

Peer Review File

Changes in cortical manifold structure following stroke and its relation to behavioral recovery in the male macaque



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Reviewer #1 (Remarks to the Author):

Review

In this work, Nashed et al. used an animal stroke model in macaques to investigate functional connectivity alterations and their importance for recovery. To assess large-scale networks, they used a low-dimensional manifold analysis and found shifts of various sensorimotor and higher-order transmodal cortices in their embedding in the networks from pre-stroke to post-stroke. Particularly, changes in default mode and limbic networks were related to recovery within 30 days after stroke.

In general, this is an interesting paper. It adds some interesting animal data to ongoing research how the brain and its functional networks react onto an acute stroke lesion and which network changes inform about recovery.

In general, this paper could be potentially interesting for this journal. However, before further consideration, various critical issues should be addressed by the authors:

1. In the abstract, the authors use “predicted”. This is not the case. All analyses are correlative in nature.
2. In the introduction, the authors state that in human stroke recovery research, little attention has been paid to brain changes outside the motor network including transmodal cortices such as on the parietal lobe. Actually, this is not true 1-5. In this regard, there are many more important human stroke studies suitable for citation than the actual ones.
3. In the discussion section it is stated: „we found that it was changes in connectivity of transmodal areas, and not sensorimotor regions, that was associated with differences in spontaneous recovery.” Could this be just a consequence of excluding almost all sensorimotor cortices of the ipsilesional hemisphere given the fact that the sensorimotor network is bilateral? This is a crucial limitation which needs further consideration.
4. The authors should objectively deduce how many PCs should be considered in the analyses. Approaches such as silhouette measure or elbow criterion based on cluster validity index. What about PCs 4 and 5? At the moment, 50% of the variance are unexplained. What variance do these PCs explain? Can such PCs add to the picture presented?
5. Page 6 / Text and Figure 2: I would like to suggest performing statistical work-up of the changes in pre/post gradients for PC1-3, e.g., by means of t-testing (paired, two-tailed). Which regions do show significant changes in PC loadings between both time points?
6. Could the more widespread distribution of points might be simply due to noise?
7. How stable are the manifolds in animals not undergoing MCAO? The inference that the change in manifold is stroke-related remains speculative unless proven that pre-stroke patterns remain unchanged in two measurements with the same time interval.
8. Eccentricity was correlated with measures of network integration and segregation. I ask the authors to assess nodal efficiency as a local measure of integration. On the global level, I would be interested to see the following correlations: mean ECC ~ modularity, mean ECC ~ global efficiency. Also, such measures should be related to the recovery trajectories as well.
9. In this context, the authors should explain what the advantage of the present manifold analyses is compared to large-scale graph metrics as repeatedly conducted in human stroke studies. If the authors are convinced that their approach really adds a novel perspective, they would have to compare manifolds with global network topologies of integration and segregation

for outcome inference. The present animal data would be of greatest interest for human stroke recovery research applying network topographic analyses to understand outcome variability after stroke given homogenous lesions and temporal trajectories 6,7.

10. Figure 3: Please add the t-statistics and the change in ECC into this figure.

11. Fig 4C: The distribution of % regions might be simply driven by the mere distribution of N regions or volumes per category (according to YEO) in the entire brain. Please show the distribution when corrected for the N of brain regions per category.

12. Myelin concentrations and receptor gradients are simple visualizations. It remains unclear whether the change in ECC is really correlated with such covariates. (see <https://www.sciencedirect.com/science/article/pii/S1053811922009284>)

13. I don't understand the selection of seed regions for further FC analyses. The relevant analysis in Fig 5D would come much later. Changes in ECC and FC-outcome association are very different analyses. In fact, Fig 4B would indicate, by bright yellow colour, that other regions are even more susceptible for ECC changes after stroke. These should be assessed in more detail as well.

14. For 4D, please provide statistically thresholded maps as well. Which FCs do show significant changes? An un-thresholded map might be misleading.

15. "For the left PCC seed region, associated with the DMN, we observed the most prominent post-stroke increases in connectivity with bilateral posterior and mid cingulate regions, as well as several frontal regions of the DMN (Fig. 4D, left)." What is the statistical basis for this interpretation? This same question would arise for other conclusions in this paragraph.

16. Initial neurological deficit also shows a relevant variability, Fig 5B. Please give more insights, please show raw data points. Are max NIHPS and d30 NIHPS correlated? As d7 fMRI data is missing, I would speculate that ECC would be simply a direct reaction of the brain to the lesion (see also point 19), would be correlated with initial NIHPS and would not be necessarily related to recovery at all. Is change in ECC linked to initial NIHPS? Is ECC significantly related to d30 NIHPS when taking initial NIHPS into account?

17. The maps in Fig 5F und G show quite sparse and distributed network alterations. Please show individual data points for FC for the clusters presented. Any outliers or influential points? A leave-one-animal out analysis could enhance the robustness of the findings in addition to FDR correction.

18. Given recent evidence for hemispheric specialization 8, and that the ipsilesional hemisphere seems to contribute only marginally to all statistical analyses (not only due to directly affected cortex but also due to widespread "subcortical" disconnections by the stroke lesions), at least for review purposes I encourage that authors to limit the analyses to the contralesional hemisphere. Findings would inform us about the influence of interhemispheric connectivity for changes in manifolds and outcome relationships. Can the authors show a lesion map?

19. Is stroke volume comparison influenced by one outlier? The authors state: "We observed no significant differences in lesion size between the good and poor recovery groups ($t(22)=-1.91$, $p=0.081$; see Supplementary Fig. 4), suggesting that differences in functional recovery, and the associated changes in brain connectivity patterns, cannot be solely explained as a difference in total stroke volume". I have some doubts with this interpretation. An additional analysis looking for a correlation between [stroke volume ~ changes in ECC / stroke volume ~ follow-up ECC / stroke volume ~ changes in regional FC] might provide helpful insights in this issue.

20. Finally, as a minor point, there is no need to include 10 refs++ per one sentence. Please be specific and only include highly suitable references after proper selection.

References

1. Reibelt A, Quandt F, Schulz R. Posterior parietal cortical areas and recovery after motor stroke: a scoping review. *Brain Commun.* 2023;5:fcad250. doi: 10.1093/braincomms/fcad250
2. Idesis S, Allegra M, Vohryzek J, Sanz Perl Y, Faskowitz J, Sporns O, Corbetta M, Deco G. A low dimensional embedding of brain dynamics enhances diagnostic accuracy and behavioral prediction in stroke. *Sci Rep.* 2023;13:15698. doi: 10.1038/s41598-023-42533-z
3. Baldassarre A, Ramsey LE, Siegel JS, Shulman GL, Corbetta M. Brain connectivity and neurological disorders after stroke. *Curr Opin Neurol.* 2016;29:706-713. doi: 10.1097/WCO.0000000000000396
4. Olafson E, Russello G, Jamison KW, Liu H, Wang D, Bruss JE, Boes AD, Kuceyeski A. Frontoparietal network activation is associated with motor recovery in ischemic stroke patients. *Commun Biol.* 2022;5:993. doi: 10.1038/s42003-022-03950-4
5. Lee J, Park E, Lee A, Chang WH, Kim DS, Kim YH. Alteration and Role of Interhemispheric and Intrahemispheric Connectivity in Motor Network After Stroke. *Brain Topogr.* 2018;31:708-719. doi: 10.1007/s10548-018-0644-9
6. Schlemm E, Schulz R, Bonstrup M, Krawinkel L, Fiehler J, Gerloff C, Thomalla G, Cheng B. Structural brain networks and functional motor outcome after stroke-a prospective cohort study. *Brain Commun.* 2020;2:fcaa001. doi: 10.1093/braincomms/fcaa001
7. Siegel JS, Seitzman BA, Ramsey LE, Ortega M, Gordon EM, Dosenbach NUF, Petersen SE, Shulman GL, Corbetta M. Re-emergence of modular brain networks in stroke recovery. *Cortex.* 2018;101:44-59. doi: 10.1016/j.cortex.2017.12.019
8. Rohrig L, Rosenzopf H, Wohrstein S, Karnath HO. The need for hemispheric separation in pairwise structural disconnection studies. *Hum Brain Mapp.* 2023;44:5212-5220. doi: 10.1002/hbm.26445

Reviewer #2 (Remarks to the Author):

Summary:

Thank you for the opportunity to review this manuscript. The authors used functional neuroimaging in a non-human primate (macaque) model to explore the relationships between ischemic stroke, whole-brain connectivity and functional recovery. The authors show that changes in default mode network and limbic network, contribute to stroke behavioral recovery at 30 days. The findings are relevant for understanding the involvement of higher-order transmodal networks. Indeed, these findings are important to understand mechanisms of recovery post-stroke and must be explored further, in addition to existing sensorimotor regions.

Introduction:

Authors need to provide some context on why spontaneous behavioral recovery is important in the context of stroke recovery.

a) What implications does this have for treatment planning and interventions?

b) Is there a distinction between true neurological recovery, spontaneous recovery and recovery

through compensatory mechanisms?

c) Why did the authors choose to focus on spontaneous behavioral recovery is not clear.

Materials and Methods:

Line 521: change to 'FSL's FAST'. FAST is an abbreviation and hence should be capitalized.

Lines 617-625: It is not clear why the seed connectivity analyses was performed. Authors need to be more clear on how this analyses adds to eccentricity analyses. In addition, why significant differences in the good v/s poor outcome groups were observed for selected seed regions is not discussed.

Neurological assessment: The authors need to describe in further detail why the NHPSS was performed on the specific chosen days. Why was a 30-day recovery period chosen over a 90-day recovery period? Why was the NHPSS chosen over the Neurological Scale for MCA for NHPs or other outcome measures?

Discussion:

The authors need to discuss why specifically MCAO strokes were correlated to less altered connectivity in DMN and limbic areas, and whether the specific connectivity patterns of these areas are related to the type of stroke. MCA territory strokes are the most common types of ischemic strokes, however, is there a correlation between MCA strokes and DMN/ limbic connectivity that has been discussed in the literature?

Lines 345-348 and supplemental figure 4: The authors need to provide further explanation on why the total stroke volume was chosen as an indicator rather than the lesion voxel size. Did the authors track/notice changes in lesion volume progression over time?

Lines 375 - 378: The authors need to discuss how the generation of a broad stroke mask, excluding ipsilesional areas of stroke infarct in individual animals can potentially impact the interpretation of the results related to ipsilesional functional connectivity or discuss individual subject differences further/ add this as a limitation.

Reviewer #2 (Remarks on code availability):

The code page is not opening/ unavailable. GitHub search did not yield any repositories.

We would first like to thank the reviewers for their helpful and constructive feedback. There is no doubt that the suggestions provided have substantially improved the manuscript. Our response will address the specific comments made by each reviewer point-by-point. Throughout our response, we will use the following format:

- (1) black text in boldface is used when we directly quote the reviewers;**
- (2) black plain text is excerpts from the original manuscript;
- (3) black text is our response; and
- (4) red text is used when quoting from the revised manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

In this work, Nashed et al. used an animal stroke model in macaques to investigate functional connectivity alterations and their importance for recovery. To assess large-scale networks, they used a low-dimensional manifold analysis and found shifts of various sensorimotor and higher-order transmodal cortices in their embedding in the networks from pre-stroke to post-stroke. Particularly, changes in default mode and limbic networks were related to recovery within 30 days after stroke.

In general, this is an interesting paper. It adds some interesting animal data to ongoing research how the brain and its functional networks react onto an acute stroke lesion and which network changes inform about recovery.

In general, this paper could be potentially interesting for this journal. However, before further consideration, various critical issues should be addressed by the authors:

1. In the abstract, the authors use “predicted”. This is not the case. All analyses are correlative in nature.

Author Reply:

We thank the review for pointing this out and have altered the text to reflect this point, line 29-31 now states:

“Further analyses revealed that it was changes in default mode and limbic network regions, and not **preserved** sensorimotor cortical regions, were **associated** with animal behavioral recovery post-stroke.”

2. In the introduction, the authors state that in human stroke recovery research, little attention has been paid to brain changes outside the motor network including transmodal cortices such as on the parietal lobe. Actually, this is not true 1-5. In this regard, there are many more important human stroke studies suitable for citation than the actual ones.

Author Reply:

We thank the reviewer for the references provided and have altered the text to appropriately include these citations. We have also altered the previous text to now state:

“To date, the majority of the work on this spontaneous recovery has tended to focus on the neural reorganization of the motor and premotor cortices^{11,17-23}, reflecting the inherent link between motor improvements and spontaneous recovery. **However, there is a growing body of research highlighting that changes also occur outside the motor system, such as in various frontal and parietal regions of transmodal cortex²⁴⁻²⁸.**”

3. In the discussion section it is stated: “we found that it was changes in connectivity of transmodal areas, and not sensorimotor regions, that was associated with differences in spontaneous recovery.” Could this be just a consequence of excluding almost all sensorimotor cortices of the ipsilesional hemisphere given the fact that the sensorimotor network is bilateral? This is a crucial limitation which needs further consideration.

Author reply:

We agree with the reviewer that this is a crucial point worth discussing, which is why we had dedicated the second paragraph of our Discussion to addressing this point. However, upon re-reading this text, we realize that we may not have been as clear and direct as we could have been in centering our interpretation around ‘preserved’ sensorimotor cortex, as well as emphasizing the distinction between the ipsilesional and contralesional hemispheres. We have now modified our Discussion paragraph to better emphasize these points. In our Discussion, we now write the following:

“As an improvement in patients’ motor control is the defining hallmark of spontaneous behavioral recovery, it is understandable that previous studies have tended to focus on the role of motor-related brain regions (e.g., primary and premotor cortex) in driving this recovery process^{11, 17-23}. It is crucial to note that our findings do not contradict this perspective. Indeed, it is expected that the ipsilesional motor and premotor cortices will have played substantial roles in the recovery of our animals. However, the nature of our analysis, which involved inter-subject manifold alignment, required excluding any brain regions affected by stroke in any single animal, leading to a broad stroke mask (see Fig. 2A), and thus the omission of many ipsilesional regions. Consequently, our findings are unable to speak to the nature of functional reorganization in these areas or the neighboring penumbra, known to aid in recovery⁶⁵⁻⁶⁷. Nevertheless, our findings indicate that *differences* in subject recovery are not actually related to changes in the connectivity of **preserved sensorimotor areas, like those in the contralesional hemisphere. This is an important observation, as several studies have suggested a prominent role for the contralesional sensorimotor cortex in stroke recovery⁶⁸⁻⁷¹. Rather, our findings suggest that individual differences in subject recovery are actually** related to the connectivity of DMN and limbic brain areas (i.e., PCC and OFC). In the context of prior work, this finding is noteworthy, as these regions—and transmodal cortex more generally—are thought to act as key hubs for cortical integration and behavioral flexibility^{39, 41, 72-74}.”

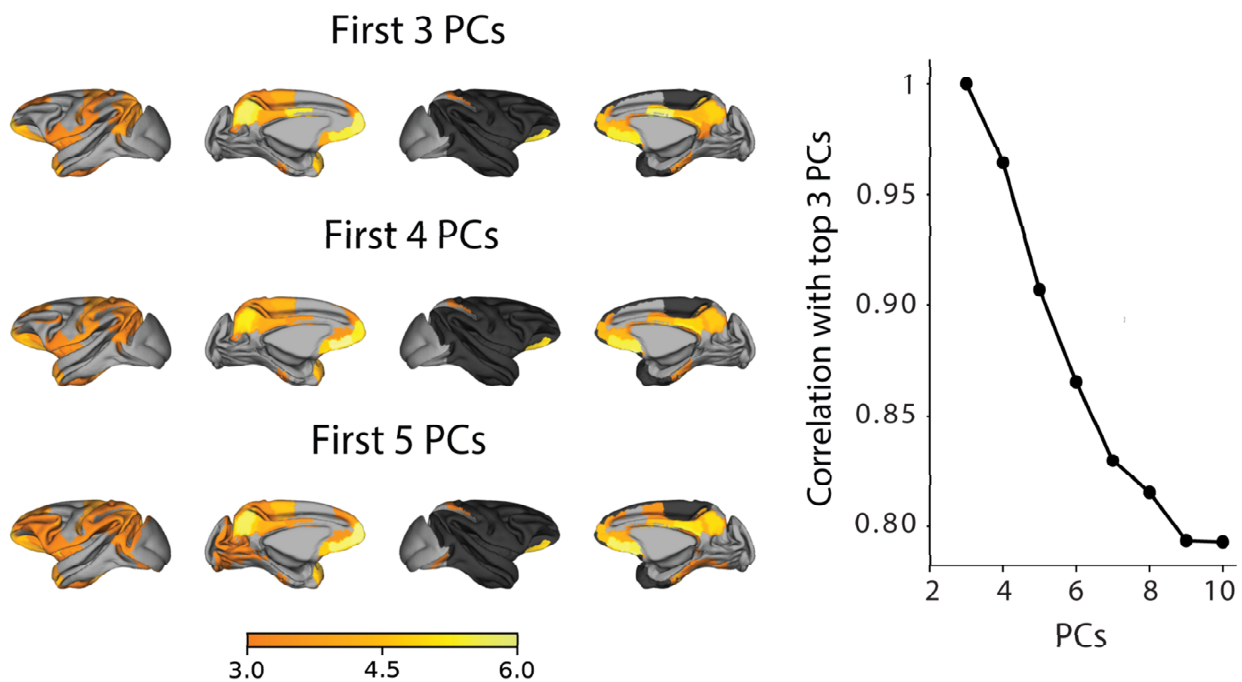
4. The authors should objectively deduce how many PCs should be considered in the analyses. Approaches such as silhouette measure or elbow criterion based on cluster validity index. What about PCs 4 and 5? At the moment, 50% of the variance are unexplained. What variance do these PCs explain? Can such PCs add to the picture presented?

Author reply:

We appreciate this comment from the reviewer. While it is true that approximately 50% of the variance remains unexplained by the first three PCs, this is not uncommon (and actually quite good) in complex biological data, which includes individual variability and measurement noise. The first few PCs typically capture the most prominent patterns in the data, whereas the different sources of variation (e.g., experimental noise, biological heterogeneity) are instead captured by the subsequent PCs, which individually explain smaller proportions of variance. Because the primary goal of our PCA was to identify the main sources of variance that contribute to the observed effects, we focused our analysis on the first three PCs (following from our previous work, e.g., Gale et al., 2022 PNAS; Nick et al., 2024 eLife).

Nevertheless, the reviewer is correct that PCs 4 and 5 do explain some portion of the variance (10.7% and 7.8%, respectively), and that the variance explained for each component clearly ‘elbows’ from PC 5 and beyond (see Fig. 2B). To examine whether these additional PCs can add anything to the overall picture presented by our study, we reperformed our main group-level analyses including both PC4 (i.e., including PCs 1-4) and PC5 (i.e., including PCs 1-5), and have added the results of this analysis to our supplement

(we also include the figure below). As can be seen in this analysis, the inclusion of PC4 and PC5 had a negligible effect on our overall results. Specifically, we find that the F-stat maps associated with Post > Pre stroke for each of PCs 1-4 and PCs 1-5 were near perfectly correlated (both $r > 0.9$) to the results we obtained using our PCs 1-3 approach. This result is entirely sensible, as the loadings of each brain region onto each of the lower variance-explained components (e.g., PCs 4 and 5) are incrementally weaker, which thus mitigates their overall influence on our eccentricity measure (which is respect to the manifold centroid).



We believe that the results of this supplemental analysis validate our approach of focusing on the first 3 PCs in our study. Nevertheless, for readers that might have similar questions to those of the reviewer, we have also included this supplementary analysis in the revised manuscript, as well as provide details about the variance explained by PCs 4 and 5. In our Results, we now write the following:

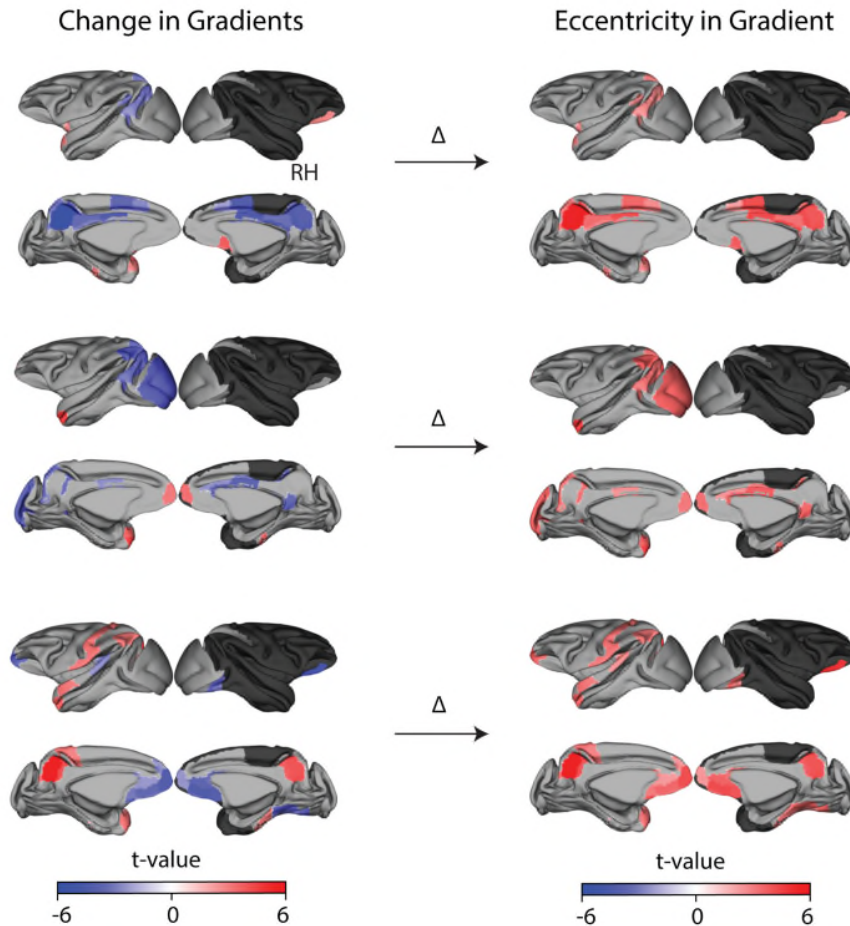
“We should note that although we only retained the top three PCs for our further analyses, it is important to highlight that the inclusions of PC 4 and PC 5, which explains 10.7% and 7.8% less variance, respectively (10.7%) than PC 3 (13.4%), does not change our overall results and interpretations; See supplemental Fig 1”

5. Page 6 / Text and Figure 2: I would like to suggest performing statistical work-up of the changes in pre/post gradients for PC1-3, e.g., by means of t-testing (paired, two-tailed). Which regions do show significant changes in PC loadings between both time points?

Author reply:

At this reviewer’s request, we have now conducted individual paired t-tests for each principal component (PC) to complement our eccentricity analysis. We have included the results of this analysis in the supplemental material (Supplementary Fig. 2), and below for the reviewer. In this figure, we show both the directional change in PC loading from the Pre- to Post-stroke scans (i.e., increase or decrease in loading along each PC axis; left panel), as well as the absolute change in PC

loading ('eccentricity along each PC; right panel). As can be seen by this figure, these t-tests produced largely similar whole-brain patterns of loading changes that were obtained using our eccentricity method, indicating consistency across the different analytical approaches (this, of course, is to be expected as the later method, eccentricity, is a cumulative measure based on the former method, changes in individual PCs). Our preference is to focus on eccentricity in our study given that it is simpler measure that aggregates changes across the individual PCs; nevertheless, we include these results in the supplement for interested readers.



In our Results, we now reference the inclusion of these results with the following:

“When compared to the pre-stroke connectivity gradients, we found that the post-stroke gradients exhibited much stronger loadings onto the individual PCs. For instance, for regions that exhibited the highest absolute component scores in PCs 1-3 pre-stroke (regions in dark red and blue in the left column of Fig. 2A), we observed that these same regions tended to now exhibit even higher component scores post-stroke (i.e., even darker red and blue colors, Fig. 2A right column). In addition, for many of the PCs, areas exhibiting the highest component loadings also tended to increase in number following stroke (compare left and right columns in Fig. 2A). For instance, for post-stroke PC1, the negative loading (in blue) of regions along the PCC extended to include more anterior regions, into the mid cingulate cortex; and similarly, for post-stroke PC2, the negative loading of the PCC (in blue) expanded posteriorly to now encompass the entirety of visual cortex.

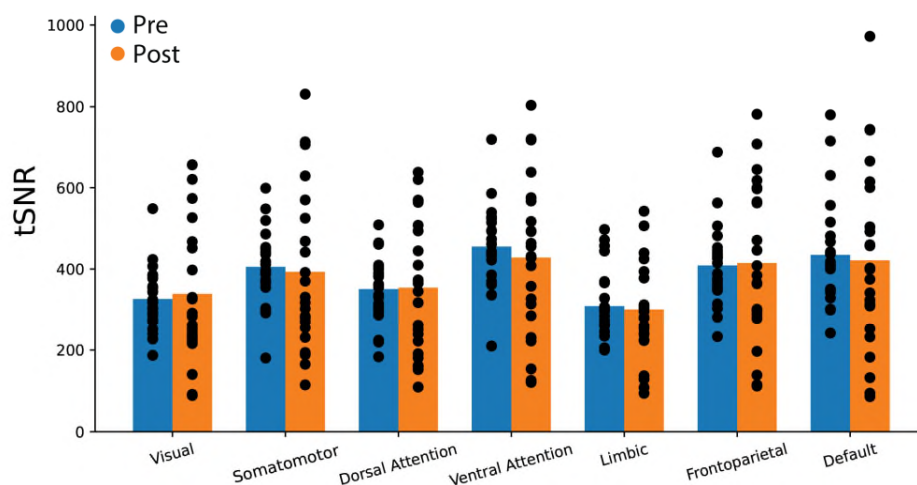
For a direct statistical comparison of the changes in regional loadings for each PC, see Supplemental Fig 2).”

6. Could the more widespread distribution of points might be simply due to noise?

Author reply:

This is an interesting question from the reviewer. Of course, when deciding upon a final analysis pipeline we considered this issue by ensuring that only regions of interest (ROIs) that were unaffected by the stroke across all animals were included in our analysis. However, if the reviewer’s point is more about the *quality* of the fMRI signal in the preserved/unaffected ROIs in the Pre- versus Post-stroke scans, then this above feature of our analysis does not address this concern.

Thus, to address this concern directly, we have now performed a formal analysis of the temporal signal-to-noise ratio (tSNR, calculated by taking the signal mean and dividing by its standard deviation) across different sets of unaffected brain regions for both the Pre- and Post-stroke fMRI scans. Given that the majority of our interesting/important results are related to the manifold changes in the DMN/limbic areas, we have averaged the tSNR values for individual brain regions according to functional brain networks defined by the Yeo atlas. We have included the results of this tSNR analysis below.



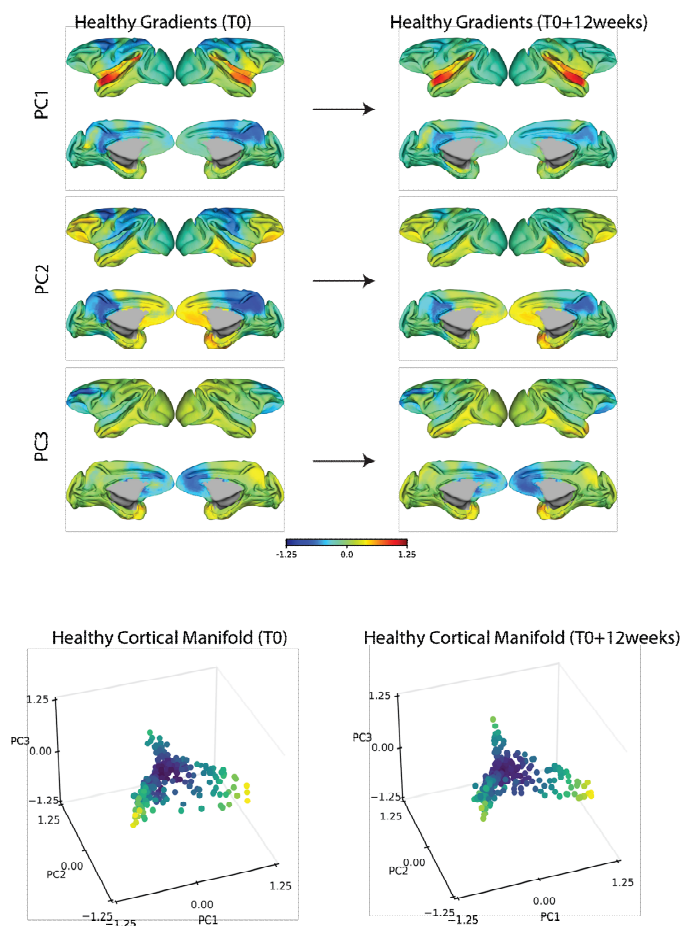
As can be clearly seen in the figure above, when we averaged the tSNR across ROIs according to their network assignments, we observed no significant differences in the tSNR between the Pre- and Post-stroke scans for either the DMN/limbic regions or any other network. Given the importance of these results to the interpretation of our findings, we have opted to include these results in our Supplemental material (see Supplementary Fig. 3) for other interested readers. Moreover, to flag these results for the reader, in our Results section we now write the following:

“Importantly, these shifts in manifold embedding do not appear to be simply attributed to noise. A formal analysis of the temporal signal to noise ratio (tSNR) across networks revealed no significant differences from pre- to post-stroke. Specifically, the tSNR within the regions with the largest shifts in manifold embedding including the DMN ($t = 0.269$, $p = 0.79$) and limbic networks ($t = 0.233$, $p = 0.81$) did not show any significant changes, nor did any other network ($p > 0.05$; See Supplemental Fig 3).

7. How stable are the manifolds in animals not undergoing MCAO? The inference that the change in manifold is stroke-related remains speculative unless proven that pre-stroke patterns remain unchanged in two measurements with the same time interval.

Author Reply:

Though it is unlikely that the large changes in manifold structure that we observe are completely *unrelated* to the stroke, it is true that without having control results in an independent sample, our findings remain somewhat speculative. Prompted by this reviewer's comments, we have obtained a dataset from a separate NHP study conducted at our MRI Centre in which 13 NHPs were scanned at two separate time points, 90 days apart (i.e., day 0 and day 90), in the absence of any experimental intervention during this 90-day period (i.e., the animals were neurologically healthy and awaiting participation in a separate future study). Using this independent data set we performed an identical set of whole-brain analyses in our NHPs to that performed in our stroke study, and Procrustes-aligned the control animals' whole-brain manifolds to the Pre-stroke scans from our current study so that the two sets of findings (the stroke and control datasets) could be directly compared. The results of this analysis, and the average whole-brain manifolds for both time points are shown in the figure below.



As can be clearly seen in this above figure, we observed only negligible changes in the PCs between the two timepoints, and no obvious discernible change in the organization of the whole-brain manifold. To directly quantify this visual observation, we performed region-wise paired samples t-

tests for each region's change in eccentricity between the two time points and performed an FDR correction ($q < 0.05$) — i.e., exact same analysis performed in Fig. 3 of our paper. This contrast revealed that no individual brain region changed its manifold eccentricity from the first to second time point.

We hope the reviewer will agree that this is an important observation, as it indicates that the findings in our stroke study are unlikely to arise simply due to the passage of time. Indeed, if this were the case, then we would certainly expect such effects to be manifest at a time interval of 12 weeks (i.e., in our control study) if they were to be manifest at just 30 days in our stroke study.

Given the importance of these findings to the interpretation of our presents results, we have included the above findings as a new figure in our supplement (Supplemental Fig. 6), and we now also highlight these important findings in our Results section with the following text:

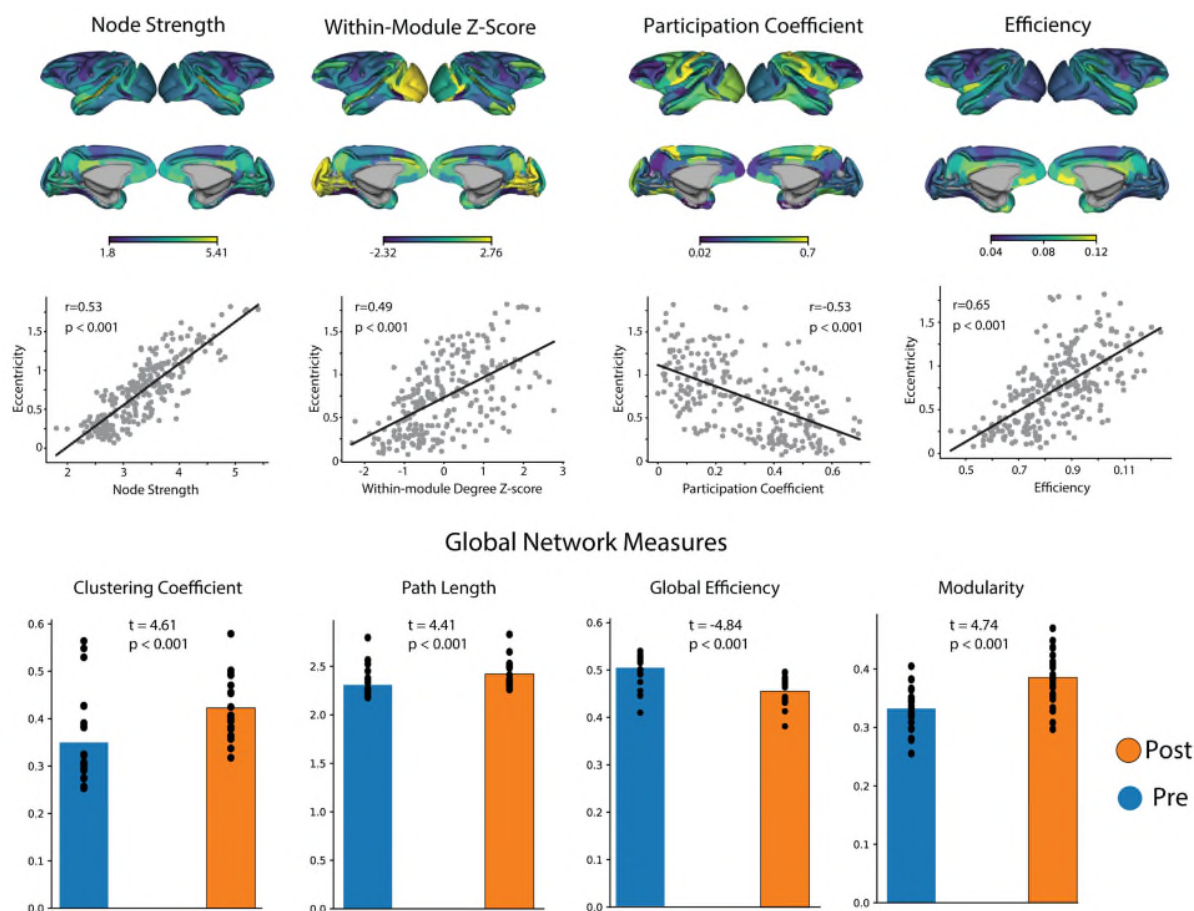
“One potential alternative explanation of the above results is that, instead of reflecting a stroke, the manifold changes might simply reflect unrelated changes in manifold structure over time and/or a byproduct of the manifold alignment procedure (e.g., the Procrustes alignment of the Post-stroke scans to the Pre-stroke scan). To address this possibility, we obtained a separate imaging dataset recently collected at our research facility (for a separate study) in which 13 neurologically healthy male cynomolgus macaques underwent scanning at two separate time points, 90 days apart. In this separate NHP sample we performed the identical set of analyses and aligned each animals' whole-brain manifolds to the Pre-stroke scans from the current data set. We then performed region-wise paired-samples t-tests to test for the change in eccentricity between the 0 day and 90 day timepoints (see Supplementary Figure 6). The results of this analysis did not identify any significant changes in eccentricity over the 90 days, indicating that the current findings (in Fig. 3D) are likely to reflect changes in cortical organization due to the stroke.”

We thank the reviewer for this suggestion, as we think the results from this control analysis have strengthened the paper considerably.

8. Eccentricity was correlated with measures of network integration and segregation. I ask the authors to assess nodal efficiency as a local measure of integration. On the global level, I would be interested to see the following correlations: mean ECC ~ modularity, mean ECC ~ global efficiency. Also, such measures should be related to the recovery trajectories as well.

Author reply:

We appreciate the reviewer's suggestions regarding the analysis of eccentricity and its relationship with network integration and segregation. Based on this reviewer's requests, we have now assessed the correlation between nodal efficiency, a local measure of integration, and regional eccentricity. We show the results of this analysis below, and have also updated our Supplemental Figure 4 to include these findings. As can be seen in the figure, we observed a strong positive relationship between eccentricity and nodal efficiency.



As for the requested global analyses, we have also performed these analyses and included them below. We observed a significantly positive correlation between whole-brain mean eccentricity and modularity ($r = 0.774$, $p = 1.46e-05$), suggesting that eccentricity reflects the compartmentalized network structure indicated by modularity. As for the correlation between whole-brain mean eccentricity and global efficiency, we observed a significant negative relationship ($r = -0.506$, $p = 0.014$). This inverse relationship implies that the network becomes less globally efficient as the eccentricity increases, indicating more segregated communication pathways. Together, these results suggest that several features of our manifold analyses can capture important properties of the whole-brain network, as revealed through graph theoretic measures.

Finally, we also examined how these network measures relate to animal recovery. However, in each case, we did not observe a correlation between our behavioral measure of recovery derived through fPCA and the different global brain measures (modularity, $r = -0.032$, $p = 0.886$; global efficiency, $r = 0.009$, $p = 0.968$, change in eccentricity, $r = -0.171$, $p = 0.437$). This may suggest that animal recovery is specifically related to changes in the organization of individual brain regions rather than global changes, at the whole-brain level.

9. In this context, the authors should explain what the advantage of the present manifold analyses is compared to large-scale graph metrics as repeatedly conducted in human stroke studies. If the authors are convinced that their approach really adds a novel perspective, they would have to compare manifolds with global network topologies of integration and segregation for outcome inference. The present animal data would be of greatest interest for

human stroke recovery research applying network topographic analyses to understand outcome variability after stroke given homogenous lesions and temporal trajectories 6,7.

Author reply:

We appreciate the opportunity to highlight what we think are some of the intrinsic benefits of manifold analyses (below), but would like to emphasize that we believe the dimension reduction approach complements, rather than replaces, traditional graph theoretic methods.

Capturing multivariate changes in brain organization: Dimension reduction techniques, such as Principal Component Analysis (PCA), aim to capture the most significant patterns of variance in the data, reflecting the global multivariate dynamics of brain activity. These methods are particularly effective in identifying underlying low-dimensional structures within high-dimensional fMRI data, providing a comprehensive view of how different brain regions co-vary over time. In contrast, graph metrics often focus on the connectivity patterns between discrete brain regions, emphasizing local and global network properties such as integration, segregation, and modularity. While these metrics provide valuable insights into the structural and functional architecture of the brain, they may not fully capture the more continuous, multivariate aspects of brain activity.

Interpretability and Visualization: By reducing the dimensionality of fMRI data, manifold learning simplifies complex datasets into lower-dimensional representations that are easier to visualize and interpret. This can help identify principal components or intrinsic brain networks that are most relevant to specific cognitive or motor functions, thereby facilitating a clearer understanding of brain-behavior relationships. Graph metrics, while powerful, often involve complex calculations and interpretations that can be less intuitive and amenable to direct visualization.

In summary, while graph theoretic approaches provide detailed insights into network properties and connectivity, manifold analyses offer a powerful means of capturing and interpreting the global dynamics of multivariate brain activity. Together, these methods can provide complementary perspectives that enrich our understanding of brain function and recovery following stroke.

Now, to address the reviewers' request for a comparison between our manifold techniques and global network topologies of integration and segregation, we have performed an analysis focusing on several key graph theoretic metrics pre- and post-stroke (shown in the figure above). As can be seen in this figure, the results demonstrate significant differences in several key network measures, underscoring the added value of our manifold learning approach.

Eccentricity is a multivariate index of each region's three-dimensional embedding. Essentially, distal regions situated at the anchors of the manifold have greater eccentricity than proximal regions within the manifold core. Overall, eccentricity showed a significant change pre to post-stroke ($t = 5.44$, $p = 1.81e-05$), highlighting the sensitivity of manifold techniques in detecting variations in network structure. High eccentricity values post-stroke suggest nodes that have become more segregated from one another. Correspondingly, both the modularity ($t = 4.74$, $p = 0.00018$) and clustering coefficient ($t = 4.61$, $p = 0.00018$) showed significantly higher values post-stroke as compared to pre-stroke. Higher modularity suggests that manifold techniques reveal more cohesive and integrated modules in the brain network, reflecting the reorganization of brain regions into more cohesive functional groups post-stroke. Similarly, an increase in the clustering coefficient indicates a higher tendency for nodes to form tightly-knit groups, reflecting localized network changes that manifold techniques are adept at uncovering. Interestingly, global efficiency ($t = -4.84$, $p = 7.69e-$

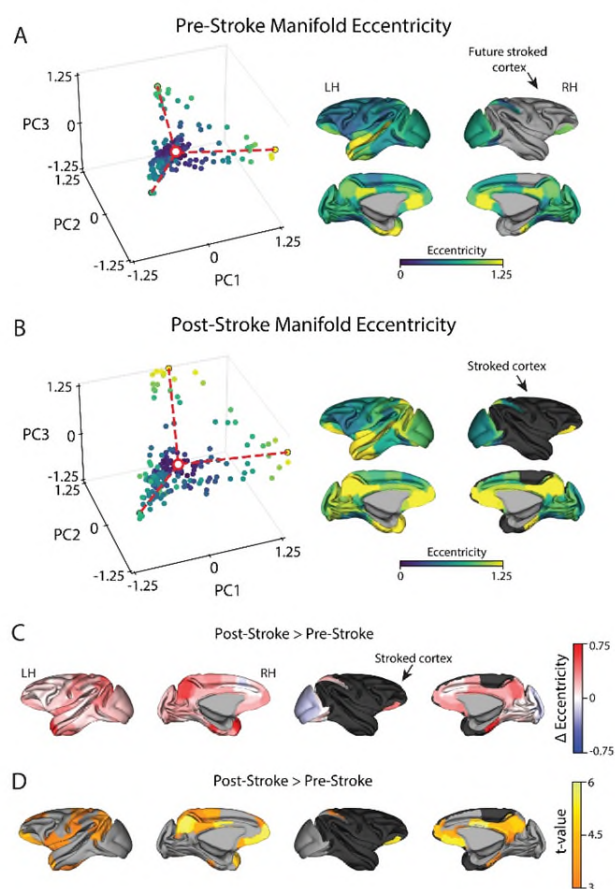
05) was lower post-stroke compared to pre-stroke. This suggests that information transfer within the network is reduced. This can be due to the creation of more segregated functional modules, a phenomenon that manifold techniques reveal through their comprehensive structure-preserving mappings. The path length ($t = 4.41$, $p = 0.00022$) showed a significant increase post-stroke, indicating longer communication pathways within the brain network stroke. An increased path length suggests that the average communication distance between brain regions has grown, which manifold techniques, by capturing the intricate layout of the multivariate data, highlight effectively.

These findings demonstrate that manifold techniques are particularly adept at capturing the subtleties of brain network changes pre- and post-stroke. By identifying these subtle patterns and variations within the data, manifold techniques provide a nuanced understanding of brain networks, which is critical for advancing our understanding of stroke recovery and outcome variability. This different perspective enhances the analysis and interpretation of complex brain data, particularly in understanding stroke recovery.

10. Figure 3: Please add the t-statistics and the change in ECC into this figure.

Author reply:

Thanks for this suggestion. This re-organization does make a lot more sense. Please find the revised figure below.



11. Fig 4C: The distribution of % regions might be simply driven by the mere distribution of N regions or volumes per category (according to YEO) in the entire brain. Please show the distribution when corrected for the N of brain regions per category.

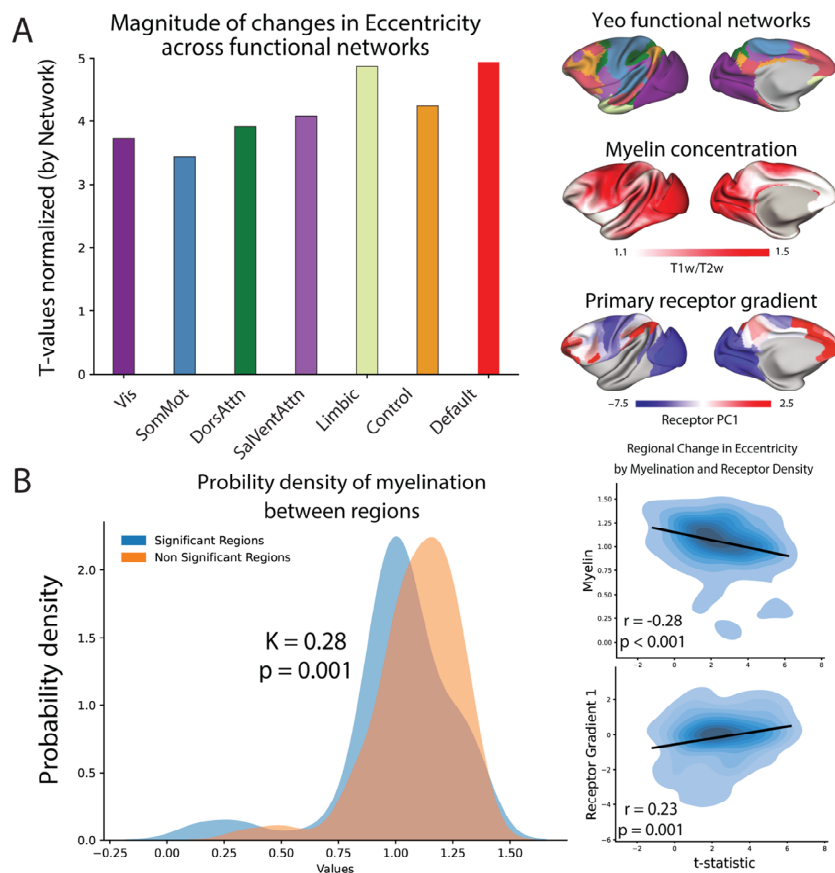
Author reply:

The reviewer is absolutely correct that the % distribution of regions across networks could simply reflect the fact that some networks have a higher number of brain regions. Indeed, when we corrected for this fact in our data (i.e., by normalizing each network by the total number of regions per network), we found that — as suspected by the reviewer — this explained our pattern of effects in the DMN and limbic areas (i.e., DMN and limbic areas tend to have a larger number of brain regions than other function networks).

However, this foray prompted us to reconsider the purpose of this analysis. Specifically, we now appreciate that our original analyses were emphasizing the wrong aspects of the data; that is, while our previous analysis on % distributions solely focused on *which* regions exhibited significant changes from Pre- to Post-stroke, it did not actually emphasize the *magnitude* of those changes along the manifold. Indeed, what is clear when looking at the Pre- and Post-stroke whole-brain manifolds in Fig. 3A and B, is that some brain regions exhibit large shifts in their manifold embedding whereas others exhibit much smaller shifts (you can also see this by comparing the yellow versus more orange regions, respectively, in Fig. 3D). To better capture this *magnitude* aspect of the changes, we have now performed a more direct analysis (included in Fig. 4A, see below) where we simply average the t-values (based on the Post > Pre comparison) across regions according to network assignment. Thus, in this analysis, networks that contain regions that exhibit larger extent shifts along the manifold will have higher average t-values than those that exhibit smaller extent shifts. As can be seen in Fig. 4A, we found that it was regions of the DMN and limbic networks that exhibited the largest changes in their manifold embedding on average, whereas it was regions in visual and somatomotor cortex that exhibited the smallest changes on average. We now reference these findings in the Results with the following text:

“With respect to the latter, we observed particularly prominent post-stroke increases in brain areas belonging to the DMN and limbic networks, such as the bilateral PCC, OFC, and anterior and medial temporal cortex. Consistent with this visual observation, we found that brain regions that exhibited the largest average changes in their manifold embedding tended to lie within the DMN and limbic networks (Fig. 4A).”

We kindly thank the reviewer for pointing out this error in our original analysis.



12. Myelin concentrations and receptor gradients are simple visualizations. It remains unclear whether the change in ECC is really correlated with such covariates. (see <https://www.sciencedirect.com/science/article/pii/S1053811922009284>)

Author reply:

What a great idea to explore this in the current study! We have now done this type of analysis for both the myelination maps and the receptor density maps, and report these new findings in Fig. 4B. The upshot is that these results have turned out beautifully; we observed significant correlations between the whole-brain changes in eccentricity versus both myelination and receptor density. We now reference these findings in the Results with the following text:

“According to prior studies, brain regions that comprise the DMN and limbic networks are positioned at the very apex of the cortical processing hierarchy, which extends from lower-order unimodal sensory and motor areas to higher-order regions in transmodal cortex⁴¹. Consistent with this interpretation, the DMN and limbic networks have been shown to (1) exhibit less myelination, thus allowing for greater synapse formation and experience-dependent plasticity⁵³, and (2) have a higher density of neurotransmitter receptors per neuron, thus allowing for greater cognitive flexibility^{54,55}; see Fig. 4BC). Given these observations, we naturally wondered whether the regional changes in manifold structure that we observed following stroke (in Fig. 3D) might relate to differences in the distribution of myelin content and the density of neurotransmitter receptors across cortex. To explore this, we tested for a spatial correlation across brain regions between the t-statistic

reflecting with the change in eccentricity from Pre- to Post-stroke versus each of the following: (1) a macaque brain map capturing regional differences in cortical myelination^{56,57} and, (2) a macaque brain map that captures total neurotransmitter receptor density per neuron⁵⁴. The results of these analyses are shown in Fig. 4B. As can be seen in these plots, brain regions that exhibited a larger, more significant change in manifold eccentricity from Pre to Post-stroke were associated both with lower myelin content and with a greater density of neurotransmitter receptors. We address the implications of these important results in our Discussion.”

In addition, we now also discuss the important implications of these findings in our Discussion section, with the following text:

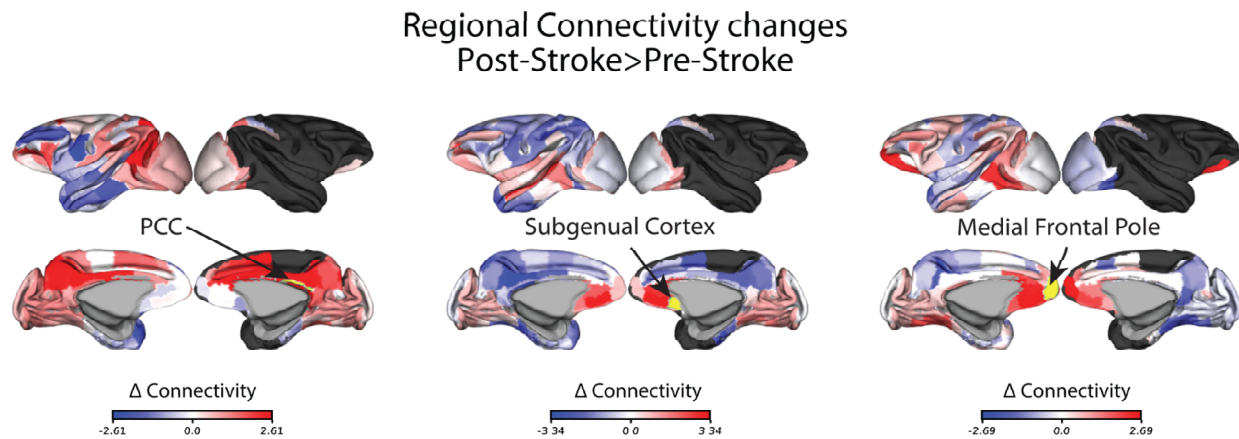
“According to contemporary perspectives on functional brain organization, the DMN and limbic networks sit atop a global processing hierarchy that extends from lower-order brain systems in primary, unimodal cortex (visual and somatomotor networks) to higher-order brain systems situated in multi-modal, transmodal cortex^{29, 75-79}. Congruent with this viewpoint, DMN and limbic areas differ anatomically from sensory and motor systems in important ways. First, in both humans and macaques, these areas have a much higher density of neurotransmitter receptors per neuron (54,55). This presumably allows these regions to contribute to more complex cortical functions, and flexibly integrate information across distributed sources^{54,55}. Second, DMN and limbic areas contain significantly less myelin compared to unimodal cortical areas^{41, 54,55} but display larger dendrites, and far more intricate patterns of dendritic branching and arborization^{80,81}. Given that myelin inhibits synapse formation⁵³, such complexity is thought to enhance their potential for neural plasticity^{53,82,83}, such as when the cortex faces damage or trauma. **Notably, when we directly compared the cortical changes following stroke to brain maps of these different structural features, we found that the regions that exhibited the largest changes Post-stroke were also regions that tended to exhibit both higher receptor density and lower levels of myelination. One interpretation of these results is that these structural features of the DMN and limbic networks — i.e., the convergence of different neurotransmitter signals, their dendritic complexity, and their lower myelin content — make these regions particularly susceptible to severe brain trauma. A compelling alternative interpretation is that these structural features might make these brain areas more adaptable and capable of re-organizing their neural connections in response to damage, which in turn might facilitate recovery and compensation. As our current results are compatible with both interpretations, future work will be required to disentangle these different possibilities.**”

13. I don't understand the selection of seed regions for further FC analyses. The relevant analysis in Fig 5D would come much later. Changes in ECC and FC-outcome association are very different analyses. In fact, Fig 4B would indicate, by bright yellow colour, that other regions are even more susceptible for ECC changes after stroke. These should be assessed in more detail as well.

Author reply:

We appreciate the opportunity to clarify this. Our original logic for selecting the seed regions was simply to show the connectivity patterns of different ROIs that would later be shown in our individual differences analyses (originally in Fig. 5D). Simply, we wanted to enhance redundancy, and thus lower the reader's cognitive load, across the figures. However, we appreciate that this approach may not have been the most effective in highlighting the areas that exhibit the largest changes in eccentricity after stroke. Thus, to better emphasize the latter, we have selected 3 new seed regions corresponding to the 3 largest changes in eccentricity from Pre- to Post-stroke in Fig. 3D. The results of these new seed connectivity analyses are shown in Fig. 5, and are included below

for the reviewer. We have also updated our descriptions of the changes in functional connectivity in the main text to account for these new seed regions.



14. For 4D, please provide statistically thresholded maps as well. Which FCs do show significant changes? An un-thresholded map might be misleading.

Author Reply:

We reply to point #14 in our response to point #15 below, which addresses a similar question.

15. “For the left PCC seed region, associated with the DMN, we observed the most prominent post-stroke increases in connectivity with bilateral posterior and mid cingulate regions, as well as several frontal regions of the DMN (Fig. 4D, left).” What is the statistical basis for this interpretation? This same question would arise for other conclusions in this paragraph.

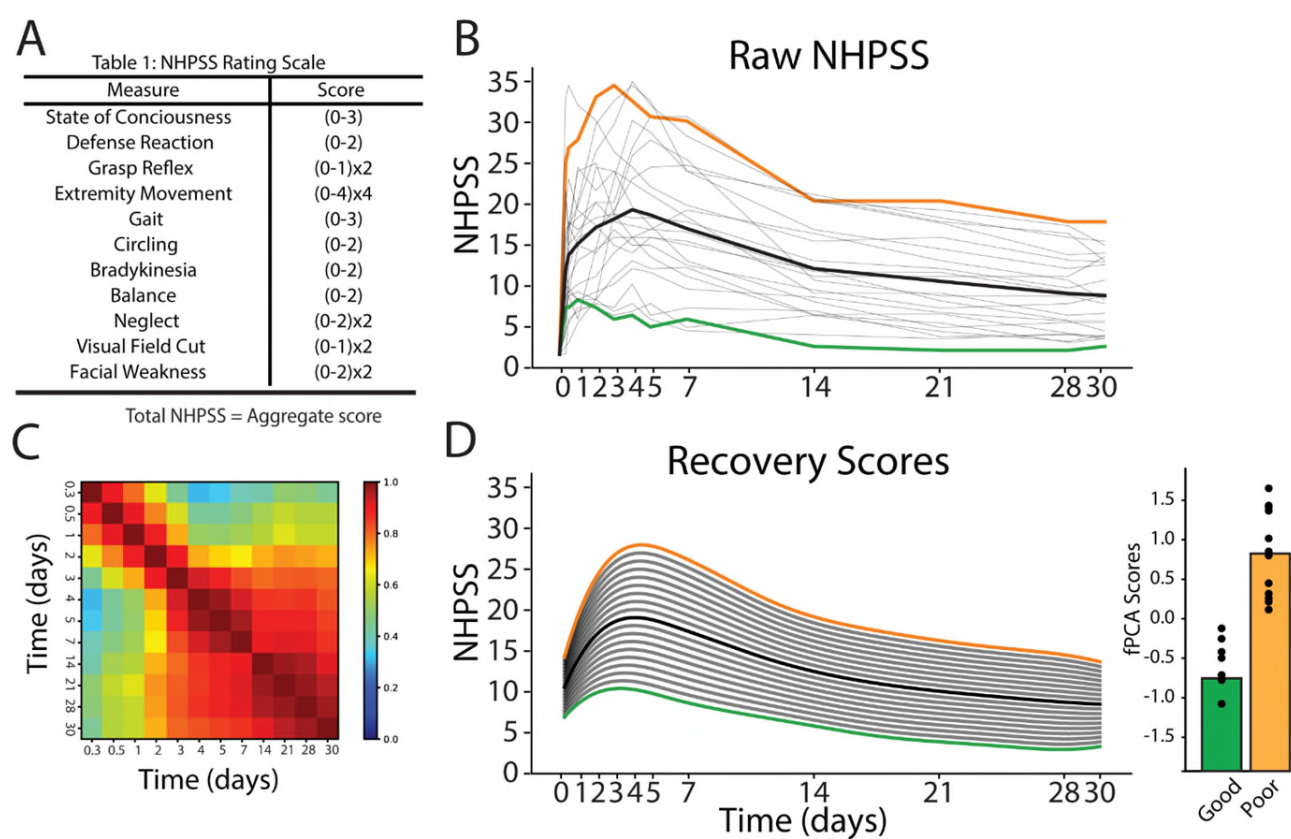
Author Reply to both 14 and 15:

There was no statistical basis for this interpretation *per se* — we were merely visually describing the whole-brain patterns of changes in functional connectivity from Pre- to Post-stroke. Because eccentricity reflects a *multivariate* index of a region’s overall whole-brain connectivity profile, from our perspective it is more sensible to *characterize* the general patterns contained in the unthresholded maps. That is, it is not obvious the extent to which a multivariate effect like eccentricity should be solely interpreted with respect to a series of individual univariate (statistically significant) changes in FC. Note also that we are not making any statistical claims about these maps, merely characterizing their cortical topography to give the reader a sense of what types of multivariate changes may underlie the changes in eccentricity.

16. Initial neurological deficit also shows a relevant variability, Fig 5B. Please give more insights, please show raw data points. Are max NIHPSS and d30 NIHPSS correlated? As d7 fMRI data is missing, I would speculate that ECC would be simply a direct reaction of the brain to the lesion (see also point 19), would be correlated with initial NIHPSS and would not be necessarily related to recovery at all. Is change in ECC linked to initial NIHPSS? Is ECC significantly related to d30 NIHPSS when taking initial NIHPSS into account?

Author Reply:

We are immensely grateful to the reviewer for forcing us to ‘go down the rabbit-hole’ on this point, as it forced us to reconsider our analysis of animals’ recovery data. In short, yes, both max NHPSS and d30 NHPSS are correlated...but then so are day 4, 5, 7, 14, 21, and 28 (see figure below). That is, there is a robust temporal autocorrelation across the timepoints on animals’ recovery curves, which isn’t terribly surprising (e.g., animals that have a lower NHPSS score following the stroke also have a lower NHPSS score at day 30). In some of the co-authors’ other work on motor learning, we see the exact same thing in subjects’ learning curves (e.g., subjects who are able to reduce their errors quickly during learning also end up obtaining a better end state during learning). This auto-correlative feature of both recovery and learning curves means that it is difficult (and potentially confounding) to relate any single time point (e.g., day 5) to brain data and instead the recovery curve must be treated as a unit, in and of itself. Fortunately, some of our co-authors have developed analysis tools for dealing with exactly this kind of problem in the motor learning domain, and we have now applied these tools in the analysis of the animals’ NHPSS trajectories of stroke recovery data. The upshot is that we now have a much more mathematically-grounded approach for dealing with time course behavioral data, but which nevertheless reconstitutes a nearly identical set of results to those reported in our original paper. We have included the results of this new analysis below.



In addition, we now provide justification for this new analytic approach, and its corresponding Results, in the Results section with the following text:

“In examining changes in the manifold structure from pre- to post-stroke, we have thus far considered the mean changes across all individuals. It is widely recognized, however, that individual differences in the trajectories of stroke recovery are substantial⁵⁸⁻⁶⁰. Although the group-averaged recovery curve depicted in Fig. 1 suggests a stereotypical rapid increase and subsequent gradual decrease in NHPSS across the 30 days, this representation obscures significant intersubject variability. For example, Fig. 6A illustrates the learning trajectories of all subjects, highlighting two distinct cases — one individual who exhibits a relatively small increase in NHPSS scores and subsequently better recovery (green trace) and another individual who exhibits a strikingly large increase in NHPSS scores and a subsequently poorer recovery (red trace).

The pronounced variability in recovery trajectories across individuals prompted us to identify a single recovery measure that could adequately encapsulate these individual differences in recovery profile, and thus simplify our examination of the relationships between manifold changes and behaviour. On this point, a particularly salient feature of individuals’ learning curves is their temporal autocorrelation; i.e., individuals who exhibit good recovery at 30 days (prior to the post-stroke scan) are also the same individuals who exhibit relatively smaller increases in NHPSS score immediately following the stroke (i.e., within days 1-5; see Fig. 6B). This temporal covariation results in a strong positive correlation between NHPSS scores obtained immediately following the stroke with those obtained at 30 days (see correlation matrix in Fig. 6C). Importantly, this covariation in time series data lends itself well to temporal dimension reduction techniques, such as functional principal component analysis (fPCA), which are able to extract the dominant patterns of covariation across time (61). Building on our previous work using these techniques^{52,62}, we employed fPCA on the recovery trajectories across animals (see Methods for details; see also Areshenkoff et al., 2022). The fPCA revealed that a single component — representing overall recovery — accounted for the majority (88%) of the variability in animals’ recovery curves (Fig. 6D). We thus utilized individuals’ scores on this primary component as a singular measure describing their overall recovery profile across the 30 days. [Note that the two subjects exemplified in Fig. 6B, are actually those with the lowest (in green) and highest (in red) loadings on the fPCA component]. In sum, our application of fPCA allowed us to distill complex, time-varying patterns of individuals’ NHPSS scores over the 30 days into a single metric that effectively describes their spontaneous behavioral recovery profile.

Next, we divided the animals into two recovery groups — a good and poor group — based on a median split of their z-scored fPCAscores. Under this division, animals with fPCA scores below the median were considered to have good recovery (N=11; -0.87 +/- 0.14; Fig. 6D), whereas animals with scores above the median were considered to have poor recovery (N=12; 0.797 +/- 0.18; Fig 6D)...

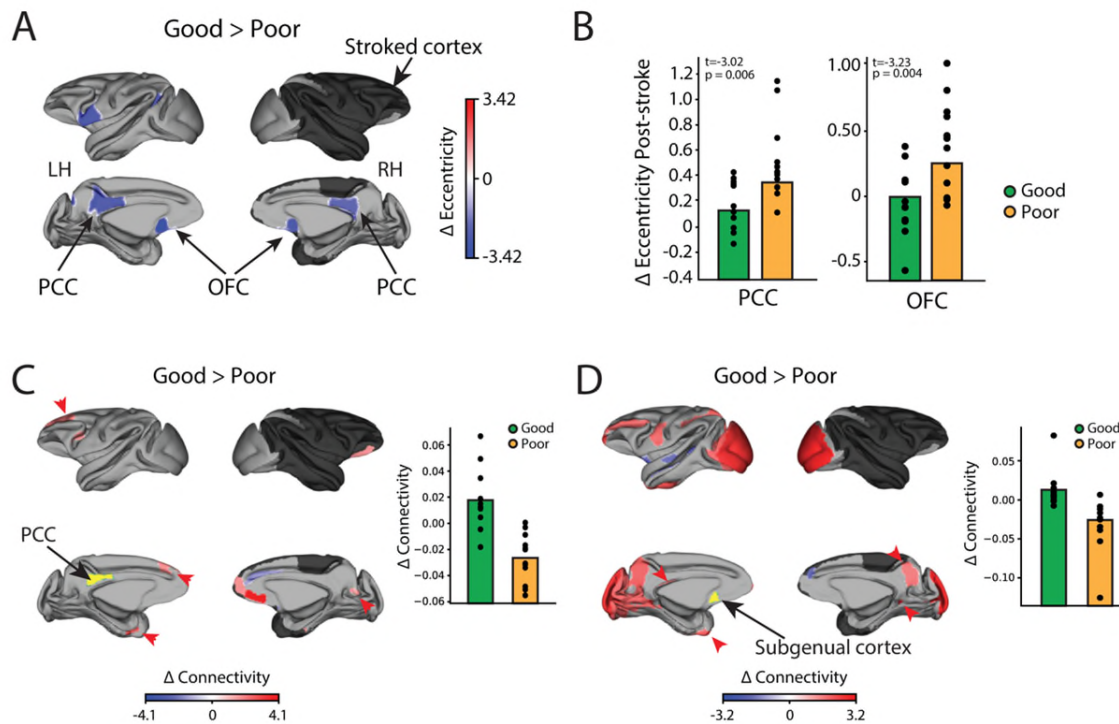
Note that we have also updated our Methods section to describe the analytical procedures used for this analysis (please see that text if interested).

17. The maps in Fig 5F und G show quite sparse and distributed network alterations. Please show individual data points for FC for the clusters presented. Any outliers or influential points? A leave-one-animal out analysis could enhance the robustness of the findings in addition to FDR correction.

Author reply:

To assuage this reviewer’s concerns, we have averaged data points across individual significant regions in the figures requested (note we opted for this averaged approach because the figure looked

far too busy and complex when showing this data for each ROI cluster individually). As can be seen in Fig. 7 (see below), it does not appear that any individual subject outliers are present that drive significance in these regions.

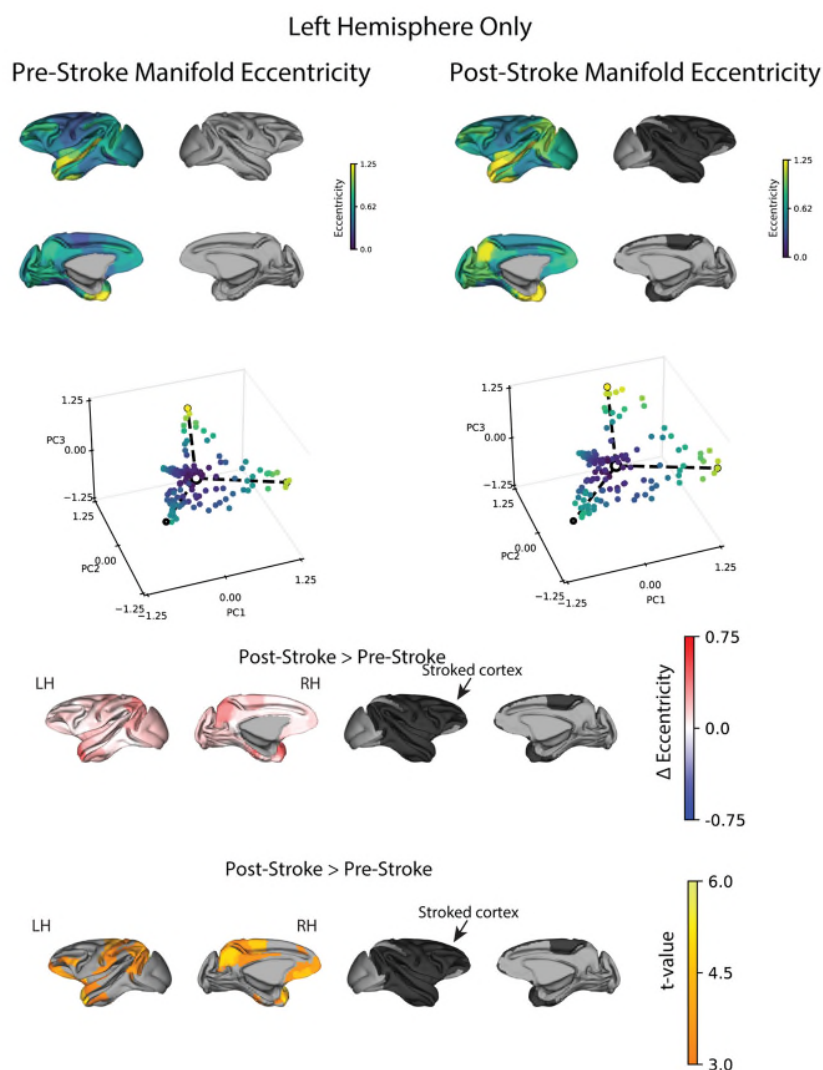


We did not perform the LOO analysis, as we are unsure of specifically what the reviewer had in mind re: its implementation and the outcome of this analysis. Were they referring to a support vector machine classification and a plotting of the distribution of ROI weights? In any case, it was unclear to us how such an analysis would enhance the robustness of our results, above and beyond the use of an FDR correction. Moreover, there does not appear to be any indication above that our results are being driven by an outlier subjects.

18. Given recent evidence for hemispheric specialization 8, and that the ipsilesional hemisphere seems to contribute only marginally to all statistical analyses (not only due to directly affected cortex but also due to widespread “subcortical” disconnections by the stroke lesions), at least for review purposes I encourage that authors to limit the analyses to the contralesional hemisphere. Findings would inform us about the influence of interhemispheric connectivity for changes in manifolds and outcome relationships. Can the authors show a lesion map?

Author reply:

This is an interesting suggestion, and one that we are happy to provide. As can be seen in the figure below, when we focused our analyses exclusively on the contralesional (left) hemisphere, we observed virtually the same left-hemisphere results as we had obtained when including both hemispheres. These findings add further support to the robustness of our findings presented in the main paper.



On the topic of lateralization, we should also note that NHPs might not exhibit the same degree of hemispheric specialization as humans for several reasons (e.g., evolutionary divergence and development of language and complex cognitive functions, structural differences the size and density of callosal connections, etc.). See some of the references below for more details on this.

Finally, with respect to the reviewer's request for a lesion map, we have included reference to the % overlap lesion map presented in Figure 1, which provides a clear visual representation of the affected areas in our subjects (Fig 1 A). We now indicate this with the following text change:

“Each animal's individual stroke mask was combined and averaged to produce a final group-average stroke template (See Fig 1A)”

References:

Rilling JK, Glasser MF, Jbabdi S, Andersson J, Preuss TM. Continuity, divergence, and the evolution of brain language pathways. *Front Evol Neurosci.* 2012 Jan 3;3:11. doi: 10.3389/fnevo.2011.00011. PMID: 22319495; PMCID: PMC3249609.

Rilling, J. K., & Insel, T. R. (1999). Differential expansion of neural projection systems in primate brain evolution. *NeuroReport*, 10(7), 1453-1459.

Aboitiz, F., Scheibel, A. B., Fisher, R. S., & Zaidel, E. (1992). Fiber composition of the human corpus callosum. *Brain Research*, 598(1-2), 143-153.

Hopkins, W. D., Tagliatela, J., & Meguerditchian, A. (2017). Evolution of Hemispheric Specialization in Primates. In J. H. Kaas (Ed.), *Evolution of Nervous Systems (Second Edition)*, pp. 413-422. Academic Press.

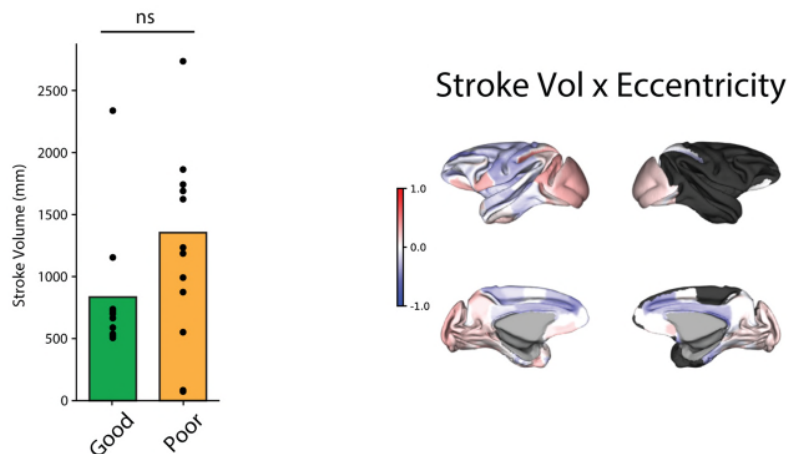
Corballis, M. C. (2009). The evolution and genetics of cerebral asymmetry. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1519), 867-879.

Phillips, K. A., Stimpson, C. D., Smaers, J. B., Raghanti, M. A., Jacobs, B., Popratiloff, A., Hof, P. R., & Sherwood, C. C. (2015). The corpus callosum in primates: processing speed of axons and the evolution of hemispheric asymmetry. *Proceedings of the Royal Society B: Biological Sciences*, 282(1818), 20151535.

19. Is stroke volume comparison influenced by one outlier? The authors state: “We observed no significant differences in lesion size between the good and poor recovery groups ($t(22)=-1.91$, $p=0.081$; see Supplementary Fig. 4), suggesting that differences in functional recovery, and the associated changes in brain connectivity patterns, cannot be solely explained as a difference in total stroke volume”. I have some doubts with this interpretation. An additional analysis looking for a correlation between [stroke volume ~ changes in ECC / stroke volume ~ follow-up ECC / stroke volume ~ changes in regional FC] might provide helpful insights in this issue.

Author reply:

We appreciate your concern about the possibility of an outlier affecting our stroke volume comparison. However, we should note that with the new subdivision of animals based on our much more principled fPCA analysis, the change in stroke volume between the good and poor groups is still non-significant ($t=1.63$, $p=0.11$, see new Supplementary Fig. 5 below). It is probably true that if you ‘removed’ a few data points from each group that you could then obtain a significant difference, but we would have no basis for performing such removal based on the data.



Next, the suggestion to explore correlations between stroke volume and measures of eccentricity is an important one to further ensure that stroke volume is not driving the changes in ECC. To address this, we have conducted additional analyses to examine the correlations between stroke volume and (1) Post > Pre changes in global eccentricity, and (2) Post-stroke (follow-up) global eccentricity. Critically, both of these analyses failed to reveal a significant relationship: 1) Correlation between Stroke Lesion Size and global change in eccentricity: $r = -0.14, p = 0.49$; 2) Correlation between stroke lesion size and Post-stroke global eccentricity: $r = 0.15, p = 0.49$. The lack of significant correlations suggests that the observed differences in functional recovery and associated changes in brain connectivity patterns at the whole-brain level cannot be solely attributed to variations in total stroke volume.

Additionally, we examined, for each brain region, the correlation between regional change in eccentricity (depicted in Fig. 3D) and total stroke volume. The results of this particular analysis are shown in the unthresholded statistical map in Supplemental Fig. 5 above (right). Critically, following FDR correction, no regions passed statistical significance for this region-wise. We believe this is a further important result that bolsters the present findings.

We have included these additional analyses in our supplemental material, and now also refer to these findings in our Results with the following text:

“We observed no significant differences in lesion size between the good and poor recovery groups ($t(22) = -1.63, p = 0.116$; see Supplementary Fig. 5). Furthermore, we observed no significant correlations between lesion size and the change in eccentricity for any single brain region (Supplementary Fig. 5), indicating that differences in functional recovery, and the associated changes in brain connectivity patterns, cannot be solely explained as a difference in total stroke volume. Rather, the findings indicate that the degree of post-stroke functional changes in transmodal cortical regions (e.g., PCC and OFC in particular) may be an important biomarker of spontaneous behavioral recovery.”

20. Finally, as a minor point, there is no need to include 10 refs++ per one sentence. Please be specific and only include highly suitable references after proper selection.

Author reply:

Thanks for this comment. This has been duly noted and implemented throughout.

References

1. Reibelt A, Quandt F, Schulz R. Posterior parietal cortical areas and recovery after motor stroke: a scoping review. *Brain Commun.* 2023;5:fcad250. doi: 10.1093/braincomms/fcad250
2. Idesis S, Allegra M, Vohryzek J, Sanz Perl Y, Faskowitz J, Sporns O, Corbetta M, Deco G. A low dimensional embedding of brain dynamics enhances diagnostic accuracy and behavioral prediction in stroke. *Sci Rep.* 2023;13:15698. doi: 10.1038/s41598-023-42533-z
3. Baldassarre A, Ramsey LE, Siegel JS, Shulman GL, Corbetta M. Brain connectivity and neurological disorders after stroke. *Curr Opin Neurol.* 2016;29:706-713. doi: 10.1097/WCO.0000000000000396
4. Olafson E, Russello G, Jamison KW, Liu H, Wang D, Bruss JE, Boes AD, Kuceyeski A. Frontoparietal network activation is associated with motor recovery in ischemic stroke patients. *Commun Biol.* 2022;5:993. doi: 10.1038/s42003-022-03950-4
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7. Siegel JS, Seitzman BA, Ramsey LE, Ortega M, Gordon EM, Dosenbach NUF, Petersen SE, Shulman GL, Corbetta M. Re-emergence of modular brain networks in stroke recovery. *Cortex.* 2018;101:44-59. doi: 10.1016/j.cortex.2017.12.019
8. Rohrig L, Rosenzopf H, Woehrstein S, Karnath HO. The need for hemispheric separation in pairwise structural disconnection studies. *Hum Brain Mapp.* 2023;44:5212-5220. doi: 10.1002/hbm.26445

Reviewer #2 (Remarks to the Author):

Summary:

Thank you for the opportunity to review this manuscript. The authors used functional neuroimaging in a non-human primate (macaque) model to explore the relationships between ischemic stroke, whole-brain connectivity and functional recovery. The authors show that changes in default mode network and limbic network, contribute to stroke behavioral recovery at 30 days. The findings are relevant for understanding the involvement of higher-order transmodal networks. Indeed, these findings are important to understand mechanisms of recovery post-stroke and must be explored further, in addition to existing sensorimotor regions.

Introduction:

Authors need to provide some context on why spontaneous behavioral recovery is important in the context of stroke recovery.

a) What implications does this have for treatment planning and interventions?

Author reply:

While current rehabilitation efforts often focus on sensorimotor cortex and motor control, our findings suggest that higher-order networks also play a significant role in recovery. Because the default and limbic networks are thought to sit atop the global processing hierarchy, our results in the context of stroke suggest that these regions might play a role in mobilizing cortical resources to improve motor outcomes following stroke. By understanding the role of higher-order transmodal networks in spontaneous recovery, we could potentially devise better therapies and treatments that directly target these particular brain networks. This could lead to the development of novel interventions aimed at promoting plasticity and functional connectivity in these areas, potentially improving outcomes for stroke patients.

In our Introduction section, we have now emphasized the potential implications of this work with the following text:

“Yet, the implications of these changes in higher-order transmodal cortex, in terms of patient impairment and contribution to spontaneous recovery, remain unclear. **Understanding the neural basis of spontaneous behavioral recovery in an important endeavor, as it not only establishes a baseline to distinguish natural improvements from those due to interventions, but also holds the potential to identify novel brain targets for rehabilitation and profoundly enhance therapeutic strategies.**”

b) Is there a distinction between true neurological recovery, spontaneous recovery and recovery through compensatory mechanisms?

Author reply:

This is a good question. In the context of the current work, True Neurological Recovery and Spontaneous Recovery are one in the same. It involves the repair or regeneration of damaged neurons and the re-establishment of neural pathways. Spontaneous recovery is the natural improvement in function that occurs without formal rehabilitation interventions. This type of

recovery typically happens in the early stages post-stroke and can be attributed to the brain's inherent neural plasticity. Mechanisms underlying spontaneous recovery may include resolution of initial edema, reorganization of neural networks, and natural healing processes.

By contrast, recovery through compensatory mechanisms involves the brain and body adapting to the loss of function by using *alternative* strategies or pathways to achieve a given outcome. For example, a patient may learn to use their non-dominant hand to perform tasks previously done with the affected hand or, alternatively, engage new brain circuits to accomplish certain behavioral functions. That is, while compensatory mechanisms do not restore the original intended function *per se*, they enable individuals to regain independence and improve their quality of life.

Given the complexity, and potential overlapping nature of these processes, we thought it important to highlight these differences (and the challenge in disentangling them) in our Discussion section. To this end, in our methodological limitations paragraph (at the end of the Discussion section) we now write the following:

“Finally, while our study has focused on spontaneous recovery, it is important to acknowledge that we cannot fully disentangle the extent to which this natural recovery reflects purely intrinsic neural rewiring processes versus more compensatory mechanisms involving the engagement of additional neural circuits to restore motor function. Future work will be required to distinguish between these different processes, which itself will be important for developing targeted interventions that can enhance both spontaneous and compensatory recovery pathways.”

c) Why did the authors choose to focus on spontaneous behavioral recovery is not clear.

Author reply:

Understanding spontaneous behavioral recovery is the critical first step in establishing a foundation for future intervention research. It allows us to identify the natural recovery processes, which is essential for distinguishing between improvements due to these processes and those resulting from specific interventions. This foundational knowledge is crucial for developing and evaluating effective therapeutic targets and strategies.

Another important aspect of understanding spontaneous behavioral recovery is its relevance to global health. Many individuals worldwide do not have access to comprehensive stroke rehabilitation interventions due to various barriers such as inadequate healthcare infrastructure, economic constraints, and geographical challenges (Feigin et al., 2022). In numerous low- and middle-income countries, specialized rehabilitation services are scarce, and the cost of these services is often prohibitive for many patients. By focusing on spontaneous behavioral recovery, our study aims to identify the brain's inherent recovery mechanisms. Understanding these mechanisms can aid in designing interventions that enhance or mimic natural recovery processes, potentially benefiting a broader range of patients. Furthermore, insights gained from studying spontaneous recovery can inform healthcare policies and practices, particularly in resource-limited settings, by emphasizing the importance of early detection and support for natural recovery processes.

As we noted in our response to point A above, we have revised the text in our Introduction to highlight the importance of focusing on spontaneous recovery for establishing a baseline of natural recovery.

Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, Fisher M, Pandian J, Lindsay P. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. *Int J Stroke*. 2022 Jan;17(1):18-29. doi: 10.1177/17474930211065917. Erratum in: *Int J Stroke*. 2022 Apr;17(4):478. PMID: 34986727.

Materials and Methods:

Line 521: change to 'FSL's FAST'. FAST is an abbreviation and hence should be capitalized.

Author reply:

Done.

Lines 617-625: It is not clear why the seed connectivity analyses was performed. Authors need to be more clear on how this analyses adds to eccentricity analyses. In addition, why significant differences in the good v/s poor outcome groups were observed for selected seed regions is not discussed.

Author reply:

We appreciate the opportunity to clarify this point. As we wrote in our Results, the goal of the seed-based functional connectivity analyses was to understand the changes in brain connectivity that ultimately underlie the changes in manifold eccentricity. We have now better clarified our rationale for this analysis with the following text changes:

“While significant shifts in the eccentricity of an individual brain region reflect alterations in its overall connectivity pattern, this metric does not clarify which specific aspect of its connectivity has undergone change. **That is, although changes in eccentricity are indicative of a change in how a brain region functionally interacts with other areas, it does not reveal the precise nature of these connectivity alterations.** To address this particular question, we conducted seed connectivity analyses...”

As for the significant differences in FC between the good vs. poor outcome groups, the reviewer is correct that while we did mention these results, we did not offer any interpretation of what the changes in connectivity mean. We have now included this interpretation in the text, along with the addition of some new analyses that highlight the increase versus decrease in connectivity with respect to the pre-stroke scan. In the text, we now write the following:

The results of the above analyses naturally beg the question as to the key differences in functional connectivity patterns of PCC and OFC regions that may account for the observed group differences (good versus poor recovery). To investigate this, we performed independent sample t-tests between the two groups on the pre- to post-change in connectivity of representative seed regions from Fig. 7A , and then corrected for multiple comparisons using FDR correction ($q < 0.05$). **For the left PCC seed region, we found that the good group had significantly higher post-stroke connectivity with several distributed brain regions including areas in the left medial frontal gyrus, dorsomedial and dorsolateral prefrontal cortex, right medial and ventrolateral prefrontal cortex, and bilateral anterior temporal cortex (see all red areas in Fig. 7C).** By comparison, we observed that the poor recovery group only had higher post-stroke connectivity in the right dorsomedial prefrontal cortex (see blue area in Fig. 7C). **Likewise, for the left subgenual seed region, we found that the good group had significantly higher post-stroke connectivity across several distributed regions including the left superior parietal cortex, lateral somatomotor cortex, DLPFC and anterior temporal cortex, as well as bilateral posteromedial cortex, retrosplenial cortex and visual cortex**

(see all red areas in Fig. 7D). By comparison, we observed that the poor recovery group only had higher post-stroke connectivity in the left temporal cortex and right dorsomedial prefrontal cortex (see blue regions in Fig. 7D). Note that when we averaged the changes in connectivity across the different significant regions for each of the PCC and OFC seed regions we found that, whereas in the poor group, these regions reflected an average decrease in connectivity with respect to the Pre-stroke scan (connectivity below zero), for the good group, these regions reflected an average increase in connectivity with respect to the Pre-stroke scan (connectivity above zero). That is, the connectivity of these different regions highlighted in Fig. 7C and D were increased in the good group following the stroke. Taken together, these seed-based results suggest that the success of animals' post-stroke behavioral recovery was related to increases in the integration of PCC and OFC areas with other brain areas; that is, animals that had better post-stroke outcomes (good group), tended to exhibit greater, and much more extensive connectivity between these PCC and OFC areas with other distributed brain areas.

Neurological assessment: The authors need to describe in further detail why the NHPSS was performed on the specific chosen days. Why was a 30-day recovery period chosen over a 90-day recovery period? Why was the NHPSS chosen over the Neurological Scale for MCA for NHPs or other outcome measures?

Author reply:

The Non-Human Primate Scoring System (NHPSS) was performed on the chosen days to align with key post-intervention milestones. These specific time points were selected based on preliminary studies and the typical progression of neurological recovery observed in similar experimental models [see references 1,2,3 below]. The 30-day recovery period was chosen based on previous studies that demonstrated significant recovery and outcome stabilization within this timeframe. Specifically, previous experiments in cynomolgus macaques with 90 min MCAO showed an initial increase in NHPSS scores, similar to human patients, and gradually declined and plateaued between 14 and 30 days, indicating that the behavioral impairment had become stable at one-month post stroke [1-3]. While a 90-day period could provide additional insights, the 30-day period allowed for a focused and resource-efficient assessment of the primary outcomes.

The NHPSS was selected for its comprehensive evaluation of neurological function and its suitability for the specific neurological deficits expected in our model. The NHPSS is akin to the NIH Stroke Scale in humans and is used to quantify stroke severity based on measures aspects of consciousness, grasp reflex, extremity movement (upper limbs and lower limbs), neglect, hemianopia, and facial weakness. Importantly, the NHPSS been previously validated in the exact animal model and stroke syndrome used in this study [1,2,3]. Furthermore, the NHPSS' evaluation of stroke deficits has been deemed comprehensive yet simple; however, one criticism is that the division of scoring rules may occasionally not be clear (which is why we had two independent raters) [4].

Importantly, the Neurological Scale for Middle Cerebral Artery Infarction has been shown to capture much of the same information as the NHPSS, suggesting that the two methods of assessing behavioural recovery are highly similar. However, the criticism of the Neurological Scale for Middle Cerebral artery infarction is that, while comprehensive, it is quite complex and highly resource-intensive – requiring professional testers and video re-evaluation, which was not an option in our study. Finally, it has been suggested that the NHPSS is perhaps more appropriate for models with a pterional approach to perform the MCA occlusion, which was the case in our study [4].

We appreciate the opportunity to clarify these points and we have now explicitly stated why the NHPSS was used in the current study in our Methods section:

Before and after the MCAO, the neurological functioning of each animal was evaluated using the Non-Human Primate Stroke Scale (NHPSS). **The NHPSS was selected as a recovery measure over other outcome measures for two main reasons: (1) It has been previously validated in the exact animal model used in this study^{43,44,46}. (2) It is a comprehensive outcome measure that is simple to administer (99).** This scale was applied at 8h and 12h post-stroke and on days 1, 2, 3, 4, 5, 7, 14, 21, 28, and 30, **as was performed in previous studies^{43,44,46,100,101,102}**

References:

- 1) Cook DJ, Tymianski M. Nonhuman primate models of stroke for translational neuroprotection research. *Neurotherapeutics*. 2012a;9(2):371-9.
- 2) Cook DJ, Teves L, Tymianski M. Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. *Nature*. 2012b;483(7388):213-7.
- 3) Roitberg B, Khan N, Tuccar E, Kompoliti K, Chu Y, Alperin N, et al. Chronic ischemic stroke model in cynomolgus monkeys: behavioral, neuroimaging and anatomical study. *Neurol Res*. 2003;25(1):68-78.
- 4) Lin X, Wang H, Chen J, Zhao P, Wen M, Bingwa LA, Jin K, Zhuge Q, Yang S. Nonhuman primate models of ischemic stroke and neurological evaluation after stroke. *J Neurosci Methods*. 2022 Jul 1;376:109611. doi: 10.1016/j.jneumeth.2022.109611. Epub 2022 Apr 26. PMID: 35487315.

Discussion:

The authors need to discuss why specifically MCAO strokes were correlated to less altered connectivity in DMN and limbic areas, and whether the specific connectivity patterns of these areas are related to the type of stroke. MCA territory strokes are the most common types of ischemic strokes, however, is there a correlation between MCA strokes and DMN/ limbic connectivity that has been discussed in the literature?

Author reply:

We appreciate the opportunity to elaborate on the correlation between MCAO (Middle Cerebral Artery Occlusion) strokes and the connectivity observed in the Default Mode Network (DMN) and limbic areas. Although previous studies have observed DMN alterations post-stroke (largely reduced activation), these studies have incorporated individuals with considerable heterogeneity in lesion locations. We could not find any specific literature on MCA strokes and their direct relationship to DMN/limbic connectivity.

Nevertheless, we can think of a few of potential reasons for why the connectivity of the DMN and limbic areas might be better preserved (or appear to be better preserved) at the day 30 post-stroke time point, and we have written a Discussion paragraph that deals with these different possibilities. We now write the following:

“In addition to the functional arguments above, there are at least a few other potential reasons for why the DMN and limbic areas in our study appeared to exhibit less altered connectivity 30 days post-MCAO stroke. Firstly, this may relate to the vascular distribution that these regions receive. MCA territory strokes predominantly affect the lateral aspects of the frontal, temporal, and parietal lobes due to the extensive distribution of the MCA. However, the DMN and limbic areas may exhibit relatively preserved connectivity because of their partial vascular supply from the anterior and posterior cerebral arteries. This secondary vascular distribution might contribute to the resilience observed in these networks post-MCAO. Secondly, many DMN and limbic regions are generally further neuroanatomically removed from the stroke location, which could potentially preserve their connectivity patterns more effectively. Lastly, an alternative possibility is that, rather than the DMN and limbic areas being less affected by the stroke, these regions may simply reinstantiate their patterns of connectivity more quickly during the 30-day recovery period. As noted above, this could be attributed to the lower myelin content of these regions^{53, 54, 55, 56, 57}, which is thought to enhance their capacity for neural plasticity. Together, these possibilities highlight the complex interplay between vascular supply, neuroanatomical distance from the stroke site, and intrinsic structural properties of the different cortical tissues in determining post-stroke connectivity patterns.”

Lines 345-348 and supplemental figure 4: The authors need to provide further explanation on why the total stroke volume was chosen as an indicator rather than the lesion voxel size. Did the authors track/notice changes in lesion volume progression over time?

Author reply:

We would like to clarify that, in our context, lesion voxel size and stroke volume are synonymous, as both metrics can be used interchangeably to quantify the extent of brain tissue affected by the stroke. Indeed, we calculated total stroke volume from the lesioned voxels. Note that using stroke volume allows us to standardize our measurements and provide a metric-system based assessment of the infarcted area.

We have added a line to the methods to make this clear:

“Finally, we also calculated the average stroke volume of each recovery group at 30 days to test whether lesion volume alone could explain the differences in animal recovery. Importantly, we calculated stroke volume for each animal by quantifying the lesion voxel size and multiplying it by the voxel dimensions (length x width x height), often in mm³.”

Regarding the tracking of lesion volume progression over time, this would require additional imaging sessions (beyond the pre- and post-stroke scans used in the current study). This is a goal for our future work.

Lines 375 - 378: The authors need to discuss how the generation of a broad stroke mask, excluding ipsilesional areas of stroke infarct in individual animals can potentially impact the interpretation of the results related to ipsilesional functional connectivity or discuss individual subject differences further/ add this as a limitation.

We acknowledge that excluding ipsilesional areas of stroke infarct in individual animals was a conservative analysis approach that might introduce variability in the data and potentially affect the assessment of functional connectivity within the lesioned hemisphere. To address this limitation, we have expanded the discussion in the manuscript to include a detailed examination of how

individual subject differences and the use of a broad stroke mask might influence our findings. Specifically, we have now written the following:

“Finally, the exclusion of ipsilesional stroke infarct areas when generating the stroke mask could potentially obscure or alter the interpretation of connectivity changes in the ipsilesional hemisphere. This exclusion may lead to an underestimation of the true extent of connectivity disruptions or compensatory mechanisms that occur post-stroke. We acknowledge that while the broad stroke mask approach simplifies analysis across subjects and affords direct comparisons, it may not fully capture the complexity of individuals’ lesion characteristics and their effects on functional connectivity.”

Remarks on code availability:

The code page is not opening/ unavailable. GitHub search did not yield any repositories.

We apologize, we have now provided a reference to the code availability:

https://github.com/jnash22/Stroke_Gradients

Reviewer #1 (Remarks to the Author):

The authors have thoroughly addressed all of my points.
Congratulations! I have no further comments.

Reviewer #2 (Remarks to the Author):

Introduction:

Line 60: Suggest dropping the word 'profoundly' as there is insufficient evidence to suggest whether making the distinction has directly impacted therapeutic intervention planning

Results:

Lines 180-194: The authors need to elaborate more on how the Euclidean distances were calculated to obtain manifold eccentricity. It is still not very clearly understood from the figures provided.

Reviewer #3 (Remarks to the Author):

It is an interesting paper. Choosing a patient-related animal model and using functional neuroimaging, authors explored how ischemic stroke affected whole-brain cortical architecture and its relation to spontaneous behavioral recovery. They found that several regions in both sensorimotor cortex and higher-order transmodal cortex exhibited significant shifts in their manifold embedding from pre- to post-stroke. Further analysis indicated that changes in default mode and limbic network regions were associated with animal behavioral recovery post-stroke. These findings were important to understand mechanisms of recovery post-stroke, however, several important questions need further analysis.

1. NHP Stroke Scale score (NHPSS) is a composite of ratings (consciousness, grasp reflex, extremity movement, etc) for monkeys, similar to National Institutes of Health Stroke Scoring (NIHSS) for patients with stroke. NIHSS scoring is widely accepted to evaluate neurological deficit for stroke patients in the acute stage. mRS is the most widely used scale for measuring the degree of disability or dependence in the daily activities of stroke patients. mRS is widely used in clinical trials. Thus, using a scale, which is similar to mRS, might be more suitable for the study using nonhuman primates to evaluate recovery (Stroke. 2020; 51: 1620-1623; Nat Protoc. 2022; 17: 2054-2084).

2. Monkey models recovered spontaneously after stroke onset. Recovery is also a complex process that might be influenced by many factors, including but not limited to initial damages, activities after stroke, microcirculation (both artery and vein), behavioural compensation, and true recovery (due to changes in local environments) [Nat Rev Neurosci 2009, 10:861-72; elife. 2024 30;12:RP90080; J Cereb Blood Flow Metab 2020; 40:3-22.]. Thus, it is difficult to define a spontaneous behavioral recovery (line 337).

3. It is also an important question what is the great value of findings in this study. I have a little suggestion to get stroke patients data and prove the findings in the study in patients. Although it might be difficult, it will be great interest to scientists and doctors in this field.

4. It is important to give a brief description that models have similar basic profiles, such as the blocking site (inducing MCA occlusion), infarct sizes, damaged brain regions, reperfusion status, NHPSS during first 7 days, ect.

We would first like to thank the reviewers for their helpful and constructive feedback. There is no doubt that the suggestions provided have substantially improved the manuscript. Our response will address the specific comments made by each reviewer point-by-point. Throughout our response, we will use the following format:

- (1) **black text in boldface is used when we directly quote the reviewers;**
- (2) black plain text is excerpts from the original manuscript;
- (3) black text is our response; and
- (4) red text is used when quoting from the revised manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have thoroughly addressed all of my points. Congratulations! I have no further comments.

Author Reply:

We are delighted and thank the reviewer for their positive comments. There is no doubt that their review of the manuscript has substantially strengthened the paper.

Reviewer #2 (Remarks to the Author):

Introduction:

Line 60: Suggest dropping the word 'profoundly' as there is insufficient evidence to suggest whether making the distinction has directly impacted therapeutic intervention planning

Author Reply:

We thank the review for pointing this out and have altered the text and removed the word “profoundly” to reflect this point, line 57-60 now states:

“Understanding the neural basis of spontaneous behavioral recovery in an important endeavor, as it not only establishes a baseline to distinguish natural improvements from those due to interventions, but also holds the potential to identify novel brain targets for rehabilitation and enhance therapeutic strategies.”

Results:

Lines 180-194: The authors need to elaborate more on how the Euclidean distances were calculated to obtain manifold eccentricity. It is still not very clearly understood from the figures provided.

Author Reply:

We appreciate the reviewer pointing this out, we have now included the relevant equations and descriptions in the document for clarity, line 743-747 now states:

“Briefly, for each brain region (N), the regional eccentricity (Ecc) was then defined as the square root of the sum of the squared differences between the coordinates of the individual brain regions and the centroid giving a length value for each region's vector from the origin (Eq. 1).

$$Ecc = \sqrt{(N_x)^2 + (N_y)^2 + (N_z)^2} \quad (1)$$

Reviewer #3 (Remarks to the Author):

It is an interesting paper. Choosing a patient-related animal model and using functional neuroimaging, authors explored how ischemic stroke affected whole-brain cortical architecture and its relation to spontaneous behavioral recovery. They found that several regions in both sensorimotor cortex and higher-order transmodal cortex exhibited significant shifts in their manifold embedding from pre- to post-stroke. Further analysis indicated that changes in default mode and limbic network regions were associated with animal behavioral recovery post-stroke. These findings were important to understand mechanisms of recovery post-stroke, however, several important questions need further analysis.

1. NHP Stroke Scale score (NHPSS) is a composite of ratings (consciousness, grasp reflex, extremity movement, etc) for monkeys, similar to National Institutes of Health Stroke Scoring (NIHSS) for patients with stroke. NIHSS scoring is widely accepted to evaluate neurological deficit for stroke patients in the acute stage. mRS is the most widely used scale for measuring the degree of disability or dependence in the daily activities of stroke patients. mRS is widely used in clinical trials. Thus, using a scale, which is similar to mRS, might be more suitable for the study using nonhuman primates to evaluate recovery (Stroke. 2020; 51: 1620-1623; Nat Protoc. 2022; 17: 2054-2084).

Author Reply:

We appreciate the reviewer's suggestion regarding the use of the primate Rankin Scale (pRS)[1], a primate adapted version of the modified Rankin Scale (mRS), for evaluating recovery in non-human primates (NHPs). We are familiar with the pRS and have recently adopted this scale in ongoing translational studies that aim to match conditions of planned clinical trials for pharmaceutical therapies. We agree that the pRS offers a direct correlate to the mRS, the most commonly used outcome for human stroke studies and regulatory applications. Similarly, the National Institutes of Health Stroke Scale (NIHSS) is frequently used as a stroke outcome in human studies and in regulatory applications and is an accepted measure of neurological outcome. The mRS measures the degree of disability or dependence after stroke whereas the NIHSS measures the neurological effects of stroke. The distinction between neurological effect/deficits and functional outcome are true of the NHPSS and pRS as well. NIHSS and mRS have been shown to correlate closely in human studies, demonstrating the relationship between neurological deficits [2].

Unfortunately, the present study data do not include pRS for comparison and retrospective extrapolation of pRS is not feasible. Our choice of the Nonhuman Primate Stroke Scale (NHPSS) in the present study was primarily driven by its previous validation in the specific stroke model used in our study [3,4,5]. The goal of the present study was to characterize the relationship between cortical manifold structure and behavioural recovery. The NHPSS captures neurological function and deficits with subsections for sensory, motor and brainstem function providing a more granular scale than the pRS. The NHPSS is required for the present study because tracking neurological deficits with sufficient granularity to detect change. Because the pRS score changes only when functional outcome shifts neurological recovery within a given domain without significantly affecting functional recovery the data would not be recorded.

We acknowledge the potential benefits of incorporating an mRS-like scale in future studies to complement the NHPSS. This dual approach would provide a more comprehensive evaluation of both neurological deficit and functional recovery in NHP models, enhancing our understanding of the relationship between brain function, neurological deficits and functional recovery post-stroke.

In our current study, the NHPSS effectively captured the changes we sought to measure and pRS was not measured. For ongoing and future research we are collecting both scales to provide a more holistic view of recovery processes in NHPs following stroke.

- 1) Wu, D., Wu, L., Chen, J., Huber, M., He, X., Li, S., ... & Ji, X. (2020). Primate version of modified rankin scale for classifying dysfunction in rhesus monkeys. *Stroke*, 51(5), 1620-1623.
- 2) Nimbvikar AA, Panchawagh S, Chavan AP, Ingole JR, Pargaonkar Y, Pai R. Modified rankin scale is a reliable tool for the rapid assessment of stroke severity and predicting disability outcomes. *J Family Med Prim Care*. 2024 Mar;13(3):1085-1090.
- 3) Cook DJ, Tymianski M. Nonhuman primate models of stroke for translational neuroprotection research. *Neurotherapeutics*. 2012a;9(2):371-9.
- 4) Cook DJ, Teves L, Tymianski M. Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. *Nature*. 2012b;483(7388):213-7.
- 5) Roitberg B, Khan N, Tuccar E, Kompoliti K, Chu Y, Alperin N, et al. Chronic ischemic stroke model in cynomolgus monkeys: behavioral, neuroimaging and anatomical study. *Neurol Res*. 2003;25(1):68-78.

We have now highlighted the potential utility of the primate Rankin Scale, line 602-604 now states:

“Despite the potential utility of other neurological scoring system, including the primate Rankin Score and Neurological Scale for Middle Cerebral Artery Infarction^{102,103,104}, the NHPSS was selected as a recovery measure over other outcome measures for two main reasons: (1) It has been previously validated in the exact animal model used in this study^{43, 44, 46}. (2) It is a comprehensive outcome measure that is simple to administer⁹⁹.”

2. Monkey models recovered spontaneously after stroke onset. Recovery is also a complex process that might be influenced by many factors, including but not limited to initial damages, activities after stroke, microcirculation (both artery and vein), behavioural compensation, and true recovery (due to changes in local environments) [Nat Rev Neurosci 2009, 10:861-72; elife. 2024 30;12:RP90080; J Cereb Blood Flow Metab 2020; 40:3-22.]. Thus, it is difficult to define a spontaneous behavioral recovery (line 337).

Author Reply:

We agree that recovery post-stroke is influenced by multiple factors. In our updated manuscript, we have revised the discussion on spontaneous behavioral recovery to reflect this complexity. We have included the additional references you suggested and clarified that our definition of spontaneous recovery encompasses these multifaceted processes. This amendment emphasizes that our observations of behavioral recovery are likely the result of a combination of these factors rather than a singular spontaneous event and that future studies will be required to disentangle these factors.

We have now highlighted these points on lines 564-568 now states:

“Finally, while our study focuses on spontaneous recovery, it is important to acknowledge that this process is influenced by numerous factors including initial damage, post-stroke activities, microcirculation, and behavioral compensation, making it difficult to distinguish between intrinsic neural rewiring and compensatory mechanisms involving additional neural circuits.”^{99,100,101}”

3. It is also an important question what is the great value of findings in this study. I have a little suggestion to get stroke patients data and prove the findings in the study in patients. Although it might be difficult, it will be great interest to scientists and doctors in this field.

Author Reply:

We appreciate your suggestion to validate our findings in stroke patients. We agree that translating our results from NHP models to human patients would significantly enhance the impact of our study. However, obtaining pre-stroke functional imaging data from human subjects is exceedingly difficult due to the non-predictability of stroke onset except in rare complications following cerebrovascular and cardiac procedures. It is out of the scope of the current project and would add substantial time to the current study. One of the strengths of our study and our use of an animal model is the availability of pre-stroke fMRI data, which is rarely obtainable in human studies. This aspect underscores the uniqueness and strength of our research.

We recognize that projects like the UK Biobank, which aim to longitudinally track subjects, may eventually help in obtaining such pre-stroke data in humans, providing valuable insights into the progression and recovery from stroke. We plan to design future studies aimed at validating our findings in clinical settings. This will involve collaborating with clinical researchers and leveraging existing patient data to draw parallels between NHP and human stroke recovery mechanisms.

4. It is important to give a brief description that models have similar basic profiles, such as the blocking site (inducing MCA occlusion), infarct sizes, damaged brain regions, reperfusion status, NHPSS during first 7 days, ect.

Author reply:

Thank you for your comment regarding the basic profiles of our models. We appreciate the importance of providing clear and comprehensive methodological details. A comprehensive review of the specific model presented in our Methods/Results section (**Methods: 582-600; Results: lines 81-91**). If certain aspects of these descriptions are insufficient or unclear we would greatly appreciate more specific guidance on which areas need elaboration or what additional details would be most valuable to include. We're committed to improving the quality and clarity of our manuscript and look forward to your further input.