Comprehensive Estimates of Potential Synaptic Connections in Local Circuits of the Rodent Hippocampal Formation by Axonal-Dendritic Overlap

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A quantitative description of the hippocampal formation synaptic architecture is essential for understanding the neural mechanisms of episodic memory. Yet the existing knowledge of connectivity statistics between different neuron types in the rodent hippocampus only captures a mere 5% of this circuitry. We present a systematic pipeline to produce first-approximation estimates for most of the missing information. Leveraging the www.Hippocampome.org knowledge base, we derive local connection parameters between distinct pairs of morphologically identified neuron types based on their axonal-dendritic overlap within every layer and subregion of the hippocampal formation. Specifically, we adapt modern image analysis technology to determine the parcel-specific neurite lengths of every neuron type from representative morphologic reconstructions obtained from either sex. We then compute the average number of synapses per neuron pair using relevant anatomic volumes from the mouse brain atlas and ultrastructurally established interaction distances. Hence, we estimate connection probabilities and number of contacts for >1900 neuron type pairs, increasing the available quantitative assessments more than 11-fold. Connectivity statistics thus remain unknown for only a minority of potential synapses in the hippocampal formation, including those involving long-range (23%) or perisomatic (6%) connections and neuron types without morphologic tracings (7%). The described approach also yields approximate measurements of synaptic distances from the soma along the dendritic and axonal paths, which may affect signal attenuation and delay. Overall, this dataset fills a substantial gap in quantitatively describing hippocampal circuits and provides useful model specifications for biologically realistic neural network simulations, until further direct experimental data become available.

Key words: CA1; CA3; dentate gyrus; entorhinal cortex; interneuron; network

Significance Statement
The hippocampal formation is a crucial functional substrate for episodic memory and spatial representation. Characterizing the complex neuron type circuit of this brain region is thus important to understand the cellular mechanisms of learning and navigation. Here we present the first numerical estimates of connection probabilities, numbers of contacts per connected pair, and synaptic distances from the soma along the axonal and dendritic paths, for more than 1900 distinct neuron type pairs throughout the dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex. This comprehensive dataset, publicly released online at www.Hippocampome.org, constitutes an unprecedented quantification of the majority of the local synaptic circuit for a prominent mammalian neural system and provides an essential foundation for data-driven, anatomically realistic neural network models.

Introduction
The hippocampal formation is a group of cytoarchitectonically distinct adjoining subregions linked by a largely unidirectional neuronal pathway (Andersen et al., 1971): the dentate gyrus (DG), cornu ammonis (CA3, CA2, and CA1), subiculum (Sub), and entorhinal cortex (EC). Like other cortical areas, the hippocampus is characterized by a high degree of neuronal interconnectivity and considerable cellular diversity. A single neuron is typically targeted by thousands of afferents from multiple neuron types, whereas the efferent output of an individual neuron contacts thousands
of postsynaptic neurons of multiple types (Halasy et al., 1996; Ali et al., 1999; van Strien et al., 2009).

Proper hippocampus functioning requires each subregion to integrate the received information before propagating it to adjacent areas (Freund and Katona, 2007; Pelkey et al., 2017; Nilsen et al., 2018). The local circuits of all hippocampal subregions are comprised of a glutamatergic principal neuron type and a wide range of largely GABAergic interneurons, which regulate neural activity mainly by feedback inhibition and local axon input (Sohal et al., 2009; Quattrocchio and Maccarferi, 2014; Schmidt-Hieber et al., 2017). Many interneurons can subserve both mechanisms, thus providing a functional link between afferent input patterns and resulting outputs (Bartos et al., 2010; Chamberland et al., 2010; Tyan et al., 2014). Neuron types exhibit distinct axonal and dendritic laminar distributions, defining specific connectivity patterns (Freund and Buzsáki, 1996; Klausberger and Somogyi, 2008; Booker and Vida, 2018). For example, CA1 oriens-lacunosum moleculare (O-LM) interneurons innervate only the apical tuft of pyramidal cells (PCs) in stratum lacunosum-moleculare (SLM) and receive inputs only in stratum oriens (SO) (McBain et al., 1994; Losonczy et al., 2002; Zemankovics et al., 2010).

Local interneuron circuits can generate recurrent activity patterns and transmit these rhythms onto principal cells for broader network propagation (Tukkér et al., 2007; Gulyás et al., 2010; Quilichini et al., 2010). These oscillations underlie mechanisms of spatial navigation (Ekstrom et al., 2003; Colgin, 2016) and memory (Bauer et al., 2007; Jensen et al., 2007), implicating various interneuron types in different roles (Csicsvari et al., 2003; Szabó et al., 2010). Several extrinsic afferent pathways from the brainstem and forebrain nuclei also provide neuron type-selective modulation (Halasy and Somogyi, 1993; Witter et al., 2017; Kinnavane et al., 2018), powerfully shaping any subsequent output.

The online knowledge base www.Hippocampome.org identifies >120 neuron types throughout the hippocampal formation (Wheeler et al., 2015) based on axonal and dendritic morphology, intrinsic and synaptic electrophysiology (Komendantov et al., 2019; Venkadesh et al., 2019), and molecular expression (White et al., 2020). This public resource links each property to available experimental evidence annotated from the peer-reviewed literature (Hamilton et al., 2017).

Despite the wealth of knowledge regarding the rodent hippocampus, quantitative estimates of connection probabilities among specific neuron types are extremely sparse, accounting for only 5% of the estimated 3120 pairs of potentially connected neuron types (“potential connections”). Local circuits make up more than three-fourths of all potential connections in the hippocampal formation (Rees et al., 2016). Although much of these data can be derived in principle from electron microscopy, the painstaking amount of time, effort, and resources required for that approach has so far limited the connectivity quantification to a minor proportion of the circuit (Megías et al., 2001; Mishchenko et al., 2010; Schmidt et al., 2017).

Here we present a data-driven pipeline to estimate most local connection probabilities among www.Hippocampome.org neuron types. Using a custom image processing approach, we quantify the layer-specific distributions of axonal and dendritic lengths for >200 representative neuron morphology reconstructions from all subregions of the hippocampal formation. From these data, in conjunction with available volumes for each anatomic parcel and ultrastructural reports of synaptic interaction distances, we estimate the probability of spatial overlap between potential presynaptic axons and postsynaptic dendrites. Hence, we calculate the average number of synapses for almost 2000 pairs of neuron types (see Fig. 1) as well as their mean synaptic distances from the soma along the axonal and dendritic paths.

**Material and Methods**

**Data sources.** We analyzed 1-5 reconstructed morphologies per identified neuron type in www.Hippocampome.org (for representative examples, see Fig. 2A): 35 reconstructions for 18 neuron types in DG, 35 for 22 neuron types in CA3, 8 for 5 neuron types in CA2, 85 for 40 neuron types in CA1, 6 for 3 neuron types in Sub, and 56 for 27 neuron types in EC. Each two-dimensional reconstruction corresponds to a published neuronal tracing image in the peer-reviewed literature (detailed in Extended Data Figs. 2-1, 2-2, 2-3, 2-4, 2-5, 2-6; Gupta et al., 2012; Liu et al.,
With sometimes multiple reconstructions coming from the same publication. We selected the reconstructions based on five inclusion criteria: (1) the neuron type was present in www.Hippocampome.org version 1.7 to

Figure 2. Morphologic reconstructions of hippocampal neuron types and their image processing. Details regarding the full dataset and sources are reported in Extended Data Figures 2-1–2-10. A, Representative tracings of selected neuron types from different subregions of the hippocampal formation: black represents glutamatergic; gray represents GABAergic. A1, DG HIPP (Hosp et al., 2014); A2, CA3 Granule (Szabadics et al., 2010); A3, EC LII Basket Multipolar Interneuron (Tahvildari et al., 2012). B, Exemplary cases of image processing for pixel count and neurite path tracing. B1, CA1 Basket cell reconstruction (S. Y. Lee et al., 2011): red represents axons; black represents dendrites. B2, Extraction of the SR (yellow frame) dendrites from the original image. B3, Estimation of mean distance from the soma along the dendritic path. We traced and averaged up to five dendrites per parcel. C, Connection probability estimation. C1, CA1 LMR-Projecting presynaptic interneuron. Reproduced from Klausberger et al. (2005). C2, CA1 Neurogliaform postsynaptic interneuron. Reproduced from Price et al. (2005). C3, Axonal-dendritic overlap in SO: green represents bistratified axons; blue represents O-LM dendrites. Orange sphere represents the zoomed-in volume of interaction (radius = 2 μm). A1, A2 and A3 100 μm; A3, B1–B3, 50 μm, C2 and C3 25 μm. B, C, Images have been modified from the originals to enhance colors.
obtain maximum coverage of the knowledge base; (2) the image contained a calibration scale bar and clear demarcations of relevant layer and subregional boundaries; (3) the traced dendritic and axonal distributions as well as soma location corresponded to the textual description in the source publication; (4) the reconstruction included both axons and dendrites, except for perisonmatic-targeting neuron types (basket and axo-axonic cells), for which only dendrites were considered; and (5) axons and dendrites could be unambiguously discerned and ascribed to a single neuron type (for this reason, we excluded reconstructions from paired recordings).

It is important to note that the 225 used reconstructions come from 96 different publications encompassing a broad variety of experimental preparations (Extended Data Fig. 2–7; Hamam et al., 2000, 2002; Kispersky et al., 2012; Leão et al., 2012; Lee et al., 2010; Lübke et al., 1998; Szabó et al., 2014; Martínez et al., 1996; Gulyás et al., 1993; Canto et al., 2012a; Ferrante et al., 2016; Glickfield et al. 2006; Harris et al., 1999; Burgalossi et al., 2011; Hájos et al., 1998; Garden et al., 2008; Canto et al., 2012b; Ferraguti et al., 2005; Bell et al., 2013; McQuiston et al., 1999; Tahvildari et al. 2005; Middleton et al. 2008; Gloveli et al. 2001; Svoboda et al. 1999; Oliva et al., 2000; Soriano et al., 1993; Spruston et al. 2007), including differences in animal species, strain, age, and sex (both females and males were used), as well as histologic details, such as slice orientation, section thickness, recording technique, and labeling method. This heterogeneity of data sources requires adequate data normalization strategies, described in the next section, and extreme caution in interpreting the results (see Discussion).

Axonal and dendritic lengths. In order to extract parcel-specific neurite lengths, we isolated the axonal and dendritic reconstructions of each neuron type according to the different layers of each subregion (see Fig. 2B). Starting from the original unmodified image, we manually segregated the axons and dendrites within each anatomic parcel using the GNU Image Manipulation Program (GIMP 2.8; www.gimp.org), and separately saved the axonal and dendritic domains in distinct files for every layer and subregion. Next, we dissected each image into different channels for white (background) and nonwhite (neurites) pixels with a custom-made MATLAB algorithm (www.github.com/Hippocampome-Org/QuantifyNeurites), which returns the neurite pixel count. We carefully inspected each separate image channel to confirm visually that the algorithm isolated the appropriate signals. The length in pixels of the calibration scale bar was combined with the neurite pixel count. We carefully inspected each separate image channel to confirm visually that the algorithm isolated the appropriate signals. The length in pixels of the calibration scale bar was measured with the open-source program Plot Digitizer (www.plotdigitizer.sourceforge.net).

The conversion from pixel numbers to parcel-specific length requires three logical steps. The first accounts for the nonuniform tracing widths across images: often neuronal arbors (especially dendrites) are reconstructed with variable branch thickness ranging from single to multiple pixels. To solve this issue, we randomly selected three locations for every neuron image, parcel, and neurite domain, measured the branch width in pixels at each location, and averaged the three values. The second logical step requires calculating the pixel length in physical units, which is simply the nominal calibration scale-bar value (in microns) divided by the measured bar length in pixels. Consequently, the parcel-specific neurite length is obtained by multiplying the pixel count for that neurite in the given parcel by the physical pixel length and dividing the result by the average branch width in pixels. The final step corrects for the artifactual flattening of three-dimensional arbors into two-dimensional images by combining the parcel-specific lengths $L_p$ with the reported section thickness $t$ using Pythagoras’ formula as follows:

$$L_p^2 = L_p^2 + t^2,$$

where $L_p$ represents the final corrected length.

The majority of the available reconstructions (78%) were from rat, and most of those (56% of the rat data) came from adult (≥1-month-old) animals. For several neuron types, however, tracing data were only available for young adult (defined as 13–30 d old, consistent with www.Hippocampome.org) rats (34%) or from mice (21%). Normalizing all lengths to the adult rat setting requires two scaling factors: one for young to adult and another for mouse to rat. Seminal studies have measured the differences in arbor size between adult and juvenile rats in the hippocampus and elsewhere: Bannister and Larkman (1995) reported a length ratio of 1.10 for CA1 PCs, but this may be an underestimate because of nonuniform histologic processing, and pointed to parallel studies suggesting a ratio of 1.24 for the same neuron type (Ishizuka et al., 1995). Also investigating CA1 PCs, but solely focusing on the basal dendrites, Juárez et al. (2008) provided lengths corresponding to a ratio of 1.30. The same authors also included data for principal cells in the PFC (adult/juvenile ratio of 1.14) as well as GABAergic neurons from the NA (ratio of 1.20).<ref> Taking this evidence into consideration, we chose to correct the length measurements ($L_p^*$) from young animals by the multiplicative factor of 1.20. In order to normalize mouse length values to rats, we used the cubic root of the parcel-specific volume scaling factor, which is described at the end of the next section (see below and compare Extended Data Fig. 2–8).

Average number of synapses per neuron pair. We calculate the average number of synapses per pair of presynaptic and postsynaptic neurons, $N_s$, from their parcel-specific axonal and dendritic lengths by separately estimating the numbers, $N_{s,ax}$, of axonal-dendritic overlaps in each parcel $x$ (Amirikian, 2005; DeFelipe, 2015). For any $x$, the value $N_{s,ax}$ can be derived as the product of three factors: the probability that presynaptic and postsynaptic elements occur within a given interaction distance $r$, the number of presynaptic elements (axonal boutons) in the given anatomic parcel, and the number of postsynaptic elements (dendritic spines or shafts) in the same parcel. The first factor is given by the ratio between the volume of the interaction sphere within which the two elements must be and the volume of the entire parcel, $V_x$. The second factor is given by the presynaptic axonal length in parcel $x$, $L_{ax}$, divided by the average distance between consecutive presynaptic boutons, $b_p$. The third factor is given by the postsynaptic dendritic length in $x$, $L_{ds}$, divided by the distance between postsynaptic elements, $s_d$ as follows:

$$N_{s,ax} = \frac{4\pi r^3}{3V_x} \cdot \frac{L_{ax}}{b_p} \cdot \frac{L_{ds}}{s_d}.$$

The total count of synapses per directed neuron pair, $N_s$, is just the sum of the numbers per parcel, $N_{s,ax}$, over all parcels.

The above calculation requires a series of realistic assumptions regarding the needed parameters. We obtained the average dendritic distance between postsynaptic elements, $s_d$, and axonal distance between presynaptic elements, $b_p$, from available ultrastructural measurements in the rodent hippocampus (1.09 and 6.2 μm, respectively: Extended Data Figs. 2–9, 2–10; Armstrong et al., 2011; Vida, 2010; Sik et al., 1994; Gould et al., 1990; Moser et al., 1994; Pierce et al., 2011; Papa and Segal.
average, the volume of overlap in parcel $x$ is therefore given by the following:

$$V_o = \frac{1}{4}(V_{dx} + V_{ax}).$$

To measure $V_{dx}$ and $V_{ax}$, we extract the convex hull areas in parcel $x$ from the isolated axonal and dendritic images for each reconstruction, using the Shape Analysis plug-in (Wagner and Lipinski, 2013) of the open-source software Fiji. Then we convert the area measurements from pixel units to $\mu m^2$ and multiply the resultant values by the reported slice thicknesses.

The overall number of contacts per connected pair is the sum of the contacts in each parcel augmented by one, reflecting the initial assumption that the neuron pair is connected as follows:

$$N_c = 1 + \sum_x N_{cx}.$$
contacts per connected pair with values previously reported for a subset of CA1 neuron types (Bezaire and Soltesz, 2013).

Once we pooled several neurite measurements to determine the means and SDs of the neurite lengths and convex hull volumes, we used SE-propagation formulas for sums and differences as follows:

\[ C = x + y - z \]

\[ \Delta C = \sqrt{(\Delta x)^2 + (\Delta y)^2 + (\Delta z)^2}, \]

and products and quotients as follows:

\[ C = \frac{x \cdot y}{z} \]

\[ \Delta C = \left| \frac{C}{z} \right| \sqrt{\left( \frac{\Delta x}{x} \right)^2 + \left( \frac{\Delta y}{y} \right)^2 + \left( \frac{\Delta z}{z} \right)^2}, \]

to calculate the final uncertainties for the average number of synapses per neuron pair, for the number of contacts per connected pair, and for the connection probabilities. Factors, such as the volume of interaction and the parcel volumes, were considered constants. In instances where there was only a single example of a presynaptic neuron type and only a single example of a postsynaptic neuron type, we assigned a value of "N/A" to the associated uncertainties.

Resource sharing. All results and underlying experimental data are freely available through the www.Hippocampome.org web portal. Specifically, the following values are available at www.Hippocampome.org/php/synapse_probabilities.php: means and SDs of dendritic and axonal lengths for all neuron types and parcels; means, SDs, minima, and maxima of the synaptic distances from the soma along the axonal and dendritic paths, for all potentially connected neuron-type pairs and parcels; and means and SDs for the connection probabilities, average numbers of synapses per neuron pair, and numbers of contacts per connected pair, for all pairs of potentially connected neuron types. Each set of values is linked to the morphologic reconstruction images from which they were extracted. Additionally, we also provide an online tool at www.hippocampome.org/php/conprob.php to recalculate the connection probability and number of contacts per connected pair for any pair of neuron types on altering the assumptions regarding interbouton and interspine distances as well as interconnection radius.

Results

Potential connectivity

The 122 neuron types identified by www.Hippocampome.org volume 1.7, across the six subregions of the rodent hippocampal formation (Fig. 1A), give rise to 3120 potential synaptic connections based on spatial colocation of presynaptic and postsynaptic elements (Moradi and Ascoli, 2020). Over 71% (2220) of these connections arise from dendritic-targeting axons in local circuits, with the rest consisting mainly of projections between different subregions (23%) and a minority of perisomatic contacts from basket and axo-axonic cells (6%). Only for 167 potential connections (5%) has the synaptic interaction been experimentally established (Rees et al., 2016): the remaining, including 2120 local dendritic connections, so far lack any quantitative assessment of synaptic connectivity (Fig. 1B). Our approach allows for estimating the synaptic connectivity parameters (connection probability and number of contacts per connected pair) of 1970 potential synapses: 1870 for which no data were previously reported and 100 for which at least partial data exists in the literature. The last 250 local dendritic connections cannot be estimated because of the unavailability of morphologic reconstructions for the presynaptic or postsynaptic neurons. Together, the present work fills nearly two-thirds of the missing data in the hippocampal formation, increasing the available knowledge >11-fold and leaving only a minority (<35%) of the potential synaptic connections unknown. Notably, prior information was mainly limited to DG and area CA1, whereas residually absent estimates are restricted to EC and CA3 (Fig. 1C).

Axonal and dendritic length validation

We validated our pixel-count neurite-length-estimation pipeline (Fig. 2) with two independent approaches. First, we compared

<table>
<thead>
<tr>
<th>Area</th>
<th>Neuron type</th>
<th>Reported length</th>
<th>Estimated length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG</td>
<td>Granule</td>
<td>D: 2793 ± 74 µm</td>
<td>D: 3538.9 ± 328.4 µm</td>
<td>Cloborne et al., 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D: 3337 ± 88 µm</td>
<td></td>
<td>Buckmaster et al., 1992</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D: 3221 ± 78 µm</td>
<td></td>
<td>Patton and McNaughton, 1995</td>
</tr>
<tr>
<td></td>
<td>Mossy</td>
<td>D: 5392 ± 313 µm</td>
<td>D: 4792.9 ± 395.9 µm</td>
<td>Kowalski et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Basket</td>
<td>D: 9800 µm</td>
<td>D: 6583.4 ± 4277.1 µm</td>
<td>Bartos et al., 2001, 2002</td>
</tr>
<tr>
<td></td>
<td>MOCAp</td>
<td>D: 1108 µm</td>
<td>D: 1258.3 µm</td>
<td>Markwardt et al., 2011</td>
</tr>
<tr>
<td></td>
<td>A: 15,750 µm</td>
<td>A: 14,438.3 µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HICAP</td>
<td>A: 9700 ± 200 µm</td>
<td>A: 7745.2 ± 642.0 µm</td>
<td>Mott et al., 1997</td>
</tr>
<tr>
<td></td>
<td>HIPP</td>
<td>D: 2500-3200 µm</td>
<td>A: 3839.3 ± 7.3 µm</td>
<td>Yuan et al., 2017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 12,250-26,800 µm</td>
<td>A: 23,708.7 ± 1683.5 µm</td>
<td>Sik et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 25,780 µm</td>
<td></td>
<td>Mott et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Total molecular layer</td>
<td>D: 2500-3200 µm</td>
<td>A: 3074.3 µm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A: 22,500-26,800 µm</td>
<td>A: 21,136.4 µm</td>
<td></td>
</tr>
<tr>
<td>CA3</td>
<td>Pyramidal</td>
<td>D: 12,481 ± 2998.9 µm</td>
<td>D: 14,624.6 ± 4604.0 µm</td>
<td>Ishizuka et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Mossy fiber-associated</td>
<td>A: 20.3-28.6 mm</td>
<td>A: 21,750.9 ± 1714.2 µm</td>
<td>Vida and Frotscher, 2000</td>
</tr>
<tr>
<td></td>
<td>Trilaminar</td>
<td>A: 99,770 µm</td>
<td>A: 67,180.4 ± 31,195.4 µm</td>
<td>Sik et al., 1997</td>
</tr>
<tr>
<td>CA2</td>
<td>Pyramidal</td>
<td>D: 15,405.8 ± 949.7 µm</td>
<td>D: 11,111.0 ± 4237.0 µm</td>
<td>Ishizuka et al., 1995</td>
</tr>
<tr>
<td></td>
<td>SP-SR</td>
<td>D: 4200 µm</td>
<td>D: 4152.9 µm</td>
<td>Mercer et al., 2012</td>
</tr>
<tr>
<td>CA1</td>
<td>Pyramidal</td>
<td>D: 11,915 ± 1030.0 µm</td>
<td>D: 10,957.4 ± 1669.4 µm</td>
<td>Bannister and Larkman, 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D: 13,424.2 ± 1060.9 µm</td>
<td>D: 7745.2 ± 598.4 µm</td>
<td>Ishizuka et al., 1995</td>
</tr>
<tr>
<td></td>
<td>Basket CCK</td>
<td>D: 6538 µm</td>
<td>D: 7372.6 ± 598.4 µm</td>
<td>Maliyá et al., 2004</td>
</tr>
</tbody>
</table>

*See also Extended Data Table 1-1, Buckmaster, 2012.
our estimations with independent reports of axonal and dendritic length for the same neuron types (Table 1). The results show a tight correspondence between our estimations and the original measurements (ratio = 0.96 ± 0.19; Pearson’s correlation = 0.99, p < 0.001). Second, we identified a subset of reconstructions for which the authors also directly reported neurite lengths (Extended Data Table 1-1), again demonstrating a tight alignment (ratio = 0.89 ± 0.12; Pearson’s correlation = 0.90, p < 0.005). While these data are largely sourced from slice preparations, we include for completeness a comparison with in vivo reconstructions, when available (Extended Data Table 1-2; Scorcioni & Ascoli, 2005). As expected, the traceable extent of axons intracellularly labeled in vivo is substantially longer and more variable because of factors, such as the duration of current injections (Li et al., 1993).

Laminar distributions of axons and dendrites

The distribution of local axonal and dendritic lengths across layers of the hippocampal formation varies by neuron type and subregion. Here we report the relative proportions (Fig. 3) as well as the means and SDs (Extended Data Figs. 3-1–3-6) for a representative subset of neuron types in each subregion. The comprehensive data for all neuron types are available at www.hippocampome.org/php/synapse_probabilities.php. For neuron types whose axon invades different subregions, we only account for the local collaterals in this analysis. For example, only 78.3 ± 8.3% of the DG Granule cell (GC) axons remain in the hilus, while the remaining 21.6 ± 9.6% (not shown) cross over into CA3 stratum lucidum (SL) and stratum pyramidale (SP). DG interneurons regulate GC activity by innervating specific parcels (Fig. 3A; Extended Data Fig. 3-1). For instance, DG basket cells innervate stratum granulosum (SG), whereas HICAP neurons extend their axons into the inner one-third of stratum moleculare. Both interneuron types spread their dendrites across all four DG layers, although nearly half of the Basket cell dendrites (48%) are found in the outer two-thirds of stratum moleculare (SMo), whereas HICAP distribution is more uniform (27% in SMo, 41% in hilus, and 32% between SG and inner one-third of stratum moleculare). Other interneurons, such as HIPP and MOPP, restrict their axon terminals within SMo and are differentiated by their dendrite localizations: HIPP in hilus and MOPP in SMo.

For the CA3 local circuit, we illustrate 8 of 22 neuron types (Fig. 3B; Extended Data Fig. 3-2), with the remaining data reported online. The CA3 principal cells are the PC, whose local axons form recurrent collaterals in stratum radiatum (SR) and SO and dendrites invade in different proportions all layers: SLM, SR, SL, SP, and SO. Our estimations confirm previous reports indicating that basal dendrites account for ~42%-45% of the whole dendritic tree (Ishizuka et al., 1990). Whereas both CA3 Ivy and Bistratified cells have axons and dendrites extending...
from SR to SO, the present analysis reveals that bistratified cells are 3- to 4-fold longer than ivy in both axonal (55.6 mm vs 18.4 mm) and dendritic trees (14.3 mm vs 3.3 mm). Other CA3 interneurons (e.g., O-LM or Interneuron-Specific Oriens and Radiatum cells) restrict their dendrites to SO, while CA3 radia-
tum cells confine into a single layer both their axons and dendrites.

In area CA2 (Fig. 3C; Extended Data Fig. 3-3), PCs also dis-
tribute their dendrites across all layers but concentrate their
axons in SO (63.6 ± 24.0%), with smaller proportions in SR and SP, again corroborating earlier reports (Ishizuka et al., 1995). CA2 Bistratified neurons display quantitatively similar axonal and dendritic patterns from SR to SO, aligned with the arbor dis-
tributions of homologous cell types in different hippocampal subregions (Mercer et al., 2007).

www.Hippocampome.org volume 1.7 identifies 37 distinct interneuron types in subregion CA1 (compare Somogyi and
Klausberger, 2005), 8 of which were further investigated by
Bezaire and Soltesz (2013). CA1 PCs have dendrites spanning all
layers in proportions comparable to previous reports (Bannister
and Larkman, 1995; Ishizuka et al., 1995) but axons restricted to
SO (Fig. 3D; Extended Data Fig. 3-4). Axo-axonic, fast-spiking
Basket, and Basket CCK+ (BC CCK+) types innervate only SP,
directly modulating CA1 PC somatic activity, similarly to previous
reports (Fuchs et al., 2007). Our analysis shows that among the
dendritic-targeting interneurons, Bistratified and Schaffer
Collateral-associated Axons mainly innervate SR, whereas CA1 Ivy
cells show a strong preference (~68%) for SO. Other neuron types
exclusively target SLM (e.g., Neurogliaform and O-LM cells).

www.Hippocampome.org identifies three neuron types in the
Sub (Fig. 3E; Extended Data Fig. 3-5). The EC-Projecting and
CA1-Projecting PCS show qualitatively similar, but quantitatively
distinct, dendritic and local axonal distributions, whereas subic-
ular Axo-axonic cells have dendrites restricted to SM and axons to
SP. Interestingly, the local subicular circuit includes axons in the
polymorphic layer, despite the dearth of identified postsynaptic
elements in www.Hippocampome.org volume 1.7. This under-
scores the prospect of discovering new neuron types in this sub-
region to receive the transmitted signals (Kinnavane et al., 2018)
(see Discussion).

The EC is traditionally divided into lateral and medial (MEC)
components, respectively, implicated by electrophysiological evi-
dence in objects representations and spatial tasks and underlying
distinguishable connectivity patterns (Masurkar et al., 2017). Despite
this functional and architectural partition, several neu-
ron types are common to both areas (Canto et al., 2008), such as
LI-II Multipolar-Pyramidal, LI-II Pyramidal-Fan, LIII Pyramidal,
each with their own quantitatively distinct axonal-dendritic distri-
butions (Fig. 3E; Extended Data Fig. 3-6). Stellate cells are the
most numerous neuron type in MEC, with dendrites equally dis-
tributed across layers I and II and axons spread across all six
layers, according to our analysis. In addition, our results indicate
that two identified interneuron types have axonal innervations re-
stricted to the superficial layers of MEC: LII Basket, LIII
Superficial Multipolar Interneuron; however, the former preferen-
tially target LII (>75%), whereas the latter target LIII (>83%). We
report further quantitative data for all additional neuron types at

Connection probability
Based on the dendritic and axonal length distributions, we esti-
mated the connection probability P (Eq. 8), for each directional
pair of neuron types in the local circuit (Fig. 4). This probability
can be interpreted as the average fraction of neurons of the post-
synaptic type contacted by a given neuron of the presynaptic

type or, equivalently, the average fraction of neurons of the presynaptic type contacting a given neuron of the postsynaptic type.

Figure 4 illustrates the complete set of probabilities by presynap-
tic sources and postsynaptic targets for the main hippocampal subregions. Here we summarize the mean values and coefficients
of variation (CV) for each “connection category”: excitatory to
excitatory (E-E), inhibitory to excitatory (E-I), excitatory to
inhibitory (E-I), and inhibitory to inhibitory (I-I). Specifically,
within each subregion, we average the individual connection probabilities for all pairs of neuron types corresponding to each
connection category; for example, the E-E category includes all
pairs of excitatory presynaptic neuron type and excitatory post-
synaptic neuron type as long as the presynaptic axons share at
least one parcel with the postsynaptic dendrites (defining a
potential connection) and unless that connection has not been
refuted experimentally (Rees et al., 2016).

The local DG circuit (Fig. 4A), which includes five different
 glutamatergic types, including GCs (that do not have potential connections with each other), exhibits a greater
 connection probability for E-E pairs (0.66 ± 0.10%, CV range = 0.18-1.60, n = 21 pairs of neuron types) than for I-E pairs (0.59 ± 0.08%, CV range = 0.15-2.45, n = 44), with intermediate
 values for E-I (0.61 ± 0.08%, CV range 0.11-2.09, n = 53) and I-I (0.59 ± 0.06%, CV range 0.20-4.04, n = 110). In
 contrast, connectivity analysis in CA3 (Fig. 4B) reveals a
higher probability from inhibitory cells (I-I: 0.81 ± 0.11%, CV range = 0.04-4.08, n = 38; I-I: 0.59 ± 0.04%, CV range = 0.07-
3.54, n = 213) than from the three types of excitatory cells (E-E:
0.40 ± 0.09%, CV range 0.04-2.94, n = 8; E-I: 0.35 ± 0.05%, CV range = 0.04-2.98, n = 48). The probability of CA3 PCs to CA3
PCs alone, is 1.0 ± 0.41% (well matching the 0.9% experimental
value measured by Guzman et al., 2016). This relative trend
holds true for CA2 (not shown in Fig. 4 but included in the
online data at www.Hippocampome.org) and CA1, too, although
with dramatic and opposite differences in absolute values: nearly
an order of magnitude greater in CA2 (I-I: 5.5 ± 1.5%, CV
range = 0.36-0.80, n = 8; E-I: 5.3 ± 3.0%, CV range = 0.43-0.70,
n = 2; E-I: 3.6 ± 2.1%, CV range = 0.80-1.40, n = 4; and E-E:
3.1 ± 3.3%, CV = 1.04, n = 1) and smaller in CA1 (I-I: 0.32 ±
0.02%, CV range = 0.02-4.99, n = 852; I-E: 0.38 ± 0.09%, CV
range = 0.02-4.01, n = 62; E-I: 0.09 ± 0.04%, CV range = 0.68-
6.23, n = 69; and E-E: 0.07 ± 0.13%, CV range = 1.19-5.30, n = 5).

Because of the small number of neuron types discovered in the
Sub to date, there are no characterized local axonal-dendritic in-
hibitory synaptic connections, and the data are very sparse for
the excitatory connections (E-E: 1.4 ± 0.2%, CV range = 0.21-
0.65, n = 4; E-I: 0.28 ± 0.09%, CV range = 0.35-1.06, n = 2). The
EC displays a pattern of synaptic-connection probabilities with

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This text is an excerpt from a scientific paper discussing the connectivity patterns of neurons in different hippocampal subregions. It analyzes the distribution of axonal and dendritic lengths, identifies distinct neuron types, and quantifies the probabilities of synaptic connections between neuron pairs. The text is rich with scientific data and references to previous studies, highlighting the complex connectivity patterns within the hippocampus.
estimated probability ranges derived by our analysis. The remaining discrepancies may be explained by differences in preparation details. Specifically, the probabilities previously reported for DG mossy to DG granule included polysynaptic connections, whereas our analysis is specific for monosynaptic connections, and the CA1 PC to CA1 PC connectivity may be highly sensitive to slice orientation and intersomatic distances because of substantial cross-lamellar connectivity along the longitudinal axis (Yang et al., 2014).

**Number of contacts per connected neuron pair**

The number of synaptic contacts, $N_c$, per pair of directionally connected neurons estimated with our approach (Eq. 6), differs broadly by presynaptic neuron type and postsynaptic target but remains within a biologically reasonable range (1-15) for the vast majority (>95%) of neuron-type pairs (Fig. 5). Over all 1970 local dendritic potential synapses examined, the number of contacts per connected pair had a mean ± SD of 5.7 ± 5.3, a median of 4.1, and a range of 1.1-62.8 (Fig. 5A).
The relative patterns concerning the number of contacts was generally consistent across all hippocampal subregions. The average number of contacts per connected pair was systematically larger for inhibitory than for excitatory presynaptic types (DG: $6.7 \pm 0.9$, $n = 154$ pairs vs $3.5 \pm 0.7$, $n = 74$ pairs; CA3: $8.8 \pm 0.7$, $n = 251$ pairs vs $4.0 \pm 0.7$, $n = 56$ pairs; CA2: $8.9 \pm 2.9$, $n = 10$ pairs vs $7.2 \pm 4.3$, $n = 5$ pairs; CA1: $5.8 \pm 0.4$, $n = 914$ pairs vs $2.5 \pm 0.8$, $n = 74$ pairs; EC: $11.6 \pm 3.3$, $n = 420$ pairs vs $5.7 \pm 0.7$ onto inhibitory cells ($n = 234$ pairs). Moreover, in areas CA3, CA2, CA1, and Sub, the number of contacts onto excitatory cells was larger than onto inhibitory cells ($9.5 \pm 1.2$, $n = 120$ pairs vs $5.8 \pm 0.3$, $n = 1196$ pairs), consistent with other studies (Deuchars and Thomson, 1996; Booker and Vida, 2018). The EC and DG, however, displayed an opposite and milder trend with an average of $4.3 \pm 0.4$ contacts onto excitatory cells ($n = 420$ pairs) and $5.7 \pm 0.7$ onto inhibitory cells ($n = 234$ pairs).

Next, we compared our data with a previous estimation of the CA1 circuit (Bezaire and Soltesz, 2013). This earlier study focused on seven dendritic-targeting neuron types: Pyramidal, Bistratified, Ivy, Neurogliaform, Perforant Path-Associated, Schaffer Collateral-Associated. For every presynaptic type, they reported the total number of axonal boutons contacting two distinct postsynaptic targets: PCs and interneurons. From these data, it is possible to calculate the average number of synapses per neuron pair from each of the seven specified neuron types onto PCs and interneurons. This calculation requires an estimate of the numbers of PCs and interneurons. The aforementioned study assumes a count of 311,500 PCs and 38,500 interneurons.
interneurons in the adult rat CA1. Whereas the former value is consistent with contemporary literature (Herculano-Houzel et al., 2011; Murakami et al., 2018), recent reports suggest a more than doubled abundance of interneurons (Erö et al., 2018). We thus used a number of 77,000 interneurons and compared the so-derived average number of synapses per neuron pair with the corresponding quantities independently obtained in the present work (Extended Data Fig. 5-1). The two sets of values are significantly correlated ($R = 0.73$, $p < 0.0002$), and the best fitting line with zero intercept has a slope close to unity (0.91 with the data derived from Bezaire and Soltesz, 2013 as ordinate and the data from current study as abscissa).

We had previously estimated the number of contacts per connected pair in selected neuron types of the CA3 circuit based on in vivo morphologic reconstructions embedded in a 3D model of the hippocampal formation (Ropireddy and Ascoli, 2011). That earlier study had quantitatively differentiated two distinct components of the “recurrent collateral” connectivity between CA3 PCs. Specifically, postsynaptic PCs were found to receive a higher number of contacts from presynaptic PCs located in the distinct subfield CA3c (5.6 ± 0.2) than from CA3 PC in the rest of area CA3 (3.4 ± 0.3). The approach given in the present work confirms the same qualitative difference, with the number of contacts from CA3c PCs exceeding those from other CA3 PCs in 16. In most other cases, the synaptic connections were confirmed to exist, but not quantified.

On the one hand, this compilation identifies a set of “unknown parameters” that are important for computational simulations but have yet to be measured experimentally. On the other, it may allow the community to extend, update, and complement the existing quantitative knowledge of the hippocampal network. According to previous measurements of the CA1 local circuit in vitro (Takács et al., 2012), 29.3% of CA1 PC boutons contact other CA1 PCs, 65.9% contact interneurons, and 4.9% of local synapses are made onto unknown targets. However, experimental evidence from cells labeled in vivo yields different percentages (46.2% interneurons, 39.2% CA1 PCs, 6.9% unknown targets) and more heterogeneous distributions. In terms of extrinsic inputs, the same study shows that Schaffer collaterals preferentially target CA1 PCs (92.9%) over interneurons (7.1%), whereas EC tempo-ammonic axons in CA1 SLM predominantly contact PC dendrites (90.8%).

Quantification of trisynaptic circuit

The connection probabilities and numbers of contacts per connected pair allow us to derive a quantitative summary of synaptic connectivity by layer onto the principal cells of the main subregions of the hippocampal formation (Fig. 6). While this analysis is limited to axonal-dendritic interactions in the local circuit (and the Fig. 6 schematic only illustrates selected presynaptic neuron types), a full understanding of the hippocampal network also requires similar data for projection and perisomatic presynaptic neuron types. For completeness, we thus collated the available experimental evidence for these complementary cases (Extended Data Fig. 6-1; Szabadics et al., 2009; Ganter et al., 2004; Gloveli et al., 2005b). Of 60 pairs of neuron types with available data, connection probabilities were reported or derivable in only 10 cases, and numbers of contacts per connected pair in 16. In most other cases, the synaptic connections were confirmed to exist, but not quantified.

Laminar distributions of axonal and dendritic path distances from the soma

To determine the distance from the soma of potential synapses along the presynaptic and postsynaptic neural arbors, we measured the layer-specific distributions of the minimum, mean, and...
maximum axonal and dendritic paths distances from the somatic origin for every neuron type. Here we illustrate the results for representative cells in DG, CA3, CA2, CA1, and EC (Fig. 7). The measurements for all analyzed neuron types are available at www.hippocampome.org/php/synapse_probabilities.php. As expected, path distances tended to increase moving away from the somatic layer. However, the range of distances within each layer was substantially broad and, in most cases, larger than the difference between layers.

We then compared the estimated postsynaptic somatic distances of CA3 PC with the distribution of potential synapses onto dendrites of the same neuron types previously derived from embedding morphologic reconstructions into a 3D hippocampal model (Ropireddy and Ascoli, 2011, their Figs. 5C, 6E). In SOM, the synaptic distance from the soma along the dendritic path of CA3 PCs obtained in the present work (128.5 ± 51.2 μm) tightly matched the distribution of potential synapses on CA3 PC basal dendrites from the earlier study (110-160 μm). In SR, the dendritic distance from the present work (256.4 ± 64.7 μm) also yielded an excellent correspondence with the Ropireddy and Ascoli (2011) distribution of potential synapses on CA3 PC apical dendrites (200-320 μm). Last, in SLM, the dendritic distance from the soma derived here (507.1 ± 130.1 μm) was consistent with the distribution of inhibitory synapses from GABAergic interneurons on the apical tuft of CA3 PCs (500-750 μm).

Public digital resource and online data accessibility
All connection probabilities and numbers of contacts per connected pair, for each of the 1970 pairs of neuron types analyzed here, as well as the (644) parcel-specific axonal and dendritic lengths and (568) somatic path distances for every neuron type, are publicly posted on www.Hippocampome.org, increasing the count of Pieces of Knowledge of this resource by 5152. The published evidence underlying all values, including 1181 neurite lengths, 3663 path lengths, 1091 convex hull volumes, 94 slice thicknesses, and 26 parcel volumes, are also directly accessible through the web portal, amounting to a total of 6055 additional Pieces of Evidence in this public knowledge base.

Specifically, the “Synapse Probability” option in the drop-down menu of the www.Hippocampome.org “Browse” tab provides a compendium of companion web pages associated with this research that allow users to interactively explore the results (Fig. 8). Users can find specific data related to their research interests, such as the distances along dendrites and axons measured from the soma, by selecting a value associated with the desired neuron type and parcel (Fig. 8A). Each value is linked to an evidence page, which displays the supporting quotes, figures, and measurements (in this specific example, of somatic distances) found in each individual supporting article (Fig. 8A1,A1b). By selecting a presynaptic neuron type and a postsynaptic neuron type (Fig. 8B), researchers can gain information about connection probabilities and numbers of contacts per connected pair in each parcel (Fig. 8B1) and inspect the evidence used to generate the data (Fig. 8B2), such as neurite lengths (Fig. 8B2a, B2b) and convex hull volumes. All the estimations are available to download in comma-separated value files that contain matrix values and further statistics (Fig. 8C). Finally, we provide a tool allowing investigators to choose their own desired values for the ultrastructural parameters (distance between axonal boutons, distance between dendritic spines or postsynaptic locations, and interaction radius) and then obtain parcel-specific connection probabilities and numbers of contacts per connected pair for any selection of neuron types (Fig. 8D,D1).

Discussion
Despite a continuous growth of information pertaining to the morphology, electrophysiology, and gene expression of neurons in mammalian brains, direct experimental evidence for quantifying neuron type-specific synaptic connectivity remains exceedingly sparse, even in the most intensively investigated neural systems, such as the rodent hippocampal formation. Here, we have introduced a novel pipeline to produce a zero-order
Figure 8. Interactive browsing and downloading of data associated with the quantification of neurites and potential synaptic connectivity on www.Hippocampome.org. A, Representative screenshot of a www.Hippocampome.org data matrix. The drop-down menu (light blue box) provides options to display dendritic and axonal lengths, somatic distances, average numbers of potential synapses, numbers of contacts per connected pair, and connection probabilities. Green-outlined box represents the Connection Probabilities tool. Red box highlights the download section for the data. The representative entry in the brown box, enlarged in the dark blue box, corresponds to the somatic distances along the dendrite for a CA1 Radiatum Giant cell in SLM.D:

B1 Number of Contacts

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<td>SmI</td>
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<td>H</td>
<td>3.54</td>
<td>1.78</td>
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Figure 2. Heterogeneity of non-FS INs. A, A non-FS cell (A1), a putative CB1R+ IN, with the axonal distribution within the IML (bottom; density plot) exhibited asynchronous release onto GCs (A2, arrow) and DII (A3).

C Neuron,Parcel,Avg,SD,Count,Min,Max

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<td>CA1 Radiatum Giant,CA1:SLM:D,534.86,166.8,12.82,84.10,49.40</td>
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<td>CA1 Radiatum Giant,CA1:SO:A,432.65,97.41,8,264.22,965.20</td>
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D Presynaptic

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<tr>
<td>Dendritic spine distance (μm)</td>
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<td>Inter-bouton distance (μm)</td>
<td>4.7</td>
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<td>Radius of interaction (μm)</td>
<td>5</td>
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approximation of the connection probability, number of contacts per connected pair, and presynaptic and postsynaptic somatic distances for most pairs of potentially connected neuron types in the local circuits of the hippocampus and EC. While this initial order-of-magnitude estimate of connectivity will undoubtedly require successive quantification refinements, our results nonetheless constitute a remarkably comprehensive characterization of nearly 2000 neuron-type connections. This dataset provides a useful placeholder for computational modeling and network analyses until firmer empirical measurements are collected and released (Ascoli and Atkeson, 2005). Notably, biologically realistic simulations of the hippocampal circuit will also require a complete neuron census, that is, the count of neurons within each type (Attili et al., 2019).

From morphology to function: synaptic count and implications for local circuits

The framework introduced in this study focuses on axonal-dendritic connections, thus excluding perisomatic synapses that only constitute a minority (~6%) of the hippocampal connectivity (Megías et al., 2001). Moreover, our approach is limited to the local circuit, as captured by typical slice preparations from which most available morphologic data are derived. While recent technological advancements now allow the systematic 3D reconstruction of long-range single-neuron axonal projections (Winnebust et al., 2019), that process is still too slow to yield dense coverage of the needed data, and currently only provides access to a small proportion (~4%) of neuron types in the mouse hippocampal formation. In contrast, representative local two-dimensional morphologic tracings are available for 115 of the 122 distinct neuron types identified by www.Hippocampe.org. The seven missing reconstructions (three in CA3 and four in EC) account for 250 connections, representing only 11% of the 2206 neuron type pairs potentially connected by local axonal-dendritic synapses. At the same time, as additional neuron types are likely to be discovered in the future, continuous screening of neuronal morphologies will be required to maintain this information up to date.

Peters’ rule assumes that the overlap of presynaptic axons with postsynaptic dendrites is equivalent to the probability of synaptic connectivity in the absence of targeting preference (Peters and Feldman, 1976). Experimental evidence in the neocortex indicates that interneuron axons have smaller branch lengths and higher tortuosity compared with PCs, suggesting greater specificity in their targeting of postsynaptic neurons (Stepanyants et al., 2004). Furthermore, while the number of actual synapses made by PCs is just a fraction (10%-30%) of all axonal-dendritic overlaps (Stepanyants et al., 2002), our estimated count of presynaptic and postsynaptic elements based on interbouton and interspine distances, respectively, already corrects for this factor.

Importantly, axonal and dendritic morphology in the rodent hippocampal formation has been proven to correctly predict circuit connectivity at the neuron-type level (Rees et al., 2017), at an accuracy of 99%, when exceptions are made for axo-axonic and interneuron-specific connections. For example, the predominant local connections from CA1 PCs to CA1 O-LM cells and other interneurons with horizontal dendritic arbors at the border of SO and the alveus (Maccafferri, 2005) are a consequence of Peters’ rule: CA1 O-LM cells and these other SO interneurons receive ~70% of their glutamatergic input from CA1 PCs (Blasco-Ibáñez and Freund, 1995). Still, proven synaptic specificity, such as that for axo-axonic and interneuron-specific connections, takes precedence over Peters’ rule in our framework: in other words, we excluded experimentally refuted connections from the calculations of connection probability and number of contacts per connected pair.

Among all analyzed subregions, CA2 had the highest connection probability, by nearly an order of magnitude (weighted average for CA2: 0.048, n = 15 neuron type pairs; weighted average for the rest of the hippocampal formation: 0.0053, n = 1955). This may be due, at least in part, to the smaller total volume of CA2 compared with other areas (CA2: 2.28 mm³; CA1: 30.13 mm³): we calculate connection probabilities based on axonal-dendritic overlap density, thus resulting in higher values for CA2 given similar axonal-dendritic extents. CA1 exhibits shorter axonal lengths within the local circuit on top of the greater volume. The small number of known neuron types in CA2 nonetheless urges interpretative caution. At the same time, connection probability is often correlated to synaptic amplitude (jiang et al., 2015); and amplitudes are indeed larger in CA2 than in CA1 neurons (Kohara et al., 2014; Sun et al., 2014), suggesting that our finding is not artifactual.

The neurite length analysis presented here also showed that the principal cells of the Sub, the CA1-projecting PCs, exhibit substantial axonal extensions in the polymorphic layer (4543.9 µm), although there are no known neuron types extending dendrites in that layer. This observation invites two alternative explanations. One is the existence of yet undiscovered neuron types that can receive the mentioned inputs. In general, the Sub local circuit remains largely unexplored, and there is growing evidence supporting the idea of new cell types (Menendez de la Prida et al., 2003; Witter, 2006; Kinnavane et al., 2018). The other possibility is that this layer might be a mere transition zone for axonal projections to reach other areas.

The involvement of gap junctions in the hippocampal formation is associated with neuronal synchronization during oscillatory activity, mainly between GABAAergic parvalbumin-positive interneurons (Ylinen et al., 1995; Fukuda and Kosaka, 2000; Baude et al., 2007). Recent evidence showed that gap junctions are present in the adult hippocampus between glutamatergic cells as mixed synapses but remain mainly closed under control conditions (<10%) and require acidic changes in intracellular pH to increase the opening probability (Ixmatlahua et al., 2020). Additional experimental evidence should be collected to address the role of gap junctions during nonrhythmic activity and for inclusion in circuitry estimates.

Path tracing and synaptic attenuation

Temporal neuronal dynamics depend on axonal delays as well as on the attenuation of evoked postsynaptic potentials across the dendritic tree. Because axonal spikes propagate at approximately constant velocity (Rama et al., 2018), presynaptic signal delay is essentially proportional to the somatic path distance to the release sites. Dendrites are constantly receiving synaptic inputs and signals may be enhanced or reduced according to their local conditions. Because axonal spikes propagate to approximately constant velocity, synaptic inputs and signals may be enhanced or reduced according to their local conditions. These effects are the result of multiple factors that change as a function of the distance from the soma along the arbor path, such as internal resistance, resting membrane potential, and receptor expression, activation, conductance, and modulation (Magee, 1998; Golding et al., 2005; Grillo et al., 2018). We estimated the mean axonal and dendritic distance from the soma across every layer. Another key influence on signal attenuation is exerted by branch diameter, which can vary substantially between layers (Megías et al., 2001) but has not been assessed yet
for many neuron types. The distal dendritic diameter of hippocampal neurons is often near the resolution limit of traditional light microscopy, and its measurement is notoriously unreliable (Scorcioni et al., 2004).

Limitations and confounds
Our approach to calculating linear-length estimations of axons and dendrites for all www.Hippocampome.org neuron types is constrained by the relatively small number of reconstructions available in the peer-reviewed literature. In order to circumvent this limitation, we collated neuronal reconstructions from several different strains of both mice and rats (Extended Data Fig. 2-7). In the absence of comparative morphologic data for specific neuron types, we adopted a conversion factor equal to the cubic root of the volume scaling parameter from mouse to rat (West et al., 1978). In contrast, we did not perform any correction for the different strains used, as a comparison of different rat atlases indicates that the hippocampal formation remains remarkably consistent across strains (Kjonigsen et al., 2015). Another large effect on neurite length and anatomic volume pertains to the age of the animals. To compensate for this known source of variability, we applied a scaling factor to normalize the measurements from young to adult animals (Juárez et al., 2008).

The variety of labeling methods are also likely to cause significant differences in observed morphologic features, even within the same neuron type (Farhoodi et al., 2019). New approaches to identify neuron populations, such as genetic barcoding, will provide improved specificity to describe the properties and dynamics associated with different neuron types and their functional role in neuronal circuits (Sugino et al., 2019). Techniques, such as octopule patch-seq recordings, optogenetics and labeling, and serial-block-face scanning electron microscopy, will continue to fill the gaps in the experimental acquisition of electrophysiology, connectivity, morphology, and transcriptomics for a large set of different neuron types (Jiang et al., 2015; Cadwell et al., 2016). However, the existing experimental tools still lack the scalability and specificity needed to classify all identified neuron types at www.Hippocampome.org. More accurate and unbiased labeling techniques are required to completely characterize neuron type populations and morphologies.

Another issue to consider is the completeness of the reconstructions. Tracings from slices are necessarily incomplete if the maximal span of the axonal or dendritic trees exceeds the slice thickness (Uylings et al., 1986; Parekh et al., 2015). Unfortunately, the depth from the slice surface to the recording neuron is seldom reported in peer-reviewed publications. Slicing orientation can also heavily affect the physical integrity of neural arbors. Ideally, different sectioning angles should be used to obtain optimal axonal reconstruction (E. Harris et al., 2001; Gloveli et al., 2005a). For example, hippocampal-EC slices preserve the fibers from the perforant path, mossy fibers, and Schaffer collateral-axals, whereas coronal slices only preserve Schaffer collateral fibers (Xiong et al., 2017). Interestingly, while some authors showed in silico that the completeness of axonal collaterals remains constant independent of slice orientation (Guzman et al., 2016), other studies report that axonal collateral densities in 400 μm coronal slices are discontinuous with the cell body (Li et al., 1993). In general, axonal reconstructions can rarely be considered complete in any slice preparation. Therefore, the data reported here, largely sourced from slices, are likely underestimates of in vivo connectivity (Extended Data Table 1-2).

In conclusion, detailed modeling of hippocampal circuits with biologically realistic neural network simulations requires quantitative information related to identified neuron types. Our estimations constitute the first attempt to fill in the gaps about the connection probabilities and numbers of contacts per connected pair between different neuron types in an approximate but comprehensive manner, until further direct experimental data become available. We also measured the layer-specific axonal and dendritic distances from the soma, which can be used to determine temporal integration of signals during circuit-related activity in distinct neuron types by electrotonic propagation and axonal delay along the neurites.

References
Acsády L, Görcs TJ, Freund TF (1996b) Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. Neuroscience 73:317–334.


cells generate the field potential oscillations induced by cholinergic recep-
tor activation in the hippocampus. J Neurosci 30:15134–15145.

Decrease in tonic inhibition contributes to increase in dentate semilunar

Guzman L, Commandeur F, Acosta O, Simon A, Fautrel A, Rioux-Lecercq
N, Romero E, Mathieu R, de Crevoisier R (2016) Slice correspondence
estimation using SURF descriptors and context-based search for pros-
tate whole-mount histology MRI registration. Annual International
Conference of the IEEE Engineering in Medicine and Biology Society,
p. 1163–1166.

Hájos N, Mody I (1997) Synaptic communication among hippocampal inter-
neurons: properties of spontaneous IPSCs in morphologically identified

types express m2 muscarinic receptor immunoreactivity on their den-
drites or axon terminals in the hippocampus. Neuroscience 82:355–376.

Spine timing of distinct types of GABAergic interneuron during hippo-

Hájos N, Karlóci MR, Németh B, Ulbert I, Monyer H, Szabó G, Erdélyi F,
Freund TF, Gulyás AI (2013) Input-output features of anatomically iden-
tified CA3 neurons during hippocampal sharp wave/ripple oscillation in

Halasy K, Somogyi P (1993) Subdivisions in the multiple GABAergic inner-
vation of granule cells in the dentate gyrus of the rat hippocampus. Eur J

selectivity and input of GABAergic basket and bistratified interneurons

electrophysiological characteristics of layer V neurons of the rat medial

Hamam BN, Amalar DG, Alonso AA (2002) Morphological and electrophys-
iological characteristics of layer V neurons of the rat lateral entorhinal

Hamilton DJ, Wheeler DW, White CM, Rees CL, Komendantov AO,
Bergamino M, Ascoli GA (2017) Name-calling in the hippocampus (and
beyond): coming to terms with neuron types and properties. Brain
Infor 41–12.

Han ZS, Buhl EH, Lórczínc Z, Somogyi P (1993) A high degree of spatial se-
lectivity in the axonal and dendritic domains of physiologically identified
local-circuit neurons in the dentate gyms of the rat hippocampus. Eur J

of the rat subiculum: I. Dendritic morphology and patterns of axonal arbo-

Harris KM, Stevens JK (1989) Dendritic spines of CA1 pyramidal cells in the
rat hippocampus: serial electron microscopy with reference to their bio-

Herculano-Houzel S, Ribeiro P, Campos L, Valotta da Silva A, Torres LB,
Catania KC, Kaas JH (2011) Updated neuronal scaling rules for the brains

Morpho-physiological criteria divide dentate gyrus interneurons into

projections originating from CA3 pyramidal cells in the rat. J Comp

Ishizuka N, Cowan WM, Amaral DG (1995) A quantitative analysis of the
organization of intrahippocampal

Juárez I, Gratton A, Flores G (2008) Ontogeny of altered dendritic morphol-
yogy in the rat prefrontal cortex, hippocampus, and nucleus accumbens
1747.

Kamsu JM, Constands JM, Lamberton F, Courtheoux P, Denise P, Philoxene
B, Coquetmonet M, Benard S (2013) Structural layers of ex vivo rat hippo-
campus at TT MR. aPLo 18e76133.

Karayannis T, Elfant D, Huerta-Ocampo I, Teki S, Scott RS, Rusakov DA,
Jones MV, Capogna M (2010) Slow GABA transient and receptor desen-
sitization shape synaptic responses evoked by hippocampal neuroglia-

(2014) GluN2B-containing NMDA receptors promote glutamate synapse

Collateral projections innervate the mammillary bodies and retrosplenial
cortex: a new category of hippocampal cells. eNeuro 5:ENEURO.0383-
17.2018.

Kirson ED, Yaari Y (2000) Unique properties of NMDA receptors enhance
synaptic excitation of radium giant cells in rat hippocampus. J Neurosci
20:4844–4854.

Kispert S, Fernandez FR, Economou MN, White JA (2012) Spike reso-
ance properties in hippocampal O-LM cells are dependent on refractory

Washolm Space atlas of the rat brain hippocampal region: three-dimen-
sional delineations based on magnetic resonance and diffusion tensor

Klausberger T (2009) GABAergic interneurons targeting dentrites of pyram-
idal cells in the CA1 area of the hippocampus. Eur J Neurosci 30:947–
957.

Klausberger T, Somogyi P (2008) Neuronal diversity and temporal dynamics:

Klausberger T, Márton LF, Baud A, Roberts JD, Magill P, Somogyi P
(2004) Spike timing of dentrite-targeting bistratified cells during hippo-

Klausberger T, Marton LF, O’Neill J, Hack HJ, Dalezios Y, Fuentebula P,
Complementary roles of cholecystokinin- and parvalbumin-expressing
GABAergic neurons in hippocampal network oscillations. J Neurosci

Kohara K, Pignatelli M, Riverst AJ, Jung HY, Kitamura T, Suh J, Frank D,
type-specific genetic and optogenetic tools reveal hippocampal CA2 cir-

Komendantov AO, Venkadesh S, Rees CL, Wheeler DW, Hamilton DJ,
Ascoli GA (2019) Quantitative firing pattern phenotyping of hippocam-

Kowalski J, Geuting M, Paul S, Dieni S, Laurers J, Zhao A, Drakew A,
Haas CA, Frotscher M, Vida I (2010) Proper layering is important for precisely
timed activation of hippocampal mossy cells. Cereb Cortex 20:2043–
2054.

Le B, Lasztoczi B, Letgler M, Viney TJ, Katona L, Valenti O, Hartwich K,
Borhegyi Z, Somogyi P, Klausberger T (2012) Behavior-dependent spe-

Leo RN, Mikulovic S, Leo KE, Menguba H, Gezelius H, Enijn A, Petras K,
differentially module CA3 and entorhinal inputs to hippocampal CA1

ergic neurons in hippocampal network oscillations. J Neurosci
30:7972–8000.

Lee SY, Földy C, Szabados J, Soltesz I (2011) Cell-type-specific CCK2 recep-
tor signaling underlies the cholecystokinin-mediated selective excitation
of hippocampal parvalbumin-positive fast-spiking basket cells. J Neurosci
31:10993–11002.

Lee T, Jarome T, Li SJ, Kim JI, Helmstetter FJ (2009) Chronic stress selec-
tively reduces hippocampal volume in rats: a longitudinal magnetic reso-


Ropireddy D, Ascoli G (2011) Potential synaptic connectivity of different neurons onto pyramidal cells in a 3D reconstruction of the rat hippocampus. Front Neuroninform 5:5.


