We thank the reviewers for their helpful feedback on our work. Please find our itemized, point-by-point replies below each comment (reviewers' comments are in Italics).

Review by anonymous reviewer 1, 07 Aug 2024 14:10

Summary

In the present registered report, Colombari and colleagues describe a study planned to investigate neural correlates of conscious visual perception and to isolate them from neural activity related to motor responses. To this goal, they plan to use a GO/NOGO detection task and the event-related optical signal (EROS) technique.

General impression

In my opinion, the registered report is very well written and satisfies most of the PCI RR Stage 1 criteria. First, I will shortly address each criterion, followed by a more detailed description of major and minor issues.
1A: The research questions are generally valid and convincingly derived from the literature. However, I would suggest incorporating some directly relevant previous studies.
1B: The proposed hypotheses are logical, coherent and plausible. On the other hand, the regions and especially intervals of interest are not stated clearly enough. Moreover, absence of evidence should not be interpreted as evidence of absence, and interaction effects should be tested directly.
1C: The methodology and analysis pipeline seem generally sound and feasible, and the experimental design is elegant. Concerning the sampling plan and statistical power analysis, some aspects should be clarified.
1D: The methodology is mostly described very clearly and in sufficient detail for close replication, with the exception of specific critical tests, exclusion criteria and the temporal aspects of the EROS data analysis.
1E: In my opinion, the authors have considered sufficient outcome-neutral conditions and quality checks. A more objective exclusion criterion for the EROS data quality would reduce flexibility. In the following, the abovementioned issues are described in more detail.

Major points

Introduction

Previous research:

Comment
The introduction (l. 48- 71) would profit from incorporating directly relevant previous studies which also specifically addressed the role of early posterior versus late centroparietal activity (i.e., VAN and LP) in conscious visual perception versus post-perceptual processes (e.g., Pitts et al., 2014; Shafto & Pitts, 2015; Dellert et al., 2021, 2022; Kronemer et al., 2022). In line with the authors' goals (l. 75-79), some of these studies also reported brain activity with both high spatial and temporal resolution by means of EEG and fMRI (e.g., Dellert et al., 2021; Kronemer et al., 2022). Moreover, there are additional relevant studies that specifically investigated effects of attention and response requirements on the VAN (e.g., l. 55-67 and l. 111-114), both with positive (e.g., Bola & Doradzińska, 2021; Dellert et al., 2021; Doradzińska & Bola, 2024) and negative (e.g., Koivisto et al., 2006; Dellert et al., 2022; Ciupińska et al., 2024) results.

Response

We thank the reviewer for having driven our attention to these interesting papers that have now been included in the revised manuscript at lines 58-64.

Methods

Intervals of interest:

Comment
The temporal aspect of the EROS data analysis should be described more clearly, e.g., which samples exactly are compared in the critical tests (l. 307-334). In all hypotheses and analysis plans (table in 3. Study design), the term "early temporal/time window" is too vague. All hypotheses should define specific intervals of interest or describe exactly how the intervals will be selected based on the data (e.g., in the case of collapsed localizers). Moreover, how will the statistical analyses control for multiple comparisons in the temporal domain? How were the intervals in the preliminary results (l. 405-414) selected?

Response
We thank the reviewer for this comment, following which we included in the manuscript the specific intervals of interest that will be taken into account to compute statistic comparisons (lines 372-376 and Table 3. Study design).
Concerning the correction for multiple comparisons in the temporal domain, unfortunately, this is not computed by Opt3d (i.e., the software dedicated to statistical analysis of EROS data). We understand that this limit represents a methodological drawback. For this reason, in order to reduce the risk of finding false positive results, we will select our intervals of interest based on previous literature and we will restrict our analyses to such intervals, testing specific a priori hypotheses.

Negative results:

Comment
All interpretations of negative results (column "Interpretation given different outcomes" of the study design table) are based on the absence of significant effects in frequentist null hypothesis testing. Especially given the limited statistical power of the planned sample size (n = 24), the absence of effects should be substantiated by Bayesian hypothesis testing. This also concerns the preliminary results (l. 378-382), where effects with d = .839 or -.791 are interpreted as "no difference" with n = 5.

Response
We thank the reviewer for this comment. In *Table 3. Study design* we describe the potential outcomes expected from our analyses. To test our hypotheses, we planned to employ frequentist null hypothesis testing, by performing a series of t-tests. As correctly stated by the reviewer, absence of effects should be substantiated by Bayesian hypothesis testing. However, in the case of EROS data, this is considerably challenging, as EROS statistical analyses are "limited" to the use of the only existing software for fast optical imaging analyses (i.e., Opt3d), which does not allow computing Bayesian statistics. Moreover, in order to perform such analysis, we would need a remarkable amount of data not yet available in the literature, as Bayesian statistics estimates the parameters of a probability distribution based on previously existing data.
As concern the preliminary results, the sample size is so small that no statistical inference can be made. Indeed, as stated at line 391, the aim of the pilot study was to test the experimental paradigm, thus to verify that the employed paradigm works as planned. For this reason, the results of the pilot study cannot be interpreted from a statistical point of view.

Interaction effects:

Comment
In hypothesis 2, the authors "expect to find the same activation" in LOC in GO and NOGO trials. How will this equality be tested? Do the authors refer to a significant awareness effect in each condition (GO, NOGO) or actually the absence of a difference between the two effects, i.e., of an interaction effect (i.e., (Aware-NOGO – Unaware-NOGO) – Aware-GO – Unaware-GO))? In this context, it should also be considered that the presence of an effect (e.g., Aware-Unaware) in one condition (e.g., GO) and its absence in another (e.g., NOGO) should not be interpreted as a difference between the conditions – instead, a direct interaction test should be performed (see Nieuwenhuis et al., 2011).

Response
We thank the reviewer for this comment. As correctly interpreted by the reviewer, we expect to find NO difference in awareness effect between GO and NOGO conditions. Thus, we agree with her/him that, in order to test our hypothesis 2, a direct interaction test should be performed. Since our methodology was not clearly explained, we modified the *Table 3. Study design*, by adding the Analysis 2.2 (A2.2) and the relative outcomes interpretation (O2.2.1 and O2.2.2).

Results

Figure 4 (l. 415): What exactly do the statistical maps present? What are the underlying data, units and scales?

Response
We thank the reviewer for noticing this oversight. Indeed, this information is lacking in Figure 4.
The figure shows statistical parametric maps representing the z-score difference computed between Aware and Unaware trials in the selected ROI thresholded at a specific value (+2.5). We added this information and the corresponding unit scale in the Figure.

Minor points

Introduction

Comment
L. 40: "However, which one [VAN or LP] represents the true signature of conscious vision is still under debate". This creates a false dichotomy because it could also be both or neither of them.

Response
We agree with the reviewer that this sentence could lead to misunderstanding. Indeed, as correctly stated by the reviewer, the electrophysiological signature of conscious vision could be represented by both VAN and LP or neither of them. We modified our manuscript at lines 42-43.

Methods

Sample size estimation:

Comment
L. 134-136 and 3. Study design / sampling plan): Concerning the "technical constraints of the employed dedicated software" (Opt-3d) which does not allow to calculate effect sizes, could the authors export the data in order to calculate them (at least in their own upcoming data)?

Response
We thank the reviewer for this comment. Ideally, exporting data would allow performing statistical analyses that the dedicated analysis software in its current state does not allow to compute. However, exporting EROS data would mean extracting a very huge amount of data (i.e., a matrix of data for each voxel and for each time point) which would be too difficult to be managed without a dedicated software. For this reason, EROS analyses are constrained to the use of Opt3d software, which, so far, is the only existing software developed for fast optical imaging analyses.

Comment
L. 142/151/152/346: Since 12.944 is much closer to 13 than 12, I would suggest to revise these lines.

Response
We thank the reviewer for noticing this oversight and we agree with her/him that these lines should be revised according to this comment (see revised manuscript).

Comment
L. 145/153 At this point, it is unclear what the research questions (Qx) are, so perhaps they could be paraphrased here.

Response
We thank the reviewer for this suggestion, according to which we modified our manuscript at lines 157-159 and 167-169 by explicitly specifying the research questions we are referring to.

Comment
L. 146: An alpha level of 2% seems unusual to me (see also "3. Study design"). Could the authors explain why they chose it?

Response
We set an alpha level of 2% because the Guidelines for Authors of PCI RR claim that *"authors intending to have their RR automatically accepted by a PCI RR-friendly journal should ensure that the evidential standard in their submission meets the minimum requirement of their preferred journal (e.g., in terms of power, alpha, Bayes factors, or any other conditions).*
As we chose Cortex as the journal where publishing the present manuscript if receiving in principle acceptance, we had to follow the corresponding requirements (i.e., alpha =.02 and power > 0.90, where applicable).

Perceptual threshold assessment:

Comment
Concerning the perceptual threshold assessment (l. 155-163, 181-189), it is unlikely that one of the nine stimuli is perceived in exactly 50% of the cases ("acknowledged/identified as perceived the 50% of the times"). Perhaps it would be more appropriate to frame the criterion for the final stimulus as, e.g., "the one perceived a minimum of 25%, a maximum of 75%, and closest to 50% of the times"?

Response
We thank the reviewer for this suggestion following which we modified our manuscript at lines 175-176 and 227-228.

Comment
Exclusion criteria (l. 166): The rule that "participants whose EROS signals could not be detected properly during the experiment will not be included in the analyses" and will be replaced (l. 170) is rather vague and leaves some room for arbitrary exclusions. A more objective criterion for signal quality would be beneficial.

Response
We thank the reviewer for giving us the possibility to better delineate the criteria we will adopt to exclude participants from statistical analyses. Indeed, in the manuscript, we did not specify how we judge the quality of the EROS signal. In particular, before running statistical analyses, the opacity value (i.e., the product of the scattering and absorption coefficients) is estimated separately for each participant. Based on this value, it is possible to judge the quality of the signal for each participant independently from the experimental condition. Specifically, opacity values of all participants are averaged together, providing the absorption coefficient to be used when running statistical analysis. Participants whose opacity value exceeds three standard deviations over the mean will be excluded from statistical analyses.
In order to make this crucial aspect clearer in the manuscript, we modified it at lines 188-194.

Comment
Fatigue: The very high number of trials (3120 per participant) is commendable. However, the duration of 3 hours per session (l. 197), further prolonged by the threshold assessment (20 min) on the first day, seems very long. How can adequate task performance be ensured despite potential fatigue?

Response
We thank the reviewer for this comment and we agree with her/him that the experiment is quite tough (for both the experimenter and the participant). However, we actually need a huge amount of trials in order to ensure a good signal-to-noise ratio (signal-to-noise ratio increases as a function of the square root of the number of trials). For this reason, we usually take some precautions:
-first of all, the participant is not engaged in the experimental task throughout the whole session since a large part of the time is employed for setting the montage on his/her head. Indeed, the experimenter has to place 60 optical fibers (sources + detectors) on the helmet following a specific pattern and this procedure usually takes almost one hour, during which the participant can rest;
-the task per se lasts around 1 hour and it is divided into blocks, each lasting about 3 minutes. Between blocks, participants are encouraged by the experimenter to rest and close their eyes. Participants are instructed to continue with the experiment only when they feel ready by pressing a key on the keyboard;
-at the end of the task, all the optical fibers are removed from the head of the participants and she/he can move and eat/drink something before starting the digitization process. Also in this case, during this procedure the participant is not asked to perform any tasks;
-in addition, in order to avoid fatigue for the participant, the experiment is divided into two sessions, each of which is performed on different days.
It is also important to note that both in the pilot study reported in the present work and in other previous experiments, participants' performance never decreased as a consequence of fatigue during the task session. For these reasons, we feel confident that our precautions are sufficient to assume that fatigue won't affect task performance.

Comment

Stimulus position: Why will the stimulus always be presented "in the lower right quadrant of the screen" rather than in the center (l. 212)? Does it have to do with the left-lateralized ROI? If so, this could be explicated.

Response
We thank the reviewer for this comment, which allows us to depict our experimental procedure more clearly. As correctly interpreted by the reviewer, the stimulus will always be presented in the lower right quadrant of the screen because of our lateralized EROS montage. Indeed, our EROS equipment (3 Imagent frequency-domain systems, equipped with a total of 48 light sources and 12 detectors) does not allow us to design a full head montage. For this reason, we decided to lateralize our recording montage, in order to provide a better coverage of the regions of interest of only one hemisphere. Moreover, since EROS technique is sensitive to depth, a right-lateralized stimulus ensures that it elicits activity in the left portion of the primary visual cortex, which is known to be anatomically closer to the skull compared to the right one, thus ensuring a better penetration of near-infrared light through brain tissues. Since a motivation for our decision to present a lateralized stimulus was lacking, we modified our manuscript at lines 206-213 by adding it.

Comment
Tests: Are the t-tests going to be one- or two-tailed (e.g., l. 287, l. 296)?

Response
We thank the reviewer for noticing this oversight. We added "two-tailed" in the manuscript at lines 325 and 333.

Comment
Epoch: Why does each epoch already begin at 486 ms (l. 306) specifically, and will the whole pre-stimulus segment be used for baseline correction (l. 302)? Since pre-stimulus brain activity can influence conscious perception, this baseline correction of pre-stimulus effects could potentially induce artificial post-stimulus effects.

Response
We thank the reviewer for allowing us to clarify how we epoch our EROS data. As reported in the paper, EROS signal will be segmented into epochs time-locked to the stimulus onset, lasting a total of 1484 ms. Specifically, the pre-stimulus segment will be 486 ms long (i.e., 19 sampling points, each lasting 25,6 ms) and the post-stimulus one will be 998 ms long (i.e., 39 sampling points). Focusing on the pre-stimulus segment only, this will be long enough to ensure a clean baseline, devoid of ringing artifacts induced by the epoching process. Indeed, our baseline is corrected considering the 204 ms time-window (i.e., 8 sampling points) preceding the stimulus onset.

Comment
ROIs: "Critical ROIs will be selected on the basis of the results obtained in the above-mentioned experiment (Colombari et al., under review) and by visual inspection of functional data." (l. 330). Does "functional data" refer to the new data to be collected here? If so, how is circularity / double-dipping (Kriegeskorte et al., 2009) avoided when basing ROIs on effects?

Response
We thank the reviewer for raising this point and for giving us the possibility to better clarify how we will select our Regions of Interest. Differently from fMRI where it is possible to perform both whole-brain and ROI analyses, the only way to perform statistical analyses with EROS is within specific regions of interest, that need to be necessarily selected a priori or as data-driven analyses. In particular, since Q1 aims at replicating the results obtained in our previous study (Colombari, E.,

Parisi, G., Tafuro, A., Mele, S., Mazzi, C., & Savazzi, S. (2024). *Beyond primary visual cortex: The leading role of lateral occipital complex in early conscious visual processing*. NeuroImage, 298, 120805) we planned to investigate the same ROIs tested in such study, as well as novel ROIs over areas responsible for visual processing and motor execution.

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Review by anonymous reviewer 2, 22 Jul 2024 11:08

The Stage 1 Registered Report The role of extra-striate areas in conscious motor behavior: a registered report with Fast-Optical Imaging by Elisabetta Colombari, Giorgia Parisi, Sonia Mele, Chiara Mazzi and Silvia Savazzi addresses the question of the spatiotemporal resolution of neural correlates of consciousness (NCC) and the distinction between true correlates of consciousness and

motor or task-specific processes. The authors chose a contrastive design realized by a Go/NoGo-Task and plan to measure brain activity using the EROS technique. Neural data is planned to be analyzed using the Granger causality analysis.
The report is well-written and clear, and it has the potential to make an interesting and valuable contribution to the field. In the following, I will raise some issues and questions to be thought about before realizing the study:

Abstract

Comment
The authors should reconsider the term, as it does not seem appropriate. Perhaps they should use 'distinctive' or 'special' instead.

Response
Although the term was erroneously not specified by the reviewer, we assumed that he/she was referring to the term "peculiar" (line 16). According to this, we modified the abstract by replacing "peculiar" with "distinctive" (line 16)

Introduction

Comment
i. *In the introduction section, the authors briefly summarize the state of research on NCCs without confounding concurrent neural dynamics. An overview of the field of no-report paradigms and the spatiotemporal characteristics of conscious processing is given. I would suggest considering the inclusion of Hense A, Peters A, Bruchmann M, Dellert T, Straube T. Electrophysiological correlates of sustained conscious perception. Sci Rep. 2024;14(1):10593. in the list of referenced works on sustained conscious perception. I believe it would complement the existing literature the authors have discussed.*

Response
We thank the reviewer for having driven our attention to this interesting paper. Since we agree with her/him that this work would complement the existing literature already discussed in the paper, we added it to the revised manuscript at line 60.

Comment
ii. *The authors aim to separate true consciousness effects and task effects by their paradigm. The Go/NoGo-Paradigm includes task effects. The authors should further elaborate on this issue.*

Response
We thank the reviewer for this comment. One of the aims of the present work is to isolate the processes underlying consciousness mechanisms from confounding processes related to the task. Indeed, one of the main sources of confounding that makes the search for the NCCs challenging is represented by the presence of response-related mechanisms, which occur concurrently with those underpinning consciousness. Therefore, we decided to adopt a Go/NoGo paradigm, which includes both trials requiring a motor response and trials requiring no task. In this way, we can efficiently dissociate the neural mechanisms related to response requirements from those related solely to the conscious experience. Indeed, in our work we will contrast Aware and Unaware trials in both Go and NoGo conditions, separately. In this way, we can investigate the NCCs both when the motor response is required (Aware GO VS Unaware GO) and when no task is performed (Aware NOGO VS Unaware NOGO). Importantly, in this latter analysis, all the effects related to the task will be eliminated, as participants are required not to perform any task, thus allowing to observe neural mechanisms strictly

related to the conscious experience. We modified our manuscript by adding this information at lines 112-115.

Methods

Comment

i. *I wonder why the analysis of the Catch Trials was not included in the exclusion criteria. The authors should provide some insights into this decision.*

Response

We thank the reviewer for this suggestion. Actually, analysis of catch trials is crucial to assess whether the participant is performing the task correctly and to ensure that the behavioral performance is not affected by response biases. For this reason, we followed the reviewer's suggestion by adding the analysis of catch trials among our exclusion criteria. We modified the manuscript at lines 183-186.

Comment

ii. *Is there any control of eye movement effect planned? This issue should be addressed, as the critical stimuli are presented offside the center of the screen.*

Response

We thank the reviewer for giving us the possibility to better specify this information. As EROS employs NIR light, all instrumentation that involves the use of near-infrared light is not compatible with EROS recording. Since our lab is not provided with compatible eye-trackers or cameras, in our experiment we do not use any direct method to monitor eye movements. Indeed, to address this issue, participants are trained to fixate the center of the screen by means of a fixation training performed before starting the experiment. This training, described in Guzman-Martinez, Leung, Franconeri, Grabowecky, & Suzuki (2009), ensures that participants maintain their gaze on the central fixation cross correctly.

We added this info in the manuscript at lines 235-237

Comment

iii. *The authors chose nine different stimuli to be shown before the experiment to determine the stimulus of the main experiment. Please discuss this choice. Doesn't the restriction to nine stimuli lead to a high drop-out rate if none of the stimuli leads to the desired behavior? Wouldn't the choice of a continuous parameter be more favorable?*

Response

We thank the reviewer for giving us the possibility to better explain how we selected our stimuli. The range of stimuli employed in the present study was selected based on the results of a previous pilot experiment in which a different sample of participants was presented with a wider range of thickness, and asked to perform the same task we employ in our perceptual threshold assessment. This was done to identify a smaller range of optimal stimuli thus excluding a range of thicknesses that participants reported to perceive the 0% or the 100% of times.

We thus excluded from our final sample of stimuli those thicknesses that resulted to be too easy to perceive (i.e., those stimuli that all participants reported to be perceived 100% of the time), as well as stimuli that no participants could perceive as different from the catch one. We added this important information in the manuscript at lines 219-224

Comment

iv. *The selection of the ROIs used for analysis based on a posteriori inspection of the data seems a bit arbitrary. The authors should explain in more detail what criteria will be used for ROI selection. Please explain why the full left motor cortex and posterior visual areas are not selected as ROIs.*

Response
We thank the reviewer for raising this point and for giving us the possibility to better clarify how we selected our Regions of Interest. When performing EROS analysis, statistics can be computed only within specific ROIs that, thus, need to be necessarily selected a priori or as data-driven analyses. In particular, we selected our ROIs based on our previous study, whose results we aim to replicate (Q1), and among those areas responsible for visual processing and motor execution. The reason why we did not select the full left motor cortex and the posterior visual areas as regions of interest is because ROIs necessarily need to be 2D boxes according to a technical constraint due to the software used to analyze data.

Comment
v. *The authors should explain how a correction for multiple comparisons is realized.*

Response
We thank the reviewer for allowing us to better explain how EROS data are corrected for multiple comparisons. As stated in the manuscript (lines 351-352), when performing statistical analyses, t-statistics are calculated within each ROIs, converted into Z-scores and corrected for multiple comparisons using Random Field Theory (RFT). RFT helps to deal with multiple comparison problem in functional imaging, as it allows to set the threshold above which values are unlikely to have arisen by chance. Indeed, other methodologies, such as Bonferroni correction, are not recommended when dealing with functional imaging data, as the number of independent observations is not known. Functional imaging data have a certain degree of spatial correlation, namely, the value of any voxel tends to be very similar to that of neighboring voxels. For this reason, in functional images there are fewer independent observations than there are voxels. In these instances, we apply FDR, which allows to find a threshold even when the number of independent observations is not given.

Pilot study

Comment
i. *The authors should comment on how the results were obtained. Are the effects based on an analysis across all available data or do the results refer to the mentioned ROIs?*

Response
We thank the reviewer for giving us the possibility to clarify how analyses on EROS data are performed. Unlike other neuroimaging techniques (such as fMRI or EEG), which allow for whole-brain statistical analysis, EROS allows running of statistical analysis only within specific pre-defined ROIs. Indeed, in order to perform statistical comparisons, specific ROIs have to be selected. Thus, the data presented in the pilot refer to the ROIs mentioned in the manuscript.

Comment
ii. *Again, how do the authors account for errors due to multiple comparisons?*

Response
Functional results are corrected using Random Field Theory (see comment above).

Review by anonymous reviewer 3, 24 Jul 2024 13:24

Review
This registered report proposes a study on the role of extra-striate areas in conscious motor behavior. The authors propose to conduct a study where a cartwheel stimulus with a bar at perception threshold is presented and participants have to report whether they perceive an increase in bar thickness or not. Response requirement will be counterbalanced in different blocks, in which sometimes participants press a button when aware and vice versa. Neural data will be recorded via fast optical imaging. I think the study addresses an interesting question and would expand other studies that addressed similar questions with EEG.

Comment
1. General point in the introduction*: I think the motivation for the study comes across clearly and it is true that the manipulation allows dissociating effects of motor responses from consciousness if a go/nogo paradigm is used. However, motor responses are not the only possible confounds in consciousness research, as has been shown by several studies (e.g. Dellert et al., 2021; Pitts et al., 2012; Schelonka et al., 2017; Schlossmacher et al., 2020; Shafto and Pitts, 2015) the sole task relevance of stimuli (in the absence of a motor response) can also elicit a late positivity. I think a more detailed discussion of these issues would benefit the registered report. I think the phrasing '… isolating neural activity strictly related to awareness from response-related mechanisms …' is a little too strong.*

Response
We thank the reviewer for this comment and we agree with her/him that motor responses are not the only possible confounds in consciousness research. Indeed, as correctly suggested by her/him, NCCs can be affected by several other factors, such as task relevance. We added the suggested papers in the manuscript, as we believe that they further complement the existing literature already discussed. Moreover, we agree with the reviewer that the phrasing …"*isolating neural activity strictly related to awareness from response-related mechanisms …"* could result too strong. For this reason, we modified the phrasing with the more cautious: "*disentangling neural activity related to awareness from response-related mechanisms*" (lines 111-112)

Comment
2. *What was the motivation for the use of cartwheel stimuli?* *Given that the stimuli in itself will always be consciously perceived, the awareness question is specifically for the awareness of radius thickness and not of the stimulus per se. Given the introduction/abstract I would expect e.g. Gabor gratings at the perception threshold (like in Koivisto et al. (2016)). Does this influence the interpretation of the results? Furthermore, will the first radius clockwise always be the radius that is potentially thicker? Could this lead to confounds?*

Response
We thank the reviewer for giving us the possibility to clarify this fundamental point. In the present study, the stimulus was always kept clearly visible in both Aware and Unaware conditions, but we manipulated the thickness of the first radius clockwise so that it resulted at the perceptual threshold level. Indeed, before taking part in the experiment, participants performed the perceptual threshold assessment with the aim of identifying the level of thickness that could be perceived only half the time. We have taken the decision to keep the stimulus always clearly visible and to manipulate a specific characteristic of the stimulus in order to obtain a highly reliable neural signal. Indeed, given the relatively low signal-to-noise ratio of EROS, we need to use a stimulus at a high contrast to elicit consistent neural activity in visually responsive brain areas. Specifically, we decided to use a

cartwheel stimulus instead of a Gabor patch because in the previous study (Colombari et al., 2024) which inspired the present work, we did use a Gabor patch that could be slightly tilted upward or downward, but it posed some problems. Actually, participants learned to discriminate its orientation along the task, and therefore the discrimination was no longer at the threshold level and this led to the discard of a large amount of participants from analyses. For this reason, and also with the aim of employing a more ecological stimulus, we decided to adopt the cartwheel stimulus. Importantly, we decided to keep the thicker radius constant (i.e., always the first one clockwise) in order to reduce variability in visual brain areas.

Comment

3. ***Sample size estimation****: I think taking the average sample size from previous EROS studies and increasing it, is a first step to sample size estimation in this particular study. However, using past EROS studies on unrelated topics would only partially be helpful, as not only EROS signal-to-noise ratio but also the question at hand and thus the expected effect size is (even more) important for determining sample size. I would like to suggest to the authors to obtain an effect size estimate from EROS data (e.g. from the previous study of the authors) even if such indices are not computed by the software itself. To my knowledge there are several ways to estimate effect sizes based on z-values/t-tests/F-tests/mean and SD differences. Maybe peak/mean values of such a kind can be extracted from the software and then be converted to estimate an effect size of EROS data for a power analysis in addition to the other arguments for the chosen sample size.*

Response

We thank the reviewer for this comment. We agree with her/him that taking the average sample size from previous EROS studies and increasing it, is a first step to sample size estimation but it's not the optimal approach. Indeed, obtaining an effect size estimate from EROS data would be more reliable. We understand the potential of the suggested approach in enhancing our study. However, we are not currently familiar with the application of this specific method. Would it be possible to provide us with more detailed guidance or key references to help us better understand its implementation and adaptation to our context? If so, we are ready to integrate this approach into the manuscript.

Comment

4. ***2.2.2 Exclusion criteria****: How will it be quantified whether an EROS signal could not be detected? Is there an objective way to do so?*

Response

We thank the reviewer for giving us the possibility to better specify how we judge the quality of EROS signal. Actually, this information was missing in the original version of the manuscript. Before performing statistical analysis, the opacity value (i.e., the product of the scattering and absorption coefficients) is estimated for each participant. Based on this value, it is possible to judge the quality of the signal for each participant independently from the experimental condition. Subsequently, opacity values of all participants are averaged together (providing the absorption coefficient to be used when running statistical analysis) and the standard deviation of such value is computed. Participants whose opacity value exceeds three standard deviations over the mean will be excluded from statistical analyses. In order to make this crucial aspect clearer in the manuscript, we modified it at lines 188-194.

Comment

5. ***Perceptual threshold assessment****: If I understood correctly, participants will be excluded if none of the nine predefined radii fits the 50%-threshold. Even if stimuli have to be created beforehand*

in Matlab, a more fine-grained or wider range of stimuli could probably be obtained to find stimuli that allow inclusion of more participants.

Response

We thank the reviewer for raising this point. Actually, the information that "participants will be excluded if none of the nine predefined radii fits the 50%-threshold" is a bit misleading: indeed, the phraseology "The stimulus identified as perceived a minimum of 25%, a maximum of 75%, and closest to 50% of the times during the subjective perceptual threshold assessment will be used in the experimental task," would be more accurate. For this reason, we modified our manuscript at lines 227-228.

In addition, to determine the range of stimuli to employ in the present study (and in the pilot presented in the manuscript), we conducted a pilot study in which a small sample of participants was presented with a wider range of stimuli, and asked them to perform the same task employed in the perceptual threshold assessment (see lines 219-224). In this way, we could identify a smaller range of optimal stimuli (i.e., a range of thickness that participants reported to perceive the 0% or the 100% of times). We thus excluded from our final sample of stimuli those thicknesses that resulted to be too easy to perceive (i.e., those stimuli that all participants reported to be perceived 100% of times), as well as stimuli that no participants could perceive as different from the catch one.

Comment

6. *I fear there is a possibility of* ***perceptual learning*** *that should be addressed. Given that 2 sessions á 3 hours are planned, it seems possible that the stimulus that was initially perceived at the 50% threshold will be perceived more easily due to learning. Behavioral data from the pilot study might also hint at this problem, as three participants were excluded using a slightly higher awareness threshold of 80% compared to the planned experiment (75%). One possibility would be to continuously monitor performance and decrease thickness of the radius if participants cross a predefined performance threshold.*

Response

We thank the reviewer for this suggestion and we agree with her/him that the possibility of perceptual learning is likely. However, continuously monitoring performance and decreasing thickness of the radius if participants cross a predefined performance threshold could lead to methodological constraints. Indeed, when using contrastive analysis (as we do in our study), it is fundamental that the physical characteristics of the stimulus are kept constant throughout the experiment, in order to avoid further confounds related to the processing of different features of the stimulus. If in principle, we agree that perceptual learning due to the very high number of trials could represent an issue, this is not the case for all participants. For this reason, we will discard from analyses those participants whose performance will cross a specific awareness threshold, in order to maintain the number of trials of Aware and Unaware conditions as equal (and thus comparable) as possible.

Comment

7. *I think the pilot data is very insightful, however, the interpretation of the statistical tests of the behavioral data seems somewhat premature given the small N and rather large differences in observed means, e.g. 50ms difference in reaction times between go aware and go unaware. Given a full sample of 24 participants, it seems possible that significant differences in reported aware trials as well as differences in reaction times will be observed. How would this impact the interpretation of the results? Could differences in motor responsivity measured by EROS (Q3) be attributed to differences in response times? Given that null effects are reported and interpreted, the authors could consider inclusion of Bayesian statistics as they allow a quantification of these effects.*

Response

We thank the reviewer for this comment. We agree with her/him that the sample size of the pilot study is so small that no interpretation of the statistical tests of both the behavioral and functional data can be made. However, such pilot study is insightful as it successfully replicates the trend of activations observed by Colombari et al., 2024, suggesting that the proposed study proves to be feasible in terms of methodology.
Concerning the potential differences in reaction times in Aware and Unaware conditions, we believe that this contingency is possible, but not probable. In fact, in our previous study there was no difference in RTs between Aware and Unaware conditions (mean RTs for Aware = 565.11 ms and Unaware condition = 568.86 ms, ($t(23) = -.480$, $p = .636$, Cohen's $d = .098$, 95%, CI [-.498, .304]), indicating that there was no difference in the responsiveness between the two conditions. Since the task of the present study is very similar to the one adopted in our previous study, we expect not to find significant differences in response times between conditions. However, in the event of differences between RTs, we expect that Aware and Unaware responses overlap at a certain point, thus allowing to select a common temporal window for statistical analysis.
Finally, as regards the possibility of including Bayesian statistics to quantify null effects, this is considerably challenging, as EROS statistical analyses are constrained to the use of the only existing software for fast optical imaging analyses (i.e., Opt3d), which does not allow computing Bayesian statistics. Moreover, in order to perform such analysis, we would need a remarkable amount of data not yet available in the literature, as Bayesian statistics estimates the parameters of a probability distribution based on previously existing data.

Comment
8. *I think it would be interesting to see performance of participants of the pilot study on* ***catch trials i****n order to see whether the paradigm works as planned. How will catch trials be investigated? If participants also report seeing the thicker bar on a substantial amount of catch trials this could hint at problems in the experimental stimuli/design.*

Response
We thank the reviewer for this comment. Actually, as he/she claims, analysis on catch trials is insightful as it allows investigating whether I) the employed paradigm works as planned, II) participants perform the task accurately and III) there are no biases related to the response. Indeed, in the original version of the manuscript we planned to perform such analysis (lines 327-335), but we did not perform it on our pilot data. Following the reviewer's comment, we thus performed catch analysis on pilot data, revealing that participants could perform the task accurately (i.e., on catch trials participants correctly reported not to see the thicker radius on average 97.41% of times (sd=2.31)).
We added this analysis to the manuscript (see section 4.1.1 Behavioral results, lines 427-433). We also added that participants whose behavioral performance will be affected by biases related to the response (i.e., report seeing the thicker bar on a substantial amount of catch trials) will be excluded from the analyses (lines 183-186).

Minor points:

Comment
Abstract l. 16, Introduction l.76: I think peculiar/peculiarity is not the right word to describe the experimental design/methods (see e.g. https://www.merriam-webster.com/thesaurus/peculiar)

Response
We thank the reviewer for this suggestion, according to which we replaced the word "peculiar" with the word "distinctive" (line 16).

Comment

l. 67ff This sentence seems incomplete, connector words are missing for it to make sense (like '… are consistent with considering…, however the localization …'

Response
We thank the reviewer for noticing this oversight. We added the connector word "while".
"Several pieces of evidence are consistent in considering VAN as the electrophysiological signature of phenomenal consciousness (Koivisto *et al.*, 2008; Railo *et al.*, 2015), **while** the localization of its neural generator still remains open.

Comment
Section 2.1: typo of 'and.' in the first sentence

Response
We thank the reviewer for noticing this typo. We deleted the "and" at the end of the sentence.

Comment
Matlab is written inconsistently (sometimes Matlab, sometimes MATLAB)

Response
We thank the reviewer for noticing this inconsistency. We replaced MATLAB with Matlab.

Comment
Different version number of Matlab are reported throughout the manuscript, is this intentional?

Response
Yes, it is and it does not represent a typo. EROS analyses are performed with Matlab, version R2013b, as dedicated scripts and programs were developed to run with this version.