Data-driven fine-grained region discovery in the mouse brain with transformers

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Abstract

Technologies such as spatial transcriptomics offer unique opportunities to define the spatial organization of the mouse brain. We developed an unsupervised training scheme and novel transformer-based deep learning architecture to detect spatial domains in mouse whole-brain spatial transcriptomics data. Our model learns local representations of molecular and cellular statistical patterns. These local representations can be clustered to identify spatial domains within the brain from coarse to fine-grained. Discovered domains are spatially regular, even with several hundreds of spatial clusters. They are also consistent with existing anatomical ontologies such as the Allen Mouse Brain Common Coordinate Framework version 3 (CCFv3[1]) and can be visually interpreted at the cell type or transcript level. We demonstrate our method can be used to identify previously uncatalogued subregions, such as in the midbrain, where we uncover gradients of inhibitory neuron complexity and abundance. We apply our method to a separate multi-animal whole-brain spatial transcriptomic dataset and observe that inclusion of both sagittal and coronal tissue slices in region identification improves correspondence of spatial domains to CCF.

Introduction

Hierarchical spatial organization is ubiquitous in tissue and organ biology. Systematic, high-dimensional phenotypic measurements of this organization, generated through experimental tools such as spatial transcriptomics, multiplex immunofluorescence, and electron microscopy, are becoming increasingly available as large, open datasets[2–4]. However, transforming this abundance of data into information and hypotheses can be difficult, even for fields with a wealth of prior knowledge, such as neuroanatomy.

The creation of open data resources such as the Allen Brain Cell Mouse Whole Brain (ABC-MWB) Atlas[2], a multi-million cell spatial and scRNA-seq atlas, provides a new opportunity to investigate whether computational tools can help biologists understand spatial cellular and molecular organization. However, the size of these datasets presents computational challenges for existing methods, many of which do not extend readily to a multi-section or multi-animal scenario. One reason for this is because existing methods for spatial niche or spatial domain detection often represent the entire dataset at once, for example an entire cell by gene matrix for a tissue section to be held in (GPU) memory or require computation of a prohibitively large pairwise distance matrix[5]. A significant class of methods

relies on Gaussian processes, which scale poorly with the number of observations[6]. Our method, CellTransformer, addresses this concern by focusing on a spatially constrained neighborhood representation of cells in space. We hypothesized this would be sufficient to recover important anatomically relevant low-dimensional structures in the data while remaining computationally efficient.

CellTransformer is a transformer[7], an established architectural tool in deep representation learning, which we use to compute a latent variable representation of cellular neighborhoods and their molecular features. In order to train this network without predefined region labels, we introduce a novel self-supervised training objective. During training time, the network is optimized to predict masked data for cells in the same neighborhood using the neighborhood representation, which is a compressed vector representation of the molecular and cellular characteristics of that cellular neighborhood. We interpret this objective function as quantifying the extent of spatial correlation between a cell and its cellular neighborhood. We evaluate CellTransformer on the ABC-MWB dataset, demonstrating its effectiveness in producing completely data-driven spatial domains of the mouse brain. We demonstrate that at coarse scale (small number of spatial clusters) CellTransformer regions are similar to CCFv3 in terms of cell type composition; at fine scale, we uncover previously uncharacterized subregions with unique cell type and gene expression patterns. CellTransformer is scalable to spatial transcriptomics datasets with millions of cells and also allows for integration of regions across animals.

Results

Data-driven discovery of fine-grained spatial domains in the mouse brain spatial transcriptomics data using CellTransformer

CellTransformer is a graph transformer[8] neural network that models spatial cellular neighborhoods, which we define for a given cell as any cells within a user-specified distance cutoff in microns. Our model takes the gene expression (here, MERFISH probe counts) and imputed cell type identity from the ABC-MWB scRNA-seq taxonomy for each cell and outputs a latent variable representation for each of these neighborhoods across the brain. One of the principal operations in a transformer is the self-attention operation, which computes a feature update based on pairwise interactions between elements in a sequence, which are referred to as tokens (here, cells). Accordingly, one interpretation of our model is of learning an arbitrary and dynamic pairwise interaction graph among cells. Restricting this graph to a small subgraph of the whole-tissue-section graph has benefits for both computational resource usage and biological interpretability. We interpret the size of the neighborhood as a restraint on the physical distance at which statistical correlations between the observed cells and their gene expression profiles can be directly captured. A secondary aspect is that truncating neighborhoods using a fixed spatial threshold allows the network to account for the differential density of cells in space. In this way, our framework incorporates a notion of both cytoarchitecture (relative density and positioning) and molecular variation (cell type and transcript-level variation) in the data.

We designed a self-supervised training scheme to induce our graph transformer to learn biologically relevant latent features in the data without spatial region labels. In this scheme, the model learns to compute latent variables of cellular neighborhoods such that they are useful to predict characteristics (observed MERFISH probe counts) of masked cells in the neighborhood. We define a neighborhood as a given cell (which we will call a reference cell) and its spatial neighbors. We train the model to use only information about the cell's neighbors to predict the gene expression profile of the reference cell (**Figure 1a**). Specifically, we first compute latent features for every cell in the neighborhood without the reference cell. We then aggregate these features using attention pooling (see **Methods**),

compressing features for cells across the network into a single vector latent variable representation. This vector is then concatenated to a token which represents the cell type of the reference cell and is used to compute a final prediction of the observed gene expression counts of the reference cell (**Figure 1b**). At test time, we extract the neighborhood representation for each cell and use *k*-means to identify discrete spatial domains.

The ABC-MWB spatial transcriptomics dataset is divided into two parts. Tissue from five animals was collected, with one animal processed by the Allen Institute for Brain Science with a 500 gene MERFISH panel and the others generated by the Zhuang lab (Zhang et al. (2023)[9]) with a 1149 gene panel. We initially trained CellTransformer on the Allen Institute for Brain Science mouse in the ABC-MWB dataset using cell type labels at the subclass level of the ABC-MWB cell type ontology, subsequently extracting embeddings for each neighborhood in the dataset and clustering using *k*-means. We found a high spatial consistency of domains across tissue sections and visual correspondence with CCFv3[1] (**Figure 2a**) using the preliminary registration available in the October 2023 data release. We provide representative images of spatial clusters across the brain at different *k* in **Supplementary Figures 1-3**. We qualitatively observed that increasing the number of spatial domains *k* yields subregions of higher-level regions in a neuroanatomically consistent manner. For example, at *k*=50, domains in the cortex resemble gross layers in putative anterior cingulate, motor cortex, and somatosensory domains, shown in different shades of green in the CCFv3 reference (**Figure 2a**). At *k*=100, areas such as the striatum are subdivided into the nucleus accumbens and caudoputamen, which is segmented along the dorsal-medial and medial-lateral axes. We also observe the subdivision of the major cortical layers into both superficial and deep partitions (**Figure 2b**), with different subregions appearing in motor (MO) and sensory cortices (SS). Anterior and posterior subdomains of the lamina of MO and SS also appear (sections 47 and 48), whereas the cingulate (boxed in section 47 and 48, **Figure 2b**) is not subdivided anterior to posterior. At *k*=1000, we observe subregions in areas such as pallidum, where substructures such as the diagonal band nucleus become visible. The caudoputamen also becomes segmented along the dorsal-ventral axis, which is shown more extensively in **Figure 2b**. We also observe anterior-posterior divisions within domains, for example in layers 1-3 of anterior cingulate, which are grouped into one anterior segment (sections 50 and 49) and a posterior segment (sections 48 and 47). We also observe fine-grained domains in the subcortex, such as the Islands of Calleja (**Supplementary Figure 4a**) and subregions of the caudoputamen (**Supplementary Figure 4b**).

To quantify the observation of high-level similarity with CCF, we selected three levels of granularity in CCF annotations: division (25 regions), structure (354 regions), and substructure (670 regions). We also computed corresponding regions using our method and CellCharter[10,] another deep learning model, with the same number of spatial domains for comparison. For each spatial domain (from CCF or from one of the methods), we computed a cell type composition vector using ABC-MWB taxonomy and compared the domains based on this vector. The ABC-MWB taxonomy is hierarchical, and we chose the subclass level (307 types) for computational expediency. Other methods including STACI[11] and spaGCN[5] were not used for comparison because these models required loading. We calculated the similarity between CellTransformer and CellCharter region sets with CCF by correlating the cell type composition vectors of a given pipeline's region with its best match in CCF, showingCellTransformer domains are better matches (**Figure 2c**). Particularly at higher numbers of regions, CellTransformer regions are very similar to at least one CCF region. We also computed the average correlation when the when the comparison is mutually exclusive (multiple spatial domains cannot match with the same CCF region, **Figure 2d**), which does not monotonically increase, although at the *k*=670 the overall similarity of CellTransformer regions is proportionally much higher than similarity at *k*=25 than CellCharter, which does not produce an increase in similarity at higher region number. We also developed an index of spatial consistency of domains from CCF, CellTransformer, and CellCharter (**Figure 2e**) using a nearest-neighbor approach (see **Methods**), demonstrating the favorable performance of CellTransformer.

The hierarchical grouping of domains at different choices of *k* prompted us to quantify the stability versus accuracy tradeoff in our clustering problem. To investigate this, we computed 20 clustering instances with different random initializations for a large range of choices of *k* and analyzed them using two metrics (**Supplementary Figure 5**). The first, inertia, is an overall goodness-of-fit measurement that quantifies the sum of squares errors for the points in latent space to their nearest centroid. We also used the Amari-type distance[12], which measures the stability of clusters at a given *k,* to quantify the similarity of clusters across random initializations. We note that the stability curve does not monotonically decrease and in particular shows a peak at *k*=500 and *k*=1100, whereas the inertia score plot relatively smoothly decreases and does not vary significantly within the iterates (the standard deviation is <1300 for all values of *k* and thus difficult to see). The observation that in some ranges of *k* spatial clusters can be considered interchangeable (and thus unstable) with respect to the *k*-means objective suggests the spatial autocorrelation of cellular neighborhoods is not uniform across the brain. Using these two metrics we further characterized spatial domains found at k=400 and *k*=1400.

Mapping of spatial domains in the hippocampal formation

We first tried to qualitatively determine the correspondence of CellTransformer spatial domains with known anatomical structure in the hippocampal formation, notably the subiculum (SUB) and prosubiculum (PS), which were investigated in Ding et al. (2020)[13]. We focused on this area because it is well characterized with respect to both connectivity[14] and transcriptomic composition[15]. Specifically, recent papers have noted the extensive laminar organization (superficial layers to deeper layers), which groups the components of the hippocampal formation into dorsal and ventral subcomponents. This organization has been attributed to distinct patterns of gene expression and connectivity. We qualitatively compare spatial domains discovered by CellTransformer with *k*=400 and *k*=1400 to the anatomical borders identified in Ding et al. (**Figure 3a-d**).

We contrast the domains found at the two levels of coarseness and observe general agreement in mapping the high-level structures (ventral PS, SUB are present and congruent). At low cluster number, we observe segmentation primarily along the proximal-distal axis, which further segments along the superficial-deep axis at higher cluster number. For example, at *k*=400, we observe a coarse partitioning of SUB and PS into multiple components along a proximal-distal axis, with the pyramidal (red) and polymorphic (orange) layers of PSd divided. Correspondingly, at *k*=1400, we observe a spatial partitioning into superficial and deep layers of PS and SD, particularly PS, where the pyramidal and polymophic layers emerge (red and brown labels in section 31). These results are similar to those identified in Cembrowski et al. (2018)[15], where transcriptomically defined subclasses were associated with specific adjacent domains and could be associated with specific downstream projection patterns.

By visualizing gene expression in the MERFISH panel within these cells (**Figure 3e**) we identify several genes which vary along either the superficial-deep or core-periphery directions. We observe that similarly to Cembrowski et al. (2018)[15], most genes traverse the long axis of SUB and PS. We performed a putative annotation of similar structures, which we visualize in **Figure 3f**. Using hierarchical clustering on the MERFISH cell by gene matrix for cells in these regions, we see that the regions cluster according to spatial proximity, with the two most dorsal regions (po/py-Sd [both polymorphic and pyramidal layers of dorsal subiculum] and polymorphic PS) clustering together and the ventral regions also clustering together.

The identification of spatial domains which varied in the proximal-distal and superficial-deep directions and featured similar types of gene expression gradients as existing literature suggests that CellTransformer embeddings were effective in learning useful neighborhood features. Encouraged by this result, we continued our analysis in the superior colliculus.

CellTransformer allows for quantification of laminar and columnar organization in superior colliculus

Recent studies using systematic mapping of cortico-tectal fibers in superior colliculus (SC) have identified distinct laminar and columnar structure[16], suggestive of the complex role SC plays in integration of sensory information and coordination of signals. Therefore, SC presented an excellent opportunity to identify transcriptomic and cellular correlates of connectomic variation. We observed a strong correspondence of three of our spatial clusters (*k*=1400) with three layers of superior colliculus, particularly the zonal, superficial gray, and optic layers (**Figure 4a**) across a set of tissue sections spanning ~600um from anterior to posterior (**Supplementary Figure 6a**). We also observed high spatial contiguity in areas such as the superficial layers of the dorsal and ventral retrosplenial area. We present a map of CCF regions registered to the section coordinates for reference. By visualizing the cell type composition within the top-ten most abundant types for these three spatial domains (**Supplementary Figure 6b**), we were able to identify cell types that were highly selective for our data-driven SC layers, such as, types 0879 SCsg Pde5a Glut_1, 0865 SCs Pax7 Nfia Gaba_3, and 0882 SCop Sln Glut. By counting the number of unique supertype level cell types in these regions (**Supplementary Figure 6c**), we observe a gradient of inhibitory neuron complexity, where our optic layer had the most inhibitory neuron types, and the zonal layer had the least. We also found major differences in excitatory-inhibitory composition; for example, the superficial optic layer had the fewest types of inhibitory neurons, but inhibitory neurons were the largest proportion of cells in this region.

Encouraged by these findings, we also identified subregions of the intermediate gray and intermediate white areas of the motor-related areas in superior colliculus (**Figure 4a**), where we identify regions which are not annotated in CCF. We identified selective cell types, including 0886 SCs Lef1 Gli3 Gaba_2 (enriched in the light blue region), 0678 SCig Foxb1 Glut_3 (enriched in the gray region), and 0679 SCig Foxb1 Glut_4 (enriched in the green region) by visualizing the top ten most abundant types at the supertype cell type level (**Figure 4c**). We note that particularly for the subregions of superior colliculus motor-related areas, differences between spatial domains may be attributed to differences in the composition of rare cell types (**Fig 4d**) instead of majority cell types. There were also major differences in the proportion of glial cell types such as 1027 MOL NN. We observed complex cell type abundance gradients by visualizing the supertype cell type composition and relative proportions of different neurotransmitter types (**Figure 4e**). For example, regions in tissue section 31 (more posterior) had more excitatory neuron types but the same overall proportion of inhibitory types compared to regions in section 32. Additionally, the two medial subregions of the intermediate gray layer (cyan and blue) are composed of a significantly larger number of inhibitory neurons. Overall, there are more excitatory neuron types in the regions closer to the midline (blue regions and red regions versus green and gray ones). We do not observe direct correspondence between CellTransformer regions to the four-column segmentation in experiments Benavidez et al. (2021)[16], (see **Figure 4f-g** for reproduction from Benavidez et al., granted with permission). However, the gross mapping of the projection organization onto the transcriptomic and cellular organization has been observed in regions such as CA1[14]; our ability to observe correlated patterns in the connectivity and transcriptomic data is a clear step forward in describing the potential covariance between molecular properties and other properties of subcortical brain areas.

A medial-lateral gradient of inhibitory neurons in the midbrain reticular nucleus

Next, we investigated the midbrain reticular nucleus (MRN), a subcortical structure with few anatomical annotations in CCF. MRN is highly enriched for interneurons in a dense array of connections,

CellTransformer identifies three subregions of the MRN, which are not included in the existing CCF annotation (**Figure 5a**). Plotting cell type proportions across the MRN, we identify cell types which are enriched for a given region, although all regions were predominantly glial (>25% cells in the 1027 MOL NN supertype for all three regions) (**Figure 5b**). Visualizing gene expression patterns across these subregions (**Figure 5c**), we identified genes which are similarly subregion selective (**Figure 5d**) and form dorsal-ventral expression gradients. The lateral spatial domains (green and teal colors) have a higher number of types of glutamatergic neurons, whereas the median ones have a higher diversity of GABA-ergic neurons (**Figure 5e-f**). Overall, these regions are again dominated by glia (>60% for all regions). Interestingly, the direction of the glutamatergic neuron abundance gradient (a lower complexity of types closer to the midline in the MRN) is opposite that of the superior colliculus gradient (more excitatory neuron types closer to the midline). There is also a rank-order correlation between diversity of glial cell types and inhibitory cell types (closest to midline).

CellTransformer enables scaling up to multi-animal, million cell datasets

The availability of multi-animal data in ABC-MWB from the Zhuang lab led us to investigate the possibility of performing automated spatial-domain integration across animals. To investigate this, we fine-tuned our network that was trained on the Allen Institute's mouse brain using the Zhang et al. (2023)[2] MERFISH data. Note that we initialized new projection layers and output layers in our network to enable the modeling of a larger MERFISH gene panel in the Zhuang lab data. We computed embeddings for each neighborhood as in the previous analysis and performed *k*-means similarly, concatenating representations for all mice. This provided an opportunity to examine whether CellTransformer could adapt to a multi-animal case in addition to finding spatial domains across tissue sections of the same animal.

We observed a moderate concordance between spatial domains across all mice, which we demonstrated by plotting sequential tissue sections across mice (**Figure 6a**). There is also clear anatomical correspondence between regions across coronal and sagittal mice, which can be observed by comparing animals 2 and 3; for example, sections 068 in mouse 1, 034 in mouse 2, and 013 in mouse 3, where the striatum and cortex are similarly grouped. To quantify the emergence of per-animal spatial clusters, we computed several clustering solutions with differing k and quantified how many spatial domains are not found in all four mice (**Figure 6b**). We found that at higher values of *k* the number of per-mouse spatial domains increases significantly, although we also noted that in general per-mouse domains appeared for animal two. For example, at *k*=630, 78% of per-mouse domains were found in mouse 2 (18.5% for mouse 1 and 3.7% for mouse 3; note that mouse 4, which has the fewest number of cells, did not develop per-mouse clusters at the tested parameter range).

To investigate whether animal-level differences in the embeddings might be related to overall appearance of spatially noncontiguous domains, we used two regression analyses to quantify the degree of positional and animal-specific information linearly contained in the embeddings. We regressed CCF-registered (x, y, z) coordinate position on the embeddings and separately used a logistic regression to classify animal identities. We then plotted the median absolute prediction error from the (x, y, z) coordinate regression against the predicted probabilities from the logistic regression (**Figure 6c**). Overall neighborhood level prediction of donor identity was very accurate (>90% for all animals), in contrast to median absolute prediction error, where on average error was greater than 1 mm (average Pearson correlation between coordinate position predictions and actual coordinates was 0.604). Animal 4 has the smallest number of measured cells compared to the others (162,578 cells vs the next largest, animal 2, with 1,227,408 cells).

We then investigated whether pooling across animals affected the correspondence of CellTransformer domains with CCF ones in terms of cell type composition. We compared cell type composition estimates for two sets of spatial clusters: one where neighborhood embeddings are clustered per-animal and one where neighborhood embeddings are clustered across animals by concatenating representations. We found that pooling across clusters improves overall correlation with CCF compared to per-animal clustering (**Figure 6d**) and that the overall correlation is higher than in the analysis with the Allen Institute mouse (which features more cells for a single mouse but fewer cells than the total number across all Zhuang lab mice). We also found that the high spatial smoothness of domains detected in the Allen Institute data is also observed in the Zhuang lab datasets (**Supplementary Figure 7a**). To explore the effect of a multi-view (coronal versus sagittal) in the Zhuang lab data, we performed an experiment comparing clustering using a fixed *k* using only one animal's neighborhood embeddings or concatenating them. For the concatenated clustering solution, we extracted a spatial domain by cell type abundance matrix as previously described, per animal, and compared those to CCF, finding the sagittal sections from animal 3 drive increased correspondence of CellTransformer domains to CCF (**Supplementary Figure 7b**). Finally, we visualized the differences between clustering solutions with only one animal or all animals and found a qualitative improvement from pooling (**Supplementary Figure 8**).

Discussion

In this study, we present a transformer-based pipeline to combine scRNA-seq and MERFISH atlases to extract local neighborhood representations. This representation space learned by our model can be clustered to identify progressively finer-scale regions using local cellular and molecular information alone, without predefined spatial labels. We show these regions can be interpreted at the gene or cell level. Furthermore, even when we extract a high number (several hundred or more) domains, they retain high correspondence to existing brain region ontology (CCF) and are highly spatially consistent both within and across tissue sections. This capability is intrinsic to our model, and is learned despite any conditional modeling for donor or section-level covariates, indicating the robustness of learned features.

The block structure in our quantitative comparison of CellTransformer's spatial domains to CCF domains illustrates the potential biological relevance of our method, directly demonstrating Cell Transformer's ability to detect brain areas concordant with existing observations as well as detection of novel subregions. We interpret the length and width of the blocks as a measurement of structural complexity in a group of domains from either CCF or CellTransformer. For example, CellTransformer regions similar to isocortex regions are in compact blocks (e.g., upper left hand of **Figure 2f**). Subcortical areas such as the midbrain comprise more regions in CCF than our method at a low *k* (*k*=670). However, our ability to search for more fine-grained regions at higher values of *k,* where we still observe high spatial regularity, provides an excellent opportunity to understand the structural organization of these complex areas. We performed proof-of-concept analyses identifying spatially varying gene expression and cell type abundance and complexity patterns in SC and MRN, with some regions not previously delineated in CCF.

Finally, we demonstrate that our method can be effective in a multi-animal setting in uncovering spatially smooth regions with high similarity to CCF. We found that "batch-effect"-signals such as donor identity and (x, y, z) coordinates are linearly extractable from the embeddings and vary among animals, suggesting distribution shifts among animals but not a specific cause. We also demonstrate that pooling across animals dramatically improves correlation of CellTransformer regions to CCF, which may be due to the inclusion of sagittal tissue sections in animal 3.

We highlight several advantages of our architecture and approach. Although the use of graph-structured architecture or self-attention to model cells in a neighborhood graph is not novel[17–19], we

contribute a novel self-supervised training objective that can be interpreted by the extent of spatial correlation between a cell and its cellular neighborhood. CellTransformer is also relatively computationally efficient. We incorporate a packed sequence format (see **Methods**) to reduce memory requirements, with total GPU memory usage <24 GB during training. In comparison, we could not run methods such as SpaGCN and STACI due to GPU memory requirements. The intersection of graph neural networks, transformers, and representation learning research is a rich and rapidly moving research area. Potential methodological improvements include incorporation of physical distances and coordinates (for example, via Laplacian positional encoding[20]), Perceiver-based accommodation of variable numbers of input genes[21,22], or rotationally-equivariant architectures[23]. Addendums for interpretability might include biasing of attention operations via prior knowledge about cell-cell interactions or molecular similarity.

There are also significant opportunities to extend CellTransformer's local representation framework to include other data modalities. Both cell-level (e.g. neurophysiology[24,25]) and pixel-level data (e.g. mesoscale axonal connectivity[11], and magnetic resonance imaging[26]) can be tokenized and included in our framework.

Caveats include the necessity of a user-specified spatial radius (for neighborhood computation) and choice of *k* for spatial cluster detection. The stability of detected spatial domains at a given radius or *k* poses an interesting future angle from which to study anatomical hierarchies in the brain, although we qualitatively found that a smaller radius detracted from visual quality of spatial clusters (**Supplementary Figure 9**). Users must also have access to GPUs (to allow for timely model fitting), which reduces overall accessibility. We include a small discussion of observed failure modes (see **Supplementary Note 2**), often manifesting as left-right asymmetry and high-frequency, non-uniform spatial clusters. Most often these can be isolated to a specific tissue section.

CellTransformer advances the state of the art for automated domain detection in the mouse brain, which could potentially inform a diverse set of fields. As spatially resolved transcriptomic and multi-omic studies of the brain become more prevalent[4,27,28], tools such as CellTransformer provide avenues to scalably integrate these data into iteratively refined anatomical maps of the brain and other complex organs.

Methods

Allen Brain Cell Mouse Whole Brain (ABC-MWB) dataset processing

We downloaded the log-transformed MERFISH probe counts and metadata from the Allen Institute public beta (https://alleninstitute.github.io/abc_atlas_access/intro.html) access for ABC-MWB for both the Allen Institute for Brain Science and Zhuang lab mice. Note that for our initial model fitting on the Allen Institute for Brain Science mouse, we used the cell type ontology from the "20230630" data release which was updated in the "20231215" data release. There are differences in the number of cell types at a given level of the ontology, for example 307 vs 338 subclasses. We transformed the (x, y) coordinates of each cell into microns (instead of mm as provided). Otherwise the dataset was used as-is for neural network training. All sections in this dataset are coronal sections.

Zhuang lab (Zhang et al.) dataset processing

For the Zhuang lab dataset mice we downloaded the "20230830" release of data from the Allen Institute ABC-MWB public data. Otherwise, the data were used as-is for neural network training. This dataset consists of four mice worth of data. Two animals (animals 1 and 2) were sectioned coronally and two animals (animals 3 and 4) were sectioned sagittally. Animals 1 and 2 consist of 2,846,909 cells and 1,227,409 cells, respectively. Animals 3 and 4 consist of 1,585,844 cells and 162,579 cells, respectively.

Cellular neighborhood construction
We consider cells in the same neighborhood as a reference cell if the distance between them is within a user-defined threshold. For the Allen Institute for Brain Science dataset we use a radius of 65 micron, while for the Zhuang lab datasets we used a larger radius of 85 micron due to inherent differences in the density of measured cells in the two datasets.

CellTransformer architecture
We construct a CellTransformer to generate a latent representation from a cellular neighborhood where this representation is composed of both molecular and cell type information. We represent cells as nodes in an undirected graph, $\mathcal{G} = \{\mathcal{V}, \mathcal{E}\}$ where $\mathcal{V}$ indexes the nodes in the graph (cells) and we add an edge (v_i, v_j) to the edge set $\mathcal{E}$ if $d_{i,j} < r$, with r a user-specified distance in microns. We assume also that for each node we have access to $\mathbf{x}_i \in \mathbf{R}^g$, a g-dimensional vector of MERFISH probe counts. We also assume we are given class labels $\mathbf{c} = \{c_i \in \{1, ..., C\}\}_{i=1}^N$ for each of the N cells. The user must also specify an embedding dimension and number of transformer encoder and decoder layers; in all experiments in this paper we use an embedding dimension of 384, 16 encoder layers, and 4 decoder layers. The meaning of these specific architectural details will be explained in the next paragraph.

To generate a neighborhood embedding, we first identify a particular cell which we call a reference cell. Its first degree neighbors are extracted from $\mathcal{G}$. We first apply a shallow encoder (two layer perceptron with GELU nonlinearity) function $f_\theta : \mathbb{R}^g \rightarrow \mathbb{R}^{192}$ which maps the gene expression into embedding space. We likewise construct and apply $g_\theta : \mathbb{R}^C \rightarrow \mathbb{R}^{192}$ to map one-hot encoded cell type labels to embedding space, here a simple lookup into a learnable embedding table. These representations are concatenated into a single 384-dimensional representation. We apply these transformations to each cell in the neighborhood, not including the reference cell. At this point, all operations have been per-cell without interactions. In addition to these cell tokens, we also instantiate for each neighborhood a learned extra token which we use to accumulate global information across the neighborhood. We refer to this token as a <cls>-like token in keeping with previous literature.

We then apply a transformer encoder to the cells, only allowing cells within the same neighborhood and their <cls>-like token. We use 8 attention heads with GELU activations and layer norm prior to attention and perceptron residual addition. We also use a bias for all layers. Following the transformer encoder, we use attention pooling to aggregate the cell and <cls> representations for each neighborhood into a single token with embedding dimension 384. We will call these the neighborhood representations.

At this point, we instantiate a new token from each reference cell that is a learned embedding for each cell type (separately from the encoder cell type embedding). These are concatenated to the neighborhood representations. We then apply a transformer decoder to the tokens. This decoder also has embedding dimension 384 (although this is not a requirement) and has 8 attention heads and bias terms. Only the reference cell token and its corresponding neighborhood representation are allowed to attend to each other.

During training, we extract only the reference cell tokens. We then use separate linear projections to output the mean, dispersion, scale, and zero inflation logit parameters for zero-inflated negative binomial regression. We optimize the model by minimizing the negative binomial log likelihood using an 80% train split size (random splitting across the entire dataset) and the ADAM optimizer over 40 epochs. We perform a linear warmup for 500 steps to a peak learning rate of 0.001 and use an inverse-square root learning rate scheduler to decay the learning rate continuously. We use a weight decay value of 0.00005 which we do not warm up. We trained all versions of CellTransformer on a system with 2 NVIDIA A6000 GPUs.

Spatial domain detection
Once trained, we apply CellTransformer to a given dataset and instead of extracting reference cell tokens we extract the neighborhood representation. We then cluster this representation using *k*-means. We use the cuml library to perform this operation on GPU.

Optional smoothing of embeddings
We observe spatial domains are spatially smooth. However in the case that there is a high-frequency signal that the end-user would like to filter, we optionally introduce a step prior to *k*-means where we smooth the embeddings using a Gaussian filter. For all comparisons in **Figure 1** and **2**, no smoothing was performed; also all diagrams displayed in **Supplementary Figures 1-3** are not smoothed. However, we applied a Gaussian filter with 20 micron full-width at half maxima, which seemed to improve coherence of spatial clusters.

Fine-tuning on Zhuang lab datasets
We perform full-finetuning. Because the MERFISH gene panel for the Zhuang lab data was larger than for the Allen Institute for Brain Science data, we initialized new input linear layers as well as output layers for the zero-inflated negative binomial parameters. We also initialized new cell type embedding layers, because in the period we conducted this study the ABC-MWB ontology was updated and our initial model fitting was on the older version; thus there would be small differences in the cell type labels. We use the subclass level labels and otherwise use the same learning rate scheduling and other optimization parameters as training on the Allen Institute for Brain Science data. We fine-tune for 35 epochs.

Regional matching with CCF computation
To quantify overall similarity of regions extracted using CellTransformer with CCF, we first extract cell type composition vectors for each region at a given level of the hierarchy. For all comparisons in **Figure 2**, we use the subclass level (307 cell types), resulting in *k*-region by 307 matrices. We then compute for a region derived from either CellCharter or CellTransformer the best match (maximum value of Pearson correlation, non-exclusively) with CCF (**Figure 2c**) or an exclusive match (using the Hungarian matching algorithm, also using Pearson correlation as the metric).

Nearest-neighbor smoothness computation
To quantify smoothness of the spatial domains, we use a nearest-neighbor approach. We extract approximate spatial neighbors for each cell using cuml.NearestNeighbor with 1000 neighbors, restricting neighbors to be within the same tissue section. For a given domain set, either from CCF, CellTransformer, or CellCharter, we extract the spatial domain label of the given cell and count the proportion of times that cell is observed in the 1000 neighbors. These proportions are averaged across all cells and tissues.

Linear probing experiments
We extract neighborhood representations for each of the cells in the Zhuang lab datasets. First, we regress these embeddings on the (x, y, z) coordinates. We then compute the absolute prediction error in terms of the coordinates and then report the average. We also fit a multi-class logistic regression using the mouse donor identity. For both models, we use the cuml library to accelerate computation.

Quantification of spatial contribution to gene expression
We interpret the accuracy of the gene expression predictions for a given cell as an index of correlation of an instance of a particular cell type with its surrounding neighbors. To do this, we compute a simple baseline model which predicts average gene expression (computed across the entire Allen Institute for

Brain Science mouse dataset) for each cell. We compute the average Pearson correlation for each instance of a given cell type and average across instances to obtain an average Pearson correlation. We then compute a Pearson correlation between each cell and the CellTransformer predictions, averaging similarly across instances of a given cell type. The difference between the baseline and model predictions is displayed, per cell type, and grouped across neurotransmitter types, in **Supplementary Figure 10**.

Zhuang lab dataset per-animal CCF comparison

We contrast two methods of extracting spatial domains from the Zhuang lab dataset, which consists of four mice. We first fix *k,* the number of desired spatial domains. Then we fit one *k*-means model on all of the neighborhood embeddings for all of four mice together. We also fit a *k*-means model to each of the mice separately. We then compute the similarity of these region sets using the same method used to quantify differences between CellCharter and CellTransformer by comparing the regional cell type composition vectors.

Other software

Principal software used in this work includes PyTorch[29], numpy[30], scikit-learn[31], scipy[32], scanpy[33], cuml[34], matplotlib[35], and seaborn[36].

Code and data availability

Code will be publicly available at https://github.com/abbasilab/celltransformer. All data used for this publication is available either at the Allen Institute ABC-MWB data portal (https://portal.brain-map.org/atlases-and-data/bkp/abc-atlas) or the CZI cellxgene portal[37].

Acknowledgments

The authors would like to thank Lydia Ng and Michael Kunst for their help with early access to the ABC-MWB dataset and experimental and informatics context. We also acknowledge Forrest Collman for help with Neuroglancer data visualization of spatial domains. We also acknowledge Patrick Xian for helpful discussions. AJL acknowledges Chang Kim, Parker Grosjean, Lee Rao, and Tom Nowakowski for feedback on neuroscientific and machine learning contexts of paper. RA, BT, AJL, and HZ would like to acknowledge support from the Weill Neurohub through the Weill Neurohub's Next Great Ideas Award. RA would like to acknowledge support from the National Institute of Mental Health of the National Institutes of Health under award number RF1MH128672 and Sandler Program for Breakthrough Biomedical Research, which is partially funded by the Sandler Foundation. AJL acknowledges support from the UCSF Discovery Fellowship and the ARCS Fellowship.

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Supplementary Notes

Supplementary Note 1: interpreting the CellTransformer objective as a measure of spatial dependence

One interpretation (see Methods and Supplementary Note 1 for further details) of the CellTransformer architecture is of learning two representations of cellular gene expression. The first is conditional on the spatial neighborhood information learned in the encoder portion of the network. The other is unconditional on the spatial neighborhood information and can be interpreted as a learned embedding for each cell type. We compare the increase in gene expression prediction accuracy from using the conditional spatial neighborhood representation to the unconditional one and interpret this value as an index of spatial dependence. A trivial or poorly fit optimization solution would produce a small value of this index, and so we hypothesized CellTransformer would achieve reasonable improvements in expression prediction, but also cell type-to-type differences.

Specifically, we use the change in Pearson correlation from using our model as a predictor and a trivial predictor of the per-cell type average gene expression. We observe increases in the predictive accuracy (mean 0.10 +/- 0.0701 in correlation). Moreover, there are few cell types for which there is a decrease in predictive accuracy, indicating that our model has nontrivially learned the objective (**Supplementary Figure 10a**). This accuracy has only a mild correlation with cellular density (**Supplementary Figure 10b**). Note that we compute accuracies at the subclass level (338 types). Increase in accuracy does correlate strongly with log-number of observations per cell type, (Pearson correlation of 0.71, **Supplementary Figure 10c**), possibly indicating inefficiencies in pretraining.

Supplementary Note 2: visual failure mode characterization in Allen Institute for Brain Science Mouse

We characterize two major failure modes in the Allen Institute for Brain Science mouse data.

On sections 54 and 51 there is consistently a tear artifact across the section (**Supplementary Figure 11a**, brown cluster in striatum) which does not appear in sequential sections, although some structures are found (for example, yellow sections of cortex at k=100) despite presence of this artifact. At higher k the tearing artifacts appear to subdivide into different groups.

On section 39 (**Supplementary Figure 11b**) there is a similar tearing artifact around the edges of the section, where tissue loss has occurred. Also, on section 40 we repeatedly observed across choices of k appearance of asymmetric thalamic and cortical regions (although not all are asymmetric) and we note that in other sections there can be mild symmetry breaking. Tissue sectioning variability across the midline (left and right sides appear mildly offset in anterior-posterior axis when viewing the CCF region registration) is difficult to disambiguate from errors in the algorithm.

Figures

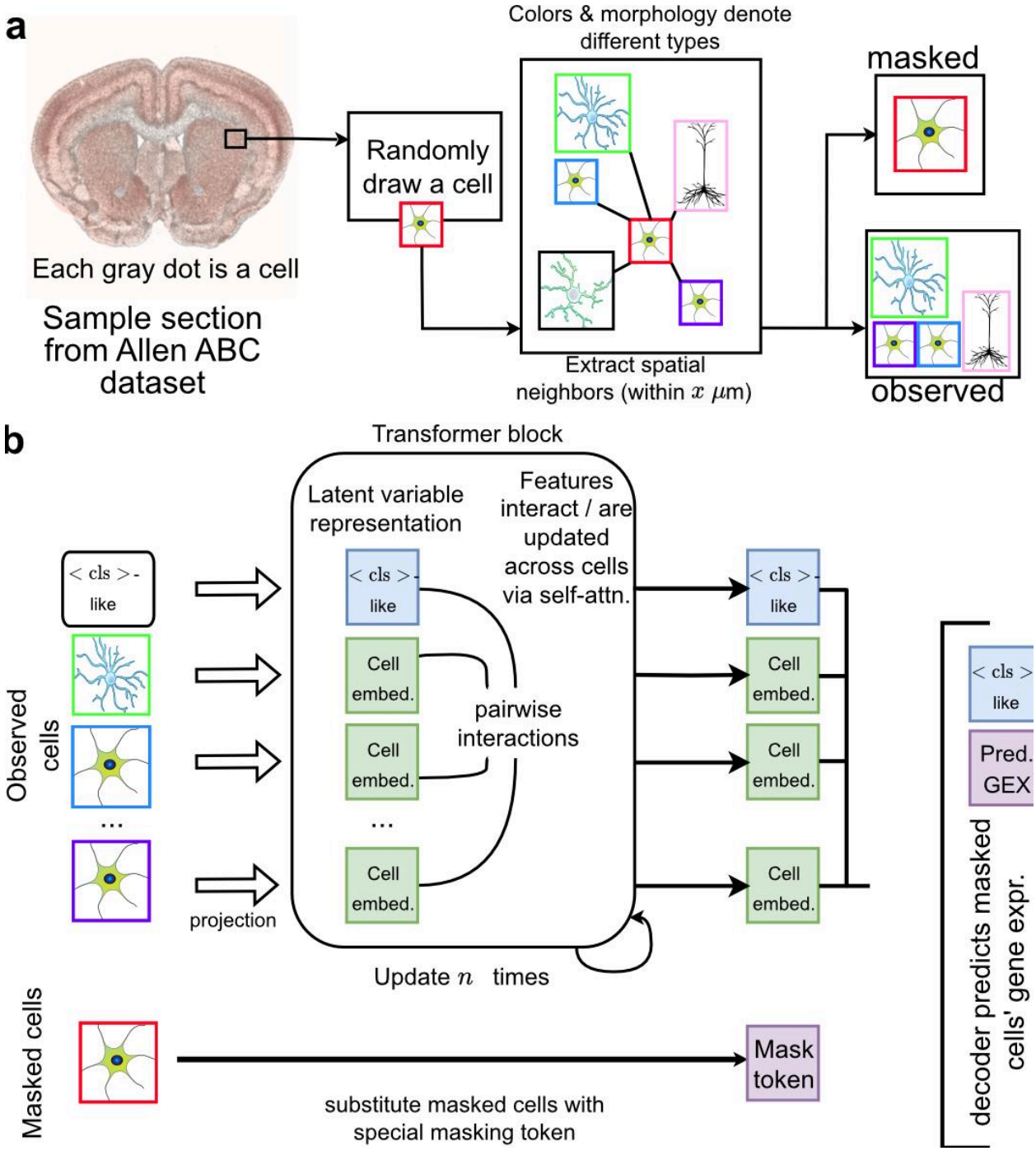


Figure 1. Overall training and architectural scheme for CellTransformer. (**a.**) During training, a single cell is drawn (we denote this the reference cell, boxed in red). We extract the reference cell's spatial neighbors and partition the group into a masked reference cell and its observed spatial neighbors. (**b.**) Initially, the model encoder receives information about each cell and projects those features to *d*-dimensional latent variable space. Features interact across cells (tokens) through the self-attention mechanism, which is repeated *n* times. These per-cell representations and an extra token are then aggregated into a single vector representation, which we refer to as the neighborhood representation. This representation is concatenated to a mask token which is cell type specific and chosen to

represent the type of the reference cell. A shallow transformer decoder (dotted lines) further refines these representations and then a linear projection is used to output parameters of a negative binomial distribution modeling of the MERFISH probe counts for the reference cell.

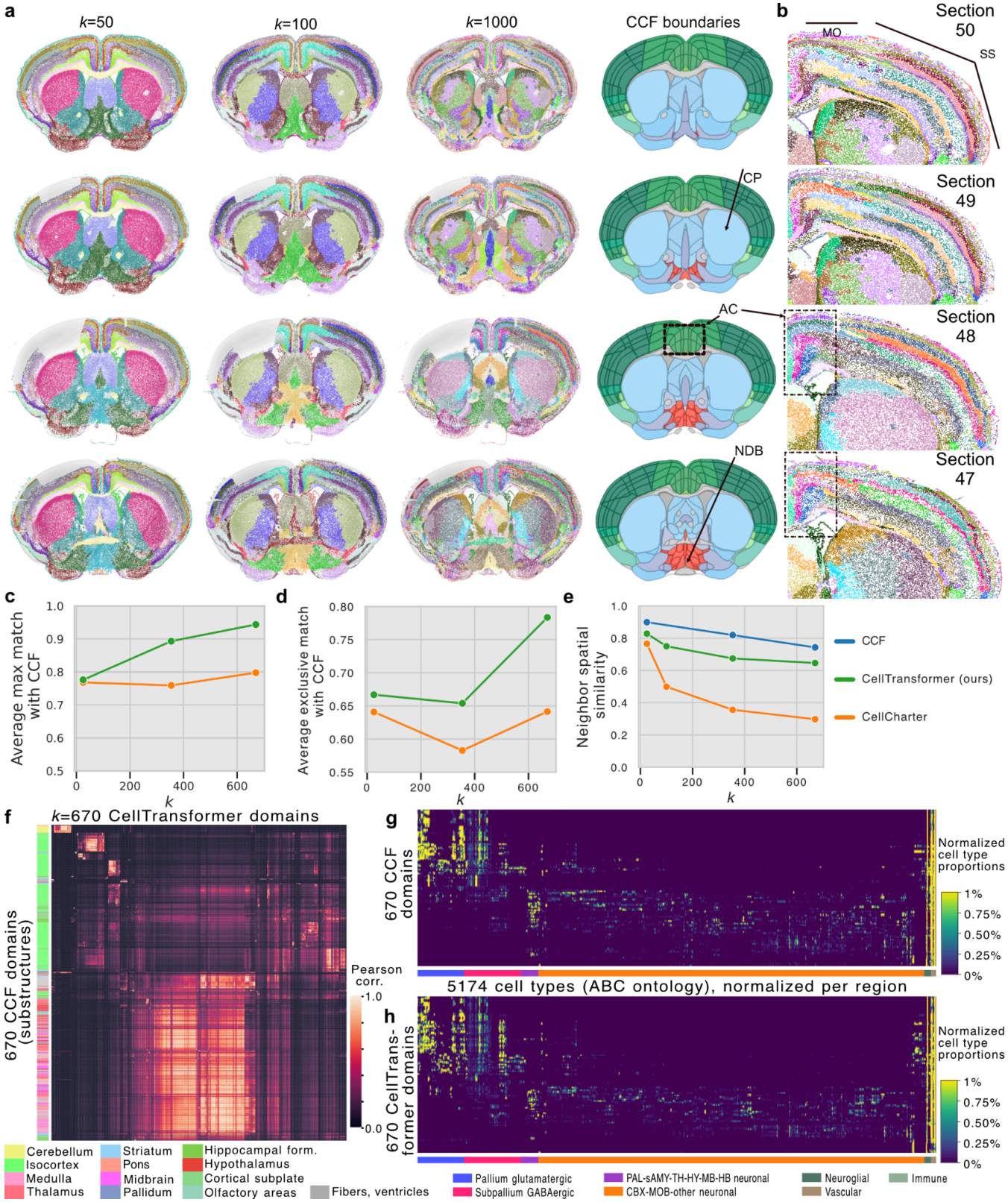


Figure 2. Representative images of spatial domains discovered using CellTransformer and comparison to CCF. (**a**.) Four sequential tissue sections (note that the inter-section distance is roughly 200 μm) from anterior to posterior. In the first three columns, each dot is a cellular neighborhood colored by the cluster label when *k*-means is conducted

with k = 50, 100, and 1000. Spatial domain labels are depicted with the same colors across sections. Fourth column shows CCF region registration to the same tissue section. The anterior cingulate (AC) is boxed in the third row. We also point out caudoputamen (CP) and the diagonal band nucleus of the pallidum (NDB) (**b.**) Single hemisphere images of same tissue sections in **a.** at k = 1000, zoomed in on cortical layers of MO and SS. (**c.**) Comparison of cell type composition of automated CellTransformer and CellCharter[10] domains with CCF ones, computed by comparing the Pearson correlation of each CCF region's cell type composition with its best match (nonexclusively) in a set of CellCharter or CellTransformer domains. (**d.**) Same as **c.** but quantifying a maximum similarity of any given CellCharter or CellTransformer region to any CCF region, exclusively. (**e.**) Comparison of CCF, CellCharter, and CellTransformer by quantifying number of nearest neighbors with the same spatial region label. (**f.**) Region-by-region Pearson correlation matrix comparing cell type composition vectors from 670 CCF regions (at substructure level) with 670 spatial domains from CellTransformer. The CCF regions are shown on the left with their structure annotations from CCF at division level. (**g.**) Cell type (cluster level) by region matrix for 670 CCF regions at substructure level. Rows are normalized to sum to 1. (**h.**) Cell type (cluster level) by region matrix for 670 CellTransformer regions. Rows are normalized to sum to 1. Colors along x-axis in both **g.** and **h.** show high level class annotations from ABC-MWB cell type ontology. Rows of both **g.** and **h.** are grouped using clustering to produce approximately similar structure.

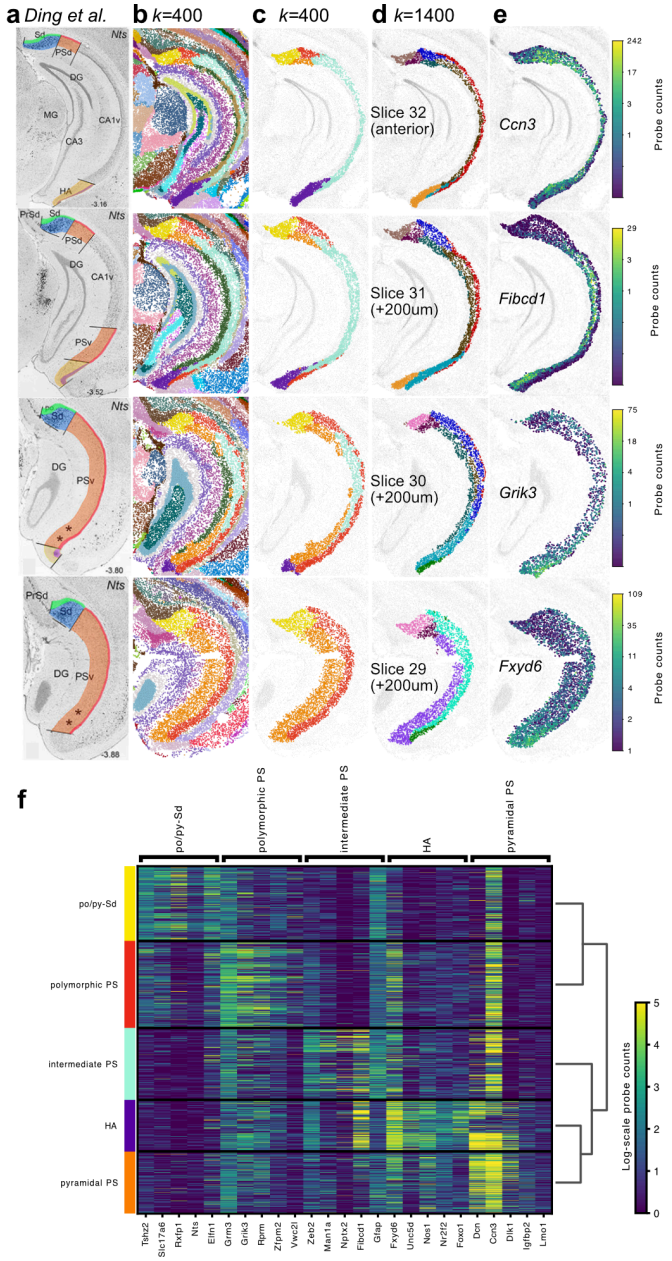


Figure 3. Comparison of CellTransformer domain sets with *k*=400, 1400 with comprehensive region set found in Ding et al. (2020). (**a.**) Representative images reproduced from Ding et al. of region boundaries in prosubiculum (PS) and subiculum (SUB). (**b.**) Images from hippocampal formation across 4 sequential tissue sections (anterior to posterior). Each dot is a cellular neighborhood colored using cluster labels with *k*=400 (across the entire brain). (**c.**) Same as **b.** but with only SD/PS shown in color. (**d.**) Same as **c.** but with spatial domain coloring come from a spatial clustering set with *k*=1400. (**e.**) Gene expression patterns visualized at the corresponding tissue section, where only cells within SD/PS are shown. Units are in raw probe counts. (**f.**) Gene expression heatmap of putative subregions, with approximate anatomical annotation (Sd: subiculum dorsal, PS: prosubiculum, HA: hippocampal amygdalar transition area). Dendrogram from hierarchical clustering in gene expression space is shown to the right.

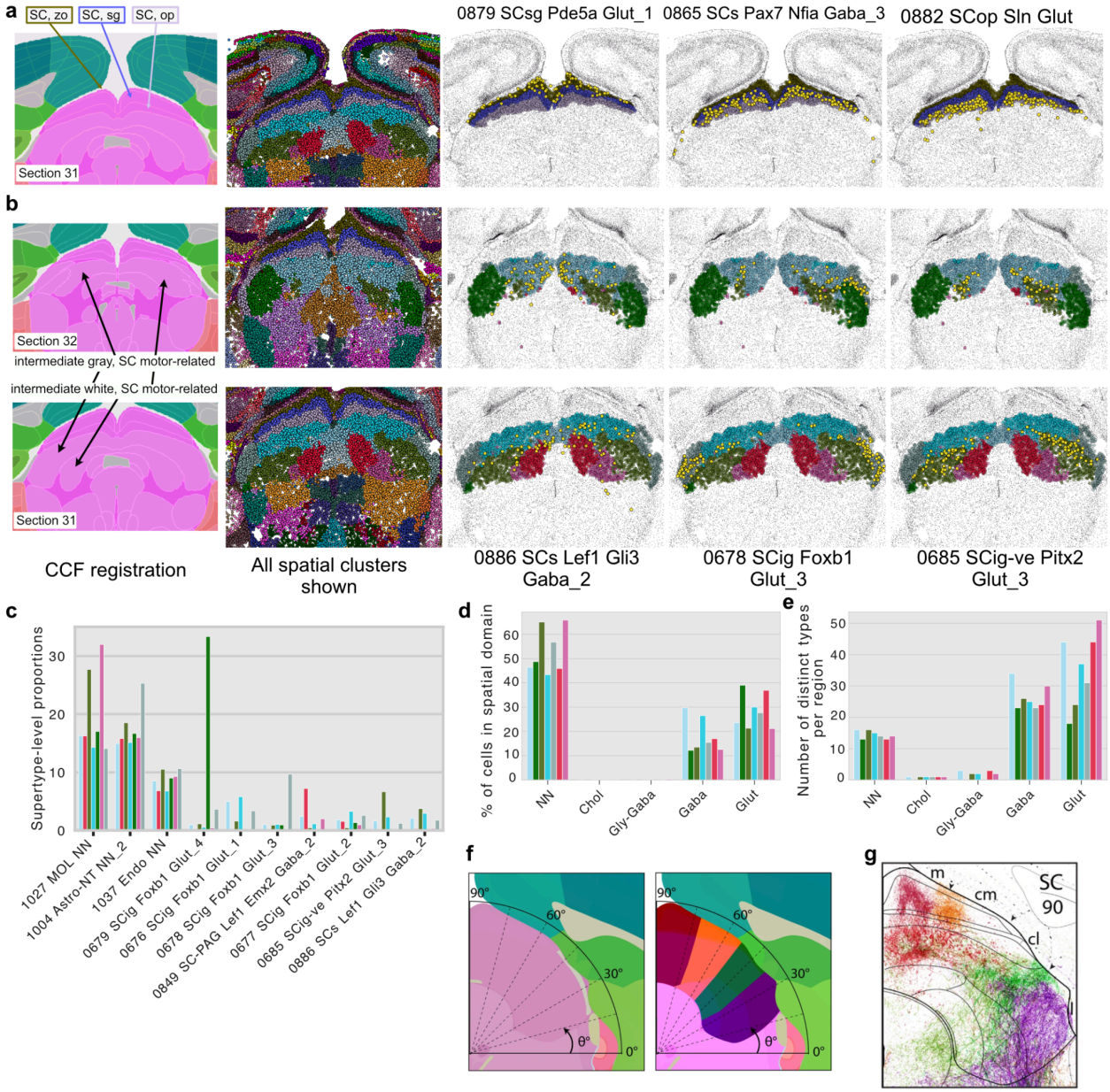


Figure 4. Examination of putative dorsal and ventral subregions of superior colliculus using CellTransformer. (**a.**) Putative subregions of sensory layers of superior colliculus in tissue section 32. CCF registration in the first column, with the zonal, superficial gray, and optic layers labeled by the color of their putative CellTransformer cluster in third, fourth, and fifth columns. The second column shows all cells in the field of view with color labels from their spatial domain from CellTransformer at *k*=1400. Third column shows only cells we identify as inside our putative zonal, superficial gray, and optic layers of superficial colliculus, with the supertype level cell type 0879 SCsg Pde5a (enriched in the light blue domain) shown in yellow. (**b.**) Sequential tissue sections (32: anterior, 31: posterior) shown similarly to (**a.**) but visualizing putative subregions of the intermediate gray and intermediate white layers, which are shown in black arrows in the CCF registered annotation image. (**c.**) Proportions of different supertype level cell types for top-ten most abundant types in different spatial domains. Colors refer to the same spatial cluster label in (a) and (b). (**d.**) Barplot of the percentage of cells for a given neurotransmitter type found in a given region. (**e.**) Number of unique cell types at supertype level in

ABC-MWB ontology found in each region. (**f.**) Figure reproduction with permission from Benavidez et al. (2021)[16], showing proposed schema of layer-specific anterograde connectivity patterns distributed across medial-lateral zones (labeled red, orange, green and purple) of superior colliculus. (**g.**) Figure reproduction from Benavidez et al. (2021) showing example anterograde connectivity patterns from regions projecting to superior colliculus.

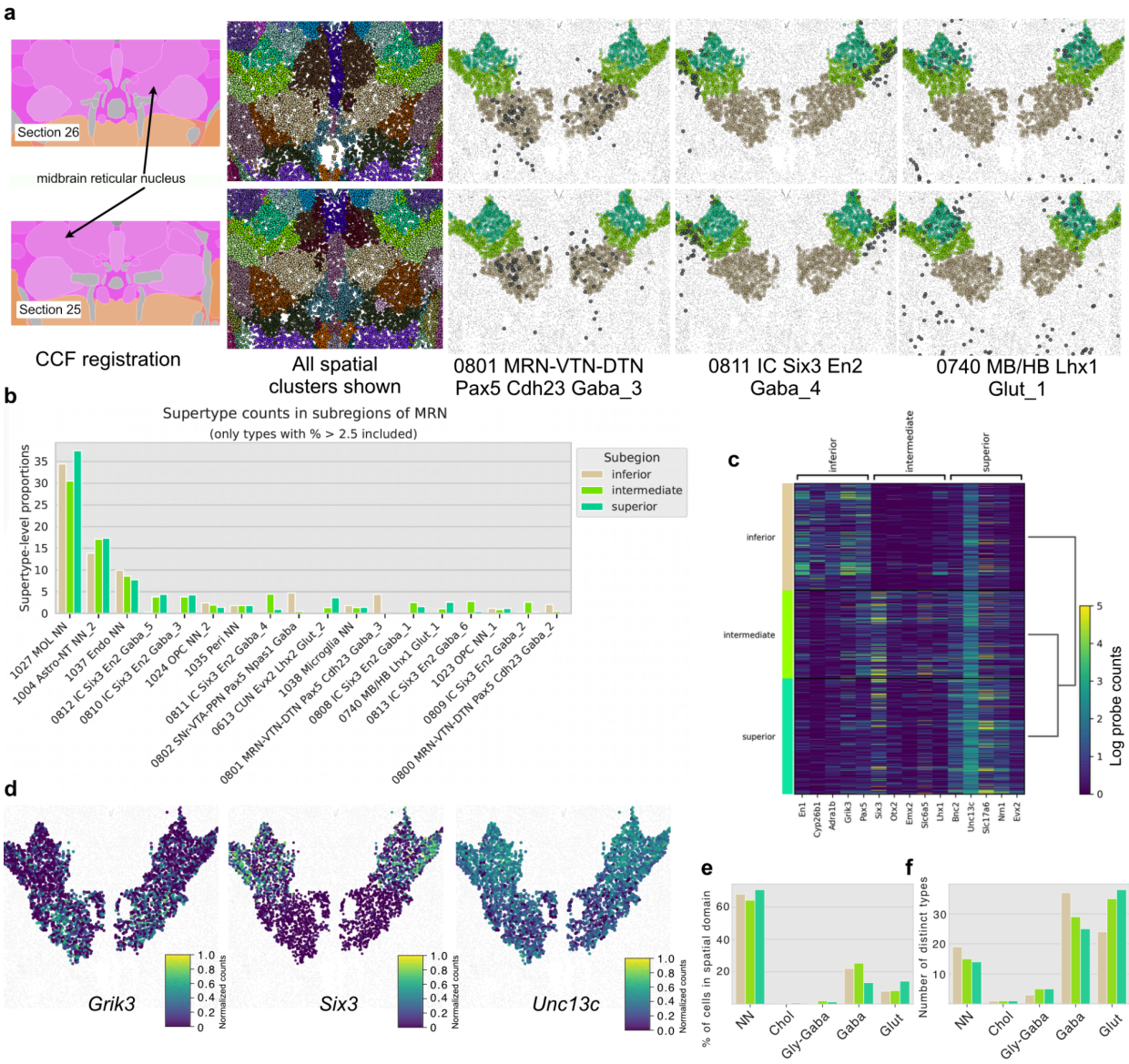


Figure 5. Subregions of midbrain reticular nucleus discovered using CellTransformer. (**a.**) Sequential tissue sections (26 and 25, anterior to posterior). First column: CCF registration with approximate location of midbrain reticular nucleus shown with arrows. Note that registration is not exact and can differ across hemispheres. Second column: all cells in field of view, with color from spatial clusters determined with *k*=1400. The rest of the columns show only cells located in the putative MRN, and each column shows a different supertype level cell type in gray. (**b.**) Supertype level cell type proportions for top ten most abundant types across the MRN subregions. (**c.**) Top 5 differentially expressed genes across regions. (**d.**) Gene expression gradients across tissue section 25 for *Grik3*, *Six3*, and *Unc13c*, showing specificity for each of the putative MRN subregions. Intensity of color is 0-1 normalized after log scaling raw probe counts. Each dot is a cell, and the color shows the relative transcript count. We show only cells within the subregions to make it visually easier to distinguish the relevant cells. (**e.**) Bar plot of the percentage of cells for a given neurotransmitter type found in each domain. (**f.**) Number of unique cell types at supertype level in ABC-MWB ontology found in each domain.

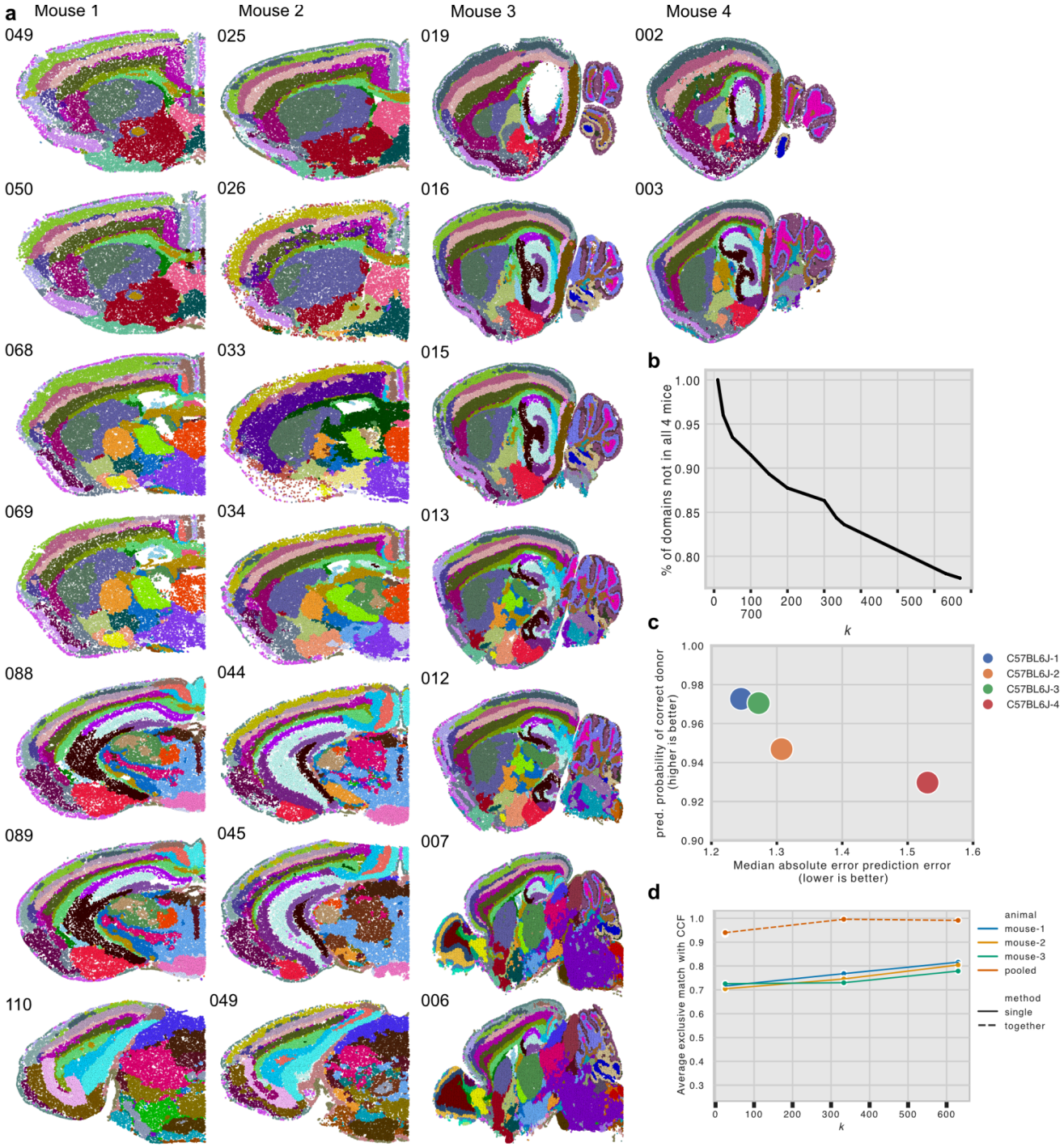
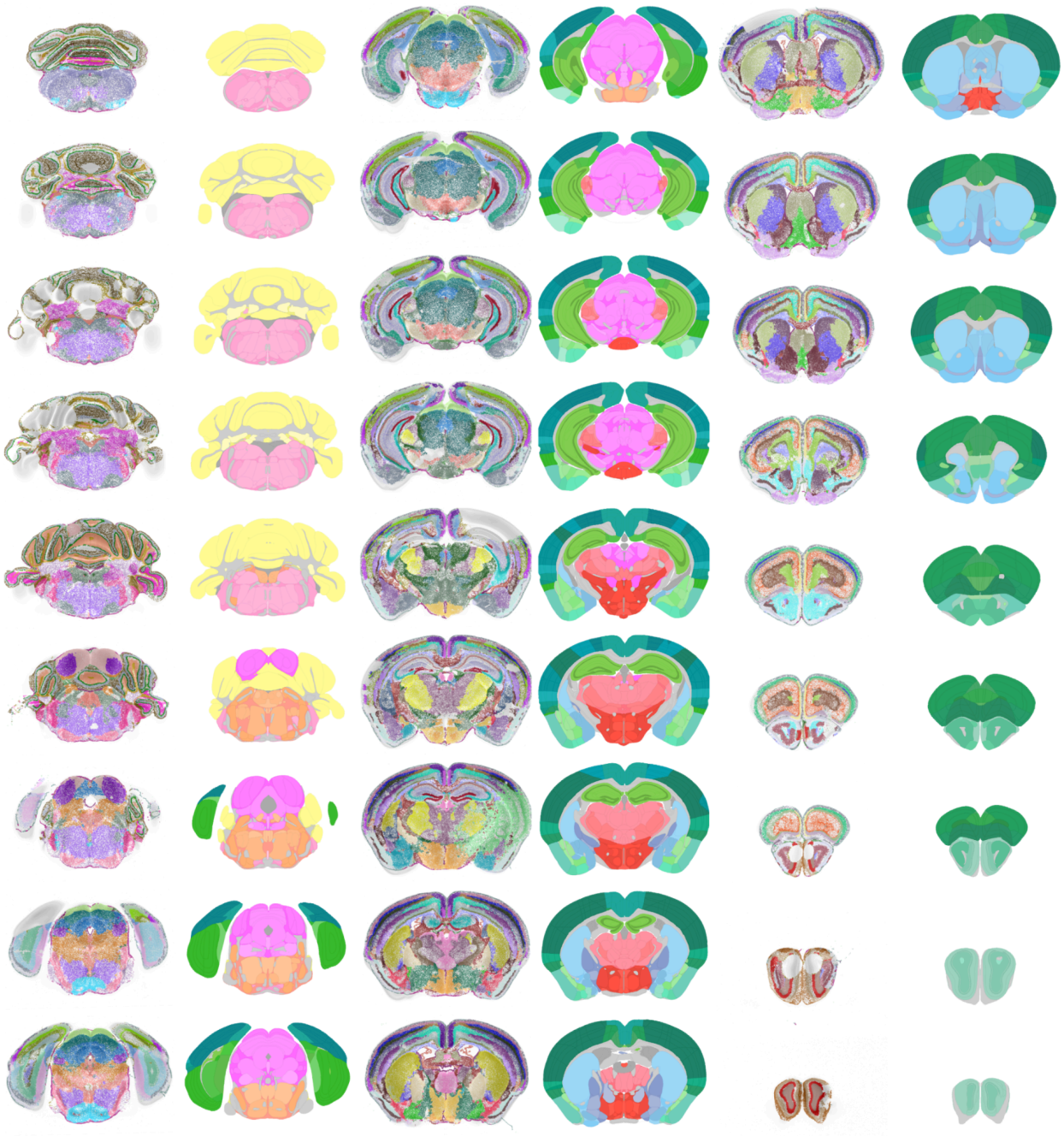


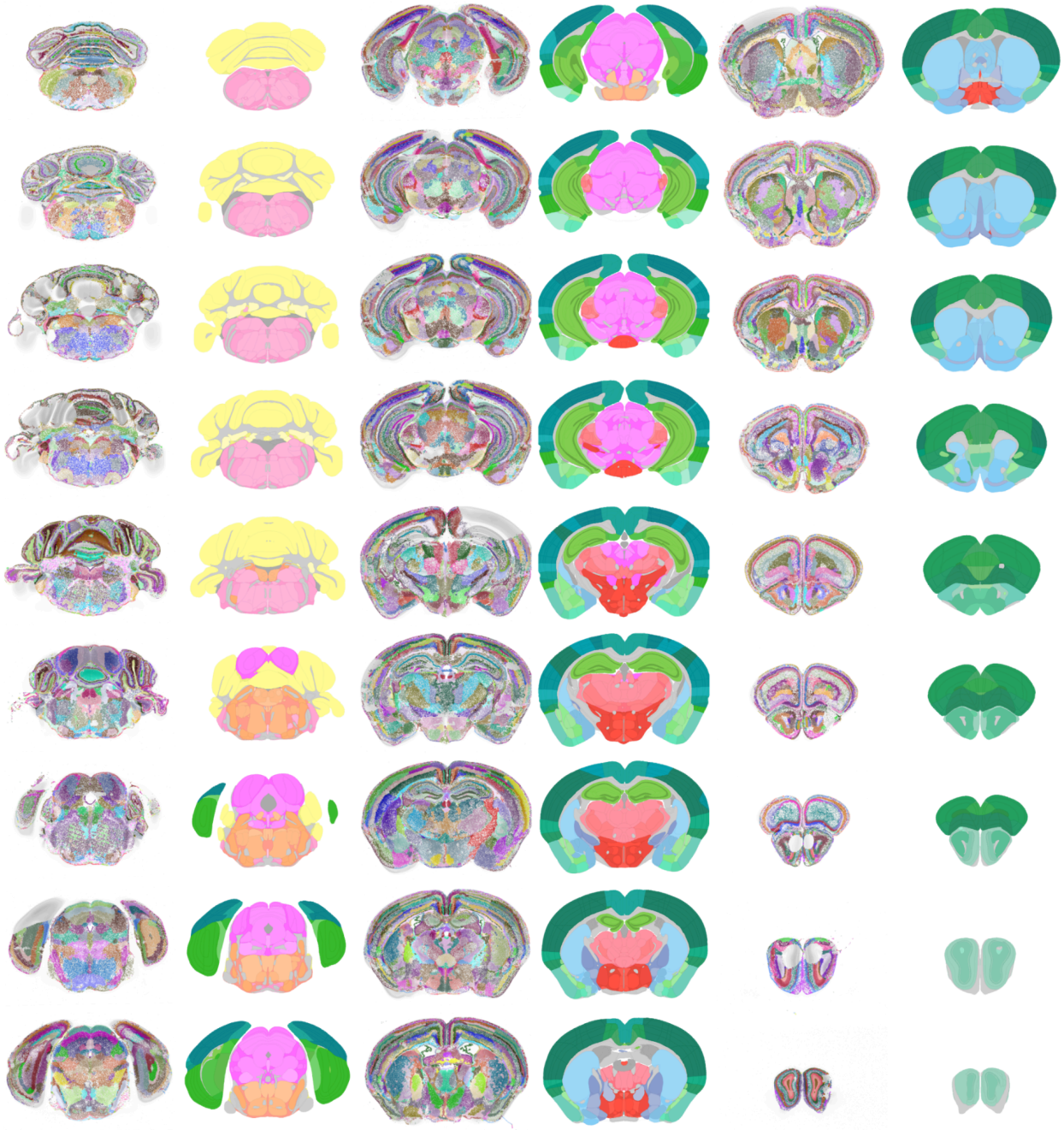
Figure 6. Investigation into performance of CellTransformer on all four Zhuang lab animals. (**a.**) Representative images of all four mice arranged by column. The section number for each mouse is shown in the upper left of each image. Note that mouse 4 only had three sections. For each image, each dot is a cell neighborhood and colors come

from a spatial clustering with *k*=100 fit by concatenating embeddings across mice. (**b.**) Quantification of number of per-mouse specific spatial clusters, computed by clustering at different *k* and computing the number of clusters not found in every animal. Note that because the sections are sampled irregularly some brain areas will have marginally higher coverage in one brain or another. (**c.**) Quantification of subject-level information present in embeddings using linear regression. The median absolute prediction error (x-axis) quantifies accuracy in predicting the (x, y, z) coordinates of a neighborhood from its embeddings. The y-axis quantifies accuracy when predicting mouse identity from embeddings using logistic regression. Values are averaged across instances per mouse. (**d.**) Average correlation of the cell type composition of brain regions computed CellTransformer to CCF regions, computed using the Hungarian matching algorithm (exclusively matching regions from one set to the other). Dotted lines indicate results when fitting using all three mice with >1M cells together. Solid lines indicate results when computing spatial clustering on each mouse in isolation.

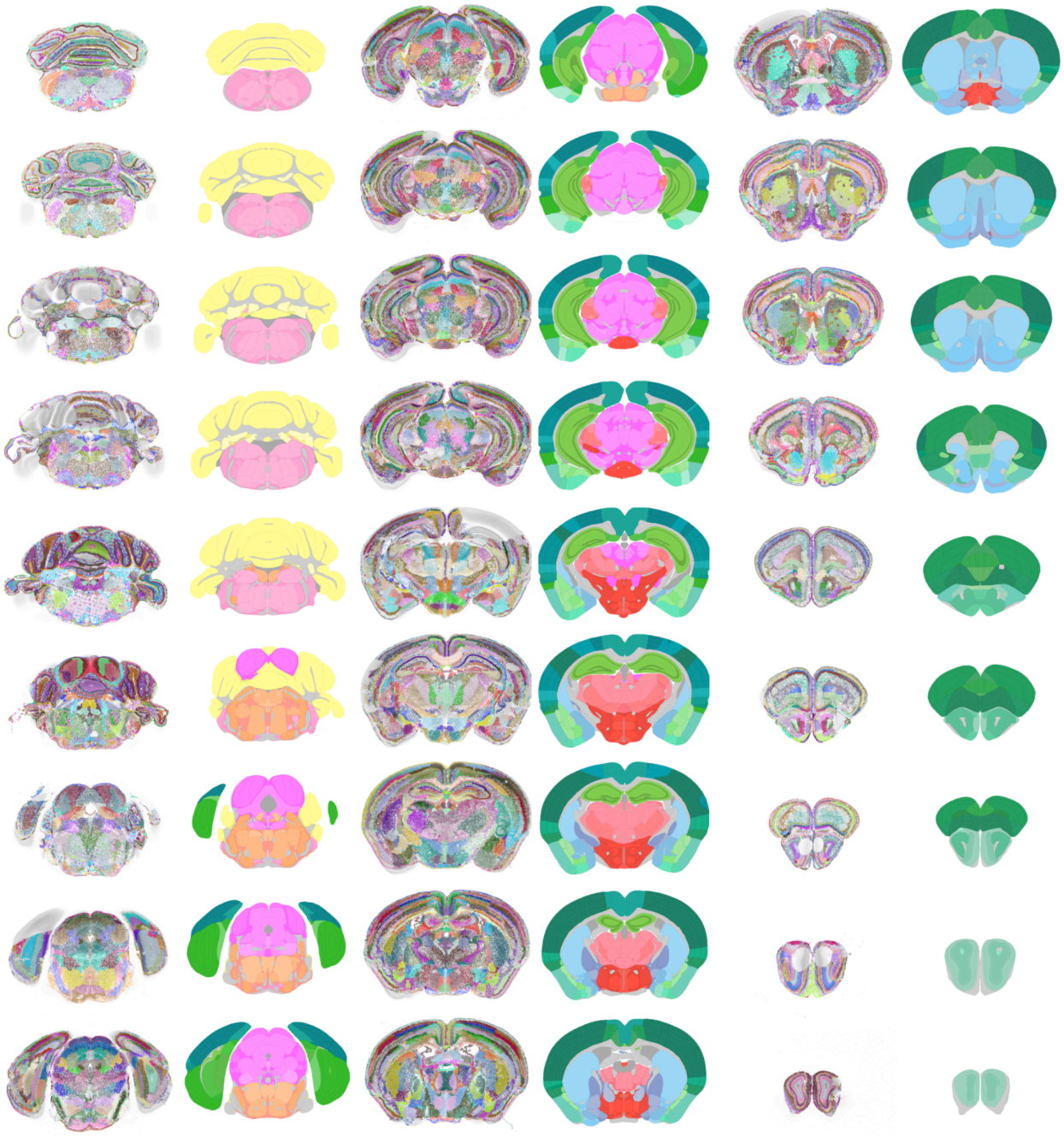
Supplementary Figures



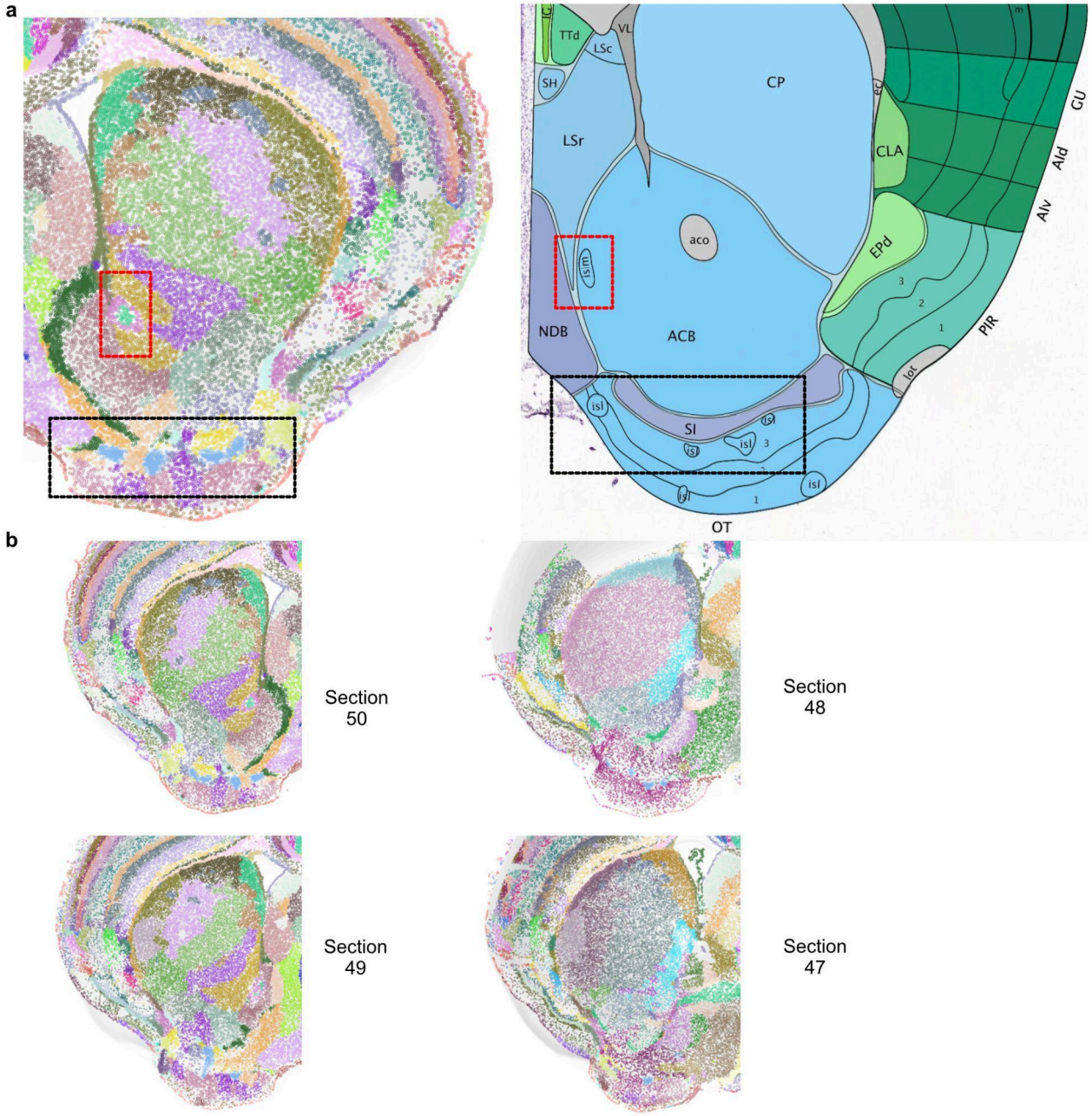
Supplementary Figure 1. Roughly half of the sections in the Allen Institute Brain Science mouse, approximately every other section. For each section color labels come from *k*-means with *k*=100 clusters and the cartoon image to the right is the CCF registered region set.



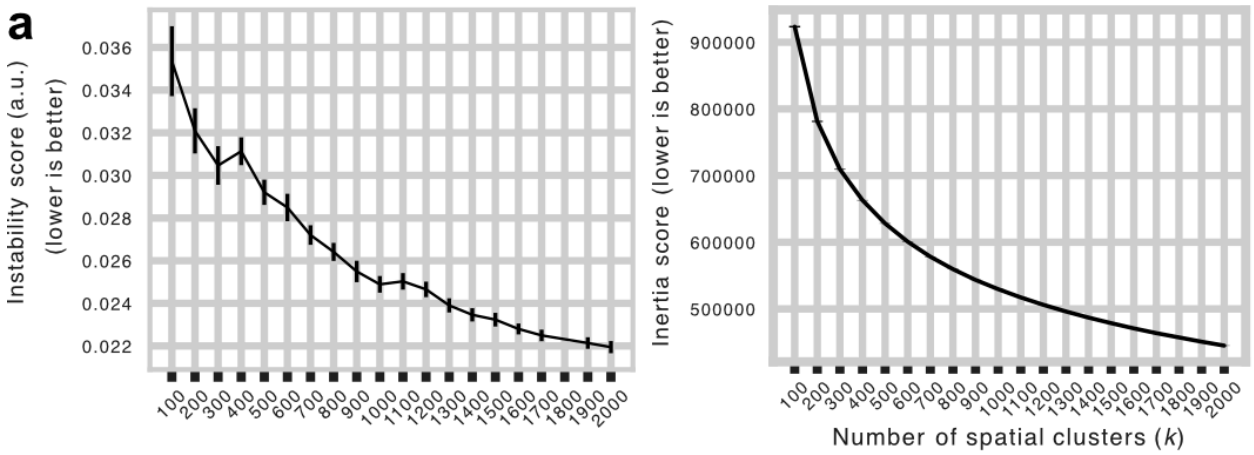
Supplementary Figure 2. Roughly half of the sections in the Allen Institute Brain Science mouse, approximately every other section. For each section color labels come from *k*-means with *k*=1000 clusters and the cartoon image to the right is the CCF registered region set.



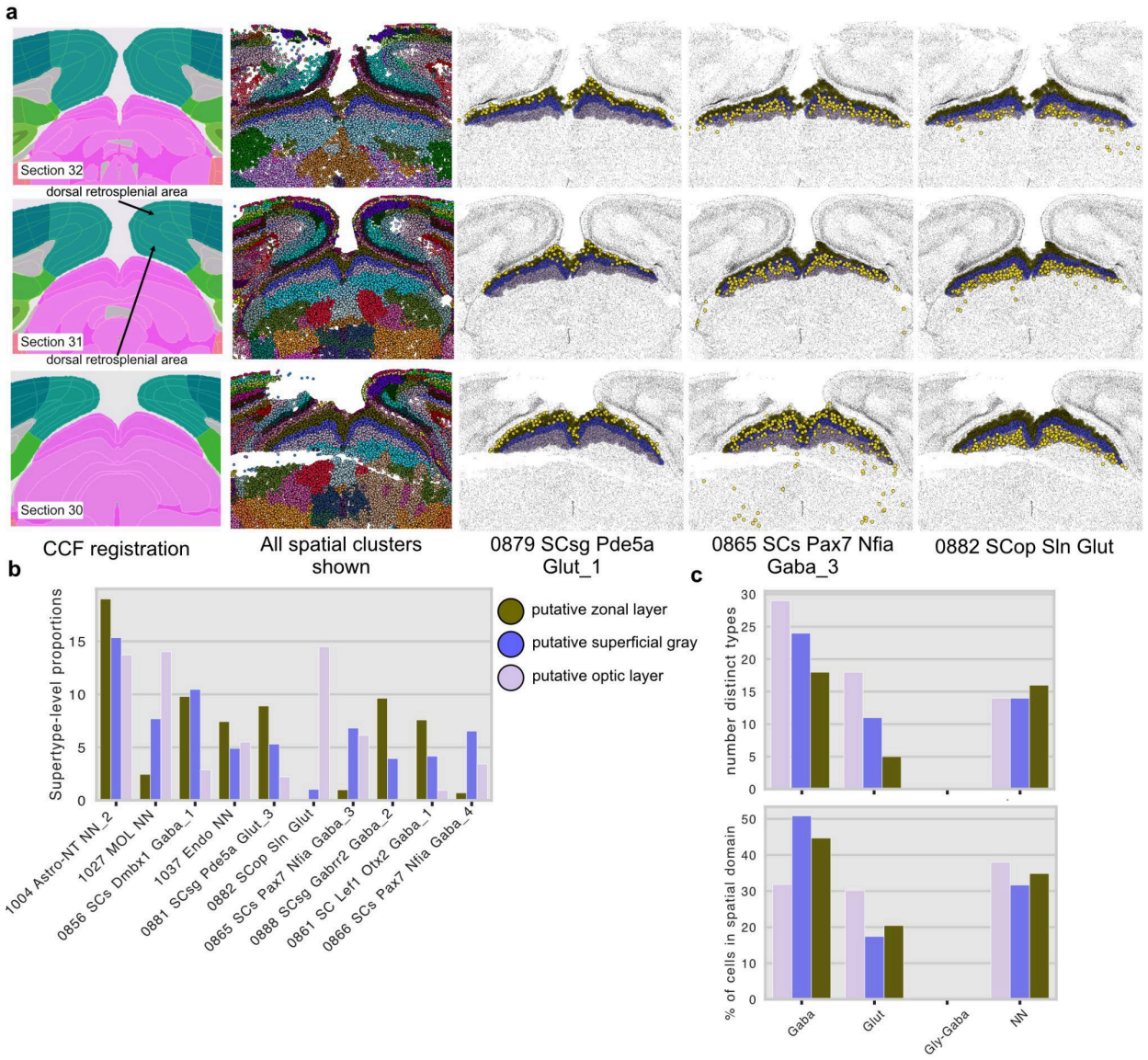
Supplementary Figure 3. Roughly half of the sections in the Allen Institute Brain Science mouse, approximately every other section. For each section color labels come from *k*-means with *k*=1400 clusters and the cartoon image to the right is the CCF registered region set.



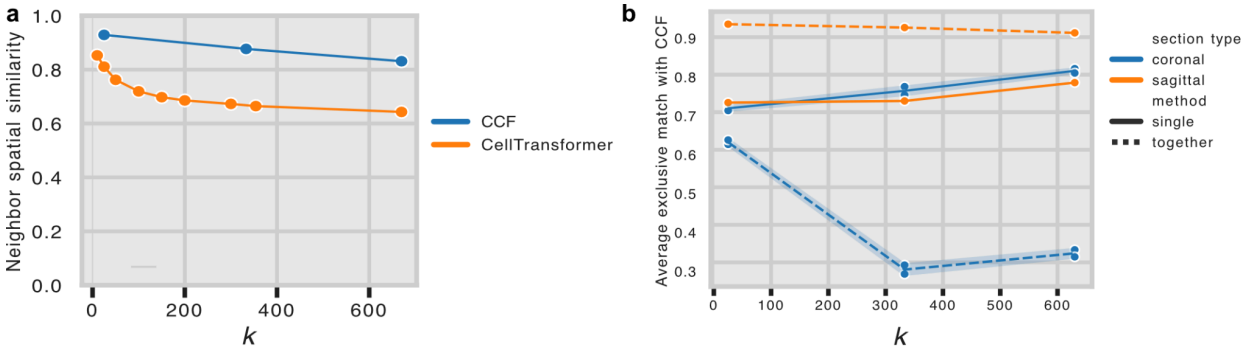
Supplementary Figure 4. (**a.**) Left: spatial cluster assignments (k=1400). Right: approximate CCF position (ARA 43/132, P56 Coronal). Black boxes indicate Islands of Calleja and red boxes indicate Major Islands of Calleja. (**b.**) Zoom in on left hemisphere of sections 50, 49, 48, and 47 (50 is most anterior section), showing segmentation into fine-grained subregions.



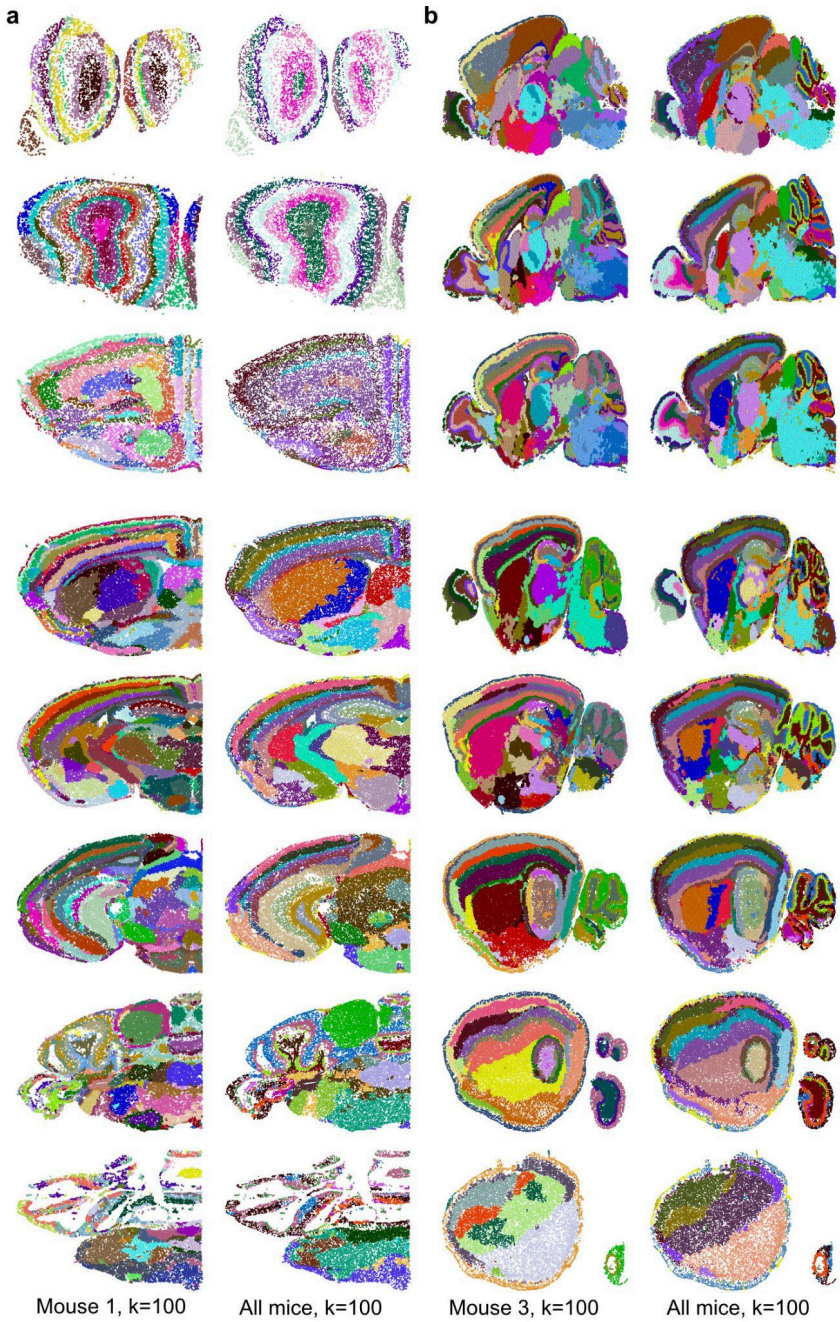
Supplementary Figure 5. Left: instability scores (see **Methods**) calculated for different clustering solutions. Error bars are standard deviation. Right: inertia (sum of squares errors for each cluster centroid) calculated for different clustering solutions. Error bars are calculated but not visible due to scale.



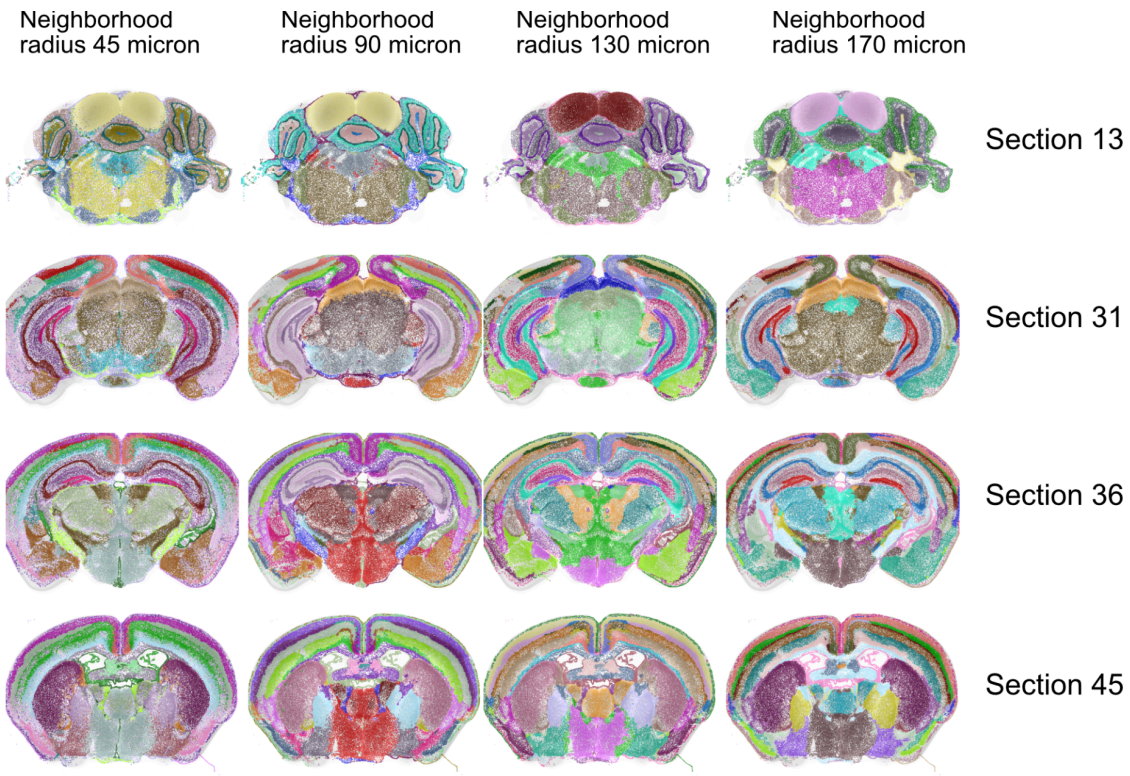
Supplementary Figure 6. Comparison of best fit spatial domains from CellTransformer with layers of the superior colliculus, sensory related area. (**a.**) Sequential tissue sections (32, 31, 30, from anterior to posterior) showing in first column CCF registration and borders of relevant areas. Second column: all cells in field of view, colored by spatial domain from CellTransformer. Third column: only visualizing cells inside our putative matches for the zonal, superficial gray, and optic layers in the superior colliculus. Also visualized is the 0879 SCsg Pde5a Glut_1 cell type (supertype-level) in yellow. Fourth column: same as third but visualizing the 0865 SCs Pax7 Nfia Gaba_3 cell type. Fifth column: same as third and fourth but visualizing the 0882 SCop Sln Glut type. (**b.**) Bar chart of cell type abundance (as a percentage) for top ten most abundant types across the putative subregions. (**c.**) Bar chart of per-region percentages of GABA and glutamatergic neurons and non-neuronal types.



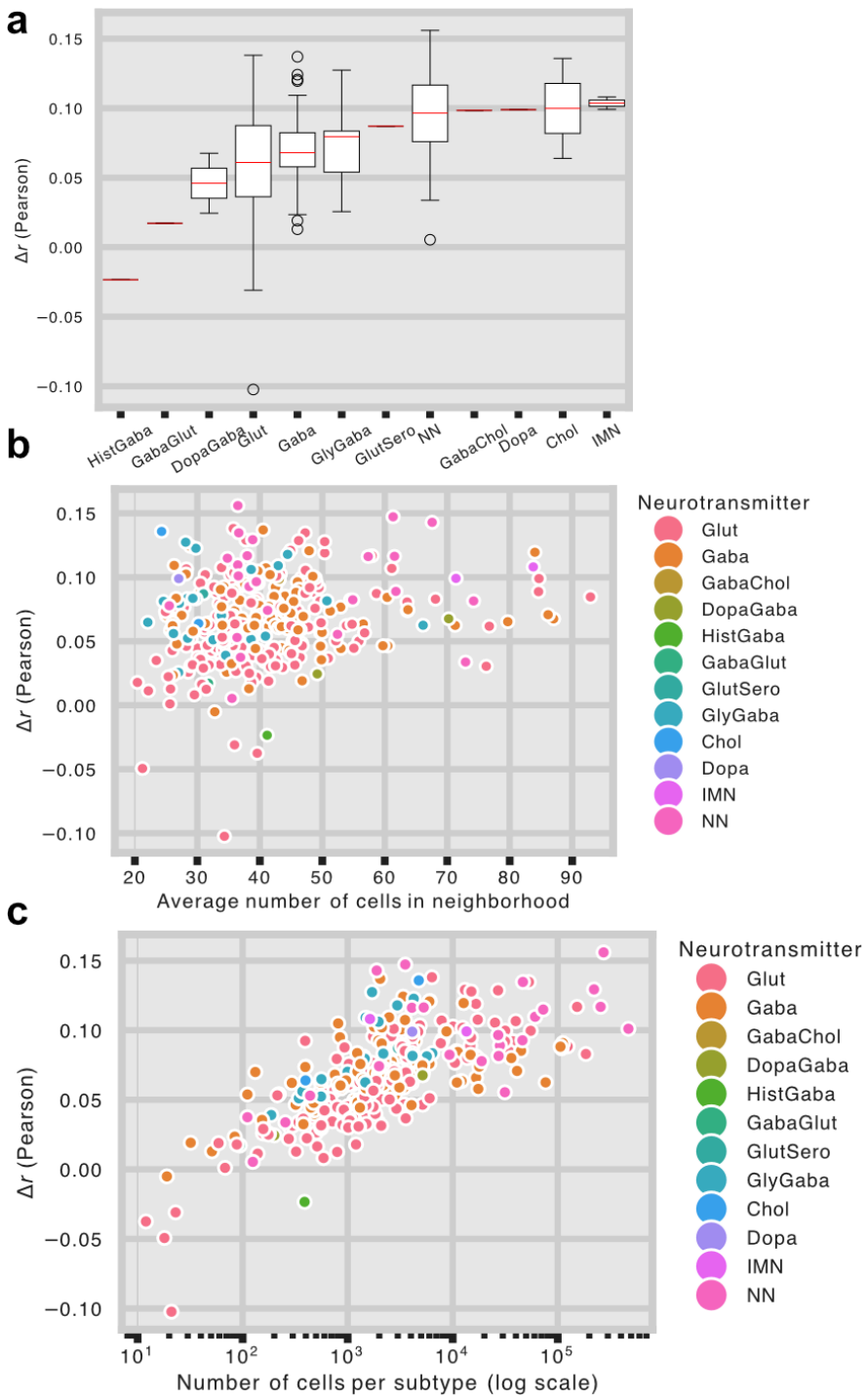
Supplementary Figure 7. Results of quantitative comparison of CCF regions and CellTransformer regions at equivalent number of regions. (**a.**) Spatial smoothness of spatial clusters as measured using a nearest-neighbors approach, computed by clustering the concatenated latent variables for neighborhoods in the Zhuang lab datasets. (**b.**) Result of comparing clustering across all animals versus individually. Solid lines indicate the match of computed spatial clusters with CCF when spatial clusters are computed at the per-animal level. Dashed area indicates standard deviation. For the analysis comparing the concatenated dataset clustering solution, we extract cell type by region matrices per animal.



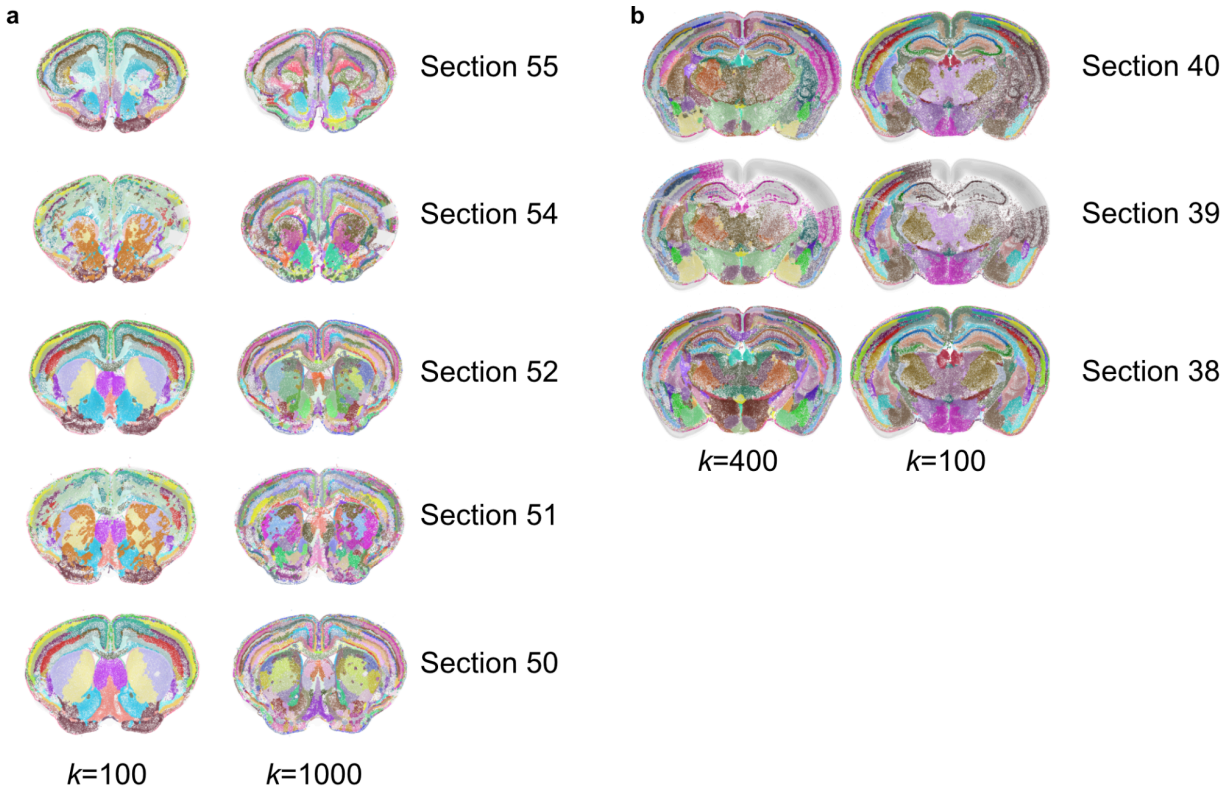
Supplementary Figure 8. Representative images of a spatial clustering solution run per-mouse on animals 1 and 3. (**a.**) Clustering results for Zhuang lab animal 1, comparing (left column) clustering with only one animal of embeddings and with all mice together (right column). (**b.**) Clustering results for Zhuang lab animal 3, comparing (left column) clustering with only one animal of embeddings and with all mice together (right column).



Supplementary Figure 9. Representative images of spatial clustering from CellTransformer models trained with different neighborhood radius parameters.



Supplementary Figure 10. (**a.**) Increase in Pearson correlation from per-cell type (at subclass level) average expression. Red lines show medians per distribution. (**b**.) Scatterplot of increase in average Pearson correlation per subclass level cell type against average neighborhood size for reference cells of that type. **c**. Scatterplot of increase in average Pearson correlation per subclass level cell type vs the number of cells of that type in log scale.



Supplementary Figure 11. Representative failure mode images.