Simulation-based inference for efficient identification of generative models in computational connectomics

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Abstract

Recent advances in connectomics research enable the acquisition of increasing amounts of data about the connectivity patterns of neurons. How can we use this wealth of data to efficiently derive and test hypotheses about the principles underlying these patterns? A common approach is to simulate neural networks using a hypothesized wiring rule in a generative model and to compare the resulting synthetic data with empirical data. However, most wiring rules have at least some free parameters, and identifying parameters that reproduce empirical data can be challenging as it often requires manual parameter tuning. Here, we propose to use simulation-based Bayesian inference (SBI) to address this challenge. Rather than optimizing a single rule to fit the empirical data, SBI considers many parametrizations of a wiring rule and performs Bayesian inference to identify the parameters that are compatible with the data. It uses simulated data from multiple candidate wiring rules and relies on machine learning methods to estimate a probability distribution (the 'posterior distribution over rule parameters conditioned on the data') that characterizes all data-compatible rules. We demonstrate how to apply SBI in connectomics by inferring the parameters of wiring rules in an *in silico* model of the rat barrel cortex, given *in vivo* connectivity measurements. SBI identifies a wide range of wiring rule parameters that reproduce the measurements. We show how access to the posterior distribution over all data-compatible parameters allows us to analyze their relationship, revealing biologically plausible parameter interactions and enabling experimentally testable predictions. We further show how SBI can be applied to wiring rules at different spatial scales to quantitatively rule out invalid wiring hypotheses. Our approach is applicable to a wide range of generative models used in connectomics, providing a quantitative and efficient way to constrain model parameters with empirical connectivity data.

Author summary

The brain is composed of an intricately connected network of cells—what are the factors that contribute to constructing these patterns of connectivity, and how? To answer these questions, amassing connectivity data alone is not enough. We must also be able to efficiently develop and test our ideas about the underlying connectivity principles. For example, we could simulate a hypothetical wiring rule like "neurons near each other are more likely to form connections" in a computational model and generate corresponding synthetic data. If the synthetic, simulated data resembles the real, measured data, then we have some confidence that our hypotheses might be correct. The challenge, however, lies in finding all the potential wiring rules, or equivalently, all the parameters of the computational model that can reproduce the observed data, as this process is often idiosyncratic and labor-intensive. To tackle this challenge, we introduce an approach that combines computational modeling in connectomics, deep learning, and Bayesian statistical inference in order to automatically infer a probability distribution over the model parameters likely to explain the data. We demonstrate our approach by inferring wiring rules in a detailed model of the rat barrel cortex and find that the inferred distribution identifies multiple data-compatible model parameters, reveals biologically plausible parameter interactions, and allows us to make experimentally testable predictions.

Introduction

Connectomics investigates the structural and functional composition of neural networks to distill principles of the connectivity patterns underlying brain function (***Chklovskii et al., 2004***; ***Sporns et al., 2005***). Over the last years, advances in imaging and tracing techniques enabled the acquisition of increasingly detailed connectivity data (***Osten and Margrie, 2013***; ***Kornfeld and Denk, 2018***; ***Macrina et al., 2021***) and led to significant insights (***Motta et al., 2019***; ***Valdes-Aleman et al., 2021***; ***Loomba et al., 2022***). These advances in data acquisition necessitate new computational tools for analyzing the data and testing hypotheses derived from it (***Jain et al., 2010***; ***Sporns and Bassett, 2018***; ***Peyser et al., 2019***). A recent computational approach for testing hypotheses in connectomics has been to use so-called *generative models* (***Betzel and Bassett, 2017***; ***Váša and Mišić, 2022***; ***Luppi et al., 2022***). The idea of generative modeling is to develop a computational model capable of generating synthetic connectivity data according to a specific hypothesis, e.g., a wiring rule (Fig. 1a, left). Subsequently, one can validate and refine the wiring rule (or the underlying computational model) by comparing the simulated with measured connectivity data (Fig. 1a, right). Examples for this approach range from large-scale generative models of functional connectivity in the human cortex (***Vértes et al., 2012***; ***Betzel et al., 2016***), system-level network models of the mouse visual cortex (***Billeh et al., 2020***), and generative models of cortical microcircuits (***Reimann et al., 2015***).

As a specific example, we here consider a generative model for simulating hypothesized wiring rules in the rat barrel cortex (***Udvary et al., 2022***). The model is based on reconstructions of axon and dendrite morphologies from *in vivo* recordings (***Narayanan et al., 2015***) and reconstructions of the barrel cortex geometry, cytoarchitecture, and cellular organization (***Meyer et al., 2010***, ***2013***). These anatomical features were combined into a 3D model to obtain a quantitative and realistic estimate of the dense neuropil structure for a large volume of the rat barrel cortex (***Egger et al., 2014***; ***Udvary et al., 2022***). Thus, by applying a hypothesized wiring rule to the structural features of the model, one can generate a corresponding synthetic barrel cortex connectome and compare it to empirical data to test the validity of the wiring rule. For example, ***Udvary et al.*** (***2022***) used the barrel cortex model to show that a wiring rule that only takes into account neuron morphology predicts connectivity patterns that are consistent with those observed empirically in the barrel cortex.

However, building generative models that accurately reproduce connectivity measurements can be challenging: Suppose a hypothesized wiring rule does not reproduce the data. In that case, a common approach would be manually refining the rule, e.g., by introducing parameters and

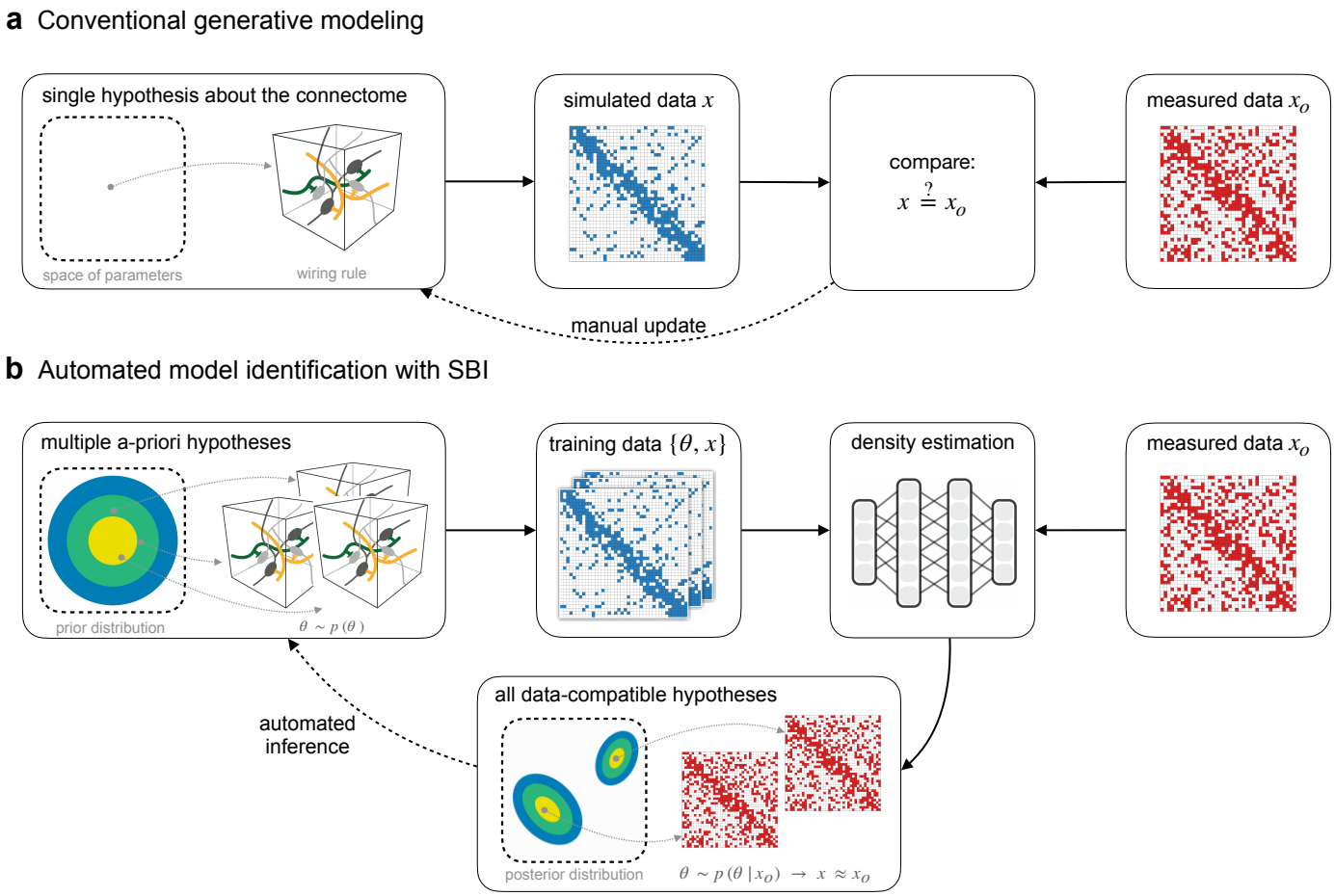

Figure 1. Enhancing generative modeling in connectomics with simulation-based inference. (**a**) Generative modeling is a common approach for testing hypotheses about the connectome: One implements a hypothesized wiring rule as a computational model that simulates connectivity data x (left) and then tests and manually refines the rule by comparing simulated with measured data x_o (right). (**b**) Our goal is to make this approach more efficient using simulation-based Bayesian inference (SBI): By equipping the generative model with parameters $\boldsymbol{\theta}$, we define a space of multiple *a-priori* hypotheses (left) from which we can generate multiple simulated data x (middle). We then use the simulated data to perform density estimation with artificial neural networks to estimate the *posterior distribution* over model parameters conditioned on the measured data, i.e., $p(\boldsymbol{\theta}|x_o)$. The inferred posterior distribution characterizes *all* wiring rule parameters compatible with the measured data, replacing the manual refinement of single wiring rules in the conventional approach (bottom).

repeating the simulate-and-compare-to-measurements loop (Fig. 1a), which can be laborious and inefficient. Additionally, identifying one specific wiring rule configuration for which simulated and empirical data match might not be enough: Given that the available empirical connectivity data is sparse compared to the structural and functional complexity of the connectome, it is likely that there are many data-compatible wiring rules and we would need to repeat the search to identify them all.

To address these challenges, we propose a new approach that employs Bayesian inference to replace the manual comparison of individual wiring rules (Fig. 1a) with the automated inference of multiple wiring rules (Fig. 1b). We achieve this by taking two conceptual steps: First, we equip the generative model with parameters $\boldsymbol{\theta}$ and interpret different parameter combinations as variants of the underlying hypothesis, e.g., variants of the wiring rule. Second, we define a probability distribution over the model parameters such that each parameter configuration corresponds to a different candidate wiring rule, i.e., a *prior distribution* $p(\boldsymbol{\theta})$ (Fig. 1b, left), and use Bayesian inference to infer all data-compatible parameters. Given measured connectivity data x_o and a parametrized generative model, we infer the conditional probability distribution over the model parameters given the

measured data, i.e., the *posterior distribution* $p(\boldsymbol{\theta}|x_o)$. The posterior distribution characterizes *all* parameter configurations (wiring rules) likely to explain the measured data, in contrast to conventional approaches that often optimize for one best-fitting parameter. For example, by sampling different parameters from the inferred posterior we would obtain different wiring rule configurations all of which are likely to generate data similar to the measured data (Fig. 1b, bottom). Additionally, the posterior distribution also allows us to quantify the correlations between the parameters, which can help to reveal parameter interactions and potential compensation mechanisms in the model.

On a technical level, standard Bayesian inference methods usually require access to the likelihood function of the model. However, generative models employed in computational connectomics are often defined as computer simulations for which the likelihood may not be easily accessible. Therefore, we propose using simulation-based inference (SBI, ***Cranmer et al., 2020***; ***Gonçalves et al., 2020***; ***Papamakarios and Murray, 2016***; ***Lueckmann et al., 2017***; ***Greenberg et al., 2019***). SBI enables Bayesian inference using only simulated data from the model, i.e., without requiring access to the likelihood. In particular, SBI performs conditional density estimation with artificial neural networks: It uses data simulated from the model to train an artificial neural network that takes data as input and predicts an approximation to the posterior distribution. Once trained on simulated data, the neural network can be applied to the measured data to obtain the desired posterior distribution $p(\boldsymbol{\theta}|x_o)$ (Fig. 1b, right).

We demonstrate our approach using the example of constraining wiring rules in the structural model of the rat barrel cortex introduced above. First, we show how to reformulate wiring rules as parametrized models to make them amenable to Bayesian inference. The resulting generative model consists of the parametrized wiring rule applied to the structural model to generate a simulated connectome of the rat barrel cortex. Second, we show that SBI can identify all parameter configurations that agree with measured connectivity data. When testing our approach in a scenario with simulated data and a known reference solution, we find that SBI performs accurately. In the realistic setting with measured connectivity data, SBI identifies a large set of rule configurations that reproduce observed and predict unobserved features of the connectome. Importantly, analyzing the inferred posterior reveals that this set of plausible rules is highly structured and reflects biologically interpretable interactions of the parameters. Finally, we illustrate the flexibility of the SBI approach by inferring two proximity-based wiring rules at different spatial scales to quantitatively show that Peters' rule cannot explain connectivity measurements in the barrel cortex.

Our approach provides a new quantitative and efficient tool for constraining model parameters with connectivity measurements and is applicable to many generative models used in connectomics. For example, it sets the stage for building generative models based on dense reconstructions of brain tissue (e.g., ***MICrONS-Consortium et al., 2021***; ***Shapson-Coe et al., 2021***; ***Turner et al., 2022***) and inferring underlying connectivity principles using SBI. We are making all software tools required for applying SBI available in an open-source software package (`sbi`, ***Tejero-Cantero* et al., 2020***), facilitating its use by researchers across the field.

Results

Formulating wiring rules in the rat barrel cortex as simulation-based models

To demonstrate the potential of simulation-based inference (SBI) for connectomics, we selected the problem of constraining wiring rules in the rat barrel cortex with empirical connectivity data. Applying SBI requires three ingredients: a simulation-based model with free parameters, a prior distribution over the parameters, and measured data (see Methods & Materials for details). Our analyses are based on a digital model of the dense neuropil structure of the rat barrel cortex (***Egger et al., 2014***; ***Udvary et al., 2022***), which we extended to obtain a simulation-based model. The model contains reconstructions of the number and distribution of somata, axon and dendrite morphologies, and subcellular features like pre-synaptic boutons and post-synaptic dendritic spines.

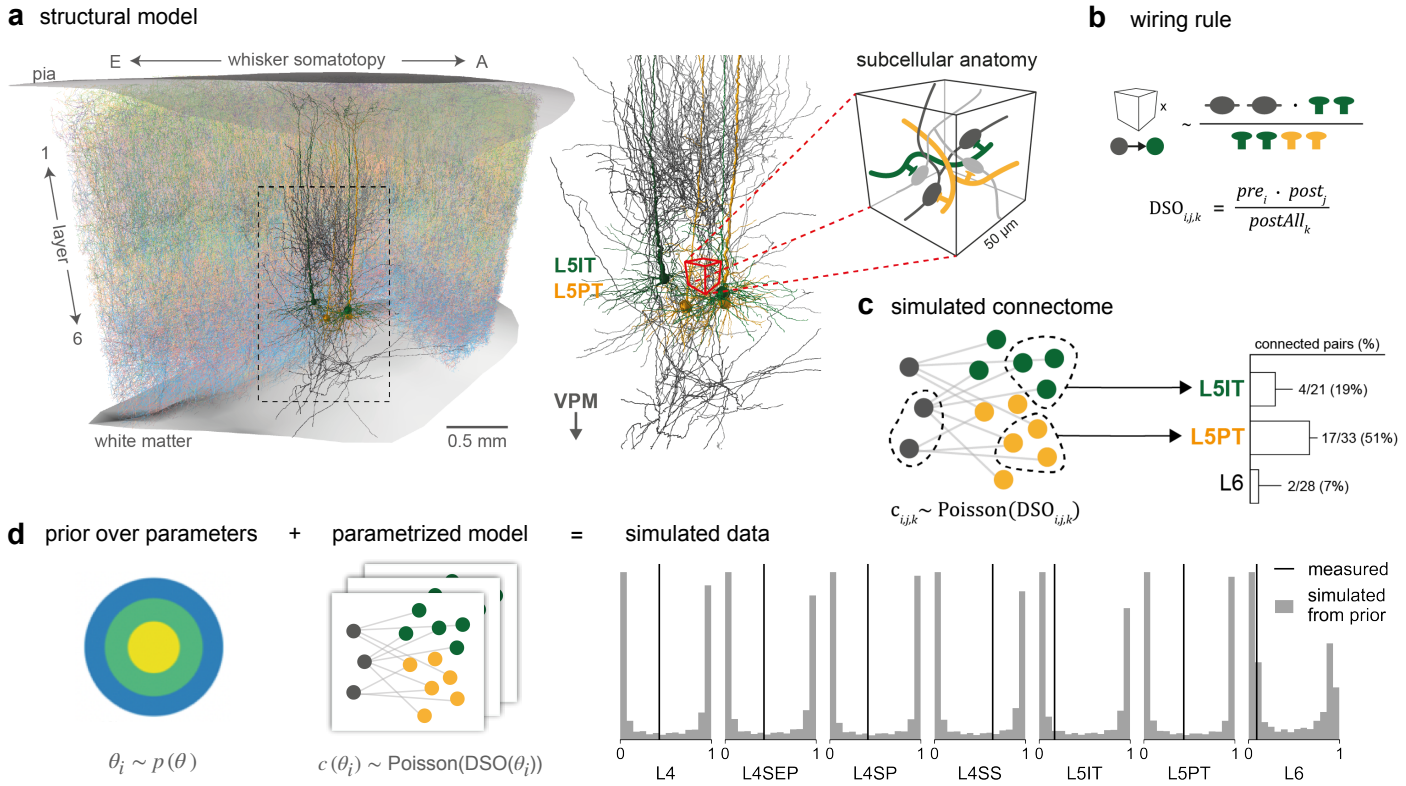

Figure 2. Formulating wiring rules in the rat barrel cortex as simulation-based models. (**a**) The structural model of the rat barrel cortex contains digital reconstructions of position, morphology, and subcellular features of several neuron types in the barrel cortex and the ventral posterior medial nucleus (VPM) of the thalamus. (**b**) We formulate a wiring rule that predicts the probability of a synapse between two neurons from their *dense structural overlap* (DSO), i.e., the product of the number of *pre*- and *post*synaptic structural features, normalized by all postsynaptic features in a given subvolume (*postAll*). (**c**) By applying the wiring rule to every neuron-pair subvolume combination of the model to connection probabilities and then sampling corresponding synapse counts from a Poisson distribution (left), we can simulate a barrel cortex connectome. To compare the simulated data to measurements, we calculate population connection probabilities between VPM and barrel cortex cell types as they have been measured experimentally (right). (**d**) To obtain a simulation-based model, we introduce parameters to the rule and define a prior distribution over parameters (left) such that each parameter combination corresponds to a different rule. Simulating data with random parameters from the prior covers the entire range of probabilities (right, gray), including the measured data (black, ***Bruno and Sakmann, 2006***; ***Constantinople and Bruno, 2013***).

These anatomical features were collected for several neuron types in the barrel cortex and projecting neurons from the ventral posterior medial nucleus (VPM) of the thalamus and arranged in 3D model (Fig. 2a, see Methods & Materials for details). Thus, by applying a wiring rule that predicts the connectivity of each neuron pair in the model from the structural features, one can construct a simulated connectome of the entire barrel cortex (***Egger et al., 2014***). ***Udvary et al.*** (***2022***) proposed a parameter-free wiring rule acting solely on structural features of the model, e.g., the pre-synaptic boutons and postsynaptic dendritic spines. Here, we extended this wiring rule to a parameterized version that allows systematic analysis of how such structural features could interact and be predictive of connectivity.

A wiring rule for the rat barrel cortex

The parameter-free wiring rule introduced by ***Udvary et al.*** (***2022***) proposes that the probability of two neurons forming a synapse is proportional to a quantity called *dense structural overlap* (DSO). The DSO is defined as the product of the number of presynaptic boutons and postsynaptic contact sites (e.g., dendritic spines), normalized by the number of all postsynaptic targets in the neighborhood (denoted as pre$_i$, post$_j$ and postAll$_k$ for each neuron i, neuron j and subvolume k in the model, Fig. 2b):

$$\mathrm{DSO}_{i,j,k} = \frac{pre_i \cdot post_j}{postAll_k}. \tag{1}$$

To simulate a connectome corresponding to the DSO wiring rule, one applies the rule to every subvolume-neuron-pair combination of the structural model and then samples synapse counts from a Poisson distribution using the calculated synapse probabilities (Fig. 2c, left, see Methods & Materials for details). The resulting simulated connectome can then be used to calculate summary statistics in the format recorded in experiments, e.g., *in vitro* or *in vivo* paired recordings or dense reconstructions at electron microscopic levels (Fig. 2c, right).

Udvary et al. (***2022***) showed that the DSO wiring rule can reproduce measured network characteristics at different scales. However, in its current form, the DSO rule assumes that the pre- and postsynaptic features have the same relative weight in determining the probability of a synapse and that these weights are the same across all barrel cortex cell types. Is this specific combination of pre- and postsynaptic features in the DSO rule is the only valid choice? A common approach to testing this question would be to iteratively modify the rule, e.g., by adding a scaling factor to the postsynaptic features or introducing different scaling factors for every cell type. However, this approach can be inefficient because any changes to the rule would require rerunning the procedure of generating simulated data and manually comparing it to measured data.

Defining a wiring rule simulator

In order to test different variations of the DSO rule efficiently, we introduced three parameters to the DSO rule: θ_{pre} for scaling the presynaptic bouton counts, θ_{post} for scaling the postsynaptic target density, and $\theta_{postAll}$ for scaling the normalizing feature (Fig. 2d, left). The parametrized DSO rule for a presynaptic neuron i and postsynaptic neuron j positioned in a subvolume k is then given by

$$\mathrm{DSO}_{i,j,k}(\boldsymbol{\theta}) = \frac{pre_i^{\theta_{pre}} \cdot post_j^{\theta_{post}}}{postAll_k^{\theta_{postAll}}}, \tag{2}$$

(see Methods & Materials for details). The three parameters represent the relative weight with which each local subcellular feature contributes to forming connections.

The next step towards applying SBI is selecting measured data x_o to constrain the rule parameters. We selected seven connection probabilities of neuronal populations mapping from the ventral posterior medial nucleus (VPM) of the thalamus to different layers and cell types in the barrel cortex, as proposed by ***Udvary et al.*** (***2022***): layer four (L4), layer four septum (L4SEP), layer four star-pyramidal cells (L4SP), and layer four spiny stellate cells (L4SS) (***Bruno and Sakmann, 2006***), layer five slender-tufted intratelencephalic cells (L5IT), layer five thick-tufted pyramidal tract cells (L5PT), and layer six (***Constantinople and Bruno, 2013***).

Overall, one simulation of the wiring rule consisted of three steps: First, applying the rule with a given set of parameters to the structural features of every combination of neuron-pair-subvolume to obtain connection probabilities; second, sampling synapses from the Poisson distribution given the probabilities; and third, calculating the summary statistics matching the measured VPM-barrel cortex population connection probabilities (Fig. 2a-d; see Methods & Materials for details). As prior over the parameters $p(\boldsymbol{\theta})$, we selected a Gaussian distribution such that sampling random parameter values from the prior resulted in simulated population connection probabilities that covered a broad range of possible values, including the measured values (Fig. 2d, right). This set of sampled model parameter values and their corresponding simulated connection probabilities constituted the training dataset for running SBI.

SBI performs accurately on simulated data

Before applying SBI to infer the parameters of the DSO rule given measured data, we validated its accuracy. As a first step, we considered a variant of the DSO rule simulator for which it was possible to obtain a ground-truth reference posterior distribution (see section Methods & Materials for

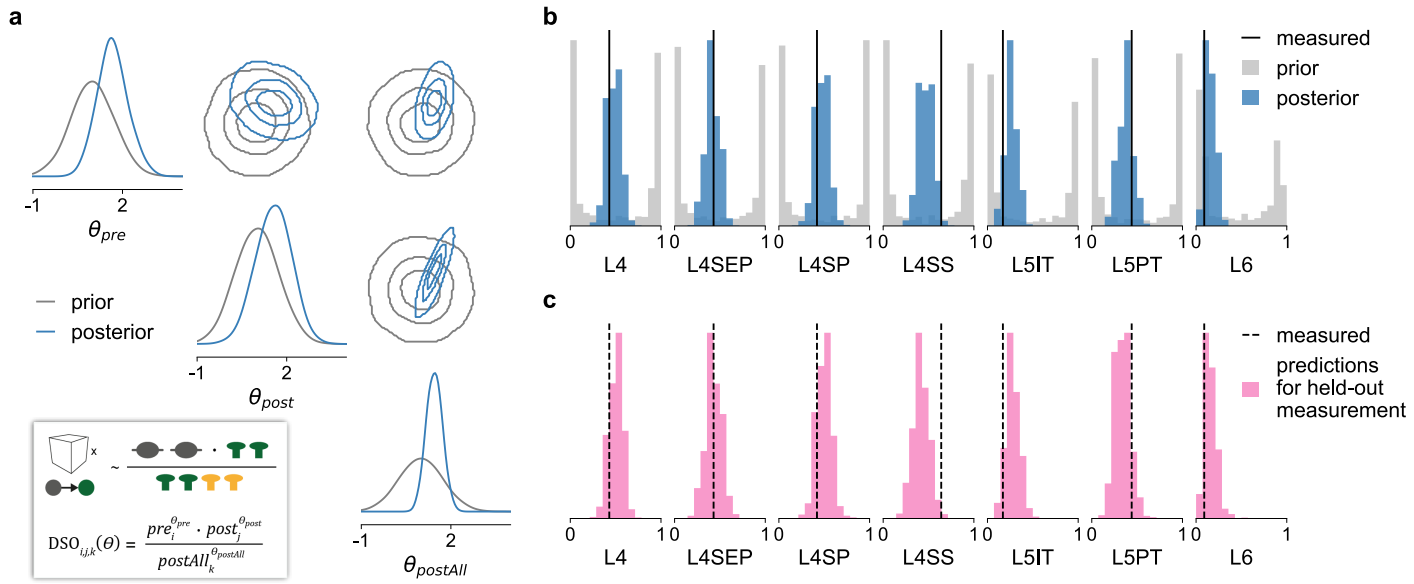

Figure 3. SBI posterior reveals parameter interactions and predicts unseen data. (**a**) The posterior over the three wiring rule parameters scaling the DSO features (see inset) inferred with SBI (blue) and the initial prior distribution over parameters (gray). The corner plot shows the one-dimensional marginal distribution of each parameter on the diagonal and the pairwise two-dimensional marginals on the off-diagonal (contour lines show the 34%, 68% and 95% credible regions). (**b**) Comparison of measured connection probabilities (black, ***Bruno and Sakmann, 2006***; ***Constantinople and Bruno, 2013***) with those simulated with parameter values sampled from the inferred posterior (blue) versus from the prior (gray). (**c**) Each panel shows the predictions for one held-out measurement generated from a posterior that was trained and conditioned only on the other six measurements, i.e., each panel refers to a different posterior.

details). Using this reference solution, we checked whether SBI infers the posterior accurately and how many training simulations it requires. We compared three SBI algorithms: Sequential Neural Posterior Estimation (SNPE, ***Papamakarios and Murray, 2016***; ***Lueckmann et al., 2017***; ***Greenberg et al., 2019***), which performs SBI sequentially over multiple rounds focusing inference on one particular observation; its non-sequential variant NPE; and a classical rejection-sampling-based approach called Sequential Monte Carlo (SMC, ***Sisson et al., 2007***; ***Beaumont et al., 2009***). We found that all three methods can accurately infer the reference posterior distribution (Suppl. Fig. S1a,b) but that they differ in terms of simulation efficiency: SNPE was slightly more efficient than NPE, and both were substantially more efficient than SMC (Suppl. Fig. S1b). As a second step, we performed two checks to validate SBI on the realistic version of the simulator for which no reference solution was available. First, we used simulated-based calibration (SBC, ***Talts et al., 2020***) to check whether the variances of posterior distributions inferred with SBI were well-calibrated, i.e., neither too narrow (overconfident) nor too wide (conservative). We found that SNPE and NPE run with *simulated* observed data inferred well-calibrated posteriors for all three parameters (Suppl. Fig. S1c). Second, we checked the predictive performance of SBI by generating simulated data using parameter values sampled from the inferred posterior. We found that the predicted data resembles the (simulated) observed data (Suppl. Fig. S1d, see Methods & Materials for details).

SBI identifies many possible wiring rules and reveals parameter interactions

After evaluating SBI with simulated data, we applied it to infer the posterior over DSO rule parameters given the seven measured VPM-barrel cortex connection probabilities (***Bruno and Sakmann, 2006***; ***Constantinople and Bruno, 2013***). Our analysis of the inferred posterior distribution revealed three key insights.

The posterior identifies many data-compatible wiring rule configurations.

We found that the inferred posterior distribution was relatively broad, suggesting many parameter combinations with a high probability of explaining the measured data (Fig. 3a): The one-dimensional posterior marginal distributions of the three parameters (Fig. 3a, blue in diagonal subplots) showed maxima at parameter values of $\theta_{pre} = 1.57$, $\theta_{post} = 1.51$ and $\theta_{postAll} = 1.38$, and marginal variances of $\hat{\sigma}^2_{pre} = 0.26$, $\hat{\sigma}^2_{post} = 0.35$ and $\hat{\sigma}^2_{postAll} = 0.07$. These values indicated that $\boldsymbol{\theta} = [1.57, 1.51, 1.38]$ was the most likely weighting of the DSO rule features (see Fig. 3a, inset) but that there are also several other parameter combinations with a high posterior probability. For example, sampling parameter values from the posterior for θ_{post} would return values lying mostly in an interval as broad as $[0.33, 2.69]$ (95% posterior credible interval). Despite this relatively broad range of plausible parameters values, we still found that simulating these parameters with the DSO rule resulted in connection probabilities close to the measured data and substantially different from those simulated with the prior (Fig. 3b, blue versus gray). How can so many parameter configurations from such broad ranges all result in similar data?

Posterior analysis reveals biologically plausible parameter interactions.

Having access to the full posterior distribution and its covariance structure allowed us to answer this question. Inspection of the two-dimensional marginals of each parameter pair indicated a correlation structure substantially different from the uncorrelated prior distribution (Fig. 3a, off-diagonal subplots). To quantify this, we estimated the Pearson correlation coefficients of $10,000$ parameter values sampled from the posterior distribution. We found a negative correlation between θ_{pre} and θ_{post} (Pearson correlation coefficient $\rho = -0.23$) and positive correlations between $\theta_{postAll}$ and θ_{pre} as well as θ_{post} ($\rho = 0.35$ and $\rho = 0.81$, respectively). These correlations are plausible given the design of the DSO rule (see equation 2). For example, the negative correlation between θ_{pre} and θ_{post} indicated that when increasing the value of θ_{pre}, we would have to decrease θ_{post} in order to obtain the same overall number of connections for a particular cell type. This suggests that a stronger influence of presynaptic boutons on the connection probability requires a weaker influence of postsynaptic target targets on the connection probability.

The correlations further suggested that all three structural features are relevant in predicting the connection probabilities: Once one parameter is fixed, the values of the other parameters are strongly constrained. Having access to the full posterior distribution allowed us to quantify this by calculating the *conditional* correlations between the parameters. We obtained the conditional correlations by conditioning the posterior on one parameter dimension—i.e., holding it at a fixed value—and calculating the correlation between samples drawn from the resulting two-dimensional conditional posterior, once for each of the three parameters θ_{pre}, θ_{post} and $\theta_{postAll}$ (see Methods & Materials for details). The resulting correlation coefficients were substantially higher than without conditioning: -0.99 between θ_{pre} and θ_{post} and 0.99 between the other two parameter combinations (see Suppl. Fig. S3 for a visualization of the conditional posteriors). This result confirmed that while the overall range of data-compatible wiring rule parameters is relatively large (Fig. 3a), once one parameter is fixed, the other two are constrained to a very small range of values. Furthermore, the strong conditional posterior correlations indicated that the DSO rule with three parameters is overparametrized, i.e., a parametrization of the DSO rule with only two parameters likely suffices to explain the measured data (see Supplementary material for details). Collectively, the inferred posterior suggested that the number of presynaptic boutons and the number of postsynaptic contact sites (and, by extension, axonal and dendritic path length) are sensitive and strongly interdependent structural features for predicting synaptic connectivity.

SBI posterior predicts unobserved connection probabilities.

To demonstrate the utility of SBI-enabled generative models as a tool for hypothesis generation, we investigated how one can make predictions on unobserved data. Above, we used SBI to constrain the wiring rule parameters with only the seven measured connection probabilities. However,

in principle, the structural model provides access to the entire (simulated) connectome of one barrel cortex column. Thus, it allows us to make predictions about other features of the connectome that were not measured yet. To test this approach, we repeated the SBI training procedure seven times, holding out each connectivity measurement once from the training data set, i.e., we trained the posterior estimator on pairs of parameters and data, $(\boldsymbol{\theta}, x)$, where x has six entries instead of seven. After training, we obtained seven different posteriors, each conditioned on six of the seven measured connection probabilities. We then sampled parameter values from every posterior, simulated the corresponding barrel cortex connectomes, and calculated *all seven* connection probabilities.

The predictions for held-out measurements clustered around the actual measurement values for most of the seven connection probabilities (Fig 3c) and closely resembled the predictive distributions of the posterior inferred given all measurements (Fig 3b). Quantitatively, we found that the predictions were within one standard deviation of the measurements (given the sample size used in the experiments, see Methods & Materials for details). A classifier trained to distinguish between the predictions of the posterior inferred from all measurements and predictions for held-out measurements achieved an accuracy of 0.68 for L4SS, 0.58 for L5PT, and < 0.55 accuracy for all other measurements (0.5 being the chance level). This cross-validation approach indicated that the structural model paired with the SBI-enabled wiring rule enables us to make experimentally testable predictions. For example, one could predict connection probabilities of cell types different from the seven measured here or other connectivity features of the rat barrel cortex available in the structural model (see below).

Using SBI to rule out invalid wiring hypotheses

Above, we demonstrated that SBI provides a quantitative way to identify valid wiring rule configurations from a large set of hypothesized wiring rules. SBI can also be used to rule out invalid hypotheses, e.g., to show that an existing hypothesis does not agree with empirical data. One such debated hypothesis in connectomics is the so-called *Peters' rule* (***Peters and Feldman, 1976***; ***Braitenberg and Schüz, 1991***). According to this hypothesis, neurons form connections whenever their axons and dendrites are in close proximity, i.e., Peters' rule can be formulated as "axo-dendritic proximity predicts connectivity" (***Udvary et al., 2022***). However, several empirical and theoretical approaches found substantial evidence against Peters' rule (e.g., ***Mishchenko et al., 2010***; ***Kasthuri et al., 2015***; ***Rees et al., 2017***; ***Udvary et al., 2022***).

Here, we show that SBI provides an alternative, quantitative way to discard this hypothesis for the rat barrel cortex. We formulated two wiring rules that implement the proximity hypothesis in the structural model of the barrel cortex at two spatial scales: one predicting connections on the neuron-to-neuron level (Fig. 4) and one predicting synapse counts at the subcellular level (Fig. 5). Both wiring rules have one free parameter, i.e., they incorporate many different proximity hypotheses, but there is one particular parameter value corresponding to Peters' rule. We used SBI to infer the posterior distribution over the rule parameters given the seven measured VPM-barrel cortex connection probabilities. Subsequently, we compared inferred parameter values and their predictions with those corresponding to Peters' rule.

Neuron-level rule

At the neuron-to-neuron level, we defined the proximity of two neurons as the number of subvolumes v they share in the structural model and introduced a threshold parameter acting on the proximity: Neurons i and j form a connection $c_{i,j}$ if the number of subvolumes v_{ij} that contain presynaptic structures of neuron i *and* postsynaptic structures of neuron j, exceeds a threshold parameter θ_{thres}:

$$c_{i,j}(\theta_{thres}) = 1 \text{ if } v_{ij} > \theta_{thres} \text{ else } 0. \tag{3}$$

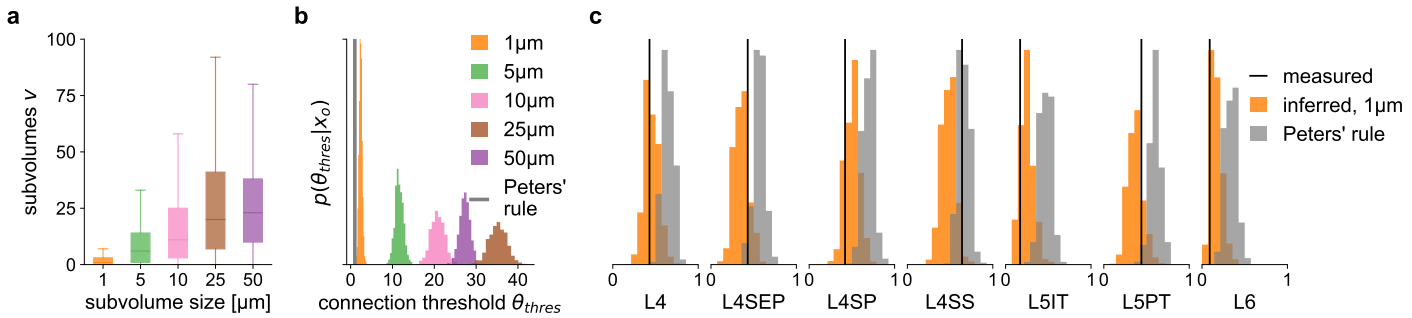

Figure 4. Neuron-level wiring rule inferred with SBI differs from Peters' rule. We used SBI to infer a proximity-based wiring rule at different spatial resolutions of the rat barrel cortex model and compared its predictions to that of Peters' rule. (**a**) Distributions of the shared subvolumes v between neurons in the barrel cortex model for each spatial resolution (subvolume edge length, see legend in (b)). (**b**) SBI posteriors inferred over the connection threshold parameter of the wiring rule (θ_{thres}, number of shared subvolumes required to form a connection), shown for each spatial resolution (colors), and for Peters' rule assuming $\theta_{thres} = 1$ (gray). (**c**) Connection probabilities simulated from the inferred posterior (orange) and Peters' rule (gray) compared to the measured connection probabilities (black).

To compare the resulting pair-wise connections between neurons in the barrel cortex model to the measured connection probabilities, we mapped them to the corresponding population connection probabilities as described above (see section Methods & Materials for details).

The structural feature used in this rule is the number of shared subvolumes between two neurons (in contrast to the *subcellular* features used in the DSO rule above). This feature directly depends on the spatial resolution of the structural model, i.e., the edge length of the subvolume used to construct the model. Therefore, we calculated the structural features at five different spatial resolutions: 50, 25, 10, 5, and $1\,\mu\text{m}$. We found that the overall number of shared subvolumes among neurons increased with edge length, reflecting the increase in subvolume size (Fig. 4a).

When applying Peters' rule to the barrel cortex model at the neuron level, a connection occurs whenever two neurons share at least one subvolume, i.e., the connection threshold would be $\theta_{thres} = 1$. Does this assumption hold for the barrel cortex at the neuron-to-neuron level as well? To answer this question quantitatively, we used SBI to infer the threshold parameter θ_{thres} of the neuron level rule for each edge length. We observed that the inferred threshold parameters shifted to larger values with increasing edge length, e.g., the higher spatial resolution, the fewer common subvolumes were required to obtain a connection (Fig. 4b). This was in line with our observation that the overall number of shared subvolumes available in the structural model increased with increasing edge length (Fig. 4a). However, irrespective of the spatial resolution, all inferred threshold parameters were substantially larger than the $\theta_{thres} = 1$ of Peters' rule, reaching from $\theta_{thres} \approx 3$ (posterior mean) for the $1\,\mu\text{m}$-subvolume model (Fig. 4b, orange) to $\theta_{thres} \approx 28$ for the $50\,\mu\text{m}$-subvolume model (Fig. 4b, violet). Accordingly, the comparison of the predictive performance of the inferred rule and Peters' rule showed that only the data simulated from the inferred rule centered around the measured data (Fig. 4c, orange and gray, respectively).

Synapse-level rule

We repeated this test of Peters' rule at the subcellular level as well. Here, we defined a probabilistic rule: Whenever a presynaptic structure of neuron i and a postsynaptic structure of neuron j are present within the same subvolume k, they form a synapse with probability θ_{prob}:

$$c_{i,j,k}(\theta_{prob}) \sim \text{Bernoulli}(\theta_{prob}) \text{ if axon of } i \text{ and dendrite of } j \text{ are present in } k. \tag{4}$$

This rule predicts synapses for every neuron-pair-subvolume combination using the structural model with subvolumes of $1\,\mu\text{m}$ edge length. To compare the simulated synapse counts to the measured connection probabilities, we calculated simulated connection probabilities as described above. The posterior distribution over the connection probability parameter θ_{prob} inferred with SBI

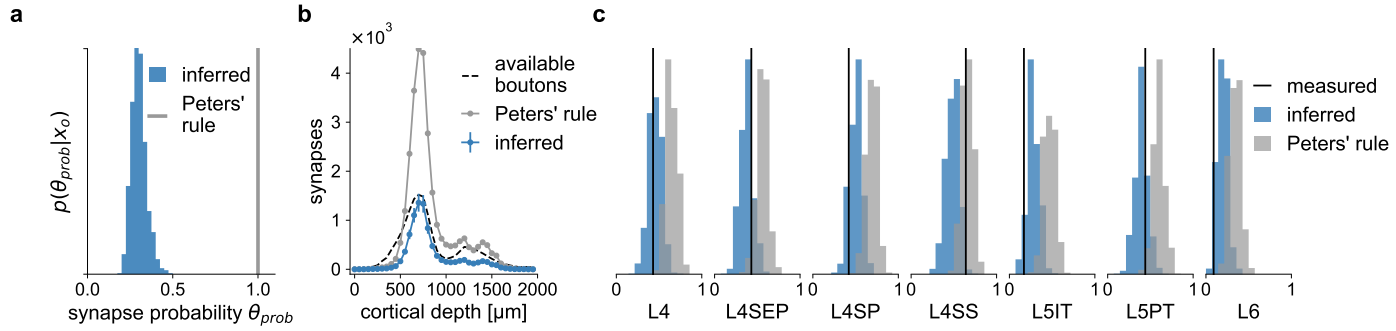

Figure 5. Synapse-level wiring rule inferred with SBI differs from Peters' rule. We compared an SBI-inferred parametrized wiring rule predicting synapse counts on the subcellular level with a corresponding formulation of Peters' rule. (**a**) SBI posterior for the wiring rule parameter θ (probability of forming a synapse if two neurons are close), compared to Peters' rule assuming $\theta = 1$ (gray). (**b**) Number of synapses predicted by the inferred posterior (blue) and Peters' rule (gray) compared to the number of presynaptic boutons realistically available in the structural model (dashed black), plotted over the entire cortical depth of the barrel cortex column. (**c**) Connection probabilities simulated from the inferred synapse level posterior (blue) and Peters' rule (gray) compared to the measured connection probabilities (black).

centered around $\theta_{prob} \approx 0.3$ (Fig. 5a). This result suggests that in only thirty percent of the locations where axon and dendrite are close to each other (shared $1\,\mu m$ subvolume), the rule predicts a synapse, which is substantially lower than the value of $\theta_{prob} = 1$ corresponding to Peters' rule. Accordingly, simulating parameter values sampled from the posterior resulted in connection probabilities closer to the measured ones than those predicted by Peters' rule (Fig. 5c, blue vs. gray).

For another comparison of Peters' rule with the inferred wiring rule at the synapse level, we leveraged the predictive properties of the structural model and the SBI posterior. In particular, the structural model provides access to estimates of the number of biologically available boutons across the cortical depth of the barrel cortex column (***Udvary et al., 2022***). The SBI posterior allowed us to simulate data according to the inferred wiring rule parameters. Thus, it was possible to compare the estimate of the number of empirically available boutons of each presynaptic VPM neuron with the number of simulated synapses from the inferred wiring rule and Peters' rule. We found that the inferred rule predicted synapses close to or below the total number of available boutons (Fig. 5b), in contrast to Peters' rule, which predicted more synapses than biologically plausible.

Our results demonstrate how SBI can be applied to different wiring rules to quantitatively rule out a specific invalid hypothesis: One incorporates the hypothesis into a parametrized model and compares the SBI-inferred parameters to those corresponding to the hypothesis. In the case of Peters' rule, the inferred posteriors showed that axo-dendritic proximity alone cannot predict connectivity observed empirically in the rat barrel cortex—it consistently predicts too many connections (Fig. 4c,d). This finding is in line with previous results showing that the number of dendrites and axons close to each other exceeds the number of synapses by 1-2 orders of magnitude (***Udvary et al., 2022***). Thus, we can conclude that to explain connectivity in the rat barrel cortex, wiring rules cannot be based solely on axo-dendritic proximity. They also have to take into account subcellular features like pre- and post-synaptic structures along axons and dendrites.

Discussion

What principles are behind the complex connectivity patterns of neural networks that shape brain function? Connectomics aims to answer this question by acquiring detailed data about the structural and functional composition of the brain. Over the last few years, the development of new computational approaches for analyzing the resulting large amounts of data and testing the derived hypotheses gained momentum (***Triesch and Hilgetag, 2016***; ***Peyser et al., 2019***). One computational approach is to leverage generative models for testing hypotheses about the connectome, e.g., to implement a hypothesized wiring rule in a computational model and ask whether model

simulations can reproduce the measured connectivity patterns of a specific brain region (***Váša and Mišić, 2022***). However, identifying the free parameters in the wiring rule that reproduce measured connectivity data can be challenging.

We introduced a method that renders the generative modeling approach to connectomics more efficient, enabling us to systematically infer *all* data-compatible parameters of a given computational model. Instead of manually refining a specific generative model to match the data, we equipped it with parameters such that it represents several candidate hypotheses. We then used Bayesian parameter inference to infer the posterior distribution over model parameters conditioned on the measured data. The inferred distribution represents all candidate parameter configurations, i.e., hypotheses, capable of reproducing the measured data. By relying on simulation-based inference (SBI) methods that do not require access to the likelihood function of the model, we were able to apply our approach to the simulation-based generative models commonly used in computational connectomics.

To demonstrate the utility of this approach, we employed it to constrain several wiring rules—at different spatial scales—with connectivity measurements from the rat barrel cortex. We first showed that the inference method is accurate in a scenario with a ground-truth reference solution. Next, in the realistic setting with measured connectivity data, we retrieved many different wiring rule configurations that could explain the measured data equally well. Analyzing the geometrical structure of the inferred posterior distribution revealed strong correlations between wiring rule parameters that are in line with their biological interpretations. Importantly, we were able to accurately predict held-out connectivity measurements, demonstrating the method's utility in making experimentally testable predictions. Finally, we used our approach to quantitatively show that a wiring rule based solely on axo-dendritic proximity cannot explain barrel cortex connectivity measurements. Overall, these results demonstrate the potential benefits of the Bayesian inference approach, i.e., having access to the full posterior distribution over model parameters rather than manually optimizing for individual parameters one hypothesis at a time and the flexibility of SBI in requiring only simulated data to perform inference.

Related work

The problem of identifying parameters of computational models that reproduce experimentally observed data has been addressed in computational connectomics before. For example, ***Vértes et al.*** (***2012***) built a model of functional connections between brain regions and used optimization methods to find single best-fitting parameters capturing the functional MRI data measured in humans. ***Klimm et al.*** (***2014***) and ***Betzel et al.*** (***2016***) used Monte Carlo sampling methods for optimizing the parameters of synthetic networks of structural connectivity to match the topological properties of human connectomes recorded with MRI. In contrast to our approach, these studies do not perform Bayesian inference but rely on optimization techniques that identify single best-fitting solutions, potentially ignoring other parameters that fit the data equally well.

While there have been Bayesian approaches to computational connectomics, they differed from the approach we proposed here. ***Jonas and Kording*** (***2015***) built a probabilistic model of cell type-dependent connectivity in the mouse retina and proposed a non-parametric Bayesian algorithm that automatically predicts cell types and microcircuitry from connectomics data. ***Klinger et al.*** (***2021***) performed Bayesian model comparison using a rejection-sampling approach (***Toni et al., 2009***) to compare a set of competing local circuit models in layer 4 of the mouse primary somatosensory cortex based on purely structural connectomics data. In contrast to our approach, they inferred the probabilities of several models whose parameters are fixed (i.e., model comparison) and did not infer the parameter of individual models. Moreover, their approach relied on classical rejection-sampling techniques, which are less simulation-efficient compared to the neural-network-based SBI we employed and will likely not scale to higher-dimensional inference problems (Figure S1b; for a detailed comparison, see ***Lueckmann et al., 2021***). However, combining both approaches, e.g., using more efficient neural-network-based model comparison techniques (***Boelts***

et al., 2019; *Radev et al., 2021*) followed by parameter inference with SBI, would be a promising direction for future research.

Aside from the above examples, computational models in connectomics are often implemented as complicated computer simulations that can generate simulated data but for which the underlying likelihood functions are not accessible, thus limiting our ability to perform Bayesian inference. To account for this limitation, we employed simulation-based inference (SBI, *Cranmer et al., 2020*) methods which only require simulations from the model to perform Bayesian inference. SBI has been applied previously in various fields, ranging from genomics (*Bernstein et al., 2021*), evolutionary biology (*Ratmann et al., 2007*; *Avecilla et al., 2022*), computational and cognitive neuroscience (*Gonçalves et al., 2020*; *Oesterle et al., 2020*; *Deistler et al., 2022b*; *Groschner et al., 2022*; *Sabbagh et al., 2020*; *Hashemi et al., 2022*), to robotics (*Marlier et al., 2021*), global health (*de Witt et al., 2020*) and astrophysics (*Alsing et al., 2018*; *Dax et al., 2021*). For the wiring rule examples presented here, we used sequential neural posterior estimation (SNPE, *Papamakarios and Murray, 2016*; *Lueckmann et al., 2017*; *Greenberg et al., 2019*), which performs neural-network-based conditional density estimation to estimate the posterior distribution from simulated data. Neural-network-based SBI approaches build on recent advances in probabilistic machine learning (*Papamakarios and Murray, 2016*; *Le et al., 2017*; *Papamakarios et al., 2021*) and exhibit several advantages compared to more classical rejection-sampling-based techniques, commonly termed Approximate Bayesian Computation (ABC, *Sisson et al., 2018*). For example, in contrast to ABC approaches, they do not require selecting a criterion for quantitatively comparing simulated with measured data. Furthermore, they can leverage the ability of neural networks to exploit continuities in the parameter space or automatically learn informative summary features from raw data. As a consequence, they are often more simulation-efficient (Suppl. Fig. S1b) and scale better to problems with more model parameters and high-dimensional data (*Lueckmann et al., 2021*).

Applicability and limitations

In this work, we applied SBI to the specific problem of inferring wiring rules that can generate observed connectivity data. A key advantage of SBI is that only data simulated from the model are required to infer the posterior distribution over model parameters. Thus, it is generally applicable to any computational model capable of simulating data from a set of parameters. To give specific examples, one could make the algorithms for generating synthetic functional connectomes proposed by *Vértes et al.* (*2012*), *Klimm et al.* (*2014*), or *Betzel et al.* (*2016*) amenable to SBI by introducing parameters of interests and then use SBI to efficiently identify all data-compatible parameter values.

Posterior-targeting SBI approaches, like the NPE algorithm we used, have the additional advantage that they can obtain the posterior distribution for new data points without retraining the underlying artificial neural networks, i.e., they perform amortized inference (see *Papamakarios and Murray, 2016*; *Gonçalves et al., 2020*, for details). Another advantage of SBI is that it can leverage the ability of neural networks to automatically learn informative summary features from observations (see *Lueckmann et al., 2017*; *Chan et al., 2018*; *Greenberg et al., 2019*; *Gonçalves et al., 2020*; *Ramesh et al., 2022*, for examples). While we did not exploit this feature for the low-dimensional measured data in the wiring rule examples presented here, we believe that it will be essential for future applications of SBI in computational connectomics, e.g., when dealing with high-dimensional dense reconstructions of electron-microscopy data (*Shapson-Coe et al., 2021*; *MICrONS-Consortium et al., 2021*; *Turner et al., 2022*).

SBI's dependency on simulated data and neural network training also entails several limitations: the inferred posterior distributions are only approximations of the unknown actual posterior distribution. Therefore, applying SBI requires careful evaluation of every problem at hand. In theory, SBI does recover the unknown posterior distribution when given enough training data (*Papamakarios and Murray, 2016*). In practice, however, the complexity and dimensionality of the posterior distribution determine how many training simulations are required for an accurate approximation

of the posterior. Previous studies have successfully applied SBI in scenarios where the simulator has a runtime on the order of seconds and with up to thirty parameters (***Gonçalves et al., 2020***; ***Deistler et al., 2022b***; ***Ramesh et al., 2022***), but these numbers strongly depend on the problem and available computational resources.

Another limitation of SBI is the problem of model misspecification. SBI generally assumes that the generative model is well-specified, i.e., that it can simulate data that is very similar to the measured data. If this is not the case, then the inferred posterior can be substantially biased (***Frazier et al., 2019***; ***Cannon et al., 2022***). We recommend performing prior predictive checks to detect model misspecification, i.e., generating a large set of simulated data with parameters sampled from the prior distribution and checking whether the measured data lies inside the distribution of simulated data, as demonstrated in the wiring rule example (Fig. 2d, see Supplementary material for details). Recent methodological work in SBI addresses this problem, e.g., by automatically detecting model misspecification (***Schmitt et al., 2022***) or by explicitly incorporating the model mismatch into the generative model (***Ward et al., 2022***).

More generally, applying SBI to new inference problems requires several choices by the practitioner, from prior predictive checks and model-checking to selecting suitable neural network architectures and validating the inferred posterior distribution. As a general guideline, we recommend following the steps we performed for the wiring rule example: First, we investigated the accuracy of SBI and estimated the required number of training simulations by testing it in a scenario with a known reference solution. Second, we ensured that the inferred posterior distribution has well-calibrated uncertainty estimates using simulation-based calibration (***Talts et al., 2020***). Third, we checked whether the parameter values identified by SBI accurately reproduced the measured data (see Methods & Materials for details). Additionally, we recommend guiding hyperparameter choices by resorting to well-tested heuristics and default settings available in open-source software packages developed and maintained by the community. We performed all our experiments, evaluation steps, and visualization using the `sbi` toolkit (***Tejero-Cantero* et al., 2020***).

Conclusion

We present SBI as a method for constraining the parameters of generative models in computational connectomics with measured connectivity data. The key idea of our approach is to initially define a probability distribution over many possible model parameters and then use Bayesian parameter inference to identify all those parameter values that reproduce the measured data. We thereby replace the iterative refinement of individual model configurations with the systematic inference of all data-compatible solutions. Our approach will be applicable to many generative modeling scenarios in computational connectomics, providing researchers with a quantitative tool to evaluate and explore hypotheses about the connectome.

Methods & Materials

Code availability

Data and code for reproducing the results are available at https://github.com/mackelab/sbi-for-connectomics, including a tutorial on how to apply SBI in computational connectomics in general. For running SBI using SNPE, posterior visualization, and posterior validation, we used the `sbi` package at https://github.com/mackelab/sbi (***Tejero-Cantero* et al., 2020***). The benchmarking of SNPE and SMC-ABC methods, including the generation of reference posteriors, was performed using the `sbibm` package at https://github.com/sbi-benchmark (***Lueckmann et al., 2021***).

Bayesian inference for computational connectomics

We introduced Bayesian inference as a tool to identify model parameters of generative models in computational connectomics, given experimentally observed data. Bayesian inference takes a probabilistic view and defines the model parameters and data as random variables. It aims to infer

the conditional probability distribution of the model parameters conditioned on the observed data, i.e., the *posterior distribution*. Bayes' rule defines the posterior distribution as

$$p(\boldsymbol{\theta}|x_{\text{obs}}) = \frac{p(x_{\text{obs}}|\boldsymbol{\theta})\, p(\boldsymbol{\theta})}{p(x_{\text{obs}})}, \tag{5}$$

where $p(x_{\text{obs}}|\boldsymbol{\theta})$ is the likelihood of the data given model parameters, $p(\boldsymbol{\theta})$ is the prior distribution over model parameters and $p(x) = \int_{\boldsymbol{\theta}} p(x|\boldsymbol{\theta})p(\boldsymbol{\theta})d\boldsymbol{\theta}$ is the so-called *evidence*. Thus, performing Bayesian inference requires three components:

1. Experimentally observed data x_{obs}.
2. A *likelihood* $p(x_{obs}|\boldsymbol{\theta})$, which defines the relationship between model parameters and data. In our setting, the likelihood is implicitly defined by the computational model, i.e., by the simulator generating connectomics data x given model parameters $\boldsymbol{\theta}$. The simulator needs to be stochastic, i.e., when repeatedly executed with a fixed parameter $\boldsymbol{\theta}$, it should generate varying data. Technically, given a fixed parameter value θ, the likelihood defines a probability distribution over x, and simulating data corresponds to sampling $x \sim p(x|\boldsymbol{\theta})$.
3. A *prior* distribution $p(\boldsymbol{\theta})$. The prior incorporates prior knowledge about the parameters $\boldsymbol{\theta}$, e.g., biologically plausible parameter ranges or known parameter correlations.

The posterior distribution $p(\boldsymbol{\theta}|x_{obs})$ inferred through Bayes' rule characterizes all model parameters likely to reproduce the observed data. For example, model parameters with a high probability under the posterior distribution will result in data close to the observed data. In contrast, parameters from low probability density regions will likely generate data different from the observed data.

In most practical applications, it is hard to obtain an analytical solution to Bayes' rule because the evidence $p(x) = \int p(x|\theta)p(\theta)d\theta$ is challenging to calculate. There exists a large set of methods to perform approximate inference, e.g., Markov Chain Monte Carlo sampling (MCMC, ***Rosenbluth and Rosenbluth, 1955***; ***Hogg and Foreman-Mackey, 2018***). MCMC methods can be used to obtain samples from the posterior distribution. However, they require evaluation of the likelihood function of the model, and computational models in connectomics are usually defined as scientific simulators for which no analytical form of the underlying likelihood is available or numerical approximations are computationally expensive.

Simulation-based inference

Simulation-based inference (SBI, ***Cranmer et al., 2020***) allows us to perform Bayesian inference without numerical evaluation of the likelihood by requiring only access to simulations from the model. The idea of SBI is to generate a large set of pairs of model parameters and corresponding simulated data and use it as training data for artificial neural networks (ANN). The employed ANNs are designed to approximate complex probability distributions. Thus, they can be used to approximate the likelihood to then obtain posterior samples via MCMC (***Papamakarios et al., 2017***; ***Lueckmann et al., 2019***; ***Hermans et al., 2020***; ***Boelts et al., 2022***) or the posterior distribution directly (***Papamakarios and Murray, 2016***; ***Lueckmann et al., 2017***; ***Greenberg et al., 2019***). Once trained, the neural networks are applied to the experimentally observed data to obtain the approximate posterior. In our work, we used an SBI approach called *sequential neural posterior estimation* (SNPE, ***Papamakarios and Murray, 2016***; ***Greenberg et al., 2019***).

Neural posterior estimation

Neural posterior estimation (NPE) uses an artificial neural network $F(x)$ to learn an approximation of the posterior from training data pairs $\{(\boldsymbol{\theta}_i, x_i)\}_{i=1}^{N}$, where $\boldsymbol{\theta}$ is sampled from a prior $\boldsymbol{\theta}_i \sim p(\boldsymbol{\theta})$, and x is simulated from the model $x_i \sim \text{simulator}(\boldsymbol{\theta}_i)$. The density estimator $F(x)$ is trained to construct a distribution that directly approximates the *posterior*. It is usually defined as a parametric family q_ϕ with parameters ϕ, e.g., a mixture density network (MDN, ***Bishop, 1994***), or a normalizing flow (***Papamakarios et al., 2021***). For example, suppose q is a mixture of Gaussians, then F would

take the data as input and predict the parameters ϕ, $\phi = F(x)$, where ϕ contains the means, the covariance matrix, and the mixture weight of each mixture component. $F(x)$ is trained to predict the parameters ϕ from x by minimizing

$$-\frac{1}{N}\sum_{i=1}^{N}\log q_{\phi=F(x_i)}(\boldsymbol{\theta}_i|x_o).$$

This training loss implicitly minimizes the Kullback-Leibler divergence between the true posterior and the approximation $q_\phi(\boldsymbol{\theta}|x)$. It will converge to zero, i.e., NPE will infer the true posterior, in the limit of infinite training data and given density estimator that is flexible enough (***Papamakarios and Murray, 2016***; ***Le et al., 2017***). Algorithm 1 summarizes the algorithmic steps of NPE; see ***Greenberg et al.*** (***2019***) for details.

Once NPE is trained on simulated data, it can be applied to the actual observed data x_{obs}, e.g., $\phi = F(x_{\text{obs}})$, to obtain an approximation to the desired posterior:

$$q_\phi(\boldsymbol{\theta}|x_{\text{obs}}) \approx p(\boldsymbol{\theta}|x_{\text{obs}}). \tag{6}$$

Importantly, NPE applies to any newly observed data without retraining the density estimator, i.e., the inference with NPE is *amortized*. There is also a sequential variant of NPE called SNPE, where the training is performed over several rounds to focus the density estimator on a specific observation x_{obs}. In each new round of SNPE, the new training data is not generated with parameters sampled from the prior but from the posterior estimate of the previous round. While the sequential approach can be substantially more sampling-efficient compared to NPE, i.e., requiring fewer training simulations to obtain a good posterior approximation for a given x_{obs} (***Lueckmann et al., 2021***), it comes with two caveats. First, it requires retraining for every new x_{obs}. Second, using a proposal distribution different from the prior for simulating new data requires a correction, resulting in additional algorithmic choices and challenges. Over the last few years, different approaches have been proposed to perform this correction (***Papamakarios and Murray, 2016***; ***Lueckmann et al., 2017***; ***Greenberg et al., 2019***; ***Deistler et al., 2022a***). We used SNPE with the correction proposed by ***Greenberg et al.*** (***2019***).

Algorithm 1: Single round Neural Posterior Estimation as in ***Papamakarios and Murray*** (***2016***)

input simulator $p(\mathbf{x}|\boldsymbol{\theta})$, prior $p(\boldsymbol{\theta})$, observed data x_{obs}
for $j = 1 : N$ **do**
  Sample $\boldsymbol{\theta}_i \sim p(\boldsymbol{\theta})$
  Simulate $\mathbf{x}_i \sim p(\mathbf{x}|\boldsymbol{\theta}_i)$
end
$\boldsymbol{\phi} \leftarrow \arg\min -1/N \sum_i^N \log q_{F(\mathbf{x}_i,\boldsymbol{\phi})}(\boldsymbol{\theta}_i)$
Set $\hat{p}(\boldsymbol{\theta}|\mathbf{x}_o) = q_{F(\mathbf{x}_{\text{obs}},\boldsymbol{\phi})}(\boldsymbol{\theta})$
return Samples from $\hat{p}(\boldsymbol{\theta}|\mathbf{x}_{\text{obs}})$; density estimator $q_{F(\mathbf{x},\boldsymbol{\phi})}(\boldsymbol{\theta})$

Posterior validation

In theory and with unlimited training data, NPE will converge to the true (unknown) posterior distribution. However, training data is limited in practice, and the underlying posteriors can be high-dimensional and complex. Thus, it is essential to validate the approximate posterior. There are two common techniques for validating SBI even in the absence of a reference posterior: predictive checks (***Gelman et al., 2020***) and calibration checks, e.g., simulation-based calibration (SBC, ***Cook et al., 2006***; ***Talts et al., 2020***).

Predictive checks

Predictive checks can be applied to either the prior or the posterior. The *prior predictive check* is applied before the inference. It checks whether the model can produce data close to the experimentally observed data x_{obs}, i.e., that the distribution obtained by sampling from the prior and simulating the corresponding data contains x_{obs}. If this is not the case, the model or the prior could be misspecified and should be refined before applying SBI. We performed the prior predictive check for the DSO rule simulator by sampling 100,000 parameters from the prior and ensuring that the resulting distribution of simulated connection probabilities covers the seven measured values (see Prior predictive checks for details).

The posterior predictive check tests the predictive performance of the posterior. It should be applied after the inference by simulating data using parameters sampled from the posterior:

$$x_p \sim \text{simulator}(\boldsymbol{\theta}_p) \text{ where } \boldsymbol{\theta}_p \sim p(\boldsymbol{\theta}|x_{\text{obs}}).$$

The simulated data should cluster around the observed data with a variance on the order of the variance expected from the simulator. We performed this check for all inferred wiring rules by simulating 1,000 data points using 1,000 parameters sampled from the corresponding SBI posterior.

Simulation-based calibration

The variance of the posterior distribution expresses the uncertainty in the parameters. Simulation-based calibration (SBC) provides a way to check whether these uncertainties are, on average, well-calibrated, i.e., that the posterior is (on average) neither too broad (under-confident) nor too narrow (over-confident). The basic idea of SBC is the following. Suppose one uses an SBI method to obtain $i = 1, \ldots, N$ different posteriors $p(\boldsymbol{\theta}|x_i)$ for different observations x_i generated from different parameters $\boldsymbol{\theta}_i$ sampled from the prior. If one determines the rank of each parameter $\boldsymbol{\theta}_i$ among samples from its corresponding posterior $p(\boldsymbol{\theta}|x_i)$, then the posteriors obtained with this SBI method have well-calibrated uncertainties if the collection of all N ranks follows a uniform distribution (***Talts et al., 2020***). To check whether the posterior obtained with SBI is well-calibrated, we repeated the inference with NPE $N = 1000$ times (no retraining required), using data generated from the simulator with parameters sampled from the prior. Subsequently, we performed a visual check for uniformity of the corresponding SBC ranks by comparing their empirical cumulative density function against that of a uniform distribution.

A generative structural model of the rat barrel cortex

We demonstrated the utility of SBI for computational connectomics by constraining wiring rules in a structural model of the rat barrel cortex with connectivity measurements. To fulfill the prerequisites of Bayesian inference defined above, we set up a simulation-based model for simulating wiring rules in the barrel cortex model. The wiring rule simulator has three components:

1. a *structural model* that provides features (Fig.2a),
2. a parametrized *wiring rule* that is applied to the features to simulate a connectome (Fig.2b),
3. calculation of *summary statistics* from the simulated connectome to match the available measurements (Fig.2c).

The structural model

The structural model is a digital reconstruction of the rat barrel cortex constructed from detailed measurements of cell types and their morphologies, locations, and sub-cellular features, including single boutons and dendrites, obtained from several animals (***Meyer et al., 2010***, ***2013***; ***Egger et al., 2012***, ***2014***; ***Narayanan et al., 2015***; ***Udvary et al., 2022***). These measurements and their digital reconstructions were copied and arranged according to measured cell type distributions in all cortical layers to obtain a realistic estimate of the structural composition of a large part of

the entire barrel cortex. The model contains around 477,000 excitatory and 77,000 inhibitory neurons, resulting in more than 5.5 billion synaptic sites. Furthermore, it incorporates the projections from the ventral posterior medial nucleus (VPM) of the thalamus to all cortical layers. The model gives access to several cellular and subcellular structural features, including presynaptic boutons and postsynaptic target counts (spine densities). These features were collected for every neuron segment in every subvolume of the model. The model does not contain synaptic connections but only the structural features. Thus, it allows to simulate the effect of different wiring rules by applying the rule to the structural features and comparing the resulting connectome to experimental measurements (***Udvary et al., 2022***).

Dense structural overlap wiring rule

We applied a wiring rule to the structural model to turn it into a simulation-based model that can generate simulated connectomes of the rat barrel cortex. As a wiring rule, we used the dense structural overlap (DSO) rule introduced by (***Egger et al., 2014***; ***Udvary et al., 2022***), which proposes that two neurons form a synapse depending on their locally available structural subcellular features summarized as DSO. The DSO is the product of the numbers of pre- and postsynaptic structures, *pre* and *post*, that a presynaptic neuron i and a postsynaptic neuron j contribute to a subvolume k relative to the total number of postsynaptic structures contributed by all neurons, *postAll* (Fig.2b):

$$\mathrm{DSO}_{i,j,k} = \frac{pre_i \cdot post_j}{postAll_k}. \tag{7}$$

We assumed that the number of connections between any neuron pair (i,j) within a subvolume k is given by a Poisson distribution with the DSO as the rate parameter (***Egger et al., 2014***, Fig.2c):

$$c_{ijk} \sim \mathrm{Poisson}(\mathrm{DSO}_{i,j,k}).$$

The DSO rule is stochastic, i.e., it samples different synapse counts every time it is applied to the structural model. However, the contribution of each structural feature to the DSO is fixed. To allow for more flexibility in the relative weighting of each feature, we generalized the DSO rule by introducing three scaling parameters for the three structural features, θ_{pre}, θ_{post}, and $\theta_{postAll}$:

$$\mathrm{DSO}_{i,j,k}(\boldsymbol{\theta}) = \frac{pre_i^{\theta_{pre}} \cdot post_j^{\theta_{post}}}{postAll_k^{\theta_{postAll}}}. \tag{8}$$

We rewrote the parametrized rule as a Poisson *generalized linear model* (GLM, ***Nelder and Wedderburn, 1972***) by transforming the features to the logarithmic space, stacking them as column vectors in a feature matrix $X_{ijk} = [\log pre(i,k); \log post(j,k); -\log postAll(k)]$ and arranging the scaling parameters in a vector $\boldsymbol{\theta}$:

$$\begin{aligned}
\mathrm{DSO}_{i,j,k}(\boldsymbol{\theta}) &= \exp\left(\log\left(\frac{pre_i^{\theta_{pre}} \cdot post_j^{\theta_{post}}}{postAll_k^{\theta_{postAll}}}\right)\right) \\
&= \exp\left(\theta_{pre} \log pre_i + \theta_{post} \log post_j - \theta_{postAll} \log postAll_k\right) \\
&= \exp(\boldsymbol{\theta}^\top X_{ijk}) \\
c_{ijk}(\boldsymbol{\theta}) &\sim \mathrm{Poisson}(\exp(\boldsymbol{\theta}^\top X_{ijk})).
\end{aligned}$$

The generalized version of the DSO rule takes parameter combination $\boldsymbol{\theta}$ and generates simulated connectomes in the format of synapse counts. Each new parameter setting corresponds to a different variant of the DSO rule that could have generated the measured data. Note that setting $\boldsymbol{\theta} = [1, 1, 1]^\top$ would result in the expression introduced in equations 7 and 8.

Mapping from simulated connectomes to measured connectivity data

The generalized DSO rule and the chosen prior distribution jointly define a space of simulated connectomes associated with the DSO rule. The last step for constructing a simulation-based model is to map the simulated connectomes to the type of data that can be measured empirically. The measurements available for the barrel cortex are connection probabilities estimated from pairwise recordings between neurons in the ventral posterior medial nucleus (VPM) of the thalamus and different layers and cell types in the cortex: to layer 4 (L4), layer 4 septum (L4SEP), layer 4 star pyramidal cells (L4SP), and layer 4 spiny stellate cells (L4SS) (all measured by ***Bruno and Sakmann, 2006***), layer 5 slender-tufted intratelencephalic cells (L5IT), layer 5 thick-tufted pyramidal tract cells (L5PT), and layer 6 (all measured by ***Constantinople and Bruno, 2013***).

While the simulated connectome provided access to all neuron-pair-subvolume combinations in the structural model, the measurements were given only as estimated connection probabilities for different cell types. To calculate these connection probabilities from the simulated connectome, we identified the pairs from the presynaptic and postsynaptic neuron populations used in the experiments and calculated the connection probabilities from the corresponding simulated synapse counts. The number of probed neuron pairs was relatively small in the experiments, e.g., around 50 (***Bruno and Sakmann, 2006***). We tried to mimic this experimental setting in the simulation by selecting a random sample of 50 pairs from the thousands of possible pairs available in the simulated connectome. We then checked how many of those neuron pairs connected with at least one synapse for each of the seven populations. Algorithm 2 summarizes the steps for calculating the summary statistics.

Algorithm 2: Wiring rule simulator and summary statistics calculation

input structural model S, wiring rule $R(\theta)$, parameter $\theta \sim p(\theta)$, measured connectivity data x_{obs}

Simulate:
generate synapse counts for each neuron-pair i, j in each subvolume k:
$c_{ijk} \sim \text{simulator}(S, R, \theta)$

Summarize:
for *each population m measured in x_{obs}* **do**
  Find index set of neuron pairs Π_m belonging to population m
  Sample 50 random neuron-pair indices $\pi \sim \Pi_m$
  Estimate population connection probability as average over connected pairs:
  $\sum_{(i,j)\in\pi} \frac{\mathbb{I}(i \to j)}{|\pi|}$
end
return simulated connectivity data x

Overall, this provided us with a setup to perform Bayesian inference as defined above, to constrain the parameters of a hypothesized wiring rule in the rat barrel cortex:

1. observed data x_{obs} given by seven measured connection probabilities between VPM and barrel cortex
2. a stochastic simulation-based model that generates simulated data x according to the hypothesized DSO rule, given parameters θ
3. a prior over model parameters θ

This setup readily extends to other observed data, other simulation-based models, or priors.

Experimental settings

Simulation and SBI settings

For performing SBI on the DSO rule parameters, we used a Gaussian prior over the three parameters:

$$\boldsymbol{\theta} \sim \mathcal{N}(\mu_0 = [1, 1, 1]^\top, \Sigma_0 = 0.5\,\boldsymbol{I}). \tag{9}$$

We chose the variance of the prior such that the distribution of simulated connection probabilities covered the range $[0, 1]$ densely (see Supplementary material for details).

For the inference on the distance-based wiring rules, we used a uniform prior over the shared subvolume threshold parameter θ_{thres} of neuron-level rule

$$\theta_{thres} \sim \mathcal{U}(0, 100),$$

and a Beta distribution prior over the synapse probability parameter θ_{prob} of the synapse level rule

$$\theta_{prob} \sim \text{Beta}(\alpha = 2, \beta = 2).$$

The settings for generating training simulations for SBI were the same for all results: We drew 1,000,000 parameter values from the prior and simulated the corresponding synapse counts or neuron-level connections, followed by the summary step (except for the simulator used for benchmarking, see below).

To perform SBI, we used (S)NPE with Neural Spline Flows (NSF, ***Durkan et al., 2019***) as density estimator. The NSF hyperparameters were: five transforms, two residual blocks of 50 hidden units each, ReLU non-linearity, and ten spline bins, all as implemented in the public *sbi* toolbox (***Tejero-Cantero* et al., 2020***). The training was performed with a training batch size of $1,000$, a validation set proportion of 10%, and a convergence criterion of 20 epochs without improvement of validation loss. We used the different versions of NPE or SNPE as follows: For the benchmark of SBI against the MCMC reference posterior, we compared the non-sequential (NPE) and sequential version (SNPE). For evaluating SBI with simulated data, we used the NPE to leverage its ability to perform inference repeatedly for many different observations without retraining as needed for running simulation-based calibration. For the inference on the DSO rule, we used SNPE with ten rounds to focus the posterior on the measured data. For the inference on the distance-based rules, we used NPE.

Validating SBI on the wiring rule simulator

We performed two validation steps to ensure that SBI performs reliably when inferring wiring rules in the structural model of the rat barrel cortex. First, we set up a simplified version of the DSO rule simulator for which it was possible to obtain a high-quality reference posterior. In particular, we reduced the number of neuron-pair-subvolume combinations from 130 million available in the original structural model to only ten. Additionally, we omitted the summary step, such that running one simulation corresponded to applying the rule to the structural features of the ten neuron-pair-subvolume combinations and sampling synapse counts from the corresponding Poisson distribution. As a consequence, the likelihood of this simplified simulator was accessible, i.e., it was given by the Poisson distribution, and it was possible to obtain accurate posterior samples using standard approximate Bayesian inference MCMC sampling (***Hogg and Foreman-Mackey, 2018***).

We implemented this reduced simulator in the SBI benchmarking framework `sbibm` (***Lueckmann et al., 2021***) and obtained references posterior samples via slice sampling MCMC (***Neal, 2003***) using ten parallel chains and sequential importance reweighting (***Rosenbluth and Rosenbluth, 1955***; ***Lueckmann et al., 2021***), all as implemented in `sbibm`. We compared three algorithms:

SNPE, which estimates the posterior in multiple rounds focusing on one specific observation; the single-round variant NPE, which works for many observations without retraining; and a classical sequential rejection-sampling-based algorithm called SMC-ABC (***Sisson et al., 2007***; ***Beaumont et al., 2009***). As a measure of posterior accuracy, we used the classifier-2-sample-test score (C2ST ***Lopez-Paz and Oquab, 2018***), defined by the classification accuracy of an artificial-neural-network classifier trained to distinguish the approximate and reference posterior samples. For running SNPE and NPE, we used the `sbi` toolbox (***Tejero-Cantero*** *et al., 2020***), and for SMC-ABC, we used the implementations provided in `sbibm`. Generating the training data for the SBI algorithm by simulation data from the model can be a crucial computational factor. To investigate the simulation efficiency of different SBI algorithms for our inference problem, we performed a quantitative comparison of the number of training simulations and the resulting accuracy of the approximate posterior by repeating inference with SMC-ABC, NPE, and SNPE for a simulation budget of 1,000; 10,000; 100,000 and 1,000,000 simulations.

As a second validation step, we applied SBI to the original version of the DSO rule for which no reference posterior was available. For this setting, we tested the validity of NPE applied to simulated observed data. First, we performed simulation-based calibration (SBC) to check the calibration of the posterior uncertainties inferred by NPE. To run SBC, we trained NPE once on 1,000,000 simulations and then obtained 1,000 different posteriors $p(\theta|x_i)$ for different observations x_i, where x_i was generated from different parameters $theta_i$ sampled from the prior. We then collected the individual ranks of the underlying parameter θ_i under their posterior and tested whether these ranks were uniformly distributed by visually inspecting their empirical cumulative density functions. Second, we checked whether the parameters identified by the NPE posterior distribution could reproduce the (simulated) observed data. To perform this check, we sampled 1,000 parameters from the posterior inferred given a simulated example observation, simulated corresponding connection probabilities using the DSO rule simulator, and compared them to the observed data.

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Author contributions

J.B. and J.H.M. conceived the methodology; J.B. conducted the experiments, data analysis, and software development with help from P.H. and F.Y.; D.U. created the rat barrel cortex model; J.B. created the visualizations with help from P.H., R.G., and J.H.M.; J.B. wrote the paper with help from R.G., J.H.M., and all other authors. J.H.M. provided the principal supervision with help from M.O., H.H., and D.B.

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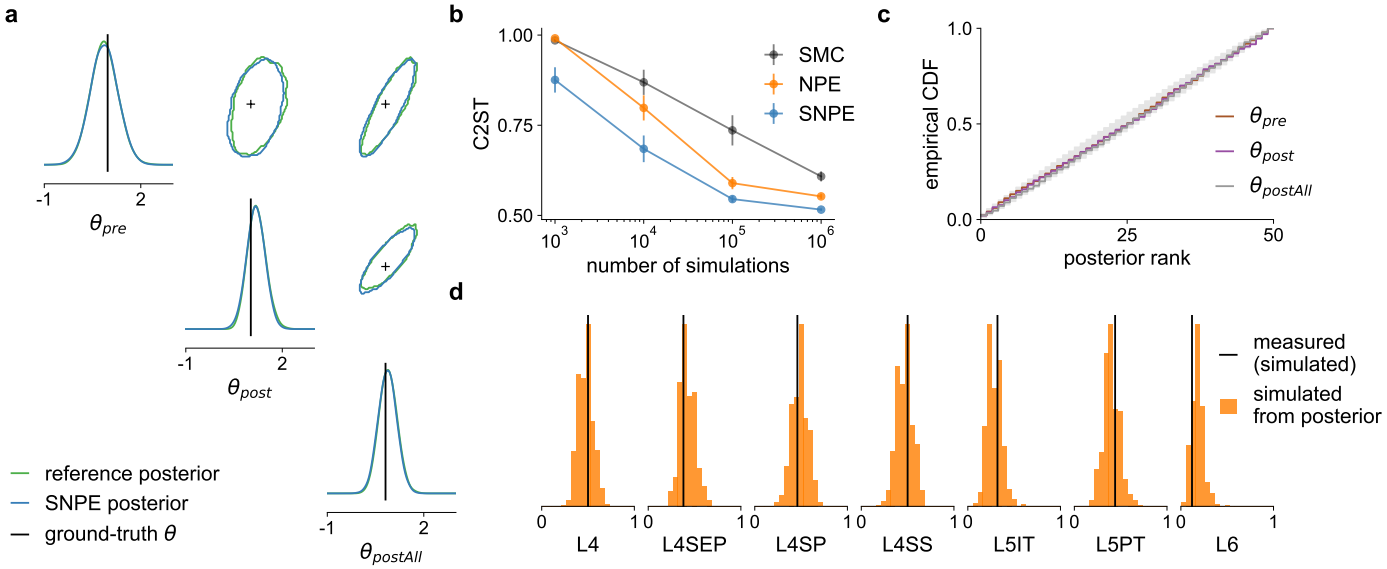
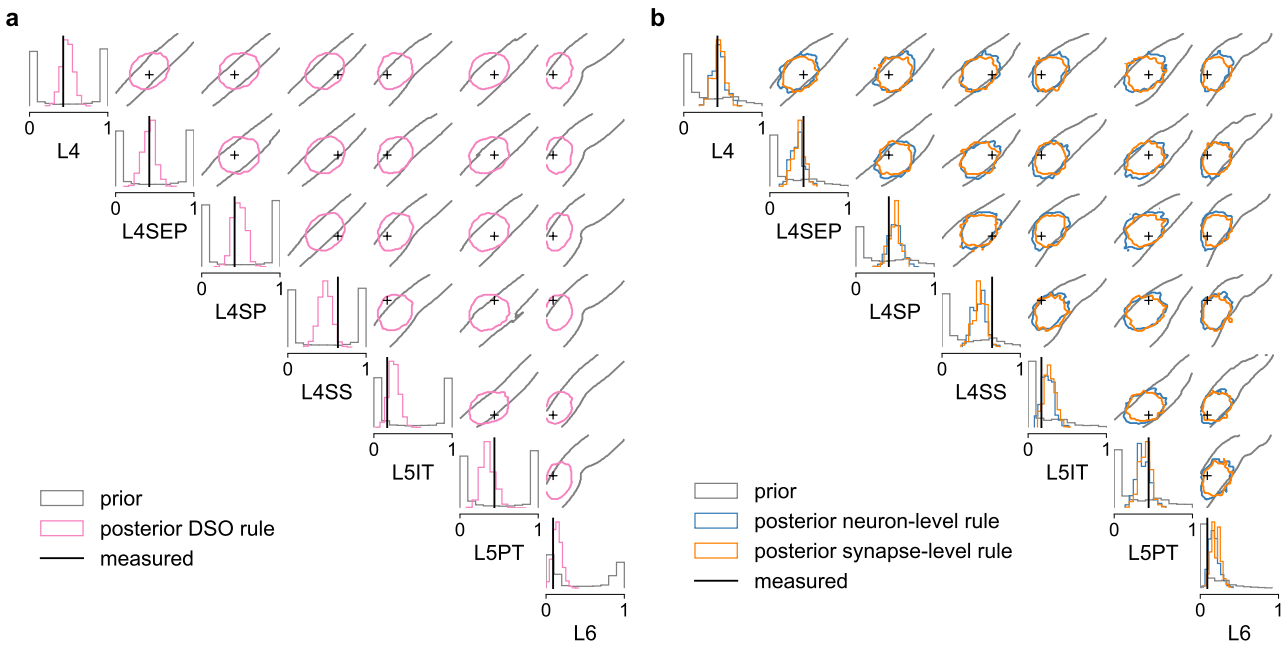
Supplementary figures



Supplementary Figure S1. Validating SBI over wiring rule parameters with simulated data. (**a**) Comparison of the posterior over wiring rule parameters inferred with SBI (using the SNPE algorithm, blue) and the reference solution (ground-truth parameters in black). (**b**) Inference accuracy in terms of classifier-2-sample-test accuracy (C2ST) between reference solution and three SBI algorithms, plotted as a function of training simulations (0.5 is best, error bars show standard error over ten different observations). (**c**) Distributions of posterior ranks from Simulation-based calibration, obtained for each parameter separately from NPE applied to the full version of the DSO rule simulator. A well-calibrated posterior should have uniformly distributed ranks, as indicated by the area shaded gray. (**d**) Comparison of data simulated with samples drawn from the NPE posterior (posterior predictive distribution, orange) and the underlying observed data (simulated, black).



Supplementary Figure S2. Predictive distributions. Comparison of connection probabilities simulated from the prior (gray), the SBI posterior (colors), and the measured data (black). Shown for the dense structural overlap rule (DSO) (see Results) in (**a**) and for the proximity-based wiring rules on the neuron level and the synapse level in (**b**). The prior predictive distributions cover a wide range of values, whereas the posterior predictive distributions cluster around the measured connection probabilities taking into account the measurement noise.

Supplementary material

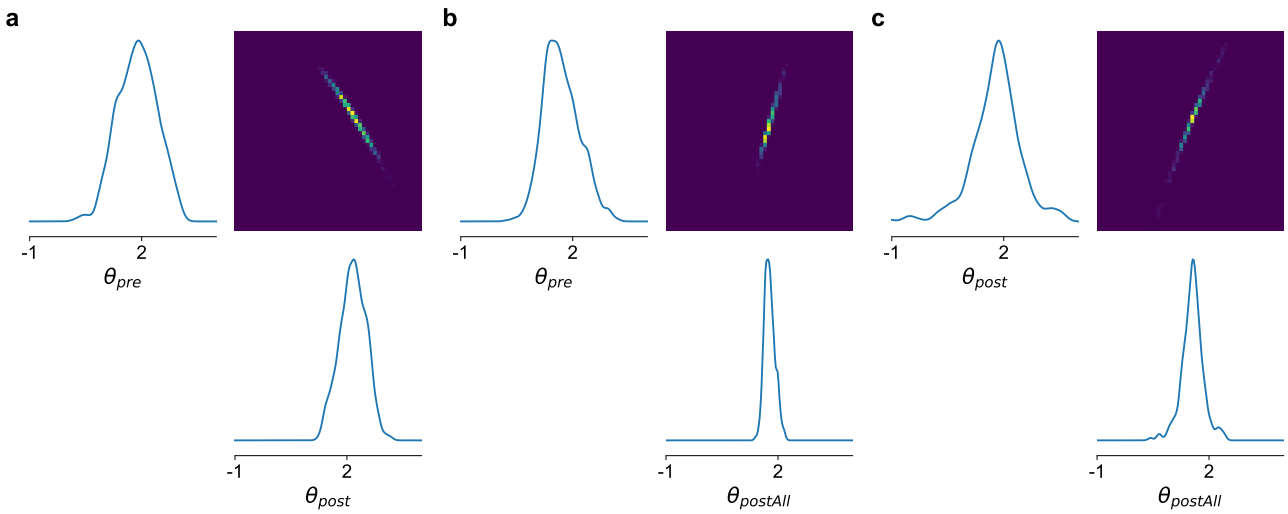
Prior predictive checks

An essential requirement for a generative model is that it can actually generate the measured data, i.e., that the model is not misspecified. The prior predictive distribution shows all data that can be generated by the model when sampling parameters from the prior. Thus, this distribution provides a tool to check for misspecification. For the wiring rule simulator, the prior predictive is a seven-dimensional distribution (Fig. S4b).

We found that with the chosen setting of the prior, simulator, and summary statistics (see Algorithm 2), the prior predictive distribution covers a large range of plausible values, including the experimental measurements (Fig. S4b). The two-dimensional marginals show strong positive correlations between all seven connection probabilities and additional blocks of stronger correlations within layer 4 (L4, L4SEP, L4SP, L4SS) and layer 5 (L5PT, L5IT), visible in the correlations matrix (Fig. S4a). These correlations are plausible because all populations share the same source populations in the thalamus, and the blocks of stronger correlations correspond to connection probabilities within the same target layer in the cortex. Furthermore, depending on the number of neuron pairs used to calculate connection probabilities (see Mapping from simulated connectomes to measured connectivity data), the simulator accurately matches the empirical variance expected from the measured data (Fig. S4c). These results indicated that a subsampling of 50 pairs was adequate to model the experiments.

Alternative parametrizations of the dense structural overlap rule

The strong conditional correlations between the three parameters of the dense structural overlap rule (DSO, see Posterior analysis reveals biologically plausible parameter interactions) indicated that an alternative parametrization of the DSO rule, e.g., using only two parameters, could also be able to explain the measured data. We tested this hypothesis by changing the DSO rule as follows.



Supplementary Figure S3. Conditional posterior distributions. We obtained conditional posterior distributions for the DSO rule (see Results) by conditioning one of the three parameters to a value drawn from the posterior. The resulting two-dimensional posterior over θ_{pre} and θ_{post} (**a**), θ_{pre} and $\theta_{postAll}$ (**b**) and θ_{post} and $\theta_{postAll}$ (**c**), showed strong correlations as visible on the off-diagonal two-dimension marginals.

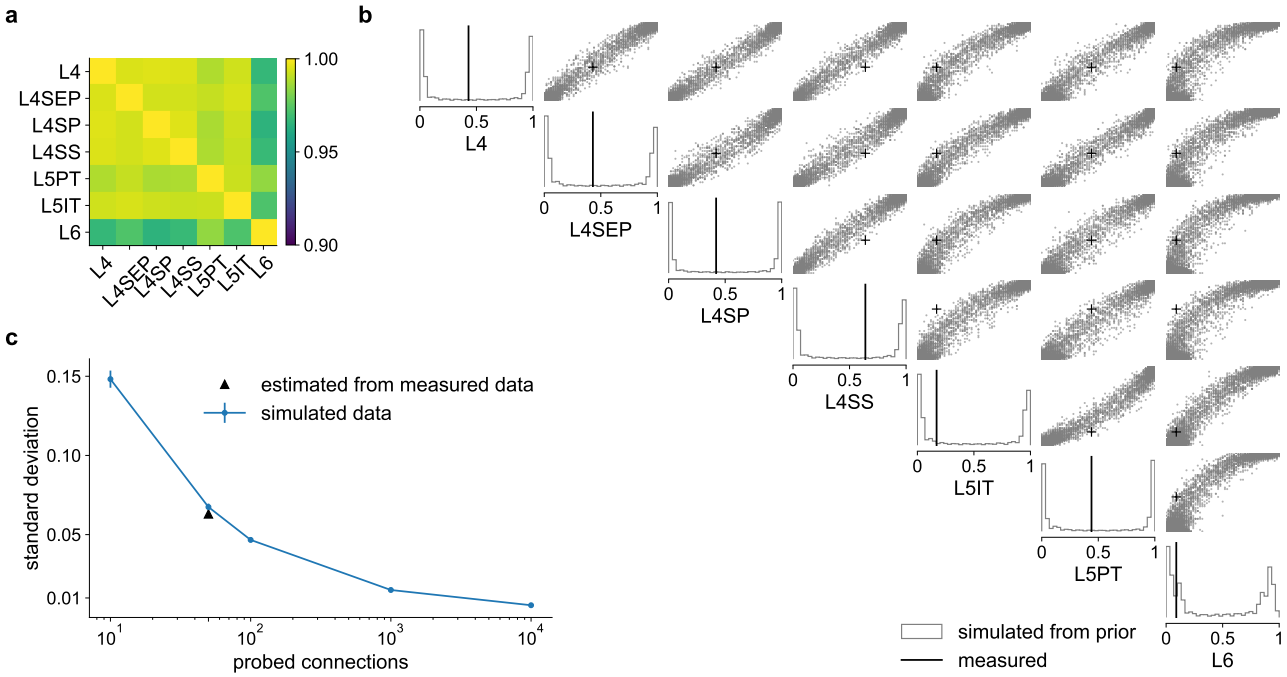
The initial formulation of the rule is given by

$$\text{DSO}_{i,j,k}(\boldsymbol{\theta}) = \frac{pre_i^{\theta_{pre}} \cdot post_j^{\theta_{post}}}{postAll_k^{\theta_{postAll}}}. \tag{10}$$

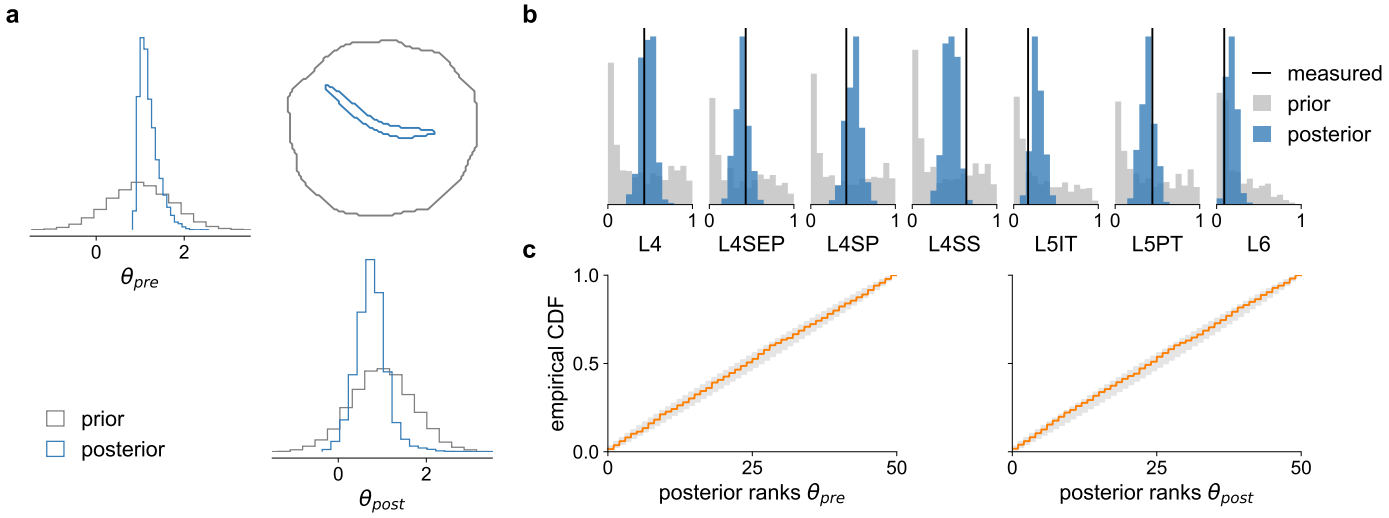
The *postAll* structural feature in the denominator contains the post-synaptic target densities of *all* post-synaptic structures in a given subvolume k of the structural model, including the $post_j$ features. It acts as a normalizing factor for the rule, e.g., when the weight θ_{post} increases, a corresponding increase in $\theta_{postAll}$ can maintain a similar overall DSO value. We changed the DSO rule by removing the parameter $\theta_{postAll}$, but including the *scaled* features $post_j^{\theta_{post}}$ into *postAll*, instead of only taking *post*. By taking the scaled version, we maintained the normalizing property of the denominator of the DSO rule: If θ_{post} was high, in the initial three-parameter formulation of the DSO rule, this could be compensated by a corresponding increase in $\theta_{postAll}$. While this was not possible anymore in the two-param version, the compensation occurred implicitly by including the scaled $post_j$ features:

$$\text{DSO}_{i,j,k}(\boldsymbol{\theta}) = \frac{pre_i^{\theta_{pre}} \cdot post_j^{\theta_{post}}}{(post_j^{\theta_{post}} + postAll_{k,\neg j})}. \tag{11}$$

We performed SBI using the same settings as before to obtain the posterior distribution over the two parameters of the reduced DSO rule (Fig. S5a). The resulting posterior predictive distribution showed similar accuracy to the one of the three-parameter DSO rule (compare Fig. S5b versus Fig. 3b). This result indicates that the two-parameter DSO rule provides an alternative to the three-parameter rule.



Supplementary Figure S4. Prior predictive distribution for the DSO rule. (**a**) Estimated correlation matrix of the seven connection probabilities generated from the model (note the restricted range of the color map). (**b**) Marginal plot of the prior predictive distribution showing data simulated from 10k samples from the prior; one-dimensional marginal as histograms on the diagonal and two-dimensional marginals as scatter plots on the upper triangular subplots; literature data in black. (**c**) Size of the random subset of neuron pairs used to calculate the connection probabilities plotted against the resulting standard deviation (std) in the simulated connection probabilities. Calculated from 1,000 simulations given the same parameters, averaged over the seven connection probabilities (blue); compared with the empirical variance expected from the sample sizes used in the experiments in the literature (black).



Supplementary Figure S5. SBI results for the two-parameter DSO rule. (**a**) Posterior distribution (blue) over the two parameters of the reduced DSO rule, inferred with SBI given the seven measured connection probabilities and the corresponding prior distribution (gray). (**b**) Connection probabilities simulated with parameters sampled from the posterior (blue) and the prior (gray), compared to the measured connection probabilities (black, ***Bruno and Sakmann, 2006***; ***Constantinople and Bruno, 2013***). (**c**) Distribution of posterior ranks for every rule parameter, calculated with simulation-based calibration (orange), compared to the desired uniform distribution (gray area on the diagonal).