From Morphology to Computation: How Synaptic Organization Shapes Place Fields in CA1 Pyramidal Neurons

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Abstract

The synaptic mechanisms driving feature selectivity in specific neuron types remains a fundamental and unresolved challenge in neuroscience. In hippocampal CA1 pyramidal neurons (PNs), the development of place selectivity, manifested as place fields, is believed to result from dendritic integration of spatially distributed inputs combined with behavioral time scale plasticity (BTSP). BTSP involves dendritic spikes that temporally regulate synaptic potentiation and depotentiation. However, the role of excitatory (E) and inhibitory (I) synaptic distributions in the emergence of place specificity in CA1 PNs remains unclear, due to the lack of detailed synaptic reconstructions in vivo. Here, we present full synaptic reconstructions from individual CA1 PNs in the mouse hippocampus, revealing that these neurons receive approximately 10,000–15,000 E synapses and 900–1,400 I synapses. Computational modeling of biologically relevant E and I synaptic distributions shows that spatial tuning is preserved with co-tuned, spatially clustered E synapses, but disrupted when E distributions are randomized. Moreover, synapse clustering has a different contribution to spatial tuning in apical vs. basal domains. Our results reveal a complex and finely-tuned interplay between presynaptic input patterns and the spatial organization of their postsynaptic targets in dictating neuronal output in CA1 PNs.

Keywords

dendritic computation, synaptic clustering, CA1, pyramidal neuron, biophysical model, morphology, dendritic nonlinearity, dendritic domains

Introduction

The hippocampus and particularly its CA1 region plays a crucial role in spatial navigation. CA1 pyramidal neurons (PNs), specifically the spatially tuned ‘place cells’, are widely hypothesized

to construct cognitive maps of the environment by combining spatial information and contextual cues[1–3]. Studies have shown that place cells are highly dynamic, undergoing remapping when an animal encounters a new or altered environment[2,4]. During remapping, CA1 place cells reorganize their spatial firing patterns in response to environmental changes, effectively encoding a new cognitive map[5]. In familiar settings, place cells exhibit stable firing fields, supporting the retrieval of established memories, while in novel contexts, rapid remapping enables the encoding of new spatial information.

This flexibility stems from the integrative properties of CA1 PNs, which allow them to adjust the salience of specific input streams based on the environmental context[6,7]. However, the electrophysiological and structural variability of these neurons hinders our in-depth understanding of their computational capabilities[8]. Numerous studies have attempted to characterize how these properties, along with the organization of synaptic inputs, collectively shape the input-output dynamics of CA1 PNs[8–14]. Yet, the impact of the spatial distribution of E and I synaptic inputs on the formation and functional features of place cells remain largely unexplored. Synaptic organization along the dendritic arbor heavily influences the integrative properties of neurons. For example, projections from the CA3 region predominantly target proximal perisomatic dendrites, while long-range inputs from the entorhinal cortex (EC) innervate the distal apical dendrites[15,16]. This anatomical segregation allows both the independent processing of distinct information streams and their conditional integration, thus dynamically shaping the neuron's feature selectivity[17–19]. Additionally, differential ion channel distributions and morphological features of dendrites across these CA1 layers modulate synaptic efficacy, such that identical inputs can have variable impacts on neuronal output depending on their dendritic location[20,21]. A comprehensive exploration of how different E and I incoming inputs shape the local dendritic properties is essential for elucidating the mechanisms underlying neuronal information processing.

Previous results suggested that rat CA1 PNs are characterized by non-uniform synaptic distributions at the sub-branch level[9]. This type of synaptic arrangement can greatly affect the

dendritic input-output function[22,23]. For example, synchronous activation of synapses within the same branch was shown to drive strong nonlinear dendritic responses[24–26], a property that generalizes to other cell types[27–29]. These nonlinear dendritic responses were eliminated when the same synapses were distributed across different dendrites[24,26], highlighting the importance of synaptic distribution on CA1 input-output mapping.

The abovementioned effects are underlined by voltage-dependent ionic conductances in dendrites, which enable the induction of local regenerative events, turning the neuron into a complex processing system with enhanced computational capabilities[22,24,26,30–32]. As the spatio-temporal organization of synaptic inputs is essential for triggering these local events, synapse location truly matters[24,26,32]. Localized within dendritic compartments that host numerous nonlinear mechanisms, synapses engage in dynamic interactions, amplifying, attenuating, or gating signals based on their dendritic positions, which suggests that the formation and maintenance of synaptic contacts is not a randomized process but an actively regulated mechanism[33–38]. Recent findings further reveal that co-tuned inputs frequently converge onto spatially clustered synapses, creating dendritic “hotspots” that can more easily produce non-linear dendritic events[39,40], as suggested by earlier theoretical work[26,32,41]. Therefore, it is clear that to fully understand how CA1 PNs compute a place field, it is essential to consider the spatiotemporal organization of their incoming inputs.

In this study, we combined experimental and computational modelling approaches to map the spatial distribution of all E and I synapses in a subset of mouse CA1 PNs in order to investigate how the spatial distribution of E and I synapses affects the output of spatially tuned CA1 PNs. We first employed an advanced sparse labeling technique to reconstruct the complete morphologies of CA1 PNs with high precision. We meticulously traced the dendritic arborizations and mapped the locations and morphological features of both inhibitory and excitatory synapses. We found a non-uniform distribution of excitatory synapses across the dendritic arbor, in contrast to a more homogeneous arrangement of inhibitory synapses. Specifically, terminal dendrites in both apical and basal dendritic domains had higher spine

density than intermediate ones, which resulted in a higher probability of finding large spines located in close proximity (i.e., spine clustering) within terminal dendritic compartments. These data were used to build biophysical models that were extensively validated against electrophysiological data and used to investigate how the spatial distribution of synaptic inputs influences the output of place cells during simulated exploration of a linear track. Models predicted that the spatial allocation of excitatory inputs strongly influences both the spatial tuning and the place cell properties of simulated neurons. Specifically, spatial tuning that was present in simulations using co-tuned and spatially clustered excitatory synapses but was disrupted when target locations were randomized. Moreover, our models suggest that there might be a set of optimal, domain-specific input patterns that impinge on the reconstructed synapses and turn these neurons into high quality place cells. Specifically, we predict that inputs to the distal apical tuft are more effective when activated in large clusters whereas inputs to the basal dendrites are more effective when synaptic clusters are distributed over multiple branches. Together, our findings reveal a complex and finely-tuned interplay between presynaptic input patterns and the spatial organization of their postsynaptic targets in specifying the output properties of CA1 PNs.

Results

Reconstruction of CA1 Pyramidal Neuron Morphology

To reconstruct the dendritic morphology and synaptic distribution of all excitatory and inhibitory synapses of mouse dorsal CA1 PNs at single-cell resolution *in vivo*, we deployed a combination of sparse reporter expression, imaging, and computational tracing methods that we previously developed[42]. We labeled dorsal CA1 PNs through *in utero* electroporation of plasmids expressing Cre-dependent Flex-tdTomato (red cell filler) Flex-EGFP-Gephyrin (inhibitory synaptic marker) along with low levels of a Cre recombinase plasmid for targeted,

sparse labeling[43,44] (**Figure 1A**). After high-resolution confocal imaging (**Figure 1B**), we used open-source reconstruction software Vaa3D[45] to obtain a trace of the dendritic tree (**Figure 1C**). Through the Vaa3D Synapse Detector toolkit[42], we annotated all excitatory and inhibitory synapses located within each segment of the dendritic tree. Given that the majority of excitatory synapses in CA1 PNs occur on spines[9], we used them as a structural proxy of all excitatory synapses in our neurons. Inhibitory synapses were identified by co-localizing EGFP-Gephyrin puncta with cytosolic tdTomato (**Figure 1B**). Using this method, we obtained detailed information about each synapse's position and shape, including features like volume, spine head diameter, and spine neck length. We also identified the relative location of inhibitory synapses, whether on the dendritic shaft or a dually innervated spine. Finally, Vaa3D algorithms linked all synaptic features to the corresponding branch segment node, and neuron trace fragments of the same neuron from serial tissue sections were stitched together to create complete morphological reconstructions. Through this method, we obtained the dendritic arbor morphology and excitatory (**Figure 1D**) and inhibitory (**Figure 1E**) synapse mapping of six CA1 PNs (with one that lacks the inhibitory synapse map). After implementing the synaptic reconstruction pipeline, we applied a custom smoothing algorithm to dendritic diameter measurements to eliminate minor artifacts introduced by the reconstruction software (**see Methods, Figure S1**). From the structural analysis of these neurons, we observed, on average, 11,796±2,212 (mean±std, n=6 neurons, std: standard deviation) spines and 741± 545 inhibitory synapses, distributed over 7,164±982 µm of average total dendritic length. Consistent with previous studies[46], the average spine density was estimated to be 1.41±0.18 while the inhibitory synapse density was 0.10±0.07[47] (**Figure 1F**).

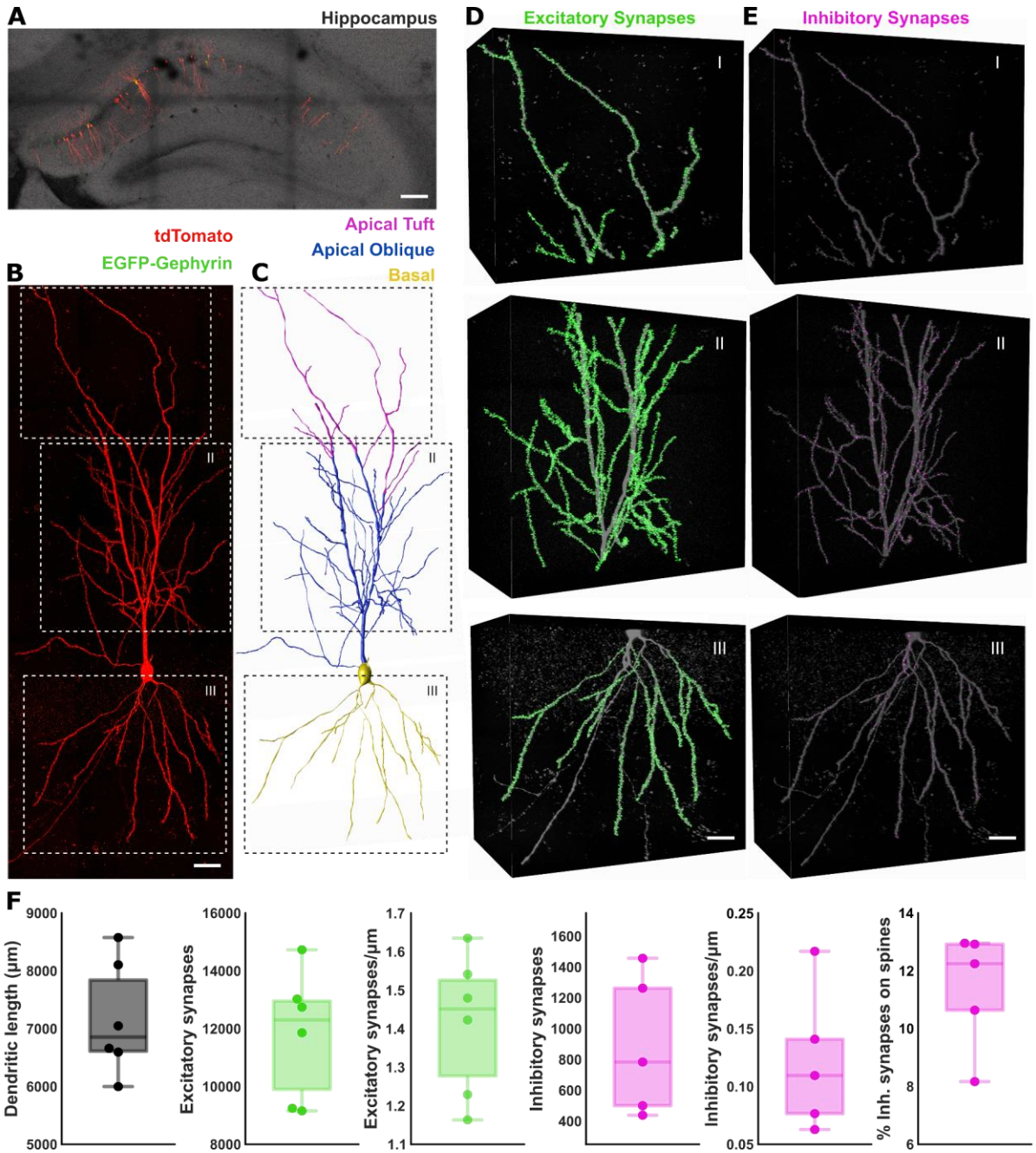

Figure 1: Reconstruction and analysis of all excitatory and inhibitory synapses across CA1 pyramidal neurons. A. Sparsely labeled pyramidal neurons expressing tdTomato (red) and EGFP-Gephyrin (green) in hippocampal CA1. Grey: tissue autofluorescence using 488nm excitation. Scale bar: 300 µm. **B**. Single-synapse resolution image of a CA1 pyramidal neuron expressing tdTomato (red) and EGFP-Gephyrin (green). Scale bar: 60 µm. **C**. Dendritic arbor of whole CA1 PN shown in panel B, annotated to highlight apical tuft dendrites (magenta), apical oblique and apical trunk dendrites (blue) and basal dendrites (yellow). **D-E**. All dendritic spines (excitatory synapses; D, green) and Gephyrin+ inhibitory synapses (E, purple) annotated throughout the dendritic arbor (grey) of the example CA1 PN reconstructed within insets from (B-C). Scale bars: 50 µm. **F.** Quantitative analysis of all CA1 PNs reconstructed showing dendritic structure and synaptic distribution. From left to right: total dendritic length

distributions (µm); total number of excitatory synapses and their density (syn/µm); total number of inhibitory synapses and their density (syn/µm); percentage of inhibitory synapses localized on a spine (dually innervated spines).

Domain-Specific Analysis of Synaptic Organization in CA1 PNs

CA1 PNs exhibit significant variability in the structural and electrophysiological properties of their dendritic arbors, which are also targeted by different presynaptic sources[8,9,48,49]. Consequently, we opted to analyze the synaptic organization by subdividing the dendrites into four distinct domains, namely *Basal*, *Apical Trunk*, *Oblique*, and *Tuft* dendritic domains. Within each domain, we further distinguished intermediate and terminal branches based on their relative branch order, referred to as *subdomains* (**Figure 2A**). We found that peri-somatic and intermediate dendritic regions exhibit a substantially lower spine density than more terminal branches (**Figure 2B**), which is in line with the literature[9,46] while inhibitory synapses show no significant variation across subdomains (**Figure 2B**).

Within these dendritic subdomains, we also characterized the size of synaptic connections in order to infer the degree of potentiation as a function of the dendritic subdomain. Synaptic volume corresponds to spine head size or postsynaptic density size for excitatory and inhibitory synapses, respectively, and is linearly correlated with synaptic strength[22,50,51]. As described in **Iascone et al.** (**2020**)[42] for layer 2/3 PNs in the mouse primary somatosensory cortex, we used the Synapse Detector tool to map the distribution of synaptic strengths across all different subdomains. Large synapses were defined as those exceeding the 80th percentile of the synaptic volume distribution of each cell. We found similar distributions of large synapses across dendritic subdomains, with the exception of the terminal Tuft, which exhibited a greater percentage of large spines compared to its intermediate counterpart (**Figure 2C**).

Another structural characteristic that may indicate an important function is the increase of synaptic density in a branch-specific way. During learning, new spines tend to form in proximity to already existing spines, or appear in small clusters, and to be more stable, marking

clustered potentiation at branch level[38,52–56]. To check this possibility, we compared the experimentally observed variation (standard deviation, in short STD) in E and I synapse density within dendritic subdomains to the synaptic densities of a subdomain-wise null distribution (**see Methods**) for each neuron. Dendritic subdomains with STD higher than that of the null distribution exhibit this branch-specific enrichment. We found branch-specific enrichment of excitatory synapses in the Trunk and Intermediate Basal domains, whereas inhibitory synapses were overall uniformly distributed (**Figure 2D**).

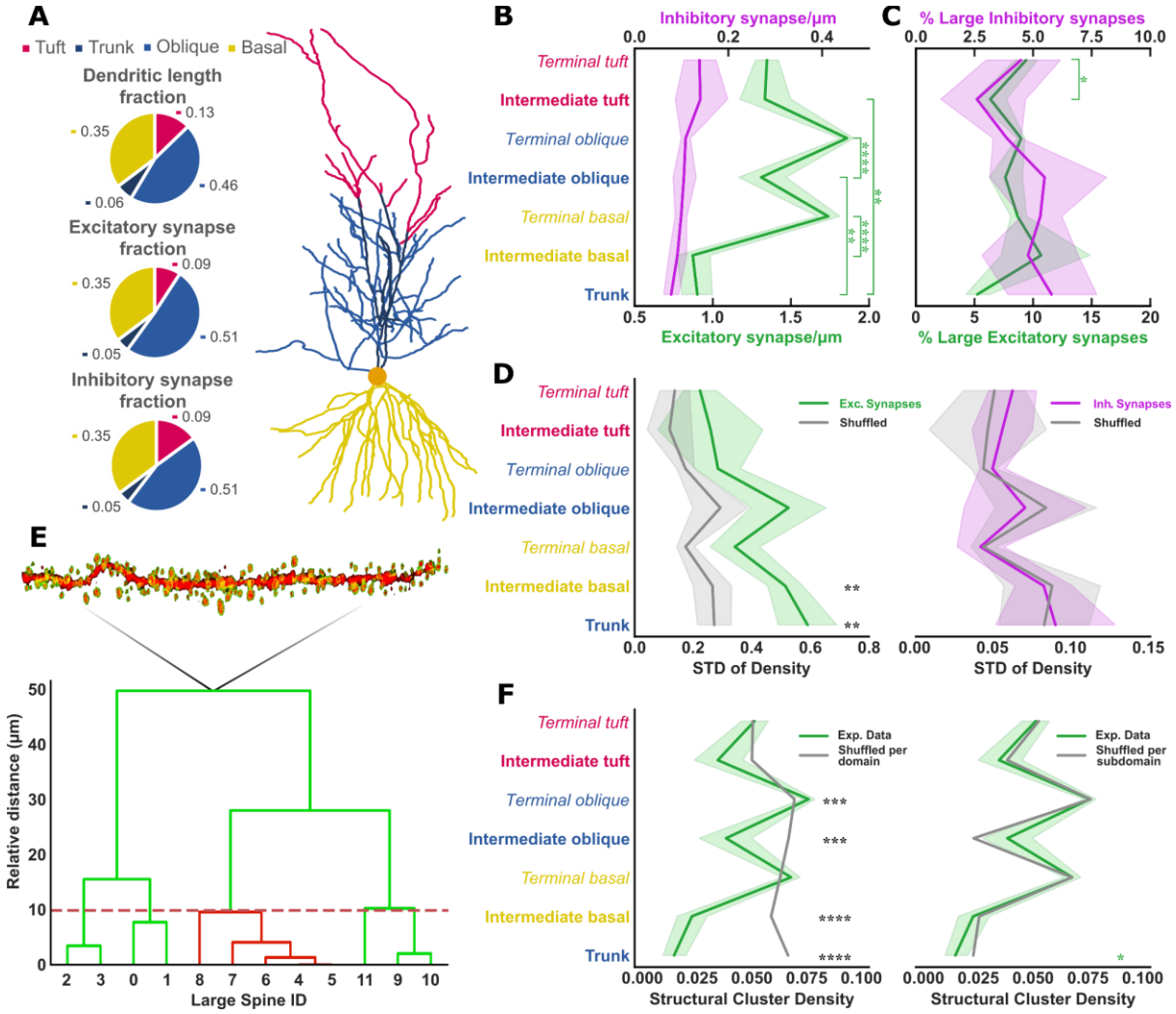

Figure 2: Analysis of Dendritic Subdomains and Synaptic Distributions. A. Left: pie charts showing the percentage of excitatory (E) and inhibitory (I) synapses located in different domains. Right: schematic representation of a CA1 PN, with distinct dendritic domains highlighted in different colors. These include the Basal

(green), Tuft (pink), Trunk, and Oblique (blue), further subdivided into intermediate and terminal subdomains using darker and lighter colors, respectively. **B.** Density of E and I synapses across different subdomains. **C.** Percentage of large spines (i.e., spines with a volume above the 80th percentile of the size distribution) for each subdomain. **D.** Variation (standard deviation, STD) in E and I synapse densities for each subdomain. Experimentally observed STDs (green/purple) are compared to those expected from a random redistribution of synapses within their specific subdomains (grey). Specifically, synapses were randomly shuffled across branches within the same subdomain, and excitatory/inhibitory synaptic density variation was recalculated. Excitatory -but not inhibitory- synapse variability is significantly different from the shuffled distribution (2-way ANOVA for spines density std: distribution: F(1, 4228)=374.74, p=4.63e-80; domain: F(6, 4228)=794.07, p=0.00e+00; distribution x domain: F(6, 4228)=10.66, p=8.71e-12). **E.** Schematic illustration of the hierarchical clustering algorithm used to identify spine clusters. The algorithm detects groups of large spines (with volumes above the 80th percentile) that are located within 10 micrometers of each other. The dashed line indicates the 10 µm distance cutoff. Red dendrogram strokes represent a spine cluster. **F.** Density of spine clusters across subdomains. Left: experimentally observed distribution (green) vs. a domain-wise shuffled distribution (grey). Right: experimentally observed distribution vs. a branch-wise shuffled distribution. Statistically significant differences are found only between the experimentally observed distribution and the shuffled-per-domain distribution. (2-way ANOVA for cluster density between `Exp. Data` and `Shuffled`: protocol: F(1, 775761)=136.83, $p<10^{-30}$; domain: F(6, 775761) = 3412.82, $p<10^{-80}$; protocol x domain: F(6, 775761)=58.33, $p<10^{-70}$). When shuffling within subdomains, the two distributions substantially overlap, with the exception of the Trunk (2-way ANOVA for cluster density between `Exp. Data` and `Shuffled per subdomain`: protocol: F(1, 775761)=1.06, p=0.303; domain: F(6, 775761)=82383.08, $p<1e^{-6}$; protocol x domain: F(6, 775761) = 4.58, p=1.19e-04). Pair-wise two-sided t-tests are shown in all plots. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, corrected for multiple comparisons using Bonferroni's method. Error-bands in **B**, **C**, **D**, and **F** represent 95% confidence interval (CI) of the mean.

Given that excitatory synapses are non-uniformly distributed, we next asked whether there is a finer patterning of synaptic connections within the different subdomains. Over the past decades, several studies demonstrated that the volume of spines tends to increase with the strengthening of the synapse[57–59]. Spines, however, do not grow in isolation but rather interact with neighboring synapses, often resulting in the formation of the so-called synaptic clusters. Indeed, several studies investigated how non-linear dendritic mechanisms promote plasticity

of nearby excitatory synapses[24,54,55,60,61]. For this reason, we investigated the presence of structural clusters in the different dendritic subdomains. To do so, we first defined a putative structural cluster as a group of three or more large (as defined previously) synapses within a branch segment of ~10 µm[62]. This approach is based on the assumption that heterosynaptic plasticity drives co-tuned nearby synapses to grow together and deviate from the average size[60,63–66]. We used a hierarchical clustering algorithm for the identification of these putative structural clusters (**Figure 2E**). Our analysis revealed a higher density of structural clusters in the terminal domains, a result that was significantly different from the density distribution obtained through domain-wise shuffling of synapses (**Figure 2F**). However, when comparing these observations with a subdomain-wise null distribution, the differences were no longer significant (**Figure 2F**). This finding suggests that the observed variations in cluster density between domains are primarily driven by differences in synaptic density within the corresponding subdomains.

Taken together, our results show that the spatial distribution of excitatory synapses in CA1 PNs is not random, showing a clear enrichment for terminal branches in basal and apical oblique dendrites, but not in apical tuft dendrites. However, this fine, domain-specific organization is not found for inhibitory synapses, creating an asymmetric E/I balance.

From Reconstruction of Synaptic Distribution to Modeling of Neuronal Functional Properties

To investigate how E and I synaptic distribution may influence the emergence of spatial tuning in CA1 pyramidal neurons, we constructed a biophysical model for each reconstructed neuronal morphology (**Figure 3A**) and simulated their activity during a virtual linear track exploration task (**Figure 3B**). The models were implemented using the NEURON simulation environment[67], adhering to the channel distribution parameters established by **Poirazi et al. 2003**[26] **(see Methods**).

Given the high synaptic density in our reconstructed neurons, we employed a fine spatial discretization by increasing the number of segments in the models. This ensured enhanced spatial resolution, enabling us to capture the detailed biophysical interactions between adjacent synapses (**Figure 3C**). To ensure the accuracy and biological relevance of our models, we validated both their passive and active properties against experimentally observed values. Passive properties, including input resistance ($\mathrm{R_{in}}$), membrane time constant (τ_m), Sag Ratio, and dendritic attenuation, were within physiological regimes[8,68,69] (**Figure 3D-4A; see Methods**). Similarly, the active properties, such as somatic and dendritic spiking activity, Frequency-Current (F-I) curve, and dendritic nonlinearities, were validated with widely used protocols[8,24] (**Figure 4B-E; see Methods**).

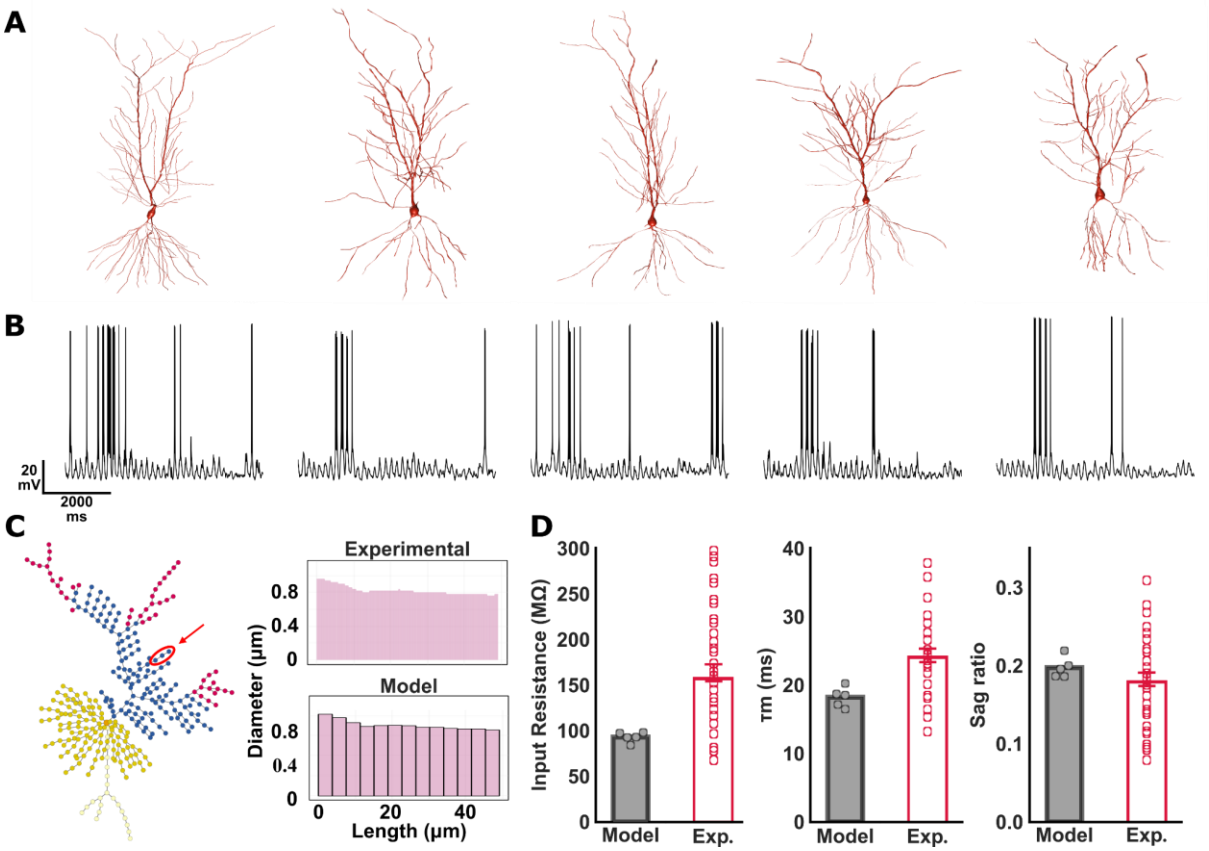

Figure 3: From morphologies to biophysical models. A. Reconstructions of dendritic morphology of the five CA1 pyramidal neurons used to generate the computational models. **B.** Somatic voltage traces produced by a place-tuned stimulation protocol used on the corresponding neuronal reconstruction. **C.** Left: schematic illustration

of the cell's segmentation used in the NEURON simulation environment (obtained using DendroTweaks[70]). Right: example of a branch structure from the data and its corresponding segmentation in the simulator. **D.** Passive membrane properties of CA1 PN models match experimental values. From left to right: input resistance, membrane time constant, and sag ratio. Experimental barplots redrawn from Masurkar et al., 2020[8].

The excitatory synaptic weights were calibrated based on experimental findings to ensure physiological relevance[71]. Specifically, we implemented the principle of synaptic democracy, which posits that synaptic inputs across the dendritic tree contribute equally to somatic depolarization, regardless of their location[72]. To achieve this, proximal synapses were parameterized to elicit a 0.2 mV depolarization at the soma[20]. For distal synapses, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor conductance was progressively increased as a function of the distance from the soma[71,73]. This adjustment ensured that while distal synapses generated larger dendritic excitatory postsynaptic potentials (EPSPs), the resulting somatic depolarization remained constant at 0.2 mV (**Figure 4F**; **see Methods**). Moreover, considering the large variability of spine sizes observed in our analysis, we decided to account for it in the calculation of the synapse strength by linearly varying the validated weight according to the spine head volume[74] (**see Methods**).

Inhibitory synapses were validated differentially based on their presynaptic sources to ensure an accurate representation of their functional characteristics. For this study, inhibitory synapses were assigned to six distinct locomotion active interneuron types, neurogliaform (NGF), oriens-lacunosum moleculare (OLM), parvalbumin-expressing basket (PVbasket), apical dendrite targeting cholecystokinin-expressing (CCK), Bistratified, and Ivy cells, based on their known post-synaptic target distributions across the dendritic tree[75–77]. Synaptic weights, as well as rise and decay times, were calibrated by replicating experimental protocols reported in the literature[78–82] (**Figure 4G**). Similar to their excitatory counterparts, inhibitory synapses were also scaled linearly with the synaptic volume. These rigorous validations

enhanced the reliability of the models and ensured they accurately reflected the physiological characteristics of CA1 PNs.

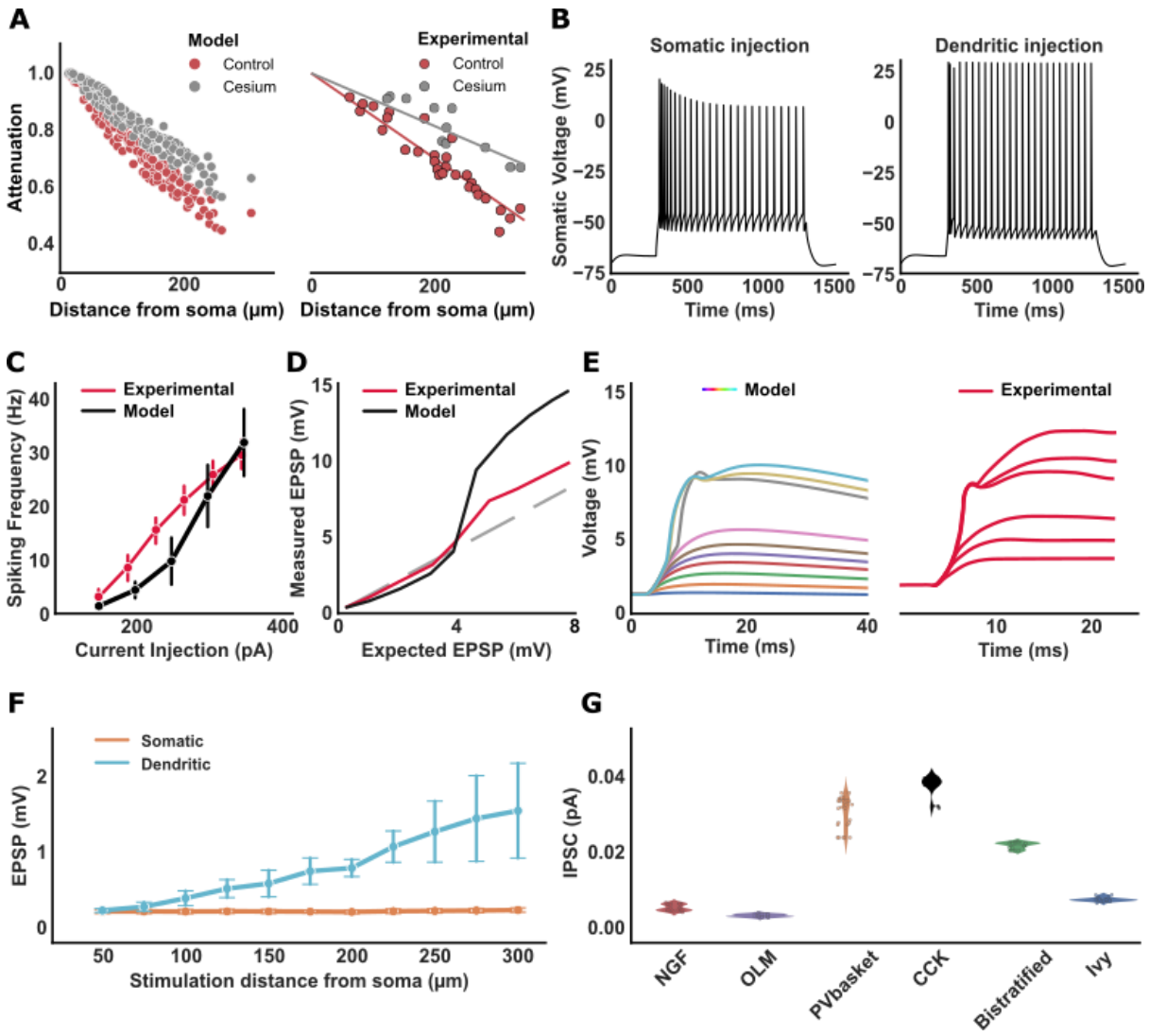

Figure 4: Electrophysiological Properties of CA1 PN Models Match Experimental Data. A. Dendritic attenuation of somatically injected negative current in control conditions (red) or simulating extracellular cesium (grey, 80% reduction of the h-current). Experimental data redrawn from Golding et al., 2005 are similar to model outputs. **B.** Examples of somatic voltage traces induced by a 300 pA current injection in the soma (left) or in a dendrite (right) located at ~250 μm from the soma. **C.** The input-output (Frequency-current (F-I)) curves of the models resemble experimental ones from Masurkar et al., 2020[8]. **D.** Peak amplitude of EPSPs measured at the soma as a function of the expected arithmetic sum of individual EPSPs. Experimental example curve redrawn from Losonczy and Magee, 2006[24]. **E.** Somatic depolarization upon simultaneous excitation of a progressively increasing

number of synapses. Left: stimulation of 1 to 30 simulated synapses on an oblique dendrite. Right: spines stimulated through glutamate uncaging, redrawn from Losonczy and Magee, 2006[24]. **F.** Dendritic and somatic excitatory postsynaptic potentials (EPSPs). Synaptic AMPA conductance strength increases with path distance from the soma, ensuring invariant somatic depolarization. **G.** Inhibitory postsynaptic currents (IPSCs) originating from various interneurons, including NGF (neurogliaform cells), OLM (oriens-lacunosum moleculare cells), PVbasket (parvalbumin-expressing basket cells), CCKbasket (cholecystokinin-expressing cells), Bistratified and Ivy cells. Validated to match experimentally observed data. Errorbars in **C**, and **F** represent 95% confidence interval (CI) of the mean.

After validating the passive and active properties of the individual PNs, we constructed the inputs to reliably reproduce the dynamics of incoming synaptic activity under a linear track exploration task. To achieve this, we produced spike trains that simulate excitatory inputs originating from CA3 and the entorhinal cortex (EC), capturing the neural patterns associated with spatial navigation. Specifically, we modeled convergent EC inputs as multiple grid representations covering a virtual linear track, from which CA3 place-like inputs were derived[6] (**see Methods**). The two resulting input streams were subdivided into a total of 12 place fields, each containing hundreds of co-tuned spike trains. In addition, background synaptic noise was simulated by introducing independent Poisson-distributed, and Gaussian-filtered inputs to CA1 PNs (**Figure 5B**).

Inhibitory inputs, conversely, were modeled as theta-filtered, phase-shifted Poisson spike trains, with each presynaptic source tailored to match the specific characteristics reported in the literature[76] (**Figure 5C; see Methods**). All inputs were distributed across synapses located in the anatomically relevant regions of the dendritic tree, ensuring that the simulated activity closely approximated the complex synaptic interactions observed during spatial navigation (**Figure 5A**). In particular, each interneuron targeted a synapse based on a probability derived from a non-linear function that depends on the distance from the soma. (**Figure S2**).

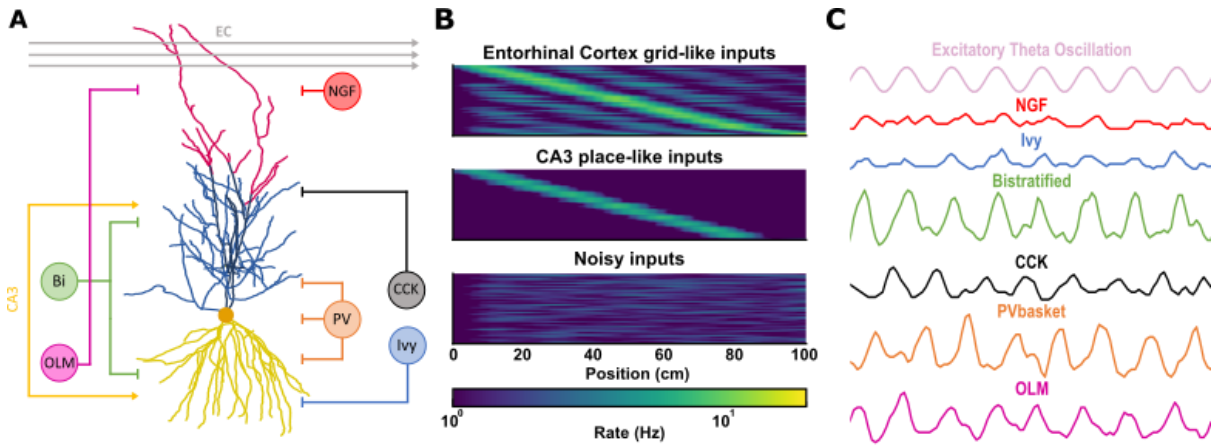

Figure 5: Patterns of Excitatory and Inhibitory Inputs to CA1 Pyramidal Cells. A. Schematic representation of the model neuron depicting excitatory and inhibitory input sources that are organized according to anatomical findings. **B.** Different types of excitatory inputs to the model. From top to bottom: Heatmap of EC grid-like, CA3 place-like and random noise spike trains. Each row represents a different presynaptic input neuron. The colormap is the same across heatmaps and brighter colors denote higher firing rates. **C.** Population activity of simulated interneuron spike trains compared to the Excitatory Theta Oscillation. Firing frequencies and theta phases are based on experimental findings.

Functional Role of Input Clustering in Place Cell Computation

After confirming that our model accurately reproduces the biophysical and electrophysiological properties of CA1 PNs given realistic synaptic input dynamics, we set out to investigate how spatial organization of excitatory synaptic inputs influences place-tuning properties. Specifically, we investigated how the clustering of excitatory synaptic inputs alters the computational capabilities of the neuron in a simulated navigational task.

A recent glutamate imaging study has characterized the spatiotemporal patterns of synaptic inputs to CA1 place cells during navigation on familiar linear tracks[39]. This study revealed that synapses receive inputs across all track locations, but the excitatory drive is significantly higher within the somatic place field than outside it. Additionally, the synapses that are co-tuned with the soma exhibit a greater tendency to form spatial clusters. Given these findings,

we generated 1,000 simulated connectivity patterns for each neuronal reconstruction designed to emulate the spatial organization of synaptic inputs that have been observed experimentally. Specifically, each connectivity pattern included a subset of structural clusters, as defined previously, which were preferentially targeted by place-tuned inputs. Inputs associated with a selected place field targeted a higher number of clustered synapses, which determined the somatic place-tuning, while the remaining synapses either received place-tuned or noisy inputs based on a uniform distribution.

This allocation protocol resulted in two distinct synaptic populations: (1) co-tuned clustered synapses, referred to as *Functional Clusters*, which were exclusively place-tuned, and (2) dispersed synapses, which were either place-tuned or targeted by noisy inputs. This configuration represented our Clustered condition (**Figure 6A, Control**). To assess the role of synaptic clustering in neuronal computation, we generated alternative synaptic distributions by shuffling the input targets under three different constraints. First, we generated a configuration where the total number of activated synapses was preserved, but inputs were randomly reassigned, disregarding the original preference for clustered synapses, which are larger and stronger (**Figure 6A, Shuffled** – Same Synapse Number (SSN)). Second, since synaptic strength scales with size, we generated a configuration in which the total synaptic volume rather than the number of inputs was preserved. This ensured a closer approximation of the excitatory drive comparable to the Clustered configuration but caused a much larger number of synapses to get activated (**Figure 6A, Shuffled** – Same Synapse Volume (SSV)). Lastly, we generated a configuration where the cumulative synaptic weight was preserved. This configuration considers the volume while also accounting for the “synaptic democracy” adjustment where synaptic conductance scales with the distance from the soma (**Figure 6A, Shuffled** – Same Synapse Weight (SSW)). These three shuffled configurations allowed us to disentangle the influence of synaptic clustering, size, and weight in shaping neuronal place computations.

To verify that the shuffling protocols disrupted input clustering, we quantified the number of functional synaptic clusters across dendritic domains for each input allocation scheme. As expected, the Clustered configuration exhibited a significantly higher number of functional clusters across all dendritic domains, particularly when compared to the SSN condition, which preserved the number of inputs but disrupted their spatial organization (**Figure 6B**). The SSV protocol, while maintaining a relatively high number of functional clusters due to the increased number of activated synapses, resulted in clusters with a smaller average size and lower total synaptic volume (**Figure S3**). These findings confirm that random shuffling alters the structural properties of synaptic clustering.

Next, we evaluated how these different input allocation protocols influenced the simulated activity of CA1 PNs. We found that neurons in the Clustered condition exhibited the characteristic place-field activity, while all shuffling protocols led to its disruption (**Figure 6C**). To quantify the extent of disruption, we assessed specific place field metrics such as in-field and out-field firing frequency, field size and spatial information[83]. We found that only the Clustered configuration generated place-field features, such as In-Field Firing Frequency (**Figure 6D**) and Spatial Information (**Figure 6E**), that aligned qualitatively with experimental results from **Mizuseki et al.** (**2012**)[84]. The SSN configuration resulted in substantially lower firing activity, leading to a marked reduction in Spatial Information. In contrast, both the SSV and SSW configurations led to a much higher firing frequency, with increased spike occurrences outside the place field. This widespread activity diminished the spatial selectivity of neuronal firing, ultimately reducing the overall Spatial Information. These results suggest that synaptic clustering plays a critical role in maintaining the spatial tuning of place cells and perturbing clustering disrupts single-cell encoding of spatial information.

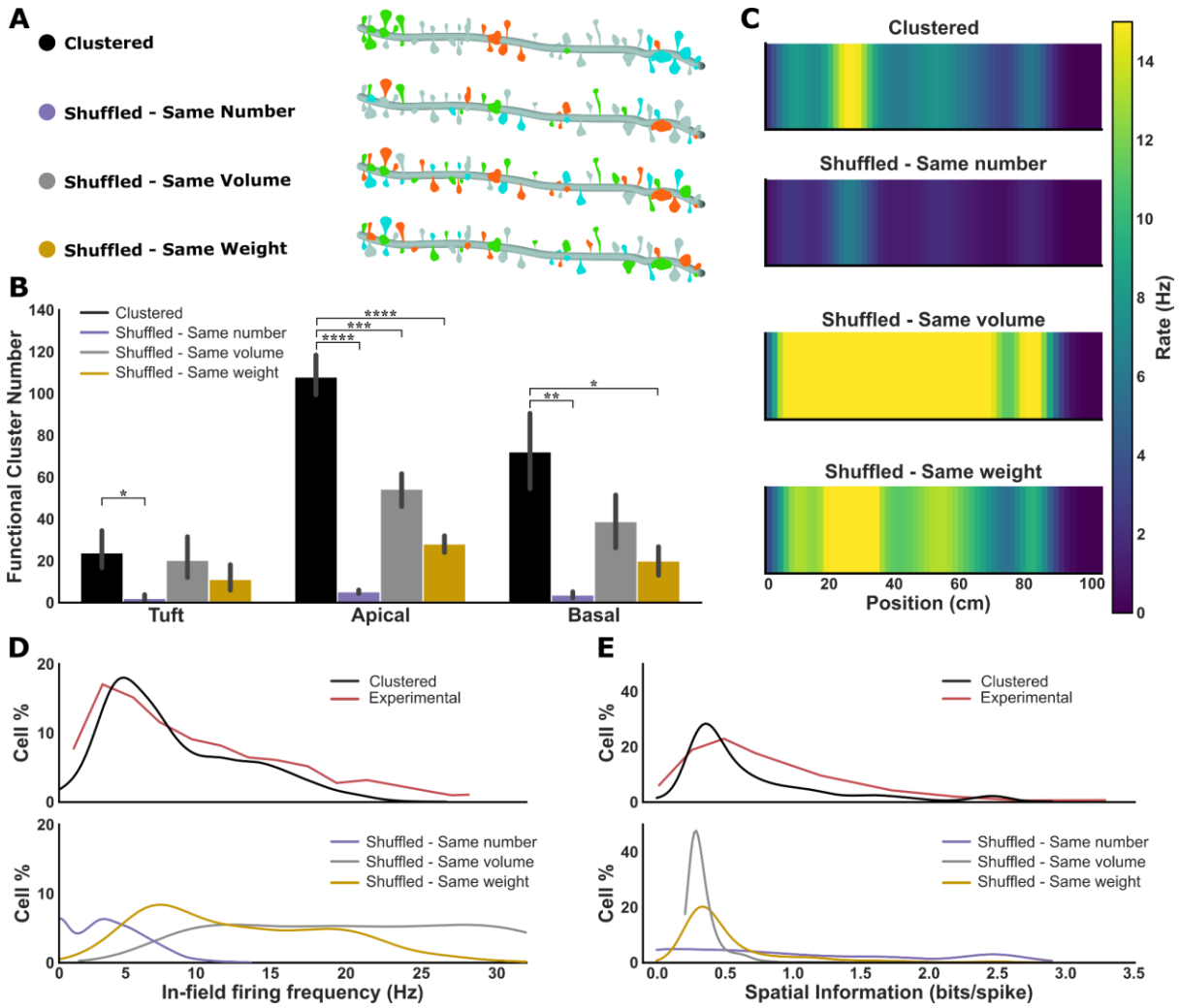

Figure 6: Effect of Synapse Clustering on Spatial Tuning. A. Schematic representation of four different synaptic configurations. Colors represent the tuning of synaptic inputs. Clustered: Synapses within pre-identified structural clusters are more likely to receive co-tuned inputs. Shuffled - Same Number: Inputs are randomly distributed while maintaining the same number of activated synapses as in the Clustered configuration. Shuffled - Same Total Weight: Inputs are randomly distributed while preserving the total synaptic strength of the activated inputs as in the Clustered configuration. Shuffled - Same Total Volume: Inputs are randomly distributed but the total volume of activated synapses remains the same as in the Clustered configuration. **B.** Number of activated functional synaptic clusters (co-tuned synapses located within a 10 µm spatial window) across dendritic domains (Basal, Apical, and Tuft) for each configuration. Statistically significant differences are indicated (2-way ANOVA for the number of Functional Clusters in the Tuft domain: cluster protocol: $F(3, 16)=5.16$, $p=1.10e-02$; Apical domain: cluster protocol: $F(3, 16)=142.96$, $p=9.19e-12$; Basal domain: cluster protocol: $F(3, 16)=19.16$, $p=1.52e-05$). Pair-wise t-test between Control and Shuffled protocols are shown in the plot. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, corrected for multiple comparisons. Error-bars represent 95% confidence interval (CI) of the mean. **C.** Average activity heatmaps showing place tuning of a population of CA1 PN models stimulated under the different

synaptic configurations. Input shuffling significantly disrupts the output place fields. **D.** Distribution of in-field firing frequencies. Top: The Clustered configuration qualitatively replicates the experimentally observed spatial firing patterns of CA1 PNs as reported by Mizuseki et al. (2012)[84]. Bottom: Firing frequency distributions of the shuffling configurations. **E.** Distribution of spatial information content (measured in bits per spike). Top: The Clustered configuration aligns qualitatively with the experimentally observed spatial firing patterns described by Mizuseki et al. (2012)[84]. Bottom: Spatial information distribution for the shuffling configurations.

Dendritic domain-specific clustering differentially contributes to spatial tuning

While global synaptic clustering alters the place-specific properties of our model CA1 PNs, its role in domain-specific integration remains unclear. To address this question, we probed the contribution of domain-specific clustering features to neuronal output. Specifically, we asked *how* synaptic clusters which are co-tuned with the soma ("in-field" clusters), and are located in the Basal, Apical or Tuft dendrites, contribute to neuronal spatial tuning. We focused on three clustering features: the *Total Cluster Volume* (the cumulative volume of all synapses within in-field-tuned clusters), the *Average Cluster Size* (the mean number of synapses of these clusters), and the *% Dendrites with In-field-Tuned Clusters*, thereby capturing both the strength and spatial distribution of synaptic inputs (**Figure 7A**).

We then applied a machine-learning regression algorithm to evaluate how well these features can predict the spatial tuning properties previously mentioned (**Figure 7B**). While the model accurately predicted in-field and out-field firing rates, suggesting that the structural properties of synaptic clusters strongly influence firing frequency, it poorly predicted field size, indicating that this property may involve more complex mechanisms (**Figure 7C**).

Notably, analysis of feature importance revealed domain-specific strategies for influencing somatic output. In the Tuft domain, *Total Cluster Volume* was the most critical predictor of spatial tuning quality. This suggests that larger clusters or bigger spines may be required to drive local spikes, thus overcoming signal attenuation caused by a large electrotonic distance

from the soma (**Figure 7D**) and exert a significant influence on somatic firing[85–87]. In contrast, for the Basal domain, the *% Dendrites with In-field-Tuned Clusters* was the most important predictor of spatial tuning quality. These findings suggest that parallel input streams across multiple branches may represent an optimal strategy for reliably modulating somatic output, highlighting the integration advantages conferred by proximity to the soma (**Figure 7D**). This interpretation is further supported by the application of the SHAP (SHapley Additive exPlanations) framework to explain the output of our Random Forest results[88]. This approach provides a per-feature and per-sample attribution to each individual predictor, highlighting the contribution of each input to the corresponding output value (**Figure S4**).

Interestingly, none of the three clustering features could reliably predict somatic output for the Apical domain, a region that in our analysis encompassed both Trunk and Oblique domains. This suggests that the apical compartment may rely on alternative mechanisms to shape spatial tuning that are not captured by the abovementioned clustering features.

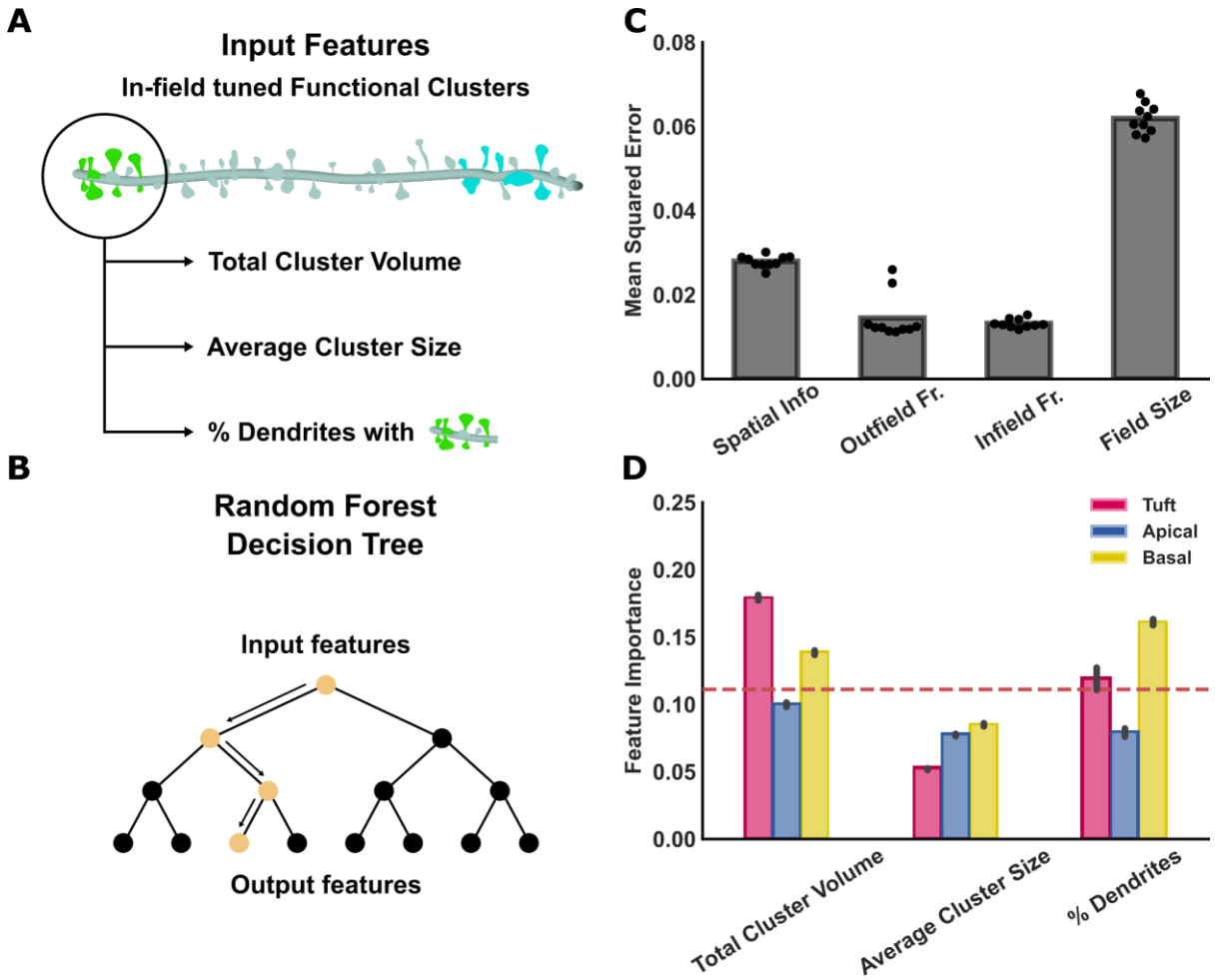

Figure 7: Cluster features Predict Spatial Tuning Quality *A.* Cluster features used as input to the multivariate random forest model to predict spatial tuning quality. The latter is assessed by the spatial information, out-field firing frequency, Infield firing frequency and field-size metrics. For each dendritic domain (Basal, Apical, and Tuft), cluster features include: the volume of in-field clusters, the size of in-field clusters, and the percentage of branches containing at least one in-field cluster. **B.** Schematic representation of the random forest methodology. The model combines predictions from multiple decision trees, each trained on subsets of the data (800 simulations used for training, 200 used for testing), to enhance robustness and accuracy in predicting model outputs. **C.** Mean Squared Error (MSE) of the output predictions on the testing set, providing a quantitative measure of the model's prediction accuracy. **D**. Feature importance in predicting simulation outputs for the Tuft, Apical, and Basal dendritic domains. The dashed line indicates the chance level of feature importance (~1.11). Error-bars represent the 95% confidence interval (CI) of the mean.

Together, these findings suggest that synaptic integration in spatially-tuned CA1 PNs is not uniform across dendritic domains. The Tuft appears to capitalize on large, high-volume clusters to initiate powerful nonlinear events, whereas the Basal domain favors a strategy of parallelized integration across multiple dendrites to collectively drive somatic depolarization. The apical domain's mechanisms remain more enigmatic, hinting at a rich, multidimensional mode of integration that may involve other variables such as temporal coordination or dynamic gating of the inhibitory inputs. These findings advance our understanding of how the organization of synaptic inputs shapes neuronal processing and underscore the importance of dendritic compartmentalization in spatial navigation.

Discussion

In this study, we combined high-resolution anatomical mapping with biophysical computational modeling to investigate how the spatial distribution of excitatory synapses influences the integrative properties of spatially-tuned CA1 PNs. Although the structural and electrophysiological characteristics of these neurons have been well-documented *ex vivo*[8–10], linking detailed anatomical properties of both E and I synapses to functional outcomes has remained challenging. Our study reveals that excitatory synapses onto CA1 PNs are organized in a non-uniform, domain-specific manner across the dendritic tree, and the patterned activation of these synapses has a marked impact on the place-tuning properties of these neurons.

Specifically, our comprehensive mapping of morphological and synaptic features reveals marked differences in spine density between dendritic domains. In line with prior work, we found that terminal dendrites exhibit a higher spine density compared to intermediate dendrites, whereas inhibitory synapses are distributed uniformly throughout the dendritic tree[9]. As a result, the probability of finding spine clusters is much higher in terminal dendritic compartments as opposed to intermediate ones. This disparity raises important questions about the mechanisms that enable the preferential formation of spines toward terminal

regions. We propose that the smaller diameter and sealed ends of terminal dendrites lead to elevated depolarization, thereby increasing the likelihood of synaptic plasticity events and subsequent spine formation, a specialization that may enhance the neuron's ability to encode spatial information[10,89,90]. This hypothesis is supported by prior modelling and experimental work, whereby activation of inputs in terminal dendrites facilitates the induction of NMDA spikes, resulting in persistent firing in L5 prefrontal cortical neuron models[91]. Similarly, in L2/3 pyramidal neurons of the primary visual cortex, NMDA spikes in the tuft dendrites facilitate orientation tuning[92]. Models predict that such NMDA spikes are more effectively induced by co-activated spines that are organized in clusters[93].

Previous studies inferred patterns of synaptic organization by analyzing E and I synaptic density in individual branches and extrapolating respective distributions by multiplying synaptic density to cumulated dendritic length[9]. Instead, our approach provides, for the first time, a complete, neuron-wide reconstruction of E and I synapses in multiple mouse CA1 PNs, as previously done for mouse layer 2/3 cortical PNs[42]. This unprecedented level of detail allowed us to capture the full statistical distribution of synapses across entire dendritic trees, revealing organizational principles that might have been overlooked in more localized analyses. Prior work has focused primarily on identifying synaptic clusters by observing newly formed spines during behavior[33,35,38]. These studies, while more relevant for linking synaptic clustering to behavioral outcomes, lack accessibility to synaptic clustering at the whole-cell level, as imaging techniques allow visualizing just a small proportion of dendrites *in vivo*. Our dataset provides access to high-resolution anatomical features of all E and I synapses found within all dendritic compartments of a CA1 PN. While providing only a static snapshot of synaptic structure, it allows us to probe the functional impact of synaptic structure on single-neuron computations, something not possible without this detailed information.

In addition to the structural mapping of E and I synaptic distribution across the entire dendritic arbor, our work has generated heavily validated computational models of 5 different reconstructed CA1 PNs using a common set of biophysical parameters. These models

account for both E and I synapse distributions and their respective input dynamics and serve as powerful tools for investigating questions that go beyond place cell tuning. Several important predictions came out of our models: first, we predict that place cells are more efficiently generated (higher quality place tuning) when co-tuned inputs are delivered to clusters of large spines. Indeed, randomizing the allocation of co-activated synapses -while keeping their number, total volume or total strength fixed-, resulted in degraded place field activity. This finding is likely due to enhanced signal propagation, reduced signal-to-noise and increased energy efficiency by activating spines in clusters[41,94,95]. Achieving the same, spatially-tuned, somatic output with small and randomly distributed synapses would require more contacts, increasing energy consumption and reducing available space for additional synapses[96,97]. This strategy aligns with principles of economical wiring and metabolic efficiency, suggesting that neurons may have evolved to optimize both their structural and functional features in order to better solve complex cognitive tasks[98].

Second, our models predict that the influence of synaptic clustering on neuronal spatial tuning varies depending on the dendritic domain. While tuft dendrites may require larger clustered synapses to impact somatic activity, in basal dendrites, distributed integration of multiple clustered inputs plays a more important role. These observations are in line with studies demonstrating strong compartmentalization of dendritic processing in CA1 PNs both *in vitro*[68,85,99] and *in vivo*[14,100]. In particular, clusters of strong, NMDA-rich synapses primarily found in the apical tuft, which are capable of generating plateau potentials, are more likely to contribute to hippocampal feature selectivity during navigation than basal synapses[99–101]. The activation of these large events is also known to initiate global plasticity events like the newly found BTSP[102,102,103].

Notably, the proportion of basal dendrites containing in-field clusters emerged as the most critical factor influencing spatial tuning. Previous studies have reported a strong correlation between basal dendritic and somatic activity, suggesting that neurons parallelize basal inputs to more efficiently regulate somatic output[14,104]. Additionally, the strong coupling between

basal dendrites and the soma may allow backpropagating action potentials to more effectively induce plastic changes, aligning basal dendritic tuning with somatic responses[14,105,106].

In sum, our results provide an integrated approach whereby detailed anatomical mapping and computational modeling are combined to unravel how E and I synaptic organization and its driving forces, which operate at the subcellular scale, shape cellular computations underlying spatial navigation. Specifically, it highlights the complex interplay between co-tuned activation of excitatory synapse clusters and dendritic domain properties in modulating place cell tuning. By linking such morphological features to computational outcomes, this work advances our understanding of the fundamental mechanisms through which structural and functional synaptic distributions modulate neuronal processing.

Resource availability

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Panayiota Poirazi poirazi@imbb.forth.gr

Materials availability

This study did not generate new, unique reagents.

Data and code availability

The source code that generates all simulations and Figures, as well as the data that support this study will be freely available upon publication on GitHub.

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Author contributions

P.P., A.L., F.P., S.C., D.M.I., and S.T. conceived the study. D.M.I. performed the in-utero electroporation, prepared the tissue, and acquired the confocal images. L.H. developed a script enabling image deconvolution of the raw PN images. Y.K. and D.M.I. manually edited semi-automated synaptic reconstructions and participated in data analysis.

S.T. refined the morphological traces, developed and validated the model, implemented the simulation code, and analyzed the data. S.C. and S.T. generated the input patterns. S.T. wrote the manuscript, with editing support from P.P., A.L., F.P., S.C., and D.M.I.

Declaration of interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this work, the authors used large language models (ChatGPT-4o) for spelling and grammar checks. The author(s) reviewed and edited the content as needed and take full responsibility for the final publication.

Materials and Methods

Mice

All animals were handled according to protocols approved by the Institutional Animal Care and Use Committee at Columbia University, New York. Postnatal day 98 C57BL/6 mice (strain code: 000664; Jackson Laboratory) were used for all experiments. Timed-pregnant female mice were maintained in a 12-hour light/dark cycle and obtained by overnight breeding with males of the same strain. For timed-pregnant mating, noon after mating is considered E0.5.

Constructs

The tdTomato reporter was previously generated by the Polleux lab[42]. Briefly, the tdTomato insert was subcloned into the pAAV-Ef1a-DIO eNpHR 3.0-EYFP plasmid (Addgene 26966) between the AscI and NheI cloning sites. The inhibitory synapse reporter EGFP-Gephyrin (clone P1) was obtained from H. Cline (TSRI, La Jolla, USA) and subcloned into the pUBC-DIO Teal-Gephyrin plasmid (Addgene 73918) between the AscI and NheI cloning sites.

In utero electroporation

In utero electroporation targeting the dorsal hippocampus was performed using a triple-electrode setup as previously described in E15.5 mouse embryos[13,107,108]. Endotoxin-free DNA

was injected using a glass pipette into one ventricle of the mouse embryos. Electroporation was performed at E15.5 using a square wave electroporator (ECM 830, BTX) with two anodes (positively charged) placed laterally on either side of the head and one cathode (negatively charged) rostrally at a 0° angle to the horizontal plane. The electroporation settings were: 5 pulses of 40V for 50 ms with 500 ms intervals. Plasmids were used at the following concentrations: Flex-tdTomato reporter plasmid: 1 µg/µl; Flex-EGFP-Gephyrin 0.45 µg/µl; NLS-Cre recombinase: 150 pg/µl.

Tissue preparation

Animals at the indicated age were anaesthetized with isoflurane before intracardiac perfusion with PBS and 4% PFA (Electron Microscopy Sciences). As previously described[42], 130 µm coronal brain sections were obtained using a vibrating microtome (Leica VT1200S). Sections were mounted on slides and briefly dehydrated at room temperature to reduce section thickness before being coverslipped in Fluoromount-G (SouthernBiotech).

Confocal imaging

Confocal imaging was performed as previously described[42]. Briefly, images of electroporated neurons in slices were acquired with a Nikon AXR confocal microscope equipped with a 100x, 1.49 NA Apochromat oil-immersion objective and controlled by NIS-Elements (Nikon Instruments Inc., Melville, NY). Image volumes were acquired using galvano scanning with a 1024x1024 scan region, corresponding to a lateral pixel size of 110 nm and a z-step size of 100 nm. To compensate for signal attenuation caused by tissue scattering and spherical aberration with increasing depth, laser power was linearly increased as a function of depth to normalize the mean fluorescent intensity of pixels from image planes throughout the stack[42]. Following acquisition, image stacks were deconvolved using the GPU-based Richardson-Lucy

deconvolution algorithm within NIS-Elements. Deconvolution was performed with a theoretical point spread function based on imaging acquisition parameters and used 20 iterations. Individual z-stacks were subsequently stitched together using Fiji's Grid/Collection stitching tool[109] to generate a single three-dimensional volume representing the entire neuron. Dendritic spines and inhibitory synapses were quantified based on tdTomato fluorescence and EGFP-Gephyrin puncta fluorescence, respectively. All quantifications were performed in CA1 hippocampus in sections of comparable rostro-caudal position.

Neuron trace reconstruction

Neuron traces, digital reconstructions of the dendritic tree, are an effective representation of neuronal topology and geometry. The traces described using a tree graph and consist of 3-D point coordinates, diameters, and connectivity between points. This representation enables an extensive quantitative analysis of the geometrical organization of the neurons they represent including total length, branching angles, distribution statistics and cumulative distance from the soma[110]. In this paper, the initial reconstructions are obtained using the automatic tracing methods built into the open-source 3D visualization and analysis tool Vaa3D[45]. Then, experts manually proofread the traces and make adjustments with the built-in proof-editing tools.

Synapse detection

Excitatory and inhibitory synapses were annotated with the Spine Detector and IS Detector toolkits within Vaa3D software as previously described[42]. Briefly, excitatory synapses are identified as dendritic spines segmented by the Spine Detector toolkit using the image and neuron trace as inputs[111,112]. Because the dendrite traces represent the dendrites with a series of overlapping nodes[113], information about the volume and distance from the dendrite of each

spine can be associated with its nearest node to assign a location within the spatial context of the dendritic arbor. Users have the ability to accept or reject potential spines and adjust their volume.

We labeled inhibitory synapses using the scaffolding protein Gephyrin tagged with EGFP as a marker[114]. Because these synapses can only occur on the dendrites or the spines of neurons of interest, IS Detector uses the image from the cell-fill channel containing the dendrites and the spines as a mask image to extract the relevant region for the inhibitory synaptic marker. Then, signal beyond user-input parameters for minimum/maximum voxel count and distance from the trace is excluded and potential inhibitory synapses from the resulting image are identified based on a user-input intensity threshold. Users have the ability to accept or reject potential inhibitory synapses, adjust their volume, and assign them as dendrite-targeting or spine-targeting.

Dendritic diameter correction

To improve the accuracy of the morphological data and ensure the physiological plausibility of the neuronal reconstructions, a custom smoothing algorithm was applied to correct artifactual fluctuations in dendritic diameters (**Figure S1**). This algorithm utilized a weighted moving average filter to smooth out large changes in diameter while preserving the overall structural integrity of the neuron. The filter was designed to account for local variations in diameter by applying larger weight to nearby points, reducing the influence of outliers and providing a consistent tapering pattern along the dendrites.

Prior to smoothing algorithm, a minimum diameter threshold was applied to all diameters to address the occurrence of bottlenecks. These bottlenecks could significantly impact simulations by increasing the axial resistance and altering signal propagation. The minimum

diameter threshold was domain-specific, reflecting the physiological constraints of different neuronal compartments.

Synaptic distribution analysis

Synaptic null distribution: within-domain and within-subdomain randomization

For each reconstructed morphology, synapses were randomly reassigned within branches of the same domain or sub-domain, ensuring a uniform probability distribution. Specifically, we first extracted the nodes of interest from the reconstructed arbor trace and then reassigned the annotations describing spine features to new, randomly selected locations within the same domain. This process preserved the overall structure of the arbor while randomizing synaptic positions. We repeated this procedure 1,000 times to create the null distribution, conducting the necessary analysis on each randomized dataset.

Structural cluster analysis

Putative structural clusters were identified using a hierarchical clustering algorithm applied solely to large spines, i.e., those above the 80th percentile of the spine volume distribution. For each branch, we selected the large spines and clustered them based on their relative distance from the soma (i.e., path length). Specifically, we used the SciPy hierarchical clustering linkage with *complete* method to calculate the Euclidian distance. A threshold of 10 micrometers was then used to define clusters, after which we collected their characteristics (e.g., spine count, total volume). Since the clustering was performed separately for each branch, any potential clusters spanning branching points were excluded from the analysis.

Computational Modeling

The CA1 PN models, implemented using the Python version of the NEURON simulation environment[67], and were inspired by prior work by using the ionic mechanism and their distribution throughout the dendrosomatic axis[26]. The ionic channels, written using NMODL

language, used for this project include: inward transient sodium current (I_{Na}), outward delayed rectifier potassium current (I_{Kdr}), A-type potassium current (I_A), Sodium persistent current (I_{Nap}), Hyperpolarization-activated current (I_h), Slowly activating muscarinic potassium current (I_m), Calcium current from T-type channel (I_{CaT}), Calcium current from L-type channel (I_{CaL}), Calcium current from R-type channel (I_{CaR}), Slow calcium-dependent potassium current (I_{sAHP}), Medium calcium-dependent potassium current (I_{mAHP}). Minor modifications were implemented in the kinetics and distribution of some channels. To facilitate the rapid and reproducible generation of models, we developed a Python Class capable of processing neuronal morphologies in .swc file format and automatically incorporating the appropriate biophysical properties and channel distributions.

To ensure high spatial resolution in the simulations, we determined the number of segments ($\mathrm{n}_{\mathrm{seg}}$) for each neuronal section using the lambda rule, which is based on the Alternating Current (AC) length constant (λ). A ten-fold higher frequency was used in the calculation to obtain a finer spatial discretization of each section. Specifically, we employed the following equation (Eq. 1):

$$\mathrm{n}_{\mathrm{seg}} = 2 \cdot \left\lfloor \frac{\frac{L}{d_\lambda \cdot \lambda_f} + 0.999}{2} \right\rfloor + 1 \ (1)$$

where L is the length of the section, d_λ the fraction of the AC length constant (typically 0.1), $\lambda_f f$ the AC length constant at the chosen frequency f, and $\lfloor\cdot\rfloor$ denotes a function that converts a specified value into an integer number. This approach ensured that all segments were small enough to disentangle inputs coming from most of nearby synapse couples, providing accurate spatial and biophysical representation of the model.

Modeling Synapses

We incorporated both glutamatergic (excitatory) and GABAergic (inhibitory) synapses. All dendritic spines identified in the reconstructed neurons were assumed to be excitatory synapses containing both AMPA and NMDA receptors, while inhibitory synapses were modeled as $GABA_A$-mediated inputs. Synaptic strength was adjusted based on the synapse size measured from the original reconstruction and, for a subset of excitatory synapses, by the distance from the soma. Specifically, for excitatory synapses, validated conductance values for both NMDAR and AMPAR components were first calculated. The AMPA conductance was then scaled by a volume-dependent scaling factor (Eq. 2). Both AMPA and NMDA components for synapses in Basal, Trunk and Oblique compartments were further adjusted by a distance-dependent scaling factor[71,73]. In contrast, inhibitory synapses were scaled solely by the volume-dependent factor (Eq. 3).

$$AMPA\ Volume\ scaling = 0.6 \cdot \frac{Spine\ volume_i}{Average\ spine\ volume} + 0.4 \quad (2)$$

$$GABA\ Volume\ scaling = \frac{PSD\ volume_i}{Average\ PSD\ volume} \quad (3)$$

AMPA & $GABA_A$ definition

AMPA and $GABA_A$ synapses were simulated with a two-state kinetic model, defined by rise and decay times. The synaptic current is calculated according to the following equations (Eqs. 4, 5):

$$I_{syn} = \mathrm{g}_{syn}(t) \cdot \left(V_m - E_{syn}\right), syn\ \in \{\mathrm{AMPA}, \mathrm{GABA_A}\} \quad (4)$$

$$\mathrm{g}_{syn}(t) = f_{norm} \cdot \bar{\mathrm{g}}_{peak} \cdot \left(\exp\left(-\frac{t}{\tau_d}\right) - \exp\left(-\frac{t}{\tau_r}\right)\right) \quad (5)$$

where I_{syn} is the synaptic current, V_m the membrane potential, g_{syn} the receptor conductance, g_{peak} the peak conductance and E_{syn} its reverse potential. τ_r and τ_d represent the rise and

decay time constants, respectively. The factor f_{norm} normalizes the difference of exponentials so that the peak of g_{syn} equals $\bar{\mathrm{g}}_{peak}$ and its calculated using the formula below (Eq. 6).

$$f_{norm} = \frac{1}{\mathrm{g}_{syn}(t_{peak})}, t_{peak} = \frac{\tau_d \tau_r}{\tau_d - \tau_r} \ln \frac{\tau_d}{\tau_r} \quad (6)$$

NMDA definition

The NMDA component of the excitatory synapses was also simulated as a two-state kinetic model but with an additional factor that accounts for the voltage dependent removal of the Magnesium blockage. The mechanism is described by the following equations (Eqs. 7, 8):

$$I_{NMDA} = \mathrm{g}_{NMDA}(t) \cdot \ h(V_m) \cdot (V_m - E_{NMDA}) \quad (7)$$

$$h(V_m) = \frac{1}{1 + \eta[\mathrm{Mg}^{2+}]_o \exp\left(-\gamma\left(V_m - V_{half}\right)\right)} \quad (8)$$

where $h(\cdot)$ is a sigmoid function, η denotes the Magnesium sensitivity of unblock, $[\mathrm{Mg}^{2+}]_o$ the Magnesium extracellular concentration, γ the slope factor that determines how steep the transition is around the midpoint, and V_{half} the membrane potential of the midpoint of the sigmoid. $\mathrm{g}_{NMDA}(t)$ is calculated with the same equations as the other synaptic currents (Eqs. 5, 6).

Pyramidal neuron model validation

The parameters that defined the biophysical properties and channel distributions were carefully validated to match the cell's behavior with experimental data. We ensured that using the same values for all morphologies would still adhere to the experimental values for each cell, while also introducing variability expected in a heterogeneous population of pyramidal

neurons. We used hyperpolarizing step current sweeps to validate passive properties as the input resistance ($\mathrm{R_{in}}$), membrane time constant (τ_m), sag ratio and the dendritic attenuation, following the protocols illustrated in **Magee (1998)**[68]**, Golding et al. (2005)**[69] for the voltage attenuation and **Masurkar et al. (2020)**[8] for $\mathrm{R_{in}}$, τ_m and sag ratio. We then used a depolarizing step current sweeps to validate the frequency-input (F-I) curve and the adaptation from **Masurkar et al. (2020)**[8]. For the synaptic strength validation, we followed the protocol used in **Magee and Cook, 2000**[71], stimulating average size synapses at increasing distance from the soma and maintaining the 0.2 mV somatic depolarization observed experimentally.

Inputs design

Grid-like and Place-like inputs

In accordance with the formulation described in **Shuman et al. (2020)**[115], entorhinal cortex layer III (ECIII) grid-like inputs were modeled as the summation of three sinusoidal wave components, following the approach of **Solstad et al. (2006)**[116]. In contrast, place-like inputs from CA3 were generated by integrating these grid-like inputs. Specifically, grid-cell activity was represented as the sum of three two-dimensional sinusoidal gratings, each defined by a distinct wave vector. The resulting grid-like function was computed as (Eq. 9):

$$\mathrm{g}_w(x,y) = \frac{2}{3}\left(\frac{1}{3}\sum_{i=1}^{3}\cos\left(k_i\,(r-r_0)\right)+\frac{1}{2}\right) \quad (9)$$

where the parameter set $w = (\lambda, \theta, r_0)$ defines the grid spacing λ, orientation θ, and the spatial location of a theoretical place cell $r_0 = [x_0, y_0]^{\mathrm{T}}$, while the variable $r = [x, y]^{\mathrm{T}}$ represents the current position, and k denotes the wave vectors arranged at angular intervals of $\frac{\pi}{3}$ and $\frac{2\pi}{3}$, respectively (Eq. 10).

$$k_i = \frac{1}{\lambda}\left[\cos\left(\theta + (i-1)\frac{\pi}{3}\right), \sin\left(\theta + (i-1)\frac{\pi}{3}\right)\right]^{\mathrm{T}} \quad (10)$$

These grid-like functions were used to compute the probability of spike generation as a function of the animal's position and the theoretical place fields. To incorporate theta rhythmic modulation, a sinusoidal filter was applied to these probabilities (Eq 11):

$$p(t) = \mathrm{g}_w(x,y)\frac{\sin\left(2\pi \cdot f_\theta \cdot \left(\frac{t}{1000}\right) + \varphi_{theta}\right) + 1}{2} \quad (11)$$

where $f_\theta = 8\mathrm{Hz}$ and $\phi_\theta = 0^o$ correspond to the theta-cycle frequency and phase, respectively, and t is expressed in milliseconds. A spike was generated for a given grid cell whenever $p(t)$ exceeded a random value sampled from a normal distribution with mean $\mu = 0.7$ and standard deviation $\sigma = 0.05$ or a random value from a uniform distribution used to introduce baseline noise. This process was repeated across all grid-like cells to construct the entire set of theoretical place fields.

To model CA3 place-like inputs, we combined the generated grid-like fields from EC inputs, ensuring proper gamma filtering and theta-phase locking. Similarly to EC inputs, a spike was generated when the probability $p(t)$ was greater than a normally distributed threshold value or a noise baseline. Additionally, a 17 ms delay was introduced to account for the natural temporal delay between EC and CA3 input streams. Finally, each spike train was filtered to a function that simulate the effect of synaptic failure based on published data[117].

Noisy inputs

To account for non-place-tuned inputs, we generated a set of noisy inputs modeled as Poisson-distributed spike trains with low baseline firing rates. To introduce temporal

fluctuations, these spike trains were modulated by applying a Gaussian filter at randomly selected time points, transiently increasing the firing rate. In our approach, the probability for a spike to occur was (Eq. 12):

$$p(t) = O + H \cdot \exp\left(\frac{\left(t - t_{peak}\right)^2}{2\sigma^2}\right) \quad (12)$$

where O represents the baseline offset of the gaussian curve, H is the peak amplitude, t is the time at which the spike occurred and t_{peak} is a randomly chosen value corresponding to the mean value of the Gaussian filter. The standard deviation σ is also chosen randomly in a fixed window of values (400 ms, 720 ms) and represents the width of the window with slightly higher firing frequency.

This process ensured that noisy inputs exhibited sporadic increases of activity while maintaining an overall unstructured firing pattern, effectively capturing the background synaptic activity present in the hippocampal CA1 place cells. Similarly to place tuned inputs, the noisy spike trains were further processed to account for synaptic failure.

Inhibitory inputs

Inhibitory inputs were modeled as theta-modulated, gamma-filtered Poisson-distributed spike trains, with firing properties tailored to different interneuron subtypes as described in **Bezaire et al., 2016**[76]. To generate these spike trains, we set baseline firing rates according to known physiological values for each interneuron type. These spike trains were then filtered using a theta-modulated function, ensuring that their activity exhibited phase locking to the theta cycle at subtype-specific phases (Eq. 11).

Additionally, a gamma-frequency filtering step was applied to refine the temporal structure of the remaining spikes. The level of randomness was controlled by introducing a noise parameter, which varied across interneuron types to reflect their intrinsic firing variability.

Delays of 300 ms were incorporated to approximate transmission latencies. Specifically, inputs were tuned to replicate the activity of different interneurons neurogliaform (NGF) cells, oriens-lacunosum moleculare (OLM) cells, parvalbumin-expressing basket (PVbasket) cells, apical dendrite targeting cholecystokinin-expressing (CCK) cells, Bistratified cells, and Ivy cells with peak firing rates ranging from 2.8 Hz to 30 Hz, distinct theta-phase preferences, and varying degrees of noise, capturing the diversity of inhibitory influences in the hippocampal network.

Input distribution

Synaptic inputs were distributed in an anatomically accurate manner. For inhibitory synapses, their connection probabilities to each interneuron subtype were based on estimates from **Bezaire and Soltesz (2013)**[118], with slight upward adjustments to account for missing interneuron types. While NGF and OLM inputs were uniformly distributed across tuft synapses, the distribution of other interneurons followed a nonlinear function of the distance from the soma. This distribution pattern was informed by anatomical observations while maintaining symmetry between the apical and basal dendritic domains. Specifically, PV basket synapses were concentrated near the soma, while CCK and Ivy cells targeted more distal synapses in the apical and basal domains, respectively. Bistratified cell synapses were predominantly located in the middle regions of both domains (**Figure S2**).

ECIII grid-like inputs were directed to synapses on the tuft dendrites in the stratum lacunosum-moleculare, whereas CA3 place-like inputs primarily targeted synapses in the trunk, oblique, and basal dendritic domains, spanning the stratum oriens and stratum radiatum. At the branch level, place-tuned inputs were assigned to both clustered and dispersed synapses. Using GluSnFR-based experimental data from **Adoff et al. (2021)**[39] as a reference, we determined the probability of each synapse to receive place-tuned inputs, guided by the observed ratios of clustered vs. dispersed synapses and the relative distributions in the apical vs. basal domains.

The total number of activated clustered synapses, tuned to the somatic place field, was randomly determined using a transformation of a Gaussian-distributed variable (Eq. 13):

$$n_{in} = r \cdot \frac{n_{cs}}{n_{pf}}, r = 0.9 + \frac{z^{1.5}}{3} \quad (13)$$

Where n_{in} represents the number of clustered in-field tuned synapses, n_{cs} and n_{pf} represent the number of clustered synapses and the number of place fields, respectively. z is a normally distributed random variable with mean 0 and standard deviation 1, so that $z \sim \mathcal{N}(0,1)$, keeping only the positive values. The range r is limited to values lower than 1.6.

The Gaussian shape was chosen to replicate the distribution of place field properties observed at the population level by **Mizuseki et al. (2012)**[84]. Clustered synapses assigned to the remaining 11 place fields were selected randomly with a fixed probability of (Eq. 14):

$$n_{out} = 0.4 \cdot \frac{n_{cs}}{n_{pf}} \quad (14)$$

Where n_{out} is the number of clustered out-field tuned synapses. Dispersed synapses were also randomly selected with fixed probabilities, determining whether they would receive place-tuned or noisy inputs. To account for biological variability, we generated a set of 1,000 distinct connectivity patterns for each of the five morphologies, ensuring a realistic representation of network heterogeneity.

Inputs Shuffling

To investigate the role of synaptic clustering in shaping neuronal responses, we implemented three distinct synaptic input shuffling protocols that redistributed the incoming place-tuned inputs while preserving specific structural or functional properties of the original configuration. In the Shuffled – Same Number (SSN) condition, for each dendritic domain, we first computed the number of synapses activated in the control (i.e., clustered) configuration. The same input

patterns were then reassigned to an equal number of randomly selected synapses within the same domain, thereby preserving synapse count but disrupting spatial clustering.

In the Shuffled – Same Volume (SSV) protocol, we calculated the total volume of all synapses activated per place field in the control configuration. Synaptic locations were then randomly selected until their cumulative volume matched that total volume. As this procedure typically resulted in a greater number of activated synapses, due to the inclusion of smaller synapses, the set of input spike trains used was expanded by sampling from the pool of inputs applied in the original, clustered configuration.

The Shuffled – Same Weight (SSW) protocol further refined this approach by summing the total synaptic weight from the control configuration, accounting for both the synaptic volume and the synaptic democracy principle (i.e., equalization of synaptic impact regardless of location). Synapses were then randomly selected until their combined weights matched the original total. Although this method also led to an increased number of activated synapses compared to the control, the number was lower than that observed in the SSV condition.

Place cell quantification

To quantify the spatial tuning properties of the simulated neurons, we computed the firing rate maps for each cell across the linear track, and used them to calculate the various place field features. The Field Size identifies the spatial extent of contiguous bins around the peak firing location where the firing rate remains above a given relative threshold. Once the Place Field width was characterized, we determined the mean firing rate within and outside the identified field region, respectively named In-field and Out-field Firing Frequency, respectively. In addition, spatial selectivity was assessed using a modified version of the spatial information index[83]. This metric calculates the information content of firing in bits per second by comparing the firing rate in each spatial bin to the mean firing rate across the track, weighted by

occupancy probability, i.e., time spent in each spatial bin. The time spent in each bin was used to normalize firing rates, and the spatial information was computed (Eq. 15) as:

$$Spatial\ Info = \sum_{i=1}^{N_{bins}} p_i \cdot \frac{\lambda_i}{\lambda} \cdot \log_2\left(\frac{\lambda_i}{\lambda}\right) \quad (15)$$

Where p_i is the occupancy probability of bin i, λ_i is the firing rate in that bin and λ is the mean firing rate across all bins, N_{bins}.

Multivariate Random Forest

To assess whether the structural features of synaptic clusters could predict the functional output of the simulated neurons, we employed a supervised machine learning approach using a Random Forest regression model. Specifically, we trained a multi-output regression model to predict place field properties (i.e., Spatial Information, In-field and Out-of-field Firing Frequency, and Field Size) from cluster-derived input features (i.e., total in-field cluster volume, average in-field cluster size, and the proportion of dendritic branches containing in-field clusters). These features were normalized to [0,1] range using a Min-Max Scaler to ensure comparability across dimensions. The target output matrix comprised the aforementioned spatial metrics, which were likewise scaled prior to training. For the analysis we used simulations conducted under the Control allocation protocol, excluding those that exhibited overflow or contained missing values.

We used a Multi-Output Regressor wrapping a Random Forest Regressor (100 estimators per model). Models were trained and evaluated over 10 independent runs using an 80/20 train-test split, with mean squared error used to assess performance across both individual outputs and overall predictions. Control protocols were validated against two baselines: one involving permutation of the input features (Random training inputs, not shown) and another involving selective removal of key input features (Removal input feature, not shown) to confirm the

robustness and informativeness of the cluster descriptors. Feature importance values were computed as the average importance across all estimators within the ensemble.

SHAP-Based Feature Attribution Analysis

To investigate the contribution of synaptic cluster features to the spatial coding properties of simulated neurons, we employed SHAP (*SHapley Additive exPlanations*), a game-theory approach for interpreting machine learning models[88]. We computed SHAP values using the *TreeExplainer* provided by the SHAP Python library, which is optimized for tree-based models. SHAP values were calculated on the training set to ensure consistency with the model fitting. The *summary_plot()* function was used to visualize both the magnitude and direction of each feature's effect. Feature values were color-coded to indicate their relative magnitude (red for high values, blue for low values), enabling intuitive interpretation of whether increasing a specific feature tended to increase or decrease a given output.

To complement the qualitative visual inspection, we also computed the Pearson correlation between feature values and their corresponding SHAP values. This provided a numerical estimate of the directionality and strength of influence each feature exerted on the model output.

Simulation environment and statistical analysis

All simulations were performed on our High-Performance Computing Cluster (Rocks 7.0) with 624 cores and 3.328 TB of shared RAM under CentOS 7.4 OS[119], through the NEURON simulator[120] using Python via an Anaconda virtual environment. Neuronal models have been simulated at a sampling rate of 40 kHz (dt = 0.025 ms). For all standard statistical tests (detailed in all relevant figure legends), the α was chosen to be 0.05 for statistical significance. To correct for multiple comparisons, the α was divided by the number of tests according to the

Bonferroni's procedure. Throughout the figures, p values are denoted by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

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Supplementary figures

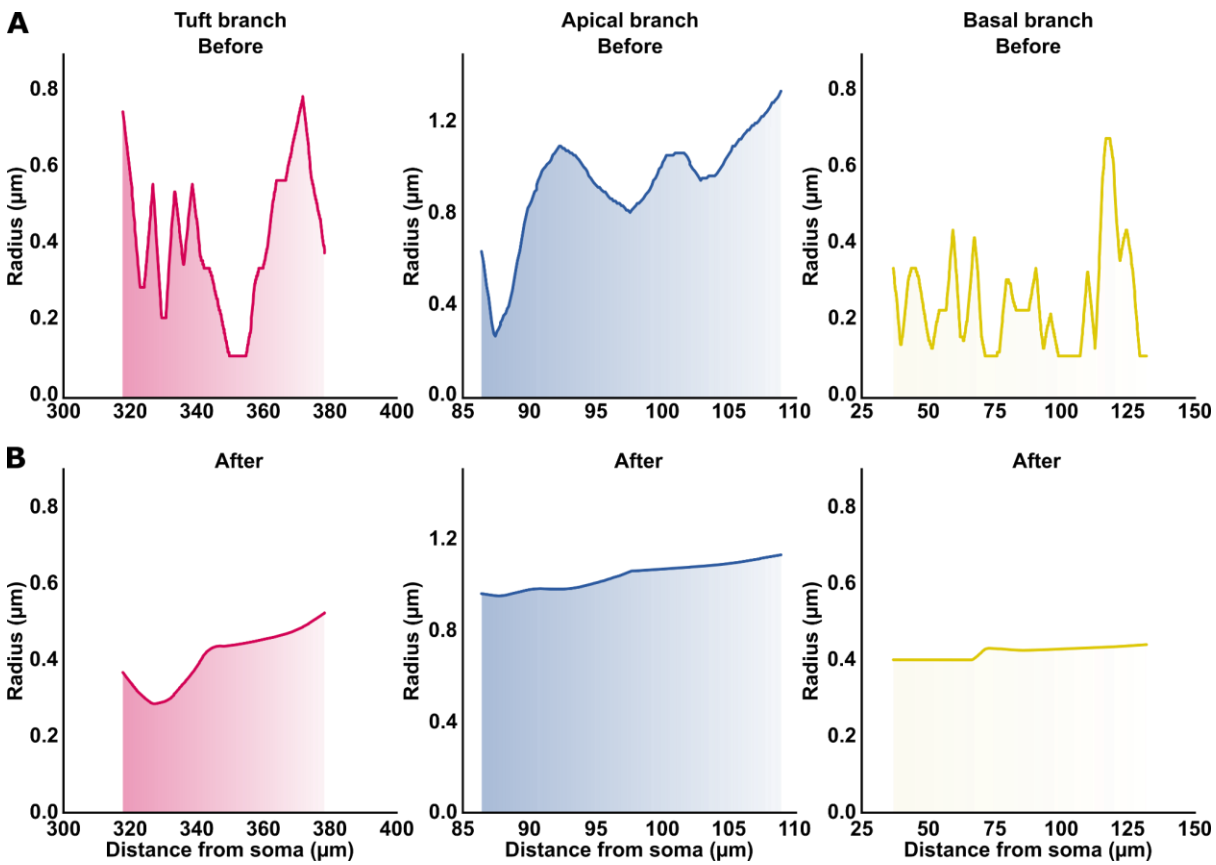


Figure S1: Dendritic diameter smoothing. *Radius profiles of three example dendritic branches before (A) and after (B) application of the smoothing algorithm. Profiles are shown for Tuft (left), Apical (center), and Basal (right) branches, with radius plotted as a function of the distance from the soma.*

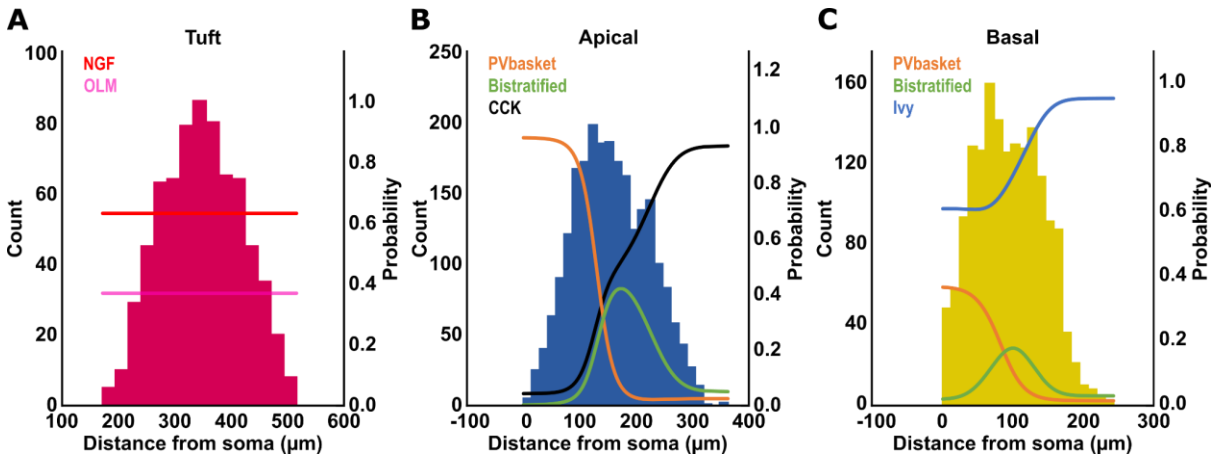


Figure S2: Probability of inhibitory input sources targeting synapses in CA1 pyramidal cells. *Distribution of inhibitory presynaptic input probabilities for synapses located on the Tuft (A), Apical (B), and Basal (C) dendrites. The probability varies with dendro-somatic path distance, capturing how different interneuron types preferentially target specific dendritic regions. Histograms show the number of synapses as a function of distance from the soma, while the overlaid curves represent the probability that a synapse is targeted by each interneuron type.*

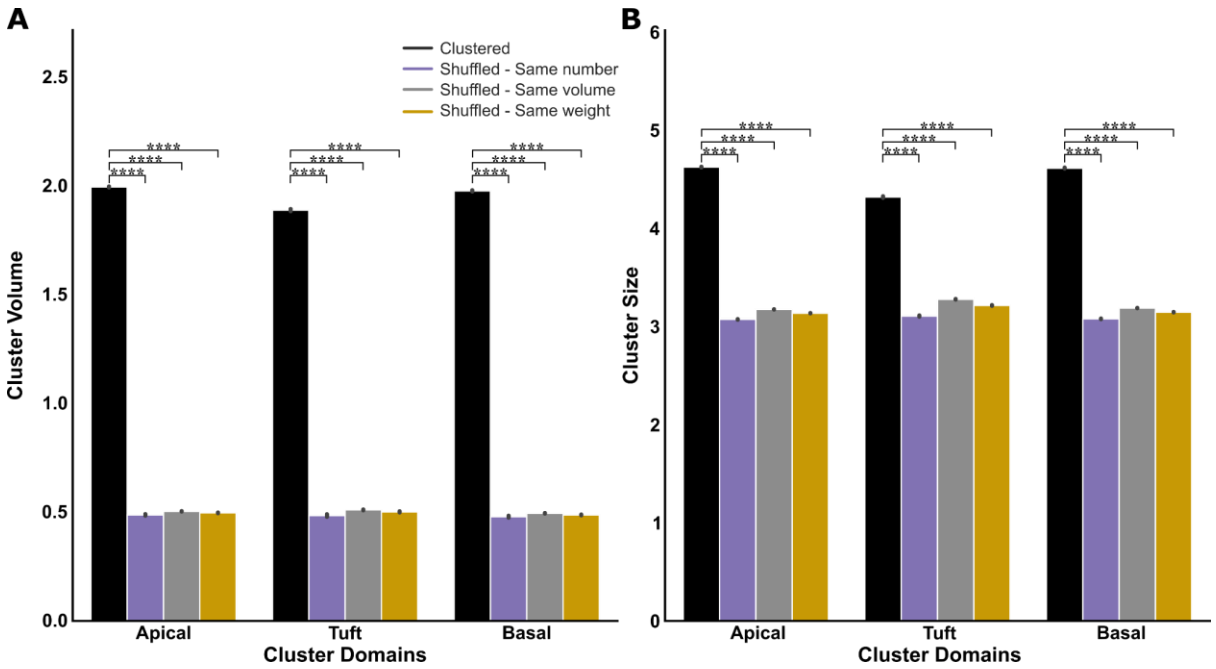
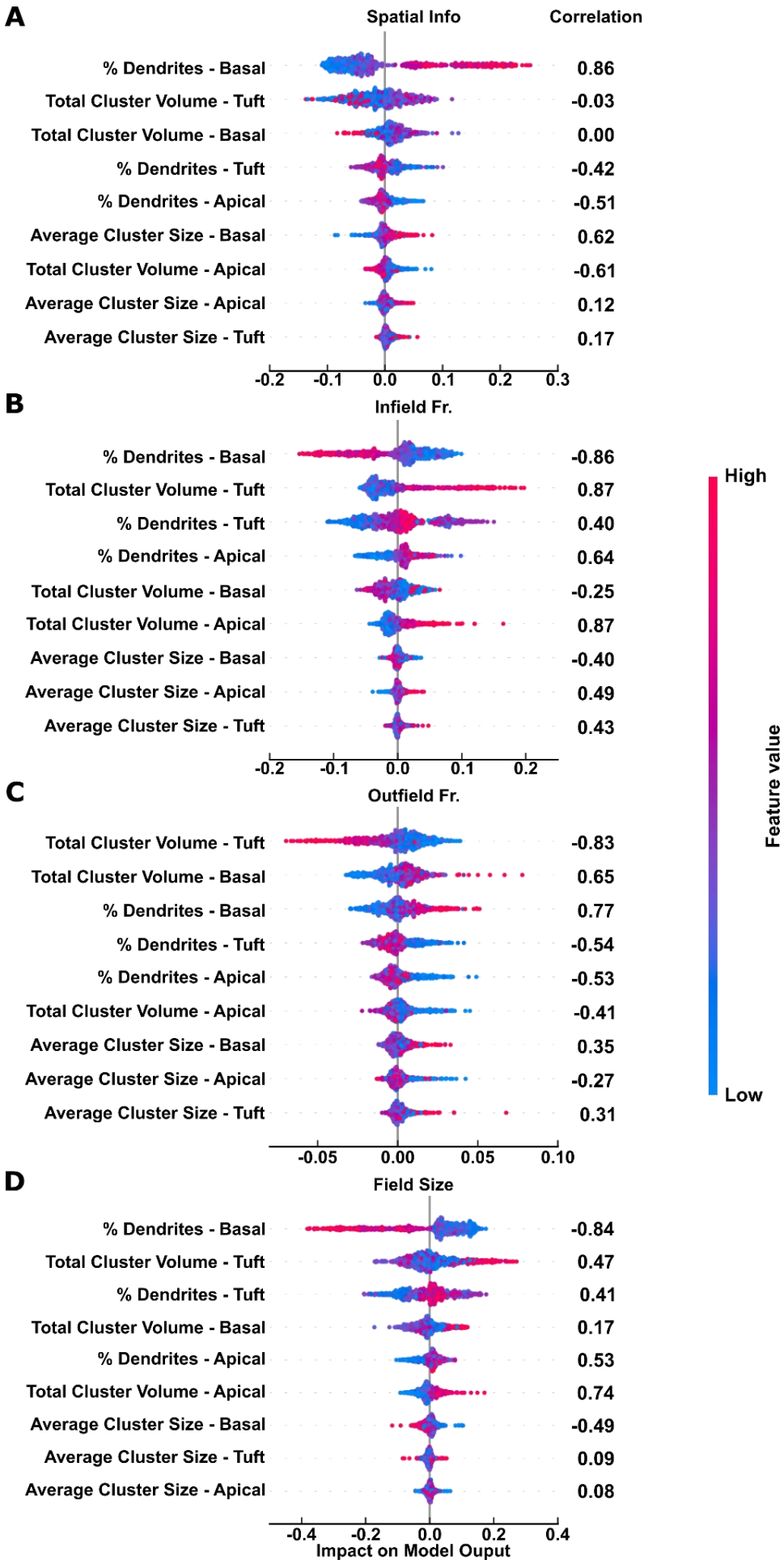
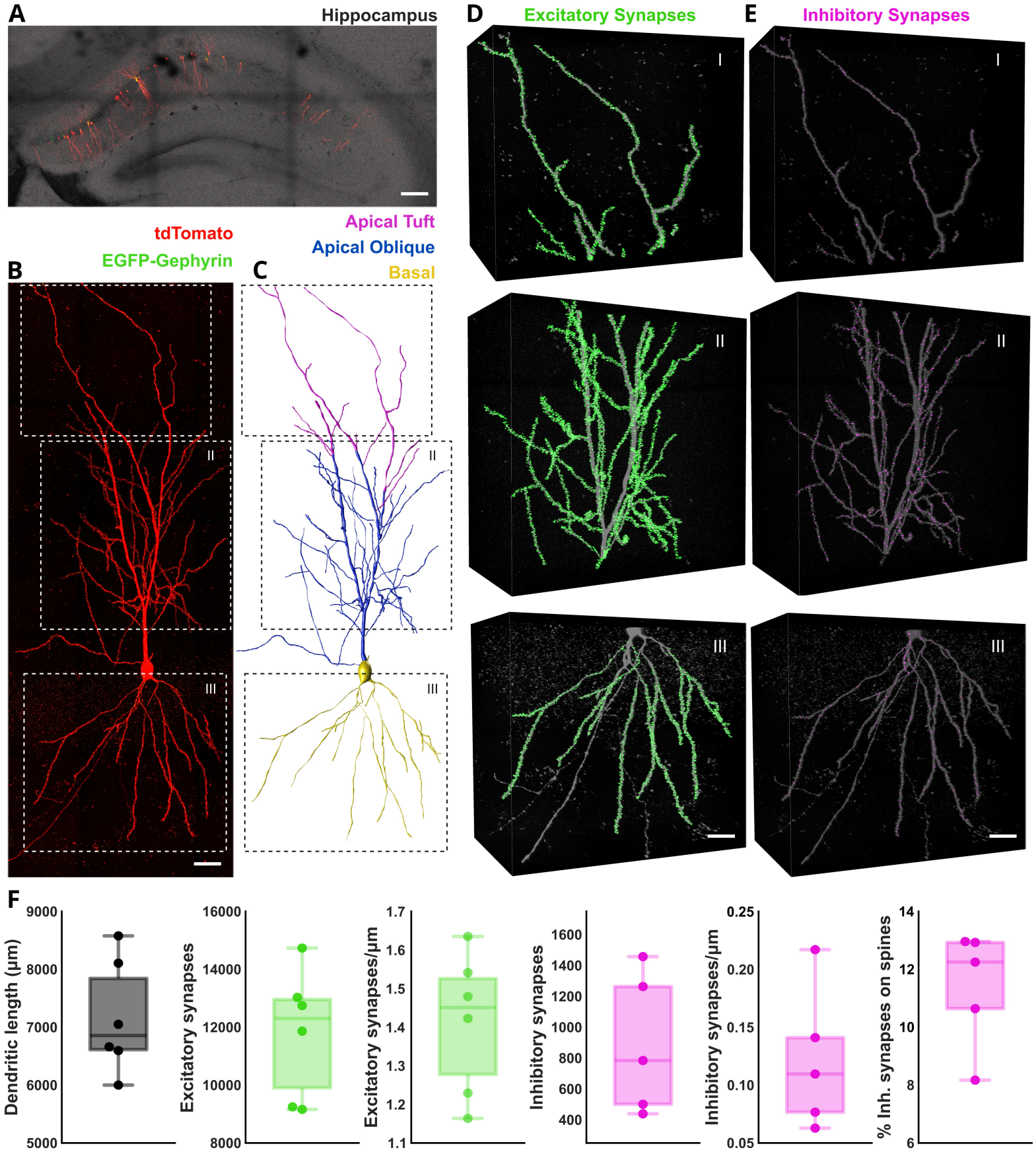


Figure S3: Characterization of synaptic clusters under alternative input allocation protocols. *Cluster volume (A) and size (B) were significantly reduced following synaptic shuffling across all protocols. Statistically significant differences were observed between the control configuration and each of the shuffled conditions, with variation maintained across dendritic domains. Pairwise t-tests between control and shuffled protocols are indicated in the plots (*p < 0.05, **p < 0.005, ***p < 0.001, and ****p < 0.0001), with corrections for multiple comparisons. Error-bars represent the 95% confidence interval (CI) of the mean.*


A
Spatial Info
Correlation
% Dendrites - Basal
Total Cluster Volume - Tuft
Total Cluster Volume - Basal
% Dendrites - Tuft
% Dendrites - Apical
Average Cluster Size - Basal
Total Cluster Volume - Apical
Average Cluster Size - Apical
Average Cluster Size - Tuft
0.86
-0.03
0.00
-0.42
-0.51
0.62
-0.61
0.12
0.17
-0.2 -0.1 0.0 0.1 0.2 0.3
B
Infield Fr.
% Dendrites - Basal
Total Cluster Volume - Tuft
% Dendrites - Tuft
% Dendrites - Apical
Total Cluster Volume - Basal
Total Cluster Volume - Apical
Average Cluster Size - Basal
Average Cluster Size - Apical
Average Cluster Size - Tuft
-0.86
0.87
0.40
0.64
-0.25
0.87
-0.40
0.49
0.43
-0.2 -0.1 0.0 0.1 0.2
C
Outfield Fr.
Total Cluster Volume - Tuft
Total Cluster Volume - Basal
% Dendrites - Basal
% Dendrites - Tuft
% Dendrites - Apical
Total Cluster Volume - Apical
Average Cluster Size - Basal
Average Cluster Size - Apical
Average Cluster Size - Tuft
-0.83
0.65
0.77
-0.54
-0.53
-0.41
0.35
-0.27
0.31
-0.05 0.00 0.05 0.10
D
Field Size
% Dendrites - Basal
Total Cluster Volume - Tuft
% Dendrites - Tuft
Total Cluster Volume - Basal
% Dendrites - Apical
Total Cluster Volume - Apical
Average Cluster Size - Basal
Average Cluster Size - Tuft
Average Cluster Size - Apical
-0.84
0.47
0.41
0.17
0.53
0.74
-0.49
0.09
0.08
-0.4 -0.2 0.0 0.2 0.4
Impact on Model Ouput
High
Feature value
Low

1382

Figure S4. SHAP summary plots for feature impact on spatial coding metrics. *SHAP value distributions for the prediction of four output features: (A) spatial information (bits/s), (B) in-field firing frequency (Hz), (C) out-of-field firing frequency (Hz), and (D) place field size (cm). Each dot represents a SHAP value for a given sample, with its color indicating the relative value of the corresponding input feature (blue = low, red = high). Features are ranked by their mean absolute SHAP value, reflecting their overall contribution to model output (Feature Importance). The numerical values on the right of each feature represent the Pearson correlation between the input feature values and their associated SHAP values, providing an estimate of the direction and linearity of the feature's effect on each output metric.*


A
Hippocampus
B
tdTomato
EGFP-Gephyrin
C
Apical Tuft
Apical Oblique
Basal
D
Excitatory Synapses
E
Inhibitory Synapses
I
II
III
F
Dendritic length (µm)
9000
8000
7000
6000
5000
Excitatory synapses
16000
14000
12000
10000
8000
Excitatory synapses/µm
1.7
1.6
1.5
1.4
1.3
1.2
1.1
Inhibitory synapses
1600
1400
1200
1000
800
600
400
Inhibitory synapses/µm
0.25
0.20
0.15
0.10
0.05
% Inh. synapses on spines
14
12
10
8
6