Supplementary Information

Laminar activity in the hippocampus and entorhinal cortex related to novelty and episodic encoding

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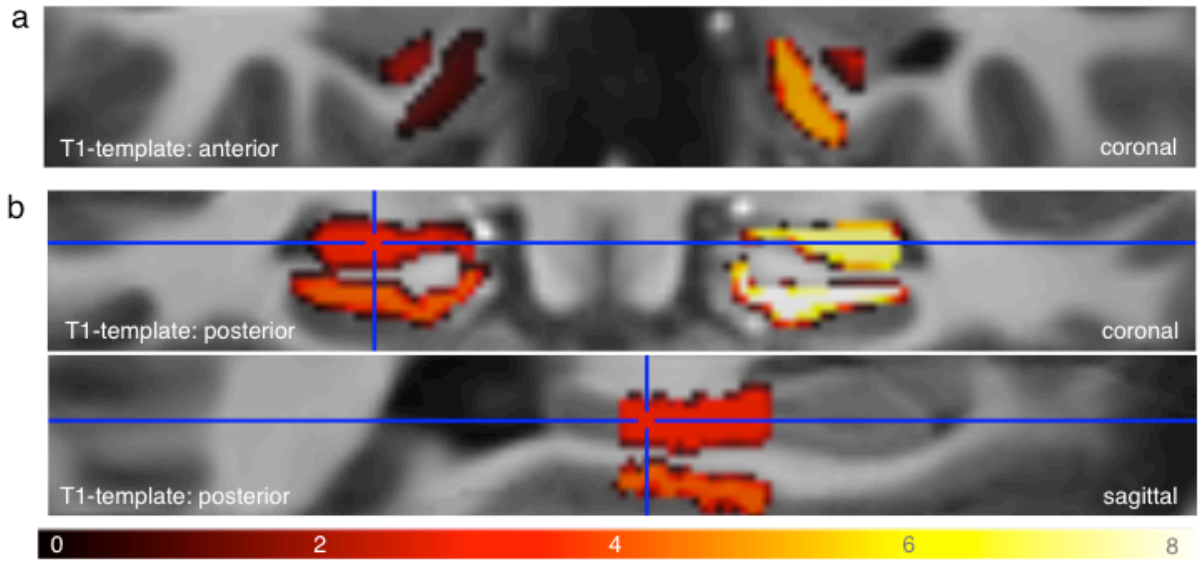
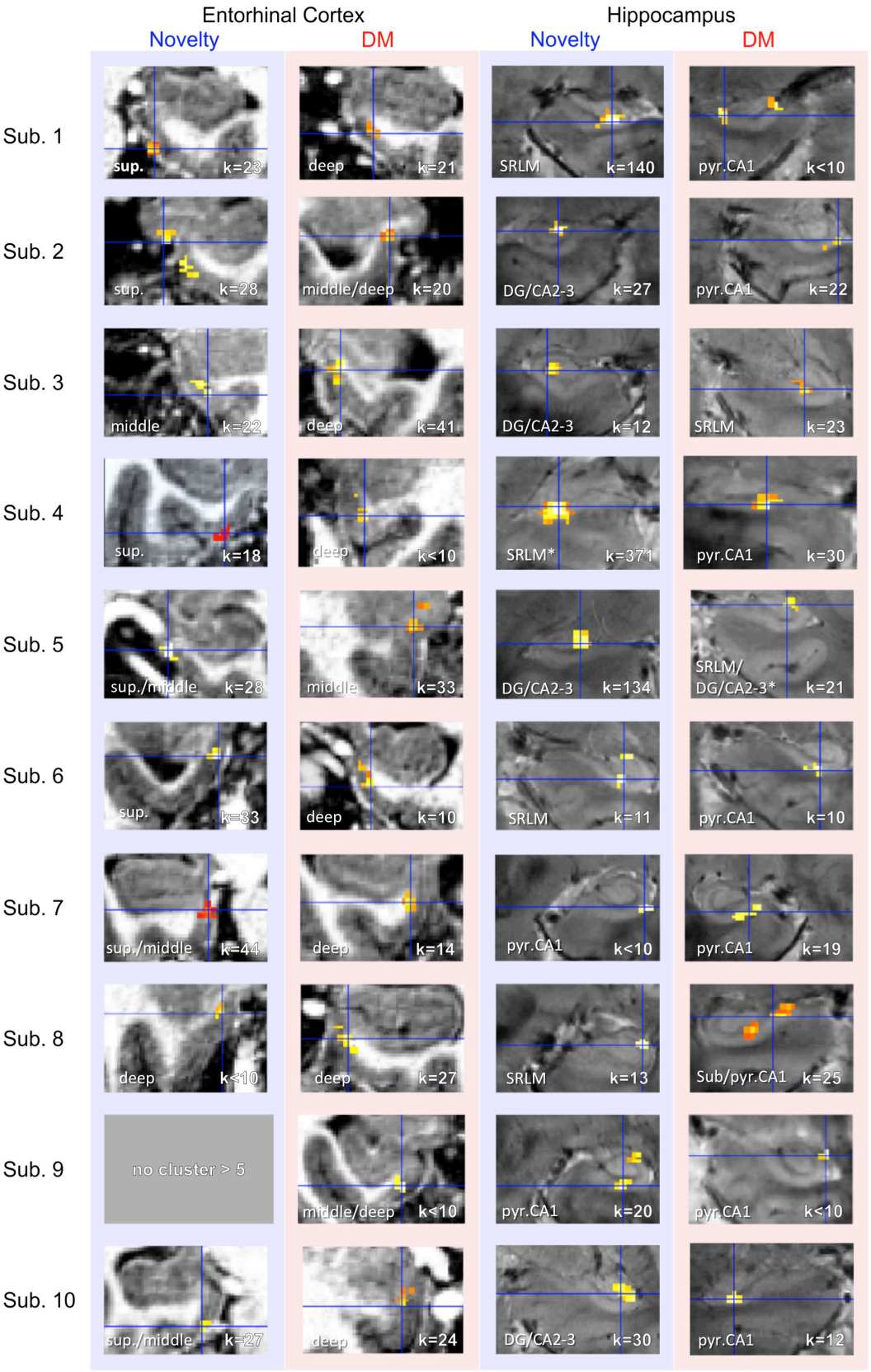
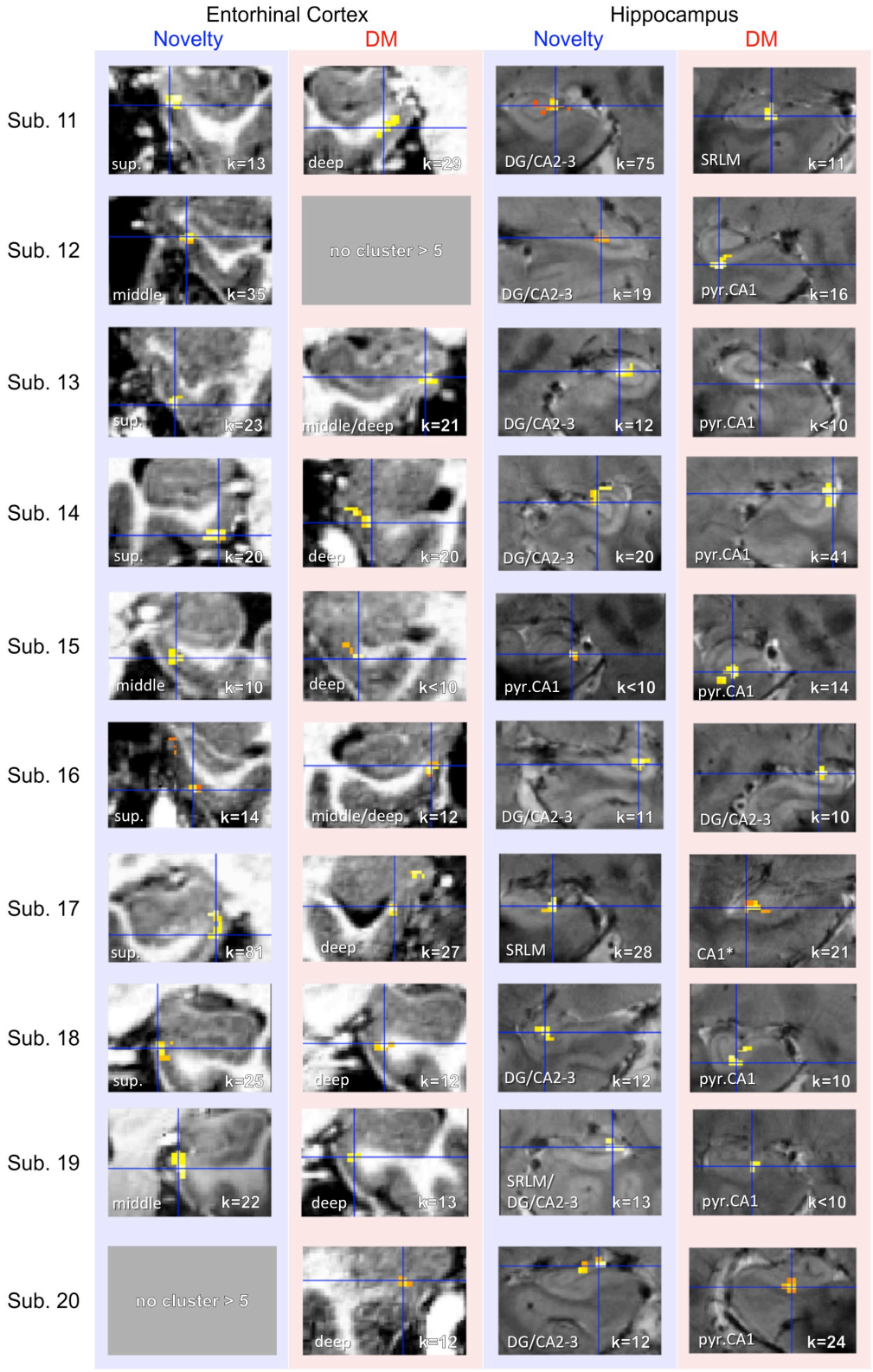
Supplementary Figures

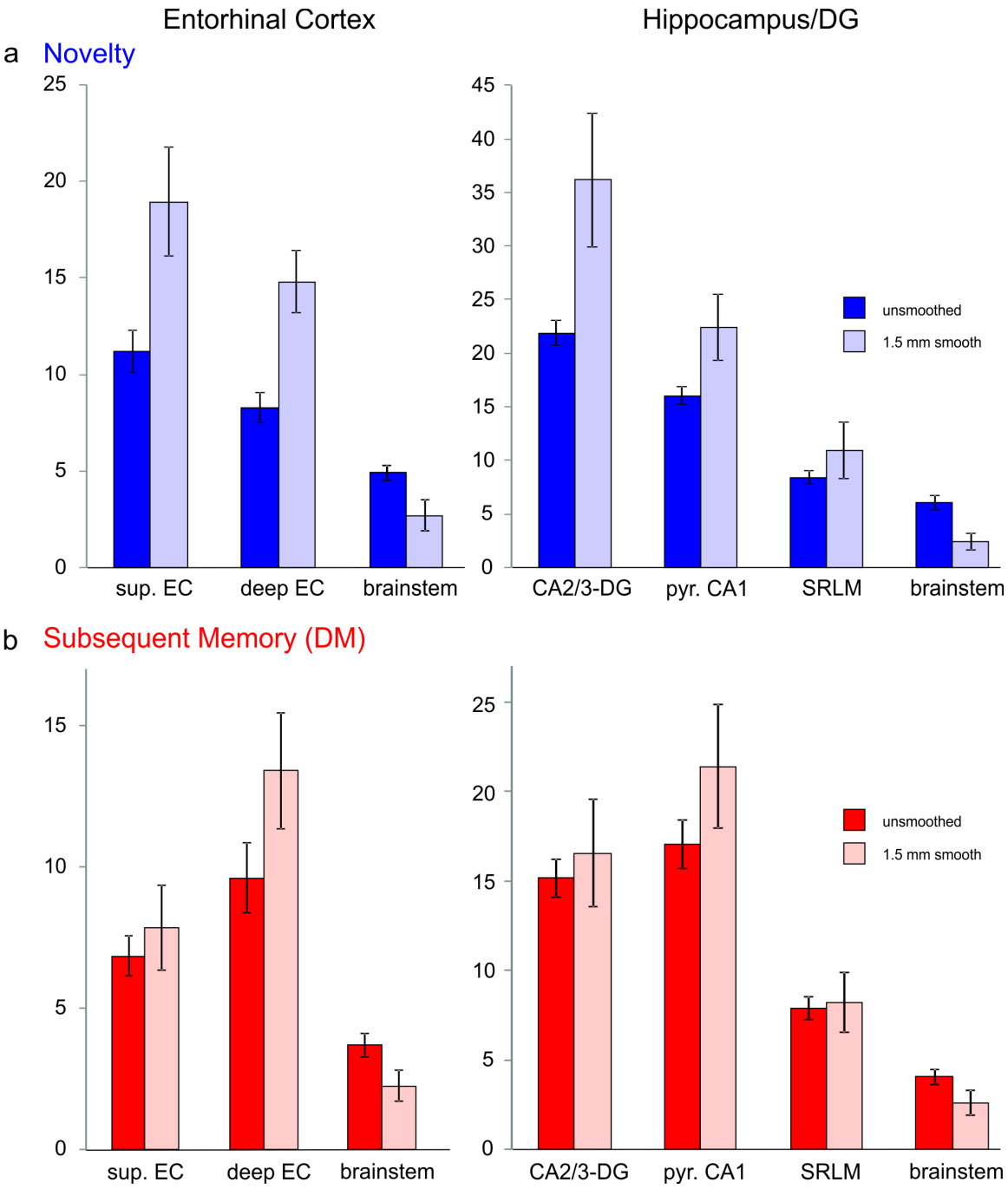


Supplementary Figure 1. T1-group template with overlaid MTL landmarks of one sample subject after label-guided diffeomorphic registration with ANTS. The high-resolution (0.6 mm^3) MPRAGE images were used to create a study-specific group template with ANTS (Advance Normalization Tools). MTL landmarks were further used to improve registration by means of label-guided alignment (expectation-based point set registration, "pse"). Therefore, MTL maps were created for all subjects and the template, in which each region was coded by a numeric value (from 1 to 8; e.g. left EC = 1, left HC head = 2, …, right PHC = 8; see different colors). To verify alignment precision, we applied the transformation parameters to the subject-specific MTL maps and checked their overlay on the template. **(a)** Registered anterior MTL regions (EC and HC head) and **(b)** posterior MTL regions (HC body and PHC) of one sample subject overlaid on the group template. Different colors denote probabilities; edges appear dark after registration due to low probability values. Please note that for one subject, alignment of EC regions failed and thus, this subject had to be excluded from all normalization-based analyses.

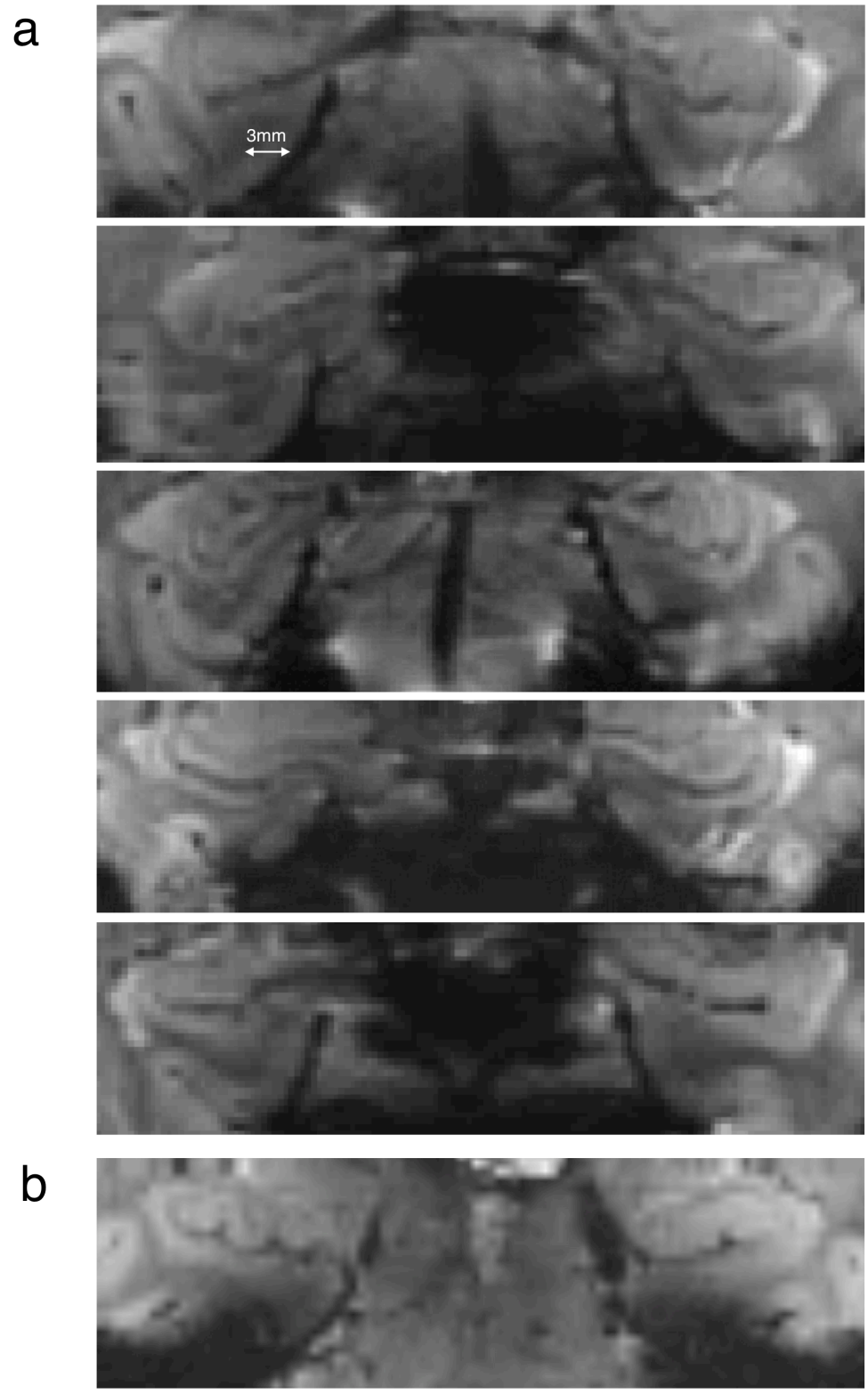

Entorhinal Cortex
Hippocampus
Novelty
DM
Novelty
DM
Sub. 1
sup. k=23
deep k=21
SRLM k=140
pyr.CA1 k<10
Sub. 2
sup. k=28
middle/deep k=20
DG/CA2-3 k=27
pyr.CA1 k=22
Sub. 3
middle k=22
deep k=41
DG/CA2-3 k=12
SRLM k=23
Sub. 4
sup. k=18
deep k<10
SRLM* k=371
pyr.CA1 k=30
Sub. 5
sup./middle k=28
middle k=33
DG/CA2-3 k=134
SRLM/ DG/CA2-3* k=21
Sub. 6
sup. k=33
deep k=10
SRLM k=11
pyr.CA1 k=10
Sub. 7
sup./middle k=44
deep k=14
pyr.CA1 k<10
pyr.CA1 k=19
Sub. 8
deep k<10
deep k=27
SRLM k=13
Sub/pyr.CA1 k=25
Sub. 9
no cluster > 5
middle/deep k<10
pyr.CA1 k=20
pyr.CA1 k<10
Sub. 10
sup./middle k=27
deep k=24
DG/CA2-3 k=30
pyr.CA1 k=12



Supplementary Figure 2. Univariate single-subject peak activation for the Novelty- and DM-contrast. Single-subject peak activations in the entorhinal cortex (EC) and hippocampus proper/dentate gyrus (HC/DG; in hippocampal body) for the Novelty- and subsequent memory (DM)-contrast overlaid on the individual coregistered MPRAGE (for EC, left panel) and T2*-image (for HC/DG, right panel). The blue cross demarcates the location of peak activation at a threshold of $p_{voxel\text{-}wise} < 0.01$ and $k \geq 10$ voxel (smoothing kernel 1.5 mm). For illustration purposes activation maps were masked with the individual EC or HC/DG ROIs. ROI labels for the location of peak activation are reported in each image (* demarcates cases where the location could not be clearly assigned). Note that for most of the subjects, several (bilateral) activations were found at this threshold. If no cluster survived $k \geq 10$ voxel, the largest cluster is shown. These subject-wise images serve to illustrate that individual (unnormalized) activation patterns related to novelty or subsequent memory are remarkably confined to hippocampal subfields/layers and to superficial or deep subregions of the EC.



Supplementary Figure 3. Univariate analyses of entorhinal and hippocampal subregion/layer-specific activation during novelty processing and successful encoding. Averaged (across individuals) numbers of voxels activated at $p_{voxel\text{-}level} < 0.01$ for **(a)** the Novelty- and **(b)** the DM-contrast (subsequent memory) in subregions/layers of the entorhinal cortex (EC) and hippocampus proper/denate gyrus (HC/DG; hippocampal body) for the unsmoothed and the 1.5 mm smoothed (both unnormalized) data. In order to confirm our findings from decoding analyses (see Fig. 3), we extracted number of significant voxels for each subject and individual ROI. Additionally, we extracted number of significant voxels from a control region (anterior ventral brainstem, same size as EC layers and SRLM, respectively) that did not show significant activation at group level. The same pattern of activation differences between superficial and deep EC subregions with respect to novelty and subsequent memory was apparent at the univariate level. These differences would be significant if paired t-tests were applied ($Novelty_{sup>deep}$: $p < 0.05$; $DM_{deep>sup}$: $p < 0.01$ for both smoothing levels). With regard to the HC/DG, number of activated voxels was highest in CA2/3-DG for Novelty and pyramidal CA1 layers for DM ($Novelty_{CA2/3\text{-}DG>pyr.CA1}$: $p < 0.05$; $DM_{pyr.CA1>CA2\text{-}3/DG}$: $p < 0.15$ for both smoothing levels). Note that region-specific differences are also apparent if 1.5 mm smoothing is applied and furthermore, that numbers of activated voxels increase in EC and HC/DG but decrease in the brainstem control region, if the data is smoothed. Error bars denote s.e.m.



Supplementary Figure 4. Sample EPI slices with and without dropouts. Shown are coronal sample slices from individual mean EPI volumes (average of 370 functional volumes, resolution: 0.8mm^3 isotropic) at the level of the hippocampal head. (**a**) Five EPI sample slices without artifacts or dropouts in the EC that were included in the analyses. (**b**) Sample EPI slice with partial dropout in the perirhinal cortex, laterally spreading into the EC (here: most prominent on the right). Dropouts occurred most frequently close to the ear canals. Overall, ca. 70% of slices covering the EC were usable for the final analysis (Left EC = 69% ± 17; Right EC = 73% ± 13).

Supplementary Note 1

Univariate layer/subregion-specific analyses of activation for novelty and subsequent memory

Multivariate Bayes decoding revealed that novelty was best predicted by activation in superficial EC regions and CA2/3-DG, whereas subsequent memory was best predicted by activation in deep EC regions and pyramidal layers of CA1 (see Fig. 3). In order to test whether the same pattern of differential activation of input and output regions is also apparent at the univariate level, we performed ROI-based analyses using the individual (unnormalized) data. While it would be nice to directly test for condition × region interactions, these are usually considered problematic in fMRI because the scaling factor of activations (i.e., the beta coefficient in the GLM) can vary across regions due to local differences in haemodynamic coupling. We therefore compared the number of activated voxels in each of our ROIs (that fell above a specific voxel-wise threshold) for both contrasts across subjects (see Supplementary Fig. 3). While we consider these additional analyses a pragmatic workaround and would continue to see our Bayesian decoding analyses (which are particularly suited for across-region comparisons) as the main basis for our conclusions, these univariate analyses confirmed the findings from MVB decoding and showed that the number of activated voxels is higher in superficial EC subregions during novelty processing and vice versa in the deep EC subregions during successful encoding.

First, we show layer/subfield-specific peak activation of each subject in the EC and hippocampus proper/dentate gyrus (HC/DG) in the hippocampal body for both contrasts (Novelty and DM; see Supplementary Figure 2) in native space (without normalization) for the 1.5 mm smoothed data. Univariate single-subject activation for the EC and HC/DG is illustrated on the individual coregistered (to the mean EPI) MPRAGE and high-resolution structural T2*-image, respectively ($p_{voxel\text{-}level} < 0.01$, $k \geq 10$ voxels; blue cross demarcates peak activation). Notably, these peak activation plots represent only a selection/cross-section of the overall activations in entorhinal and hippocampal subregions. In most subjects, several activation clusters were found bilaterally along the anterior-posterior axis. We have included these analyses to highlight that individual activation patterns related to novelty or subsequent memory can be remarkably confined to hippocampal subfields/layers and to superficial or deep subregions of the EC.

Furthermore, we extracted the number of significant voxels at a given voxel-wise threshold for each subject, contrast and region in native space for the unsmoothed raw data and the 1.5 mm smoothed data. To that end, we used the manually labeled ROIs as masks for the individual thresholded T-maps ($T > 2.33 \approx p_{voxel\text{-}level} < 0.01$) and determined the number of voxels surpassing this T-threshold. These univariate analyses confirmed the findings from Multivariate Bayes decoding and showed that the number of activated voxels is higher in superficial EC subregions during novelty processing and vice versa in the deep EC subregions during successful encoding (see Supplementary Fig.3; left). These differences hold with classical statistics (i.e. paired t-tests: $Novelty_{supEC>deepEC}$: $p < 0.05$; $DM_{deepEC>supEC}$: $p < 0.01$ for both smoothing levels). Regarding the HC/DG (see Supplementary Fig. 3; right) the same pattern of differential activation was found as revealed by multivariate decoding, with more activated voxels in CA2/3-DG than CA1 pyramidal layers for novelty processing and vice versa for successful encoding. However, these differences would be only significant for the Novelty-contrast ($Novelty_{CA2/3\text{-}DG>pyr.CA1}$: $p < 0.05$) but not the DM-contrast ($DM_{pyr.\ CA1>CA2/3\text{-}DG}$: $p < 0.15$) if paired t-tests were applied. Please note that results are similar if a more conservative voxel-wise threshold is used ($T > 2.56 \approx p_{voxel\text{-}level} < 0.005$).