We thank the reviewers for acknowledging the relevance of the study aim and the complexity of the methodologies used. We appreciate their helpful comments and suggestions that greatly improved our work. We addressed all issues raised and revised the manuscript accordingly. Please find our answers (A) detailed in red and changes (C) in the manuscript in blue.

**Reviewer #2**

**Comment of the reviewer:** Thank you for this opportunity. This research article examines the effects of ischemic stroke on the functional organization of the brain using functional connectivity gradients. The authors investigate how stroke disrupts these gradients and how this disruption relates to behavioral deficits. Importantly, they address the impact of stroke-induced hemodynamic delays on gradient accuracy, showing that correcting for these delays enhances the gradients' ability to capture meaningful functional changes. The study also explores the role of hemispheric asymmetry in stroke-induced functional reorganization, finding that the right hemisphere is more sensitive to stroke damage than the left. The researchers conclude that functional connectivity gradients provide a valuable framework for understanding the consequences of ischemic stroke on brain function and that correcting for hemodynamic delays is crucial for accurate analysis.  
  
*Major Strengths:*  
1) The study addresses a crucial methodological issue in fMRI research on stroke by explicitly correcting for hemodynamic lags. This correction enhances the accuracy of the gradients and highlights the importance of considering temporal delays in BOLD signal analysis after stroke.  
2) The study's emphasis on the second gradient, which captures the visual-somatomotor axis, aligns well with the common observation that stroke primarily affects these basic functions. This focus contributes to the understanding of how stroke disrupts the brain's fundamental functional organization.  
3) The authors thoroughly investigate functional deviations about behavioral impairments, lesion location, and hemispheric asymmetries. This multi-faceted analysis provides a comprehensive picture of the functional consequences of stroke.

(A) We greatly appreciate the positive comments on our work. Please find below the detailed replies to the concerns raised in this revision.  
  
**Point 1:** While the authors acknowledge limitations in interpreting lag correction, further discussion is needed to address concerns related to potential confounding effects on frequency domain analysis. Elaborating on strategies to mitigate these issues and future directions for research in this area would strengthen the manuscript.

(A) We thank the reviewer for the insight provided. ***SEE MY REPLY TO YOUR COMMENT ABOVE.***

(C) Following reviewer’s suggestions, we have elaborated the relevant issue in the last paragraph of the discussion (starting from line number XXX) as follows: “For example, Siegel, Ramsey, et al. (2016) argue that although lag correction improves the connectivity, it is not suggested because there is no way to fix the other differences related to the frequency domain and the signal is inherently altered. Lag correction might complicate those confounding even more. However, our findings (also theirs, Siegel, Snyder, et al., 2016) show that this is a necessary step to be able to compare the data from stroke subjects with healthy controls, and the issues in the frequency domain should be handled separately. The two steps are not mutually exclusive since shifting a time series will not alter its power-frequency distribution. Inspiration could be drawn from the literature studying brain tumors, where it has been found that local and global shifts of the spectrum carry prognostic (Park et al., 2023) and network (Falcó-Roget et al., 2024) information. Potential attempts at fixing the spectral anomalies using well-known methods such as Fourier-based reconstruction or wavelet-based denoising could only elucidate the usefulness of the method. Although the deformed signal indeed reflects the way lesioned area works and must be accepted as the way it is, if the aim is to identify the functional plasticity after stroke damage, the structural and metabolic confounds have to be accounted for before any kind of investigation.”

**Point 2:** The study finds that the right hemisphere is more sensitive to stroke-induced functional deviations. However, the underlying reasons for this asymmetry are not fully explored. Discussing potential explanations based on known hemispheric specialization and interhemispheric interactions would provide valuable insights. Would you have provided details about the dominant hand of the participants, if possible? I miss this point!

**Point 3:** The study uses Euclidean distance to assess the relationship between lesion location and functional deviation. Exploring the use of disconnectome maps, which capture the disruption of white matter pathways, could provide a more nuanced understanding of the anatomical underpinnings of functional changes after stroke.

**Point 4:** I highly suggest that the authors reduce the "Methods" section and prepare supplementary material along with the manuscript. The Methods section is too long and may weary their readers.

(A) Thank you for this suggestion. We recognize that the methods section is lengthy, as fMRI data demands extensive preprocessing. Additionally, calculating functional connectivity gradients and analyzing low-dimensional space require detailed descriptions. We believe that including these steps in the main text provides a comprehensive overview for the reader. However, we agree that some slightly more standard steps could be condensed for clarity and brevity.

(C) As such, we have reshaped the methods section with the following changes. Briefly, details about the image acquisition and preprocessing have been largely moved to the supplements. The methods related to the computation of the gradients and derivates have been left, given their crucial importance to our work. Some minor variations of the latter points have also been made.

1. The subsection *Preprocessing of imaging data* has been renamed solely “Imaging data” to include a more general description of the process (line XX).

2. An entirely new section in the Supplementary Material has been created: “Magnetic resonance imaging processing”.

2.1. It includes a subsection with the details regarding image acquisition: “Image acquisition”. These details were previously located in the first subsection of the supplements. No changes have been made to the text and it has simply been relocated to the supplements.

2.2. We also moved the *Anatomical data preprocessing* and *Functional data preprocessing* to the same section in the supplements. No changes have been made to the text and it has simply been relocated to the supplements.

2.3. Details about the nuisance regression, previously found in the subsection *Postprocessing of the functional data and generating connectivity matrices* have been moved to the subsection “Nuisance regression of functional MRI data”, in the supplements. No changes have been made to the text and it has simply been relocated to the supplements.

2.4. We have left two sentences with a very high-level description of the steps we followed in the subsection *“Imaging data”* of the Methods: “Briefly, preprocessing steps involved skull-stripping, normalization to a brain template, and nuisance regression using 36 parameters (Satterthwaite, et al., 2013). More details about the image acquisition and preprocessing can be found in the Supplementary Materials.”.

2.5. Additionally, we have shortened the sentence related to the visualization and can be found in the subsection *“Functional connectivity gradients reference topography”,* in line XX: “Visualization of the gradients and their derivatives were done using several software packages publicly available (Bayrak, 2019; Gale et al., 2021; Vos de Wael et al., 2020; Worsley et al., 2009).”

3. The subsection *“Effect of lesion location on ED”* has been modified and refined to include a much more robust statistical procedure. While the underlying idea and hypotheses remain identical.

4. Lastly, the subsection “*Relationship between and behavioral-structural measures*” has been re-written and shortened (lines XX-YY): “The behavioral examination includes five main test batteries from Corbetta, et al. (2015) to assess motor, linguistic, executive, memory, and attentional functions. These tests were grouped into three main clusters, explaining 60\% of the variance in functional abnormalities (Corbetta, et al. 2015). The first cluster (25\% variance) included language, verbal memory, and spatial memory; the second (23\%) included visual field bias, left body, and spatial memory; and the third (17\%) included right body and attention-shifting factors. Each cluster score served as a predictor for corresponding behavioral deficits. We correlated with these clusters to explore links between functional and behavioral abnormalities.”.

*Minor Points:*  
**Minor point 1:** Ensure consistency in reporting statistical results (e.g., use of p-values with or without correction for multiple comparisons).

(A) Thank you for pointing this out. We apologize for the lack of consistency.

(C) We have reported the statistical testing results in a more homogeneous manner. When applicable, the multiple hypotheses corrections were made explicit. The format we used is the following: “[...] (statistic value(s), p value, multiple comparison correction method).”. For example: “When correlated with mean gradient scores, the mean lag pattern showed a very high correlation with G2, and lower with G1 and G3 (r = 0.7, r = 0.25, r = 0.2, respectively, p values < 0.001, FDR corrected)”. Multiple instances of this were modified accordingly throughout the different results subsections.

**Minor point 2:** Consider including a table summarizing the demographics and clinical characteristics of the stroke patients, including lesion size and location.

**Minor point 3:** I found some grammatical and symbolic errors such as "400 x 400", and "...seen in (C) and (D) respectively." Correct: 400 ✕ 400 / "...seen in (C) and (D), respectively."

(A) We thank the reviewer for pointing these out.

(C) We have made the suggested formatting changes using the latex syntax “400 $\times$ 400”. Other small grammatical errors have also been corrected.

Overall, this manuscript presents a well-conducted study that makes a valuable contribution to the field of stroke research. The authors' use of lag correction and their focus on functional connectivity gradients provide novel insights into the functional consequences of stroke. Addressing the suggested areas for improvement will further enhance the scientific rigor and impact of this work.

1. We thank the reviewer for the constructive comments and the insightful suggestions.

References:

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**Reviewer #3**

**Comment of the reviewer:** The manuscript titled "Reshaped functional connectivity gradients in acute ischemic stroke" presents an intriguing exploration of functional brain reorganization following ischemic stroke using fMRI-based connectivity gradients. While the study applies advanced computational methods to understand the impacts of stroke, the current version has some major issues that need to be addressed before publication.

(A) We thank the reviewer for acknowledging our efforts. Below we reply to the issues raised hoping to further clarify and strengthen the results and conclusions in the manuscript.

**Point 1:** The study's reliance on a relatively small sample size, especially in terms of controls, limits the robustness of the findings, particularly given the complexity of the analysis. Furthermore, while the findings on functional connectivity deviations and behavioral correlations are promising, the study could provide more direct evidence linking these deviations to clinical outcomes in a more comprehensive and statistically sound manner.

(A)

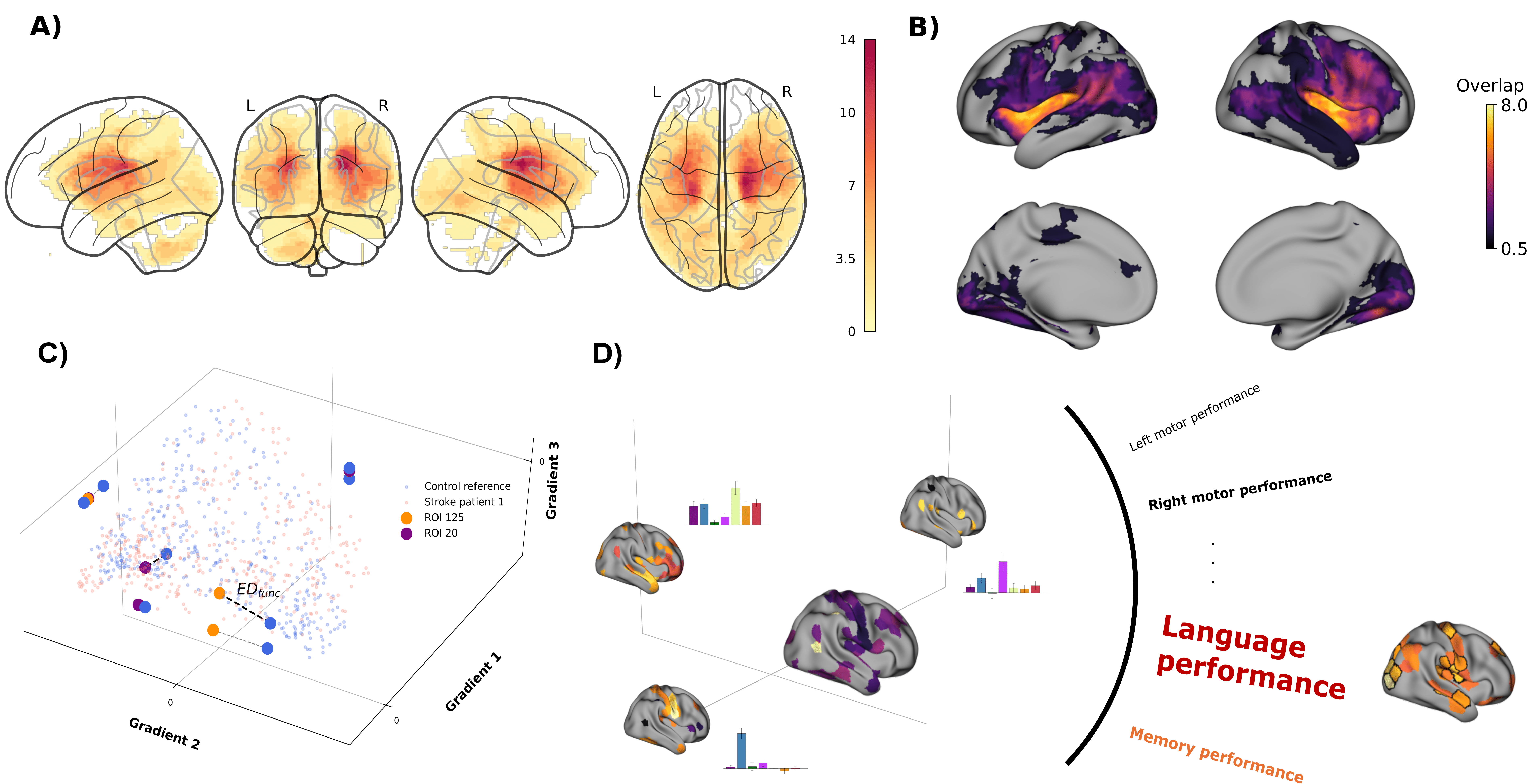
(C)  
  
**Point 2:** One of the main concerns is the limited explanation of the methodological choices, especially regarding the application of lag correction. Although this technique is essential to account for stroke-induced hemodynamic changes, the impact of such corrections on the gradient structure and subsequent clinical interpretations is underexplored. The manuscript does not provide enough detailed comparison of results pre- and post-lag correction, which could be key in validating the necessity and accuracy of the correction. Additionally, while the authors address changes in the second gradient (visual-somatomotor), they could further elaborate on why this particular gradient is more affected and how it compares with findings in previous studies of stroke patients.

(A)

(C)

1. ***METHODS AND RESULTS: See my comments on the section between anatomical and functional similarities.***

2. Furthermore, we have added two panels in Fig. 1 to clarify the methods. The goal was to devise a simple score that could capture the location and magnitude of the functional deviations caused by the stroke. The Euclidean distance measured in the “gradient space” could be projected and analyzed for each axis of brain function. When correlated these deviations with the behavioral performance scores, this gave us a clear and interpretable measure of the origins of cognitive dysfunction as well as a predictive framework. The latter, crucially, stems from the fact that a given stroke repositions each region of interest in the gradient space and this repositioning is significantly related to a certain function. Importantly, the anatomical location of the lesion does not necessarily cause the same functional realignment, thus our framework offers considerable advantage for personalized clinical models. The caption of this figure, added below has also been modified accordingly. Multiple references to this new figure have been added along the methods section to enhance the clarity of our analyses.

**Figure 1. Profiles of ischemic stroke occurrence. (A)** Overlap of the 84 manually drawn lesion masks in the subcortical and cerebellar areas. Regions around the basal ganglia and thalamus show the most overlap, especially in the right hemisphere. The colorbar shows the number of subjects. **(B)** Cortical projection of the lesion occurrences. The total number of subjects with cortical lesion occurrence is less than the total number of subjects with subcortical lesion occurrence. **(C)** Functional connectivity gradients map brain activity onto a three-dimensional space, where the position of each brain area within this manifold reflects typical brain function (blue points). Any deviation from this reference may indicate a potential source of behavioral dysfunction (orange and purple markers), with the magnitude of this deviation measurable along each axis of brain function. **(D)** This method, as described in **(C)**, can be applied across all regions of the brain to identify areas where stroke-induced changes are most pronounced. Changes in every gradient individually can also be decomposed into canonical resting state networks. These distance metrics can, in turn, help predict and elucidate specific cognitive impairments associated with a given infarct.

3. Subsection of lag correction in the results (the last one). Be more explicit on how the results are stronger after correcting for lag. The table is clear, but we could emphasize them more.

4. Discussion about the implications of lag correction on the gradient structure

5. Clinical implications of (not) correcting. If we repeat the ED vs behavior analyses, and they are stronger with the lag-we can reiterate this point. Essentially, we could say that we are tightening the functional markers to the observed clinical factors.

6. any other? Maybe add some new figures in the supplements?

- Gradients for uncorrected data

- Analyses of ED func with uncorrected data. Repeat Figure 4 basically.  
  
**Point 3:** Another major area for revision is the clarity of the results section. Although the authors provide an extensive range of analyses, including functional and structural metrics, the presentation of results can be made more accessible. Some of the statistical findings, particularly those related to the Euclidean distance metrics and their clinical relevance, are difficult to follow and could be streamlined to improve readability. The discussion would also benefit from a deeper integration of how these findings relate to existing literature and a clearer articulation of the study's contribution to the field.

(A)

(C) In line with R#2, we standardized the way we reported the statistical results. When applicable, the multiple hypotheses corrections were made explicit. The format we used is the following: “[...] (statistic value, p value, multiple comparison correction method).”. For example: “When correlated with mean gradient scores, the mean lag pattern showed a very high correlation with G2, and lower with G1 and G3 (r = 0.7, r = 0.25, r = 0.2, respectively, p values < 0.001, FDR corrected)”. Multiple instances of this were modified accordingly throughout the results.

Importantly, the results have not changed, but multiple sentences have been rewritten to try to emphasize the relevance of each analysis:

1. In the subsection *Effect of lag correction on the connectivity matrices and gradients* (line XX) we have implemented changes in the writing to accommodate our reply to Point 2 (see details above).

2. In the subsection “Intrahemispheric connectivity profiles and lateralization of ” (line XX), we are now more explicit w.r.t. the analysis method and the result reported. Additionally, we have added a short paragraph summarizing the most important result from that subsection (line XX): “***SUMMARY PARAGRAPH ALREADY INDICATED IN THE LATEX***”.

Given these concerns, I recommend a major revision with a focus on expanding the methodological details, improving result clarity, and enhancing the discussion on clinical implications.