nature portfolio

Peer Review File

Evolutionarily conserved fMRI network dynamics in the mouse, macaque, and human brain

Reviewer #1 (Remarks to the Author):

Gutierrez-Barragan et al. introduce a concept called C-mode, building on their previous work with Coactivation Pattern (CAP) analysis. This method employs a frame-wise approach that pairs each CAP with its anti-correlated counterpart, significantly streamlining the analysis of functional brain dynamics and enhancing the quantitative assessment of brain connectivity. This approach is simple, flexible, and intuitive, distinguishing it from traditional static connectivity-based methods. By utilizing this innovative tool, the authors effectively demonstrate its comparability with conventional approaches across different species and have identified evolutionarily conserved fMRI network dynamics.

The findings are logically robust, having been rigorously cross-validated across multiple species using established analytical methods. This research marks a notable advancement in neuroscience, suggesting that C-mode could serve as a more quantitative approach for assessing brain connectivity, offering new insights that might be missed by conventional methods. The methodology is exceptionally thorough, with a meticulous selection and application of statistical methods to ensure the results are both solid and reproducible.

Despite the overall strength of this manuscript, there are a few areas that could benefit from further refinement by the authors. My specific observations and recommendations are detailed below.

- Major
1. While the study presents groundbreaking findings in the cross-species analysis of C-modes, there are concerns regarding the determination of regions of interest (ROIs) in mice. The foundational papers cited provide a basis for understanding network topography, yet they offer no clear rationale for the specific delineation of the mice network map conducted in this study. Particularly concerning is the division of the default mode network (DMN) into anterior and posterior segments to align with those of other species. This segmentation includes regions such as the Caudate Putamen (CPu) in the anterior DMN, and the Superior Cingulate (SC) in the posterior DMN. In my opinion, these regions are not traditionally recognized as part of their respective networks in other species. While there is evidence from previous studies indicating these regions' involvement in the networks, the major aim of cross-validation might be compromised by including them without clear justification. This inclusion does not accurately reflect homologous structures across species, raising questions about the suitability of these regions for cross-species comparison. It is recommended that the authors provide further clarification on the criteria used for determining mice ROIs in this study. Specifically, an explanation for the inclusion of certain subcortical regions only in mice would be beneficial, or alternatively, addressing these concerns by offering additional supporting information to justify their decisions.

2. In Figure 1D, the labeling of the C-modes for macaque and mice is reindexed in 1E-G based on their potential correlation with human C-modes, highlighting a need for clarity. Although Figure 1D illustrates the corresponding matching among species, the authors' decision to reorder these labels, using inconsistent indexing within the same label style, introduces confusion. This is particularly noticeable as it is re-labeled again for mice in Figure 2, suggesting a clearer labeling strategy could enhance comprehension. For example, adopting an alphabetical order for the initial labeling of CAPs followed by integer indexing for subsequent

references might mitigate confusion, particularly when labels are reassigned in figures such as Figure 2.

3. In Figure 1E-G, clarity is needed regarding the matching of C-modes across species, especially considering the foundational definition of C-modes includes the existence of anti-correlated counterparts. This raises a question about whether strongly anti-correlated features across species might yield more accurate matches. Specifically, there are concerns about the matching results for C-mode 4 between humans and macaques, as well as for C-mode 4 (labeled as 3 in this figure) between macaques and mice.

4. In Figure 2, the authors categorize each C-mode with descriptors such as DMN-SMN, DMN-Limbic, SMN-VIS, and VIS-FPN, which denote the primary features of each C-mode. However, the basis for these classifications is not explicitly defined. Specifically, referring to the rightmost column of Figure 2A, C-mode 3 is determined by the Z-scored C-mode maps profiles from predefined ROIs, revealing the most pronounced peaks (a positive peak for SMN and a negative peak for VIS). This approach to identification raises questions, as the methodology for distinguishing the other C-modes appears not to adhere uniformly to this principle. To improve clarity and facilitate the reproducibility of the findings, a detailed explanation of the criteria used to define each C-mode within the methods section is recommended.

5. On page 10, from line 21, the authors introduce functional connectivity gradients, aligning them with C-modes based on their occurrence and relevance. However, the manuscript introduces functional connectivity gradients without substantial background information, merely mentioning in the conclusion that these gradients represent the principal axes of variance in static fMRI activity. To enhance the narrative and analytical depth, incorporating a detailed description of functional connectivity gradients in an earlier section would clarify their significance and the rationale behind their integration with C-modes. Further elaboration on their importance in the discussion and conclusion sections would not only clarify this measure's compatibility with traditional approaches but also highlight how the frame-wise C-mode approach serves as a comprehensive tool for examining brain functional dynamics across species.

6. In addition, in Figure 6, while most matches between C-modes and gradients across species seem valid, certain pairings, such as C-mode 3 with Gradient 4 in humans and C-mode 3 with Gradient 2 and 4, appear less ideal. This concern arises from the use of the Hungarian algorithm, which, although it ensures an optimal match within the matrix leading to one-to-one pairings, might not fully capture the depth of the relationship between C-modes and functional gradients, both quantitatively and qualitatively. The critical issue is utilizing these matched pairs without further validation of their interconnection. As the authors have shown in Figure 2A with cross-species matching, a more detailed exploration into the alignment of C-modes with functional gradients is essential. This step is crucial to rectify potential mismatches and solidify the associations' credibility, especially considering Figure 6B uses these pairings to assess their linear relationship. This evaluation is pivotal as discrepancies in pairing could skew the interpretation of linearity tendencies, underscoring the need for meticulous validation to ensure the accuracy and relevance of the observed correlations.

- Minor

7. On page 5, 13th line from the bottom, there appears to be an extra "that" in the sentence 'These results suggest that >that< a few...' Removing the redundant "that" would clarify the statement.

8. On page 6, line 11, the authors mention that the sets of ROIs are based on evolutionarily conserved resting-state networks, citing references 28-31. While these references demonstrate the existence of RSNs across species, the specific term 'evolutionarily conserved' might benefit from additional citation. Reference 51, which delves into the evolutionary aspects of these networks, could provide a more robust foundation for this claim.

9. On page 6, line 24, the description of the authors' methodology as 'spatial correlation' might not fully capture the essence of their approach. Given the analysis seems to involve calculating correlations between vectors representing the topographic profiles of each C-mode's predefined RSNs, the term 'topological correlation' might more accurately describe the method.

10. On page 6, line 29, there is a reference to Figure 2C, which appears to be a mislabeling since Figure 2C does not exist. This could likely be a reference to Figure 2A?

11. In Figure 2A, it appears there is a formatting issue for the 'Z-scored' which is currently in bold.

12. In Figure 2B, enhancing the bar graph and scatter plot to include the polarity of each occurrence would provide a clearer and more informative presentation of the data.

13. On page 12, 4th line from the bottom, consider adding a hyphen to 'interareal', changing it to 'inter-areal' to enhance readability.

14. On page 17, line 19, it would be advisable to replace the punctuation with a comma for the thousand marks in the number of voxel counts, ensuring consistency with standard numerical formatting.

Reviewer #2 (Remarks to the Author):

This work provides much needed evidence supporting the notion that the dynamic organization of resting-state fMRI networks in mammalian species is governed by species-invariant principles. These results not only provide insights into the fundamental principles underlying brain network dynamics but also offer opportunities to relate fMRI network findings across different species, facilitating a better understanding of brain evolution and function.

This study is important to improve translational research efforts aimed at understanding brain function and dysfunction associated with resting-state networks using animal models. Although the matching of network patterns across species is challenging, the authors employed a strategy that allowed them to qualitatively compare across species, despite the anatomical differences.

I leave some comments below, which I would like the authors to adress:

Introduction:
1. The introduction is very well written and covers the state of the art. I just find this statement outdated: 'RSN mapping typically entails the computation of time-averaged statistical dependencies between fMRI time-series under the assumption that temporal structure of this activity is time-invariant over a time window of minutes.'. Indeed, the reference used is from 2013, and over the last decade the field has been gradually moving to dynamic analysis of functional connectivity, so I suggest rephrasing.

2. In the sentence: ' and compare the dynamic organization of fMRI in the mammalian brain.' I suggest adding 'signals' after fMRI.

Results:
3. 'Previous work has shown that CAPs embody rich fMRI topographies that can be reliably matched into mirrored coactive and anti-coactive pairs characterized by opposite BOLD polarity'.

While I understand what the authors mean by 'opposite BOLD polarity' it is important to highlight that the mechanism for this anti-polarity detected in fMRI signals remains to be fully linked with the BOLD response (especially since anti-phase locking has been detected between CSF in ventricles and gray matter voxels), As such, to avoid making unsupported assumptions, and given that the current study is not aiming to disambiguate the origin of the signals, but rather their organization principles, I would suggest rephrasing to 'opposite polarity in fMRI signals' to be more general.

4. 'Human to macaque matching gave consistent results for both HNU and MSC datasets, with the spatial of topography of human C-modes 1-4 being best aligned to corresponding macaque C-modes 1-4.' just a typo, remove 'of' between spatial and topography.

5. 'Thus, the temporal structure of C-modes in humans was biased towards a greater occurrence of polymodal integrative metastates (C-modes 1 and 2), with mice (and possibly macaques= exhibiting instead a greater occurrence of sensory-oriented network modes (C-modes 3 and 4).'
Could the authors explain better the expression 'polymodal integrative metastates'? Is there a reference? Also please correct the typo '=' should be ')'.

6. 'The power spectra of the resulting "C-mode timeseries" revealed that C-modes undergo infraslow fluctuations in all species, with most of the power peaking within the 0.01-0.03 Hz range'

Given that all the fMRI signals were band pass filtered between 0.01 and 0.1 Hz, the lower boundary of the power spectra shown in Figure 3 is strongly affected by the filtering. If the authors shuffle the spatial modes, would the temporal signatures still peak in the same range? (this would be a way to verify if the peak at 0.01Hz is really signature of resonance of the modes or if it is an artefact from the filtering. But perhaps the best solution would be: once having the spatial modes obtained with the current approach, compute the temporal signatures from the fMRI timeseries but considering this time the fMRI data before band-pass filtering. This would allow having an unbiased view of the power spectra of the modes.

7. ‘We found that the most recurring C-modes (1-2 in humans, 3-4 in macaques, and 3-4 in mice) were sinks of preferred directional transitions ($p < 0.01$, black crosses in Figure 5A), which can as such considered as state attractors.’

While I appreciate the term 'sinks' borrowed from dynamical systems theory, perhaps it would be good to add a short explanation about what is understood as a sink attractor. Also please add ‘be’ considered as state attractors. Moreover, it is to note that given that the states are not stable over time, they could be termed pseudo-attractors, or metastable attractors or even ghost attractors.

Discussion:

8. ‘Over the last decade, several influential mathematical models have described resting state activity in terms of networks of coupled oscillators. This work provides important empirical support to this modelling as it shows, from data and without making assumptions about the mechanism, that transition probabilities between different brain states can be described in terms of coupled C-Modes oscillating at infraslow frequencies.’

While I agree that this work provides important insights for modelling works, the specific work cited in this paragraph (Deco et al, 2008) used oscillators in the gamma frequency range 40 Hz and did not report ultra-slow oscillations (only ultra-slow fluctuations in synchrony degree, which, unlike oscillations, do not have sustained periodicity). Perhaps it would be better to cite here other works, by the same group, who used coupled infra-slow oscillators, such as https://doi.org/10.1371/journal.pcbi.1004100. Moreover, it would be interesting to add that the results from this work also align with the emerging hypothesis that the oscillations are not generated at the local level, but represent the slow frequencies of macroscopic modes of resonance in brain anatomy (https://doi.org/10.1038/s41586-023-06098-1 and https://doi.org/10.1038/s41467-023-36025-x).

We would like to thank the Reviewers for their positive evaluations, constructive comments, and for the opportunity to submit a revised manuscript. We highly value the insightful feedback provided, and we believe that the resulting paper has been significantly improved based on their suggestions.

Please find below a point-by-point response to the reviewers' comments (our response in blue typeface). We have also added below the text edits we have introduced in the manuscript in response to the reviewers' comments using yellow highlight.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Gutierrez-Barragan et al. introduce a concept called C-mode, building on their previous work with Coactivation Pattern (CAP) analysis. This method employs a frame-wise approach that pairs each CAP with its anti-correlated counterpart, significantly streamlining the analysis of functional brain dynamics and enhancing the quantitative assessment of brain connectivity. This approach is simple, flexible, and intuitive, distinguishing it from traditional static connectivity-based methods. By utilizing this innovative tool, the authors effectively demonstrate its comparability with conventional approaches across different species and have identified evolutionarily conserved fMRI network dynamics.

The findings are logically robust, having been rigorously cross-validated across multiple species using established analytical methods. This research marks a notable advancement in neuroscience, suggesting that C-mode could serve as a more quantitative approach for assessing brain connectivity, offering new insights that might be missed by conventional methods. The methodology is exceptionally thorough, with a meticulous selection and application of statistical methods to ensure the results are both solid and reproducible.

We thank the reviewer for the positive appraisal of our work and for appreciating the importance of the questions addressed.

Despite the overall strength of this manuscript, there are a few areas that could benefit from further refinement by the authors. My specific observations and recommendations are detailed below.

- Major

1. While the study presents groundbreaking findings in the cross-species analysis of C-modes, there are concerns regarding the determination of regions of interest (ROIs) in mice. The foundational papers cited provide a basis for understanding network topography, yet they offer no clear rationale for the specific delineation of the mice network map conducted in this study. Particularly concerning is the division of the default mode network (DMN) into anterior and posterior segments to align with those of other species. This segmentation includes regions such as the Caudate Putamen (CPu) in the anterior DMN, and the Superior Cingulate (SC) in the posterior DMN. In my opinion, these regions are not traditionally recognized as part of their respective networks in other species. While there is evidence from previous studies indicating these regions' involvement in the networks, the major aim of cross-validation might be compromised by including them without clear justification. This inclusion does not accurately reflect homologous structures across species, raising questions about the suitability of these regions for cross-species comparison. It is recommended that the authors provide further clarification on the criteria used for determining mice ROIs in this study. Specifically, an explanation for the inclusion of certain subcortical regions only in mice would be beneficial, or alternatively, addressing these concerns by offering additional supporting information to justify their decisions.

We thank the reviewer for this comment, which gives the opportunity to clarify and expand on how we matched mouse network to macaques' and humans'. We begin our reply by acknowledging that our methods section lacked reference to an important study we used to guide selection of regions used for mouse to monkey matching, i.e. the paper by Whitesell et al (Whitesell et al., 2021). In this manuscript we systematically described the anatomical regions that in the mouse belong to the DMN as probed with resting-state fMRI and anterograde axonal tracing. This investigation revealed that the DMN in the mouse brain incorporates *dorsal* components of the caudate putamen, a reflection of the strong reciprocal innervation of these regions with medial prefrontal regions. The involvement of dorso-striatal areas is especially pronounced in awake conditions, a finding we recently described in greater details elsewhere(Gutierrez-Barragan et al., 2022).

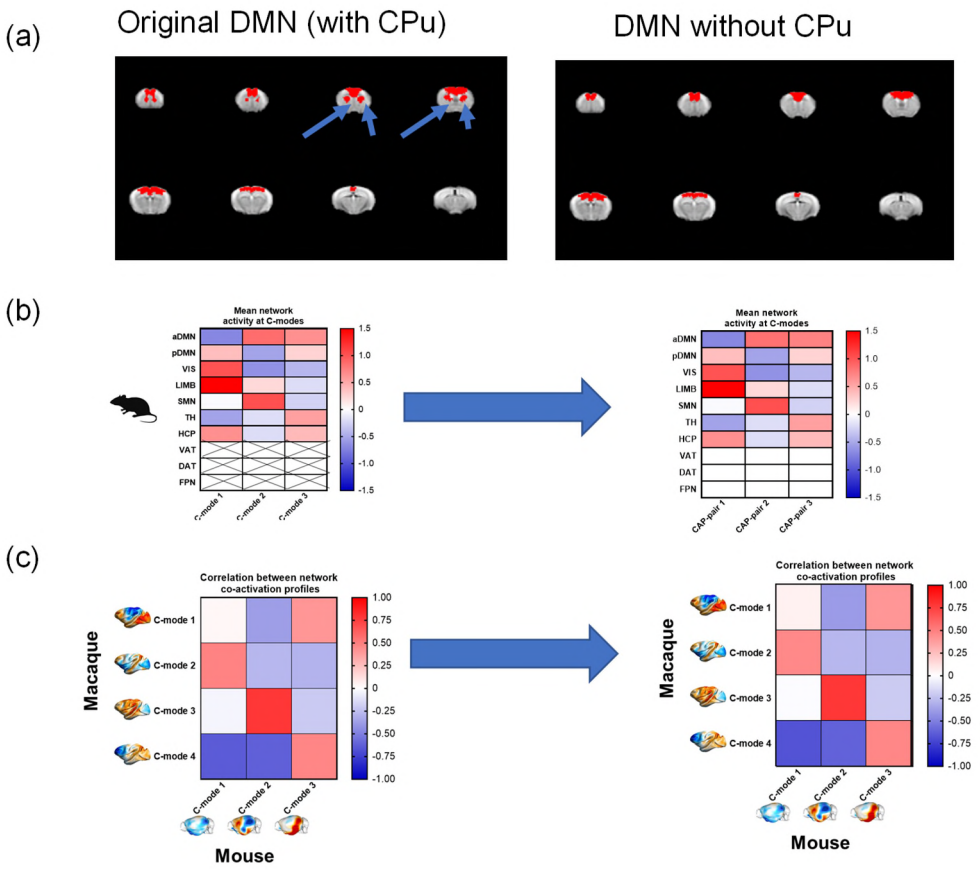
Our decision to split the DMN in anterior and posterior parts reflects emerging evidence that in primates this network is less cohesive than its human counterpart and often splits into two separate antero-posterior components when probed for example using independent component analysis (Garin et al., 2022; Gutierrez-Barragan et al., 2022).In rodents this is especially apparent in awake conditions, where parietal and peri-hippocampal cortical components that are synchronous with the mouse medial PFC under anesthesia tend to form two dissociable networks (see Figure 2 in Gutierrez-Barragan et al., 2022, and Rebuttal Figure 1 below). Our antero-posterior DMN split was thus informed by anatomical information obtained by these previous studies. In the anterior DMN (aDMN) we included the anterior cingulate, which in rodents serves as a hub for this network (Liska et al., 2015). In the posterior DMN (pDMN) we instead included the retrosplenial cortex (the region the reviewer referred to as "superior cingulate", we believe). To properly contextualize this choice, it may be useful to remember that rodents do not have a cytoarchitectural homologue of the posterior cingulate cortex (PCC)(Vogt & Paxinos, 2014). However, humans, macaques and rodents do possess a retrosplenial cortex, which in rodents is configured as a caudal extension of the anterior cingulate. Importantly, in both humans and macaques, the retrosplenial cortex is located ventro-medially to (and neighbors with) the PCC. Owing to these characteristics, the retrosplenial cortex is regarded by many as a "rodent precursor" of the primate PCC (Gozzi & Schwarz, 2016; Vogt & Paxinos, 2014). We thus believe that this distinction is anatomically plausible, and supported by prior functional mapping of the DMN.

We include below a full list of the ROIs that we included in the mouse DMN for the reviewer's perusal.

ACRONIM	NAME	network
MOp	Primary motor area	aDMN
MOs	Secondary motor area	aDMN
SSp-ll	Primary somatosensory area, lower limb	aDMN
SSp-ul	Primary somatosensory area, upper limb	aDMN
SSp-un	Primary somatosensory area, unassigned	aDMN
VISam	Anteromedial visual area	aDMN
ACAd	Anterior cingulate area, dorsal part	aDMN
ACAv	Anterior cingulate area, ventral part	aDMN
PL	Prelimbic area	aDMN
ILA	Infralimbic area	aDMN
ORB	Orbital area	aDMN
STRd	Striatum dorsal region	aDMN
SSp-bfd	Primary somatosensory area, barrel field	pDMN
SSs	Supplemental somatosensory area	pDMN
AUDd	Dorsal auditory area	pDMN
AUDp	Primary auditory area	pDMN
AUDpo	Posterior auditory area	pDMN
AUDv	Ventral auditory area	pDMN
VISpor	Postrhinal area	pDMN
RSPagl	Retrosplenial area, lateral agranular part	pDMN
RSPd	Retrosplenial area, dorsal part	pDMN
RSPv	Retrosplenial area, ventral part	pDMN
PTLp	Posterior parietal association areas	pDMN
TEa	Temporal association areas	pDMN
STRv	Striatum ventral region	pDMN

Given that our work focused on how large-scale rsfMRI networks interact in time and are represented within single C-modes, we deemed that the best approach to match C-modes between species was through network-averaged BOLD intensity within a C-mode map. In our manuscript we termed these maps ‘network profile vectors’ (Figure 1C), which are computed by summing mean BOLD signal of voxels that belong to predefined network at each C-mode. As correctly inferred by the reviewer, the aDMN map in mice also included the whole caudate putamen (CPu) which we decided to maintain in its entirety (as opposed to including the sole dorsal part of this area) for simplicity, given there is no consensus anatomical division of this region between posterior and ventral portion at present.

To make sure this arbitrary choice did not bias our cross-species matching, we have now repeated cross-species C-mode matching after removing the entire CPu from the mouse DMN. We report the results we obtained in Rebuttal Figure 1 below. We found that the corresponding network coactivation profile was virtually unaffected by the presence (or lack thereof) of the CPu, and that the resulting macaque-to-mouse matching was also unchanged.



Rebuttal Figure 1. *Macaque-to-mouse matching after removing the Caudate Putamen from the (anterior) DMN of the mouse brain. (a): Anatomical distribution of the mouse DMN (in original EPI resolution). This map guided ROI selection in our original manuscript. On the right we report the corresponding version of the network upon removal of dorsal CPu (blue arrows). (b) This panel reports the original mouse network profile (left, from Figure 1 of our original manuscript) and on the right, the corresponding profiles computed by removing the CPu. In (c) we report macaque-to-mouse matching using original DMN (Figure 1 of our original submission) and corresponding Hungarian algorithm-based matching obtained upon removal of the CPu from the mouse DMN. Note that the resulting matching was unchanged compared to original analyses.*

Because the inclusion of this regions did not affect pour results, in the revised manuscript we have left the CPu as an integral part of the DMN as per our original work. We have now revised our manuscript to more explicitly describe in the methods section the reasons behind our choice to split the DMN as follows:

Methods: Coactivation Modes and cross-species matching

*"Given that C-modes represent unique configurations between known resting state networks[16,17,19], we extracted the mean BOLD values from voxels within masks of previously described, evolutionarily conserved[31,55] cortical RSN in humans[28], macaques[29] and mice[30,71], as well as hippocampal and thalamic masks[72,82] (**Figure 1C**). To best match DMN organization across species, we split this network into an anterior and a posterior component. This choice was made to account for recent primate and mouse evidence of rostro-caudal disassembly of this network in some datasets and species[30,83]. In rodents, this is especially apparent in awake conditions, where parietal and peri-hippocampal cortical components that are synchronously connected to the mouse PFC (in anesthesia) tend to form dissociable networks in awake animals[30]. For consistency with previous studies, we included the caudate putamen in the mouse DMN (but not in primate and human DMN) as in rodents this region is proximal and highly synchronous with anterior cingulate hubs of the DMN both under anesthesia and in awake conditions[12]. Pilot studies showed that the inclusion (or lack thereof) of the CPu in mouse DMN did not alter monkey-to-human C-Mode matching."*

2. In Figure 1D, the labeling of the C-modes for macaque and mice is reindexed in 1E-G based on their potential correlation with human C-modes, highlighting a need for clarity. Although Figure 1D illustrates the corresponding matching among species, the authors' decision to reorder these labels, using inconsistent indexing within the same label style, introduces confusion. This is particularly noticeable as it is re-labeled again for mice in Figure 2, suggesting a clearer labeling strategy could enhance comprehension. For example, adopting an alphabetical order for the initial labeling of CAPs followed by integer indexing for subsequent references might mitigate confusion, particularly when labels are reassigned in figures such as Figure 2.

We thank the reviewer for highlighting this potential source of confusion. Now in Figure 1 we name human C-modes "H1-H4", macaque C-modes "M1-M4", and mouse C-modes R1-R3 (R for rodent). Moreover, not until Figure 2 do we number them as per our original manuscript. New text in the revised version of our manuscript now includes:

RESULTS section: fMRI C-modes exhibit evolutionarily-conserved functional organization

"… *A key exception of note is the lack of established phylogenetic precursors of the VAT, DAT, and FPN in the rodent brain[10]. For this reason, these networks were not included in the coactivation profile in mouse data. For matching purposes, we ordered C-modes for each species in decreasing joint occurrence rates, and labelled them H1-H4 for humans, M1-M4 for macaques, and R1-R3 for rodents (mice).*

…

Human to macaque matching gave consistent results for both HNU and MSC datasets, with the spatial topography of human C-modes H1-H4 being best aligned to corresponding macaque C-modes M3, M4, M2, M1, respectively (see also ***Figure 2A****). Because the human HNU dataset included more subjects and was performed at daylight hours as animal scans, we describe our results hereafter for the HNU as main dataset and present a summary of the obtained MSC results as supplementary figure* ***(Figure S7).*** *Macaque to mouse matching linked C-modes M1 to R1, M2 to R2, and M4 to R3, respectively (****Figure 1G, Figure 2A****). The resulting overall cross-species matchings yielded an organization linking C-modes H1-M3, H2-M4-R3, H3-M2-R2, and H4-M1-R1. For brevity, we henceforth refer to these as "C-mode 1", "C-mode 2", "C-mode 3", and "C-mode 4", respectively. As human and macaque C-mode 1 was not preferentially matched to any mouse C-modes, we refer to mouse C-modes as 2, 3, and 4 for consistency with those mapped in higher species throughout the manuscript."*

3. In Figure 1E-G, clarity is needed regarding the matching of C-modes across species, especially considering the foundational definition of C-modes includes the existence of anti-correlated counterparts. This raises a question about whether strongly anti-correlated features across species might yield more accurate matches. Specifically, there are concerns about the matching results for C-mode 4 between humans and macaques, as well as for C-mode 4 (labeled as 3 in this figure) between macaques and mice.

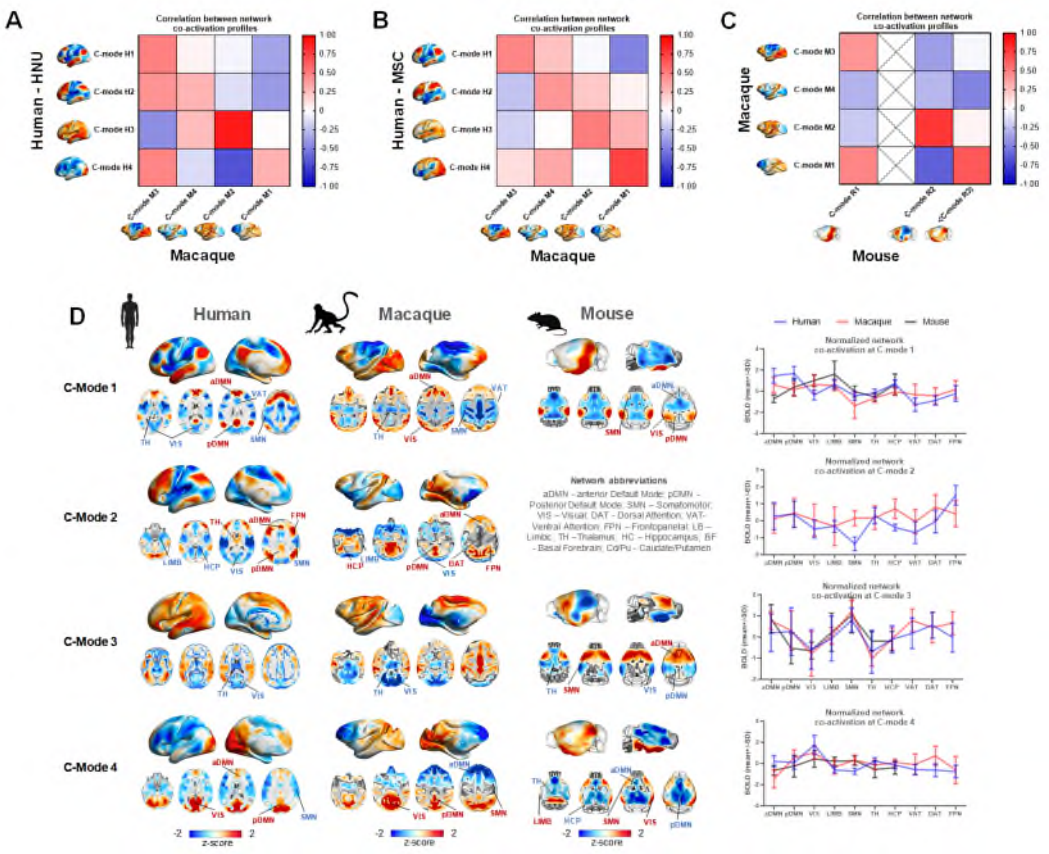
We thank the reviewer for this important comment. The problem of how to best match coactivation topology across evolutionary-distant species like macaques and rodents is a challenging one, and one that lends itself to possibly multiple solutions. We expand below on the reasoning behind the specific C-mode matching we opted for in our article.

As previously mentioned, CAPs and hence C-modes are instances of network interaction for which we believe cross-species matching via a comparison of network coactivation profiles is a viable approach. In our original work, matching of coactivation vectors was done using the Hungarian Algorithm, using signed correlation as a distance metric. As pointed out by the reviewer, this approach may bias matching toward positive correlations. Though we recognize there are other

matching methodologies, our intention was to utilize a quantitative approach based on network coactivations that was in line with observations of overall network organization across species, and that could highlight salient evolutionarily-relevant dynamic properties of brain networks.

As suggested by the reviewer, in human HNU dataset there is evidence for possible alternative solutions to the proposed C-Mode H4 to M1 human-to-macaque pairing (Figure 1E). Our choice to select the specific matching we used in the manuscript over possible alternatives (e.g. C-Mode H3 to M2) is the result of several converging considerations. First, the spatial (anti)correlation between C-modes H4 and M2 (e.g., a possible alternative to our matching) is lower in magnitude (|r|= 0.61) than the one observed with original H3 to M2 matching (|r|= 0.89). This suggests that C-mode H3 is overall a better match to C-mode M2 than any other human C-mode.

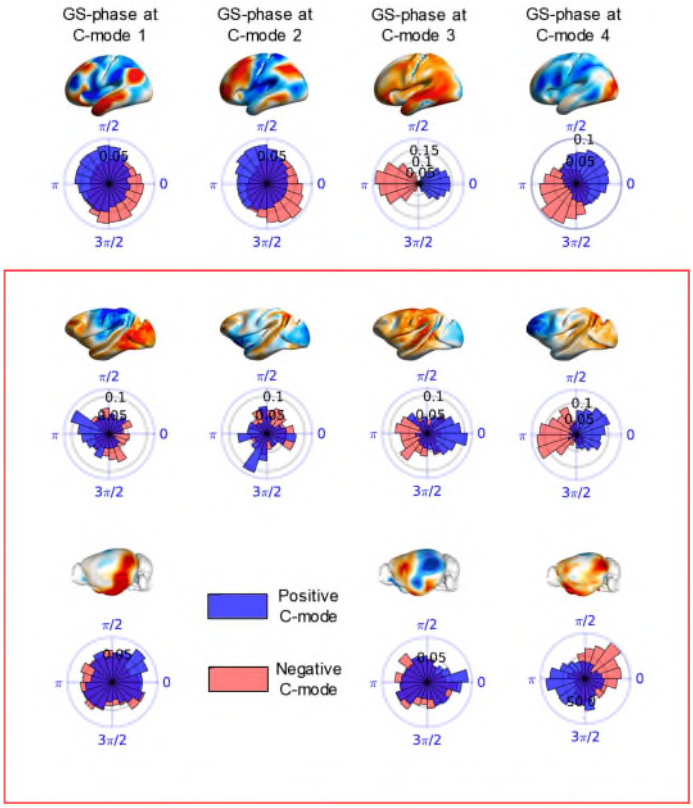
In keeping with this notion, when we replicated human-to-macaque C-mode matching in an independent dataset (MSC – Figure 1F), we found that the best matching solution once again paired C-modes H3 to M4, and H4 to M1. Moreover, in this second dataset there was no evidence of the anti-correlation between C-modes H3 and M2 as seen in the HNU dataset. Importantly, the validity of our proposed matching was also independently supported by *a posteriori* observation of highly concordant phase distribution of C-Modes 3 and 4 in both humans and macaques (Figure 4B). Taken together, these results suggest the validity of the proposed human-to-macaque matching. This notion is further corroborated by additional human-to-macaque C-mode matching that we carried out using absolute valued correlations as per the reviewer's suggestions (Rebuttal Figure 2, below). As reported in panels A, and B of this figure, the resulting human-to-macaque matching confirmed our original pairings in both the HNU and MSC scans.



Rebuttal Figure 2. C-mode matching using absolute value correlations as distance metric. (A) Correlation between matched C-modes from humans (HNU dataset) and macaques, **(B)** between humans (MSC dataset) and macaques; and **(C)** between macaques and mice. **A-B** matching between humans and macaques remains the same, but there is a switch between the associated C-modes in mice **(C)**. **(D)** C-mode maps after changing mouse matching and inverting C-mode R3. Right panels show the normalized network coactivation profiles. Abbreviations: aDMN - anterior Default Mode; pDMN - Posterior Default Mode; SMN – Somatomotor; VIS – Visual; DAT - Dorsal Attention; VAT-Ventral Attention; FPN – Frontoparietal; LIMB – Limbic; TH –Thalamus; HC – Hippocampus; BF - Basal Forebrain; Cd/Pu - Caudate/Putamen. P-values: *p < 0.05, **p < 0.01, *** p < 0.001, **** p < 0.0001.

Owing to the large evolutionary distance that exists between macaques and rodents, it is not surprising that C-mode matching between these two species exhibited a higher degree of degeneracy, with evidence of a possible redundancy in the matching between C-mode 1M and C-modes R1-3 in the mouse (Figure 1F). In our work, we selected the solution produced by the Hungarian algorithm using only absolute valued correlations as a distance metric, which paired C-modes M4, M2 and M1 to C-modes R3, R2 and R1, respectively. This solution has the advantage of producing a plausible and distinct matching for all the three mouse C-modes, highlighting key correspondences in coactivation profiles of C-modes and their constituent networks across species (Figure 2). More importantly, this matching also broadly recapitulates the dominant phase distribution observed when comparing corresponding human, macaque and mouse C-modes (see C-modes 3 and 4, Figure 4). This latter observation indirectly supports the plausibility of our matching in the presence of potential alternative solutions, which failed to replicate these phase correspondences.

Specifically, when we recalculated the macaque-to-mouse C-mode matching using absolute value correlation (Rebuttal Figure 2, below), we found a new configuration where the sign of mouse C-mode R3 is switched, resulting in a better alignment with macaque C-mode M1. This adjustment would lead to C-mode 2 being unique to macaques and humans. While this alternative matching may be spatially plausible, it nevertheless failed to highlight consistent C-mode phase distribution across species (see Rebuttal Figure 3 below). We therefore believe that the macaque to mouse matching we chose for our original submission, while not spatially unequivocal, provides a plausible representation of key evolutionarily-relevant dynamic features of C-modes. We have therefore decided to retain our original matching in the revised manuscript.



Rebuttal Figure 3. C-mode occurrence within fMRI global signal cycles when macaque C-modes are matched to mouse C-modes using absolute valued correlation. (A) Group-level power spectral density (mean+/- SEM) of the GS. **(B)** Distribution of GS-phases at the occurrence of each C-mode. Blue and red distributions correspond to GS phases sampled from the positive and negative C-mode time courses, respectively. All distributions significantly deviate from circular uniformity (Rayleigh test, $p < 0.05$, FDR corrected). Note lack of concordance (or open discordance, C-mode 4) of phase distributions between macaque and mouse (with respect to Figure 4).

With the goal of keeping the manuscript within editorial limits and limit the technical content of the paper we have decide to report these findings (i.e. those depicted in Rebuttal Figure 2 and 3) solely within the confines of this rebuttal letter. We remain open to reconsideration should the reviewers think the inclusion of these considerations and additional analyses may significantly add to our work.

In the revised manuscript we have expanded the description of our matching strategy, and we now mention these results as part of the pilot studies used to guide the matching strategy employed, as follows:

Results:

*Human to macaque matching gave consistent results for both HNU and MSC datasets, with the spatial topography of human C-modes H1-H4 being best aligned to corresponding macaque C-modes M3, M4, M2, M1, respectively (see also **Figure 2A**). Because the human HNU dataset included more subjects and was performed at daylight hours as animal scans, we describe our results hereafter for the HNU as main dataset and present a summary of the obtained MSC results as supplementary figure **(Figure S7).** Macaque to mouse matching linked C-modes M1 to R1, M2 to R2, and M4 to R3, respectively (**Figure 1G, Figure 2A**). he resulting overall cross-species matchings yielded an organization linking C-modes H1-M3, H2-M4-R3, H3-M2-R2, and H4-M1-R1 For simplicity, we henceforth refer to these as "C-mode 1", "C-mode 2", "C-mode 3", and "C-mode 4", respectively."*

Methods:

*For each C-mode, we computed their occurrence rates as the average occurrence of the CAP and anti-CAP conforming the C-mode. We then organized the vectorized network coactivation profiles of each C-mode in descending occurrence rate order, and matched C-mode profiles of humans (C-mode H1-4) to macaques (C-mode M1-4), then macaques to mice (C-mode R1-3, rodent) using the Hungarian Algorithm[32]. This matching was conducted using vector-correlations between profiles as distance metrics for the cost function to be minimized (**Figure 1D**). We represented the similarities between network coactivation profiles of C-modes in the human HNU dataset and macaques (**Figure 1E**), as well as with the MSC dataset and macaques, confirming our matching (**Figure 1F**). Finally, we matched macaque C-mode network coactivation profiles with those of mice, without accounting for Fronto-Parietal (FPN), Ventral (VAT) and Dorsal Attention (DAT) networks, as these have not been described in the mouse brain (**Figure 1G**). We note that since C-mode 1 was not reliably identified in mice, most likely due to the lack of weighting by higher order VAT, DAT, and FPN, we hereafter exclude C-mode 1 from this species.*

Because C-modes fundamentally include an anticorrelated counterpart, in pilot studies we probed cross-species matching using absolute value correlations as opposed to positive correlation only. We found that only macaque M4 and mouse R3, as well as M1 and the inverse of R1, produced a different match when absolute value correlation was used as distance metrics. Matching between human and macaque C-modes remained unchanged. However, while this alternative matching appeared to be spatially plausible, only matching using positive spatial correlation as a cost function resulted in a plausible cross-species preservation of phase coupling with fMRI global signal cycles in macaques and mouse. For this reason, we retained positive correlation-based matching as reference dataset for the present manuscript, as we believe it provides a more plausible representation of key evolutionarily-relevant dynamic features of C-modes.

4. In Figure 2, the authors categorize each C-mode with descriptors such as DMN-SMN, DMN-Limbic, SMN-VIS, and VIS-FPN, which denote the primary features of each C-mode. However, the basis for these classifications is not explicitly defined. Specifically, referring to the rightmost column of Figure 2A, C-mode 3 is determined by the Z-scored C-mode maps profiles from predefined ROIs, revealing the most pronounced peaks (a positive peak for SMN and a negative peak for VIS). This approach to identification raises questions, as the methodology for distinguishing the other C-modes appears not to adhere uniformly to this principle. To improve clarity and facilitate the reproducibility

of the findings, a detailed explanation of the criteria used to define each C-mode within the methods section is recommended.

We agree with the reviewer that the employed criteria were too arbitrary, and not properly explained. We have also realized these labels did not add to the narrative of our work, and were scarcely mentioned in the remainder of our manuscript. For these reasons, we have now removed these descriptors throughout the text and figures of our revised manuscript.

5. On page 10, from line 21, the authors introduce functional connectivity gradients, aligning them with C-modes based on their occurrence and relevance. However, the manuscript introduces functional connectivity gradients without substantial background information, merely mentioning in the conclusion that these gradients represent the principal axes of variance in static fMRI activity. To enhance the narrative and analytical depth, incorporating a detailed description of functional connectivity gradients in an earlier section would clarify their significance and the rationale behind their integration with C-modes. Further elaboration on their importance in the discussion and conclusion sections would not only clarify this measure's compatibility with traditional approaches but also highlight how the frame-wise C-mode approach serves as a comprehensive tool for examining brain functional dynamics across species.

We acknowledge the reviewer's observation regarding the brevity of our description of gradients. This was a deliberate decision aimed at preventing our manuscript from exceeding editorial limits of Nature Communications. In the revised manuscript we expanded our discussion of how gradients offer a way to better static and dynamic organization of fMRI network activity due to their ability to conflate high dimensional information in connectomes into manifold representations. We have therefore added the following text to our initial description:

RESULTS: fMRI C-mode occurrence predicts ranking of connectivity gradients in humans, macaques and mice

"To further investigate the relationship between C-mode dynamics and static fMRI connectivity, we next inquired whether C-mode occurrence could also be related to the organization of fMRI connectivity gradients[36,37]. Gradient approaches were favored here because of their ability to chart the high dimensional static functional connectome into low-dimensional manifold representations. In humans, the principal gradient of resting state fMRI delineates a hierarchical organizational axis that reconstitute unimodal-transmodal spectrum in cortical regions. Crucially, this arrangement spatially recapitulates key myeloarchitectural, cytoarchitectural and transcriptional features of the human and animal neocortex[38,39]. Higher-order gradients have similarly been linked to axes of differentiation between sensory cortical modalities. Here, we examined how the dynamic organization of fMRI activity relates to the spatial organization of gradients. We posited that C-mode occurrence rate may be linked to the ranking of functional connectivity gradients, with dominant (i.e. most occurring) C-modes aligning with the gradients that explain the most variation in fMRI connectivity."

We have also expanded discussion of gradients as follows:

DISCUSSION:

"Our results also highlight cross-species correspondences in the relationship between the dynamic organization of fMRI activity, and principal axes of variance of static fMRI activity as probed with connectivity gradients. We found that temporally dominant C-mode spatially reconstitute the principal axis of static functional organization. This positive relationship – i.e. the occurrence of C-mode and the order the functional gradient – is preserved across species, suggesting a fundamental conservation of how dynamic states underpin static functional organization across species. Interestingly, in previous studies the transmodal-unimodal hierarchy gradient was commonly

identified as the dominant organizational axis of static fMRI connectivity in humans[36]. However, this gradient has not been universally observed as the foremost gradient in other species[36,39,69,70]. By examining static functional organization through the lens of the functional dynamics, our study provides a deeper understanding of this discrepancy, suggesting that it can be attributed to the different occurrence of underlying C-mode dynamics. Taken together, these results the macroscale organization of the functional connectome is critically shaped by the occurrence of its constituting spatiotemporal modes, a notion supported also by complementary conceptualization of fMRI dynamics[67]."

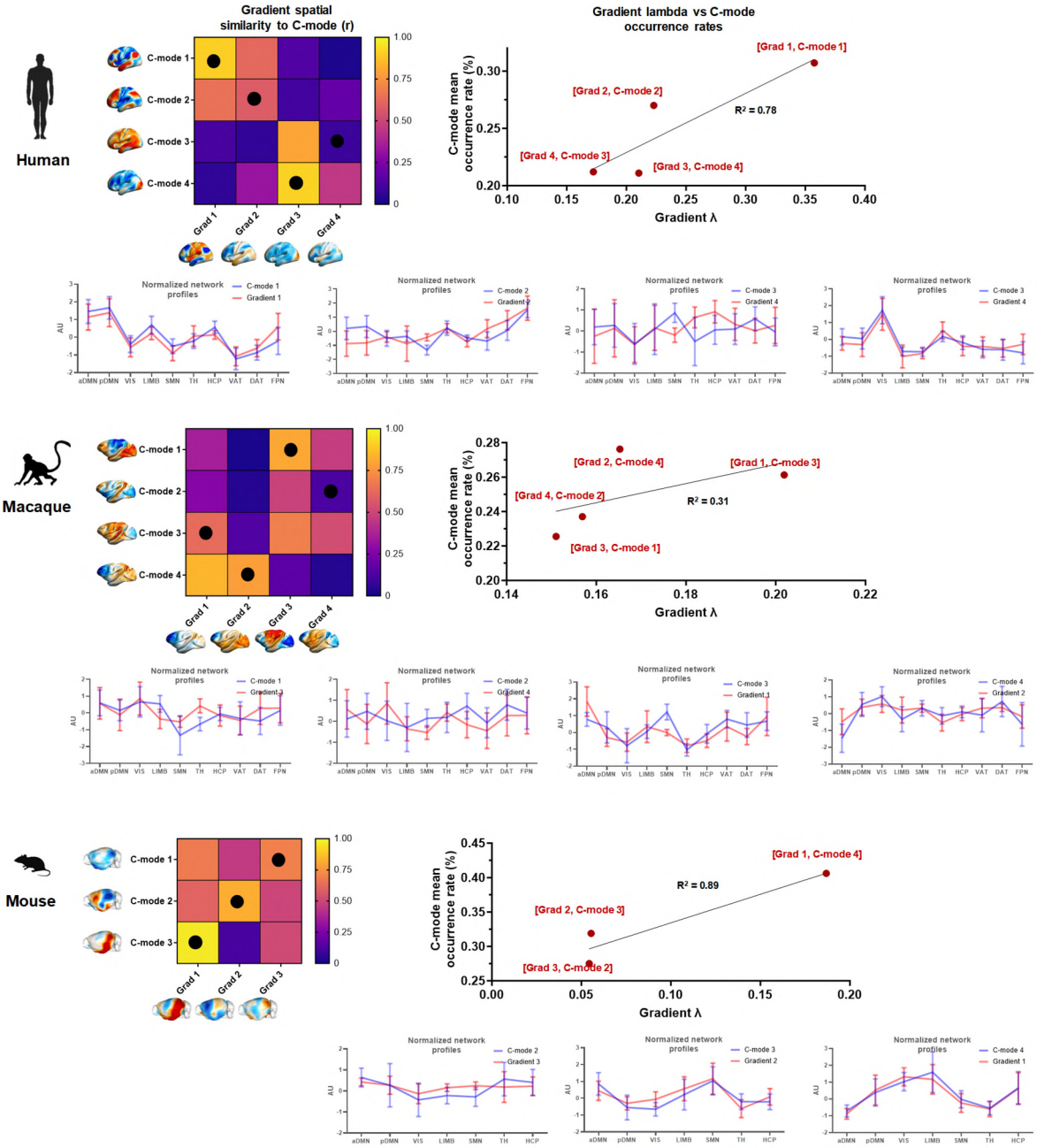
6. In addition, in Figure 6, while most matches between C-modes and gradients across species seem valid, certain pairings, such as C-mode 3 with Gradient 4 in humans and C-mode 3 with Gradient 2 and 4, appear less ideal. This concern arises from the use of the Hungarian algorithm, which, although it ensures an optimal match within the matrix leading to one-to-one pairings, might not fully capture the depth of the relationship between C-modes and functional gradients, both quantitatively and qualitatively**.** The critical issue is utilizing these matched pairs without further validation of their interconnection. As the authors have shown in Figure 2A with cross-species matching, a more detailed exploration into the alignment of C-modes with functional gradients is essential. This step is crucial to rectify potential mismatches and solidify the associations' credibility, especially considering Figure 6B uses these pairings to assess their linear relationship. This evaluation is pivotal as discrepancies in pairing could skew the interpretation of linearity tendencies, underscoring the need for meticulous validation to ensure the accuracy and relevance of the observed correlations.

We thank the reviewer for giving us the opportunity to further expand on these findings. We agree that the one-to-one correspondence between C-modes and gradients we proposed was not perfect. The problem pertained mostly C-mode 3 in humans and macaques, for which the matching suggested by the Hungarian algorithm appear to be potentially non-univocal. This finding is not surprising if we consider that C-mode 3 represents a spatiotemporal pattern characterized by an almost global coactivation, which we found in all species to be temporally in phase with fMRI GS cycles (Figure 4). Probably due to the involvement of this specific C-mode in GS-related coactivation (as shown in mice (Coletta et al., 2020), macaques (Xu et al., 2020), and humans – this work), we did not find a gradient axis in which most of the brain networks act in unison. We therefore did not expect to find a similar matched 'global' gradient in either species. However, despite this potentially imperfect matching, we have observed a consistent cross-species trend where the most dominant gradients (i.e. those that explain the most variance) are more likely to correlate with C-modes that have a higher occurrence (Fig. 6B). Our reasoning was therefore to maintain a quantitative matching, for each species, and to allow the algorithm to suggest which was the gradient most similar spatially to this particular C-mode 3 (hence the low correlation values in the corresponding elements of matrices in Figure 6A). We are now adding some relevant points as limitations in the Discussion section of the manuscript (see below).

Regarding the matching itself and its corroboration across species, in Figures 1 and 2 we matched C-modes between species using network profiles as the different CAP maps do not belong in the same voxel space. All other within species comparisons were done at the voxel level to take into account all of the spatial variability offered by this resolution. This also led us to compare C-modes and gradient maps the same way.

To qualitatively inform our matching and corroborate our previous solution, following the reviewer's suggestion, we have now performed an additional C-mode to Gradient matching using the previously identified resting-state network profiles. To this end, for each species we took the network profiles from each C-mode in Figure 2, and also extracted the average value of the voxel within each network in each gradient map. We then computed the matching through the Hungarian Algorithm between these network profiles. We report the obtained correlation matrices between C-mode and gradient network vectors below for the reviewer's perusal (**Rebuttal Figure 4,** dots

highlight the matched maps). Below these matrices we depict the network profiles for each matching between a C-mode (blue), and its corresponding gradient (red), as well as the linear relationship between the occurrence rate of a C-mode and the variance explained by its matched gradient. The obtained results show that for humans and mice, matching remained identical as with our voxelwise method and that network profiles show high correlations, especially for C-modes with preferred occurrences ($r > 0.75$). In macaques, however, the network profile matching switched the pairing of C-mode 3 to gradient 1 and C-mode 4 to gradient 2, exchanging the associated gradients between these C-modes. This change drastically lowered the variance explained of the linear model to $R^2 = 0.31$, instead of its original 0.81. This discrepancy suggests that our voxelwise matching better captures the fine-grained spatial covariation of gradients with respect to the anatomy of C-modes. For this reason, we think it is best to leave our original matching scheme intact, while openly acknowledging the limitations of our method.



Rebuttal Figure 4. C-mode to gradient matching using network profiles for humans (top), macaques (middle), and mice (bottom). Each specie's panels includes the C-mode to gradient network profile correlation matrix (top-left), with a black dot denoting the match pairs (Hungarian Algorithm, absolute correlation distance). **(top-right)** Scatterplot of the variance explained by each gradient (lambda) versus the match C-modes average occurrence rate. R-square from a linear fit ($p < 0.02$). **(bottom)** Network profiles from each C-mode and its matched gradient, denoting the average (+/- SD) intensity of voxels within a network in each map.

With this in mind, we added the following section to our discussion:

DISCUSSION

"It should be noted that C-mode 3 exhibited low correlation to its assigned gradient in both human and macaque datasets. This C-mode involves simultaneous co-activation of most brain regions and is temporally aligned with fMRI GS cycles. As such, C-mode 3 reflects a state of global activity for which an axis of sorts is not the leading dimension explaining variance in the functional connectomes. In keeping with this, previous studies in mice[71], macaques[37], and humans[36] did not identify an organizational axis in which most of the brain networks act in unison. We therefore did not expect to find a similar 'global' gradient in either species. To maintain a semi-quantitative matching, we nonetheless allowed the algorithm to suggest which was the gradient most spatially similar to this C-mode. Future studies will warrant dedicated investigations of how periods of near whole-brain coactivation can be best accounted for as variance descriptors of static connectomes."

Minor

7. On page 5, 13th line from the bottom, there appears to be an extra "that" in the sentence 'These results suggest that >that< a few...' Removing the redundant "that" would clarify the statement.

We have now fixed this typo.

8. On page 6, line 11, the authors mention that the sets of ROIs are based on evolutionarily conserved resting-state networks, citing references 28-31. While these references demonstrate the existence of RSNs across species, the specific term 'evolutionarily conserved' might benefit from additional citation. Reference 51, which delves into the evolutionary aspects of these networks, could provide a more robust foundation for this claim.

We appreciate the suggestion and have added ref. 7 (Pagani et al. 2023(Pagani et al., 2023), Communications Biology Review) as well as other references to support the claim.

9. On page 6, line 24, the description of the authors' methodology as 'spatial correlation' might not fully capture the essence of their approach. Given the analysis seems to involve calculating correlations between vectors representing the topographic profiles of each C-mode's predefined RSNs, the term 'topological correlation' might more accurately describe the method.

We thank the reviewer for this suggestion. We have now used "topological correlation" to refer to calculation of correlations between our coactivation vectors.

10. On page 6, line 29, there is a reference to Figure 2C, which appears to be a mislabeling since Figure 2C does not exist. This could likely be a reference to Figure 2A?

We thank the reviewer for spotting this mistake. Our reference was to the network coactivation profiles alongside the C-mode maps in Figure 2A.

11. In Figure 2A, it appears there is a formatting issue for the 'Z-scored' which is currently in bold.

We have corrected this formatting issue.

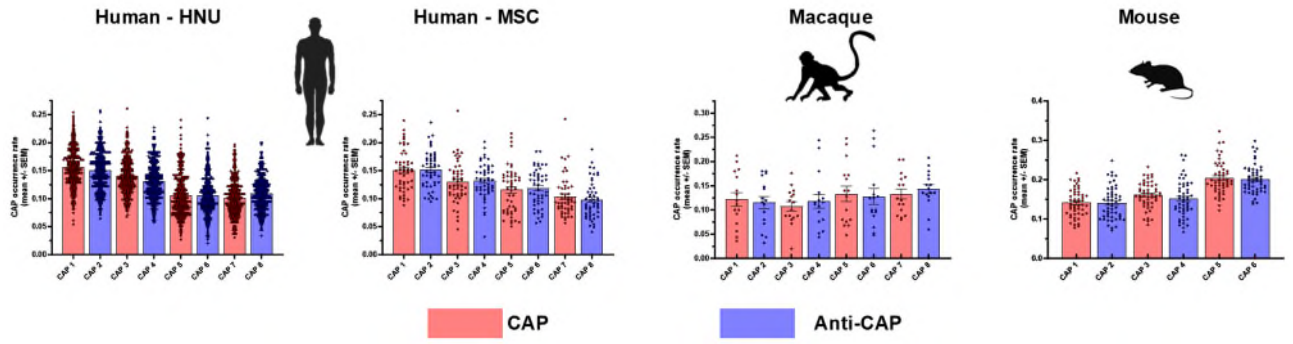
12. In Figure 2B, enhancing the bar graph and scatter plot to include the polarity of each occurrence would provide a clearer and more informative presentation of the data.

We thank the reviewer for the opportunity to expand on our initial results and probe the independent occurrence rates of the CAPs that compose C-modes. We have now plotted the occurrence rate of

CAPs composing each C-mode as Supplementary Figure S6 in the revised manuscript**.** We report the figure below for the reviewer's perusal. A high concordance in the occurrence rate of CAP pairs belonging to the same C-mode is apparent. As part of this analysis, we also tested whether there were significant differences between the occurrence rate of each CAP and anti-CAP. We found no statistically significant differences in all species ($p > 0.2$, signed-rank test). The results of these investigations suggest that C-modes encapsulate key dynamic properties of their constituting CAPs, and support the use of C-modes to reduce dimensionality in CAP analyses.

RESULTS: fMRI C-modes exhibit evolutionarily-conserved functional organization

*"To further corroborate these results, we additionally computed the occurrence rates of individual CAPs (**Figure S6**) and compared them with their corresponding anti-CAP (both which compose a C-mode), finding no significant differences between these pairs of distributions in any of the analyzed datasets ($p > 0.2$, signed-rank test). This result supports the notion that C-modes reliably encapsulate key dynamic properties of their constituting CAPs."*



Supplementary Figure S6. *Individual CAP occurrence rates. CAPs are organized in CAP and anti-CAP pairs, composing a C-mode as per the order of Figure 2B. Signed-rank tests between CAP and anti-CAP show no significant differences in occurrence rate distributions ($p > 0.2$).*

13. On page 12, 4th line from the bottom, consider adding a hyphen to 'interareal', changing it to 'inter-areal' to enhance readability.

We have now used inter-areal throughout out manuscript

14. On page 17, line 19, it would be advisable to replace the punctuation with a comma for the thousand marks in the number of voxel counts, ensuring consistency with standard numerical formatting.

We have now fixed this typo.

Reviewer #2 (Remarks to the Author):

This work provides much needed evidence supporting the notion that the dynamic organization of resting-state fMRI networks in mammalian species is governed by species-invariant principles. These results not only provide insights into the fundamental principles underlying brain network dynamics but also offer opportunities to relate fMRI network findings across different species, facilitating a better understanding of brain evolution and function.

This study is important to improve translational research efforts aimed at understanding brain function and dysfunction associated with resting-state networks using animal models. Although the matching of network patterns across species is challenging, the authors employed a strategy that allowed them to qualitatively compare across species, despite the anatomical differences.

We are very grateful to the reviewer for the positive and constructive appraisal of our work, and for underscoring the importance of the questions addressed.

I leave some comments below, which I would like the authors to adress:

Introduction:
1. The introduction is very well written and covers the state of the art. I just find this statement outdated: 'RSN mapping typically entails the computation of time-averaged statistical dependencies between fMRI time-series under the assumption that temporal structure of this activity is time-invariant over a time window of minutes.'. Indeed, the reference used is from 2013, and over the last decade the field has been gradually moving to dynamic analysis of functional connectivity, so I suggest rephrasing.

We thank the reviewer for spotting this and agree that this statement is outdated, though it helps introduce the concept of resting-state networks. We added the following phrase to clarify that the current trend is towards time-varying approaches and cite relevant reviews on these techniques:

INTRODUCTION:

"Spontaneous fluctuations in resting fMRI signals have been consistently shown to be temporally synchronized across multiple functional systems, delineating a set of reproducible topographies often referred to as Resting State Networks (RSNs)[1,2]. While RSN mapping typically entails the computation of time-averaged statistical dependencies between fMRI time-series to produce maps of "static fMRI connectivity"[3], the last decade has brought forth a series of alternative time-varying approaches to investigate the temporal features of RSN activity[4,5]."

2. In the sentence: ' and compare the dynamic organization of fMRI in the mammalian brain.' I suggest adding 'signals' after fMRI.

We have now reworded this sentence as suggested.

Results:
3. 'Previous work has shown that CAPs embody rich fMRI topographies that can be reliably matched into mirrored coactive and anti-coactive pairs characterized by opposite BOLD polarity'.

While I understand what the authors mean by 'opposite BOLD polarity' it is important to highlight that the mechanism for this anti-polarity detected in fMRI signals remains to be fully linked with the BOLD response (especially since anti-phase locking has been detected between CSF in ventricles and gray matter voxels), As such, to avoid making unsupported assumptions, and given that the current

study is not aiming to disambiguate the origin of the signals, but rather their organization principles, I would suggest rephrasing to 'opposite polarity in fMRI signals' to be more general.

We thank the reviewer for this thoughtful comment, and we apologize for the confusion. It is of paramount importance that readers understand that we are making no claims about BOLD polarity and about what 'negative BOLD' is or is not. We have now changed 'BOLD polarity' to 'opposite (patterns of) BOLD coactivation' throughout the paper.

4. 'Human to macaque matching gave consistent results for both HNU and MSC datasets, with the spatial of topography of human C-modes 1-4 being best aligned to corresponding macaque C-modes 1-4.' just a typo, remove 'of' between spatial and topography.

We have now corrected this sentence.

5. 'Thus, the temporal structure of C-modes in humans was biased towards a greater occurrence of polymodal integrative metastates (C-modes 1 and 2), with mice (and possibly macaques= exhibiting instead a greater occurrence of sensory-oriented network modes (C-modes 3 and 4).' Could the authors explain better the expression 'polymodal integrative metastates'? Is there a reference? Also please correct the typo '=' should be ')'.

The '=' was a typo: we thank the reviewer for spotting this mistake. We also fully agree with the reviewer that our definition of the leading C-mode in humans as 'polymodal integrative metastates' is difficult to interpret. Our intention was to differentiate C-modes which are characterized by the engagement of polymodal *associative* networks such as the DMN and FPN. Since we only refer to them as "metastates" in this sentence to denote they are transients of DMN-led activity, we decided to remove this word, and rewrite the sentence as follows:

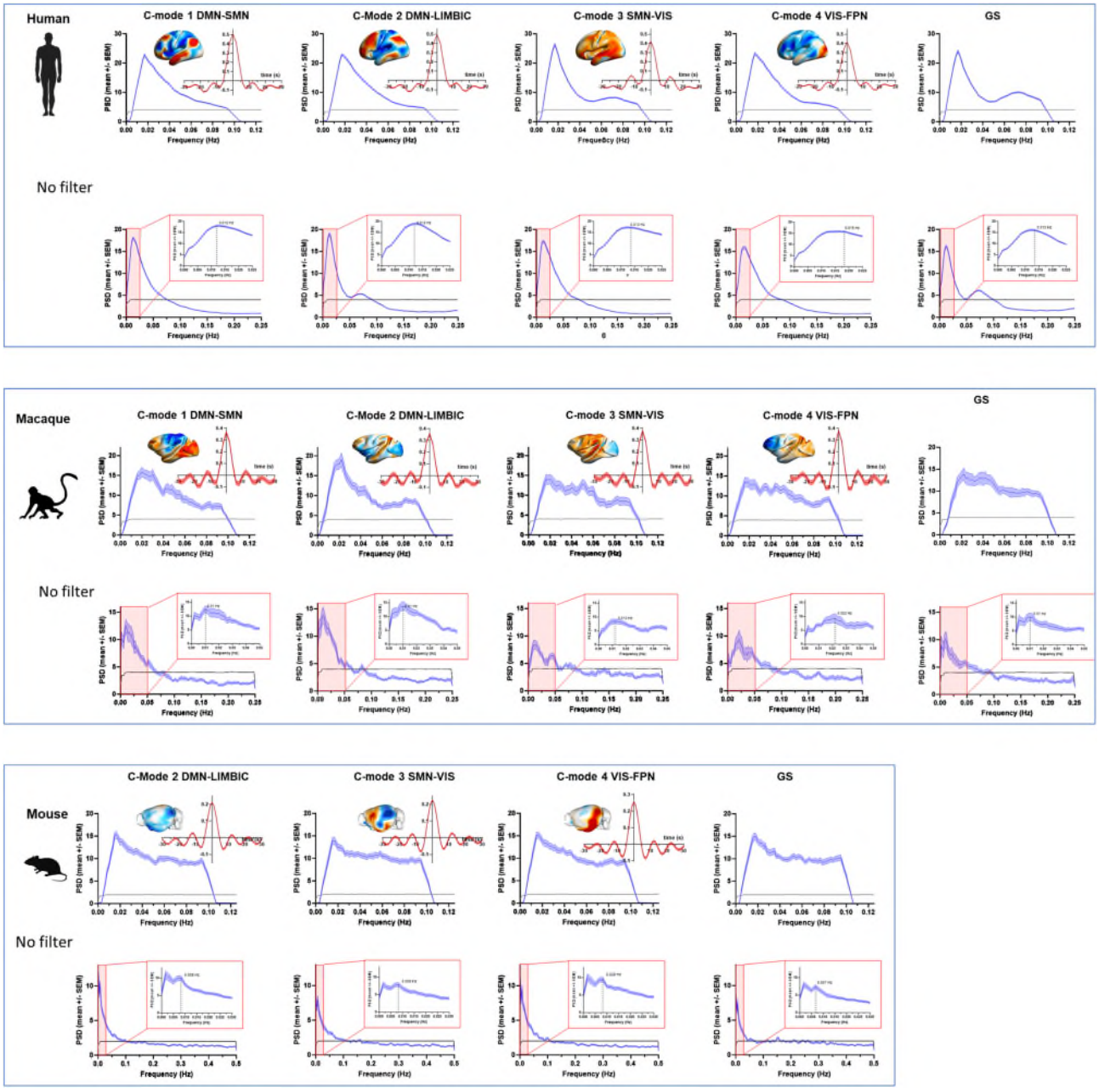
RESULTS: fMRI C-modes exhibit evolutionarily-conserved functional organization

"Thus, the temporal structure of C-modes in humans is a preferred occurrence of configurations led by associative cortical networks such as the DMN (C-modes 1 and 2), with mice and possibly macaques exhibiting instead greater occurrence of C-modes encompassing coactivation of sensory unimodal areas (C-modes 3 and 4)."

6. 'The power spectra of the resulting "C-mode timeseries" revealed that C-modes undergo infraslow fluctuations in all species, with most of the power peaking within the 0.01-0.03 Hz range' Given that all the fMRI signals were band pass filtered between 0.01 and 0.1 Hz, the lower boundary of the power spectra shown in Figure 3 is strongly affected by the filtering. If the authors shuffle the spatial modes, would the temporal signatures still peak in the same range? (this would be a way to verify if the peak at 0.01Hz is really signature of resonance of the modes or if it is an artefact from the filtering. But perhaps the best solution would be: once having the spatial modes obtained with the current approach, compute the temporal signatures from the fMRI timeseries but considering this time the fMRI data before band-pass filtering. This would allow having an unbiased view of the power spectra of the modes.

This is a crucial observation which we have explored in our previous work in anesthetized mice (Gutierrez-Barragan et al., 2019, Current Biology – ref.16). We thank the reviewer for giving us the opportunity to further corroborate our findings with the suggested methods. Specifically, we have now computed power spectral density (PSD) after randomly shuffling the timepoints in the C-mode correlation timeseries for each subject, for each session, and averaged for each frequency (1000 iterations). We show that for our band of interest (0.01-0.04Hz), the true PSD is significantly above the null distribution ($p = 0.001$, black traces). We have now added to **Figure 3** a black trace depicting the null PSD, and we added the relevant description in Results and Methods sections (see below).

In **Rebuttal Figure 4** below, we show the new PSD plots we report in the manuscript (with null model), as well as the corresponding PSDs computed for unfiltered data, showing that even without the critical filtering step (which we carried out to remove scanner drift as well as high-frequency noise), C-modes PSD peaks near 0.01Hz for all species. These analyses suggest that infraslow fluctuations of C-modes are not a consequence of the bandpass filter applied and that their peaks are above and beyond chance level.



Rebuttal Figure 5. Remake of Figure 3, and addition of PSD without band-pass filtering.

To comply with editorial limits, we have not included this new figure and analysis in the text. We have instead changed the text in methods as follows:

METHODS: C-mode infraslow dynamics, formation, and temporal structure

"We investigated the temporal evolution of C-modes by generating a time-course of instantaneous C-mode to fMRI frame spatial correlation (hereafter referred to as "C-mode time-courses") for each subject/animal, and then computing the power spectrum of these time-courses as well as that of the Global fMRI signal (GS, i.e., the average of all voxels at each frame). We next computed the group

mean +/- SEM power spectra. C-mode time-courses were normalized to standard deviation units and mean zero. The significance of the observed infraslow portion of C-mode spectra was assessed by calculating the power spectrum of surrogate C-mode timeseries obtained after jointly shuffling the timepoints of the original C-mode time-courses using the same random sequence of time points. This method preserves the signal amplitude distribution and the correlation structure of original timeseries[16]. We next carried out, for each frequency, a one-tailed two-sample t test (FDR corrected for comparisons within the 0.01-0.1Hz band) comparing the power of the original C-mode PSD with the mean of 1000 iterations of the surrogates. "

RESULTS: fMRI C-modes exhibit infraslow fluctuations in humans, macaques and mice

*"The power spectra of the resulting "C-mode timeseries" revealed that C-modes undergo infraslow fluctuations in all species, with most of the power peaking within the 0.01-0.03 Hz range (**Figure 3**). Power within the infraslow band was significantly greater than the mean power of surrogate timeseries generated from shuffling the joint C-mode timeseries 1000 times (**Figure 3**, black traces)."*

7. 'We found that the most recurring C-modes (1-2 in humans, 3-4 in macaques, and 3-4 in mice) were sinks of preferred directional transitions ($p < 0.01$, black crosses in Figure 5A), which can as such considered as state attractors.'

While I appreciate the term 'sinks' borrowed from dynamical systems theory, perhaps it would be good to add a short explanation about what is understood as a sink attractor. Also please add 'be' considered as state attractors. Moreover, it is to note that given that the states are not stable over time, they could be termed pseudo-attractors, or metastable attractors or even ghost attractors.

We agree that our previous text was unclear, and we thank the reviewer for giving us the opportunity to improve it. We have now revised the text to better emphasize the actual finding (i.e. the observation that the most recurring C-modes are those that have a higher probability of being transitioned to) and better explain the dynamical system interpretation (as sink). We also agree that the interpretation as attractor may be overstated. This interpretation as attractors is not necessary to the interpretation of our data, and therefore we have removed it. The revised sentence reads as follows:

RESULTS: Coupled oscillatory activity explains C-modes transition dynamics

*"We found that the most recurring C-modes (1-2 in humans, 3-4 in macaques, and 3-4 in mice) were those with the with the highest probability of being transitioned to. ($p < 0.01$, black crosses in **Figure 5A**). Thus, they can be conceptualized as "sinks" of preferred directional transitions."*

Discussion:

8. 'Over the last decade, several influential mathematical models have described resting state activity in terms of networks of coupled oscillators. This work provides important empirical support to this modelling as it shows, from data and without making assumptions about the mechanism, that transition probabilities between different brain states can be described in terms of coupled C-Modes oscillating at infraslow frequencies.'

While I agree that this work provides important insights for modelling works, the specific work cited in this paragraph (Deco et al, 2008) used oscillators in the gamma frequency range 40 Hz and did not report ultra-slow oscillations (only ultra-slow fluctuations in synchrony degree, which, unlike

oscillations, do not have sustained periodicity). Perhaps it would be better to cite here other works, by the same group, who used coupled infra-slow oscillators, such as https://doi.org/10.1371/journal.pcbi.1004100. Moreover, it would be interesting to add that the results from this work also align with the emerging hypothesis that the oscillations are not generated at the local level, but represent the slow frequencies of macroscopic modes of resonance in brain anatomy (https://doi.org/10.1038/s41586-023-06098-1 and https://doi.org/10.1038/s41467-023-36025-x).

We thank the reviewer for this correction, and for suggesting to cite these highly relevant publications. We have now changed the corresponding paragraph as follows:

DISCUSSION:

[…] *"Moreover, the fact that the state transitions can be reliably described as coupled oscillators in all species suggests that a functional architecture based on coupled oscillating networks offers important evolutionarily advantageous computational benefits. These include the possibility of rapidly reconfiguring coordination and communication between different brain regions, and effectively transferring information across scales and brain areas[21,55,68]. Importantly, our work also aligns with the emerging hypothesis that oscillations are not generated at the local level, but represent slow frequencies of macroscopic modes of resonance in brain anatomy[66,67]."*

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Reviewer #1 (Remarks to the Author):

I appreciate the authors' significant efforts in addressing my comments from the previous review. They have successfully clarified sections that could have caused confusion and validated all the details I pointed out.

All responses were rigorously evaluated and handled with care, addressing every comment I had.

For my final minor comment, I have identified a small inconsistency in the use of some terms in the manuscript, such as "anti-CAP" and "antiCAP," "GS-phase" and "GS phases," or "GS cycle(s)" and "GS-cycle(s)." I suggest correcting these for consistency.

Overall, the manuscript demonstrates rigorous research. The authors have presented their findings clearly and convincingly. This outstanding study provides a robust tool to quantify evolutionarily conserved features from fMRI network dynamics through cross-validation and rigorous methods. It will be of great interest to researchers and make a significant contribution to the field of translational neuroimaging research.

Reviewer #2 (Remarks to the Author):

The authors have adequately addressed most of my concerns, and I have only two comments.

Comment 1:

My 3rd comment was not adequately addressed and needs revising, given that the authors did not understand my point and only changed the term 'polarity' to 'opposite patterns of coactivation', but still kept the reference to the BOLD response, which was precisely my point.

The point is that it has not been fully demonstrated (at least to my knowledge) that the 'opposite patterns of co-activation' (which is the same as 'opposite polarity') in fMRI signals are linked with an opposite pattern of oxygenation in hemoglobin (i.e. when blood oxygenation increases in one side it decreases in the other and vice versa). So keeping the term BOLD is making the assumption that the fMRI signals with opposite polarity are associated exclusively to blood oxygenation.

There are several works showing that fMRI signals have other strong contributors, which are not yet fully understood. One particular example is the finding that the fMRI signals in the cortex are locked in phase with the fMRI signals in the ventricles, but there are others.

10.1126/science.aax5440
https://doi.org/10.1371/journal.pbio.3001233
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So the authors should at least state in this work that they are making the assumption that the fMRI signals detected in this study 'are assumed to be exclusively driven by the BOLD response', and therefore use BOLD signal as a synonym to fMRI signal.

'3. ‘Previous work has shown that CAPs embody rich fMRI topographies that can be reliably matched into mirrored coactive and anti-coactive pairs characterized by opposite BOLD polarity’.

Previous review: While I understand what the authors mean by ‘opposite BOLD polarity’ it is important to highlight that the mechanism for this anti-polarity detected in fMRI signals remains to be fully linked with the BOLD response (especially since anti-phase locking has been detected between CSF in ventricles and gray matter voxels), As such, to avoid making unsupported assumptions, and given that the current 14 study is not aiming to disambiguate the origin of the signals, but rather their organization principles, I would suggest rephrasing to ‘opposite polarity in fMRI signals’ to be more general.

Author reply: We thank the reviewer for this thoughtful comment, and we apologize for the confusion. It is of paramount importance that readers understand that we are making no claims about BOLD polarity and about what ‘negative BOLD’ is or is not. We have now changed ‘BOLD polarity’ to ‘opposite (patterns of) BOLD coactivation’ throughout the paper.

Comment 2:

In the response to point 7. there are 2 typos in the sentence added to the manuscript:
' were those with the with the highest ' repeated
' transitioned to. ($p < 0.01$, black crosses in Figure 5A).' remove the dot

If the authors address these points, I am happy to endorse publication.

We thank the Reviewers for their additional feedback and words of appreciation. Below is a point-by-point response to their comments (our response in blue typeface). We have also added below the text edits we have introduced in the manuscript in response to the reviewers' comments using yellow highlight.

REVIEWER COMMENTS

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We highly appreciate the effort to detect these inconsistencies. We have corrected these terms throughout the paper and used "anti-CAP", "GS-phase" and "GS-cycles" as preferred terms.

Reviewer #2 (Remarks to the Author):

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Comment 1:

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We thank the reviewer for clarifying the previous comment and agree that our research does not intend to address the implication or origins of negative BOLD responses. Since in our analyses we used normalized fMRI time-series, we have now removed the term BOLD all-together and replaced it with “fMRI signal”. We have now also added a discussion paragraph where we explain that we used normalized preprocessed fMRI signals, and hence cannot claim to be exclusively driven by positive or negative changes in BOLD activity.

“Because our fMRI timeseries were preprocessed and normalized, the polarity of our averaged fMRI signals cannot be assumed to directly reflect positive or negative BOLD activity. Uncertainty as to the determinants of intrinsic BOLD activity remains, with recent evidence suggesting that it may be influenced also by non-neural signals, including cerebrospinal fluid pulsations[72,73]. Models of neurovascular coupling are still being validated to help interpret what BOLD activity implies at the neurovascular level[74,75], and establishing a direct physiological link between peaks and throughs of fMRI activity in C-modes and the actual polarity of the underlying BOLD signal was beyond the scope of this investigation. We however corroborated the oscillatory nature of dominant large-scale network configurations in the mammalian brain, and showed how their dynamic features are species-invariant. Future studies employing concurrent widefield neural activity readouts and fMRI will be crucial to expand this interpretation.”

Comment 2:

In the response to point 7. there are 2 typos in the sentence added to the manuscript:
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' transitioned to. ($p < 0.01$, black crosses in Figure 5A).' remove the dot
If the authors address these points, I am happy to endorse publication.

We thank the reviewer for detecting these typos, which were fixed.