Cell

Supplemental Information

Reconstruction and Simulation of Neocortical Microcircuitry

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Supplemental Experimental Procedures

MORPHOLOGICAL DIVERSITY

Reconstruction of morphologies

3D reconstructions of biocytin stained neuronal morphologies were obtained from whole-cell patch-clamp experiments on 300 µm thick brain slices from juvenile rat hind-limb somatosensory cortex, following experimental and post-processing procedures as previously described (Markram et al., 1997).

Neurons were chosen for 3D reconstruction that were high contrast, completely stained, and had few cut arbors. Reconstruction used the Neurolucida system (MicroBrightField Inc., USA) and a bright-field light microscope (Leica DMRB, Wetzlar, Germany) at a magnification of 100x (oil immersion objective, 1.4-0.7 NA) or of 60x (water immersion objective, 0.9 NA). The finest line traced at the 100x magnification with the Neurolucida program was 0.15 µm. The slice shrinkage due to staining procedure was approximately 25% in thickness (Z-axis) and approximately 10% anisotropically along the X- and Y-axes. Only the shrinkage of thickness was corrected at the time of reconstruction (X-Y shrinkage is corrected later by the unraveling step). Reconstruction resulted in a connected set of points traced from the morphology, each having a 3D (x, y, z) position and diameter.

Classification of morphologies

Following reconstruction, neuronal morphologies were classified into one of 55 different morphological types (m-types) based on the layer containing their somata, and their anatomical and electrical features (see Figure 2 for a complete listing of m-types) as described below.

Excitatory m-types in layers 2 to 6

Pyramidal cells (PC) in different cortical layers were mainly characterized by their apical dendrites, since their large axonal clusters were cut in the slice preparation. The classification of excitatory neurons in layers 2 to 6 was combined with quantitative analysis of 3D computer reconstructions of biocytin stained neurons. We attempted to correlate layer 6 PC m-types with their long-distance axonal projections by performing retrograde labeling experiments. The PC m-types defined here are consistent with previously reported subtypes that have distinct long-distance projections (Van Aerde and Feldmeyer, 2015; Marx and Feldmeyer, 2013; Mercer et al., 2005; Oberlaender et al., 2012; Zhang and Deschênes, 1997).

Layer 2/3 PCs: As reported previously (Feldmeyer, 2006; Holmgren et al., 2003), PCs in layer 2/3 were simply pooled together according to their location in the supragranular layer.

Layer 4 PCs: There were 3 excitatory m-types in layer 4 (Feldmeyer et al., 1999): 1) L4PC (tufted PC) had an apical dendrite with a small tuft that often did not extend to layer 1. 2) L4SP (untufted PC, or Star PC) had a slender apical dendrite without a tuft. 3) L4SS (Spiny Stellate cell) had an apical dendrite with one or few branches, having a radial length similar to basal dendrites. In comparison with the L4PC and L4SP m-types, L4SS had tortuous and thicker basal dendritic segments.

Layer 5 PCs: There were 4 PC m-types in layer 5 (Frick et al., 2007; Kasper et al., 1994; Markram et al., 1997; Romand et al., 2011): 1) L5TTPC1 (Thick-tufted PC 1) had a thick apical dendrite with a big tuft that bifurcated at the distal half of the apical dendrite. 2) L5TTPC2 (Thick-tufted PC 2) had a thick apical trunk that bifurcated at the proximal half of the apical dendrite into multiple apical dendrites that further bifurcated respectively forming a smaller tuft in layer I. 3) L5STPC (small tufted PC) had a thin apical dendrite with a small tuft. 4) L5UTPC (untufted PC) had a thin apical dendrite without a tuft. The L5TTPCs had bigger and more typical pyramidal-shaped somata compared to L5STPC and L5UTPC. While L5TTPC1 and L5TTPC2 were bigger neurons with basal dendritic clusters, L5STPC and the L5UTPC were smaller with similar basal dendritic clusters.

Layer 6 PCs: There were 5 PC m-types layer 6: 1) L6TPC (Tufted PC) had an apical dendrite with a tuft terminating in either layer 1 or 4. 2) L6UTPC (untufted PC) had an apical dendrite without a tuft. 3) L6IPC (Inverted PC) had large dendrites inverted towards the white matter with more branches than other basal dendrites. 4) L6BPC (Bipolar PC) had a typical apical dendrite towards the pia with or without a small tuft and a big inverted dendrite (towards the white matter) with more branches than other basal dendrites. According to retrograde labeling experiments, L6BPCs were similar to cortico-claustral PCs projecting to the ipsilateral claustrum; L6TPCs, L6UTPCs, and L6IPCs corresponded to cortico-cortical PCs projecting to the contralateral somatosensory cortex (also see (Kisvárday et al., 1990)).

Inhibitory m-types in layers 2 to 6

Interneurons were classified according to axonal and dendritic features, especially the characterized axonal morphological features, as published previously (Markram et al., 2004; Wang et al., 2002, 2004) . With the exception of layer 1, all other layers shared a similar set of m-types, with variations in the shape and size of dendritic and axonal clusters.

Large basket cell (LBC): LBCs had multipolar or bitufted dendrites, an axonal cluster characterized by straight and long axonal segments with low density of boutons, and long collaterals giving branches from both sides. Layer 2/3 LBCs commonly had descending axonal collaterals while those in deep layers 5 and 6 often had ascending axonal collaterals reaching layer 2/3 or even layer 1.

Nest basket cells (NBC): These cells correspond to Arcade or Willow cells, having multipolar or bitufted dendrites without long collaterals. NBCs in supragranular layers were in general smaller in size than those in infragranular layers.

Small basket cell (SBC): SBCs had multipolar or bitufted dendrites and axon collaterals with numerous curved or straight short axonal segments. The axon collaterals contained a high density of boutons and formed a dense local axonal cluster around the soma.

Chandelier cell (ChC): ChCs had multipolar or bitufted dendrites, and were distinguished by pre-terminal axon branches that form short vertical rows of boutons resembling candlesticks. These interneurons were also referred to as axo-axonic cells as they form synaptic contacts on the axon initial segment of target PCs (Somogyi et al., 1982).

Martinotti cells (MC): MCs had multipolar, bitufted, or bipolar dendrites, and were distinguished by ascending axons that commonly gave rise to two axonal arbors, one near the cell body and another in layer 1 except those in layer 6. Layer 6 MCs had a second axonal cluster formed below layer 1. The second axonal plexus of MCs were very dense (axonal tuft) or diffused. Axonal collaterals of MCs were characterized by spiny boutons while dendrites contained sparsely distributed spines that were uncommon for other interneuron types.

Double bouquet cell (DBC): DBCs had multipolar or bitufted dendrites, and were distinguished by a horse-tail like axon bundle. The axon bundle consisted of one to few long descending/ascending vertical axonal collaterals from which many short branches emerged, or by tightly intertwined bundles of long descending vertical collaterals (corresponding to horse-tail like neurons; (Somogyi and Cowey, 1981)). Layer 2/3 DBCs commonly had descending axonal collaterals while those in deeper layers 5 and 6 often had ascending axonal collaterals reaching layer 2/3 or even layer 1.

Bipolar cell (BP): BPs had bipolar dendrites that emerged from the two poles of a small vertical spindle like soma. A few BPs could have a third dendrite emerging from the middle of the soma. Their axonal clusters were narrow and vertically oriented.

Bitufted cell (BTC): BTCs had bitufted dendrites and were characterized by long axonal segments, typically forming a large translaminar or transcolumnar axonal cluster.

Neurogliaform cell (NGC): NGCs had multipolar dendrites and were characterized by very small and dense local axonal arborization around the soma, and short axonal segments bearing a high density of small boutons.

Inhibitory m-types in layer 1

Layer 1 contained six m-types - Neurogliaform Cells with dense (NGC-DA) and sparse local axonal arborization (NGC-SA), Horizontal Axon Cell (HAC), Descending Axon Cell (DAC); Large Axon Cell (LAC) and Small axon cell (SAC). Except NGCs, other m-types were specific only to layer 1. Cajal-Retzius cells were not included as they exist mainly in the embryonic stage before birth and are no longer present by post-natal day 11 (P11) in rodent neocortex (Hestrin and Armstrong, 1996)

Neurogliaform cells with dense axonal arbors (NGC-DA): NGC-DAs were very similar to the NGC cells reported in other cortical layers showing small compact axonal arborization (Kawaguchi and Kubota, 1997; Kisvárday et al., 1990; Szabadics et al., 2007). These neurons also typically displayed the shortest dendritic segments among all layer I neurons.

Neurogliaform cells with sparse axonal arbors (NGC-SA): Visually, NGC-SAs appeared as a sparser variation of NGC-DAs. They displayed similar axonal branching patterns in terms of segment length, tortuosity, and branch angles. However, NGC-SAs differ from NGC-DAs in that they displayed significantly smaller vertical arborizations with fewer and shorter axon collaterals. On the other hand, the dendritic arborization extended further horizontally with longer dendritic segments and smaller branching angles.

Horizontal axon cells (HAC): HACs were characterized by extensive horizontal axonal arborizations as compared to vertical extents, with long axonal segment lengths. Their horizontal/vertical extent ratio (often > 4 fold) was the highest among all other m-types in layer 1.

Descending axon cells (DAC): DACs were easily distinguished due to the presence of one to a few descending axon collaterals that reached layers 4, 5, and occasionally 6 (Hestrin and Armstrong, 1996). Their other striking distinguishing feature was the large horizontal (often > 1 mm) and vertical (often > 0.5 mm) extents of their axonal arborization, which was the largest of any m-type in layer 1.

Large axon cells (LAC): LACs appeared visually similar to the HACs. On closer inspection, however, the axonal segments were shorter and projected more radially compared with HACs. Particularly, many short branches emerged from long axonal collaterals. They displayed the longest total length of axon, the highest number of segments, and the highest maximum branch order among layer 1 m-types. The axonal collaterals of more than 50% of LACs were often seen to project vertically into layers 2 and 3. Their dendrites also displayed the highest segment number indicating frequent branching.

Small axon cells (SAC): SACs had the smallest axonal arborization reflected in the lowest total axonal lengths among layer 1 m-types. The axonal arbor also displayed the lowest number of axonal segments and the lowest maximum axonal branch order. The axons, however, had the largest axonal branch angles with straight axonal segments. About a third of SACs projected one or two axonal collaterals into layers 2 and 3.

Unraveling morphologies

The histological processing performed on brain slices results in tissue shrinkage, which leads to an increase in arbor tortuosity. This leads to a decrease in the overall reach of the neuron, while presumably maintaining a constant total arbor length (Jaeger, 2000). In order to correct for this increased tortuosity and reduction in overall reach, we developed an unraveling procedure to smoothen and extend the reach of arbors while maintaining their overall length. The unraveling process used a centered moving window algorithm for each arbor branch. Assuming a window size of *N* points, running on a branch of *M* points, the algorithm started on the first point of the branch to be unraveled (call this point's index *p*). The points in the window were fitted using principal component analysis (PCA) by a 3D direction vector, $\vec{D}_p$. The moving window ran on the tortuous neuron, computing the PCA direction vectors for each point. The algorithm then updated the position of each point sequentially to align them with the PCA direction. The coordinates of the point *p+1* then became:

$$\begin{pmatrix} \hat{x}_{p+1} \\ \hat{y}_{p+1} \\ \hat{z}_{p+1} \end{pmatrix} = \begin{pmatrix} \hat{x}_p \\ \hat{y}_p \\ \hat{z}_p \end{pmatrix} + \left\|\vec{V}_p\right\| \times \frac{\vec{D}_p}{\left\|\vec{D}_p\right\|}$$

where

$$\vec{V}_p = \begin{pmatrix} x_{p+1} - x_p \\ y_{p+1} - y_p \\ z_{p+1} - z_p \end{pmatrix}$$

and (*x, y, z*) are the coordinates of the point before unraveling, $(\hat{x}, \hat{y}, \hat{z})$ are the coordinates after unraveling, and $\left\|\vec{V}_p\right\|$ is the distance to the previous point. The unraveling thus preserved the overall length of the arbors while decreasing the tortuosity (increasing the smoothness) and increasing the range of the neuron. At the boundaries (if $p < N/2$ or $p+N/2 > M$), the window was truncated to the points available. The window size could be adjusted to achieve a desired increase in the range of the neuron. We found empirically that using a window size ($N = 5$) resulted in a range increase of 10% on average, and this value was used for unraveling.

Repairing morphologies

Because neuronal morphologies were reconstructed from brain slices, some reconstructions were truncated at the slice edges. To restore the severed arbors we developed repair algorithms to mitigate the slicing artifacts. The repair process, described here briefly, was based on previously published methods (Anwar et al., 2009). The first step of this process was to determine if the morphology was actually truncated at the two cut-planes. Our coordinate system for reconstructing neurons defined the cut plane as XY (perpendicular to the Z-axis) and the origin as the center of the soma. Thus, the first step of cut-detection was to determine the maximum extent of arbors in the positive and negative Z directions. In order for a cut-plane to be detected, there must be a minimum number of terminal points within 30 µm of the maximum Z extents (five points by default, or the total number of terminal points divided by four if there are fewer than 20 terminal points). In the case of finding two cut-planes in this manner, we imposed an additional requirement of having a minimum span between maximum Z extents of 200 µm. If the span was less than this value, the side with the most terminal points

within the 30 μm range was considered the only cut side. Any terminal points further than 30 μm from the maximum Z extents were eliminated as cut points. Additionally, if any point along the arbor path from the soma to a terminal point was more distal in Z than the terminal point itself, this terminal point was eliminated as a cut point (i.e. the arbor loops back, or is re-entrant). This process resulted in reconstructed morphologies with a certain number of terminal points tagged as cut-points. These cut branches were then repaired using separate algorithms for dendrites and axons.

The dendrite repair process "regrows" cut dendrite branches in order to recover the portions severed during slicing (Anwar et al., 2009). This process was not intended to recover the original dendritic morphology, but rather recovered the overall morphology in a statistical manner, using the assumption of statistical symmetry. The dendrite repair process analyzed the properties of the intact dendrites of a single morphology and uses that data to stochastically generate "virtual" dendrites at its identified cut points. This process was developed on the basis of existing models (Ascoli and Krichmar, 2000; Ascoli et al., 2001; Burke et al., 1992; Donohue and Ascoli, 2008; Hillman, 1979; Pelt and Uylings, 2003). Our model utilized the behavior of uncut branches as a function of branch order and straight-line distance from the root. For basal dendrites the root was defined as the point where the dendrite emerges from the soma, for apical oblique dendrites the root was defined as the point where the dendrite emerges from the main apical trunk, and for apical tuft dendrites the root was defined as the point where the apical trunk begins forming the tuft. Probability density clouds for continuing, bifurcating, and terminating events were calculated in a series of spherical shells (Sholl, 1953) for each branch order. $P(E|O,S)$ was the probability of event E occurring, knowing that the branch is of order O and was in shell S. The event probabilities were calculated as

$$P(E|O,S) = \frac{N_{E,O,S}}{N_{O,S}}$$

where $N_{E,O,S}$ was the number of branches of order O undergoing event E in shell S and $N_{O,S}$ is the total number of branches of order O in shell S. At each cut point, the behavior of the branch was stochastically sampled utilizing the calculated event probabilities. If the branch is to continue, it was regrown up to the next spherical shell. If it was to bifurcate, a random number of points were added before the bifurcation, and the daughter branches were grown up to the next spherical shell, with the angle between the daughters randomly sampled from the daughter angle distribution of all bifurcations in the neuron. If the branch was to terminate, a random number of points were added and the branch terminated. The direction in which branches are re-grown was allowed to vary randomly from the current direction by 5 -10%.

The axonal repair process differed from dendrite repair in that it did not assume that the axonal arborization was symmetrical (Anwar et al., 2009). Neocortical axons are known to exhibit laminar preferences, with lateral extent dependent upon depth within the microcircuit (Larsen and Callaway, 2006). Thus, our axonal repair algorithm attempted to maintain the laminar structure. Due to the greater complexity of axonal arborization, instead of regrowing branches at cut points, the algorithm selected and pasted intact subtrees from the particular m-type. The first step was to create a pool of intact subtrees (those without any cut branches) from all reconstructed morphologies of a given m-type. For each axonal cut-point, the nearest intact tree became the target subtree. From the pool of intact subtrees, the one with the closest matching overall length was chosen and pasted onto the cut point.

Cloning morphologies

Morphological diversity is an important component of robust and invariant connectivity patterns (Ramaswamy et al., 2012). In order to increase morphological diversity, we performed several procedures on our population of reconstructed and repaired neurons.

Neurons reconstructed from brain slices lose all arbors outside of the cut planes, which is particularly problematic for m-types with extensive axonal arborizations. Even after our repair process, some of our neuronal morphologies exhibited impoverished axonal arborizations despite having excellent dendrite arborizations, or vice-versa. A mix-and-match procedure mitigated this problem by separating neurons at the axon, discarding poor dendrite and axonal reconstructions, and then recombining good dendrite and axonal reconstructions in all possible permutations, thus increasing utilization of reconstruction data and increasing the number of unique morphologies available for microcircuit building. We manually annotated the dendritic and axonal arborizations as acceptable or not and perform the procedure on all pyramidal cell m-types in layers 2-6 as well as on layer 4 spiny stellate neurons.

For placement of morphologies within the simulated neocortical column, a specific m-type was determined for each somatic location and then the morphology of that m-type which best fit the location was chosen, as described below. However, for some locations, especially near the layer boundaries, the best-fit morphology did not fit particularly well. This was assumed to be due to a combination of choice bias during the

experiments (experimenters looking for a specific cell type search near the middle of the layer) and variability in cortex height between different animals. To ameliorate this issue, the repaired morphologies were duplicated and scaled vertically (Y-axis) by ± 2.5% and ± 5.0%.

In order to further increase morphological diversity, a cloning algorithm was implemented. This algorithm took each morphology and injected noise into branch lengths and rotations, leaving the overall branching structure unchanged, but resulting in completely unique space-filling for each clone. For each branch in the current morphology, a random number was sampled from a Gaussian distribution of mean 0% and standard deviation 20%, and the branch's length was then scaled by this amount. At each bifurcation point, there were two subtrees, each of which were rotated by a degree sampled from a Gaussian distribution of mean 0° and standard deviation 10°. The rotation occurred around the vector determined by the point at the base of the subtree and the first principal component of all arbor lengths in the subtree. This generally resulted in unique cloned morphologies that maintained the general structure of the m-type (e.g. laminar structure). However, in L1HACs and Martinotti cells in all layers, this process resulted in a degradation of laminar structure, and thus cloning was not performed on these cells.

Some m-types occur very infrequently, resulting in small numbers of reconstructions for these types (e.g. neurogliaform cells, chandelier cells, and bipolar cells). As such, we allowed these cell types to be used in layers next to the layer in which they are found. Thus, L23NGCs could also be placed in layer 6, L6NGCs could be placed in layer 5, L5ChCs could be placed in layers 4 and 6, and L5BPs and L5DBCs could be placed in layer 6. Martinotti cells were also allowed to be placed in the layer above and below the layer in which they were found, with two exceptions: layer 1 did not contain Martinotti cells and layer 6 Martinotti cells were not allowed to be placed in layer 5.

Objective classification and validation of cloning

Following the unraveling, repair and cloning/scaling of the reconstructed morphologies, an objective classification was performed to ensure that the repaired cells and the clones belong to the same classes assigned by the expert classification. Of the original 55 m-types, 43 were objectively classified. The remaining classes (L23BP, L23NGC, L4BP, L4NGC, L4ChC, L5BP, L5NGC, L6BTC, L6ChC, L6DBC, L6NGC, L6BP) were not classified because too few biological reconstructions were available. The 583 repaired morphologies of 43 m-types were classified using the method of leave-one-out Linear Discriminant Analysis (Rao, 1948) provided by the open-source Python-based machine learning library Scikit-learn (Pedregosa et al., 2011).

The morphologies were divided into 9 superclasses in two steps. The first step distinguished the interneurons from the pyramidal cells depending on the absence or presence of an apical dendrite, and the second step categorized the cells of different layers, according to the expert layer assignment based on the layer containing their somata. An initial classification was performed with 105 anatomical features for pyramidal cells and 75 (apical features are not included) anatomical features for interneurons (see Table S4) that were extracted through NeuroM. The results of this classification showed 99% accuracy between the expert and the objective classification. However, randomization of classes within layers only reduced this accuracy to 97%, indicating over-fitting of the data. To reduce this problem, a second classification was performed using a subset of features. In order to select the most significant features for the anatomical classification of the cells a multi-objective optimization problem was designed to assign an importance score to each feature. The Pareto Front was extracted from the multi-objective optimization problem of minimizing within-group variance and maximizing between-group variance, with the constraint of the predefined 43 classes. The 15 most significant features were selected for each layer and type (Interneuron or Pyramidal cells). After the feature selection, the overall accuracy of the 43 m-types classification was around 70%, while the randomization of classes resulted in a significant decrease of the accuracy to around 40%. This shows that the expert-proposed features (see the section: Classification of morphologies) were also objectively significant.

In order to further improve the classification and minimize the effects of the sparsity of data in interneuron classes (layer 2-6), a hierarchical clustering approach was used for the group of interneurons. The local arborization of most interneurons results in similar morphometrics within their classes (BP, BTC, ChC, DBC, LBC, NBC, SBC, MC, NGC), independently of the position of their somata. This property, which is unique for interneurons, allowed the merging of interneuron classes of different layers into larger classes for classification purposes. Layer 1 interneurons were excluded from this process, because of their distinct morphologies, which cannot be integrated with the rest of the interneurons. The overall accuracy of supervised clustering with feature selection for Layer 1 interneurons is 68%. The described process increased the sample size and reduced the number of classes that could not be objectively classified to two (BP and NGC) because they still had less than 3 members. A subsequent application of hierarchical clustering, a technique commonly used in clustering problems with a large number of classes (here 7) and few members per class (here 7 to 50 members), resulted in a significant improvement of the overall classification accuracy for the interneurons (~

90%). The randomization of classes suggested terminating the hierarchical clustering at three final classes (DBC, ChC, BTC). The detailed results for the interneurons in Layers 2 to 6 are presented in Figure S2A1, A2.

The hierarchical clustering was not applied to the pyramidal cells for the following reasons. First, the pyramidal neurons are cells that commonly span larger areas and are not locally restricted to the layer of their somata. As a result, pyramidal cells of the same type with somata positions in different layers have significantly different morphologies. Second, the number of classes of pyramidal cells per layer (Layer 2/3: 1 class, Layer 4: 3 classes, Layer 5: 4 classes) and the sufficient number of members of each class did not indicate the need for hierarchical clustering. The overall accuracy of the pyramidal cells classification was around 88%. The detailed results of the classification of pyramidal cells Layer 2 to 5 are presented in Figure S2B1, B2. Layer 6 pyramidal cells are a special case, since 2 classes (IPC and BPC) can be identified by the number and the orientation of the apical trees with 100% accuracy, one class does not have sufficient members to participate in the objective classification (HPC) and the rest of the classes (TPCL1, TPCL4, UTPC) can be classified with ~60% accuracy. These results are not presented in Figure S2 since the used method is modified.

Once the performance of the classifier had been quantified, 9187 unique cloned-scaled morphologies were classified according to the described method to ensure that the cloning process did not modify the assigned cell classes. The mean score for the classification of the cloned morphologies was 81%, which is significantly similar to the accuracy of the repaired cells' classification. As a result of this objective classification, we concluded that the assignment of classes to the repaired and cloned cells agrees with the expert classification.

Morphological Structural Analysis

We generated a profile of the mean densities of arbors around the somata of all 55 m-types based on manually reconstructed and repaired morphologies in our database (N = 433), as shown in Figure S1. To generate the profile for a given morphology type we placed 10 copies of each relevant morphology at the same point in (virtual) space and rotated them between 0 and 360 degrees around the y-axis (perpendicular to the layer boundaries). Next, we converted the axons and dendrites into a point cloud by moving recursively from the soma to the tips of the arbors, placing a point in space every 1 µm. Each of these points represented 1 µm of arbor length centered at its location. The final result therefore represented density of arbor length, and not volume. The point clouds for dendrites and axons were converted into a volume separately by counting the number of axon/dendrite points that fall into a 1 µm cubic voxel. Finally, the volume was normalized to present the probability density of finding the center of a randomly picked 1 µm segment of arbor inside the voxel. To visualize the volumes, we calculated the sum along the z-axis (parallel to the layer boundaries) and mapped the densities to a color map. Axon density is shown in blue, dendrite density in red (see Figure S1).

Cell densities and layer boundaries

Slicing procedures

Layer boundaries and densities per layer were computed from light microscopic techniques on slices. P14 Wistar (Han) rats (N = 6) were anesthetized with pentobarbital (100 mg/kg) and transcardially perfused with 20 ml 0.1 M phosphate buffer followed by 100 ml of 4% paraformaldehyde (pH 7.4) prepared in the same buffer. The brains were post-fixed in the same solution for 24 h, and coronal sections were cut with a vibratome. All animals were handled in accordance with the guidelines for animal research set out in the European Community Directive 86/609/EEC and all the procedures were approved by the local ethics committee of the Spanish National Research Council (CSIC).

Immunohistochemistry

Free-floating sections were treated for 30 min with 1% H_2O_2 to deplete the endogenous peroxidase activity, and then non-specific binding was blocked for 1 h in PB with 0.25% Triton-X and 3% horse serum (Vector laboratories Inc., Burlingame, CA, USA). The sections were incubated overnight at 4°C with a mouse anti-neuron specific nuclear protein (NeuN, 1: 2000, Chemicon, Temecula, CA, USA), and they were then processed by the avidin-biotin method, using a biotinylated secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA) and the Vectastain ABC immunoperoxidase kit with 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, St Louis, MO, USA) as the chromogen. After staining, the sections were dehydrated, cleared with xylene and cover-slipped.

The specificity of the staining was controlled by processing selected sections after either replacing the primary antibody with preimmune horse serum, after omission of the secondary antibody, or after replacement of the secondary antibody with an inappropriate secondary antibody. No significant staining was detected under these control conditions.

To generate the figures, images were captured with a digital camera (Olympus DP70) attached to an Olympus BX51 light microscope (Olympus, Ballerup, Denmark), and Adobe Photoshop CS4 software (Adobe Systems, San Jose, CA, USA) was used to produce figure plates.

Estimation of layer boundaries and densities per layer in NeuN-stained sections

Neuronal density were estimated using optical dissectors (Bonthius et al., 2004; West and Gundersen, 1990), and with the aid of the Stereo Investigator software (StereoInvestigator 7.0, MicroBright Field Inc. Vermont, USA).

Optical dissectors were performed on every cortical layer (I, II, III, IV, Va, Vb, VI) from each animal. After randomly selecting a starting point, 5-6 sections (50 µm thick) were selected at equally spaced intervals in the same cortical area (HL somatosensory cortex (Paxinos and Watson, 1998)). Optical dissectors were made in an Olympus BX51 light microscope (Olympus, Ballerup, Denmark) with an oil immersion x100 objective, within a depth of 15 µm. To provide a systematic area offset, the movement of the stage was controlled through the Stereo Investigator software. A neuron was counted only if the nucleus was clearly identified in the height of the optical plane along the *z*-axis.

Correction for tissue shrinkage

To estimate the shrinkage in our samples, we measured the surface area and thickness of the vibratome sections with Stereo Investigator in three different experimental conditions: (1) before fixation, in fresh (unfixed) tissue; (2) after fixation (overnight at +4°C in 4% paraformaldehyde in 0.1M PB); (3) after processing for NeuN immunostaining. For fresh to fixed tissue, the surface area after fixation was divided by the value before fixation to obtain an area shrinkage factor of 0.929. The linear shrinkage factor for measurements in the plane of the section was therefore its square root, 0.964. From fixed tissue to NeuN-immunostained tissue, the surface area after processing was divided by the value before processing to obtain an area shrinkage factor of 0.83. The linear shrinkage factor for measurements in the plane of the section was therefore 0.91. The shrinkage factor in the z-axis was 0.47. Thus, the final values of the neuronal densities were corrected to obtain an estimation of the pre-processing values. Layer thicknesses were corrected by a factor of 0.964*0.91 to obtain an estimation of the pre-processing values.

Morphological composition

E/I ratios

A P14 Wistar (Han) rat was anesthetized with pentobarbital (150 mg/kg) and transcardially perfused with 50ml 0.1M phosphate buffer followed by 100ml of 4% paraformaldehyde (pH 7.4 in 0.1M phosphate buffer). The brain was post-fixed in the same solution for 2h, and 50 µm sagittal sections from right hemisphere were sliced with a vibratome (Leica, VT-1000-S), slices were placed into cryoprotectant (30% glycerol, 30% polyethylene glycol in distilled water) at -20°C until staining.

The non-specific binding sites of the free-floating sections were blocked for 2h in 0.1M PBS with 0.3% Triton X-100 and 1% bovine serum albumin. The sections were first incubated at 4°C for 20h first with a mouse anti-neuron specific nuclear protein 1:1000 (anti-NeuN, Chemicon, MAB377). They were then incubated with a rabbit anti-GABA (anti-GABA, Sigma-Aldrich Inc., A2052) 1:500, at room temperature for 1h followed by 24h at 4°C, with blocking steps in between (PBS-T with 3% normal goat serum). Revelation was done with a goat anti-mouse IgG Alexa488 (Alexa488, 1:1000, Molecular Probes, A11029) and with a donkey anti-rabbit Alexa568 (Alexa568, 1:200, Molecular Probes, A10042) in 0.1M PBS with 1% bovine serum albumin and 0.3% Triton X-100. DAPI was used as a nuclear counterstain (1:50'000, Sigma-Aldrich, D9542). After staining, the sections were mounted in aqueous anti-fading reagent (Dako, S3023) and cover-slipped. Negative control of staining was performed with secondary antibodies only. No significant staining was detected under this control condition.

Imaging was performed on a motorized confocal microscope (Zeiss LSM 700) with 40x magnification at 0.223 µm x 0.223 µm x 1 µm voxel size with 12-bit color depth. Stack depth was chosen to be 90 slices thick to account for possible errors in *z* alignment. A region of 640 µm x 2673 µm was imaged with 10% overlap - resulting in an image size of 2871 x 11990 x 90 voxel. Single stacks were stitched using the custom made Fiji "massive stitcher" plugin implemented by the BioImaging and Optics Platform of EPFL (http://biop.epfl.ch/).

The representative counting region was chosen to be 152.53 µm x 1800 µm x 53 µm in size inside the somatosensory area ranging from bottom of L6 to pia inside the hind-limb somatosensory cortex. Shrinkage correction was not necessary. Counting volume was re-sliced into horizontal plane to better assess nucleus position inside the slice. Counting was performed three times by independent experimenters using the Fiji cell counter plugin. All cells with matching criteria were counted. Upper and left bounding box borders were

defined as exclusion borders. Lower and right borders as inclusion borders. Cells with nuclei touching exclusion borders were not counted. Positions of counted cells were saved. Only double positive (NeuN/GABA; +/+) cells counted as inhibitory neurons. Layer boundaries were applied after counting. Volume rendering was performed with Imaris scientific visualization (Bitplane) and VTK (Kitware Inc., Visualization Toolkit).

All procedures were conducted in conformity with the Swiss Welfare Act and the Swiss National Institutional Guidelines on Animal Experimentation for the ethical use of animals. The Swiss Cantonal Veterinary Office approved the project, following its ethical review by the State Committee for Animal Experimentation.

Immunohistochemistry for Marker Visualization

Wistar rats (n=4 aged 14 days) were sacrificed by administering a lethal intraperitoneal injection of sodium pentobarbital (40 mg/kg), and they were then perfused intracardially with saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. All experiments were approved by the ethics committee of the Spanish National Research Council (CSIC) and performed in accordance with the guidelines established by the European Union regarding the use and care of laboratory animals (Directive 2010/63/EU). Brains were removed and postfixed by immersion in the same fixative for 7 h at 4ºC and then were cryo-protected in 30% sucrose solution in PB until they sank, frozen in dry ice and cut at 50 µm in the coronal plane with a sliding freezing microtome. The sections were pre-incubated for 1 h at room temperature in a stock solution containing 3% normal goat serum (Vector Laboratories, Burlingame, CA) in PB with Triton X-100 (0.25%) and then incubated for 48 h at 4 ºC in the same stock solution containing the following antibodies, alone or in combination: Mouse anti-CB (Swant 1:2000), mouse anti-PV (Swant 1:2000), mouse anti-CR (Swant 1:2000) rabbit anti-NPY (Peninsula, 1:2000), rabbit anti-Som (Peninsula, 1:2000), rabbit anti-CCK (Sigma 1:10000) and rabbit anti-VIP (Incstar 1:1000). Sections were then rinsed in PB and incubated in for 2 h at room temperature goat anti-rabbit and or goat anti-mouse coupled antibodies with Alexa 488 or Alexa 594 (1:1000; Molecular Probes, Eugene, OR). Sections were rinsed and stained with Dapi, to reveal borders between layers and cytoarchitectonical areas. The sections were then washed in PB, mounted in anti-fade mounting medium (Invitrogen/Molecular Probes, Eugene, OR) and studied confocal microscopy (Zeiss, 710). Controls were included in all the immunocytochemical procedures, either by replacing the primary antibodies with pre-immune goat serum in some sections, by omitting the secondary antibodies, or by replacing the secondary antibody with an inappropriate secondary antibody. No significant immunolabeling was detected under these control conditions. Additional quantitative staining was performed using a variation of a previously published protocol (Brionne et al., 2003).

Defining horizontal circuit dimensions

The horizontal dimensions of a microcircuit were estimated by evaluating the density of dendritic fiber at the center of the circuit, as cells are placed at successively farther distances from the center. In particular, we calculated the total length of morphological segments whose midpoint is contained inside a cylinder at the center of the circuit with a radius of 25 µm but spanning all layers. At first, we only considered dendritic segments of cells that had their soma within 25 µm horizontally of the center. We then increased that maximal distance in steps of 25 µm, each step increasing the total length in the center. We calculated the radius where 95% of the asymptotal maximal length is reached as 210 µm (linearly interpolated). Instead of a circle, we used a hexagon with identical area as the base of the microcircuit to facilitate tiling, while minimizing asymmetrical edge effects.

Soma positions

The horizontal and vertical extents yield a 3D microcircuit volume in the shape of a hexagonal prism that is 461.8 µm wide (at its widest point, side length 230.9 µm) and 2082 µm high (See Figure 3D), composed of 6 layers. This volume was then populated by assigning soma positions according to the derived layer-specific cell densities. The positions were distributed in mini-columnar arrangements using the Niederreiter space-filling algorithm (Niederreiter, 1988). Each mini-column contains around 100 neurons (depends on total numbers in circuit, which varies slightly across the different instantiations, Bio1-Bio5, BioM, according to their differing total numbers of neurons) and exactly 310 mini-columns per unitary microcircuit. Finally, each soma position was assigned an m-type according to the derived morphological composition and E-I fractions. Furthermore, each soma position was associated with a random rotation to be applied to the morphology.

Ensuring inter-laminar structure

The microcircuit contains morphologies of different sizes and shapes. Placing the soma of reconstructed morphologies in the layers they were found in during the reconstruction is insufficient to ensure a biologically correct placement. We found that there are more constraints on the morphologies than just the home layer of

the soma. For example, within a layer, a given morphology may be limited to only a small fraction of the available space, due to its axon or dendritic structure. We know for example, that the arbors of a cell cannot stretch further than the top of layer I. This means that the largest pyramidal cells of a given layer can only be placed at the bottom of that layer. Conversely, the dendrites of the smallest pyramidal cells originating from the top of layer II/III will be unable to reach layer I, if placed at the bottom of the layer.

Once a neuronal location was assigned an me-type, it was assigned a morphology from the database of morphologies provided by the morphology release process (unravel, repair, mix-and-match, scaling, cloning, substitution) according to a placement scoring algorithm designed to ensure adherence to known biological rules for laminar placement of morphological features (e.g. L1 targeting axon clusters of Martinotti axons, L4 targeting pyramidal dendrites).

A broad literature review was undertaken to identify described rules for the laminar targeting of morphological features, and individual repaired morphologies (prior to mix-and-match, scaling and cloning) were manually annotated for vertical intervals which should target specific layers. Annotations of parent intervals were carried over to mix-and-match, scaled, and cloned morphologies. For the latter, sections annotated in the parent were also annotated in the clone by correspondence of segments, and the clone was rejected if any extent of an annotated region was not within ± 60% of the parent region.

Scores were computed for each pool of m-types for bins of 10 µm along the vertical axis of the microcircuit. The score was computed as the generalized mean of the overlap of Gaussians between feature interval and target interval over all annotated rules for a given morphology. A given morphology was removed from consideration for placement if it penetrated the pia. For a given location, the candidate morphology was randomly chosen weighted by score from the pool of cells with the highest scores accounting for 8% of the total score of the m-type pool.

In the superficial layers 2 and 3, some m-types contained no exemplars that could be placed in upper regions of the layer without exiting the pia. In this case, the density of this m-type was redistributed to the sub-volume of the layer where exemplars were available to be placed.

MORPHO-ELECTRICAL DIVERSITY

Physiological recordings

Electrophysiology

The firing patterns of neurons, obtained from *in vitro* recordings in P14-16 rat somatosensory cortex, were expert-classified into one of 11 electrical types (e-type; cAC, bAC, cIR, bIR, cNAC, bNAC, dNAC, cSTUT, bSTUT, dSTUT, cAD; see Figure 4) based on their response to stimulus protocols as described below. The response properties recorded from several neurons (N = 143) were selected from our experimental database as the basis for the distributions of feature values used for electrical model optimization. The experimental procedures were published in previous studies (Toledo-Rodriguez et al., 2004).

Stimulus protocols

Neurons were stimulated with a set of previously described protocols (Le Bé et al., 2007; Wang et al., 2002, 2004). Only a subset of these stimuli was used to generate neuron models.

- **IDRest**: In the IDRest protocol the experimenter determines a hyperpolarizing offset current to keep the cell at -70 mV (before liquid junction potential correction) and applies this current during the entire protocol. After an initial period of 700 ms, a step current was applied for 2000 ms, and after the step, a final period of 300 ms is recorded. This protocol is repeated with different step currents normalized to the threshold current (i.e. the lowest current that generates one AP during the step). For this study, the current steps IDRest150, IDRest200 and IDRest250 (resp. 150%, 200% and 250% threshold) were used.
- **APWaveform:** The offset current is determined in the same way as in the IDRest protocol. To obtain a detailed profile of the action potential waveform, voltage is sampled with a period of 10 µs. After 5 ms, a step current is applied at typically 2 to 4 times the threshold step. Voltage is recorded for 50 ms, mostly generating a trace containing two to three high-resolution AP waveforms.
- **APThreshold**: The offset current is determined in the same way as in the IDRest protocol. A ramp current (from 0 pA to 4 times threshold) is injected for 2 seconds after an initial period of 100 ms

To create pyramidal cell models, only features extracted from voltage traces obtained during the IDRest protocol were calculated. For the interneuron models, APWaveform and APThreshold protocol traces were included.

Composition of e-types

The me-type fractions summarized in Figure 4C were assessed from statistics of assigned e-types from a pool of 511 m- and e-type classified inhibitory neurons. Since researchers are unable to target cells based on their electrical properties, the fractions are assumed to be unbiased. Excitatory m-types were uniformly classified as continuous adapting/accommodating (cAD). Since some m-types are infrequent, in some cases samples from different layers were pooled to have sufficient samples to assess the e-type fractions. Such pooling was done in a conservative manner, only when necessary, and only from neighboring layers if possible. For example, BTCs for layers 5 and 6 were pooled to assign the fractions in those layers. The pooling rules applied, in an m-type specific manner, are as follows: (A) L4 + L5 + L6, (B) L5 + L6, (C) L1 lumped (NGC + NGC-DA + NGA-SA), (D) L23 + L4 + L5 + L6. The application of these rules is summarized in Table S5.

RECONSTRUCTING MICROCIRCUIT CONNECTIVITY

Touch detection

After placing the morphologies in 3D space, the next step consisted of generating a structural circuit by detecting zones of geometric overlap called “touches”. This step was performed by the custom developed BlueDetector software (Kozloski et al., 2008), which implemented the following workflow: 1) Reconstructed morphologies were loaded, translated and rotated according to the assigned soma positions and rotations (see Soma positions); 2) A division of the volumetric space into sub-volumes (called "slices") was calculated in such a way that every sub-volume held the same amount of data; 3) Morphology segments were evenly distributed over available cores, based on previous slicing; 4) An in-core touch detection algorithm was independently executed on each core (which holds a slice); 5) The detected touches were then sent back to the core that holds each full morphology of the previously sliced neurons; 6) Neurons touches were then filtered according to the biological rules (Riachi, 2010); 7) The resulting set of structural touches was written in parallel to disk. The execution of such a process for a microcircuit consisting of 219k neurons and around 7.0 billion structural touches ran on 8k cores of a CADMOS IBM BG/P or BG/Q supercomputer with an execution time of approximately 1.5 hours.

Touch filtering

For details of the filtering of touches or appositions to constrain synaptic connectivity to experimental data, see the companion paper (Reimann et al., 2015).

Estimation of external input

We estimated the fraction of external inputs into all layers (Figure S7E) by comparing the spine density emerging from connections within the microcircuit to biologically characterized densities. Spine densities in the reconstructed microcircuit were calculated as the density of excitatory synapses, thus assuming one excitatory synapse per spine. Densities were calculated on dendritic segments in the most central 50 μm of the microcircuit where axonal density was highest. They were then compared to m-type specific values from the literature, calculating the fraction of external input needed to reach full, biological density. Finally, fractions were averaged per layer.

NEURONAL PHYSIOLOGY

Neuron models

Multicompartmental, conductance-based models of neurons were obtained from reconstructed morphologies in P14 rat somatosensory cortex. Up to 13 active ion channels types and a model of intracellular Ca^{2+} dynamics were incorporated in neuron models. The compartments were separated into different regions: (1) axon initial segment (AIS), (2) soma, (3) basal dendrites and (4) apical dendrites. Interneurons contained only one dendritic region. Each region received a separate set of channels ((see NMC portal;(Ramaswamy et al., 2015)). The full axon was not simulated, but only the AIS. The AIS was represented by two fixed length sections with a 30 μm length, whose diameter was obtained from the reconstructed morphology used in the model fitting process.

Action potentials were detected in the AIS, and the information was transmitted with a delay to the dendritic locations of synaptic contacts on postsynaptic neurons (see Synaptic Physiology).

Compartment discretization

Reconstructed and repaired neuron morphologies (Anwar et al., 2009) were divided into isopotential compartments of a maximal length (20 µm). The average number of compartments in all neuron models was approximately 260.

Passive properties

Membrane capacitance (C_m) was set to 1 µF/cm^2 for the soma, AIS and dendrites. In pyramidal cells a value of 2 µF/cm^2 was used for the membrane capacitance in the basal and apical dendrites to correct for dendritic spine area. Axial resistance (R_a) was set to 100 Ω·cm for all compartments. For pyramidal cells the maximal conductance of the leak current was manually set to a value that created a resting potential, membrane time constant and input resistance in accordance with reported values (Le Bé et al., 2007; Stuart and Spruston, 1998). For interneurons, the leak reversal potential and the leak conductance were set as a free parameter in the optimization algorithm, within physiological bounds.

Conductance mechanisms

We included 13 key active ionic currents known to play a role in neocortical neurons, with kinetics obtained from published ion channel models or published experimental data (transient sodium (Colbert and Pan, 2002), persistent sodium (Magistretti and Alonso, 1999), transient potassium (Korngreen and Sakmann, 2000), persistent potassium (Korngreen and Sakmann, 2000), m-current (Adams et al., 1982), h-current (Kole et al., 2006), high voltage-activated calcium (Reuveni et al., 1993), low voltage-activated calcium (Avery and Johnston, 1996), Kv3.1 (Rettig et al., 1992), d-type potassium (Shu et al., 2007), stochastic potassium (Diba et al., 2006), SK calcium-activated potassium (Köhler et al., 1996)). The kinetics of ionic conductances that were characterized at room temperature (21 °C) were adjusted to the simulation temperature of 34°C using Q10 of 2.3. The kinetics obtained from experiments where the liquid junction potential was not corrected for were shifted by -10mV. The reversal potentials for Na^+, K^+ and Ih were set to 50, -85 and -45 mV respectively.

Ion currents were modeled using Hodgkin-Huxley formalism, so that for each ion current:

$$I = \bar{g} * m^x * h^y (V - E)$$

where $\bar{g}$ is the maximal conductance (or density); *x* and y are the number of gate activation and inactivation variables, respectively; E is the reversal potential of the given ion; and V is the membrane potential. Figure S8 describes the kinetics of each ion channel conductance mechanisms used in this study. To link the calcium channels to the calcium-activated potassium channels, an exponentially decaying intracellular calcium pool was included.

Reconstructing morpho-electrical behavior

Conductance distribution

For pyramidal cell models, all ion channel mechanisms were uniformly distributed in the soma, AIS, basal and apical dendrites, except Ih, which was exponentially distributed in apical dendrites (Kole et al., 2006). For the interneuron models, all ion channels were uniformly distributed in the soma, AIS and dendrites with the exception of Ih, which was exponentially distributed in dendrites. A complete description of all the channel distributions is provided in the Neocortical Microcircuit Collaboration (NMC) Portal - https://bbp.epfl.ch/nmc-portal (Ramaswamy et al., 2015).

Optimization of neuron models

A feature-based multi-objective optimization method as previously described (Druckmann et al., 2007) was used to fit the neuron models. In brief, feature-based error functions were designed to deal with the variability of experimental responses to identical stimuli. The free parameters in the optimization were primarily the density of ion channel conductances located in the soma, AIS and the dendrites (see NMC portal).

We developed an optimizer framework in C++ to integrate the NEURON simulation environment (Carnevale and Hines, 2006), optimization library PISA (Bleuler et al., 2003), and a feature extraction library. Among different implemented optimization algorithms (NSGA, NSGA-II, SPEA2, IBEA), we used the indicator-based evolutionary algorithm (IBEA). Cell model optimization converged faster and reliably found models with summed errors better than the previously reported modified NSGA algorithm (Druckmann et al., 2007). The evolutionary algorithm was run with a population size of typically 1024 individuals on 512 cores of a BlueGene/P system for 100-200 generations. After the optimization, the best solution was the individual in the

population with the smallest sum of its objective values. Every optimization was typically run with different random seeds. The best solution among different seeds was selected as the final electrical model.

We used a set of key electrical features (e-features) of target firing behavior at the soma and dendrites (see NMC portal). For every e-feature an absolute standard score Z_i was calculated:

$$Z_i = \frac{|f_i - \mu_i|}{\sigma_i}$$

with f_i the e-feature value measured from the output traces of the models, μ_i and σ_i the experimentally measured mean and standard deviation for the e-features in the respective cell types.

These Z-scores served to define the objectives O_j to be fitted by the evolutionary algorithm:

$$\boldsymbol{O_j} = \sum_{\boldsymbol{i=0}}^{\boldsymbol{n_{O_j}}} \boldsymbol{w_i Z_i}$$

with w_i weight factors, and n_{O_j} the number of e-features in objective O_j.

Every optimization was run with an exemplar morphology that corresponded to the target electrical firing type.

In total, 14 electrical models were created: Ten models corresponding to the inhibitory firing types (cAC, bAC, cIR, bIR, cNAC, bNAC, dNAC, cSTUT, bSTUT, dSTUT), and four models corresponding to excitatory firing types (one model each for PCs in layers 2/3 to 6, and one model for L4SS). This is required due to fact that the morphology of pyramidal cells found in different layers is markedly different.

Electrical models of pyramidal neurons were generated based on a set of experimental features identified from responses to “IDRest” stimuli (see (Hay et al., 2011)). The feature value set used for electrical models of pyramidal neurons across all layers was the same; only the morphology used during the optimization differed. The experimental mean and standard deviation for features measuring the mean frequency, adaptation, CV of the ISIs, doublet ISIs, time to the first spike, AP height / width, AHP depth / time were calculated for all these traces, and were used as target data. One feature also measured the height of the back-propagating action potential in the model at two different locations in the dendrites based on the literature (Larkum et al., 2001). Every objective seen by the optimization algorithm consisted of a combination of a selected set of features. The maximal conductance of the I_h channel and the reversal potential of the leak current were fixed to values based on previous studies (Kole et al., 2006).

Inhibitory cell models were based on the features extracted from responses to APWaveform, APThreshold IDRest150, IDRest200 and IDRest250 stimuli from the experimental data (see *Stimulus protocols*). Due to the large variability of firing types of the interneurons, and due to the computational cost of the optimizations, a different strategy was used for these models. First a set of four core e-types, namely cAC, bAC, cNAC and bNAC was selected, and full optimizations were run to generate models for these firing types. The maximal conductances of Ih and the leak current and the reversal potential of the leak current were part of the optimized parameters. For these interneuron models, every objective in the optimization algorithm corresponded with exactly one feature. Since all the cells of the same e-type were obtained from different m-types and pooled together, some of the resulting features had a large experimental standard deviation. Therefore, the experimental distributions of some features were manually restricted to yield tighter constraints for the optimization.

To generate models for delayed, stuttering and irregular spiking cells, an extra stochastic potassium channel was added to the soma and dendrites of the four core e-types, creating channel noise in the models (Diba et al., 2006). A new optimization was then run to find a value for the conductance of the channel, with the remaining parameters untouched. In this manner, cSTUT, bSTUT, dSTUT, cIR, bIR were derived from cNAC, bNAC, dNAC (see below), cAC, bAC respectively. The stochasticity of the channel was ensured by implementing a unique random number generator for each compartment in these electrical models. A different seed was assigned to every cell and compartment, and the long period of the random number generators avoided conflicts between the different seeds. In a similar way, dNAC was created from the cNAC model by adding a slowly inactivating D-type potassium channel (Shu et al., 2007). The conductance of this channel was optimized using the time to the first spike as a target feature.

Quality assurance of morpho-electrical models

After creating the 14 electrical models that were optimized for specific exemplar morphologies, these models were combined with all the relevant morphologies in our database to create a large set of morpho-electrical

(me) combinations. We ran a process called Model Management to ensure the quality of thus generalized me-combinations. First, the IDRest protocol was applied to every me-combination, and the features that were used during the optimization were calculated from the traces (for IR, STUT and delayed models these were combined with the feature values of the core e-type they were derived from). The repaired version of the exemplar morphology used during the optimization was a benchmark to accept or reject other me-combinations. Combinations were accepted if the Z-scores Z_i for all individual features f_i met the following criteria: $Z_i \leq \max(5, 5 \cdot Z_{i,\text{exemplar}})$ for pyramidal cells and $Z_i \leq 5 \cdot Z_{i,\text{exemplar}}$ for interneurons. To ensure sufficient numbers of interneuron combinations were retained, the scores of certain features were given a higher acceptance threshold.

Goodness-of-fit

Z-scores in Figure 8 were calculated based on experimental features, where values that were changed to improve the optimization algorithm were omitted. Pyramidal neuron models have higher Z-scores due to less variability in the experimental data as against inhibitory neurons, mainly because for interneurons data from more m-types was pooled together. For the Z-score calculation of L23 pyramidal cell models, additional traces specific to these cells from our experimental database were used.

SYNAPTIC PHYSIOLOGY

Modeling stochastic synaptic transmission

At each synaptic location identified by the algorithm to reconstruct microcircuit connectivity, we implemented a stochastic model of synaptic transmission based on previous work (Fuhrmann et al., 2002), but also including facilitation. The implemented two state Markov model is a stochastic model of dynamic synaptic release with an ensemble average response equal to that of the phenomenological Tsodyks-Markram dynamic synapse model (Tsodyks and Markram, 1997). The model further incorporates NMDA receptor (NMDAR) kinetics (Jahr and Stevens, 1990a, 1990b) where applicable. The underlying assumptions were derived from the classical quantal model of synaptic transmission, in which a synaptic connection is assumed to be composed of *N* independent release sites (Del Castillo and Katz, 1954); (Korn and Faber, 1991), each of which has a probability of release, *p,* and contributes a quanta *q* to the post-synaptic response. Release from any particular site is independent of release from all other sites (Fuhrmann et al., 2002). We assumed the number of release sites equals the number of synapses per connection (Ramaswamy et al., 2012).

The two state Markov model to simulate stochastic synaptic transmission has the following properties:

- There can be no consumption of synaptic resources when an event fails to release neurotransmitter - i.e. during failure of synaptic transmission
- After neurotransmitter release at any given release site, there can be no further release of at that site until it recovers.

The exact synapse models as implemented are included in the neuron model packages available for download on the NMC portal.

Parameterizing synaptic kinetics

Excitatory synaptic transmission was modeled with both AMPA and NMDA receptor kinetics. For AMPA receptor (AMPAR) kinetics, the rise ($\tau_{riseAMPA}$) and decay ($\tau_{decayAMPA}$) time constants were 0.2 ms and 1.74 ± 0.18 ms, respectively (Häusser and Roth, 1997). Pathway specific values for the parameter "utilization of synaptic efficacy" (U, analogous to the probability of neurotransmitter release) were unified from various experimental studies of synaptic transmission in juvenile rat somatosensory cortex (Le Bé et al., 2007; Brémaud et al., 2007; Feldmeyer, 2006; Koester and Johnston, 2005; Markram et al., 1997; Silver et al., 2003).For NMDAR kinetics, $\tau_{riseNMDA}$ and $\tau_{decayNMDA}$ were 0.29 ms and 43 ms, respectively (Sarid et al., 2007). The time constants are consistent with several previous in vitro studies (Feldmeyer, 2006; Flint et al., 1997; Monyer et al., 1994; Rinaldi et al., 2007). The concentration of Mg^{2+} was generally set to 1 mM (Jahr and Stevens, 1990b), but was set to 0.5mM where exceptionally specified. The reversal potential of AMPA and NMDA receptors was set to 0 mV. The axonal conduction delay for each synaptic contact was computed using the axonal path distance from the soma, and a AP conduction velocity of 300 µm/ms, based on experimental estimates (Stuart et al., 1997). Furthermore, experimentally measured ratios of NMDA and AMPA conductances were used in order to model their relative contribution to unitary the EPSC (Feldmeyer, 2006; Myme et al., 2003; Rinaldi et al., 2007; Silver et al., 2003; Wang and Gao, 2009). For pathways where specific

values are lacking, we used extrapolated conductance ratios of 0.8 ± 0.1 and 0.4 ± 0.1 for E-E and E-I connections, respectively (Wang and Gao, 2009).

Inhibitory synaptic transmission was modeled with a combination of $GABA_A$ and $GABA_B$ receptor kinetics. For $GABA_A$ receptor ($GABA_AR$) kinetics, the rise ($\tau_{riseGABAA}$) and decay ($\tau_{decayGABAa}$) time constants were 0.2 ms and 10.4 ± 6.1, 8.3 ± 2.2 or 6.44 ± 1.7 ms respectively, for the 3 specific inhibitory synapse types used (Gupta et al., 2000). Synapse-type-specific values for the parameter U (analogous to the probability of neurotransmitter release) were compiled from previous experimental studies (see Table S6). The reversal potentials for $GABA_A$ and $GABA_B$ were set to -80 mV and -93 mV respectively (Mott et al., 1999; Silberberg and Markram, 2007). Due to a lack of voltage-clamp experimental data on the synaptic kinetics of $GABA_B$Rs in neocortical connections, data from hippocampal connections was used (Khazipov et al., 1995; De Koninck and Mody, 1997; Mott et al., 1999; Rovira et al., 1990). For $GABA_B$ receptor ($GABA_BR$) kinetics, the rise ($\tau_{riseGABAB}$) and decay ($\tau_{decayGABAB}$) time constants were 3.5 ms and 260.9 ms respectively (Mott et al., 1999).

Synaptic conductances were determined by optimization of in silico paired recordings in the reconstructed microcircuit to match experimentally measured PSP amplitudes where available (see Table S2). For pathways where experimental data was not available, averages computed for E-E, E-I, I-E, and I-I connection types were assigned (see Table S6).

Reconstructing synaptic dynamics

Based on experimental data, synapse types (s-types) were separated into facilitating (E1 & I1), depressing (E2 & I2), and pseudo-linear (E3 & I3) types (Beierlein et al., 2003; Gupta et al., 2000; Reyes et al., 1998; Thomson et al., 1996; Wang et al., 2002, 2006); for a review see (Thomson and Lamy, 2007). We identified and used several constraining principles based on the current state of the art to map s-types associated with specific me-type to me-type pathways (Angulo et al., 1999; Bannister and Thomson, 2007; Blatow et al., 2003; Feldmeyer, 2006; Feldmeyer et al., 1999; Frick et al., 2007; Galarreta and Hestrin, 1998; Gupta et al., 2000; Holmgren et al., 2003; Kapfer et al., 2007; Maffei et al., 2004; Markram and Tsodyks, 1996; Markram et al., 1997, 1998; Mason et al., 1991; Mercer et al., 2005; Reyes et al., 1998; Rozov et al., 2001; Silberberg and Markram, 2007; Thomson and Bannister, 1998; Thomson and Lamy, 2007; Thomson et al., 1993; Wang et al., 1999, 2002, 2006). See Figure S9 for a summary of s-type assignment rules used.

Where directly available, experimental data were applied to relevant pathways, and where unavailable the constraining principles as previously identified were applied to produce a fully constrained map of synaptic dynamics for all possible me-type to me-type pathways. Unique dynamic synaptic parameters (U, D & F) for individual synapses were prescribed from a truncated Gaussian distribution with the SD parameter determined from experiments (Gupta et al., 2000; Silberberg and Markram, 2007; Wang et al., 2002, 2006).

Spontaneous synaptic release

Spontaneous miniature PSCs were modeled by implementing an independent Poisson process (of rate λ_{spont}) at each individual synapse to trigger release at low rates. The rates of spontaneous release for inhibitory and excitatory synapses were chosen to match experimental estimates(Ling and Benardo, 1999; Simkus and Stricker, 2002). The excitatory spontaneous rate was scaled up on a per layer basis to correct for missing extrinsic excitatory synapses. The resulting spontaneous release rates for unitary synapses were low enough (0.01Hz-0.6Hz) so as not to significantly depress individual synapse.

SIMULATION

Environment

The reconstructed microcircuit was simulated using the NEURON simulation package as the core computational engine (Hines and Carnevale, 1997). A collection of tools and templates, called Neurodamus, written in the HOC and NMODL programming languages (Hines and Carnevale, 2000) were employed to handle the setup and configuration of the microcircuit on the parallel machine architecture (Hines et al., 2008a). In addition, a reporting library written in C++ handled the parallel gathering of data and output to disk (Gropp et al., 1999). Simulations were configured in a configuration file to the main run script of Neurodamus, which specified the location of key data files, and assigned stimuli and reports to designated groups of cells, referred to as targets.

Simulation Configuration

A simulation was parameterized through a configuration file. Multiple sections in the file described an aspect of the simulation: general run parameters, stimuli, report generation, synapse and connection configurations. Key-Value pairs were used to apply settings within a given section. The initial section of the configuration provided user specified general settings for a simulation, including paths to various locations on disk such as the circuit and synapse files, the morphology files, the neuron models (electrical templates), and an output directory for generated reports. Other settings included the duration of a simulation, the time increment of the numerical solver, and the load-balancing mode. Stimulus sections were created for each stimulus to apply to a set of cells. Stimuli of various patterns were available to choose from: pulse, ramp, spike train, sine, random noise, etc. Depending on the stimulus pattern, additional settings were required. For example, a pulse stimulus required specification of a current amplitude (nA), a spike train required a frequency (Hz) and so on. All stimuli required a time delay and duration. Report sections were created to record a variable from a set of cells. Reports were configured to specify the variable in the NEURON simulation environment from which to record, for a starting time until some end time. In addition, the user could choose the file output format: ASCII, hdf5, or binary. Binary format was required when using multisplit load balancing in order to handle restructuring data that has been split across CPUs (Hines et al., 2008a). Connect sections were created to customize synapse creation. Users could designate two sets of cells, the source target and destination target, and any synapses from the source to the destination were configured as specified within the section.

Neurons were grouped together as a target. Two types of targets were used for regular simulations: cell and compartment targets. Cell targets were used more generally throughout simulation configuration. Members of a cell target were typically interpreted as whole cells for the purpose of connect sections. For stimuli and reports, members were interpreted as somata. Compartment targets provided a more specific addressing capacity in order to allow for dendritic or axonal locations of a cell to be included for stimulus and report usage.

Load-balancing and multisplit

When simulating the microcircuit on a massively parallel machine, it was important that the workload be distributed as evenly as possible to minimize idle CPUs. NEURON provides hooks to compute the computation load of a cell and determine the optimal way to separate that cell in multiple pieces assigned across multiple CPUs (Hines et al., 2008b). The initial microcircuit was instantiated by Neurodamus in a straightforward manner, which may not have very good load balancing. Calls were assessed by NEURON's load balancing algorithm and the optimal splitting strategy was written to disk. The microcircuit was then cleared from memory and Neurodamus recreated it using the now available load balancing information. During later stages of setup, checks were made to ensure that actions were executed for pieces on the local CPU and skipped when the piece exists on a remote CPU. The reporting library especially took care to handle the split cell case and managed the reorganization of data into the final output report file such that any cell's data appear all together within the report.

IN SILICO EXPERIMENTS

Bath manipulation simulations

In vivo and in vitro-like conditions were reconstructed in silico by modifying ionic concentrations in the extracellular bath medium. The extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) was manipulated by changing the utilization of synaptic efficacy parameter (U) in the stochastic synapse model (see section Synaptic Physiology). The reconstructed synaptic U parameters for standard in vitro bath conditions ($[Ca^{2+}]_o$ =2.0mM) were scaled as a function of $[Ca^{2+}]_o$ with pathway specific interpolating functions. The interpolating functions were determined from experimental data on changes in PSP amplitudes (relative to PSP at $[Ca^{2+}]_o$ =2.0mM) as a function of $[Ca^{2+}]_o$ by assuming all $[Ca^{2+}]_o$ induced changes where due to changes in U, and that U and PSP amplitude scalings have a linear relationship. As depicted in Figure S12, multiple data sources on such PSP scalings were collected from literature, and found to be consistent with one of two Hill isotherms reported previously (Rozov et al., 2001), either steep or shallow dependence. Parameterizations of $K_{1/2}$=2.79 for steep and $K_{1/2}$=1.09 for shallow are from previous reports (Rozov et al., 2001).

Specificities of $[Ca^{2+}]_o$ dependencies on connection type were implemented as follows: a) synapses between excitatory neurons, and between excitatory neurons and distal-targeting interneurons (DBC, BTC, MC, BP) in both directions have a steep calcium dependency; and b) synapses between excitatory and proximal-targeting PV+ interneurons (LBC, NBC, SBC, ChC) in both directions have a shallow calcium dependency (Figure S12A) (Gupta et al., 2000; Rozov et al., 2001; Silver et al., 2003; Tsodyks and Markram, 1997). Due to a lack of experimental data, synapses between inhibitory neurons, and between excitatory neurons and all other

inhibitory neurons are assumed to have level of dependency on $[Ca^{2+}]_o$ as the average of the two extremes (Figure S12A).

Depolarization in bath manipulation experiments (e.g. to mimic increased $[K^+]_o$ in vivo, tonic glutamate, or neuromodulators in the bath) was achieved by current injection at the neuron soma. Currents were expressed in terms of percentage of the minimum step current injection required for each cell to spike at least once (rheobase).

Bath manipulation experiments using multi-electrode array

To experimentally validate the observed transitions in the network state due to changes in $[Ca^{2+}]_o$, a multi-eletrode array was used to observe changes in synchronous network activity in brain slices while allowing rapid changes in bath composition. In accordance with the Swiss national and institutional guidelines, 300 µm thick sagittal brain slices were prepared from the somatosensory cortex of postnatal day 14 to 15 Wistar rats (N=2) of either sex in iced artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 25 D-glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 1 $MgCl_2$; all chemicals from Sigma-Aldrich, St. Louis, MO or Merck, Darmstadt, Germany) using a HR2 vibratome (Sigmann Elektronik, Heidelberg, Germany). The primary somatosensory cortex was manually dissected and isolated to obtain rectangular slices of 5 – 7 mm width and containing the neocortex in its entire height. Optimal slices, with apical cell dendrites running parallel to the slice surface, were selected for recordings. Slices were incubated at 22 ºC for 30 – 60 min until mounting in the recording chamber. Slices were mounted on a 3D-MEA with 60 pyramidal platinum electrodes (electrode basis: 40 µm × 40 µm, electrode height: 50 – 70 µm, electrode interspacing: 200 µm; Qwane Bioscience SA, Lausanne, Switzerland) after evaporation of a mounting solution of 0.14 mg/L nitrocellulose in an ethanol (99%) – methanol (1%) mixture. Experiments were conducted at room temperature (22°C).

We modified the concentration of potassium $[K^+]$, magnesium $[Mg^{2+}]$ and calcium $[Ca^{2+}]$ ions present in the extracellular solution to induce transitions in the network activity. We defined 3 different conditions corresponding to 3 sets of concentrations. Baseline solution: $[K^+]$ 2.5 mM; $[Mg^{2+}]$ 1.0 mM; $[Ca^{2+}]$ 2.0 mM; solution S1: High Potassium Low Calcium: $[K^+]$ 6.25 mM; $[Mg^{2+}]$ 0.5 mM; $[Ca^{2+}]$ 1.0 mM; and solution S2: High Potassium High Calcium: $[K^+]$ 6.25 mM; $[Mg^{2+}]$ 0.5 mM; $[Ca^{2+}]$ 2.0 mM. Bath changing times were minimized by employing a pipette to remove the recording chamber solution prior to changing the subsequent solution. We perfused the baseline solution for 5 minutes then recorded the network activity in the surface chamber for 90s; then, perfused solution S1 for 5 minutes recorded the network activity for 270s; and finally perfused solution S2 for 5 minutes recorded the network activity for 270s. Several experiments were undertaken to establish the transition time using this approach, and the 5 min interval between recording times was determined to be a minimal yet sufficient time to induce a transition to bursting (baseline->S2 or S1 ->S2).

We recorded activity in a total of 12 slices (3 slices per hemisphere per animal). Experimental data analysis was performed in Matlab (The MathWorks, Inc., Natick, MA, USA) with custom scripts. Extra-cellular spikes were detected when recorded signal crossed a dynamic threshold *T*:

$$T = \dot{x} - 5 * std(X)$$

X is the recorded data chunk over the whole recording duration whereas $\dot{x}$ is a local average of the recorded data (sliding window of 40 ms). We computed the inter burst interval (IBI) when slices were perfused with S2, and oscillations lasted for 30s or more. To avoid transitions in the network activity to affect this measure, the IBI was computed over period considered as regular (IBI below a threshold = avg(IBI) + 3 std(IBI)). Data are presented as the mean ± SEM. Paired Student's ***t*** tests were applied as statistical tests, and statistical significance was asserted for: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Observations of oscillations for various solutions as follows. Baseline solution: 1/12 slices, 2 bursts (~20 Hz); S1: 2/12 slices, sparse bursting; S2: 9/12 slices, regular bursting with an IBI of 1.59 +/- 0.09 s (mean +/- SD) or 0.98 +/- 0.05 Hz.

Generating thalamic input

The hind-limb somatosensory cortex receives input from the ventro-posterolateral (VPL) nucleus of the thalamus (Kandel et al., 2000). As data for the VPL enervation pattern is scarce, we used published data from the ventral posterior medial (VPM) thalamic nucleus to generate a vertical density profile for external thalamic synaptic input (Meyer et al., 2010). In a number of successive steps, first the vertical depth profile was digitized and binned with a bin size of 25 µm. For each depth bin, we then found all morphological segments contained inside that bin (i.e. the midpoint of the segment was located inside the bin). We then continuously drew random segments from the pool and placed a synapse at their centers, until 2.6 times the biological density (oversampling) at that depth bin was reached. Synapses were then pruned using the multi-synapse rule step (see (Reimann et al., 2015)). Drawing was with replacement, i.e. a segment could be drawn more

than once. The probability of drawing a given segment was proportional to its length, i.e. longer segments will be drawn more often. The 2.6 oversampling factor was chosen to match thalamocortical synapses per connection onto L4 excitatory cells.

Dynamic synaptic parameters were drawn from distributions with means and standard deviations taken from the literature (Amitai, 2001; Gil et al., 1999).

Each external synapse was assigned a virtual presynaptic VPM cell, whose spike will activate it. To accurately capture the correlation between synaptic inputs, the mapping to virtual presynaptic cells was spatial according to minicolumns, i.e. synapses that were close together were likely to be innervated by the same virtual presynaptic cell. In particular, there was one virtual cell per minicolumn and the probability that a synapse was mapped to it was dependent on the distance to the center of the minicolumn:

$$P(S_{pre} = i) \propto e^{-\frac{\left|C_i - T_{pre}\right|}{2\sigma^2}},$$

where S_{pre} denotes the mapping of synapse S, T_{pre} its spatial location and C_i the location of the center of minicolumn i ; σ defined the degree of spatial mapping and was set to 25 µm.

Calculation of spike time correlations

The mean spike-spike correlations (Figure 17, S13) were calculated as the histogram of intervals between all spike times of two different cells (bin size 1 ms). The average was computed over 10 ’000 randomly selected cell pairs.

Detection of structural assemblies

Structural assemblies (Figure S15) were detected based on the number of common neighbors between cell pairs, i.e. the number of neurons connected pre- or post-synaptically to both cells. We calculated the number for all N^2 potential pairs, where N is the number of neurons in the circuit. The numbers were converted into a distance measure between 0 and 1, setting the distance between pairs to 1 minus the cumulative probability of the number of common neighbors. The cumulative probability was calculated once based on the number of common neighbors of all pairs involving one cell of the pair, then based on the other cell and finally the root mean square was used:

$$D(i,j) = 1 - \sqrt{\frac{C_i\left(N(i,j)\right)^2 + C_j\left(N(i,j)\right)^2}{2}},$$

where *N(i,j)* refers to the number of common neighbors between *i* and *j* and C_x to the cumulative probability function based on the number of common neighbors of *x* with any cell.

Assemblies were then detected by creating a hierarchical cluster tree based on the distance matrix using Matlab (The MathWorks, Inc., Natick, MA, USA).

Calculation of voltage correlations

Voltage correlation was computed by convolving the two voltage traces with their means subtracted.

REPRODUCTION OF *IN VIVO* EXPERIMENTS

Emergence

We performed a series of simulations on monotonically increasing circuit sizes (50, 100, 200, 300, 400 and 1000 minicolumns per microcircuit, $[Ca^{2+}]_o$ = 1.25 mM, 100% depolarization), resulting in a collection of six 20 second recordings of spontaneous *in silico* activity. Subsequently, each microcircuit’s minicolumns were sorted into spatially coherent clusters comprising an average of ten minicolumns using a k-means clustering algorithm. The result was that the six circuits were grouped into 5, 10, 20, 30, 40 and 100 clusters of 10 minicolumns, respectively. Finally, we computed the population-level firing rate PSTH for each cluster in each circuit using a time bin of 5 ms (Figures 20E,F) or 20ms (Figures 20C,D). Spatial profile plots were obtained by interpolation of the estimated PSTH for a given time slice assigned to the locations of the minicolumns using the ‘cubic’ method of scipy.interpolate.griddata. Pairwise cross-correlation coefficients between the PSTHs were computed for each possible combination of two clusters.

Temporally sequential structure during spontaneous activity of L5 neurons

To construct an in silico approximation of an in vivo UP state, the inner 20 minicolumns of the reconstructed microcircuit were stimulated by activating the afferent VPM fibers innervating these minicolumns. The stimulus was delivered 1500 ms after the commencement of each trial to ensure that the circuit dynamics had achieved a steady-state prior to stimulation. Neural responses were recorded for 500 ms following application of the stimulus. The data contained in Fig. 18A were obtained by repeating the stimulus protocol described above 25 times, and concatenating the spike trains generated by each neuron during each trial. Thus, we obtained a dataset consisting of 25 activated (UP state-like) states, each lasting 500 ms (though the time course of neural activity throughout this period was typically much shorter, on the order of 250 ms), preceded by 1500 ms of spontaneous activity.

In line with Luczak *et al.* (Luczak et al., 2007), we restricted our analysis to a randomly selected pool of 50 neurons in L5, whose average firing rate was greater than 3 Hz throughout the course of each trial. 19,600 cell trios were constructed from this pool by considering all distinct combinations of 3 neurons. The collection of triplets associated with a given trio was obtained by sequentially iterating over each spike in the raster of the trio's first cell, and calculating the time differences between the occurrence of that spike and the occurrences of all spikes in the rasters of the remaining two neurons (see Fig. 18A). Next, for each trio, we computed a count matrix of triplet structures (see Fig. 18A1), comprising a normalized 2D histogram of the trio's triplets binned at 3.2 ms, which we subsequently smoothed with a 10 ms Gaussian kernel. From each trio's count matrix, we extracted the precisely repeating triplets, defined as those triplets occurring within +/- 10 ms of the mode. Furthermore, to produce Fig. 18A2, we calculated each neuron's average latency, which we defined as the center of mass of a given cell's activated state-triggered PETH (smoothed by a 10 ms Gaussian kernel) within a 250 ms time window. Finally, we compared the results of our analysis against two competing null hypotheses (see Fig. 18A3). In the first, an independent Poisson model, we randomly permuted the spike times of each cell's spike train (thereby preserving the overall firing rate of each cell), and calculated the average probability of observing a precisely repeating triplet as a function of time from the onset of an activated state. To this end, we constructed a normalized histogram (binned at 3.2 ms) of the temporal occurrence of precisely repeating triplets produced by all trios. In the second, a "common excitability" model of triplet activity, we randomly exchanged spikes between the rasters of all 50 neurons, thereby preserving both the average firing rate of each cell and the exact spike times contained in the pooled set of raster data, and computed the average probability of observing precisely repeating triplets as described above.

Neuronal Responses to Single Whisker Deflection

In each trial, the 60 VPM fibers closest to the center of the microcircuit were stimulated with synchronous action potentials. There were 200 trials with different simulation seeds. Only the stimulated column was simulated.

Neurons for the scatter plot were randomly selected reflecting the statistics used in the in vivo study by Reyes-Puerta *et al*. (see Fig 3B in (Reyes-Puerta et al., 2015)). The overall neuron counts that were chosen in silico were: 2630 excitatory neurons (2080 in L5, 360 in L4, 100 in L3, and 90 in L2), 550 inhibitory neurons (370 in L5, 50 in L4, 20 in L3, 10 in L2). NR cells were defined as those that either did not pass the significance test ($p > 5$) or did not fire at all.

VISUALIZATION

Mesh Generation

In order to generate the meshes used in the visualization, we used the Visualization Tool Kit, an open-source software package for 3D computer graphics. The mesh generator built upon our previous technique (Lasserre, 2012). Morphology files were read in and a kernel of the appropriate radius was extruded along the morphology. At branch points, new holes were opened in the mesh and joined with the child processes. Processes were connected to a spherical soma kernel and smoothing performed. The final product was a watertight, manifold mesh suitable for simulation with subcellular simulators such as MCell as well as visualization using RTNeuron. The mesh generation step could export in a variety of formats, including ply, obj, vtk and internal formats that include information about the identity of the subcellular components and their mapping to the neuronal morphologies.

RT Neuron

Some of the high resolution images and the simulation playback movies were rendered with RTNeuron (Hernando et al., 2008, 2012). RTNeuron is a C++ rendering engine with a Python wrapping based on

OpenSceneGraph and Equalizer (for parallel rendering) (Eilemann et al., 2009), usable as either a standalone application or a Python module. It was developed in house as the result of a long-term collaboration between the BBP and the Cajal Blue Brain project. Apart from being a tool for generating presentation media, RTNeuron also allowed the interactive visualization of the structural information and the simulation results of large cortical circuits (~10K cells). These capabilities were used for debugging the model building process as well as the simulation. The interactive capabilities were used to design the color maps used to map simulation variables onto the mesh model of each cell membrane. RTNeuron implemented a fast algorithm for rendering transparent geometry that was used to generate the images showing simulation data. RTNeuron read the file formats produced by other applications from the tool chain using an object-oriented library developed for that purpose, the BBP-SDK. Apart from interactive visualization, it can write images in all file formats supported by OpenSceneGraph for monoscopic and stereoscopic visualization.

Maya

For some visualizations, we used Maya® 3D animation software (Autodesk, San Rafael, California, USA). As Maya is limited in the number of meshes it can handle, it was mainly used for creating high quality static images of small neural circuits. We built an automated workflow on top of Maya and the mesh generation software to visualize individual neurons color-coded according to their morphology types or layers that could also show the distribution of the excitatory and inhibitory synapses color-coded according to the morphology type of their pre-synaptic partners.

The neuron meshes were exported in Wavefront object format from the mesh generation stage and read on the fly into Maya. We mapped a shader onto the mesh in order to give the appearance of texturing seen in electron micrograph images. Color, glow, transparency were also altered according to the visualization scenario. In images displaying synapses and spines, the positions were assigned according to an input list, generated from the circuit. In images involving multiple neurons, the neuron meshes were rotated and translated according to their position in the circuit.

Rendering was performed on a cluster of 12 Intel Xeon X5690 cores, running at 3.47 GHz. Rendering time per frame on one node ranged from 10 seconds. Frames were integrated and post-processed using Adobe Aftereffects (Adobe Systems, San Jose, California, USA).

Volume Rendering

Some microcircuit images were generated using volume rendering. We chose this method because volumetric effects such as slicing, segmenting, volumetric noise and transparency were needed to simulate the sample images taken from laboratory microscopes. The volume rendering equations were evaluated using volume ray casting (Levoy, 1990), which calculates the absorption incurred by rays cast from the eye of the observer.

There were two stages in volume rendering: rasterization and rendering. In the first stage, meshes were rasterized into regular grids and then placed in the circuit volume. This resulted in a large regular grid data containing the density values of the rasterized meshes in each cell. The tools employed in this stage included C++ libraries and python scripts. One could set the branch order for visualizing different number of branching depths of the cell processes, the inclusion of soma, dendrites, axons, as well as shell rendering for just visualizing the iso-surface of the neuron mesh.

In the rendering stage, the engines employed were the Python-based Mayavi (Ramachandran and Varoquaux, 2011) library for easy python scripting and the Voreen tool (Meyer-Spradow et al., 2009) for easy GUI-based modifications to the rendering. The coloring and transparency of the images are evaluated through modifying the transfer functions of the rendering equation in volume ray casting.

The stained sample images taken from laboratory microscopes exhibited staining noise. This noise was mimicked computationally using the Perlin noise generator (Perlin) integrated into the Voreen tool with the Libnoise library (available from Sourceforge). The cells in these images also had noise on the soma surface, which was simulated using uniformly distributed noise in the volume multiplied by the intensities in the non-empty grid cells.

In silico Fluorescence Microscopy

A novel method was developed to visualize the fluorescent tissue models (Abdellah et al., 2015) using the spectral characteristics of fluorescent dyes including their emission and excitation spectra, quantum yield and their concentration in the tissue. This method uses physically-based volume rendering to simulate the light interaction with the fluorescent-labeled brain structures relying on the optical properties of the tissue and the fluorescent dyes. The generated renderings can accurately reflect the optical sections created from fluorescence microscopy.

Connectivity diagrams

The ribbon plot in Figure 7C was generated using Circos software (Krzywinski et al., 2009).

SOFTWARE INTEGRATION AND COMPUTING INFRASTRUCTURE

Development process and continuous integration

Data integration and post processing as well as building, simulation, analysis and visualization of neuronal network models relied on developing and integrating more than 30 software applications in complex workflows. The efficient development of such a large set of applications, by dozens of contributors, required putting together a comprehensive development environment. All major software tools are registered with a common versioning system (Git) and all modifications are traced. For the major software applications, online code review via Gerrit is implemented as well as continuous building, testing, packaging and deployment using Jenkins. The software is released on various machines and architectures and GNU modules are used to expose the installed software to the end user.

Workflows and Provenance

A significant aspect of reproducibility is the tracking of the input and output artifacts in each step in the circuit building, simulation, and analysis workflow. To this end, we constructed a collaboration portal, which provides a) functionality to register artifacts and process execution manually or automatically upon workflow execution b) a forum to view and discuss the results of simulations c) functionality to do comparative analysis of different simulations. Workflows are collections of standardized conversion, analysis and validation tasks using a custom Python-based Task component framework. The Task component framework allows the wrapping of any type of code into a source-controlled component, which automatically tracks provenance of artifacts during execution. This is accomplished by leveraging the OPM provenance model as implemented by the Karma Provenance Server (http://d2i.indiana.edu/provenance_karma).

Storage for Experimental Data

Experimental data is stored on a scalable unified storage resource delivered by a NetApp FAS3240 cluster. A unified storage architecture allows the consolidation of diverse workloads and helps maximizing the efficiency. It also allows us to support multiple network configurations and protocols. Experimental data is internally accessible via native NFS and CIFS protocols, and federated externally via iRODS system. The Integrated Rule-Oriented Data System (iRODS) is an open-source data management software in use at research organizations and government agencies worldwide. It functions independently of storage resources and abstracts data control away from storage devices and device location.

Analysis and Visualization Cluster

Computation for processing of experimental data, models or for interactive visualization sessions is provided by a fully model-managed cluster (Intel 188-cores, 39 x nVidia GTX580) with SLURM reservation system and scheduler. The model-based approach helps to manage the cluster throughout its lifecycle, from provisioning and configuration to orchestration and reporting, by enforcing a standard operating environment and thus eliminating configuration drifts.

Supercomputing

For more compute intensive workflow steps, such as cell building, circuit building, network simulation the study relied on supercomputers. Amongst the systems used were the CADMOS 4-rack IBM Blue Gene/P (until February 2013), CADMOS 1-rack IBM Blue Gene/Q (March 2013 until March 2014). The CADMOS infrastructure was interconnected with the Analysis and Visualization Cluster by using a common shared GPFS file system where data exchange between the two machines is supported by 2x10 Gib/s Ethernet links.

Building on the CADMOS-EPFL hardware integration experience, a tighter design resulted in the construction of Blue Brain IV, 100th most powerful supercomputing system (Top500, June 2015), which is operated by the Swiss National Supercomputing Center (CSCS) for the Blue Brain Project. It includes a 4-rack IBM Blue Gene/Q, IBM Blue Gene Active Storage, and a 40-node Intel cluster fully interconnected using Infiniband technology and a GPFS file system with 4.2 Petabyte raw storage (Schürmann et al., 2014).

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Author Contributions

H.M. conceived and led the study. F.S., S.L.H., I.S., J.DF. co-led the study. H.M. planned & supervised experiments, data integration, strategies and algorithms, model building, *in silico* experiments and analysis. F.S. planned & supervised the development of algorithms, software & workflows, computing infrastructure and technical integration. E.M., S.R., M.W.R., and S.L.H. drove & co-supervised the integration of the data, tools, models, simulations, and analyses.

Manuscript

H.M., S.R., E.M., and M.W.R. wrote the manuscript.

I.S., D.K., and R.W. contributed to the writing of the manuscript.

S.R., E.M., M.W.R., and H.M. generated the figures.

D.K., W.V.G., N.A., M.A., A.B., F.D., F.S., A.C., J-P.G., M.T., L.K., Y.K., R.R., C.A.R., G.C., T.H.N., M.N., E.G., J.V.D., R.D., and M.T-R. contributed to the generation of figures.

S.R. compiled and integrated all the references.

All authors provided input to the manuscript.

Data Integration

E.M., S.L.H., F.S., and M.T. co-supervised and performed data integration.

S.R. integrated synaptic data from the literature, and lead the literature consistency assessment.

Modeling

E.M., S.L.H., I.S., and F.S. co-supervised model building and analysis.

I.S., W.V.G., S.D., E.H., R.R., S.R., A.Gi., C.A.R. performed modeling of channels and cells.

E.M., S.L.H. performed modeling of cells & circuits, in silico experiments & analysis.

M.T., G.K., D.K., E.M., Y.W., F.S. performed neuronal composition modeling.

S.R., E.M., and M.W.R. performed modeling of synapses.

S.R. performed modeling of synaptic pathways.

M.W.R., S.R. and E.M. devised and implemented algorithms for modeling thalamo-cortical projections.

E.M., S.R., M.W.R., and D.K. conceived, supervised and E.M. performed network level simulations for the synchrony-asynchrony spectrum.

E.M., M.W.R., S.R., W.V.G., J.G.K, G.C., T.H.N., and M.N. conceived and performed *in silico* experiments.

E.M., M.W.R., S.R., A.C., R.P. performed circuit analyses.

J.C.S., J.G. co-supervised and J.G., G.A., L.K. co-developed algorithms and validations for cloning and morphology classification.

Software Development

J.G.K., R.R., W.V.G., K.M., M.T., G.K., R.P. co-developed software & workflows for data acquisition, databasing, and/or data integration.

J.C.S., J.G. co-supervised morphology data integration and J.G., I.R., J.K., D.K., G.A., A.K., L.K., Y.K., and E.M. co-developed algorithms, software & workflows for cell morphologies.

W.V.G., S.D., E.H., R.R., J.G.K., A.Gi. co-developed algorithms, software & workflows for cell building.

E.M., S.L.H., F.D. co-supervised and E.M., J.G.K., M.W.R., S.R., I.R., M.T., G.K., B.R.C.M., N.V.H., P.K., J.D., M.E.G., J.L.R., K.S. co-developed algorithms, software & workflows for circuit building.

M.W.R. developed the connectome algorithm.

S.R., J.G.K., E.M., and M.W.R. developed algorithms, software & workflows for synaptic pathways.

F.D. co-supervised and M.H., J.G.K, R.R., M.W.R., W.V.G., E.M., F.D., A.Gi., A.O. co- developed algorithms, software and workflows for simulation.

J.Mu. co-supervised and J.Mu., J-D.C., S.M.Z., M.T., S.A. co-developed software & workflows for the portal.

E.M., S.L.H., F.D. co-supervised and J.B.H., S.L., T.T., E.M., J.K., I.R., M.W.R., S.R., R.R., M.T., F.D., D.N., N.V.H., J.D., M.E.G., J.L.R., J.P. co-developed algorithms, software & workflows for analysis.

S.E. and L.P. co-supervised and J.B.H., S.L., T.T., M.A., A.B., S.E., D.N., M.W.R., D.K., J.V., R.D. co-developed algorithms, software & workflows for visualization.

A.A. supervised, T.H. co-supervised, and F.T., T.H., B.R.C.M., S.E. co-developed algorithms, software and workflows for spatial indexing.

Visualizations

S.E. co-supervised and M.A., A.B., J.B.H., M.T., M.W.R., S.R., N.A., J.V.D., R.D., and E.M. generated scientific visualizations and movies.

Computing Infrastructure

F.D. and N.B. co-supervised and F.D., N.B., C.A., R.S., B.R.M., planned, implemented, and operated general and high performance computing infrastructure.

Experiments

Y.W., Z.K. co-supervised cell reconstructions. Y.W., T.K.B., and H.M. performed classifications, J.Me., Y.S. coordinated and performed reconstructions and organized morphology data.

T.K.B., S.M., R.P., V.D., G.S., J.V.-LB., A.Gu. performed single and multi-cell electrophysiology experiments.

V.D. performed and analyzed multi-electrode array experiments.

M.T-R. performed single cell electrophysiology, eCode, and gene expression experiments.

J-P.G. and J.Me. performed immunohistochemistry experiments.

J.Me. organized data.

J.DF. supervised and L.A-N., A. M-P., A.M., and J.-R.R. performed experiments and analysis on synapse/cell densities, volume fractions, and somatic innervation patterns.

R.R. performed ion channel experiments.

Additional Supplementary Tables

Table S4. Complete List of Features Used in the Classification of Morphologies, Related to Figure 2.

Bifurcation Mean Branch Length (all neurites)	Bifurcation Mean Branch Length (apical dendrites)	First Moment in z axis (axon)
Mean Density (all neurites)	Mean Trunk Diameter (apical dendrites)	Second Moment in x axis (axon)
Horizontal-Vertical ratio (all neurites)	First Moment in x axis (apical dendrites)	Second Moment in y axis (axon)
Mean Horizontal Range (all neurites)	First Moment in y axis (apical dendrites)	Second Moment in z axis (axon)
Maximum Branch Order (all neurites)	First Moment in z axis (apical dendrites)	Number of Neurites (axon)
Maximum Degree (all neurites)	Second Moment in x axis (apical dendrites)	Number of Fragments (axon)
Maximum Path Length (all neurites)	Second Moment in y axis (apical dendrites)	Mean Termination Branch Length (axon)
Maximum Radial Distance (all neurites)	Second Moment in z axis (apical dendrites)	Min Termination Path Distance (axon)
Mean Trunk Diameter (all neurites)	Number of Neurites (apical dendrites)	Mean Tortuosity (axon)
First Moment in x axis (all neurites)	Number of Fragments (apical dendrites)	Number of Branches (axon)
First Moment in y axis (all neurites)	Distance of Apical Point To Soma (apical dendrites)	Total Length (axon)
First Moment in z axis (all neurites)	Mean Termination Branch Length (apical dendrites)	Total Surface Area (axon)
Second Moment in x axis (all neurites)	Min Termination Path Distance (apical dendrites)	Total Volume (axon)
Second Moment in y axis (all neurites)	Mean Tortuosity (apical dendrites)	Bifurcation Mean Branch Length (basal dendrites)
Second Moment in z axis (all neurites)	Number of Branches (apical dendrites)	Mean Density (basal dendrites)
Number of Neurites (all neurites)	Total Length (apical dendrites)	Horizontal-Vertical ratio (basal dendrites)
Number of Fragments (all neurites)	Total Surface Area (apical dendrites)	Mean Horizontal Range (basal dendrites)
Mean Termination Branch Length (all neurites)	Horizontal length of Apical Tuft (apical dendrites)	Maximum Branch Order (basal dendrites)
Min Termination Path Distance (all neurites)	Vertical length of Apical Tuft (apical dendrites)	Maximum Degree (basal dendrites)
Mean Tortuosity (all neurites)	Depth of Apical Tuft (apical dendrites)	Maximum Path Length (basal dendrites)
Number of Branches (all neurites)	Total Volume (apical dendrites)	Maximum Radial Distance (basal dendrites)
Total Length (all neurites)	Mean Vertical Range (apical dendrites)	Mean Trunk Diameter (basal dendrites)
Total Surface Area (all neurites)	Bifurcation Mean Branch Length (axon)	First Moment in x axis (basal dendrites)
Total Volume (all neurites)	Mean Density (axon)	First Moment in y axis (basal dendrites)
Mean Vertical Range (all neurites)	Horizontal-Vertical ratio (axon)	First Moment in z axis (basal dendrites)
Bifurcation Mean Branch Length (apical dendrites)	Mean Horizontal Range (axon)	Second Moment in x axis (basal dendrites)
Mean Density (apical dendrites)	Maximum Branch Order (axon)	Second Moment in y axis (basal dendrites)
Horizontal-Vertical ratio (apical dendrites)	Maximum Degree (axon)	Second Moment in z axis (basal dendrites)
Mean Horizontal Range (apical dendrites)	Maximum Path Length (axon)	Number of Neurites (basal dendrites)
Maximum Branch Order (apical dendrites)	Maximum Radial Distance (axon)	Number of Fragments (basal dendrites)
Maximum Degree (apical dendrites)	Mean Trunk Diameter (axon)	Mean Termination Branch Length (basal dendrites)
Maximum Path Length (apical dendrites)	First Moment in x axis (axon)	Min Termination Path Distance (basal dendrites)
Maximum Radial Distance (apical dendrites)	First Moment in y axis (axon)	Mean Tortuosity (basal dendrites)
Number of Branches (basal dendrites)	Total Surface Area (basal dendrites)	Mean Vertical Range (basal dendrites)
Total Length (basal dendrites)	Total Volume (basal dendrites)	Soma Cross Section Area

Table S5. Rules for m-type Specific Pooling for e-types across Layers, Related to Figures 2 and 4.
The pooling rules applied, in an m-type specific manner, are as follows: (A) L4 + L5 + L6, (B) L5 + L6, (C) L1 lumped (NGC + NGC-DA + NGA-SA), (D) L23 + L4 + L5 + L6. See Supplementary Experimental Procedures: Composition of e-types.

	BP	BTC	ChC	DBC	LBC	MC	NBC	NGC	SBC
L2/3	D		D					C	D
L4	D		D	A				C	D
L5	D	B	D	A			B	C	D
L6	D	B	D	A			B	C	D

Table S6. Prescribed Parameters for Synaptic Transmission, Related to Figures 9 and 10.

Excitatory-excitatory connections

Connection type	Synapse type	Kinetic parameters (mean ± SD)			Dynamic parameters (mean ± SD)		
		g_{syn} (ns)	τ_{rise} (ms)	τ_{decay} (ms)	U	D (ms)	F (ms)
L23PC-L23PC	Excitatory, depressing (E2)	0.68± 0.46	0.2 ± 0.1	1.7 ± 0.14	0.46 ± 0.26	671 ± 17	17 ± 5
L4Exc-L4Exc	Excitatory, depressing (E2)	0.68 ± 0.45	0.2 ± 0.1	1.7 ± 0.14	0.86 ± 0.09	671 ± 17	17 ± 5
L4SS-L23PC	Excitatory, depressing (E2)	0.19 ± 0.12	0.2 ± 0.1	1.7 ± 0.14	0.79 ± 0.04	671 ± 17	17 ± 5
L5TTPC - L5TTPC	Excitatory, depressing (E2)	1.5 ± 1.05	0.2 ± 0.1	1.7 ± 0.14	0.5 ± 0.02	671 ± 17	17 ± 5
L5STPC - L5STPC	Excitatory, depressing (E2)	0.8 ± 0.53	0.2 ± 0.1	1.7 ± 0.14	0.39 ± 0.03	671 ± 17	17 ± 5
All other E - E connections	Excitatory, depressing (E2)	0.72 ± 0.5	0.2 ± 0.1	1.7 ± 0.14	0.5 ± 0.02	671 ± 17	17 ± 5

Excitatory-inhibitory connections

Connection type	Synapse type	Kinetic parameters (mean ± SD)			Dynamic parameters (mean ± SD)		
		g_{syn} (ns)	τ_{rise} (ms)	τ_{decay} (ms)	U	D (ms)	F (ms)
L5TTPC-L5MC	Excitatory, facilitating (E1)	0.11 ± 0.08	0.2 ± 0.1	1.7 ± 0.14	0.09 ± 0.12	138 ± 211	670 ± 830
L5PC - L5BC/ L5ChC	Excitatory, depressing (E2)	0.72 ± 0.5	0.2 ± 0.1	1.7 ± 0.14	0.72 ± 0.12	227 ± 70	13 ± 24
All other E - I connections	Excitatory, depressing (E2)	0.43 ± 0.28	0.2 ± 0.1	1.7 ± 0.14	0.5 ± 0.02	671 ± 17	17 ± 5

Inhibitory-excitatory connections

Connection type	Synapse type	Kinetic parameters (mean ± SD)			Dynamic parameters (mean ± SD)		
		g_{syn} (ns)	τ rise (ms)	τ_{decay} (ms)	U	D (ms)	F (ms)
L5MC-L5TTPC	Inhibitory, depressing (I2)	0.75 ± 0.32	0.2 ± 0.1	8.3 ± 2.2	0.3 ± 0.08	1250 ± 520	2 ± 4
L23(NBC, LBC)/L23ChC - L23PC	Inhibitory, depressing (I2)	0.91 ± 0.61	0.2 ± 0.1	8.3 ± 2.2	0.14 ± 0.05	875 ± 285	22 ± 5
All other I - E connections	Inhibitory, depressing (I2)	0.83 ± 0.2	0.2 ± 0.1	8.3 ± 2.2	0.25 ± 0.13	706 ± 405	21 ± 9

Inhibitory-inhibitory connections

Connection type	Synapse type	Kinetic parameters (mean ± SD)			Dynamic parameters (mean ± SD)		
		g_{syn} (ns)	τ rise (ms)	τ decay (ms)	U	D (ms)	F (ms)
All I - I connections	Inhibitory, depressing (I2)	0.83 ± 0.55	0.2 ± 0.1	8.3 ± 2.2	0.25 ± 0.13	706 ± 405	21 ± 9