Homologous laminar organization of the mouse and human subiculum

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The subiculum is the major output component of the hippocampal formation and one of the major brain structures most affected by Alzheimer’s disease. Our previous work revealed a hidden laminar architecture within the mouse subiculum. However, the rotation of the hippocampal longitudinal axis across species makes it unclear how the laminar organization is represented in human subiculum. Using in situ hybridization data from the Allen Human Brain Atlas, we demonstrate that the human subiculum also contains complementary laminar gene expression patterns similar to the mouse. In addition, we provide evidence that the molecular domain boundaries in human subiculum correspond to microstructural differences observed in high resolution MRI and fiber density imaging. Finally, we show both similarities and differences in the gene expression profile of subiculum pyramidal cells within homologous lamina. Overall, we present a new 3D model of the anatomical organization of human subiculum and its evolution from the mouse.

The subiculum (SUB) is a stratified cortical region that is anatomically-positioned as the major output of the hippocampal formation. The SUB strata consist of a plexiform molecular layer, a pyramidal cell layer, and a deep polymorphic cell layer. Based on Golgi stained neuronal morphology, Lorente de Nó described an additional sublaminar organization within the pyramidal layer that he used to define SUB subfields including the prosubiculum (ProSUB). Although Lorente de Nó could discern these more subtle SUB cellular lamina across multiple mammalian species, mapping the complete laminar distribution of SUB pyramidal neurons and clearly identifying SUB subfield organization has remained challenging. In the last several decades, separate anatomical tract tracing studies in rats suggested both columnar and laminar organization characterized SUB neuron connectivity, but a comprehensive understanding as to how many lamina and columns existed along the whole longitudinal axis remained obscure until recently.

Our previous work creating the Hippocampus Gene Expression Atlas (HGEA) demonstrated that combinatorial gene expression patterns identify the hidden sublaminar organization of SUB pyramidal neurons and these gene expression patterns were highly related to specific connectivity labeling patterns. Unlike anatomical tracer patterns which typically label a topographic subpopulation defined by the size and placement of the injection site, in situ hybridization gene expression patterns reveal a complete laminar distribution across the entire longitudinal axis. Similar to the approach used by Lorente de Nó previously, the HGEA outlines five SUB subregions based on the representation of four identified gene expression lamina: dorsal and ventral parts of the dorsal subiculum (SUBdd and SUBvd, respectively), the prosubiculum (ProSUB), and the ventral subiculum (SUBv) along with its ventral tip (SUBvv). While the HGEA delineates the complete distribution of SUB subregion and lamina across the longitudinal axis in mice, it remains unclear how the new HGEA subregional and laminar organization is represented across other mammals, particularly humans. Previous translational studies have examined gene expression patterns to define hippocampal and SUB boundaries, but did not report a laminar organization within the SUB.

Many functional and anatomical studies have suggested that the hippocampus is generally homologous across mammals although the spatial position of the hippocampus within the brain has shifted across evolution. The hippocampal longitudinal axis (red axis in Fig. 1) is oriented dorsoventrally in mice, whereas in primates and...
humans this axis is rotated into the posterior–anterior direction. Based on this rotation, the mouse dorsal SUB is generally believed to be homologous to the human posterior SUB and mouse ventral SUB is homologous to anterior human SUB. Functional evidence supports this view as the mouse dorsal SUB and human posterior SUB are involved in visuospatial navigation10,17–19. In contrast, the mouse ventral SUB and human anterior SUB are related to limbic emotional processing and social behaviors12,20,21. Based on the anatomical and functional homology, we hypothesized that the laminar gene expression patterns that delineate mouse HGEA SUB subregions would demonstrate a similar relationship pattern in the corresponding parts of human SUB. Analyzing gene expression patterns from the Allen Human Brain Atlas in situ hybridization database, we identified genes with unique spatial distribution patterns that characterize relatively distinct and complementary lamina across the human posterior and anterior SUB. Particularly, the posterior human SUB contains three complementary gene expression layers whose distribution patterns strongly reflect the mouse SUBdd and ProSUB when accounting for the rotation of the longitudinal axis. Additionally, posterior SUB gene expression boundaries aligned with differences in fiber density microstructure demonstrated by ex vivo diffusion-weighted magnetic resonance imaging (DW-MRI). In the anterior SUB, we found these lamina continued within ventral parts of the SUB, but not in the dorsal parts of the anterior SUB, suggesting a difference between dorsal and ventral parts of the anterior SUB that was not previously known. Taking this new data together with our previous understanding of the mouse subiculum, we propose a new 3D model of the human subiculum and its homology to the rodent. Overall, our gene expression analysis provides a new understanding of the homology between mouse and human hippocampus that is critical to translational studies of hippocampal diseases such as Alzheimer’s disease, hippocampal sclerosis, and epilepsy.

Results
All in situ hybridization data was downloaded from the online Allen Brain Atlas image database (www.brain-map.org). Compared to the mouse database which includes both coronal and sagittal tissue sections, the current Allen Human Brain Atlas database contains only a limited set of in situ hybridization data in coronally-sectioned tissue (www.human.brain-map.org). Two separate tissue blocks containing hippocampus/amygdala were found in four subjects as part of the Neurotransmitter Study: one block of tissue includes the anterior hippocampal pole and another block of tissue at a more intermediate/posterior hippocampal level. We observed relatively consistent gene expression patterns in all 4 cases although because of variability in tissue dissection and sectioning quality, it was difficult to relate corresponding sections across subjects. Therefore, we present data from the tissue series with the best histological quality (H0351.1010, 28 year old Hispanic male with no known cognitive impairment) to demonstrate their patterns across adjacent rostrocaudal sections (tissue index (TI) identifies adjacent series section number). We will first describe observed gene expression patterns in the human posterior SUB followed
by analysis of anterior SUB gene expression patterns. In addition, we provide evidence from ex vivo MRI imaging data that gene expression boundary delineations correspond to differences in human imaging microstructure. Finally, we provide a comparison of mouse and human gene expression within homologous SUB lamina.

**Gene expression patterns in the posterior human SUB.** Within posterior hippocampal tissue sections, in situ hybridization gene expression patterns reveal the laminar organization of SUB pyramidal neurons. Notably, neuron expressing Nts, Chrm2, and Htr2a are broadly distributed as three complementary gene expression patterns across the CA1, ProSUB, and SUB (Fig. 2). Nts is robustly expressed within a layer of superficial SUB pyramidal neurons (adjacent to the molecular layer), whereas Chrm2-expressing pyramidal neurons are distributed as a deep thin layer directly adjacent to the alveus white matter tract. The deep Chrm2 gene expression layer is thicker laterally where Chrm2 expression also continues into the presubiculum (PRE), but gradually becomes thinner as it extends medially into the ProSUB region. In contrast to the SUB, the superficial ProSUB neurons near the molecular layer robustly express Htr2a rather than Nts. Htr2a expression is continuous within CA1 and ProSUB, but ends near the SUB border. Together, Nts, Chrm2, and Htr2a expression patterns identify three separate gene expression domains across the human SUB and ProSUB with relatively distinct boundaries. The SUB and ProSUB can be distinguished by Nts vs. Htr2a expression although each region contains a common deep layer of Chrm2-expressing neurons.

Along the longitudinal axis, the size and shape of the three SUB/ProSUB gene expression domains and their boundaries shift as the cytoarchitecture of the SUB and ProSUB changes (Fig. 3). However, the complementary arrangement of the gene expression domains to each other, as well as their relative position adjacent to the alveus and molecular layer, remains consistent. From the available consecutive tissue series covering 10 mm of the hippocampal longitudinal axis, the data suggests that the relatively segregated gene expression layers can be considered as continuous laminar sheets extending rostrocaudally across the whole SUB. Additionally, gene expression for Th and Pcp4 appear to mirror the distribution patterns of Nts and Chrm2, respectively (Fig. 3). Although it is unclear if these genes are expressed within the same individual cell types or share a similar spatial distribution, Th and Pcp4 gene expression demonstrates that the SUB molecular domains represent differences in multiple combinatorial gene expression patterns.

**Gene expression patterns in the anterior human SUB.** Compared to the posterior hippocampus, the anterior hippocampus is structurally more complex including cytoarchitectural ridges and folding. At the anterior pole of the hippocampus, the transverse axis folds around the dentate gyrus and ultimately ends near the amygdala, an area commonly referred to as the hippocampal amygdala transition area (HATA). In coronal sections of the anterior hippocampus, the CA1/SUB is present on both dorsal and ventral sides of the hippocampal sulcus. In the ventral part of anterior SUB (Fig. 4), in situ hybridization reveals that Nts-, Htr2a-, and Chrm2-expressing neurons are arranged in similar gene expression patterns to the posterior SUB levels, suggesting the ventral anterior SUB neurons are rostrocaudally continuous with neurons observed in the posterior SUB sections (Fig. 3). In contrast, the dorsal part of anterior SUB demonstrates a distinct combination of gene expression and cytoarchitecture. Examining the Nissl cytoarchitecture of this region reveals a distinct intermediate layer of densely packed and darkly stained pyramidal neurons that suggests a trilaminar organization (Fig. 4). Chrm2-expressing neurons are distributed in the deep layer adjacent to the white matter whereas Htr2a-expressing neurons form a complementary gene expression pattern and are located within both the intermediate and superficial layer (Nts expression is mostly absent).

Examining tissue sections closer to the anterior pole reveals that the dorsal and ventral parts of the SUB are continuously adjoined. The layer of Chrm2-expressing neurons in the dorsal part of anterior SUB joins with the ventral part of SUB by continuing around the medial/anterior pole of the hippocampus (Fig. 5). In addition, the Nts-expressing neuronal layer also partially extends around the anterior pole where these cells seem to be interposed between the layers of Chrm- and Htr2a-expressing neurons. At the anterior end of the SUB, Chrm2-expressing neurons are distributed as an outer ring layer with a more internal core of Nts-expressing neurons and a relative absence of Htr2a-expressing neurons. Overall, the distribution of gene expression patterns suggest that the SUB and ProSUB contain unique combinations of molecular domains composed of continuous sheets of distinct neuronal cell types spanning the entire hippocampal axis.

**Comparison of gene expression-derived histological boundaries to human MRI imaging.** To determine if the regional boundaries identified by different gene expression patterns correspond to other structural differences in connectivity microstructure, we compared our gene expression delineations to in vivo and ex vivo structural MRI and track density imaging (TDI) of hippocampal pathways (Fig. 6). At a rostrocaudal level similar to the posterior SUB described above, in vivo 7 T MRI images of human brain provide macroscopic resolution, sufficient to clearly distinguish the SUB molecular layer from the pyramidal layer, but difficult to distinguish finer details that are useful for delineating subregion boundaries (Fig. 6a,b). In comparison, ex vivo 16.4 T MRI of a dissected human hippocampal sample provides mesoscopic details. Hippocampal strata are clearly apparent, including the granule cell layer of the dentate gyrus, and the alveus can be distinguished from the angular bundle within the white matter (Fig. 6c). TDI analysis of the tissue microstructure in this sample reveals differences in the fiber pathway orientation and distribution (Fig. 6d,e) that notably align to our observed gene expression boundaries (Fig. 6f). The PRE contains dense bundles of dorsoventrally-oriented perforant path fibers that strongly contrast with more minimal fibers within the SUB. Within the boundaries of the ProSUB, thicker dorsoventrally oriented fiber bundles are apparent that contrast it from the adjacent CA1 and SUB. Finally, a mediolateral fiber bundle can be observed running along the deep ProSUB and SUB just dorsal to the alveus that appears similar to the laminar distribution of Chrm2-expressing neurons. Using the Quantitative
Figure 2. Complementary gene expression patterns in the posterior subiculum. (left) In situ hybridization staining for Htr2a, Nts, and Chrm2 in adjacent tissue sections alongside Nissl stained cytoarchitecture and corresponding atlas drawing (based on Nissl section). Htr2a is strongly expressed in the CA1 and superficial ProSUB area (outlined in orange), Nts is strongly expressed in superficial SUB cells located near the molecular layer (m; expression area outlined in red), and Chrm2 is expressed in a deep layer of cells dorsal to the alveus (alv) within the ProSUB and SUB (outlined in yellow). Tissue Index (TI) number references section number within the overall tissue series. Red and blue boxes represent zoomed in image areas of the SUB and ProSUB, respectively, shown in middle and right columns. Together, the three gene expression patterns represent disparate molecular domains as shown by the red, orange, and yellow colored regions in the atlas drawings in top row. All Nissl and in situ hybridization images downloaded from www.brain-map.org.
**Figure 3.** Gene expression domain boundaries shift across the longitudinal axis, but maintain their complementary relationship. In situ hybridization images of distribution patterns for Pcp4, Chrm2, Th, Htr2a, and Nts gene expression (outlined in yellow, red, or orange colors) at three different rostrocaudal levels spanning 8 mm of the longitudinal axis (posterior to anterior from left to right). Tissue Index (TI) numbers on each image reference section number in tissue series. Pcp4 and Th expression patterns closely mirror the distribution patterns of Chrm2 and Nts, respectively. On the top are corresponding atlas drawings demarcating each of the three laminar molecular domains (drawings based on the boundaries drawn in the Th-labeled sections). Note, at most posterior levels (TI: 99–136), the area of the ProSUB is small compared to the more anterior levels. All in situ hybridization images downloaded from www.brain-map.org.
Imaging Toolkit (QIT) software, we delineated the gene expression-based ROI across 600 μm anterior–posterior of the ex vivo MRI volume and quantified the fiber track density within each region (Fig. 6g). We found that each gene-expression defined regions of the SUB and ProSUB contained significantly different fiber density (Wilcoxon signed-ranked test (mean ± S.D.); SUB_3 = 29.2 ± 11.5, SUB_1 = 18.7 ± 6.5, SUB_4 = 38.1 ± 11.1; p<0.0001 for all comparisons). Overall, the TDI fiber analysis provides evidence that microstructural differences in human hippocampal tissue correspond to differences in genetic cell type distribution when observed with high enough resolution.

Comparative analysis of the laminar organization in mouse and human SUB. In general, our observations of human SUB and ProSUB gene expression patterns are consistent with our previous analyses of gene expression patterns in the mouse SUB and we have maintained this color scheme in the human atlas drawings (Fig. 7). The mouse HGEA defines 5 SUB subregions based on the representation and distribution of four distinct gene expression pyramidal sublayers. First, a dorsal and ventral part of dorsal subiculum (SUBdd and SUBdv) consists of a pyramidal layer with two gene expression sublayers (sublayers 1 and 4). The SUBdd most closely represents the area many studies refer to as the distal SUB, whereas ProSUB (sublayers 3 and 4) may closely correspond to proximal SUB in other studies. In contrast, the ventral subiculum (SUBv) has a trilaminar pyramidal layer (layers 2, 3, and 4). The thickness of these layers changes near the ventral tip of the subiculum (SUBvv) distinguishing this region from the SUBv. A major challenge for comparison analysis of mouse and human hippocampal gene expression data is the difference in orientation of the hippocampus between species. Because of the rotation of the longitudinal hippocampal axis across evolution, coronal mouse hippocampal sections are not in the same sectioning plane relative to the longitudinal axis as coronal human sections. Coronal

Figure 4. Complementary gene expression patterns in the anterior SUB (TI: 901–930). (middle row) In situ hybridization staining for Chrm2, Th, and Htr2a (colored outlined) in adjacent tissue sections alongside Nissl stained cytoarchitecture and corresponding atlas drawing (based on Nissl section). In anterior coronal sections, the SUB appears separated by the CA1 into a dorsal and ventral region. The ventral region of the SUB contains complementary Chrm2, Th, and Htr2a expression patterns in the SUB and ProSUB as observed in posterior hippocampal levels (zoomed in images of red boxed regions are shown in bottom row). In contrast, the dorsal region of the SUB at this anterior level contains Chrm2 and Htr2a expression, but very little Th expression (zoomed in images in blue boxed regions are shown in top row). Closer examination of the Nissl staining suggests a tri-laminar cytoarchitecture containing a distinct intermediate layer (arrow in top row Nissl image, orange domain in atlas drawing) with cells that are more darkly-stained and densely packed than the cells located deeper (yellow in atlas drawing) and more superficially (blue in atlas drawing). Based on this trilaminar organization, Chrm2-expressing cells are primarily distributed in the deep layer adjacent to the alveus (alv) whereas Htr2a-expressing cells are located in the intermediate and superficial layers near the molecular layer (m). All Nissl and in situ hybridization images downloaded from www.brain-map.org.
hippocampus sections in humans would most directly relate to horizontal sections in the mouse and vice versa. However, several key aspects of SUB organization can be understood even comparing tissue that is sectioned at different angles relative to the longitudinal axis.

In both mouse dorsal SUB and human posterior SUB, *Nts* is strongly expressed in a superficial layer of SUB pyramidal neurons closest to the molecular layer, whereas *Chrm2*-expressing neurons are primarily located in the deepest part of the SUB, forming a continuous layer adjacent to the alveus white matter tract (Fig. 7a). Note that the position of the alveus and molecular layer are switched dorsoventrally in mouse vs. human tissue. This data suggests that the superficial SUB layer in humans (containing *Nts*- and *Th*-expressing neurons) is directly homologous to SUB sublayer 1 in the mouse HGEA, whereas the *Chrm2*-expressing deep layer in humans is homologous to mouse SUB sublayer 4. Therefore, *Htr2a*-expressing human SUB neurons likely correspond to the other two mouse SUB layers 2 and 3. However, *Htr2a* expression appears almost entirely absent in the mouse CA1 and SUB and other genes that are present in both mouse and human SUB (ex. *Pcp4*) are expressed in different combinatorial patterns (Fig. 7b). Overall, annotation of gene distribution within the SUB layers of both mouse and human subiculum suggests similarities and differences in combinatorial gene expression distribution (Supplementary Table 2). Together, this data suggests that while the anatomical laminar organization is evolutionarily conserved between mice and humans, there exists both conservation and divergence of the gene expression profile for each SUB layer and their unique cell types.

Although visualizing overall structure from thin-cut tissue sections can be difficult, the relationship of mouse and human SUB is easier to visualize in 3D and we have generated a model of the human SUB based on our understanding of the changes to the mouse SUB (Fig. 8a). In addition to the rotation of the longitudinal axis, one end of the hippocampus structure (the anterior pole in human corresponding to ventral pole in mouse) has folded back against the longitudinal axis. In comparison, the mouse and human SUB laminar organization...
demonstrate a similar subregional organization across the longitudinal axis despite its rotation (Fig. 8b). The area of the posterior human SUB containing gene expression layers 1, 3, and 4 correspond remarkably well to the SUBdd and ProSUB subregions in the dorsal part of the mouse SUB. In the anterior human SUB, the ventral area containing layers 1 and 4 is homologous to the mouse SUBdv whereas the dorsal part appears homologous to the trilaminar mouse SUBv/SUBvv. Additionally, the data from the human anterior pole is similar to the organization in the caudal parts of the mouse SUB where layers 1 and 4 partly curl around the ventral tip of the dentate gyrus. Overall, the data suggests the SUB has a conserved mammalian architecture that in humans has undergone expansion and structural folding.

Discussion

Overall, this study provides many novel insights into the organization of the human SUB and its evolutionary relationship to the mouse. Previous studies have analyzed gene expression patterns to map anatomical boundaries between the human SUB and ProSUB, however these studies did not report expression patterns of Chrm2 and other genes which are present within a subset of neurons in both regions and demonstrate their laminar organization. Based on the current data, we believe that the structure of mouse and human SUB are highly conserved with respect to two major changes: (1) the rotation of the longitudinal axis and (2) the folding of the CA1/SUB at the anterior pole. The mouse and human in situ hybridization data for Nts, Chrm2, and Htr2a suggests that the anatomical laminar organization of SUB pyramidal neuron cell types is conserved between mouse and human although many similarities and differences in their gene expression profiles can be observed. Generally, we find that the posterior SUB region in humans contains two distinct complementary gene expression layers (characterized by Nts and...
**Considerations for investigations of translational mammalian neuronal cell types.** Recently, a translational gene expression study comparing mouse and human suggested a conservation of cortical cell types with divergent features across mammals. In the hippocampus, CALB1 has previously been shown to be selectively expressed in the human dentate gyrus (DG), whereas CALB1 in the mouse is expressed within the DG and CA2. These variations raise important considerations as to how to classify cell types across species: Do the CALB1-positive CA1 and CA2 neurons represent a distinctly murine neuronal cell type not found in humans or are the homologous cell types still present in the human, but simply no longer express CALB1? Although the current study focused on the SUB, we also observed several instances where the combinatorial expression of specific genes is different between mice and human across a variety of other hippocampal structures. Cross-species differences at the single-cell transcriptome level may underlie the failure to successfully translate mouse research to the clinic. Drugs that are effective in mice because they bind a receptor in specific target neurons may not work in humans because (1) either the homologous human neuron doesn’t express that receptor or (2) the receptor is expressed in additional neurons causing unexpected side effects that were not observed in the mouse experiments. Because of these potential pitfalls in cross species drug development, it is imperative to accurately define cell types across different mammalian nervous systems. The evidence presented here provides a foundation for understanding the organization of mouse and human SUB and extends the anatomical and functional homology across mouse and human SUB. We find that both mouse and human SUB pyramidal neurons contain a hidden laminar

**Conclusions.** This study establishes a new understanding of the organization of the human SUB and provides a translational perspective that corroborates and extends the anatomical and functional homology across mouse and human SUB. We find that both mouse and human SUB pyramidal neurons contain a hidden laminar...
organization of gene expression across the longitudinal axis. These findings establish a foundation for future investigations of the organization of the human hippocampus using gene expression and establishing an integrative approach with in vivo and ex vivo MRI imaging.

Methods

Allen Brain Atlas in situ hybridization data. All human and mouse in situ hybridization images used in the analysis of this study were downloaded from the Allen Brain Atlas website (www.brain-map.org). Complete detailed information about histological processing and hybridization can be found in the Allen Institute white paper (Mouse: “In situ hybridization data production”, Nov. 2011; http://help.brain-map.org/display/mousebrain/Documentation; Human: “In Situ Hybridization in the Human Brain Atlas”, Oct. 2013 v.7; http://help.brain-map.org/display/humanbrain/Documentation).

Human hippocampal in situ hybridization image datasets are part of the Neurotransmitter Study (four post-mortem subjects (H0351.1009, a 57 year old Caucasian male with hypertension; H0351.1010, 28 year old Hispanic male control; H0351.1012, 31 year Caucasian male control; H0351.1016, 55 year old Caucasian male control). All 88 probes (see Supplementary Table 1 gene list) and additional histological stains are performed in sequential tissue sections in order from anterior to posterior (Nissl stained sections occur every 25 sections). Each tissue section is hybridized to one unique probe and the procedure is repeated every 100 sections such that the rostrocaudal sequence of each gene expression tissue series is spaced 2 mm apart (100 sections x 20 μm section thickness). All image data used for analysis was openly published online and downloaded from www.brain-map.org.

Post-mortem tissue and ex vivo imaging parameters. The hippocampal specimen used for ex vivo imaging (a 55-year-old male with normal cognitive function) was acquired post-mortem from the Alzheimer's Disease Research Center (ADRC) Neuropathology Core at the Keck Medical Center of USC (Los Angeles, CA; NIA P50 AG05142). All experiments were reviewed and approved by the Institutional Review Board (IRB) of the University of Southern California (USC) and data was collected in accordance with the relevant guidelines of the USC IRB. Patient consent was obtained by the USC ADRC and de-identified prior to distribution. The specimens were immediately fixed in 10% phosphate-buffered formalin, and later dissected at the level of the lateral geniculate nucleus, including the hippocampal and parahippocampal gyri. A 1 cm extent of the hippocampal tissue was submitted for ex vivo MRI analysis.

MRI data was acquired on a 16.4 T vertical wide-bore microimaging system, running Paravision 6.0.1 (Bruker Biospin, Karlsruhe, Germany), using a micro 2.5 gradient coil (max strength 1.5 T/m) and 28 mm birdcage volume coil (M2M Imaging, Brisbane, Australia). The sample was incubated in 0.2% gadolinium diethylenetri-amino-pentaacetic acid (Gd-DTPA, Magnevist, Bayer) for 4 days, and fixed onto a plastic holder with a small volume coil (M2M Imaging, Brisbane, Australia). Track-density imaging (TDI) 40 was performed using single shell DW-MRI on the shell with b-value of 5000 s/mm², using MRtrix software (version 0.2.12; http://jdtournier.github.io/mrtrix-0.2/index.html). Voxels with FA > 0.7 were segmented and the spherical harmonic decompositions of all the resulting profiles were then averaged to estimate the response function. We then applied constrained spherical deconvolution 41 to estimate the fiber orientation distribution in each voxel using a maximum spherical harmonic of order 6. Then, 500,000 streamlines were generated using probabilistic tractography tool 23 with
the following parameters: curvature = 0.075, cutoff = 0.1, minlength = 1, length = 15, step = 0.015. TDI with voxel size of (100 mm)³ was then derived from generated streamlines. Gene expression-based domain volumes within ProSUB and SUB were manually delineated on MRI dataset from images spanning 600 μm anterior–posterior using the Quantitative Imaging Toolkit (QIT) software (SUB_1 = 379 voxels, SUB_3 = 499 voxels, SUB_4 = 276 voxels)²². The track density index is computed as the number of reconstructed streamlines in each voxel generated from the TDI technique. The average track density index of these regions was averaged across all voxels and compared using Wilcoxon signed-rank test (mean ± S.D.).

**In vivo high resolution hippocampal imaging.** A 32-year-old female was scanned using T2-weighted turbo spin echo sequences with 2 mm slice thicknesses and 340 μm (interpolated to 170 μm) in-plane resolution, 4 averages, resulting to total scan time of 13 min²⁵,⁴⁴. We used a single-channel quadrature transmit radiofrequency (RF) coil and a 32-channel receive array coil (Nova Medical Inc., MA). All methods were carried out in accordance with relevant guidelines and regulations and approved by the institutional review board of the University of Southern California. Informed consent was obtained from the volunteer, and the image was anonymized.

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**Figure 8.** A 3D translational model of mouse and human SUB laminar organization. (a) Using the 3D HGEA model of the mouse SUB (left), we developed a 3D model of the human SUB based on our observations of the gene expression-based SUB lamina and the relative position of corresponding SUB subregions. Our model suggests two changes to the SUB have occurred across evolution between the mouse and human: 1) rotation of the longitudinal axis (middle), and 2) the folding back of the anterior SUB against the long axis (right). Images on the right show the view of the human SUB model from the medial (top) or lateral perspective (bottom; see also Supplementary Movie 2 or use Schol-AR app). (b) Coronal atlas section series from the mouse HGEA with the 4 colored gene expression layers and subregions (left) with similarly corresponding coronal human atlas drawings with similarly colored gene expression layers and subregions (right). Data viewable with Schol-AR augmented reality app, for details visit https://www.ini.usc.edu/scholar/download.html.
Human SUB structural modeling and 3D rendering. The human SUB 3D model was created by morphing the anatomical positions of the previously generated HGEA mouse SUB 3D model to align with the distribution of gene expression lamina observed in the coronal in situ hybridization tissue section dataset. Using Autodesk 3ds Max animation software, vertex positions were assigned to the mouse SUB model and the longitudinal axis of the model was rotated to the orientation to correspond to the position of a reference hippocampal MRI volume and the anterior hippocampal pole was folded back to the approximate rostrocaudal level where dorsal sections of the SUB are present in the human hippocampus sections. Finally, the Morpher modifier, which is able to morph a model between several different shapes with corresponding vertices and orientation, was used to create an animated morph of the model transition state from the mouse shape and orientation to the human shape and orientation (Supplementary Movie 2).

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Author contributions
M.S.B. designed the study, performed gene expression analysis of in situ hybridization image data, wrote the manuscript text, and prepared figures. F.S. wrote manuscript text and prepared figures for MRI data. F.S. and N.D.K designed and optimized the ex vivo MRI sequence. N.D.K performed ex vivo MRI. F.S. designed and acquired in vivo MRI and analyzed all MRI data. C.A.M. acquired post-mortem human tissue samples and K.C. and C.A.M. supervised and managed the ex vivo MRI study. M.S.B., J.S., L.K., and N.K designed and created videos of the mouse and human hippocampus and subiculum. H.H. edited the manuscript and provided feedback on figure and manuscript organization. H.W.D. supervised and managed the gene expression analysis and edited the manuscript text. All authors reviewed the manuscript.

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Competing interests
The authors declare no competing interests.

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