nature portfolio

Peer Review File

Local homeostasis preserves global neural dynamics compensating for structural loss during human lifespan aging

Corresponding Author: Dr Arpan Banerjee

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Comments to the paper “Identifying the multi-scale neurocomputational principles to preserve functional integration in cortical networks across healthy lifespan aging trajectories”

Saha et al. investigate how the aging brain preserves functional coordination despite structural and neuromolecular changes, using biophysically grounded whole-brain modeling across three large neuroimaging datasets. By estimating GABA and glutamate levels through advanced model inversion techniques, they demonstrate that slow, adaptive tuning of these neurotransmitters helps maintain functional integration over the lifespan. Their findings suggest that a reduction in glutamate levels plays a crucial role in preserving whole-brain functional connectivity, offering a mechanistic explanation for previously observed age-related reorganization in functional connectivity.

The significance of using whole-brain models to probe aging-related neurocomputational changes cannot be overstated, as they move beyond traditional correlation analyses to uncover causal and mechanistic principles underlying brain function. However, as with any modeling approach, it is essential to acknowledge inherent limitations—models are only as reliable as the assumptions they rest upon. As George Box famously stated, "All models are wrong, but some are useful." My critique primarily stems from this perspective, aiming to refine and strengthen an already compelling contribution to our understanding of brain aging.

I invite the authors to consider answering the following comments.

Major comments

1 – Indeed, the working point of the brain is very important for optimal dynamics as they state several times in the introduction. And this applies even more in the context of the model. In whole-brain models this is ensured by tuning the global coupling parameter G. However, it is not clear from the manuscript what this parameter has been set to. This is a fundamental point as any results derived from the models will be dependent on this working regime set by the G parameter. Could the authors elaborate on what they chose to be the coupling? I would expect the following parameter search for the FCD and metastability in line with the following paper Figure 2A - https://www.science.org/doi/full/10.1126/sciadv.abf4752

2 – Related to the previous point. I strongly suggest for the authors to provide the functional connectivity matrices (FC) as it will allow for visual inspection on how far away the empirical and simulated data are from each other. As above, in line with the following paper Figure 2B - https://www.science.org/doi/full/10.1126/sciadv.abf4752

3 – The whole argument of the paper is not entirely clear. The way I understand it is that you posit that during aging the working point of the brain (indexed by metastability) is preserved while the SC and FC undergo changes (in terms of segregation, integration and resilience) to support this working regime. Moreover, this adaptive reorganisation is done by changes to the glutamate and gaba levels. Would that be a correct summary? If so, I really recommend turning the results around. Ie show that metastability does not change at the empirical level, while the FC and SC changes do change. And then fit the model and show that these changes are driven by the glutamate and gaba levels. Otherwise, the story seems

very convoluted.

4 – The same as point 3 applies to the first paragraph of Results. Why fitting the model first? First investigate empirical results, validate the models and then analyse the causal mechanism?

5 - I would also recommend expanding the abstract as many of the results are not reflected in there.

6 – “The metric of static functional connectivity (FC) essentially provides a temporally averaged snapshot of all covariations pairwise between brain areas and hence, once averaged across the entire brain one can consider it as a summary measure of spatial complexity.”
I am not sure we can talk of spatial complexity in this context. FC describes the connectivity profiles of different brain states. If you wish to talk about spatial complexity, I suggest to calculate the following measure that defines the normalised histogram entropy of the FC distribution as a measure of functional complexity.
https://www.nature.com/articles/srep38424

7 – Authors should add how it relates to dementia and other disorders related to ageing.

See for example - https://www.biorxiv.org/content/10.1101/2023.04.20.537688v1.abstract
And
https://www.sciencedirect.com/science/article/pii/S2213158217301961
and
https://elifesciences.org/articles/83970

Minor Comments

1 – I would not call functional connectivity distance FCD as it confuses the more established FCD fitting term functional connectivity dynamics (see again https://www.science.org/doi/full/10.1126/sciadv.abf4752 figure 2). Perhaps you can use mseFC or edge FC.

2- This is a results statement but in introduction “Indeed, we also observed that connection probability changes with age (Fig. 1(b)) for the Cambridge aging Neuroscience (Cam-CAN) cohort used in the present study.”

3- it should be obvious from the summary figure 1 what Tglu and Tgaba represent in the model. I strongly suggest to add the clear model description and mention what is being manipulated in the figure similar to figue 1 here
https://www.science.org/doi/full/10.1126/sciadv.abf4752

4 – some parts of results are more for introduction or methods. See for instance section 2.2.6. Metastability indexes.. read a lot like methods or introduction

5 -maximal whole brain fluidity? – do the author mean metastability?

6- not sure the word “inclement” is used correctly. Perhaps rephrase

Reviewer #2

(Remarks to the Author)
The paper aims to uncover the neurocomputational principles that allow the human brain to preserve functional integration despite structural decline during healthy aging. Using a multi-scale dynamic mean field (MDMF) model, the study investigates how glutamate and GABA levels adjust over the lifespan to maintain critical neural dynamics. The results, derived from three large neuroimaging datasets, reveal that while structural connectivity declines with age, functional connectivity remains stable due to compensatory mechanisms. Notably, the study finds that glutamate levels decrease in older adults while GABA remains relatively unchanged, leading to a shift in the GABA/Glutamate ratio that helps sustain functional network integration. Additionally, metastability— a measure of dynamic brain coordination— remains unchanged, suggesting that the aging brain maintains an optimal working point through neurotransmitter regulation. These findings support the neurocompensation hypothesis, emphasizing that the brain dynamically adjusts its excitatory-inhibitory balance to counteract age-related structural decline. The study’s computational framework provides a novel tool for investigating brain aging and potential applications in tracking neurodegenerative diseases and cognitive decline. Despite all the strengths, the research has some serious concerns, which might be considered for the next review step if the authors can address below issues.

1- The paper would benefit from a thorough restructuring to enhance readability, logical flow, and clarity in its scientific argument. The introduction should focus on providing a "general background" on brain aging and functional compensation, avoiding dense technical details. It should clearly state the study’s motivation and hypothesis while ensuring a smooth transition into the methods. The methods section should explicitly define key concepts, outline the computational model, and

describe how neurotransmitter levels were inferred, without assuming prior knowledge. The results section should present empirical findings objectively, avoiding repetition of model assumptions and limiting comparisons with past studies—these should be reserved for the discussion. The discussion should then summarize key results, contextualize findings within existing literature with a more detailed review of relevant studies, and provide broader theoretical interpretations. Additionally, this section should include a clear limitations subsection, discussing potential biases, methodological constraints, and areas requiring further validation, followed by future research directions and a concise conclusion. These changes would ensure each section functions independently and cohesively, reducing redundancy and improving the paper's readability. It can be ordered as, introduction, methods, results, discussion.
2- The study infers glutamate and GABA levels rather than directly measuring them. The model predicts neurotransmitter changes, but these are not validated against empirical MRS (magnetic resonance spectroscopy) data. How confident can we be that the inferred neurotransmitter concentrations reflect actual biological levels? Have you considered validating these findings with MRS or other neurochemical imaging techniques?
3- GABA and glutamate are not uniformly distributed across the brain, yet the model treats them as global parameters. Also, the functional network alterations have been explored globally. I wonder how the authors address these differences.
4- The study categorizes aging into two broad groups (18-34 and 60-85), which may oversimplify the gradual changes occurring in the brain. Would a continuous lifespan analysis (e.g., using regression models across age as a continuous variable) provide a more comprehensive view of how neurotransmitter levels and functional integration change over time?
5- The study focuses heavily on white matter loss but does not address cortical thinning or synaptic density reduction, which are also known to impact brain function in aging. How might cortical atrophy or synaptic pruning contribute to functional changes observed in aging? Could these be incorporated into the model?
6- The binarization of functional connectivity (FC) matrices depends on arbitrary threshold choices, which can significantly affect network metrics. How did you determine the optimal threshold for binarizing FC? Would alternative thresholding methods (e.g., proportional thresholding) lead to similar conclusions? Why weighted graph measures have not been used?
7- The study does not mention whether the MDMF model code or datasets will be made available for replication. Do you plan to share the code and data for others to validate and extend your findings?
8- The title is somewhat vague and overly broad, e.g. the term "multi-scale neurocomputational principles" does not clearly indicate whether the focus is on neurotransmitter regulation, functional connectivity, or dynamic interactions. A more precise title ensure that readers immediately grasp the study's main contribution. The abstract, while scientifically detailed, can be improved by providing a clearer statement of the hypothesis and ensuring that the results are stated more solidly rather than being implied. Additionally, it should state clearly how the research addresses the gap in the field. Lastly, the final sentence can emphasize the study's broader potential. A stronger title and a more structured abstract would significantly improve the paper's impact and readability.

Reviewer #3

(Remarks to the Author)
Saha et al. used a multiscale Dynamic mean field (MDMF) modeling approach to examine how (healthy) aging brains can preserve their functon(metastability) amidst the degradation of long-range white matter tracks. Through model parameter fitting, the authors have concluded that glutamate concentration reduction as a function of age is the primary explanation for the preservation of metastability in healthy aging, i.e., as a compensatory mechanism against gradual age-related degradation of long-range structural connectivity. Overall, I found the paper theoretically interesting, well-written, and mindful of the rigor (i.e., results are validated across different datasets and subsets). Nevertheless, some technical clarifications are needed before the manuscript is ready for publication. I also have other suggestions for improving the readability of the manuscript. Detailed comments are below:

1. The results section begins with the MDMF modeling (sec 2.1) and ends with MDMF modeling results (sec 2.3), with a large portion of empirical SC, FC analysis (no modeling) in between (sec 2.2). I find this organization confusing, and it is not always clear how results in sec 2.1 relate to those of sec 2.3.
a. Is the result in Figure 2G, i.e. contrast between glutamate and gaba concentration across ago groups, significant? If not, how do you reconcile the lack of significance here with the significant individual-level modeling results shown in Figure 7?
b. I think it'd be more helpful to put the network metrics of the empirical data and that of the model FC results side-by-side (e.g., merging figure 3 and 8). It would also make sec 2.2 feel lack disconnected (right now it feels like a traditional MRI paper inserted into the middle of a modeling paper).
2. Sec 2.1, line 1: FCD appears for the first time – the full name needs to be spelled out. The same with T_glu and T_gaba. The author should briefly mention the biophysical interpretation of these parameters and point to more details in methods and supplementary materials.
3. Figure 2 I, J: the star for the optimal parameters is very small, please make it bigger. There seems to be a lot of degeneracy in the model, i.e., a lot of combos of (T_glu, T_gaba) seem to have the same FCD and metastability. Please discuss why one should expect the model to converge to (near) a unique solution.
4. Section 2.2.1, 2.2.2 title: I would suggest using the language of "long-range segregation" and "long-range integration" since, by definition, modularity, and clustering coefficients imply there is high integration locally despite large-scale segregation.
5. Section 2.2.4., 2.2.5.: I appreciate the analysis, but moving them to supplementary materials would make the paper more readable (unless other reviewers strongly demand them to stay here).
a. 2.2.5: what does "consistency" refer to here? Is it consistency with specific p values considered as ground truth or between datasets? Without more clarification, I am not sure what the "inconsistency" in Figure 5b really implies.
6. The definition of transitivity: the definition in p13 (sec 3.1.2.ii) is different from the usual definitions that I have seen.

Especially, it's different from the brain connectivity toolbox code. I'm also baffled why the modularity and transitivity should be anticorrelated in your results. Please double check.
7. Figure 6: Here, the authors essentially argue that the null hypotheses are true. In this case, the authors should show Bayesian statistics to support the null hypothesis (JASP is a statistical software that is really easy to use for this purpose).
8. Section 3.1.1: there's a corrupted symbol in the FC definition. Please check.
9. Section 3.2.1:
a. would be helpful to mention that the first to fourth equations in (1) is exactly the same with Deco et al 2014, since various work in the community use it as base model.
b. Is the global coupling here G fitted? If not, please discuss how it would affect your interpretation of T_glu and T_gaba.
10. Regarding metastability:
a. A lot of people are using standard deviation of kuramoto parameters (SDKP) as a measure of metastability on fMRI data. The problem is that SDKP is only a meaningful measure of metastability when the underlying dynamics are rhythmic, and there is no such rhythmicity in the fMRI data. One can pretend there is such rhythmicity by applying a very narrow band filter on nonrhythmic data, but this should at least be discussed as a limitation.
b. The fact that SDKP cannot differentiate between metastability and noisy multistability should also be discussed.
c. I did not find the info on how model BOLD signal was filtered prior to computing SDKP. Please clarify.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
2nd round of comments to the paper "Neurocomputational principles of brain aging: Local homeostasis preserves global neural dynamics compensating for structural loss"

Thank you to the authors for addressing many of my concerns.

I wish to further highlight some of the remaining ones considering their responses.

Related to my second point on plotting the empirical and simulated FC and FCD fits. The fact that the authors reproduce the results in three independent datasets is strong, they might want to double-check the NKI dataset. From the image they provide it looks far away a from typical connectivity structure (no left, right hemispheric connectivity or visible homotopic connections in the interhemispheric part). Unlike the other two datasets where the FC structure can be clearly seen. As it stands, I would not distinguish it from a randomly generated FC matrix. Could the author double-check or comment on why would that be? Moreover, consulting the SI about the NKI dataset brings further confusion as it is not clear what parcellation has been used or why two different parcellations might have been used (Craddock and AAL). This needs to be done impeccably as the rest of the analysis builds on the data. I strongly suggest for the authors to review it.

This point further relates to the FCD plots - why only ~80-100 timepoints? For example, for the berlin dataset there are about 22 minutes of TR = 2? All these points should be consistent between the methods and plots else it creates at best a confusion in the analysis process.

Similarly, it applies to the new Figure S8. Did the authors flip the FC axis? As it seems now the interhemispheric connectivity is in the top left and bottom right quadrants which is not the case in figure 4…

Reviewer #2

(Remarks to the Author)
Thank you for the thoughtful and detailed revisions. The paper is now much clearer and better organized. My main concerns from the first review, especially about structure, methods, and interpretation, have been addressed as well. I appreciate the revision. The paper makes a strong and valuable contribution to the field, and I support its publication.

Reviewer #3

(Remarks to the Author)
My concerns have been well addressed. I am a little puzzled by why the FC of NKI dataset is so featureless (Figure 4d, second row) -- maybe the authors can make a quick clarification.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I have no longer comments.

Response to Reviews

We extend our sincere thanks to the editors and reviewers for their invaluable suggestions and insightful comments, that helped improve our manuscript. We carefully addressed each of the reviewers' comments point by point. Based on their suggestions, we have made a major overhaul of the main text with particular attention to rewriting the abstract, introduction, and method sections for improved clarity and delineating the scientific impact of our findings. In the revised version, we have thoroughly revised and reorganized the results section, revised figures and reproduced results. Further to this, we have also undertaken new analysis following reviewer recommendations.

In the revised manuscript, we have highlighted our revisions in the red-colored text. Below, we also provide a summary of major revisions carried out in the revised version of the manuscript.

1. The title has been changed.

2. Figure 1 is revised following the reviewer's suggestions for a clear description of our computational framework.

3. Figure 2 (in the previous version; **Fig. 4** in the revised text) is placed in the Model simulation section after the empirical data analysis. We redraw the figure while including the empirical and simulated static FCs as well as FC dynamics (FCD).

4. In **Figure 6** (previously **Fig. 8**), we have included results on empirical FCs on top of the model-based FC properties for better visualization and comparison.

5. Figure 4,5,9 (in the previous version), has now moved to supplemental materials; see **Figs. S2, S3, S7**.

6. The abstract and Introduction sections are revised according to the revised narrative.

7. We have rearranged the "**Results**" section in the revised manuscript. Empirical metastability and brain network properties are placed first. Next, model-based observations on group-averaged results and participant-wise results are shown. Finally, model evaluation is presented.

8. In the revised version, the "**Methods**" section is placed after the introduction section as per the reviewer's recommendation.

9. A new subsection describing how we generated simulated-BOLD signal is added, see **Section "2.6 Simulated BOLD activity".**

10. Presenting new results of **coupling strength (G)** dependency on the parameter estimations. The results are incorporated in the supplementary materials in **Fig. S8**.

Below we provide point-by-point responses to the Reviewers' comments in the same order as it appeared.

Reviewer 1:

Comments to the paper "Identifying the multi-scale neurocomputational principles to preserve functional integration in cortical networks across healthy lifespan aging trajectories"
Saha et al. investigate how the aging brain preserves functional coordination despite structural and neuromolecular changes, using biophysically grounded whole-brain modeling across three large neuroimaging datasets. By estimating GABA and glutamate levels through advanced model inversion techniques, they demonstrate that slow, adaptive tuning of these neurotransmitters helps maintain functional integration over the lifespan. Their findings suggest that a reduction in glutamate levels plays a crucial role in preserving whole-brain functional connectivity, offering a mechanistic explanation for previously observed age-related reorganization in functional connectivity.

The significance of using whole-brain models to probe aging-related neurocomputational changes cannot be overstated, as they move beyond traditional correlation analyses to uncover causal and mechanistic principles underlying brain function. However, as with any modeling approach, it is essential to acknowledge inherent limitations—models are only as reliable as the assumptions they rest upon. As George Box famously stated, *"All models are wrong, but some are useful."* My critique primarily stems from this perspective, aiming to refine and strengthen an already compelling contribution to our understanding of brain aging.

I invite the authors to consider answering the following comments.

Response: We sincerely thank you for your thorough understanding of our work. We tried to address all your concerns in the revised manuscript. Please find our detailed responses to your comments below.

Major comments

1 – Indeed, the working point of the brain is very important for optimal dynamics as they state several times in the introduction. And this applies even more in the context of the model. In whole-brain models this is ensured by tuning the global coupling parameter 'G'. However, it is not clear from the manuscript what this parameter has been set to. This is a fundamental point as any results derived from the models will be dependent on this working regime set by the 'G' parameter. Could the authors elaborate on what they chose to be the coupling? I would expect the following parameter search for the FCD and metastability in line with the following paper Figure 2A - https:// www.science.org /doi /full /10.1126 /sciadv.abf4752

Response: Thanks to the reviewer for raising this point. It is indeed a very crucial point. In the revised version, we have now plotted the FC-FC correlation and FC-FC distance against coupling strength *'G'* to display the optimal values. The *'G'* dependency on the parameter estimation is also added to the supplementary material **Fig. S8**. We have shown that simulated FC is a better fit to empirical FC at optimal G. Although, an almost similar pattern of age-related changes is observed for different G values, other than optimal G, either no statistical significance is observed or the observations are reversed in older adults. Additionally, the simulated FC is not a best fit, and excitatory firing activity fluctuates at a much higher rate when G is higher than the optimal value.

We have mentioned how we selected "G" in the '**Method'** section -- **2.5 Multi-scale Dynamic Mean Field (MDMF) model**, **page 8, lines 287-294:**
"The two free parameters, Tglu and Tgaba, capturing the neurophysiology of the excitatory and inhibitory process, are algorithmically adjusted to fit the model generated FC and empirical FC. For a fixed glutamate and GABA concentration, we select coupling strength G for which simulated and empirical rsFC distance is minimum and their correlation is maximum, while maintaining a firing rate approximately 3-4 Hz Bittner et al (2017). The fitting of G and its effects on the parameter estimation is shown in Supplemental material Fig. S8."

The **supplementary material Fig. S8** illustrating the effects of coupling strength ***G*** on parameter estimation and firing rates, below:

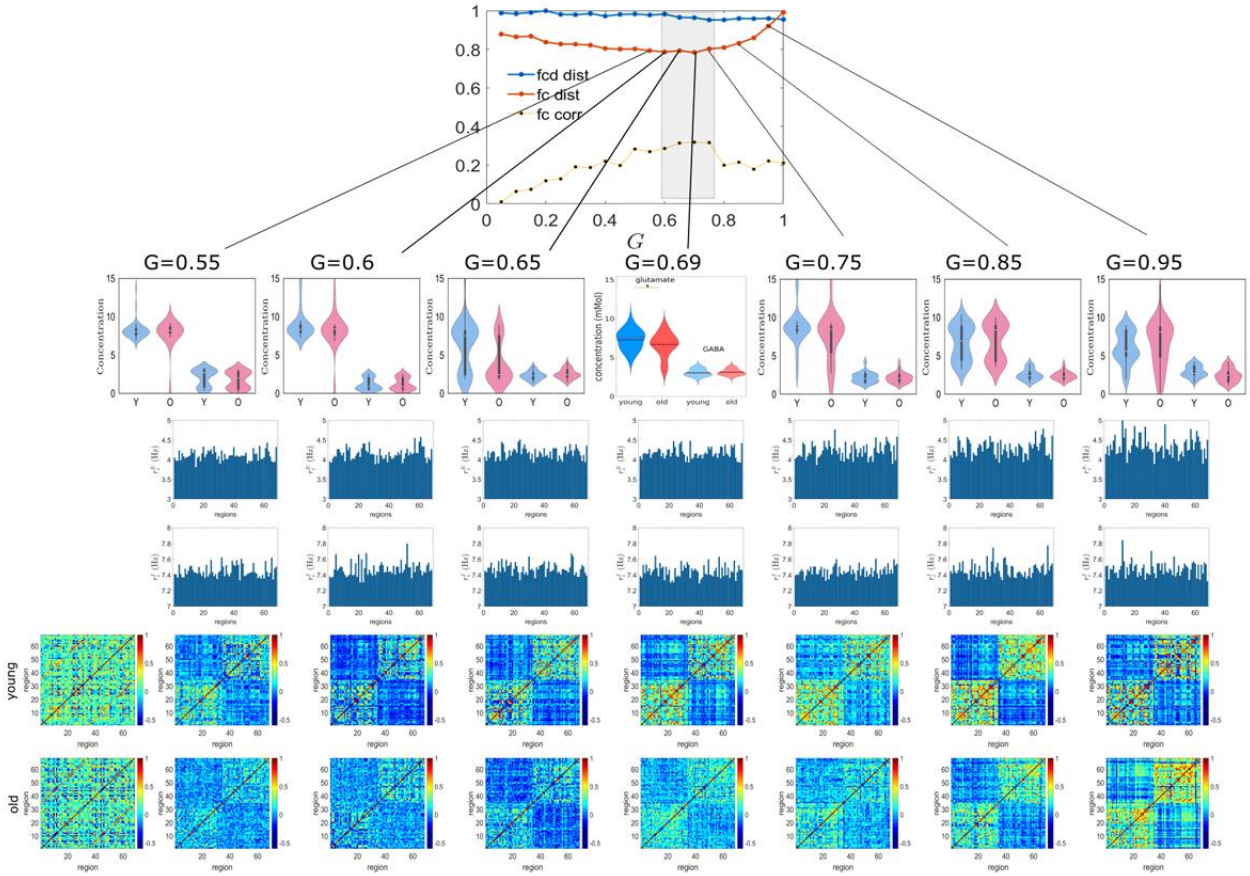

Figure S8. Effect of coupling strength (G) on parameter estimation and firing rates. The results are produced in Cam-CAN data.Top panel shows fitting of the coupling strength based on the distance and correlation between static FCs, and also dynamic FC distance. We have selected G=0.69 for our analysis in Cam-CAN data. Second row shows optimal GABA/Glutamate estimated at different G values. In the shaded range, we observe similar pattern of age-related neurotransmitter changes. Excitatory and inhibitory firing rates obtained for a single participant are plotted in the third and fourth rows, respectively. Fifth and last rows present empirical and simulated FCs from one young adult and elderly participant generated for different G values. Simulated FC is not a best fit and excitatory firing activity fluctuates at much higher rate when G is higher than optimal value.

2 – Related to the previous point. I strongly suggest for the authors to provide the functional connectivity matrices (FC) as it will allow for visual inspection on how far away the empirical and simulated data are from each other. As above, in line with the following paper Figure 2B - https:// www.science.org /doi /full/ 10.1126 /sciadv.abf4752.

Response: Empirical and simulated FCs are now presented in **Fig. 4(d)** (revised manuscript, **page 14**). The simulation is performed for the optimal parameter choices corresponding to the three distinct datasets.

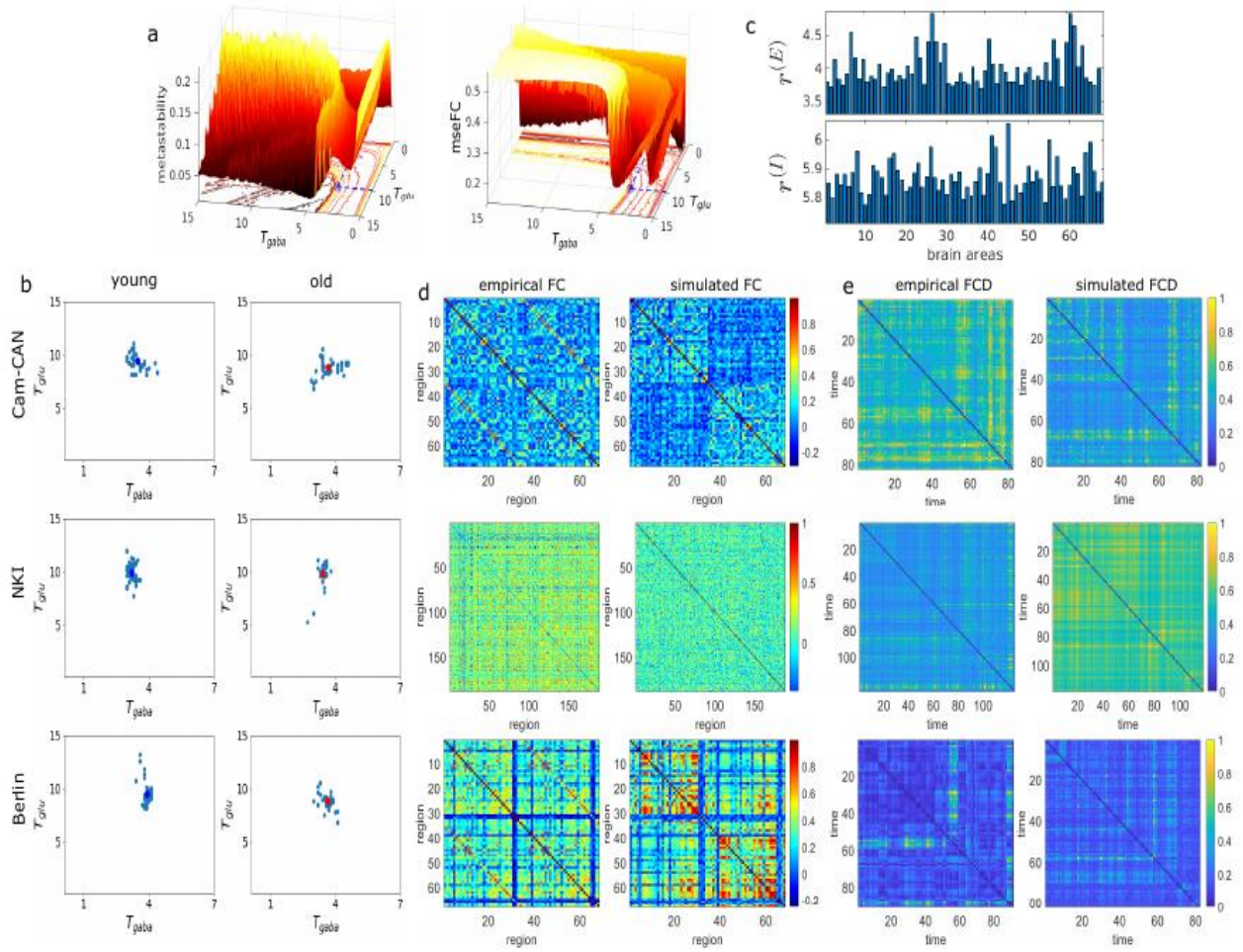

Figure 4 Model-based results obtained from averaged subjects: parameter estimations, firing and FCs. First, the SCs of individual groups are averaged, and then independent simulations are performed. (a) The 3-D maps of metastability and mean-square root-error between FCs (mseFC) are plotted on Tgaba − Tglu parameter plane. The blue stars, marked on the 2D-parameter plane, indicate optimal Tglu, and Tgaba values. (b) Clouds of estimated parameters are shown for young adults and elders, produced by 50 independent simulations. (c) Regional excitatory and inhibitory firing rates are plotted for the estimated Tglu, and Tgaba values. (d) Empirical and simulated FCs are plotted using estimated parameters, taking average over trials. (e) Empirical and simulated FC dynamics (FCD) are shown. The simulated results are produced using estimated parameter set.

3 – The whole argument of the paper is not entirely clear. The way I understand it is that you posit that during aging the working point of the brain (indexed by metastability) is preserved while the SC and FC undergo changes (in terms of segregation, integration and resilience) to support this working regime. Moreover, this adaptive reorganisation is done by changes to the glutamate and gaba levels. Would that be a correct summary? If so, I really recommend turning the results around. Lets show that metastability does not change at the empirical level, while the FC and SC changes do change. And then fit the model and show that these changes are driven by the glutamate and gaba levels. Otherwise, the story seems very convoluted.

Response: Our sincere thanks to the reviewer for such invaluable suggestions and advice. Indeed, this is the main message of the paper. Please refer to the list of summary for the changes made in this revised version. Also, please find below a short summary of the revision made in the current version after reorganizing and rewriting the texts in Introduction and Results sections. Please see below an excerpt from all the changes made in the revised version.

Introduction: The first paragraph reviews the age effects on the brain. The second paragraph describes the age-associated structural/functional markers identified by graph theoretical metrics. The third paragraph

describes coordinated dynamics using the standard measure of metastability that conceptualizes the optimal working point of the brain and connects to the homeostasis regulation. The fourth paragraph establishes connections among the structural connectivity, controlling parameters, neural dynamics and reorganized functional connectivity with age and identifies knowledge gaps. The last paragraph discusses the objectives of identifying computational principles of the healthy aging process and possible solutions to address the aim.

Results section**:** First, we present the empirical metastability, graph properties of structure/function connectivity to examine age-related changes from neuroimaging data.
3.1 Dynamic working point: empirical metastability, page 10 line 402.
3.2 Alterations in brain networks with age, page 11 line 414.
3.3 Summary of observations from empirical data, page 13, line 504.

Next, we presented model-based results, and described the computational principles of the compensation for structural loss in the aging brain--
3.4 Model based observations, page 13, line 517.
The model simulation has been described in three subsections:
3.4.1 Simulation results: Optimal parameter selection and consistency, **page 13, line 518.**
In this subsection, the results on averaged subjects from individual groups are shown in **Fig. 4** (in revised text, **page 15**). This describes that for an optimal parameter set, the model fits well to the empirical FCs and reproduces metastable. We checked the consistency of the estimated parameter values (GABA, glutamate). We conducted 50 independent runs, while keeping all other computational settings fixed. A cloud of simulation-based GABA, and glutamate values are observed.
3.4.2 The MDMF model-based glutamate-GABA concentrations, **page 14, line 534.**
In this second part, participant-wise parameter estimation is presented, see **Fig. 5**, **page 15**.
3.4.3 Model performance evaluation: Graph theoretical metrics computed from simulated FC, page 15, line 576.
In this part, the model performance has been evaluated through qualitative comparisons between the empirical and model-based FC properties, please find **Fig. 6, page 17**.

4 – The same as point 3 applies to the first paragraph of Results. Why fitting the model first? First investigate empirical results, validate the models and then analyse the causal mechanism?

Response: We completely agree with your suggestions. We have now placed the **Fig. 2** in the model simulation subsection (**Fig. 4 in the present version, page 14**) after the results from the empirical data analysis.

5 - I would also recommend expanding the abstract as many of the results are not reflected in there.

Response: Thank you for the suggestion. We have revised the **Abstract**, page 1, lines 16-32.

"Here, we seek to identify a key role of neurotransmitter in shaping brain-wide complex dynamics despite structural losses, in particular, how GABA/glutamate upholds the desired neural dynamics quantified by invariant metastability across adulthood aging. We employ biophysically-grounded multiscale modelling, constrained on human brain's structural connectivity, where the two neurotransmitters are tuned to regulate local homeostasis. The two free parameters are estimated from the state-of-the-art model inversion strategy, enabling the model to produce age-related FC changes consistent with empirical observations. We observed stable GABA and reduced glutamate as a key dynamical mechanism to support emergent neural dynamics in aging brain, subsequently reorganizes functional connectivity as evidenced from graph theoretical markers. Results are validated in three distinct datasets. The study offers an

operational framework integrating brain networks with metabolites to constrain models of large-scale brain function, providing a tool to investigate neural disorders with disrupted homeostasis."

6 – "The metric of static functional connectivity (FC) essentially provides a temporally averaged snapshot of all covariations pairwise between brain areas and hence, once averaged across the entire brain one can consider it as a summary measure of spatial complexity."
I am not sure we can talk of spatial complexity in this context. FC describes the connectivity profiles of different brain states. If you wish to talk about spatial complexity, I suggest to calculate the following measure that defines the normalised histogram entropy of the FC distribution as a measure of functional complexity.
https://www.nature.com/articles/srep38424

Response: Yes agreed. Now, we have removed the word "complexity" and rephrased the text accordingly.

7 – Authors should add how it relates to dementia and other disorders related to ageing.
See for example - https://www.biorxiv.org/content/10.1101/2023.04.20.537688v1.abstract
And
https://www.sciencedirect.com/science/article/pii/S2213158217301961
and
https://elifesciences.org/articles/83970

Response: Thank you. Indeed helpful references now discussed and included to '**Discussion'**, the end paragraph, **page 19, lines 738-749**.

"While experimental research has demonstrated that metabolite alterations aid in early Alzheimer's disease (AD) diagnosis (Huang et al (2017)) and that GABA-mediated excitation-inhibition (E-I) balance restoration can treat depression Myers et al (2014), the in-silico paradigm offers complementary mechanistic insights into disrupted neural dynamics. For instance, computational studies have revealed how noise in the left temporal lobe impairs functional connectivity in AD (Demirtas et al (2017)), a framework further extended by recent in-silico perturbation protocols for dynamic recovery Vohryzek et al (2023); Perl et al (2023). "

Minor Comments:

1 – I would not call functional connectivity distance FCD as it confuses the more established FCD fitting term functional connectivity dynamics (see again https://www.science.org/doi/full/10.1126/sciadv.abf4752 figure 2). Perhaps you can use mseFC or edge FC.

Response: FCD is now replaced by **mseFC**, and also made necessary changes in the text and figures accordingly.

2- This is a results statement but in introduction "Indeed, we also observed that connection probability changes with age (Fig. 1(b)) for the Cambridge aging Neuroscience (Cam-CAN) cohort used in the present study."

Response: Thanks for pointing this. This sentence is now removed from the Introduction section.

3- it should be obvious from the summary figure 1 what Tglu and Tgaba represent in the model. I strongly suggest to add the clear model description and mention what is being manipulated in the figure similar to figue 1 here https://www.science.org/doi/full/10.1126/sciadv.abf4752

Response: Based on your suggestions, we have now carefully revised **Fig. 1(b)** for providing greater clarity of our computational pipeline; please refer to **Fig. 1b**, **page 4, 'Method'** section:

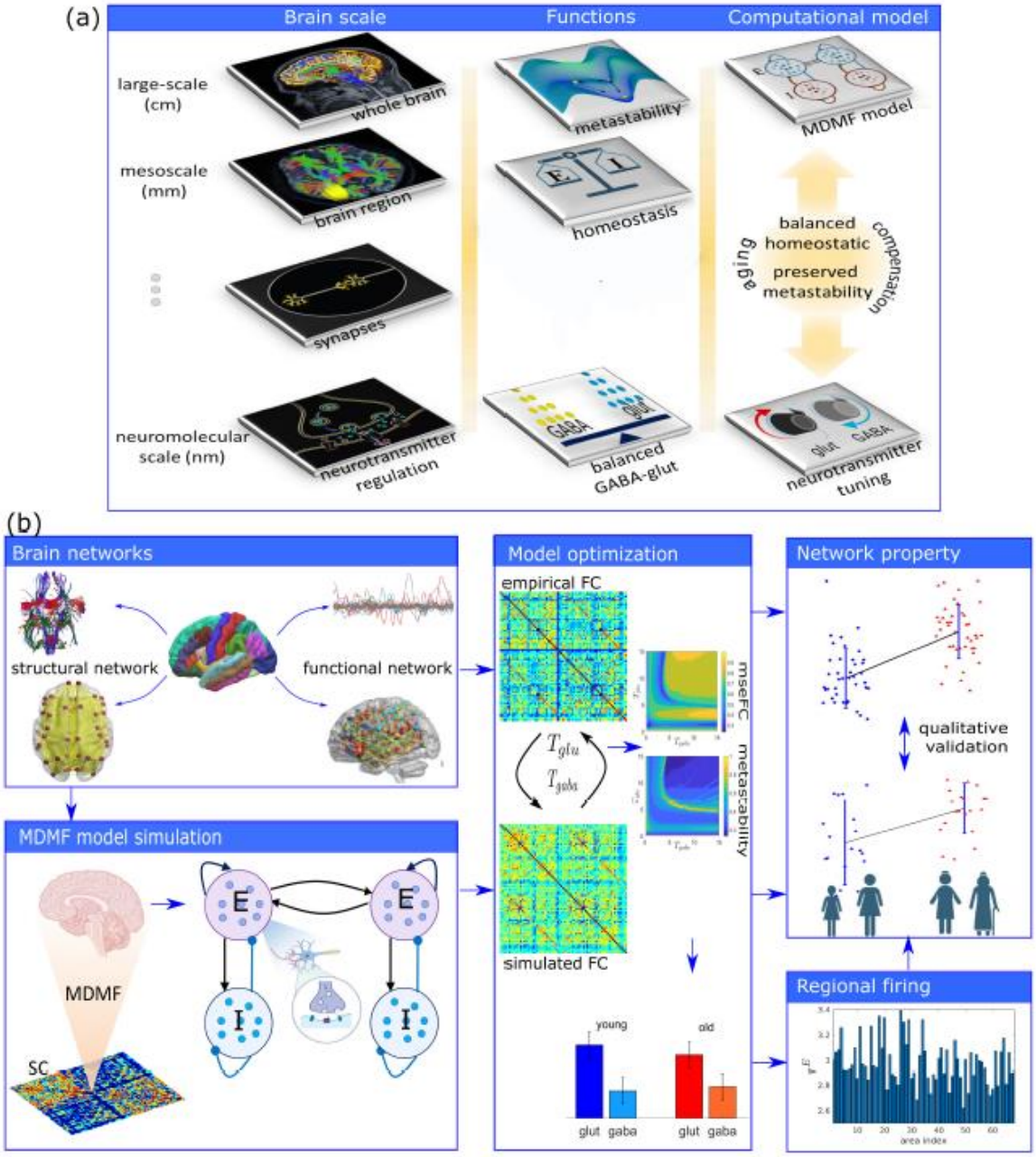

Figure 1. Workflow for model fitting and evaluation. (a) First column displays a schematic diagram of different brain scales from microscopic to macroscopic level. Second column shows functions at different brain scales. Global dynamics quantified by metastability and E-I homeostasis are observed at macroscopic and mesoscopic scales, whereas, glutamate-GABA kinetics can be observed at microscopic level. Third column presents the working principles of the computational construct. The MDMF model placed at each node of an anatomical brain network (top tile) are optimally tuned by the two parameters (glutamate, GABA) across lifespan aging while maintaining E-I balance. (b) Overview of the entire pipeline for model fitting and evaluation. Empirical structural connectivity (SC) is constrained to the MDMF model that generates simulated FCs. Two conditions, i.e., maximizing metastability and minimizing FC distance between empirical and simulated FCs, are used to estimate optimal Glutamate and GABA values of for individual participants. Graph theoretic measures of segregation and integration provide an independent qualitative validation for the robustness of our result.

4 – some parts of results are more for introduction or methods. See for instance section 2.2.6. Metastability indexes. read a lot like methods or introduction

Response: We have rearranged this part. The metastable dynamical state is described in the **Introduction** section to explain coordinated neural dynamics and the cardinal factors controlling emergent behaviour, and later defined in the method section.

Please find the text in the **'Introduction', page 2, line 67-85:**
" In parallel, complexity of neural coordination in time-varying functional networks (Deco and Kringelbach, 2016; Deco et al, 2017; Thuwal et al, 2021; Sastry et al, 2022) can also provide validation measures to quantify age-related network changes across cohorts. The brain-wide dynamics, involve transitions between "order"- when network nodes may display in-sync activity and "disorder" when nodes are weakly synchronized or display incoherent dynamical relationship (Bullmore and Sporns, 2009; Deco et al, 2011). Such transitory "in-between" dynamical state of the brain is assumed to be metastable, which does not commit to a specific attractor state, but remains perpetually prepared to internalize a stimulus that drives the transitions from one attractor state to another (Pillai and Jirsa, 2017). In characterizing global dynamics of the coordinated neural activity at rest, the measure of metastability has been extensively used across various research studies (Kelso, 2012; Deco and Kringelbach, 2016; Naik et al, 2017a; Chakraborty et al, 2025). Being in a metastable state allows the brain to maximize information processing and to promptly react to any internal or external stimulus (Haldeman and Beggs, 2005; Schneidman et al, 2006; V´azquez-Rodr´ ıguez et al, 2017). This dynamical state is often conceptualized as the optimal working point of a healthy brain (Deco et al, 2017). Emergence of such neural dynamics depends on two cardinal factors: (i) mutual influences among brain-wide neural populations mediated by their interconnecting anatomical projections (i.e., SCs), and (ii) neurotransmitters responsible for regional firing activity of excitatory and inhibitory populations. While the interconnected fibers serve as the structural foundation for synaptic communication across distributed cortical networks, the neurotransmitter kinetics regulate local excitation-inhibition homeostasis, together enabling the integration of neural activity that supports brain functions and cognitive processes."

We have defined the measure of metastability in the Method section: "**2.2 Dynamical measure of metastability", page 5.**

5 -maximal whole brain fluidity? – do the author mean metastability?

Response: Yes true. We have now rephrased the word "whole brain fluidity" in the Introduction.

6- not sure the word "inclement" is used correctly. Perhaps rephrase

Response: Thank you for your suggestion. We have now rephrased the sentence in the Introduction and Results sections.

Reviewer #2 (Remarks):

The paper aims to uncover the neurocomputational principles that allow the human brain to preserve functional integration despite structural decline during healthy aging. Using a multi-scale dynamic mean field (MDMF) model, the study investigates how glutamate and GABA levels adjust over the lifespan to maintain critical neural dynamics. The results, derived from three large neuroimaging datasets, reveal that while structural connectivity declines with age, functional connectivity remains stable due to compensatory mechanisms.
Notably, the study finds that glutamate levels decrease in older adults while GABA remains relatively unchanged, leading to a shift in the GABA/Glutamate ratio that helps sustain functional network integration. Additionally, metastability— a measure of dynamic brain coordination— remains unchanged, suggesting that the aging brain maintains an optimal working point through neurotransmitter regulation.

These findings support the neurocompensation hypothesis, emphasizing that the brain dynamically adjusts its excitatory-inhibitory balance to counteract age-related structural decline. The study's computational framework provides a novel tool for investigating brain aging and potential applications in tracking neurodegenerative diseases and cognitive decline.
Despite all the strengths, the research has some serious concerns, which might be considered for the next review step if the authors can address below issues.

Response: Thank you for your in-depth understanding of the study theme as well as insightful suggestions. We have tried our best to improve the clarity for general audience. Please find our responses below.

1- The paper would benefit from a thorough restructuring to enhance readability, logical flow, and clarity in its scientific argument.
The introduction should focus on providing a "general background" on brain aging and functional compensation, avoiding dense technical details. It should clearly state the study's motivation and hypothesis while ensuring a smooth transition into the methods.
The methods section should explicitly define key concepts, outline the computational model, and describe how neurotransmitter levels were inferred, without assuming prior knowledge.
The results section should present empirical findings objectively, avoiding repetition of model assumptions and limiting comparisons with past studies—these should be reserved for the discussion.
The discussion should then summarize key results, contextualize findings within existing literature with a more detailed review of relevant studies, and provide broader theoretical interpretations. Additionally, this section should include a clear limitations subsection, discussing potential biases, methodological constraints, and areas requiring further validation, followed by future research directions and a concise conclusion.
These changes would ensure each section functions independently and cohesively, reducing redundancy and improving the paper's readability. It can be ordered as, introduction, methods, results, discussion.

Response: Thank you for this important suggestion. Following your suggestions and as well that of other reviewers, we have now carefully reorganized and made a major overhaul of our previously submitted version of the manuscript with emphasis put on rewriting the abstract and introduction ensuring greater clarity for the readers and elucidating the motivation behind our study. We have now made the section ordering as, Introduction, Methods, Results, Discussion.

2-**The study infers glutamate and GABA levels rather than directly measuring them. The model predicts neurotransmitter changes, but these are not validated against empirical MRS (magnetic resonance spectroscopy) data. How confident can we be that the inferred neurotransmitter concentrations reflect actual biological levels? Have you considered validating these findings with MRS or other neurochemical imaging techniques?**

Response: Our work employs whole-brain computational modeling to infer (not measure) glutamate and GABA values as a theoretical construct for understanding how neurotransmitter adjustments may compensate for structural decline in the aging brain. While the model's predictions are not empirically validated against MRS data in this work, they align with available experimental observations of metabolite alterations associated with aging from various studies in the literature.

Our primary goal is to identify a neurocomputational principle—specifically, how functional reorganization could emerge from homeostatic neurotransmitter adjustments—with the understanding that this work prioritizes mechanistic insight over exact biological replication. We highly appreciate your thoughts in this direction. We also acknowledge that future validation with MRS or other neurochemical imaging (e.g., PET) would strengthen these findings, and we highlight this as a valuable direction for subsequent research. This in-silico approach provides a generalizable framework for many neuropsychiatric and neurodegenerative

disorders where E/I balance may be crucial for understanding altered large-scale brain network functional connectivity and dynamics.

We have discussed this point in the **Discussion** section--**4.2 Insight from the model simulation**, **page 18, lines 663-674.**

" It is important to note that in the absence of brain-wide MRS data on GABA/ glutamate concentrations, evaluating a goodness of fit of model estimated neurotransmitter concentrations is out of scope of the present manuscript. Rather, this work seeks to provide a deeper insight on a probable computational principle by which interaction between brain network dynamics and neurotransmitter kinetics gives rise to a complex adaptive process associated with brain aging. To gain an empirical view of neuromodulation in the aging brain, we refer to the existing literature reporting regional changes in metabolites. For example, empirical observations from healthy human adults in the posterior cingulate cortex Suri et al (2017) and rodent brain Davis and Himwich (1975); Benedetti et al (1990), revealed significant reduction in the glutamate level in the aging brain, aligned with the patterns of alteration observed in our model-based prediction. The reduced glutamate level has been interpreted as a key mechanism by which the aging brain adapts to maintain the desired regulation of I-E by down-regulating glutamate and also decreasing the expression of several other presynaptic markers Liguz-Lecznar et al (2015). "

3- GABA and glutamate are not uniformly distributed across the brain, yet the model treats them as global parameters. Also, the functional network alterations have been explored globally. I wonder how the authors address these differences.

Response: Yes, this is a crucial point and thank you for pointing this out. We have added this in the discussion and limitations. Please check the '**Methods'** section, **page 8, lines 302-312**.

"We have not considered spatial variability in the neurotransmitters' concentrations, thus, GABA and Glutamate values are homogeneously distributed across all brain regions. Hence, we will get one single pair of parameter values for each independent simulation. For the optimal choice of parameters, the excitatory firing rates are approximately 3-4Hz, concurrent with empirical observations Burns and Webb (1976); Koch and Fuster (1989); Softky and Koch (1993); Bittner et al (2017). Since the model is stochastic in nature, it is unlikely that the converge to a unique solution. Thereby, we have assessed the model performance by comparing simulated FC (generated for the estimated GABA-glutamate values) against empirical FC changes with age."

In the '**Limitation'** section in Discussion, **page 18, lines 714-723**:

"The computational framework we have argued in this article gives a bird's eye view of the neuromolecular mechanisms at the whole-brain level. Nonetheless, it is still more complex since it fails to capture spatial heterogeneity of metabolites in the cortex. In future, incorporating training models that consist of template MRS data from individual brain regions can be used to address the limitation of regional heterogeneity in the two metabolites. An atlas of whole brain metabolite map from future MRS studies can be used to tune the MDMF model for better predictions of resting state BOLD dynamics that can improve model fits to empirical FC properties. This would also require the implementation of a more advanced model inversion technique beyond two-parameter (GABA, Glutamate concentrations for this manuscript) fitting with optimized metastability and mseFC used here. In other words, machine learning models such as Bayesian model inversion can replace this step."

4- The study categorizes aging into two broad groups (18-34 and 60-85), which may oversimplify the gradual changes occurring in the brain. Would a continuous lifespan analysis (e.g., using regression models across age as a continuous variable) provide a more comprehensive view of how neurotransmitter levels and functional integration change over time?

Response: To enable clear and interpretable comparisons, we deliberately choose two extreme age groups (young adults vs. elderly). This group-wise design allowed us to capture broad, age-related differences in

brain network dynamics at the two aging boundaries and investigate how the model adjusts glutamate and GABA levels to compensate for structural loss, thus sustaining essential neural activity and critical firing rates at opposing ends of the aging spectrum. To maximize contrasts in glutamate-GABA values and their ratio, we focus on the age boundaries, providing a straightforward observational setup to identify dynamics of the neurotransmitter shifts during aging.

Please find the text in the '**Results**' section, **page 10, lines 386-392**.
"For clear and interpretable comparisons, we purposefully choose two extreme age groups (young adults (18-34) and elders (60-85)). This group-wise design allowed us to capture broad age-related differences in brain network dynamics at the two aging boundaries, and enable us to investigate how the model adjusts glutamate and GABA levels to compensate for structural loss, thus sustaining essential neural activity and critical firing rates at opposing ends of the aging spectrum. To maximize contrasts in glutamate-GABA values and their ratio, we focus on these distinct age boundaries, providing a straightforward observational framework to assess key neurotransmitter shifts in aging brain."

5- The study focuses heavily on white matter loss but does not address cortical thinning or synaptic density reduction, which are also known to impact brain function in aging. How might cortical atrophy or synaptic pruning contribute to functional changes observed in aging? Could these be incorporated into the model?

Response: The existing multiscale mean-field model MDMF, or a large-scale brain model is constrained by structural connectivity only, do not encompass all necessary complexity. Indeed, cortical thinning, synaptic and myelin density can also be included with suitable extensions on the model. While it is easier, to include myelin density and synaptic scaling (see Pathak et al 2022, Sorrentino et al 2024) in high resolution time series data, e.g MEG/ EEG with the phase oscillators models for slow BOLD signals DMF/ MDMF are method of choice which relates local E-I balance to whole brain metrics. In future it is possible conceptualize cortical thinning as well as synaptic density by combining these factors together with fiber properties in the term C_{ij} of equation 2. Myelin density can also be included by adding terms such $S_i^{(E)}(t-\tau)$ to equation (2). However, these are currently out of scope of this paper as it increases complexity considerably and the BOLD signal on a slow time scale masks the time-delay effects substantially. But certainly, these factors can be the goal of a future study possibly combining diffusion imaging and neurophysiological maeasurements such as EEG/ MEG.

6- The binarization of functional connectivity (FC) matrices depends on arbitrary threshold choices, which can significantly affect network metrics. How did you determine the optimal threshold for binarizing FC? Would alternative thresholding methods (e.g., proportional thresholding) lead to similar conclusions? Why weighted graph measures have not been used?

Response:
Indeed, the binarization process has a big impact on network metrics. We used weighted graph measures for structural connectivity (SC) networks, because the edges represent actual neural pathways containing synapses. In contrast, edges in functional connectivity (FC) networks represent functional correlations between regions. For our FC networks, we selected only significant functional correlations between brain regions. Importantly, thresholds were not arbitrarily chosen but based on statistical significance, where correlations with $p<0.05$ were considered for connected components in the FC networks.
In this context, previous studies have shown promising outcomes when inferring valuable insights from binarized resting-state functional connectivity (e.g., Shinn et al., 2023; Chen et al., 2021; Park et al., 2015; Geerligs et al., 2015). Furthermore, we cross-validated our observations using additional threshold values ($p<0.04$ and $p<0.03$) to binarize the FCs when examining functional alterations in the aging brain. Further, the robustness of our results was tested across three different datasets.

[Shinn et al 2023] Shinn, M., Hu, A., Turner, L., Noble, S., Preller, K. H., Ji, J. L., ... & Murray, J. D. (2023). Functional brain networks reflect spatial and temporal autocorrelation. Nature neuroscience, 26(5), 867-878.
[Chen et al 2021] Chen, X., Necus, J., Peraza, L. R., Mehraram, R., Wang, Y., O'Brien, J. T., ... & Taylor, J. P. (2021). The functional brain favours segregated modular connectivity at old age unless affected by neurodegeneration. Communications biology, 4(1), 973.
Kumar, R. (2016). Disrupted functional brain network organization in patients with obstructive sleep apnea. Brain and behavior, 6(3), e00441.
[Park et al 2015] Park, B., Palomares, J. A., Woo, M. A., Kang, D. W., Macey, P. M., Yan-Go, F. L., ... &
[Geerligs et al 2015] Geerligs, L., Rubinov, M., & Henson, R. N. (2015). State and trait components of functional connectivity: individual differences vary with mental state. Journal of Neuroscience, 35(41), 13949-13961.

We have mentioned the method to create FC networks (both empirical and simulated FCs) in the main text. Please find the text in the **Method** section-- "**2.3 Preparing empirical SCs and FCs for network analysis**", **page 6, line 195-205.**

"We converted the original weighted FC matrix into a undirected binary graph by applying a threshold (δ) based on the p-values obtained from pair-wise Pearson correlations while taking the pairwise correlation of the BOLD signals between any pair of brain regions. We averaged the thresholds over entire cohorts to obtain a single threshold value. Thereafter, the weighted FCs are converted into the binary network by setting the condition as, $FC_{i,j}=1$, if $abs(FC_{i,j}) > \delta$, where $\delta=0.076$; otherwise, $FC_{i,j}=0$. In other words, only the significant correlations were chosen to build the binary FC network. Next, we have derived the FC properties for various other threshold values in order to check threshold dependency on the graph theoretical metrics. In particular, the FC thresholding was defined by the minimum correlation coefficient value corresponding to the p-values < 0.04 and 0.03, i.e., $abs(FC) \geq \delta = 0.085$ and 0.091. For the three chosen thresholds, preserved edge is between 88% to 85% of the total network's edge (please see, SM Fig. S3)."

Also, please check the supplemental material **Fig. S2** and **Fig. S3** for effects of thresholding on our observations of FC properties derived from empirical data; given below:

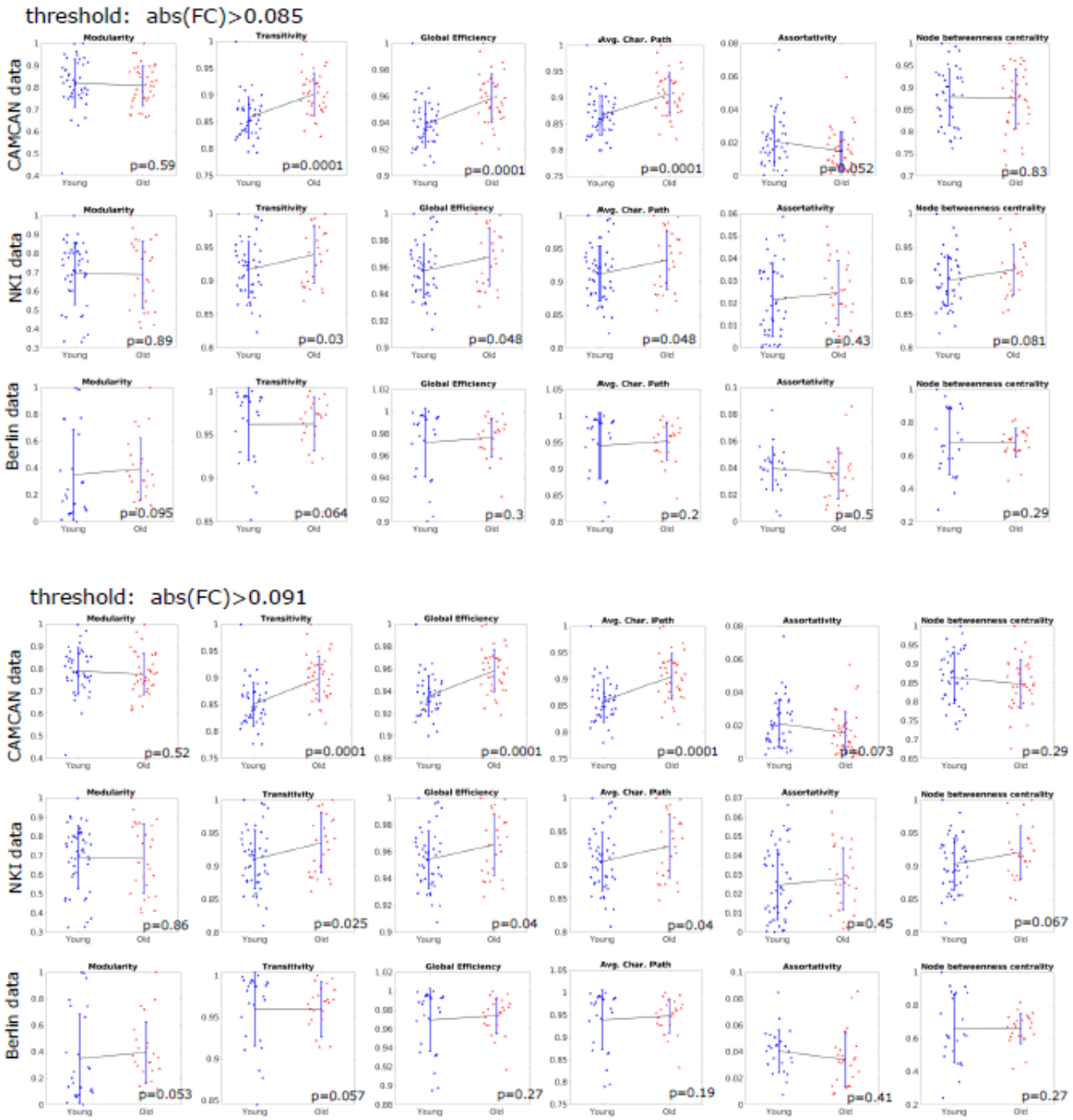

Figure S2 Effect of threshold to binarize the empirical FCs (Pearson correlation coefficient). Pattern of changes in rsFC network properties in the three datasets remains stable between two age-groups for a wide range of the threshold.

We used similar methods for simulated FCs; please find the supplemental material **Fig. S7, page 13,** also shown below:

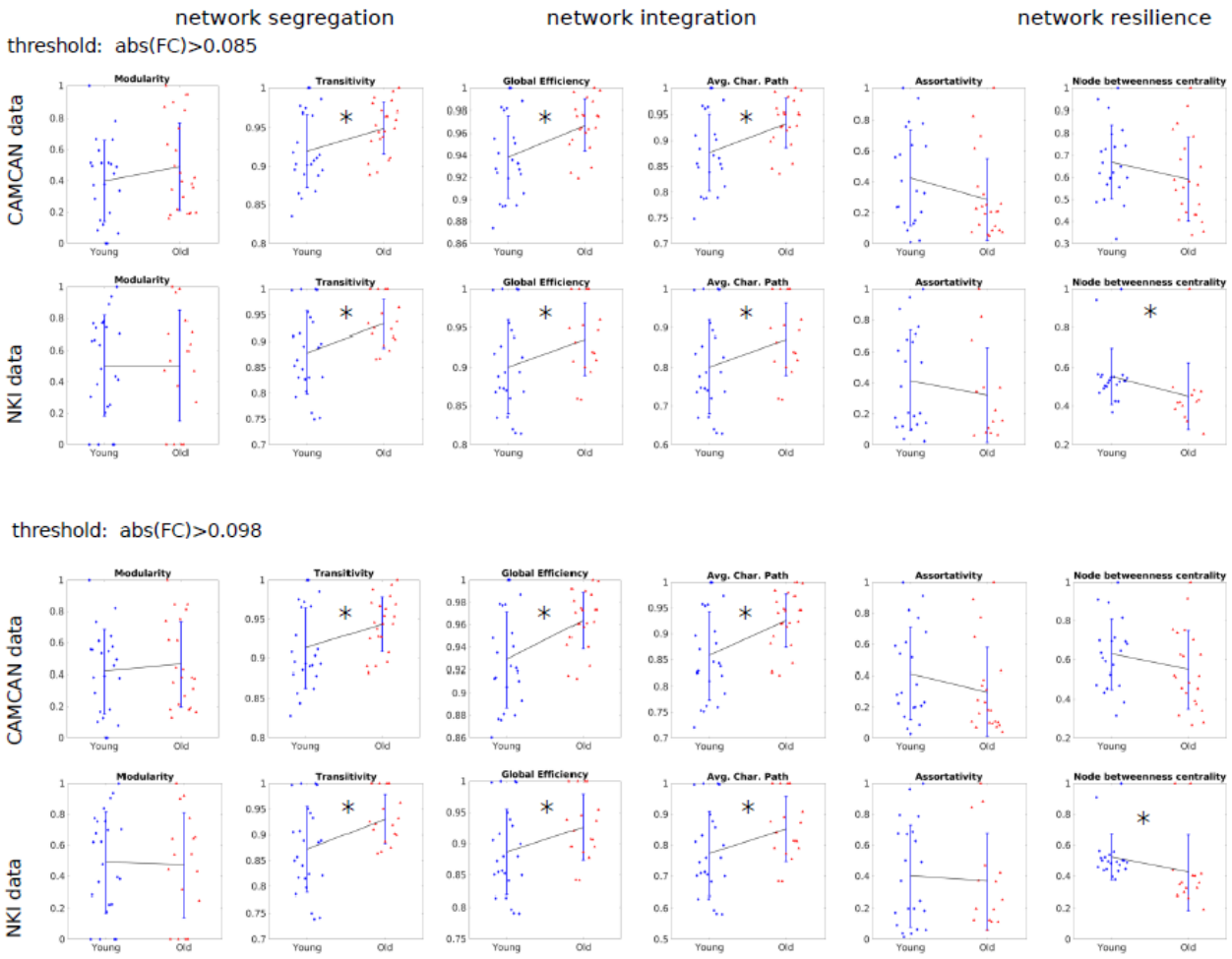

Figure S7 Effect of threshold to binarize the simulated FCs. Pattern of changes between young and old cohorts in the model based FCs properties remains almost similar to that of empirical rsFC properties for a wide range of the threshold. Only node betweenness centrality metric is not fully predicted in the test cohorts; while comparing between the age groups the statistical significance does match to the empirical observation. The star $*$ represents $p < 0.05$, otherwise not significant.

7- The study does not mention whether the MDMF model code or datasets will be made available for replication. Do you plan to share the code and data for others to validate and extend your findings ?

Response: It is mentioned in the **'Code Availability'** section. Please refer to the end of the main text, **page 19**, **line 772.**

8- The title is somewhat vague and overly broad, **e.g. the term "multi-scale neurocomputational principles" does not clearly indicate whether the focus is on neurotransmitter regulation, functional connectivity, or dynamic interactions. A more precise title ensure that readers immediately grasp the study's main contribution. The abstract, while scientifically detailed, can be improved by providing a clearer statement of the hypothesis and ensuring that the results are stated more solidly rather than being implied. Additionally, it should state clearly how the research addresses the gap in the field. Lastly, the final sentence can emphasize the study's broader potential.**
A stronger title and a more structured abstract would significantly improve the paper's impact and readability.

Response: We appreciate your excellent suggestions. We have now modified the abstract as per your suggestions. We sincerely hope that the revised title and abstract provide enhanced readability and clarity. Please find the modified text below.

Title: "Neurocomputational principles of brain aging: Local homeostasis preserves global neural dynamics compensating for structural loss"

Abstract: "Aging brain undergoes a gradual structural decline over lifespan accompanied by changes in neurotransmitter levels, leading to altered functional markers. {\color{red} Past studies have reported human resting state brain display a remarkable preservation of coordination among neural assemblies stemming from an underlying neurocomputational principles along aging trajectories, however, the true nature of which remains unknown. Here, we seek to identify the computational mechanisms with which neurotransmitters, such as altered GABA and glutamate concentrations, can preserve functional integration across lifespan aging, despite structural decline. We employ multiscale, biophysically grounded modeling, constrained by the empirically derived anatomical connectome of the human brain, where the neurotransmitter concentrations can be free parameters that are algorithmically adjusted to maintain regional homeostasis and optimal working point. The two estimated neurotransmitters can maintain critical firing rates in the brain region and mimic age-associated FC patterns, consistent with empirical observations. We identified invariant GABA and reduced glutamate as the principle computational mechanism that can explain the topological variation of functional connectivity along lifespan, validated using graph-theoretic metrics. The results are subsequently replicated on three distinct datasets. The study offers an operational framework to integrate the brain network dynamics at macroscopic scale with neurotransmitter concentration changes at molecular scales, thus, providing a tool to gain insights into the biological mechanisms underlying neural disorders arising out of disrupted homeostasis."

Reviewer #3 (Remarks to the Author):

Saha et al. used a multiscale dynamic mean field (MDMF) modeling approach to examine how (healthy) aging brains can preserve their functon(metastability) amidst the degradation of long-range white matter tracks. Through model parameter fitting, the authors have concluded that glutamate concentration reduction as a function of age is the primary explanation for the preservation of metastability in healthy aging, i.e., as a compensatory mechanism against gradual age-related degradation of long-range structural connectivity. Overall, I found the paper theoretically interesting, well-written, and mindful of the rigor (i.e., results are validated across different datasets and subsets). Nevertheless, some technical clarifications are needed before the manuscript is ready for publication. I also have other suggestions for improving the readability of the manuscript. Detailed comments are below:

1. The results section begins with the MDMF modeling (sec 2.1) and ends with MDMF modeling results (sec 2.3), with a large portion of empirical SC, FC analysis (no modeling) in between (sec 2.2). I find this organization confusing, and it is not always clear how results in sec 2.1 relate to those of sec 2.3.

Response: We extend our sincere thanks for your critical review of our submitted version.

In the revised version, we have made several changes in the '**Result**' section, including:

(1) **Figures 4, 5, 9** (in the **previous version**) are now moved to supplemental materials, **Figs. S2, S3, S7 (present version)**.
(2) We have revised **Fig. 4**, and **Fig. 6** in the main text (**present version**).

In addition, we have reorganized the **'Results'** section. We presented empirical metastability analysis first. Next, the graph theoretical properties of the empirical structural-functional networks are shown. Finally, we put the model-based analysis. The model simulation has been described in three subsections:

3.4.1 Simulation results: Optimal parameter selection and consistency, **page 14 line 518**

In this subsection, the results on averaged subjects from individual groups are shown in **Fig. 4** (in revised text). This describes that for an optimal parameter set, the model fits well to the empirical FCs and reproduces metastable. We checked the consistency of the estimated parameter values (GABA, glutamate). We conducted 50 independent runs, while keeping all other computational settings fixed. A cloud of simulation-based GABA, and glutamate values are observed.

3.4.2 The MDMF model-based glutamate-GABA concentrations, **page 14 line 535.**

In this second part, participant-wise parameter estimation is presented in **Fig. 5**, main text to test our hypothesis.

3.4.3 Model performance evaluation: Graph theoretical metrics computed from simulated FC, page 16, line 578.

In this part, the model performance has been evaluated through qualitative comparisons between the empirical and model-based FC properties, please find **Fig. 6** (in revised text, **page 16**).

a. Is the result in Figure 2G, i.e. contrast between glutamate and gaba concentration across ago groups, significant? If not, how do you reconcile the lack of significance here with the significant individual-level modeling results shown in Figure 7?

Response: The results in Fig. 2G (previous version of the manuscript) depict an averaged-subject estimation (similar to a single subject from each group), the error bars show standard deviation across trials. We averaged all the SCs within each group creating one single averaged-subject SC, and then the model was simulated to estimate those values. Because this analysis was based on only two averaged subjects from each age group, it does not allow for direct comparisons of glutamate and GABA concentrations between age groups. Instead, statistical significance between age groups is derived from the participant-wise estimated values presented in **Fig. 5** in the revised text, **page 17** (in earlier version, it was Fig. 7).

b. I think it'd be more helpful to put the network metrics of the empirical data and that of the model FC results side-by-side (e.g., merging figure 3 and 8). It would also make sec 2.2 feel lack disconnected (right now it feels like a traditional MRI paper inserted into the middle of a modeling paper).

Response: We appreciate your suggestions. We have re-organized entire Result section.
Please refer to **Fig. 6** (in revised text, **page 16**).

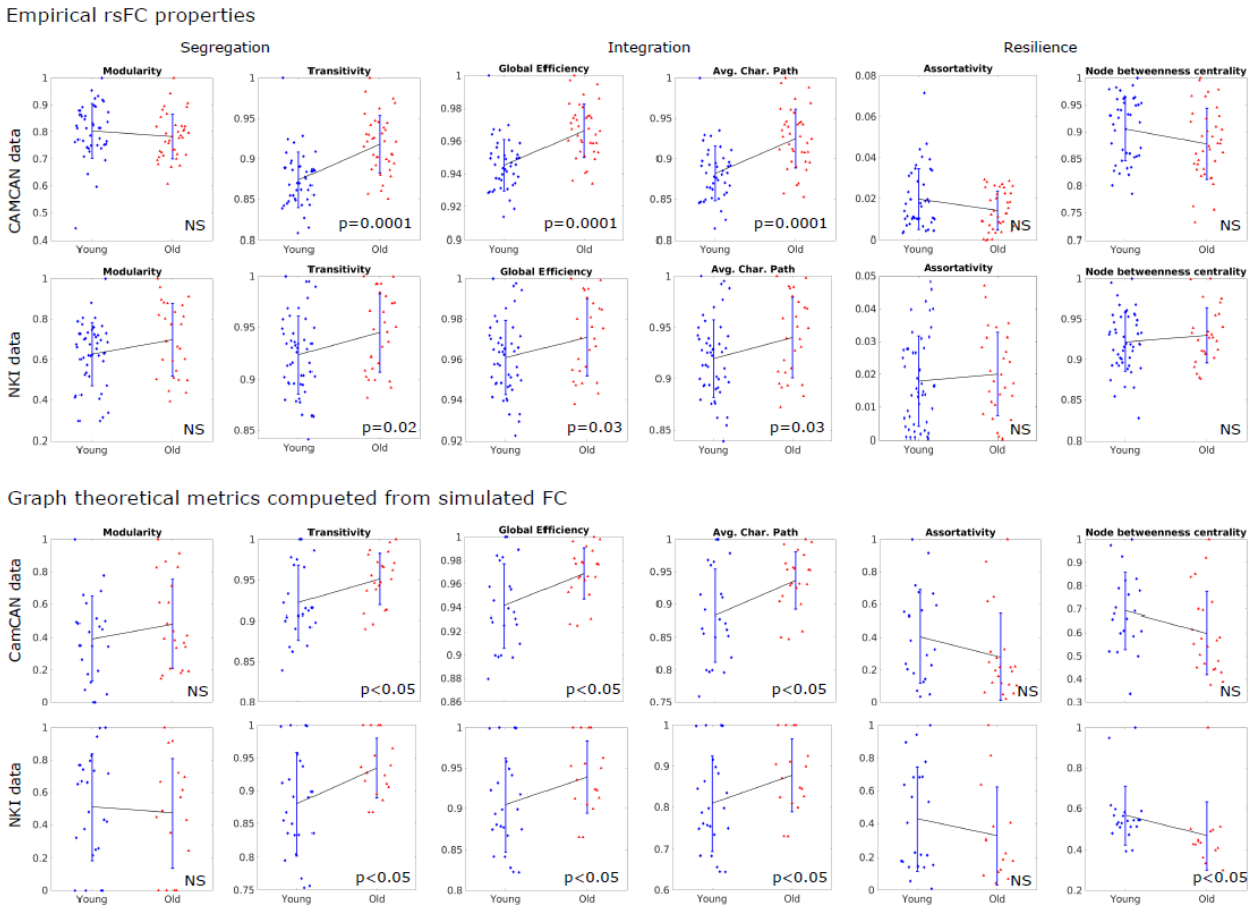

Figure 6 Qualitative comparison between empirical and simulated FC properties. Top two rows display network properties of the empirical rsFC, compared against the observations from simulated FCs (bottom two rows), which are obtained for the optimal parameter choices. The variability is coming solely from individual's SC and is mainly assessed in the test cohort. The MDMF model inversion reproduces almost similar alterations between two age groups occurring for the test cohorts, where, the modeling arameters are computed from training cohorts. Except, alterations in betweenness centrality is not fully predicted from the test subjects of NKI data. Statistical differences is assessed by Mann-Whitney U test, where $p<0.05$ implies statistically significance, otherwise not significant (NS). A solid black line connecting two mean values of younger and elderly groups, is plotted to visually tracking alterations. The errorbars show standard deviations.

2. Sec 2.1, line 1: FCD appears for the first time – the full name needs to be spelled out. The same with T_glu and T_gaba. The author should briefly mention the biophysical interpretation of these parameters and point to more details in methods and supplementary materials.

Response: Thank you for pointing this. Based on the suggestion from the reviewer 1, we have now replaced the term "**FCD**" with "mean-square root-error between FCs (**mseFC**)" and made appropriate changes in the text and figures. Here, we mention that the term **FCD** is now represents **FC dynamics** (FCD) in the present version.

The biophysical interpretation of these parameters are mentioned now. Please find 'Method' section--**2.5 Multi-scale Dynamic Mean Field (MDMF) model, page 7, line 259-274**.

Individual brain region is modeled by two distinct pools of excitatory and inhibitory neuronal populations having recurrent E-I, I-I, and I-E interactions, and coupled with neurotransmitters GABA and glutamate via NMDA (N-methyl-D-aspartate) and GABA synapses, respectively. The study by Destexhe and colleague Destexhe et al (1994) conceptualized the occurrence of neurotransmitter release into the synaptic cleft as a pulse following the arrival of an action potential at the presynaptic terminal. Accordingly, one can

consider a chemical kinetic reaction as R+T ↔T R, where T, R, and TR are the neurotransmitters released in the synaptic cleft, unbound receptors, and bound receptors of postsynaptic neuron, respectively. Subsequently, neurotransmitter release can be captured in the rate equation that describes the dynamics of probability of open channels of a specific neurotransmitter (synaptic gating variable). Detailed expressions of how the concentration of GABA and glutamate can be obtained from population-level averaging from individual neuron-level synaptic concentrations are presented in our earlier study Naskar et al (2021). The long-range connections are made between the excitatory pools of any pair of brain regions, defined as SC, which is derived from the fiber densities (interconnected fiber bundles) computed by diffusion tensor imaging data of healthy human adults. The regional excitatory pools receive following input currents: inhibitory currents from local inhibitory pools, self excitatory currents, long-range excitatory currents from the excitatory pols other brain region, and a constant external current.

3. Figure 2 I, J: the star for the optimal parameters is very small, please make it bigger. There seems to be a lot of degeneracy in the model, i.e., a lot of combos of (T_glu, T_gaba) seem to have the same FCD and metastability. Please discuss why one should expect the model to converge to (near) a unique solution.

Response: The stars are made bigger. PLease see **page 15.**

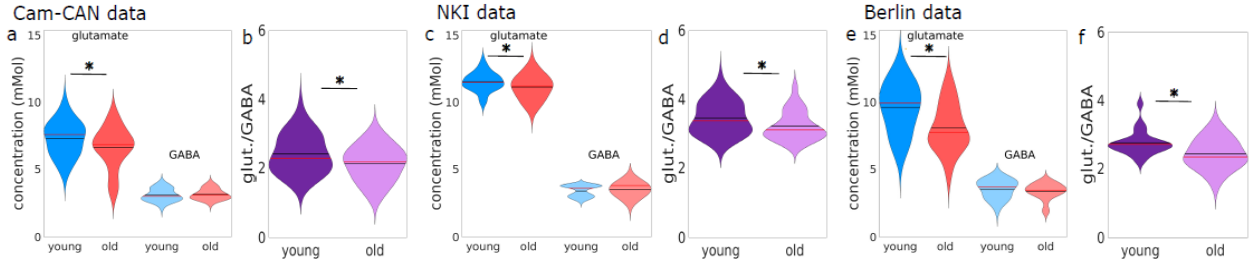

Figure 5. Model-based parameter estimation. Participant-by-participant basis GABA-Glutamate values and their ratio are shown in (a, b) for Cam-CAN, (c, d) for NKI, and (e, f) for Berlin data. The aging brain significantly tunes the glutamate level more prominently than that of GABA level, where a significant decay in GGR can also be observed. The star * indicates $p < 0.05$.

Yes, there are no unique values of the two parameters for the different trials of estimation keeping all other settings intact. The trial-wise estimation of the parameters is shown in **Fig. 4** (**page 15**), by averaging all subjects within individual groups. There is a cloud of estimated values, found within a very close proximity. To assess the model performance for the estimated parameter set, we have compared age-related variability between empirical FC and model-generated FC properties. Almost similar patterns of changes are observed, also the firing rates produced for the choice of parameters are found to be within the critical ranges, approximately **3-4Hz** for excitatory populations (please see **Fig. 4c,** and supplementary martial **Fig. S8**).

4. Section 2.2.1, 2.2.2 title: I would suggest using the language of "long-range segregation" and "long-range integration" since, by definition, modularity, and clustering coefficients imply there is high integration locally despite large-scale segregation.

Response: We understand why you are making this statement. However, we feel, using the term "the long range", without defining what is short range will make the narrative more confusing. Since we have not explicitly distinguished the scales here introducing this term may be out of scope for this study. Previous works [Shinn et al 2023**,** Chen et al 2021, Kumar, R. (2016), Park et al 2015, Geerligs et al 2015, Carbal 2011] also have used similar terminology hence we want to stick to that to avoid confusion.

[Shinn et al 2023] Shinn, M., Hu, A., Turner, L., Noble, S., Preller, K. H., Ji, J. L., ... & Murray, J. D. (2023). Functional brain networks reflect spatial and temporal autocorrelation. Nature neuroscience, 26(5), 867-878.

[Chen et al 2021] Chen, X., Necus, J., Peraza, L. R., Mehraram, R., Wang, Y., O'Brien, J. T., ... & Taylor, J. P. (2021). The functional brain favours segregated modular connectivity at old age unless affected by neurodegeneration. Communications biology, 4(1), 973.
Kumar, R. (2016). Disrupted functional brain network organization in patients with obstructive sleep apnea. Brain and behavior, 6(3), e00441.
[Park et al 2015] Park, B., Palomares, J. A., Woo, M. A., Kang, D. W., Macey, P. M., Yan-Go, F. L., ... &
[Geerligs et al 2015] Geerligs, L., Rubinov, M., & Henson, R. N. (2015). State and trait components of functional connectivity: individual differences vary with mental state. Journal of Neuroscience, 35(41), 13949-13961.
[Carbal 2011] Cabral, J., Hugues, E., Sporns, O., and Deco, G. (2011). Role of local network oscillations in resting-state functional connectivity. Neuroimage 57, 130–139. doi: 10.1016/j.neuroimage.2011.04.010

5. Section 2.2.4., 2.2.5.: I appreciate the analysis, but moving them to supplementary materials would make the paper more readable (unless other reviewers strongly demand them to stay here).

Response: Thank you for this suggestion. These subsections are now moved to the supplementary materials.

a. 2.2.5: what does "consistency" refer to here? Is it consistency with specific p values considered as ground truth or between datasets? Without more clarification, I am not sure what the "inconsistency" in Figure 5b really implies.

Response: True, the term 'Consistency' is defined based on our observations on the age-related changes (mainly by the p-values) in FCs properties for different thresholds used to binarize the FCs. We intend to examine how thresholding can affect network connectivity, i.e., reduction in edges with increased threshold values, that might affect our observation. Thus, this result helped us to draw a general interpretation of the network properties within the considered threshold ranges. The figure is moved to supplemental material **Fig. S3** (earlier version, Fig. 5b).

6. The definition of transitivity: the definition in p13 (sec 3.1.2.ii) is different from the usual definitions that I have seen. Especially, it's different from the brain connectivity toolbox code. I'm also baffled why the modularity and transitivity should be anticorrelated in your results. Please double check.

Response: We modified the definition of Transitivity in the revised text. Essentially, we used the same algorithm given by Rubinov and Sporns 2010. formula used to measure Transitivity. Please find the correction in **page 6, lines 222-227** in the **'Method'** section in main text.

(ii) Transitivity Rubinov and Sporns (2010); Newman (2003b) is a classic measure of clustering coefficient that captures the tendency of a node to cluster together. High transitivity means the network contains communities or groups of nodes that are densely connected internally. Transitivity of a network is $T = \frac{\sum_{i \in N} 2t_i}{\sum_{i \in N} k_i(k_i-1)}$, where k_i is the degree of i^{th}-node. Number of triangles around a node i is $t_i = \frac{1}{2}\sum_{j,h \in N} a_{ij}a_{ih}a_{jh}$. a is the adjacency matrix (undirected binary graph). For weighted graph, geometric mean of triangles around node i is $t_i^w = \frac{1}{2}\sum_{j,h \in N}(w_{ij}w_{ih}w_{jh})^{1/3}$.

Thank you for the suggestion. We have rechecked this for different thresholds. A possible explanation for such observation could be-- 'Transitivity' (classical metric of global clustering coefficient) measures tendency of nodes to form triangles, while, 'Modularity' captures how well a network can be divided into distinct modules (communities). A network can have high modularity but low transitivity (e.g., distinct cliques with few internal triangles). Other way round, a network can have high transitivity properties having low modularity value, e.g., a small-world network with uniform clustering but no clear communities. Ideally, in a hierarchical modular network (such as the brain), where modules themselves have high internal clustering (both measures may be high, or, in random and lattice networks, both metrics could be low), however, the overall changes between the two distinct groups might not necessarily follow similar patterns of alterations, since the two metrics capture different aspects of network organization by definition, but

can be interpreted as a common graph property capturing network segregation, in line with the description in Rubinov and Sporns 2010.

[Rubinov and Sporns 2010] Rubinov M, Sporns O, Complex network measures of brain connectivity: uses and interpretations. Neuroimage 52(3):1059–1069 (2010).

7. Figure 6: Here, the authors essentially argue that the null hypotheses are true. In this case, the authors should show Bayesian statistics to support the null hypothesis (JASP is a statistical software that is really easy to use for this purpose).

Response: Nice suggestion. We have performed the Bayesian Independent Samples ttest; one of the results is presented here, which is **BF_{10}=0.249<1** (error%=0.014) that implies non-significant changes in the empirical metastability between the two age groups.
However, we request you to check the **supplemental material Figures S4** and **S5** (**page 9**), where we have tested the validity of our observation using the surrogacy method. In particular, We test the reliability and reproducibility of our observations on empirical metastability in 645 human participants. The measured empirical metastability values are examined against the surrogate data by randomizing phases of the resting-state BOLD signals of individual participants. Additionally, we examined empirical metastability in the moving watching and sensory-motor task and tested against surrogate data to compare with the resting state results. Overall, we found no correlation between the empirical data and surrogate data, implying the measure of metastability is not coming from any random processes.

8. Section 3.1.1: there's a corrupted symbol in the FC definition. Please check.

Response: Thank you. It is rectified now.

9. Section 3.2.1:
a. would be helpful to mention that the first to fourth equations in (1) is exactly the same with Deco et al 2014, since various work in the community use it as base model.

Response: Thank you for pointing this. We have now mentioned this in the revised main text right after the model equations, **page 8, lines 282-29**1.

"The base model proposed in Deco et al (2014) is identical in form for the first four Eqs. (2-5), and the later two Eqs. (6-7) are proposed by Naskar and colleagues Naskar et al (2021), by incorporating kinetics of the two primary neurotransmitters, glutamate (Tglu) and GABA (Tgaba) within the dynamics of the two gating variables ($S_i^{(E,I)}$ (t)). Thus, the MDMF model extends computational observations beyond large-scale neuroimaging, capturing neuromolecular dynamics that govern region-specific E-I balance and neural firing. The two free parameters, Tglu and Tgaba, capturing the neurophysiology of the excitatory and inhibitory process, are algorithmically adjusted to fit the model generated FC and empirical FC. For a fixed glutamate and GABA concentration, we select coupling strength G for which simulated and empirical rsFC distance is minimum and their correlation is maximum, while maintaining a firing rate approximately 3-4 Hz Bittner et al (2017)."

b. Is the global coupling here G fitted? If not, please discuss how it would affect your interpretation of T_glu and T_gaba.

Response: Yes, the global coupling *G* was fitted, and its effects on parameter estimation are shown in the Supplemental material **Fig. S8.**

10. Regarding metastability:

a. A lot of people are using standard deviation of kuramoto parameters (SDKP) as a measure of metastability on fMRI data. The problem is that SDKP is only a meaningful measure of metastability when the underlying dynamics are rhythmic, and there is no such rhythmicity in the fMRI data. One can pretend there is such rhythmicity by applying a very narrow band filter on nonrhythmic data, but this should at least be discussed as a limitation.

Response: We have filtered both the empirical and simulated BOLD signals using a narrow band filter allowing 0.001 Hz to 0.1 Hz. Please find newly added section-- **2.6 Simulated BOLD activity, page 8-9, line 314-329**.

2.6 Simulated BOLD activity

We calculate model generated resting state BOLD signals from th emodel-based neural activity of the excitatory pool firing rate $r_i^{(E)}$ in individuals brain region i^{th} using the hemodynamic Balloon-Windkassel model in Friston et al (2003); Cabral et al (2011). In the hemodynamic model, an increase in the firing rate $r_i^{(E)}$ increases vasodilatory signal s_i subjected to autoregulatory feedback. Blood flow of the i^{th} brain region is f_i, that responds in proportion to this signal with concomitant changes in blood volume v_i and deoxyhemoglobin content q_i.

$$\dot{s}_i = r_i^{(E)} - \kappa_i s_i - \gamma_i(f_i - 1) \tag{9}$$

$$\dot{f}_i = s_i \tag{10}$$

$$\dot{v}_i = \frac{1}{\tau_i}(f_i - v_i^{1/\alpha}) \tag{11}$$

$$\dot{q}_i = \frac{f_i(1-(1-\rho_i)^{1/f_i})}{\tau_i \rho_i} - \frac{q_i v_i^{1/\alpha}}{\tau_i v_i} \tag{12}$$

ρ=0.34 is the resting oxygen extraction fraction, τ=0.98 is a time constant, and α=0.32 is the Grubb's exponent, represents the resistance of the veins. κ=0.65 and γ=0.41. The BOLD signal for i^{th} brain region is considered as a static nonlinear function of volume v_i and deoxyhemoglobin q_i comprised of volume-weighted sum of extra- and intravascular signals, given by

$$B_i = V_0[k_1(1-q_i) + k_2(1-q_i/v_i) + k_3(1-v_i)], \tag{13}$$

where $k_1 = 7\rho_i$, k_2=2, k_3=$2\rho_i - 0.2$, and V_0=0.2 is resting blood volume fraction. All the biophysical parameters are taken from Friston et al (2003). To consider functionally relevant frequency range for resting-state conditions, the simulated BOLD activity has been filtered using a bandpass filter (0.001 Hz< f <0.1 Hz). Thereafter, we detrended the simulated BOLD activity and use Pearson correlation between any pair of brain regions to generate the simulated or model FCs.

b. The fact that SDKP cannot differentiate between metastability and noisy multistability should also be discussed.

Response: This is a valid point. There is always the possibility of mis-characterizing metastability and hence must be measured over long time scale to avoid the possibility of multistability. However, please note we have never reduced the time series duration as available in each data sets to ensure the metastability is measured over long time scales.

We have discussed this in '**Limitation**' **page 19, lines 732-737,** texts are given below:

"It is crucial to note that the standard deviation of instantaneous Kuramoto order parameter as a measure of metastability has a significant limitation: it is only meaningful when applied to rhythmic dynamics. To overcome this limitation, we implemented a narrow band filter (0.001 Hz to 0.1 Hz) on both the empirical and simulated BOLD signals prior to calculating metastability. This filtering process effectively enhanced the rhythmicity of the BOLD signals, thereby ensuring that our metastability measurements remained valid and interpretable throughout our analysis"

c. I did not find the info on how model BOLD signal was filtered prior to computing SDKP. Please clarify.

Response: Excellent point. It is mentioned now. We used a bandpass filter with pass-band frequencies of 0.001 Hz to 0.1 Hz. Please see revised manuscript, **page 9 , line 326-328.**

"To consider functionally relevant frequency range for resting-state conditions, the simulated BOLD activity has been filtered using a bandpass filter (0.001Hz <f< 0.1 Hz)."

We extend our sincere thanks to the editors and reviewers for the thoughtful suggestions and comments, which greatly helped to improve our work. Based on the suggestions, we have made changes in the Fig. 4 and Fig. S8.

Reviewer #1 (Remarks to the Author):

2nd round of comments to the paper "Neurocomputational principles of brain aging: Local homeostasis preserves global neural dynamics compensating for structural loss".
Thank you to the authors for addressing many of my concerns. I wish to further highlight some of the remaining ones considering their responses.

Response: Thank you for your wonderful suggestions – they've made a tremendous difference in improving our work.

Related to my second point on plotting the empirical and simulated FC and FCD fits. The fact that the authors reproduce the results in three independent datasets is strong, they might want to double-check the NKI dataset. From the image they provide it looks far away a from typical connectivity structure (no left, right hemispheric connectivity or visible homotopic connections in the interhemispheric part). Unlike the other two datasets where the FC structure can be clearly seen. As it stands, I would not distinguish it from a randomly generated FC matrix. Could the author double-check or comment on why would that be?

Response: Thank you for pointing this out. In the previous version we used the same colorbar scale to plot the FC matrices of Fig 4. Due to the difference in time series lengths that are nascent to each data set and slightly different scan parameters, the variability in FC in each data set (eg minimum and maximum values) when plotted in same scale of colormap doesn't show qualitative similarity and in fact appears as "random" for NKI. We have modified Fig. 4 which now shows qualitative similarity across datasets when the colorbar scales were changed to reflect the range of variability nascent to the corresponding dataset.

Moreover, consulting the SI about the NKI dataset brings further confusion as it is not clear what parcellation has been used or why two different parcellations might have been used (Craddock and AAL). This needs to be done impeccably as the rest of the analysis builds on the data. I strongly suggest for the authors to review it.

Response: Every analyses in NKI dataset are conducted on the Craddock 200 atlas (188 ROIs); only metastability metric is derived using AAL atlas (116 ROIs) in NKI dataset to maintain spatial consistency with the Cam-CAN data, where we have derived metastability using AAL, besides Desikan-Kiliany parcellation scheme.

We have now mentioned this point explicitly in the Supplemental Information to avoid any confusion, below is the text.

"1.2.3 Resting-state functional connectivity using AAL parcellation :

We have performed every analysis in NKI dataset using Craddock atlas; only metastability is measured using the AAL atlas to maintain consistency with the CamCAN analyses, where we also examined the metastability metric using AAL atlas (116 ROIs), besides Desikan—Killiany atlas (see section 2.4.3, Fig. S4 for Cam-CAN results using AAL atlas). This served as a cross-validation of the metastability metric. Since metastability is a time-varying measure, qualitatively similar results are obtained whether we use Desikan (68 ROIs) or the AAL atlas (116 ROIs). "

This point further relates to the FCD plots – why only ~80-100 timepoints? For example, for the berlin dataset there are about 22 minutes of TR = 2? All these points should be consistent between the methods and plots else it creates at best a confusion in the analysis process.

Response: Previously, time-window index had been shown which was not appropriate – we agree. Now, the time windows are mapped to its actual time points (in unit of minutes) in the three datasets; please find Fig. 4, below for ease of viewing. Berlin data was temporally longer due to design considerations of collecting EEG for a long duration. Thank you for pointing this out, very important for clarity.

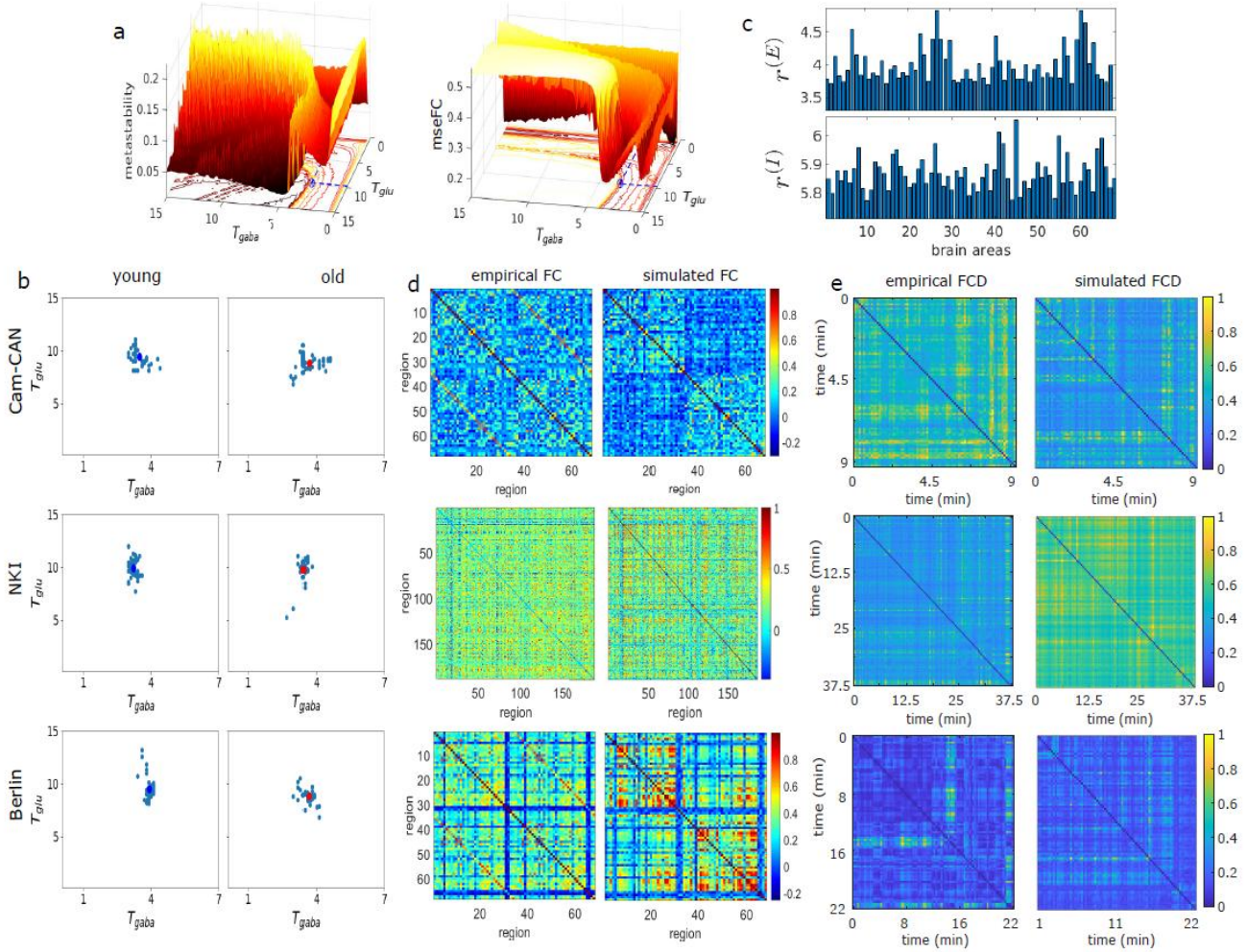

Figure 4 Model-based results obtained from averaged subjects: parameter estimations, firing and FCs. First, the SCs of individual groups are averaged, and then independent simulations are performed. (a) The 3-D maps of metastability and mean-square root-error between FCs (mseFC) are plotted on $T_{gaba} - T_{glu}$ parameter plane. The blue stars, marked on the 2D parameter plane, indicate optimal T_{glu}, and T_{gaba} values. (b) Clouds of estimated parameters are shown for young adults and elders, produced by 50 independent simulations. Blue and red circles represent the average values in young and old groups, respectively. (c) Regional excitatory and inhibitory firing rates are plotted for the estimated average T_{glu}, and T_{gaba} values. (d) Empirical and simulated static FCs are plotted using estimated parameters, taking average over trials. (e) Empirical and simulated FC dynamics (FCD) are shown in time-versus-time plot. All the simulated results are produced using estimated parameter values.

Similarly, it applies to the new Figure S8. Did the authors flip the FC axis? As it seems now the interhemispheric connectivity is in the top left and bottom right quadrants which is not the case in figure 4...

Response: Thank you for pointing this out. We have flipped the axis in Fig. S8 to align with Fig. 4.

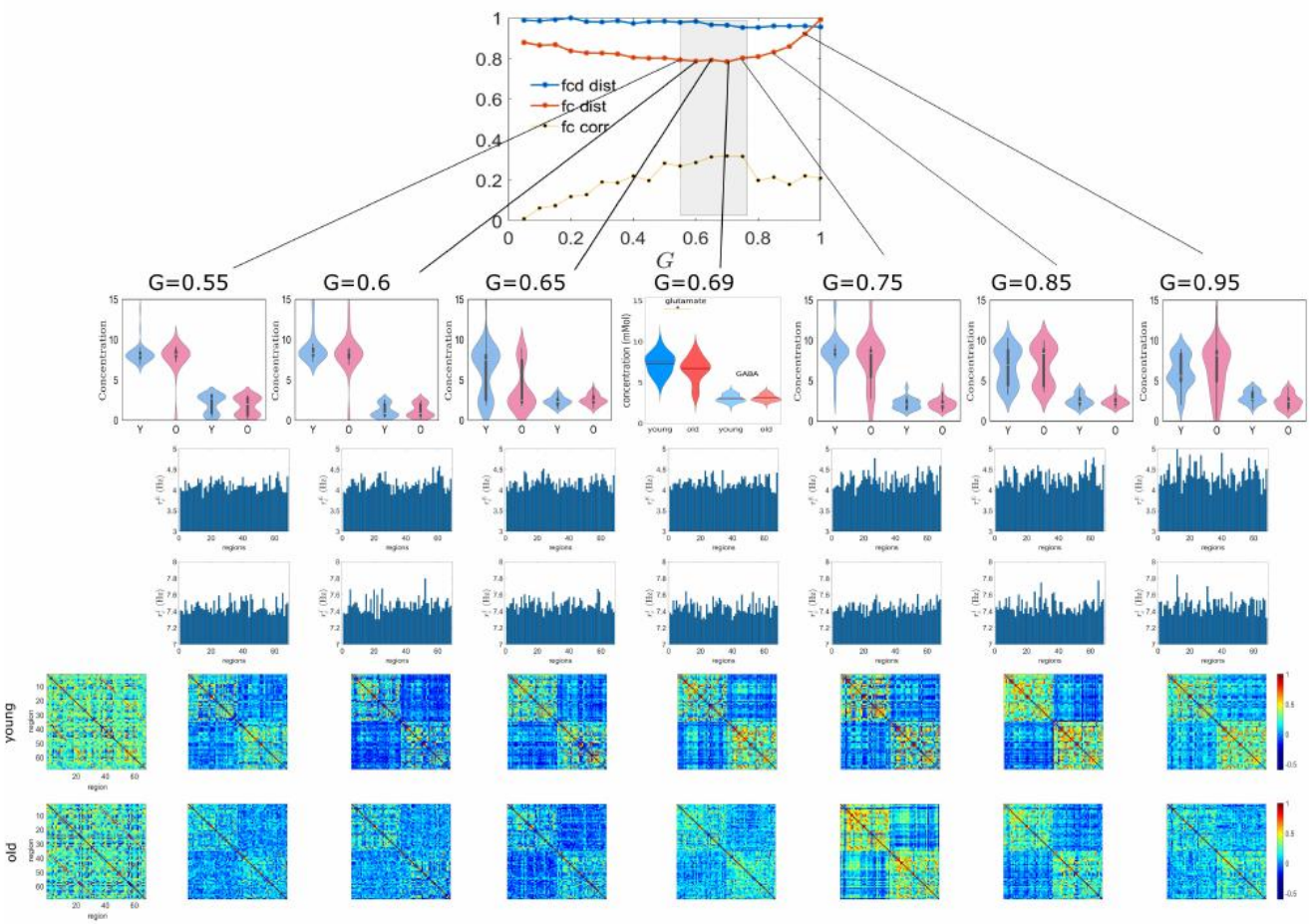

Figure S8 Effect of coupling strength (G) on parameter estimation and firing rates. The results are produced in Cam-CAN data. Top panel shows fitting of the coupling strength based on the distance and correlation between static FCs, and also dynamic FC distance. We have selected G=0.69 for our analysis in Cam-CAN data. Second row shows optimal GABA/Glutamate estimated at different G values. In the shaded range, we observe similar pattern of age-related neurotransmitter changes. Excitatory and inhibitory firing rates obtained for a single participant are plotted in the third and fourth rows, respectively. Fifth and last rows present empirical and simulated FCs from one young adult and elderly participant generated for different G values. Simulated FC is not a best fit and excitatory firing activity fluctuates at much higher rate when G is higher than optimal value.

Reviewer #2 (Remarks to the Author):

Thank you for the thoughtful and detailed revisions. The paper is now much clearer and better organized. My main concerns from the first review, especially about structure, methods, and interpretation, have been

addressed as well. I appreciate the revision. The paper makes a strong and valuable contribution to the field, and I support its publication.

Response: Thank you for your thoughtful comments that helped improve our work.

Reviewer #3 (Remarks to the Author):

My concerns have been well addressed. I am a little puzzled by why the FC of NKI dataset is so featureless (Figure 4d, second row) -- maybe the authors can make a quick clarification.

Response: Your insightful suggestions have been absolutely invaluable in helping us enhance our work. Thank you for the correction. We have corrected Fig. 4, shown above to address comment of Reviewer #1.