on accuracy.

A 2 (awareness: aware, unaware) \times 2 (target emotion: fearful, neutral) repeated-measures ANOVA was performed on the average dwell time on targets. As shown in Figure 2, a main effect of awareness was found, $F(1,31) = 201.72$, $p < 0.001$, $\eta_p^2 = 0.87$, which was modulated by a significant interaction with the emotion of the target face, $F(1,31) = 16.29$, $p < 0.001$, $\eta_p^2 = 0.34$. Follow-up comparisons showed that, on unaware fixations, no difference was found between the dwell time on fearful ($M = 304.64$ ms, $SE = 7.04$) and on neutral targets ($M = 316.01$ ms, $SE = 6.92$), $p = 0.110$. However, when participants were aware of the stimuli, dwell time on fearful targets ($M = 544.37$ ms, $SE = 21.74$) was significantly shorter than on neutral targets ($M = 595.85$ ms, $SE = 21.82$), $p < 0.001$.

FRPs

A factorial mass univariate analysis (FMUA) omnibus 2 (awareness: aware, unaware) \times 2 (target emotion: fearful, neutral) ANOVA was performed on all electrodes using the Linear Modeling of MEEG data toolbox.⁴⁹ Since unaware conditions yielded fixations that lasted on average approximately 310 ms, which were much shorter than aware fixations, we only compared the FRPs extending to 310 ms between conditions of awareness.

The main effect of awareness was found to be significant in a time window spanning from 112 to 308 ms over C3, CP5, P3, P8, PO8, PO9/10, O2, Cz, Pz, and Oz (temporal peak: 308 ms at electrode PO10; $F = 74.63$, $p = 0.001$), all $F_s > 28.37$, $p_s < 0.05$. Specifically, FRP amplitudes in the aware condition were significantly more negative than the unaware condition at posterior electrodes (P8, PO8, PO9/10, O2, and Oz) in this time window (Figure 3A). This negativity for consciously processed stimuli is consistent with a VAN that is often reported between 200 and 300 ms, peaking around 250 ms post-stimulus.^{41–43,50} Simultaneously, FRPs were found to be more positive in the aware compared to the unaware condition over centro-parietal electrodes (C3, CP5, P3, Cz, and Pz) during this time window (Figure 3B; for a raster plot of significance testing results please see Figure 3C).

The omnibus ANOVA also revealed a main effect of emotion between 173 and 261 ms over P7/8, PO8, and PO9/10 (temporal peak: 218 ms at electrode PO9; $F = 41.45$, $p = 0.005$), all $F_s > 28.28$, $p_s < 0.05$ (Figure 3D). Specifically, a fearful target was associated with more negative FRP amplitudes, compared to a neutral target, in a time window (173–261 ms) that encompasses the face-selective N170 and the VAN (Figure 3A). Although the interaction between target emotion and awareness was non-significant, $p_s > 0.05$, *a priori* pairwise comparisons were carried out to address our hypotheses. Specifically, we compared the FRPs between fearful and neutral targets in the unaware and the aware conditions, respectively, using the F -tests with an alpha level of 0.025. No emotion-related difference was found in the unaware condition ($F_s < 20.29$, $p_s > 0.025$). However, in the aware condition, a fearful target was associated with more negative amplitudes than a neutral target between 161 and 225 ms over P8, PO8, and PO10 (temporal peak: 200 ms at electrode

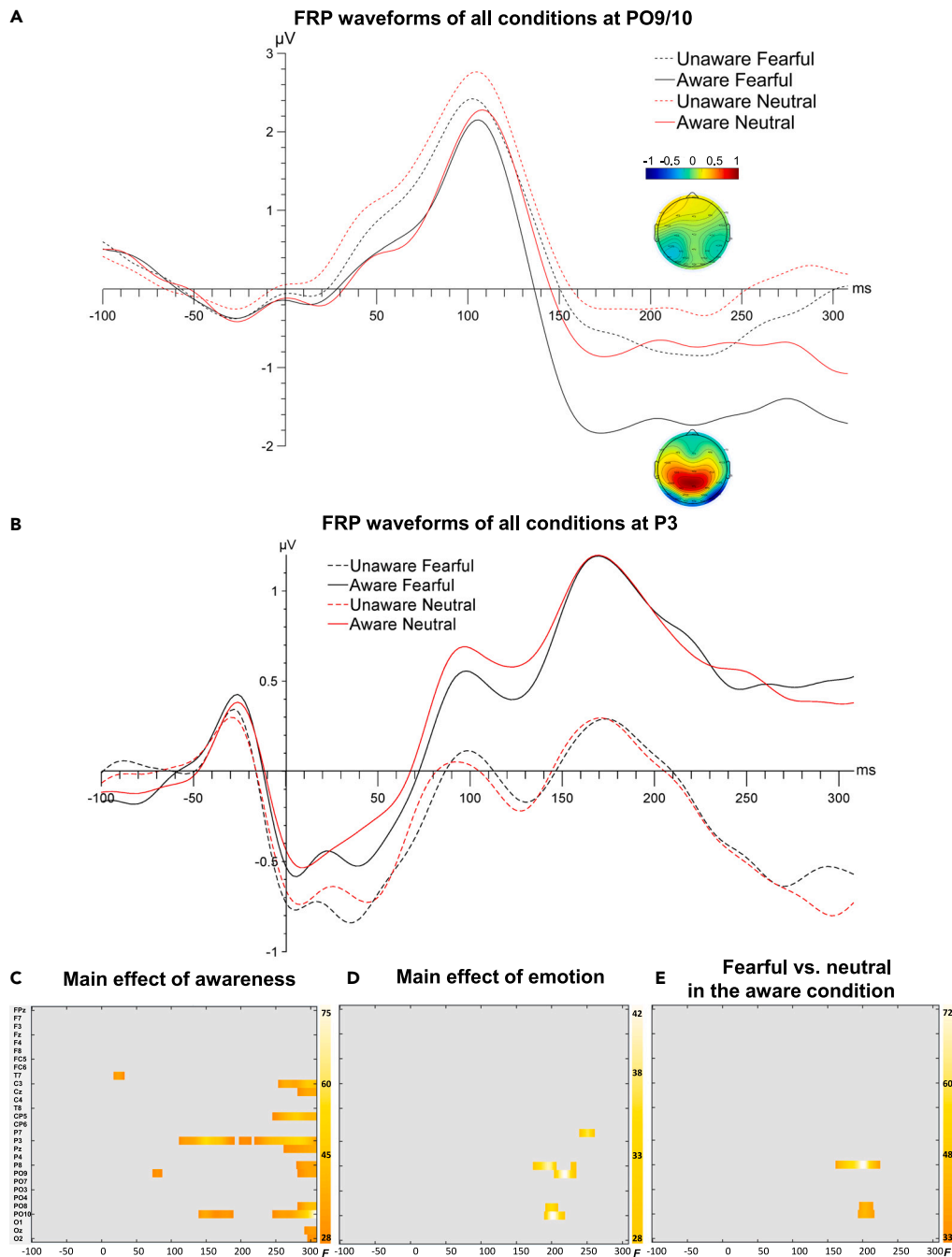


Figure 3. FRP results of the 2(awareness) x 2(target emotion) ANOVA

See also [Figure S2](#).

(A) FRP waveforms of all conditions at electrode PO9/10, the electrode sites where the maximal omnibus effects of awareness and emotion were found. Topographic maps (time range: 112–308 ms) were plotted separately for the unaware (top) and aware (bottom) conditions, collapsed across the emotion of the target face.

(B) FRP waveforms of all conditions at electrode P3, demonstrating the parietal positivity.

(C) Raster plots of the main effect of awareness.

(D) Raster plots of the main effect of emotion.

(E) Raster plots of the fearful-neutral comparison in the aware condition.

P8; $F = 71.81$, $p = 0.001$, all $F_s > 33.05$, $p_s < 0.025$ (Figure 3E). Although these follow-up tests were conducted in an *a priori* manner, they may be considered insufficiently convincing.⁵¹ Consequently, we additionally carried out a Bayesian analysis, in which we averaged data over posterior electrodes across the N170 and the VAN time range (120–310 ms; for a similar procedure please see Schindler et al.⁵²), and ran Bayesian related-samples *t*-tests between fearful and neutral targets, separately for aware and unaware conditions. The Bayes factors for the contrasts were 1.82 and 0.02 in the unaware condition and the aware condition, respectively. These Bayesian results show that there is anecdotal evidence for no difference between fearful and neutral targets in the unaware condition but there is very strong evidence for fearful-neutral differences in the aware condition. Therefore, the FRPs differed between fearful and neutral expressions in the N170 and the VAN time range, only when participants were aware of the stimuli.

Is conscious report a gradual phenomenon?

In most trials, there were multiple or repeated fixations on the target. Thus, the current paradigm provides an opportunity to investigate the electrical activity across the repeated fixations prior to the final conscious report for the target. To test whether perceptual evidence was accumulated in a gradual or an all-or-none manner leading to a conscious report, we extracted trials where participants reported seeing the target face after at least three fixations on the target. Because the average dwell time for the three fixations was 420 ms, FRP epochs were time locked to each fixation and extended 420 ms. Then, we compared the FRPs between the last three fixations on the target (i.e., last fixation, 1-prior, 2-prior). A one-way (fixation: last fixation, 1-prior, 2-prior) FMUA ANOVA was performed on all scalp electrodes. We found a main effect of awareness over CP5 and P3 between 163 and 216 ms ($F_s > 22.48$, $p_s < 0.05$; see Figures 4A and 4B). Follow-up *F*-tests with an adjusted alpha level ($\alpha = 0.017$) showed a significant positivity for the last fixation, compared to both 1-prior fixation between 119 and 203 ms at electrodes F8, CP5, and P3, $F_s > 17.43$, $p_s < 0.017$ (Figure 4C), and 2-prior fixation between 163 and 223 ms at electrodes CP5 and P3, $F_s > 19.69$, $p_s < 0.017$ (Figure 4D). Note that the FRP amplitudes for the 1-prior fixation were not significantly different from the 2-prior fixation ($F = 12.50$, $p = 0.096$).

DISCUSSION

This study examined visual awareness and the unconscious processing of fearful faces under conditions of unconstrained visual exploration, using combined EEG and eye-tracking methodologies. An electrophysiological effect of awareness was found over a time window beginning at around 110 ms and extending over the 200 ms period leading to the subsequent saccade. Differences across emotions were only found in the FRPs when fixations gave rise to awareness. However, when fixations were not associated with awareness, no differences were found between target expressions.

As describe earlier, initial studies implicated the P300 as a marker of awareness when comparing conscious and unconscious processes.^{33,47} One interpretation for this occurrence is that once a certain threshold is reached, numerous cortical regions are recruited and the sensory information becomes accessible by different information processing modules, which then leads to awareness.³³ This view has been supported notably by the observation that cortical activation during the P300 encompasses extensive parietal and frontal brain regions.^{47,53,54} However, others have argued that the P300 may be linked to post-perceptual processes subsequent to awareness, as noted above. For example, using a masking procedure in an attentional task, Del Zotto and Pegna⁵⁵ observed a negativity in the 200 ms range for consciously detected emotional faces, while the P300 was modulated depending on whether the face was a target or not, revealing that the magnitude of this marker depended on stimulus processes that are separate from awareness. Similarly, Pitts and colleagues compared the ERPs to geometric patterns that participants were aware of or not, using an inattention blindness task.⁵⁶ Compared with the unaware condition, the aware condition showed an increased negativity around 200–240 ms; however, the P300 appeared only when the geometric shapes were relevant to the task at hand.⁵⁶ These results linked the P300 to the relevance of the stimulus as a target rather than awareness *per se*.

The early onset of the awareness-related potential in our study argues against the P300 being the earliest marker of awareness. In the current study, the FRP differences between aware and unaware fixations of the target revealed a greater negativity over posterior and lateral electrodes during a time window extending roughly from 110 to 218 ms. This time course corroborates the hypothesis that an early visual awareness negativity, or the VAN, is the earliest index of conscious processing.⁴¹ A number

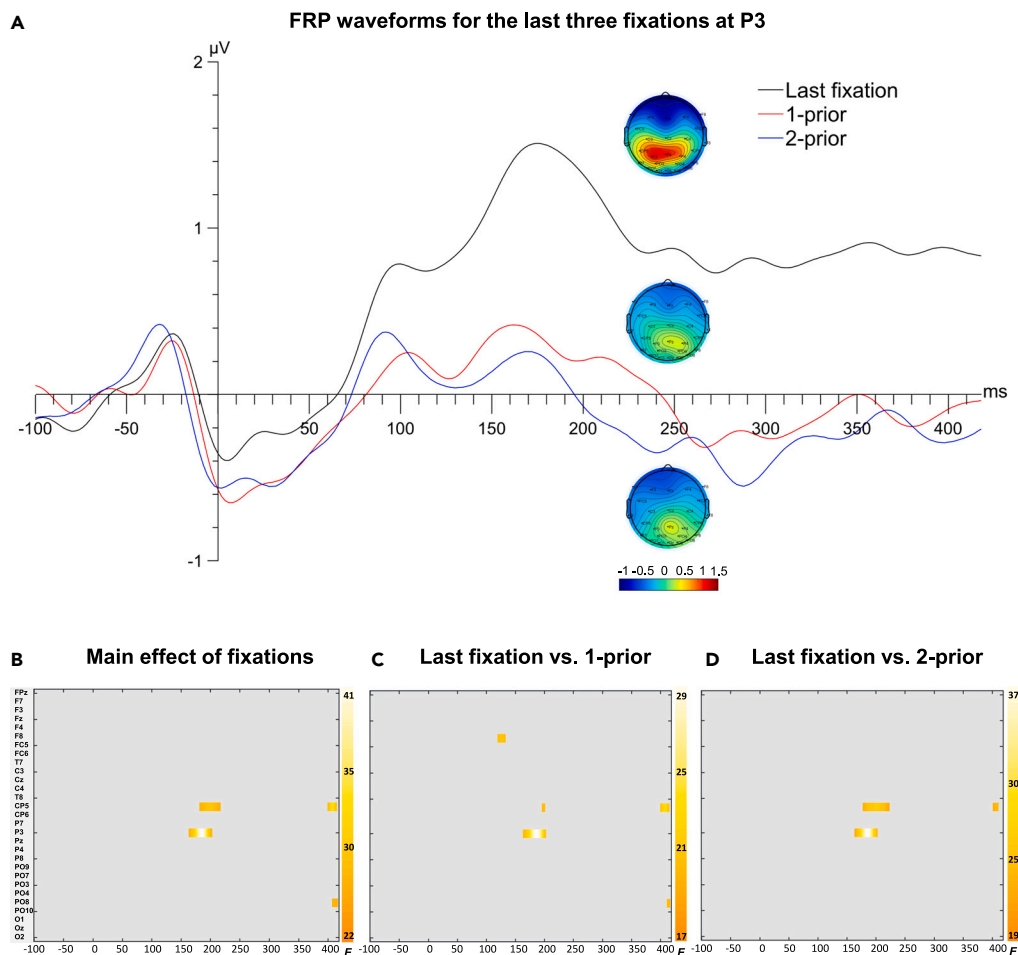


Figure 4. Non-linear increase in the P300 prior to conscious report

(A) FRP waveforms for the last three fixations at electrode P3. Topographic maps (time range: 119–223 ms) were plotted separately for different fixation conditions.

(B) Raster plots of the main effect of fixations.

(C) Raster plots of the last fixation vs. 1-prior comparison.

(D) Raster plots of the last fixation vs. 2-prior comparison.

of observations have indicated that visual awareness produces a negative ERP deflection over temporo-occipital electrodes, peaking approximately between 200 and 250 ms,^{57–59} when comparing stimuli that are consciously reported with those that are not. This negative deflection has been evidenced using different methods to interfere with awareness, including masking, stimulus degradation, attentional blink, change blindness, and bistable perception (see the study by Koivisto and Revonsuo⁶⁰ for a review).

Our findings suggest that under more naturalistic viewing conditions (i.e., without any manipulation of the stimulus presentation characteristics), awareness may begin to emerge already after approximately 100 ms. The slightly earlier onset of our awareness-related potential may seem premature. However, although the VAN has been reported to peak at around 200 ms, paradigms exploring awareness have shown that the electrophysiological differences can onset as early as 100 ms, with less discernible stimuli delaying the VAN onset.⁴¹ For example, in one study by Wilenius and Revonsuo,⁶¹ low-contrast stimuli were used to compare aware and unaware conditions. In this case, the VAN was delayed by 150 to 200 ms compared to a similar procedure using higher-contrast stimuli. This suggests that the emergence of awareness and its electrophysiological correlates are subject to a certain degree of variability depending on the low-level features affecting stimulus visibility. These observations support the current findings as we presented photographs without altering their visibility in our procedure.

Evidently, our observations do not rule out the possibility of any subsequent processing in the aware condition after approximately 300 ms. This is due to the fact that comparisons between aware and unaware conditions could only be computed over the duration of the shorter dwell time (the unaware condition; 310 ms) in this study. As a result, the electrophysiological responses could not be compared over ~300 ms after which the visual scene in the unaware condition changed. The complete expression of the P300 therefore cannot be ascertained in this paradigm and its emergence during conscious processing cannot be ruled out. In fact, because the aware condition was associated with a longer target dwell time, it seems likely that some additional processing did occur after the stimulus was consciously identified as the target. In line with this, one study attempted to distinguish the electrophysiological correlates of stimulus identification and stimulus detection,⁶² and showed that stimulus *detection*, but not its *identification*, produced a posterior negativity between 200 and 300 ms (i.e., the VAN). On the other hand, stimulus *identification* produced a later increase in the P300 component.⁶² Based on these findings, it seems possible that our early negativity is linked to the initial conscious detection of the stimulus, which may still be devoid of higher level processes. Future paradigms could be carried out to explore this eventuality.

A potential alternative interpretation of our findings may be argued. One may contend that awareness could have occurred during early fixations on targets, but participants may have continued visual exploration to confirm that no other stimuli matched the target expression. Such behavior may be caused by stimulus ambiguity, and viewers would thus have processed the stimulus consciously but pursued their search due to a level of uncertainty. However, arguing against this, a number of trials saw participants correctly localize the target expression upon their first fixation on the target. Furthermore, emotion recognition was judged as relatively easy as participants showed high accuracy scores on the task (97%). The task therefore appears to efficiently distinguish unconscious from conscious processing of facial expressions.

It is worth noting that the comparison of the P300 for the penultimate and antepenultimate fixations did not show any significant difference and only the P300 in the final fixation differed from the preceding ones. This finding is consistent with the suggestion of a non-linear or non-gradual gain for awareness. Similar non-linear effects have been reported in studies investigating the P300.^{63,64} Indeed, since the global neuronal workspace model⁴⁴ posits that consciousness results from a non-linear and sudden activation or “ignition” of neurons, a qualitative increment may be expected. That said, the distinction between graded or dichotomous awareness is arguably too simplistic, and recent suggestions point to factors such as task difficulty which may affect the levels of processing and the associated electrophysiological components.⁶⁵

As far as emotion processing is concerned, our study failed to identify any electrophysiological evidence of unconscious processing of fearful expressions. Differences between the FRP responses to fearful and neutral target expressions were found only in conditions of awareness. Specifically, fearful target faces were associated with a greater negativity over the N170 and the VAN time windows compared to neutral target faces when participants were aware of the stimuli. This finding is consistent with previous observations reporting early modulations of the N170 and an early posterior negativity (EPN) for negative emotional expressions (see the study by Schindler & Bublatzky⁶⁶ for a review), particularly in conditions where participants are aware of the stimuli.⁶⁷ The timing of the emotion effect observed here is also consistent with previous research using similar analysis procedures (mass univariate analysis⁶⁸).

Many observations have evidenced an enhanced N170 component when comparing fearful with neutral expressions.^{42,69–73} It has been hypothesized that the N170 increase may be linked to stimuli indicating the possible presence of threat and may find its origin in feedback activation from the amygdala.⁷⁴ Similarly, an enhanced EPN has also been reported for fearful expressions in tasks where participants were required to attend the facial expression.^{75–77} The EPN has been posited to reflect the early attentional selection associated with emotional significance and stimulus saliency.^{78,79} In our case, the EPN seemed to index emotional content only when the stimuli have reached consciousness. Relevant to our work, a recent study explored the ERP responses to emotional faces that were preceded by a task which varied in its perceptual load as well as in its temporal separation from the subsequent face stimuli.⁸⁰ It was found that the EPN emerged for fearful expressions only when greater intervals were inserted between the load task and the face presentations, indicating that the EPN may emerge only when sufficient attentional resources are available.⁸⁰ In our study, the electrophysiological modulation observed could well reflect awareness which then enables the processing of the emotional stimuli, giving rise to further modulations in this

time window. However, in view of the overlap in timing and in conditions of appearance, the distinction between the VAN (awareness) and the EPN (emotion) is challenging in the current study.

Finally, it should be noted that the dwell time also differed according to the target expressions in the aware condition. The dwell time on fearful target faces was shorter than on neutral target faces, while this was not the case in the unaware condition. The shorter dwell time on fearful face targets is reminiscent of previous behavioral studies exploring attentional attraction of emotional faces,^{12,81–83} indicating an easier detection of fear than of neutral expressions. Alternatively, when searching for fearful faces, the distractor faces which were composed of neutral and happy expressions may have interfered less with visual search, by allowing attentional disengagement more easily, than fearful face distractors in the search for neutral targets. Similar findings have been reported by other researchers.^{84,85} For example, Fox and colleagues found that angry distractor faces slowed down the visual search for a target emotion, compared to neutral distractors.⁸⁴ The dwell time in our case thus mirrors previous reports by showing that negative emotional expressions may slow down attentional disengagement during visual search.

In conclusion, the evidence obtained in this study suggests that during unconstrained free viewing visual search, electrophysiological correlates of awareness appear to emerge at around 110 ms, which argues in favor of an early negativity as the first index of awareness. Furthermore, while conscious processing of fear emerged in the N170 and the EPN time range, our current data argue against any unconscious processing of fearful expressions during visual search.

Limitations of the study

One limitation of the current study could be that we were not able to examine the FRPs beyond 310 ms in the main analysis. Consequently, we were not able to ascertain the complete expression of the P300. However, as explained, we restricted the FRP time windows to 310 ms to ensure that the signals being compared between unaware and aware conditions were both triggered by fixations on the target faces. Another limitation could be that we used different distractors in different conditions of target expressions. As a result, the distractors may have constituted different levels of hindrance to the task, as we acknowledged. However, this approach was necessary in the current paradigm in order to ensure a sufficient level of task difficulty. We also believe that this variability is inherent in the way emotion is processed in the real world, where emotional stimuli are typically presented in complex environments.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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 - Additional analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.107148>.

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AUTHOR CONTRIBUTIONS

Conceptualization, Z.Q. and A.J.P.; Methodology, Z.Q.; Investigation, Z.Q. and H.X.; Formal Analysis, Z.Q. and Z.H.; Writing – Original Draft, Z.Q.; Writing – Review & Editing, Z.Q., A.J.P., S.I.B., and Z.H.; Resources, A.J.P. and S.I.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
MATLAB R2020b	Natick, Massachusetts: The MathWorks Inc.	RRID: SCR_001622 https://au.mathworks.com/products/matlab.html
IBM SPSS Statistics 27	Armonk, NY: IBM Corp.	RRID: SCR_016479 https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-27
EEGLAB version 2022.1	Swartz Center for Computational Neuroscience, CA	RRID: SCR_007292 https://sccn.ucsd.edu/eeglab/index.php
EYE-EEG toolbox version 0.99	Dimigen et al. ⁸⁶	https://www.eyetracking-eeg.org/
ERPLAB version 9.0	University of California, Davis	https://erplab.org/
LIMO EEG	Pernet et al. ⁴⁹	https://github.com/LIMO-EEG-Toolbox/limo_tools
Pre-processed EEG data used in the paper	This paper	https://osf.io/9hswj
All codes used in the paper	This paper	https://osf.io/9hswj

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Zeguo Qiu (zeguo.qiu@uq.net.au).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Pre-processed data and group results have been deposited at OSF and are publicly available as of the date of publication. The DOI is listed in the [key resources table](#). Raw EEG data used in this paper will be shared by the [lead contact](#) upon request.
- All codes used in our data pre-processing and analyses have been deposited at OSF and are publicly available as of the date of publication. The DOI is listed in the [key resources table](#).
- Any additional information required to reanalyse the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The sample size was determined based on the smallest effect size reported in our previous work on awareness of fearful faces ($\eta_p^2 = 0.33$).⁵⁰ For our within-participants 2-by-2 factorial design, the minimum sample size was 16 for a significant main effect of awareness that is sufficiently powered (i.e., 90%), with an effect size of 0.33 at an alpha level of 0.05, two-tailed (calculated with MorePower Software⁸⁷). Out of an abundance of caution, especially considering that this is a novel paradigm, we doubled the sample size. Thirty-two participants ($M_{age} = 21.4$ years, $SD_{age} = 3.1$ years; 12 males, 20 females) were recruited at the University of Queensland and were compensated with either course credits or \$50 (AUD) for their participation. All participants had normal or corrected-to-normal vision and they had no history of neurological or psychiatric conditions. After data pre-processing, data from two participants were excluded for the FRP analyses (see Data pre-processing). As a result, the final sample for the FRP analyses consisted of 30 participants ($M_{age} = 21.2$ years, $SD_{age} = 2.9$ years; 11 males, 19 females). The experimental procedure was approved by the ethics committee of the University of Queensland. All participants provided informed consent prior to their participation.

METHOD DETAILS

Apparatus and stimuli

All stimuli were presented on a 19" color LCD monitor (resolution: 1280 × 1024 pixels) with a viewing distance of 65 cm. The experiment was programmed and run in PsychoPy3.⁸⁸

We obtained fearful, happy and neutral face images from 48 models (24 males, 24 females) from the Radboud Faces Database.⁸⁹ Each face image was rendered black-and-white and scaled approximately 4.2 cm × 3 cm (3.7° × 2.6° in visual angle; see [Figure 1A](#)). The average luminance was matched across images. All photo editing was done in Photoshop 2021 version 22.4.0 (Adobe Systems, San Jose, CA).

Each search array consisted of one target face and 28 distractor faces. The screen was divided into 54 (a 9-by-6 grid) virtual image holders. In each search array, images were presented at 29 image holders randomly selected by the experimental program. To make sure that the task was sufficiently difficult, we used two non-target expressions in the distractor face images ([Figure 1A](#)). Specifically, in the fearful target face block, a target fearful face was presented among 14 happy distractor faces and 14 neutral distractor faces. In the neutral target face block, a target neutral face was presented among 14 happy distractor faces and 14 fearful distractor faces.

Eye movement and EEG data acquisition

Monocular gaze position was recorded using the Eyelink 1000 plus system (SR Research Ltd., Canada) with a spatial resolution of <0.01° and a sampling rate of 1000 Hz.

Continuous EEG was acquired at 500 Hz using the BrainProducts 32-channel system (Brain Products, Germany) using the international 10–20 configuration. During recording, EEG signals were band-pass filtered between 0.01–40 Hz, and a notch filter of 50 Hz was used to reject power line noise. Recordings were referenced online to a reference electrode taped to participants' left ear. Impedances were kept below 15kΩ.

Procedure

Before the experiment began, the eye tracker was calibrated with a 9-point calibration, and participants completed five to ten practice trials with one target expression (fearful or neutral), randomly determined by the program.

As shown in [Figure S1](#), each trial started with a screen where participants were presented with a text prompt ("Press SPACE bar when you're ready."). Once participants pressed the Space bar, a fixation screen was presented. Participants were required to stably fixate at the central fixation cross for 500ms to proceed. Afterward, participants were presented with a search array and were asked to freely explore the stimuli on the screen in order to find the target face as quickly as possible. Once they found the target, they were required to fixate back at the central fixation cross and press the Space bar to activate the mouse cursor. Then, they could move the cursor to the target face and click on it to indicate its location. The purpose of requiring participants to fixate back at the central fixation prior to making a response (i.e., clicking on the target) was to prevent any motor preparations or movements from contaminating the FRP data in the time window of interest and to ensure that target detection was always followed by a saccade, as with an unaware/unreported fixation. After participants made their response, the search array was replaced by a blank screen of 1000ms, which ended the trial.

Participants performed two fearful-face-target blocks and two neutral-face-target blocks with 100 trials in each block. Breaks were allowed every 50 trials. The order of fearful and neutral blocks was randomised, separately for the first and the second half of the experiment.

Data pre-processing

Pre-processing of the EEG data was performed with EEGLAB⁹⁰ and ERPLAB.⁹¹ We interpolated individual electrodes that produced sustained noise signals throughout the experiment. Signals were filtered from 0.1 to 30 Hz, and a notch filter of 50 Hz was included to remove line noise. We re-referenced the signals to the average of all electrodes. Because the dwell time was significantly shorter in the unaware conditions than the aware conditions, FRP signals were segmented into epochs with a time window of 310ms (the average of unaware fixation dwell time) from the onset of a fixation on the target face, relative to a pre-fixation

baseline (−100 to 0ms). A fixation was identified as within the target face region if the distance between the fixation and the center of the image was smaller than half of the diagonal of the image holder (95 pixels). In visual angles, a fixation was deemed within the target face region if it situated horizontally within 2.5° and vertically within 2.4° from the center of the image.

From each trial, one or two fixations on the target could be analyzed (see Figure 1B). Specifically, a fixation on the target was identified as an aware fixation if it was immediately followed by a saccade to the central fixation point and subsequently a correct localisation of the target. The epoch time-locked to this fixation is labeled the aware condition. A fixation was identified as an unaware fixation if it was the first fixation on the target in each trial but was not followed by a saccade to the central fixation point in preparation for making a response. Both conditions were separated by the target face expression (i.e., fearful vs. neutral).

In order to correct for eye-movements or eye-blinks components in the EEG data, we ran an optimised Independent Component Analysis (ICA) guided by the eye-tracking data, using the EYE-EEG toolbox⁸⁶ and customised MATLAB scripts. Here briefly, we first parsed the eye-tracking data and re-sampled it from 1000 Hz to 500 Hz to match the EEG data sampling rate. Then, we synchronised the eye-tracking and EEG data by identifying and matching the onset of the triggers in both data. Bad eye-tracking data including intervals of eye-blinks and out-of-range eye-movements were detected and marked. Afterward, saccades and fixations were detected using the velocity-based saccade detection algorithm.^{86,92} Specifically, (micro)saccades were defined as intervals in which the velocity of the recorded eye movements exceeded six median-based standard deviations of all eye velocities for at least four samples. Additionally, for micro-saccades detection, a magnitude threshold of 1° was used, and the interval between successive saccades was set as 50ms for saccades clustering.^{86,93}

A customised ICA was then run on the EEG data that contained eye-movement information, using the infomax ICA algorithm of Bell and Sejnowski.⁹⁴ Specifically, for each participant, a copy of the participant's data (training dataset) was created and filtered using a passband edge of 2.5 Hz.⁹⁵ Samples identified as saccadic spikes in previous steps were appended to the original data so that the total data length doubled the original data length. ICA was performed on this training dataset, and the resulting ICA weights were applied to the original non-overweighted data.⁹⁵ Subsequently, eye-movement and eye-blink components were decomposed and removed from the original EEG data. Afterward, we segmented the data based on the fixation events (described above). A threshold of −80 to 80 μ V was used for automatic detection of artifacts in the segmented data. We further inspected the data and rejected epochs containing artifacts on a trial-by-trial basis. Epochs containing bad eye-tracking intervals were automatically removed. As it was important for us to remove eye-related components from the EEG data, we excluded participants with eye-related components (eye-blinks and eye-movements) identified with a likelihood of less than 50%. As a result, data from two participants were excluded from further FRP analyses.

The remaining participants had on average the following mean numbers of epochs per condition: 89 epochs (range: 53–132 epochs) for unaware fearful targets, 143 epochs (range: 85–180 epochs) for aware fearful targets, 102 epochs (range: 63–130 epochs) for unaware neutral targets, and 137 epochs (range: 101–185 epochs) for aware neutral targets.

We also obtained target face dwell time data for all conditions. Specifically, dwell time was calculated as the time between the onset of the first saccade into the target face region to the onset of the saccade leaving the target face region. Dwell times shorter than 50ms or longer than 1500ms were excluded.

QUANTIFICATION AND STATISTICAL ANALYSIS

The average dwell time data were analyzed with a 2 (awareness: aware, unaware) \times 2 (target emotion: fearful, neutral) repeated-measures ANOVA in IBM SPSS Statistics 27.

The FRP data were analyzed using the Linear Modeling for EEG data (LIMO).⁴⁹ We conducted a repeated-measures 2(awareness: aware, unaware) \times 2(target emotion: fearful, neutral) ANOVA on the FRP epochs (0 to 310ms) across all scalp electrodes. Multiple comparisons were corrected using the *F*max Statistic, which estimates a null distribution of the maximal effect (i.e., the maximal *F* value) across all electrodes and time-points using a bootstrap procedure (1,000 bootstraps).⁴⁹ The observed *F* statistic at each time point was considered significant if its value exceeded the 95th percentile of the null distribution. Because

we were particularly interested in identifying the neural activity underlying conscious and any nonconscious emotion processing, we conducted *a priori* comparisons between fearful-target and neutral-target epochs using *F*-tests with an alpha level of .025, corrected for multiple comparisons through *F*max Statistic. Uncorrected LIMO results can be found in the [supplemental information](#) (see [Figure S2](#)).

Additional analysis

In an additional analysis, we used epochs time-locked to the first target fixations from each trial and re-ran the same ANOVA on these epochs. All effects were replicated, showing that the temporal order of fixation events did not affect the results. The full results of the additional analyses are reported in [supplemental information](#) (see [Figure S3](#)).