We appreciate the detailed reviews of our manuscript and the opportunity to revise and resubmit. We have performed additional analyses suggested by the reviewers, as described below. Other revisions include edits to the text of the manuscript to more clearly describe the approach and more accurately align our work to the literature.

The reviewers' concerns are provided in blue text, with our responses in black text. Revisions are provided below and in the revised manuscript and supplementary material in *red text*.

Please also note that in undertaking the additional work for these revisions, we have included the expertise of three colleagues - Jayson Jeganathan, Bryan Paton, Nikitas Koussis - now added to the authorship byline.

Reviewer #1:

An interesting study design, with respect to both the task (naturalistic, implicit memory) and population (healthy and MCI).

Response: We thank the reviewer for their insightful review and have undertaken additional analyses and edits as described below

R1.1. My key concern, which requires additional analysis, is the suggested link between the hippocampal gradients and functional cortical networks. The descriptions in the Results and Discussion lack specificity (e.g. which parts of the DMN overlap with) and would be difficult to understand for someone not already studying cortical gradients. More importantly though, these links should be empirically tested, for example by using non-parameteric testing to evaluate whether the values in the DMN are significantly higher than null models.

Response: We have now performed non-parametric testing using a method (wavestrapping from Breakspear et al. (2004), "Spatiotemporal wavelet resampling for functional neuroimaging data." *Human brain mapping*) that preserves the spatial correlation of the cortical map while disrupting its relationship to the hippocampal gradient. This generates a null hypothesis that represents non-specific mappings from the cortex to the hippocampus with excursions from the null hypothesis that correspond to regions in the cortex that associate with the extremes of the hippocampal gradient, i.e. the head and/or tail of the hippocampus.

Interestingly, in addition to regions in the default mode, this null also identifies a peak in the mid-insula. This is described in the revised manuscript (p16), with an additional panel in Figure 6 as follows,

We used nonparametric (wavelet-based) resampling to formally identify cortical regions that specifically map to the anterior and posterior extremes of the hippocampal gradient (see Supplementary Methods). We observe that projections of the anterior extreme of the hippocampus gradient reside in the anterior pole of the temporal cortex, the angular gyrus, rostral anterior cingulate cortex, posteromedial cortex, and mid insula gyrus (Fig. 6c). The first four of these are key regions of the default mode network and mirror the peaks in gradients derived purely from cortico-cortical functional connectivity (Margulies et al., 2016). Here, we also find that the mid insula maps to the anterior pole of the hippocampal gradient.

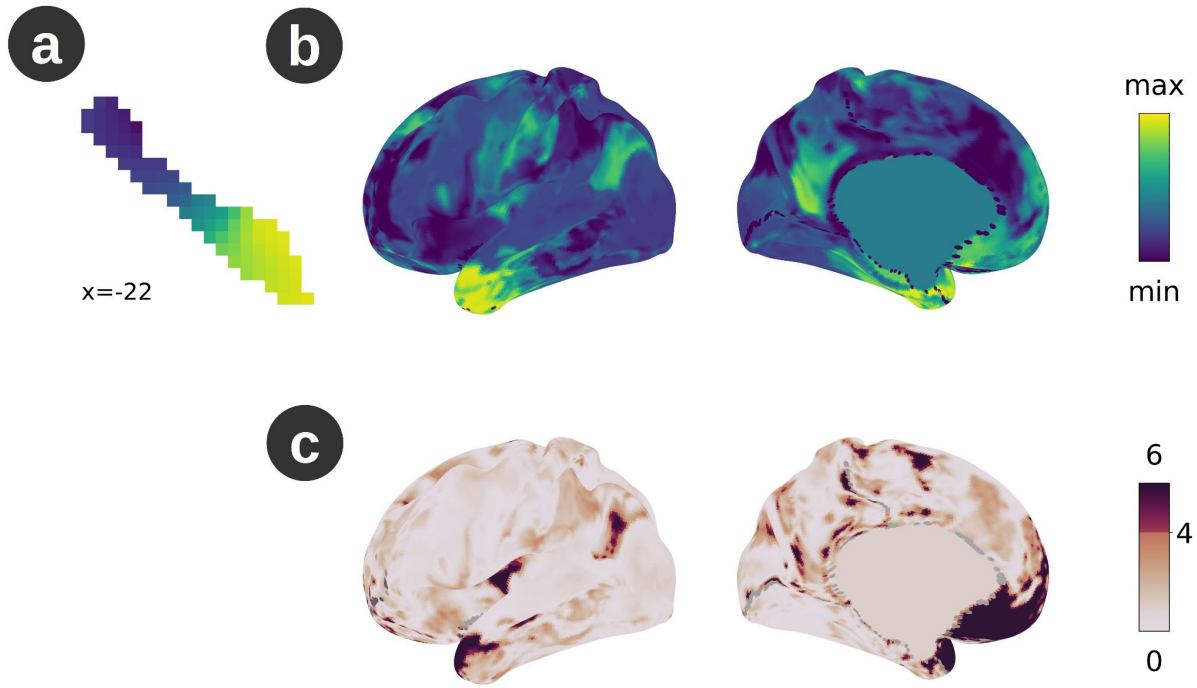


Figure 6: Projection of gradient I eigenmap within the left hippocampus onto the cortical surface (HC, continuing task). *(a) Left: The eigenmap for Gradient I in anatomical space shows the anterior-posterior organization. (b) right: The gradient eigenmap projection onto the cortical surface shows that the progressively more anterior hippocampus (red) projects to progressively more ventromedial parts of the prefrontal and cingulate cortices. The same progressive projection pattern is observed when approaching the temporal pole and the precuneus (posteromedial cortex) (c) Nonparametric wavelet resampling reveals specific projections from the anterior extreme of the hippocampal eigenmap to the anterior*

pole of the temporal cortex, the angular gyrus, rostral anterior cingulate cortex, posteromedial cortex, and mid insula gyrus. Color depicts the strength of statistical association (-log(p)). Thresholded maps are provided in Supp. Fig. 12.

In the discussion, we now note that (p18),

Likewise, the cortical projection of this gradient mirrors that of purely cortical gradients, although the mid-insula is an additional inclusion of the projection of the anterior extent of the hippocampus gradient.

We describe the test and provide the unthresholded and thresholded z-maps in the new section of the Supplement, namely **Supplementary Method - Wavelet resampling** (see Supplementary Information, p5).

R1.2 The article would also benefit from deeper investigation of what the hippocampal gradients capture in functional connectivity to the cortex. It is not clear what the hippocampal gradients represent beyond some non-specific differentiation. For example, I have no clue what is meant by "it is notable that functional connectivity gradients encompass extensive cortical networks".

Response: We have undertaken additional investigations of the task-free and task-specific functional connectivity between the hippocampus and cortex, as described below in the Results section, p15:

Cortical projection: *To study the cortical projection of the hippocampal eigenmode, we first identified the maximum functional mapping between all cortical and all hippocampal voxels. For each cortical voxel y_i, we identified the hippocampal voxel x_j with the maximum task-free GLM coefficient β_1. Group-wise, second-level inference within the healthy cohort showed that all cortical voxels possess a statistically significant mapping to one or more hippocampus voxels (Supp. Fig. 9,10) following correction for all possible pairwise coefficients (see Supp. Methods - Corticohippocampal functional networks). The corresponding functional cortico-hippocampal maps for each of the task-associated PPI coefficients are weaker, but still encompass extensive cortical networks. Approximately 66.8% of cortical voxels possess a statistically significant psychophysical interaction for the naive task (coefficient β_5), increasing to 75.3% of cortical voxels for the continuing task (PPI coefficient β_4; FDR corrected, q=0.05).*

More details of this process and the associated statistical maps are provided in **Supplementary Method - Corticohippocampal functional networks** (see Supplementary Information, p5).

We have also incorporated these new analyses into our interpretation of the hippocampal gradients in the Discussion section, p18:

We used the framework of psychophysiological interactions to decompose cortical activity into task-invariant and task-modulated functional connectivity with the hippocampus. These analyses show how task-mediated effects superimpose on a widespread task-invariant functional embedding of cortex and hippocampus. Application of gradientography reveals how these task effects modulate a functional gradient along the AP axis of the hippocampus that maps onto an extensive functional gradient across the cortex.

R1.3 Gradients are proposed to "be a fundamental property of cortical organization being present in functional and structural connectivity", but almost all the of the cited articles use non-linear dimensionality reduction on pre-smoothed data, and thereby do not evaluate whether gradients exist or not. A better argument for the fundamental nature of gradients comes from developmental work on morphogen gradients. Furthermore, the smoothness of changes along connectivity gradients is still contentious. More nuance in the argumentation would be appreciated, especially noting the influence of smoothing and resolution on gradients of the hippocampus.

Response: It is true that most prior studies apply nonlinear dimension reduction to pre-smoothed data and do not study the existence of the gradients per se. Note, however, our own team have previously disambiguated smooth versus step-like changes in connectome gradients using geometrically-constrained null hypothesis testing (Tian et al. 2020). We acknowledged this in the original manuscript where we stated, *"However, it is not clear whether functional differences along the antero-posterior axis reflect the existence of a discrete partition into segregated parcels or a continuous change along a smooth gradient (Genon et al., 2021; Tian et al., 2020)."*

We have now edited the Discussion to acknowledge that (p20),

Hippocampal functions reflect a composite of connectivity gradients and subfields (Genon et al., 2021; Vos de Wael et al., 2018) whose disambiguation is unlikely to be evident at the

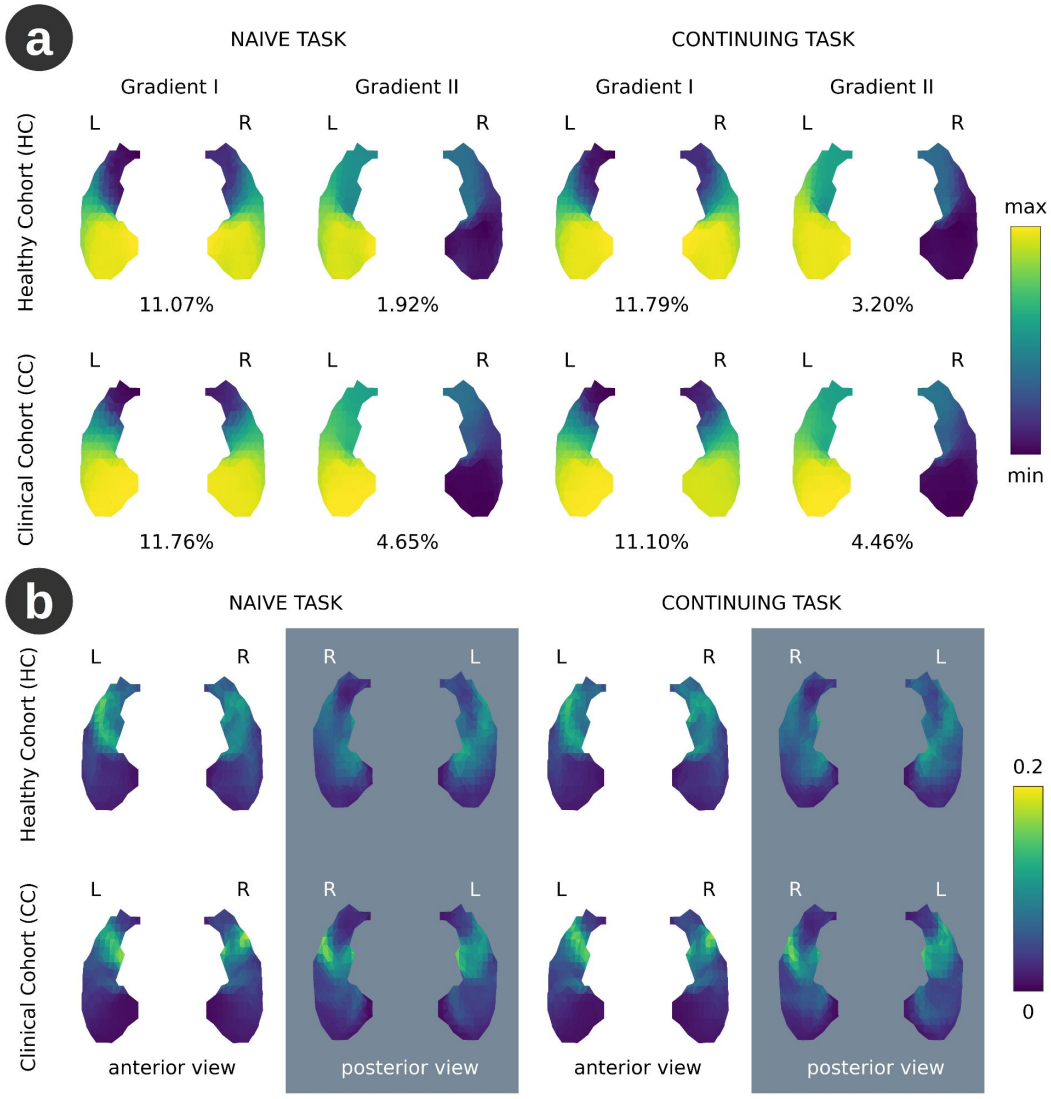
relatively coarse scales of acquisition resolution and smoothing width employed in the present study. We do, however, note that the hippocampus is considerably longer (~4-5cm) than our primary smoothing kernel (6mm FWHM) and moreover, connectivity gradients remain clearly present when the data are smoothed at 4mm FWHM. While these observations are reassuring, imaging at high field strength combined with techniques that eschew the need for spatial smoothing may assist here.

and in the limitations section (p20),

Finally, we note that while the study of functional gradients across brain systems has already revealed promising new principles of functional organization, there remains considerable validation to be achieved, including the relationship of connectivity gradients to cytoarchitecture and their robustness across different preprocessing and analytic choices (Bernhardt et al., 2022; Genon et al., 2021).

The term gradients has also been amended to "*connectivity gradients*" in the abstract.

We have now also considered the issue of spatial smoothing in more detail in the present paper by repeating the derivation of the hippocampal-cortical gradients after pre-smoothing the data at 4mm FWHM. These reveal a basic conserved gradient architecture as shown below (Supp Figure 5),



Supplementary Figure 5: Functional connectivity gradient mapping with spatial smoothing performed with a Gaussian smoothing kernel of 4mm FWHM (full width at half maximum), instead of 6mm. *(a) Gradients eigenmap (see Fig. 2 caption). (b) Gradient I magnitude (see Fig. 3 caption).*

We overview these new analyses in the Results section (p12),

To explore the effect of spatial smoothing, we repeated the gradientography pipeline after reducing the size of the data smoothing kernel from 6mm to 4mm FHWM. We observe that the total variance explained is considerably smaller (from approximately 25-30% at 6mm down to 12-16% at 4mm) and the relative variance explained by the two principle gradients switches order. This may reflect the lower signal-to-noise ratio in subcortical regions due to the surface head coil, with resulting noisier data when smoothed at 4mm FHWM, consistent

with the comparison of different smoothing kernels in resting state fMRI (Tian et al 2020). Despite these differences, the global pattern and associated magnitudes of the gradients are preserved, including the existence of a faster gradient transition between the anterior and posterior hippocampus (Supp. Fig. 5). We hereafter focus on gradients following smoothing at the default of 6mm.

R1.4 Final note on gradients, the anterior-posterior axis in the hippocampus does not have a clear cytoarchitectural basis. The authors mistakenly Paquola et al., 2020 on this point, which discusses the "iso-to-allocortical axis" of the mesiotemporal lobe.

Response: The reviewer is correct: While Paquola et al., 2020 discuss functional connectivity gradients along both iso-to-allocortical and anterior-posterior axes, a cytoarchitectural gradient is only observed along the iso-to-allocortical axis. We have corrected the text to capture this nuance which we previously overlooked (p4),

Hence functional specialization and external connectivity of the hippocampus appear to unfold along its long axis. However, the relationship of these gradients to anatomy and cytoarchitecture remain unclear. Notably, cytoarchitectural properties of the broader mesiotemporal lobe change smoothly along the iso-to-allocortical direction, orthogonal to the antero-posterior alignment of the functional and connectivity gradients within the hippocampus (Vos de Wael 2018, Paquola et al., 2020).

R1.5 More specific details on the computational analysis should be offered, in particular in the "Gradientopography" section:

- What data is it performed on?

Response: We have clarified this as follows (p7),

To study functional connectivity gradient changes in the task fMRI data, we adapted a recently developed method to map the organization of the human subcortex from large-scale functional connectivity gradients (Tian et al., 2020).

R1.6 "principal components analysis (PCA) was first used to reduce the dimensionality of whole-brain activity." - what is the input to the PCA?

Response: We have clarified this as follows (p7),

In brief, principal components analysis (PCA) was first used on temporally concatenated fMRI signals to reduce the dimensionality of whole-brain activity.

R1.7 "The whole-brain functional connectivity fingerprints were then mapped for each subcortical voxel" - what does mapped mean? Correlated with something else? Or is this the imgradientxyz function?

Response: We have reworded this as follows:

Whole-brain functional connectivity was then derived between cortex and all voxels by correlating their fMRI signals with the ensuing PCA components.

R1.8 "To disambiguate functional connectivity from the confound of task co-activation, task manipulations in the previous study were regressed from the BOLD signal prior to gradient analyses" - how was the task design regressed? What's the shape of the regressor?

Response: We have clarified this as follows (p7),

Gradientography was previously employed to investigate variation in functional boundaries of the subcortex between rest and task conditions (Tian et al., 2020). In this previous study, functional connectivity was disambiguated from the confound of task co-activation by regressing, task manipulations - represented as task block regressors convolved with hemodynamic response functions - from the BOLD signal prior to gradient analyses, consistent with previous work (Cole et al., 2014).

R1.9 I don't find key lines of code in the github repository, such as for computing gradients around line 24 of 'functions/gradientography/cont_model.m'.

Response: The comment around line 24 of the cont_model.m function was indeed confusing. We moved the "fprintf('Computing Gradient %d\n',Vn-1);" to the beginning of the function because it is the whole function that computes the gradient eigenmap and magnitude, not just the second part.

R1.10 Additionally, thresholding (applied in the code) was not mentioned in the text.

Response: The thresholding of the similarity matrix is a step in the gradientography pipeline as described below in (Tian et al. 2020),

“The similarity matrix was transformed into a sparse graph using the weighted adjacency matrix, whereby the weights of all connections with a Euclidean distance less than ε were set to zero. The connection density was determined by the smallest value of ε that ensured a connected graph 11,72,73. This threshold varied between individuals, yielding graphs with a connection density of 0.4% on average.”

We prefer not to elaborate on these details in the manuscript in order to provide an overview of the gradientography pipeline and not overburden the methods description. At the conclusion of the **Supplementary Method - Data Processing**, we now state,

Gradientography was performed on the ensuing preprocessed data (see Methods and Tian et al 2020 for full details).

R1.11 The role of the hippocampus in “linking salient events" is core to the argumentation. Could this be further unpacked? For instance, what is meant by linking? Specifically, what evidence supports this statement?

Response: We have addressed this question (also in response to suggestions by R2 and R3) with a rewritten concluding paragraph,

The hippocampus underpins relational binding, forming “rapid, continuous, and obligatory associations among disparate elements across space and time” (Olsen et al., 2012). In this vein, hippocampal neurons detect cognitive boundaries as they occur in naturalistic stimuli and reinstate neural states in response to subsequent cues (J. Zheng et al., 2022). Recent theories propose that these hippocampal functions are supported via a ‘conceptual model’ of cortex, performing a computational integration of related cognitive representations (Whittington et al., 2022). Here, we find that visual and semantic cues that link present with recent narratives lead to a modulation of the functional embedding of hippocampal activity in cortical systems, organized along hippocampal-cortical connectivity gradients. Intriguingly, neurophysiological recordings reveal that theta oscillations precede continuously along the long axis of the hippocampus as traveling waves (Lubenov & Siapas, 2009) suggesting that hippocampal gradients shape local information propagation (Kleen et al., 2021). Waves of activity also characterize cortical states across a wide variety of cognitive states and tasks (Roberts et al., 2019), including working memory (Sreekumar et al., 2021). Coupling between cortical and hippocampal waves propagating along their principal connectivity gradients is hence a candidate mechanism for the spatiotemporal integration of cortical and

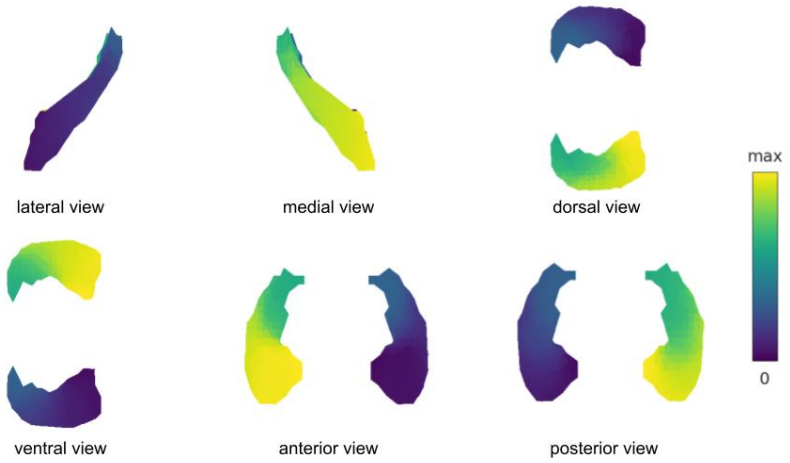
hippocampal activity.

R1.12 Regarding the Figures, colourmaps (jet in particular) are not perceptually uniform, causing difficulty in interpretation (doi:10.1038/s41467-020-19160-7).

We replaced the colormap "jet" with the colormap "viridis" as recommended in (doi:10.1038/s41467-020-19160-7).

R1.13 I do not understand the anterior vs posterior view in Figure 3. Is it more superior vs inferior? Relatedly, what are the changes in the gradients in the z-direction? The Figures only represent two dimensions.

In order to most clearly visualise the gradients in figures 2, 3, 4 and 5, we project the gradients on the surface of the hippocampus. Among the views proposed by the nilearn library (https://nilearn.github.io/dev/modules/generated/nilearn.plotting.plot_surf.html; also described here: https://www.richardsonthebrain.com/ways-to-view-the-brain), the anterior and posterior views provide the most informative rendering (see below). For completeness, we now provide complimentary views of the primary hippocampal eignemap in Supplementary Figure 3 (referred to at the end of the caption for Figure 2).



Supplementary Figure 3: Gradient I eigenmap. *Lateral, medial and ventral views to complement the anterior and posterior views shown throughout the manuscript.*

R1.14 Also. In Figure 6, what are the values projected on the surface? Is it a rho value? Please provide a colour axis.

The values projected onto the surface correspond to the eigenmap values. We have specified this in the figure legend and added a colour axis.

R1.15 Finally, there is some unclear terminology that can cause confusion. Please consider whether it's worthwhile to use field-specific lingo, such a "gradientography", "representation" and "fingerprint", all of which add vagueness rather than clarity. Additionally, there were a couple of occasions of tautology-like language, such as "variation across the topography of the subcortex" and "map the cartography".

Response: We have revised the manuscript throughout and provided definitions where the context-specific meaning may be vague (such as “gradientography” p4 and “fingerprint” p8).

Here, we address these issues using ‘gradientography’, a method for characterizing how patterns of whole brain functional connectivity change across the subcortex (Tian et al., 2020).
Each subcortical voxel in each participant therefore possesses a unique connectivity map (or “fingerprint”): Pairs of voxels with similar fingerprints are functionally connected to similar brain areas.

We have also deleted the tautologies - thank you again for your close and attentive reading.

Reviewer #2:

This manuscript provides a novel insight into task-positive hippocampal connectivity gradients (ie. the major components from dimensionality reduction of hippocampal-neocortical connectivity) and gradient "magnitudes" (ie. the approximate spatial derivatives or 'texture' of gradients). This work is similar in methodology to a recent high-impact publication (Tian et al 2020), but extends on this work by i) focusing on the hippocampus, ii) employing an episodic memory task during fMRI, and iii) additionally examining a clinical cohort with MCI or AD. In many ways the work shown here agrees with previous studies, and also finds that task conditions with a primed episodic experience show greater gradient magnitudes in anterior hippocampus than novel episodic experiences. Peak gradient magnitudes may also be shifted more posterior in the clinical cohort.

Response: We appreciate your careful reading of the manuscript and the constructive feedback which we have addressed as described below.

Major issues:

R2.1 The primary gradient in this study differs predominantly between left and right hemispheres, and then A-P. This differs from previous studies (eg. Vos de Wael et al 2018 which is similar but not cited and Tian et al 2020). This may be in part because Vos de Wael looked only at ipsilateral neocortical connectivity. This should certainly be discussed since it is a major difference with previous work.

Response: We have extracted the brief prior discussion of the symmetric and antisymmetric gradients from the limitations section of the Discussion and now consider this issue in more depth (p19),

Orthogonal decompositions of structural and functional neuroimaging data do typically yield symmetric plus asymmetric leading modes (Tokariev et al., 2019), as we indeed observe here. Through their relative contributions to the original data (through summation or subtraction) such complimentary modes can account for shared bilateral effects versus unilateral hemispheric dominance. Human hippocampi do show a degree of left-right structural asymmetry in health (Woolard & Heckers, 2012) and during neurodegenerative disorders (Wachinger et al., 2016). While we focus on the gradient magnitudes, future work could explore the relative importance of these two modes across different tasks. However, in contrast to the robustness of the gradient magnitude, spatial smoothing strongly influenced

the relative variance explained of these two modes, suggesting further validation is required before this potential approach could be exploited.

Note that we have also corrected our oversight of Vos de Wael 2018, but address that in your later more detailed relevant point (**R2.5** below).

R2.2 Between cohorts and between task conditions, gradient magnitudes are compared statistically, but gradients themselves are not. Why make this indirect comparison without directly comparing the gradients themselves?

Response: There are indeed several lines of enquiry that the "gradientography" pipeline could permit - such as the different gradients (symmetric versus asymmetric) and their relative variance explained (see above). However, the study requires a conceptual anchor. The smoothness versus step-like change in gradients (as seen in the magnitudes) speaks specifically to regions of functional differentiation and, for example, form the basis of functional boundaries in our prior work, using the approach to define a subcortical atlas (Tian *et al* 2020). It is also somewhat unclear how a circumscribed difference in a connectivity gradient could be interpreted, whereas the changes in the magnitude suggest differences in the extent of anterior versus posterior common functional alignment between hippocampus and cortex. We now better motivate our focus on gradient magnitudes in the task and group contrasts (p8),

Group and task comparison of gradient magnitudes: *These steps yield task-related connectivity eigenmaps (gradients) and their spatial rates of change (gradient magnitudes) For group and task contrasts, we focus on the gradient magnitudes as they capture functional differentiation in whole brain connectivity by task effects or group differences: For example, differences in the gradient magnitude suggest changes in the extent of anterior versus posterior functional alignment between hippocampus and cortex.*

and in the Results (p13),

The location of the peak in the gradient magnitude between the anterior and posterior hippocampus is consistent across both smoothing kernels and with the boundary previously identified using resting state gradientography (Tian et al., 2020). We next used permutation testing to study task effects and group differences in the location and extent of this magnitude peak across tasks and groups.

We have also more clearly described the definition and derivation of the gradient magnitudes in the Methods (see response to your concern, **R2.4).**

R2.3 One of the major novel aspects of this study is its departure from the resting-state. While the task condition differences are novel and interesting, could the authors also examine estimated intrinsic connectivity (beta1 in their GLM)? This may be a simple way to replicate previous work (such as Vos de Wael et al., 2019) and establish the differences between both task conditions and rsfMRI connectivity gradients.

Response: This is an excellent suggestion which we have now added to the paper (with corresponding results in Fig. 1, 2, 3, 5 and Supp. Fig. 2, 6). The following associated edits are associated with this new analyses,

Methods (p8),

The T-statistic associated with the coefficient β_1 represents the intrinsic (or task-free) functional connectivity between gray matter voxels y_i and hippocampal or subcortex voxels x_j whereas the T-statistics associated with each PPI coefficient (β_4 and β_5) correspond to the modulation of functional connectivity for each task (naïve and continuing).

Results (p11),

The eigenmaps of these two gradients are visually similar between the healthy and clinical cohorts and across the task-free and two task conditions (Fig. 2).

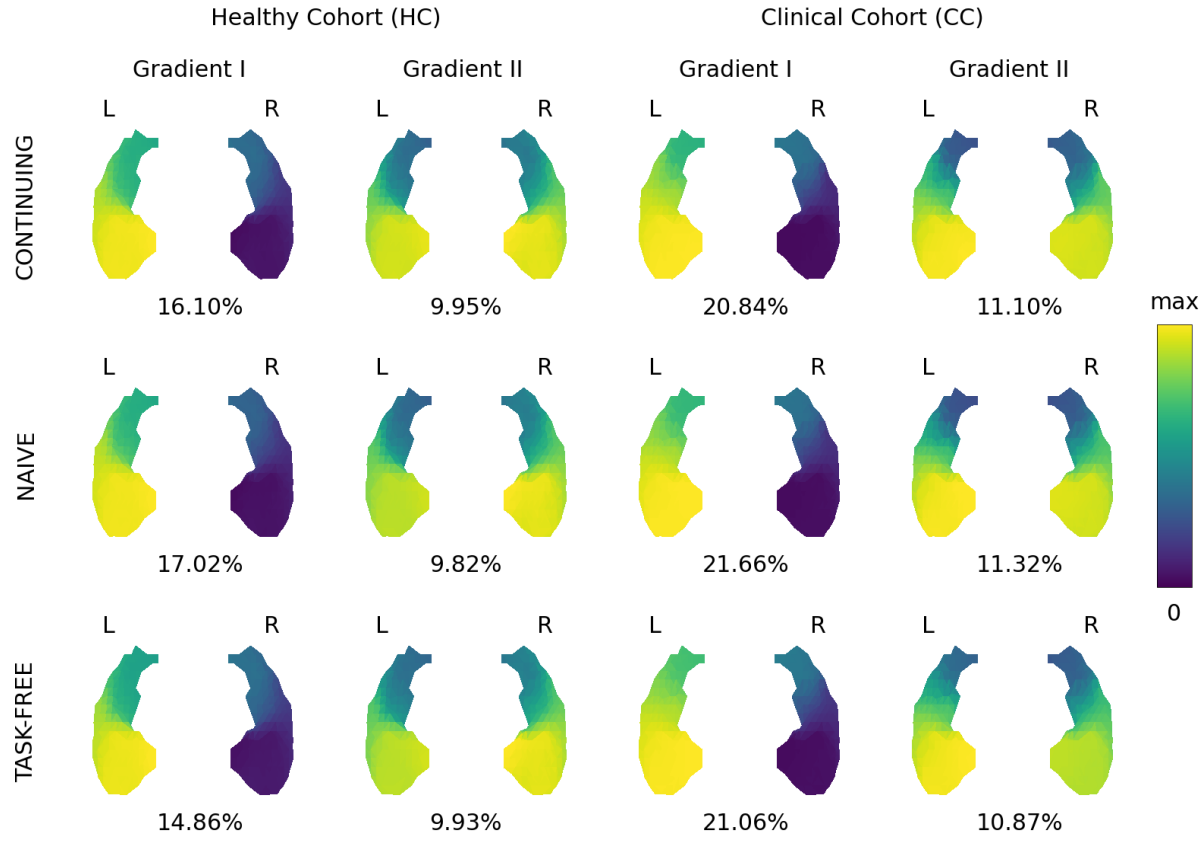


Figure 2: Gradients eigenmap. *The eigenmaps of the two gradients explaining the most variance are projected onto the hippocampus surface for each cohort and for each condition (continuing task, naive task and task-free). The percentage of variance explained is indicated below each gradient. The colormap limits are different for each projection: they range from 0 to the maximum eigenmap value (i.e. from left to right, CONTINUING: 0.054, 0.042, 0.052, 0.040; NAIVE: 0.053, 0.042, 0.052, 0.038; TASK-FREE: 0.056, 0.046, 0.052, 0.042). Alternative views of the first eigenmap are shown in Supp. Fig. 3.*

… and Results (p12),

The magnitude of Gradient I possesses a peak between the anterior and posterior hippocampus (Fig. 3), corresponding to a rapid change in hippocampus-to-whole brain task-free and task-related functional connectivity.

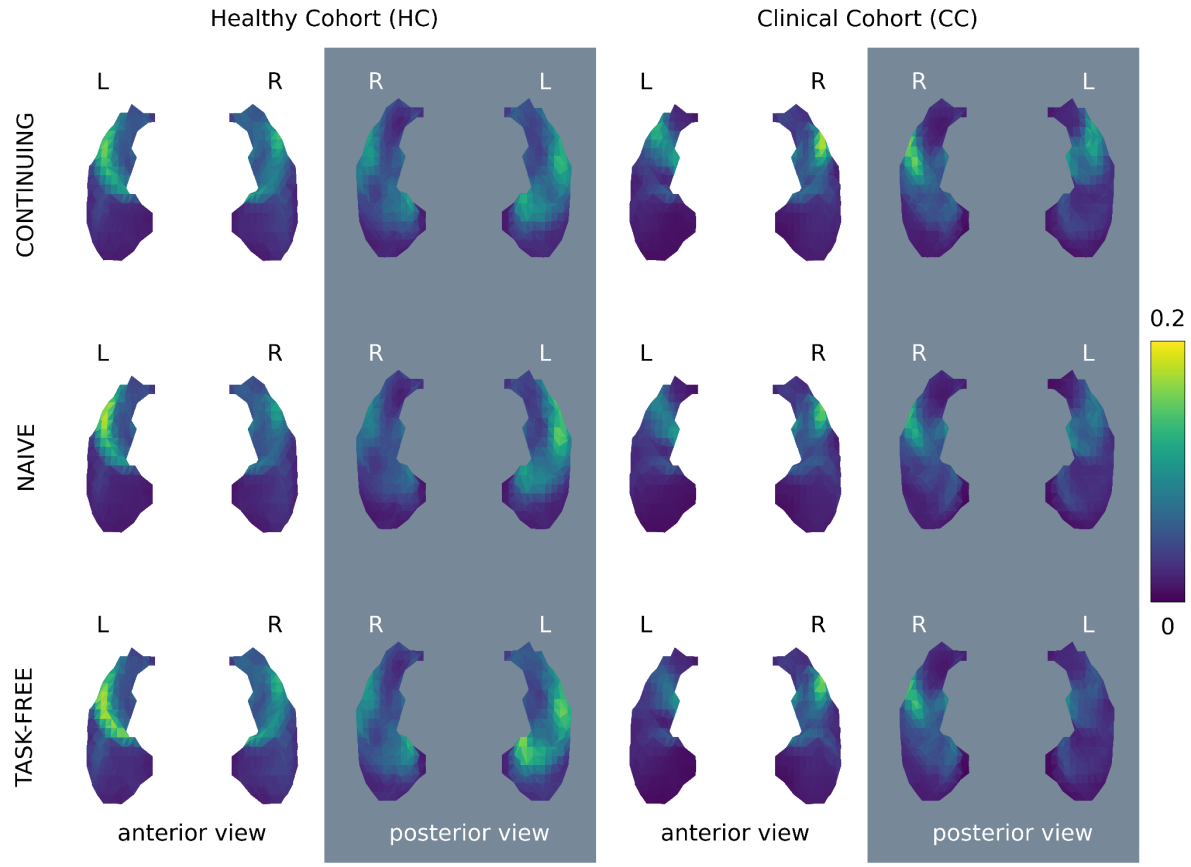
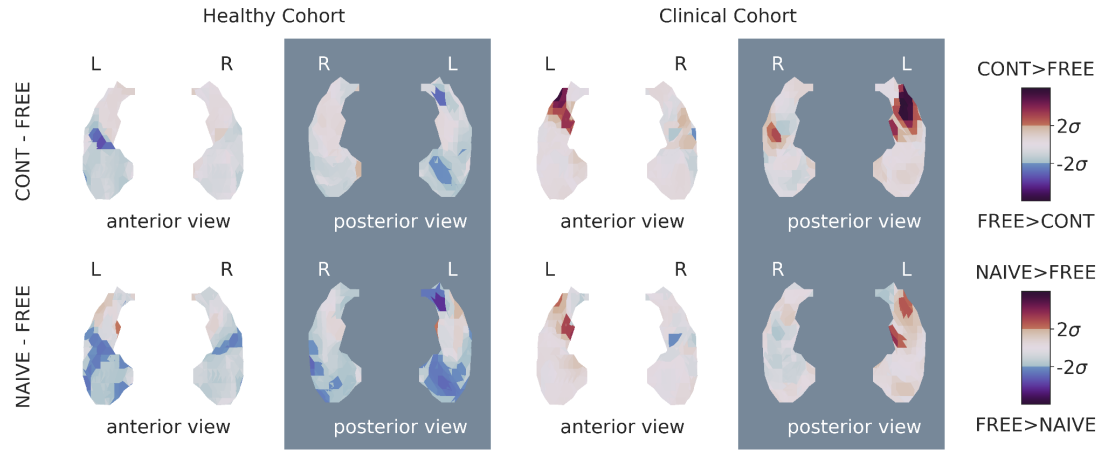


Figure 3: Gradient I magnitude. *The magnitude of Gradient I is projected onto the hippocampus surface for each cohort and for each condition (continuing task, naive task and task-free). The 1st and 3rd columns correspond to the anterior view of the hippocampus and the 2nd and 4th columns to the posterior view. The gradient magnitudes are similar between conditions. On each projection, there is a peak in magnitude on the boundary between the anterior and posterior hippocampus. This peak appears to have a more posterior location for the clinical cohort.*

and Results (p13),

We next contrasted the task-free and task-related functional connectivity gradients. In the clinical cohort, the anterior/posterior gradient magnitude is substantially stronger at the boundary of the anterior and posterior hippocampus in both task-related conditions than for the task-free regressor (Supp. Fig. 6). The opposite effect occurs in the healthy cohort, although in a spatially heterogeneous pattern.



Supplementary Figure 6: Difference in Gradient I magnitude between task-free (FREE) and tasks (CONT: continuing; NAIVE: naïve). *Panels and colorbar as per Figure 4.*

… and the group contrast now also includes the task-free-derived magnitude, which notably does not differ between groups (Results, p14),

The magnitude of the task-free connectivity does not differ across groups.

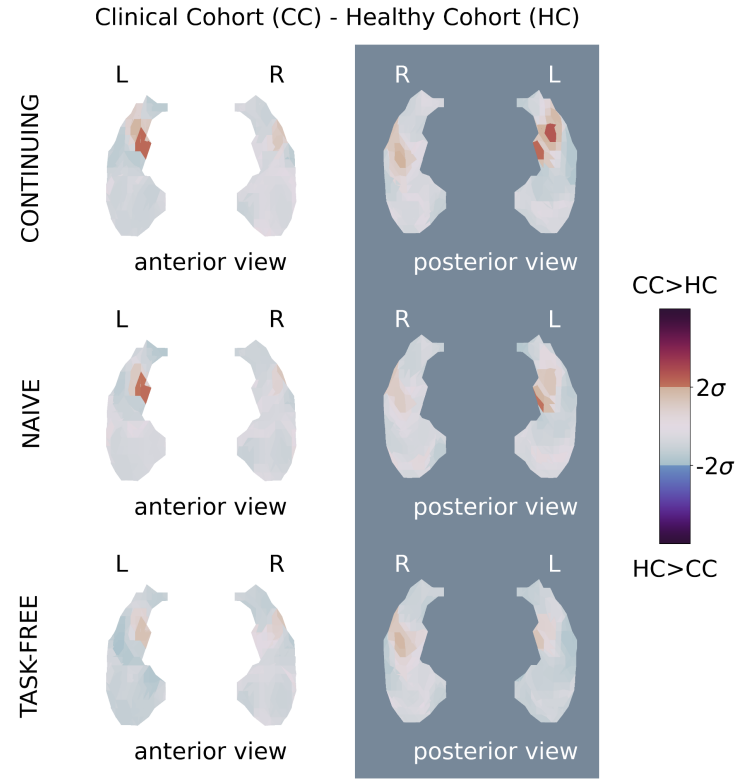


Figure 5: Between-cohort differences in Gradient I magnitude. *For each voxel, the difference in magnitude of Gradient I between the clinical (CC) and healthy cohorts (HC) was z-scored relative to the null distribution of the between-cohort permutation (n=1000). The z-scored difference is here projected onto the anterior (columns 1 and 3) and posterior (columns 2 and 4) view of the hippocampus for each task. The threshold of the color palette corresponds to +/- two standard*

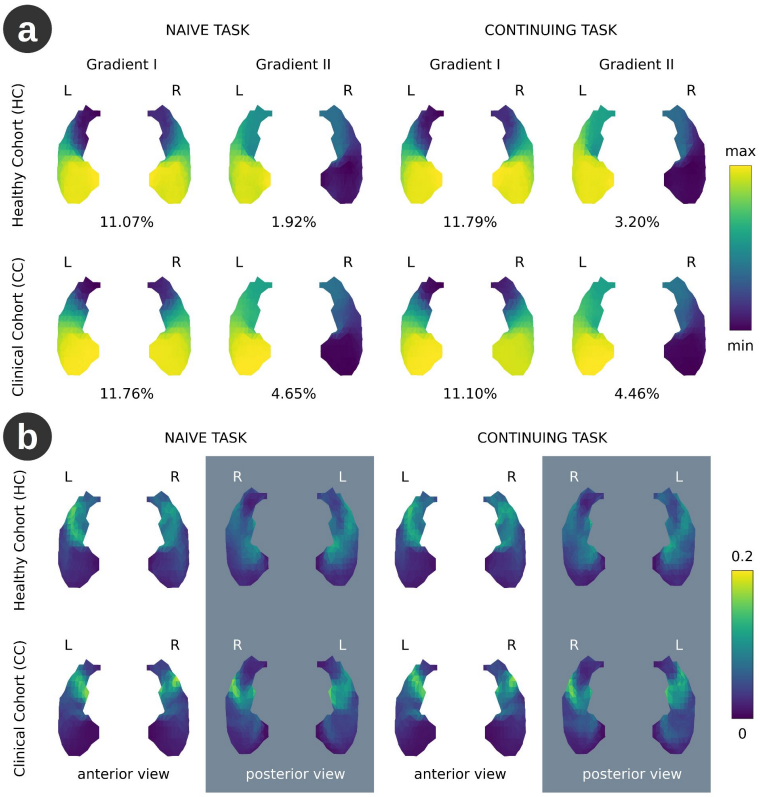
deviations of the z-scored difference. For both continuing and naive tasks, the functional boundary between the anterior and posterior hippocampus is significantly more posterior for the CC than for the HC. There are no significant group differences in the corresponding task-free-derived gradient magnitude.

The presence of a group effect in the task, but not task-free, gradient magnitude is interesting and we now note this in the Discussion (p19).

We observed a posterior shift in the location of the gradient transition in the left hippocampus, present in both task conditions in these clinical participants, but not in the task-free connectivity. Hence, in the presence of a task-related stressor, the region of the anterior hippocampus with a shared connectivity fingerprint extends further in the posterior direction in this cohort.

R2.4 In this study and its predecessor (Tian et al 2020), fMRI data are extensively smoothed (FWHM=6mm, Supp Mats) which necessarily enforces a smooth gradient of hippocampal connectivity. The hippocampus is only ~1-2cm wide and ~1cm tall, so it seems unlikely that any gradient other than A-P could have been found with these methods. A similar issue is that the same MNI space atlas is used for all hippocampi despite the fact that the hippocampus becomes smaller in older adults and especially those with MCI or AD. These points all question the spatial specificity of the current methods.

Response: We now address the choice of smoothing kernel by repeating the gradientography pipeline after spatial smoothing at 4mm FWHM (see also R1.3). These reveal a basic conserved gradient architecture as shown below (Supp Figure 5),



Supplementary Figure 5: Functional connectivity gradient mapping with spatial smoothing performed with a Gaussian smoothing kernel of 4mm FWHM (full width at half maximum), instead of 6mm. *(a) Gradients eigenmap (see Fig. 2 caption). (b) Gradient I magnitude (see Fig. 3 caption).*

We overview these new analyses in the Results section (p12),

To explore the effect of spatial smoothing, we repeated the gradientography pipeline after reducing the size of the data smoothing kernel from 6mm to 4mm FHWM. Although the relative variance explained by the two principle gradients switches order, their global pattern and associated magnitudes are preserved, including the existence of a faster gradient transition between the anterior and posterior hippocampus (Supp. Fig. 5). However, the total variance explained is considerably smaller (from approximately 25-30% at 6mm down to 12-16% at 4mm) and we therefore focus on the gradients following smoothing at the default of 6mm.

We are aware this does not fully negate the effect of smoothing, although it provides some reassurance and further insights into the related issues raised by the reviewer. We include a further consideration however in the Discussion (p20),

There are several caveats to our study. First, the gradients observed here align with the AP axis of the hippocampus and as such do not engage with other facets of hippocampal organization, including the influence of subfield divisions. Hippocampal functions reflect a composite of connectivity gradients and subfields (Genon et al., 2021; Vos de Wael et al., 2018) whose disambiguation is unlikely to be evident at the relatively coarse scales of acquisition resolution and smoothing width employed in the present study. We do, however, note that the hippocampus is considerably longer (~4-5cm) than our default smoothing kernel (6mm) and moreover, connectivity gradients remain clearly present when the data are smoothed at 4mm. While these observations are reassuring, imaging at high field strength combined with techniques that eschew the need for spatial smoothing may assist here.

R2.5 Permutation testing should ideally account for spatial autocorrelations

Response: Permutation testing across groups/tasks implicitly preserves the spatial correlations within the data (and gradients) as these are present within the individual permuted data. We added a point that clarifies this in the Methods (p9),

Note that by permuting gradient magnitudes between groups or tasks, this approach implicitly preserves the spatial correlations within the data.

For the new analysis undertaken to highlight the projection of the extreme ends of the hippocampus gradient onto the cortex (see response **R1.1** above), a null test that explicitly preserves the spatial autocorrelations of the data is indeed essential: To achieve this, we permuted data following a whitening (discrete wavelet) transform (see responses to R1 and new **Supplementary Method - Wavelet resampling**).

R2.6 I found the language used in this manuscript confusing or even misleading at times: "Gradient magnitudes" is used to refer to the Sobel operator applied to gradients but this was not defined in the manuscript (I had to look it up in Tian et al). Also the term "magnitude" could just as easily refer to the gradient eigenvector or eigenvector*eigenvalue, and doesn't, on its own, convey or imply the fact that it represents a spatial pattern or texture of a gradient. Thus I'm not sure "magnitude" is the most appropriate word for this measure, perhaps "texture" would be better. If the authors want to maintain the use of the term "magnitude" then at minimum they should clearly define what it refers to (both in the technical sense in the Methods and ideally they should provide an intuitive idea of what it means throughout the entire paper).

Response: We now provide a clearer description of the meaning and derivation of gradient magnitudes in the Methods (p7),

The scalar values of these Laplacian eigenmaps (connectivity gradients) across the hippocampus capture whole brain connectivity of the subcortex. The rate of change in these connectivity gradients captures functional homogeneity: slow gradient changes represent locally shared patterns of whole brain connectivity whereas rapid changes indicate functional differentiation. The magnitude and direction of these changes was estimated by application of the Sobel gradient operator to each subcortical voxel. The magnitudes of these changes (or simply "gradient magnitudes") capture gradual versus abrupt spatial changes (discontinuities) and their task modulation (Tian et al., 2020).

R2.7 The term "gradient" is sometimes used as if it refers to the same measure in different studies, but in fact it is merely the same (or a similar) calculation employed on different measures. For example, *"Functional connectivity with cortical and subcortical structures appears to change gradually, rather than suddenly, along the long axis of the hippocampus (Amaral & Witter, 1989; Strange et al., 2014; Vos de Wael et al., 2020). This gradient in functional connectivity predicts recollection ability (Przeździk et al., 2019) and mirrors gradients in cytoarchitecture (Paquola et al., 2020) and gene expression (Vogel et al., 2020)."* The primary gradient in Paquola et al histology is not similar to functional connectivity gradients (the latter differs A-P while the former is nearly perpendicular differing across hippocampal subfields and MTL regions). In other words, these studies use converging statistical methods with converging validity but do not reveal converging organizing principles.

Response: We have revised the text to highlight the convergence and divergence of "functional and connectivity gradients" with cytoarchitecture (also including the previously overlooked Vos de Wael paper, p4),

Hence functional specialization and external connectivity of the hippocampus appear to unfold along its long axis. However, the relationship of these gradients to anatomy and cytoarchitecture remain unclear. Notably, cytoarchitectural properties of the broader mesiotemporal lobe change smoothly along the iso-to-allocortical direction, orthogonal to the antero-posterior alignment of the functional and connectivity gradients within the hippocampus (Vos de Wael 2018, Paquola et al., 2020).

R2.8 "Gradient" means both a gradual change and also, within this paper, is a technical term for the major components from dimensionality reduction of hippocampal-neocortical connectivity. Ideally this should be defined clearly in the Intro for those not already familiar with the field of "gradientography".

Response: We now provide a definition for the term “gradientography” (p4).

Here, we address these issues using ‘gradientography’, a method for characterizing how patterns of whole brain functional connectivity change across the subcortex (Tian et al., 2020).

Minor:

R2.9 The summary of A-P differences in the hippocampus as being self- to world-centric is a bit odd since i) there are competing accounts of these differences in the literature and ii) it is easily confusable with a related field examining ego- and allo-centric cognitive maps. Thus I don't think this is a good summary of the referenced work by Plachti et al and Poppenk et al.

Response: We agree that there are multiple accounts of functional AP differences, although in our defense, Plachti do explicitly conclude that, “Behavioral profiling … indicate an emotion–cognition gradient along the anterior–posterior axis and additionally suggested a self-world-centric gradient” which is a close paraphrase of our original citation to this paper. Moreover, we don’t cite Poppenk et al in support of a self- to world centric but rather that “the anterior pole facilitates emotions and episodic memory whereas the posterior pole facilitates spatial navigation” which was derived from their Table 1.

As the reviewer notes, this is a minor point and as the main underlying point is simply to highlight the existence of functional differentiation along the AP axis we would prefer to leave the current citation to the self-to-world centric gradient, noting that we also list other accounts of functional differentiation.

R2.10 I also don't think the proceeding discussion of hippocampal theta is needed as it is not mentioned again in the manuscript.

Response: We have moved this note from the Introduction to the Discussion to assist in the integration of our findings with the notion that hippocampus binds cortical representations across space and time (see also R1.11, p20),

The hippocampus underpins relational binding, forming "rapid, continuous, and obligatory associations among disparate elements across space and time" (Olsen et al., 2012). In this vein, hippocampal neurons detect cognitive boundaries as they occur in naturalistic stimuli and reinstate neural states in response to subsequent cues (J. Zheng et al., 2022). Recent theories propose that these hippocampal functions are supported via a 'conceptual model' of cortex, performing a computational integration of related cognitive representations (Whittington et al., 2022). Here, we find that visual and semantic cues that link present with recent narratives lead to a modulation of the functional embedding of hippocampal activity in cortical systems, organized along hippocampal-cortical connectivity gradients. Intriguingly, neurophysiological recordings reveal that theta oscillations precede continuously along the long axis of the hippocampus as traveling waves (Lubenov & Siapas, 2009) suggesting that hippocampal gradients shape local information propagation (Kleen et al., 2021). Waves of activity also characterize cortical states across a wide variety of cognitive states and tasks (Roberts et al., 2019), including working memory (Sreekumar et al., 2021). Coupling between cortical and hippocampal waves propagating along their principal connectivity gradients is hence a candidate mechanism for the spatiotemporal integration of cortical and hippocampal activity.

R2.11 Hippocampus is not subcortical, but archicortical. This is not trivial as the archicortex can be modeled as a surface like, and gradually continuous with, the neocortex (DeKraker et al. 2021, Paquola et al. 2020). This issue is not specific to this paper but rather the field in general.

Response: We appreciate this which we now incorporate into the limitations sections of the Discussion (p20),

Second, we apply gradientography in three-dimensional (voxel) space, treating the hippocampus as a discrete subcortical volume - consistent with much of the prevailing analysis of hippocampal structure and function. However, the human hippocampus is more accurately α folded archicortical sheet, continuous with the adjacent medial temporal lobe (DeKraker et al., 2021). In addition to incorporating this important feature of hippocampal structure, a surface-based treatment of hippocampal gradients would also render the analysis of its associated magnitude to a more parsimonious rate of change on a two-dimensional sheet.

As the reviewer notes, the semantic inclusion of the hippocampus under the umbrella term of the “subcortex” is typical in the field, so we continue to refer to “subcortex and hippocampus” elsewhere simply as “subcortex”.

R2.12 "Gray matter voxels" or "M" or "whole brain"(Fig1) should be only neocortical gray matter voxels (unless I have misunderstood the design)

Response: As in (Tian et al., 2020), M is the number of voxels in the grey matter of the whole brain (cortex and subcortex). We did not exclude the subcortical voxels because subcortical-subcortical connectivity is also important. We are interested in the whole-brain connectivity profile of subcortical regions, not just cortical-subcortical connectivity. Subcortical regions are also densely interconnected.

We have clarified this point as follows:

As in (Tian et al., 2020), the BOLD signals for each individual were represented in a matrix of dimension T × M, where T denotes the number of time frames and M denotes the number of gray matter voxels (cortical and subcortical voxels).

I appreciated the clear limitations section, which only makes the paper stronger in my mind and benefits the entire field.

Signed,
Jordan DeKraker

Thank you for your excellent review.

Reviewer #3:

The authors computed gradients derived from hippocampal-cortical connectivity during a naturalistic episodic memory task in healthy individuals and individuals with preclinical or clinical AD. The first two gradients showed an anterior/posterior axis of hippocampal connectivity, though the first gradient primarily featured a strongly left/right asymmetry. Cortical projections showed that the more anterior hippocampus was more strongly connected to higher-order/transmodal cortical regions, while the more posterior hippocampus was more strongly connected to sensory/unimodal cortical regions. When examining the magnitude of the first gradient, the authors observed a posterior shift in the transition between the left anterior/posterior hippocampus in the clinical group compared to healthy controls.

The manuscript was well written and clear. I only have a few questions/comments

Response: Thank you for your constructive appraisal and feedback.

R3.1 Were left and right hippocampal-cortical connectivity computed separately? If yes, perhaps this could explain why the first gradient contrasts the left vs. right hippocampal connectivity. Either way, could the authors speculate on what this strong asymmetry could represent, e.g., is it more likely due to hippocampal function or methodology?

Response: This is an interesting observation, also noted above (R2.1): The gradientography pipeline was applied to the entire whole brain data. The decomposition into symmetric and antisymmetric principle gradients is a typical finding in orthogonal decompositions of both functional and structural neuroimaging data, but does also reflect underlying properties of the data. We have commented on this in the revised Discussion (p19),

Orthogonal decompositions of structural and functional neuroimaging data do typically yield symmetric plus asymmetric leading modes (Tokariev et al., 2019), as we indeed observe here. Through their relative contributions to the original data (through summation or subtraction) such complimentary modes can account for shared bilateral effects versus unilateral hemispheric dominance. Human hippocampi do show a degree of left-right structural asymmetry in health (Woolard & Heckers, 2012) and during neurodegenerative disorders (Wachinger et al., 2016). While we focus on the gradient magnitudes, future work could explore the relative importance of these two modes across different tasks. However, in contrast to the robustness of the gradient magnitude, spatial smoothing strongly influenced

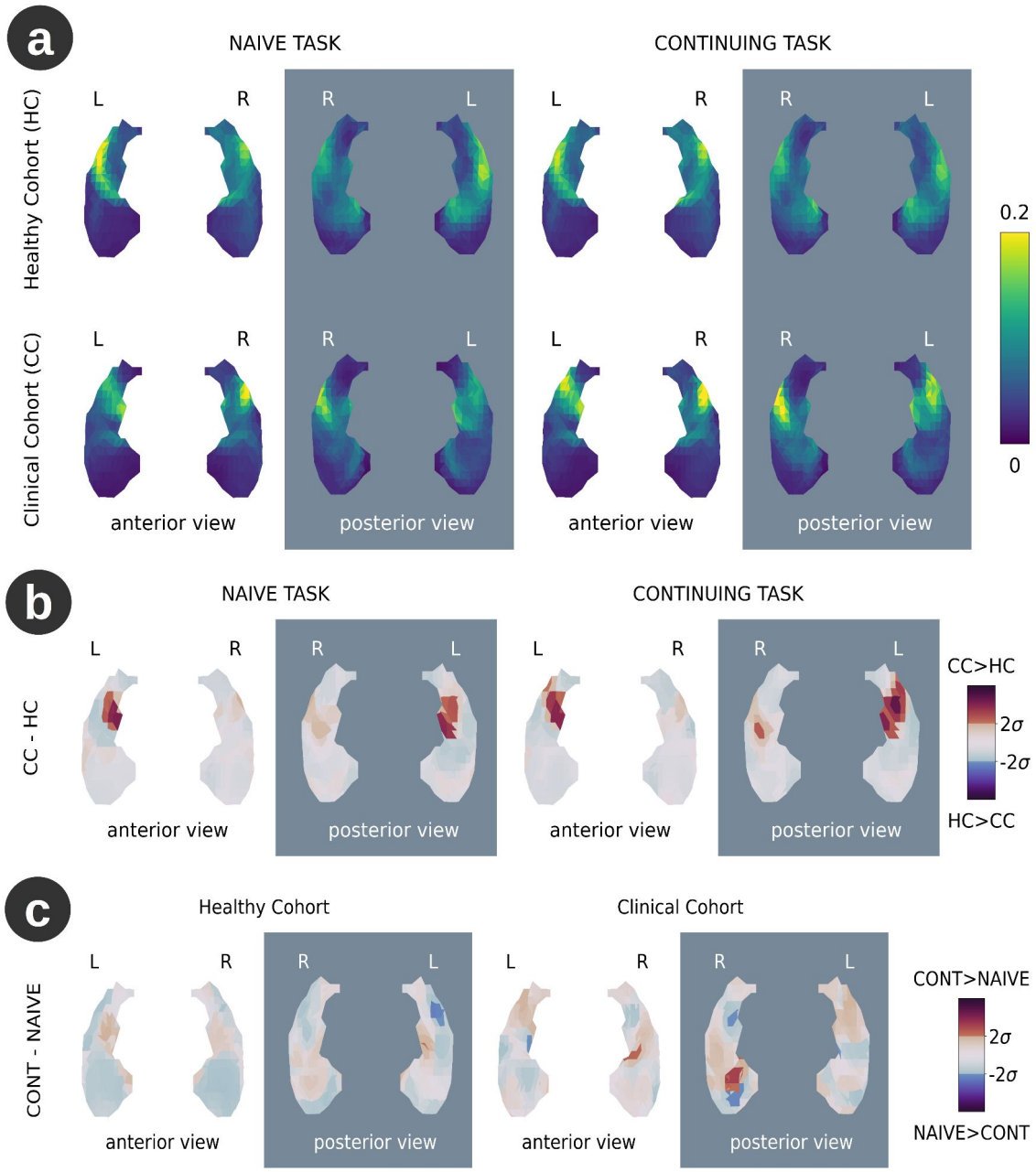
the relative variance explained of these two modes, suggesting further validation is required before this potential approach could be exploited.

R3.2 Could the authors further explain why they did not compare gradient magnitude between groups and task conditions in the second gradient? It could be a neater comparison of the anterior/posterior hippocampal connectivity between groups/conditions, than the first gradient which might be confounded by the strong left/right asymmetry.

Response: We focused on the first gradient because it explains the most variance and we sought to balance the complexity of the findings, against the depth of the underlying effects. However, we appreciate this more nuanced effects and have now added contrasts of the second gradient as follows, (Results, p13),

Task comparison: *(...) This task-related difference in gradient magnitude is not significant for gradient I in the clinical cohort (Fig. 4) nor for gradient II in either cohort (Supp. Fig. 4).*

Group comparison: *(...) This shift in the location of the magnitude in the left hippocampus is even more pronounced for gradient II (Supp. Fig. 4).*



Supplementary Figure 4: Gradient II. *(a) Gradient II magnitude (see Fig. 3 caption). (b) Magnitude differences of the Gradient II between the healthy (HC) and clinical cohorts (CC) (see Fig. 5 caption). (c) Magnitude differences of the Gradient II between naive (NAIVE) and continuing (CONT) tasks (see Fig. 4 caption).*

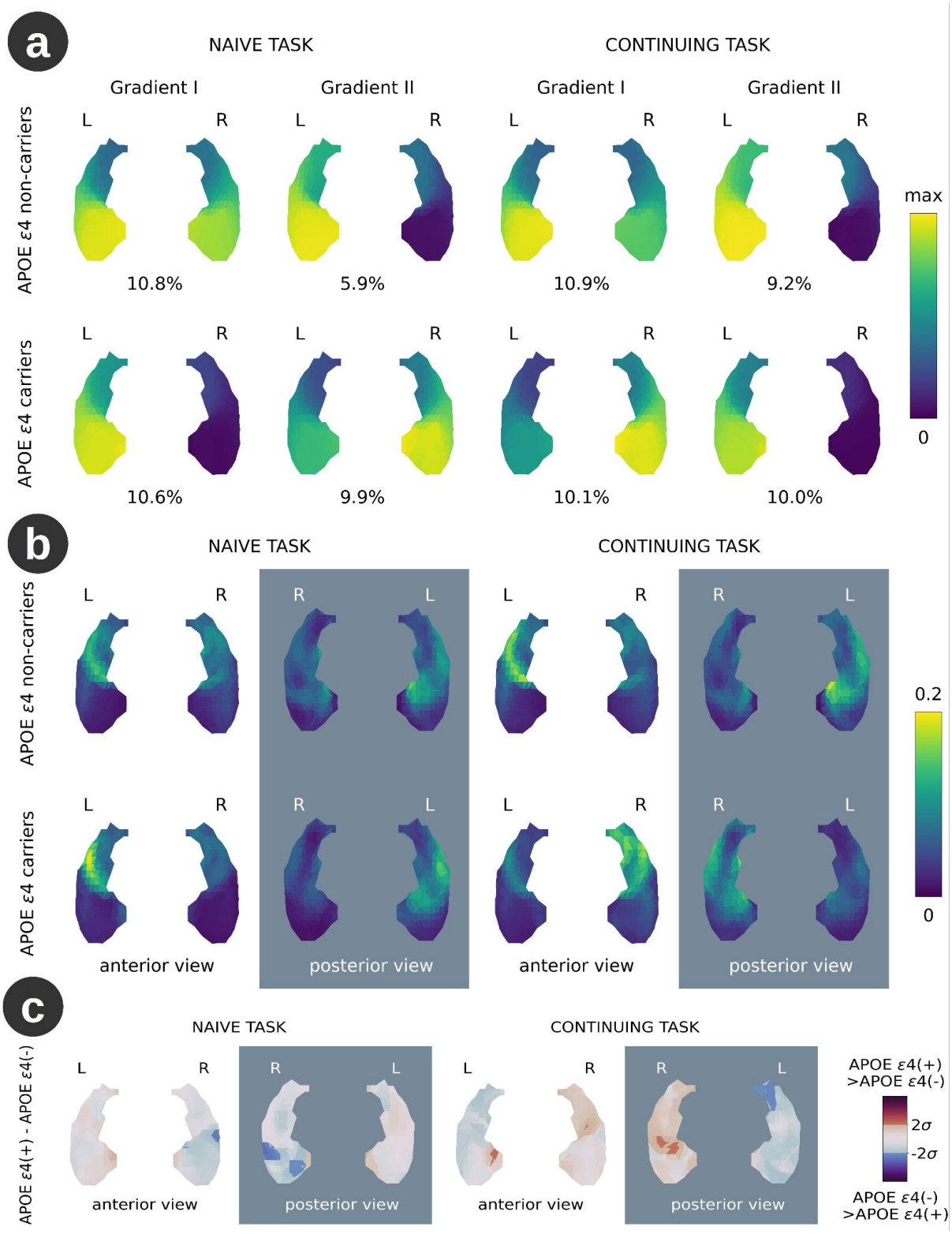
R3.3 Were amyloid and APOE4 status examined in MCI patients? We know that not all MCI patients will progress to AD, but to other dementias such as vascular dementia. Therefore, the underlying neuropathology could be affecting circuits/brain structures other than the medial temporal lobe.

Response: Yes, indeed examined amyloid and APOE4 status in the original manuscript, which we include here for completeness (p15),

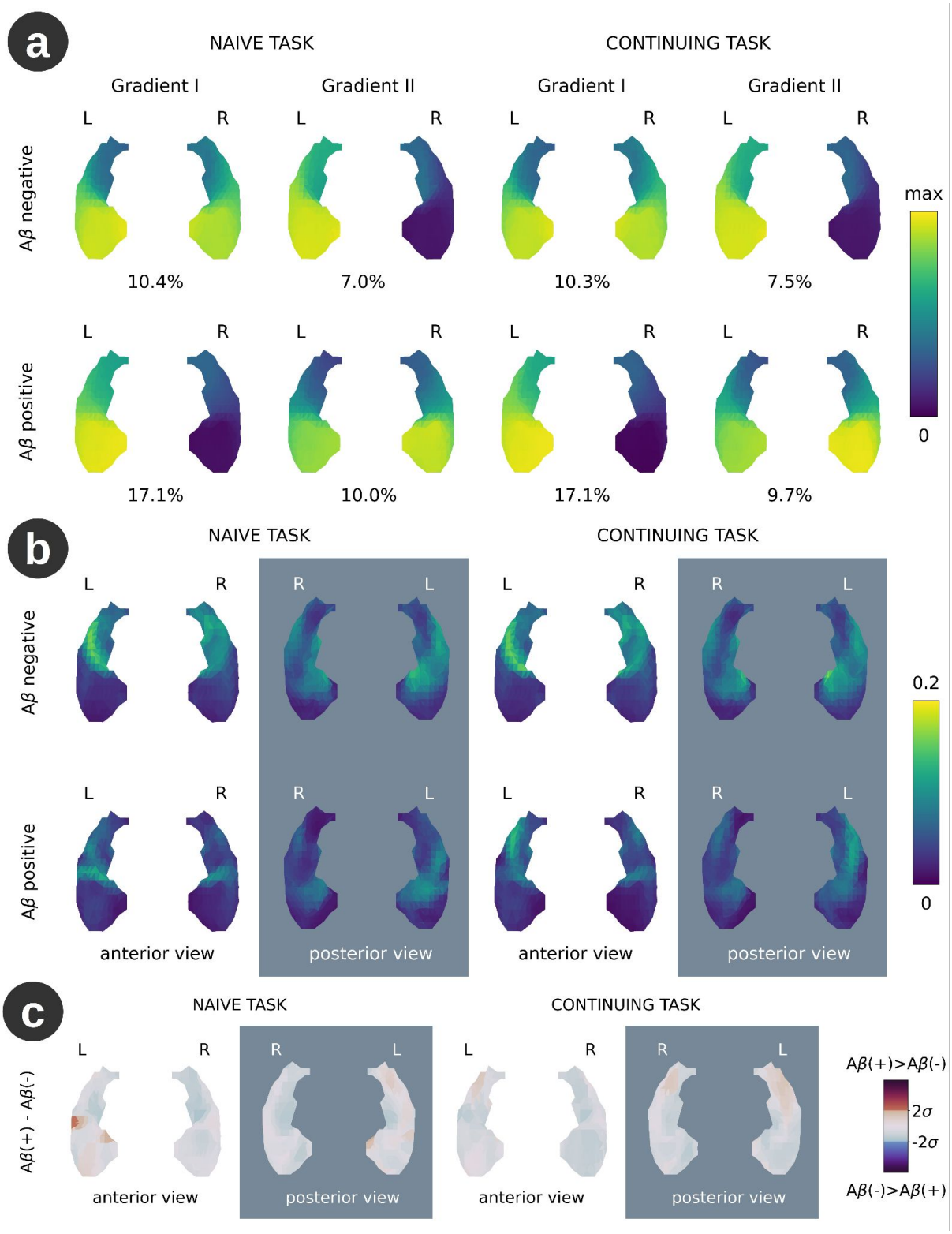
We also contrasted healthy individuals according to their amyloid and APOE status. Despite a reasonably well-powered contrast for APOE status (N=85 APOE ε4 carriers; N=73 non-carriers), there is at best an equivocal effect near the transition of the right anterior hippocampus (Supp. Fig. 7). There is no substantial effect of amyloid status across either task within the hippocampus (Supp. Fig. 8), although we note weaker power for this contrast (N=15 HC Aβ(+); N=144 HC Aβ(-)).

We have now added a brief note regarding these findings in the Discussion (p19),

Both APOE and amyloid status were also available on 158 of the healthy participants, indicating a higher risk of future neurodegeneration. We observed a patchy and somewhat equivocal effect for APOE status predominantly in the right hippocampus (Supp. Fig. 7) but no clear effect of amyloid status across either task. Our future longitudinal follow-up of this cohort may reveal stronger effects that are currently latent.



Supplementary Figure 7: Comparison of healthy APOE ε4 carriers (N=85) and non-carriers (N=73) participants. (a) Gradients eigenmap (see Fig. 2 caption). (b) Gradient I magnitude (see Fig. 3 caption). (c) Magnitude differences of the Gradient I between the healthy APOE ε4 carriers (APOE ε4(+)) and non-carriers (APOE ε4(-)) participants (see Fig. 5 caption).



Supplementary Figure 8: Comparison of healthy amyloid positive (N=15) and negative (N=144) participants. (a) Gradients eigenmap (see Fig. 2 caption). (b) Gradient I magnitude (see Fig. 3 caption). (c) Magnitude differences of the Gradient I between the healthy amyloid positive (Aβ(+)) and negative (Aβ(-)) participants (see Fig. 5 caption).