Enabling Electric Field Model of Microscopically Realistic Brain

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Abstract

Background: Modeling brain stimulation at the microscopic scale may reveal new paradigms for a variety of stimulation modalities. Objective: We present the largest map of distributions of the extracellular electric field to date within a layer L2/L3 mouse primary visual cortex brain sample, which was enabled by automated analysis of serial section electron microscopy images with improved handling of image defects ($250\times140\times90$ μm^3 volume). Methods: We used the map to identify microscopic perturbations of the extracellular electric field and their effect on the activating thresholds of individual neurons. Previous relevant studies modeled a macroscopically homogeneous cortical volume. Result: Our immediate result is a reduction of the predicted stimulation field strength necessary for neuronal activation by a factor of approximately 0.7 (or by 30%) on average, due to microscopic perturbations of the extracellular electric field—an electric field "spatial noise" with a mean value of zero. Conclusion: Although this result is largely sample-specific, it aligns with experimental data indicating that existing macroscopic theories substantially overestimate the electric fields necessary for brain stimulation.

Keywords:

Brain stimulation
Brain modeling at the microscopic scale
Electric field spatial noise
Boundary Element Fast Multipole Method (BEM-FMM)
Biophysical modeling
Multiscale brain modeling

Significance statement

Currently, there is a discrepancy between macroscopic volumetric brain modeling for brain stimulation and experimental results: experiments typically reveal lower electric intensities required for brain stimulation. This study is arguably the first attempt to model brain stimulation at the microscopic scale, enabled by automated analysis of modern scanning electron microscopy images of the brain. The immediate result is a prediction of lower electric field intensities necessary for brain stimulation, with an average reduction factor of 0.7.

Data availability statement

1. Post-processed cell CAD models (383, stl format), microcapillary CAD models (34, stl format), post-processed neuron morphologies (267, swc format), extracellular electric field and potential distributions at different polarizations (267x3, MATLAB format), *.ses projects files for biophysical modeling with Neuron software (267x2, Neuron format), and computed neuron activating thresholds at different conditions (267x8, Excel tables) are made available online through **BossDB**, a volumetric open-source database for 3D and 4D neuroscience data.

2. Computational programs (MATLAB platform under Windows) for simple examples demonstrating the concept of the electric field spatial noise in a dense microenvironment and its major feature – oscillation about a homogeneous state – are made available in online **Supplements A, B, and C**. **Supplements D and E** present additional materials supporting the main paper text.

   Supplement A:

   i. Computer code for cathodic stimulation + noise with arbitrarily varying parameters;
   ii. Computer code for stimulation with uniform electric field + noise (terminal effects);
   iii. Computer code to study generic spatial electric field noise with mean value zero.

   Supplement B:

   Full-wave computer code for cathodic stimulation + noise with finite-length cylinders and electrode potential computation.

   Supplement C:

   Full-wave computer code for computing parameters of the extracellular field in an artificial specimen mimicking a finite sample of convoluted neuronal arbor – a 3D finite cylindrical lattice.

   Supplement D:

   i. Additional materials supporting the main paper with Table D1. Post-processing parameters of the L2/L3 mouse brain sample;
   ii. Comparison with previously reported experimental and computed data for mouse primary motor cortex.

   Supplement E:

   Justification of the nested iterative BEM-FMM algorithm.

1. Introduction

Advances in brain stimulation leverage models of electric fields within the brain for understanding the brain's response to external stimuli provided by transcranial magnetic stimulation (TMS) [1], transcranial electrical stimulation (TES) [2], deep brain stimulation (DBS) [3], electroconvulsive therapy (ECT) [4], or similar excitation. However, accurately modeling these fields within the brain presents a great challenge due to the brain's complex microstructure, including the intricate networks of neurons and blood vessels [5],[6],[7],[8],[9] all of which must be included into the model.

The conventional approach of field modeling in brain stimulation [10],[11],[12],[13],[14],[15],[16] ignores all microscopic structures, with each region represented by macroscopically averaged properties (e.g., isotropic or anisotropic macroscopic conductivity). Only after the macroscopic electric field is evaluated with finite element method (FEM) [17], are isolated neurons "reinserted" to model their biophysical response.

Ignoring microstructure is a recognized limitation but treated as a *fait accompli* because of known computational intractability of FEM for very complex geometries, except for models of isolated and simplified axonal and vascular geometries (cylinders, spheres, regular polygons, etc.), where the importance is demonstrated both theoretically and experimentally [18],[19],[20],[21],[22],[23],[24],[25],[26].

Over the last few years, several brain samples with realistic anatomical microstructure enabled by automated analysis of serial section electron microscopy images were made available. Those are an L2/L3 mouse primary visual cortex brain sample ($250\times140\times90$ μm^3 volume) or IARPA Phase I [5],[6],[7] with both membrane meshes and morphological reconstructions, a 1 mm^3 mouse primary visual cortex brain sample with membrane meshes and cortical column reconstruction or IARPA Phase III [7],[8], and a 1 mm^3 human brain sample (temporal cortex) with membrane meshes and some morphological reconstructions [9]. All samples are augmented with capillary blood networks (about 230 millimeters of blood vessels in [9]).

Is it possible to electromagnetically model these samples to extract information useful for brain stimulation? The present study introduces arguably a first attempt to do so. In contrast to the common FEM, we utilize a matrix-free iterative boundary element fast multipole method (BEM-FMM) [27],[28],[29] with an additional nested iterative solution based on physical separability of different cells. The BEM-FMM charge-based formulation [30],[31],[32],[33] could presently handle up to ~200 million surface degrees of freedom simultaneously. It is readily applicable to computing electric charges induced on nonconducting cell membranes (under initial polarization) of realistic closely spaced neuronal cells and nonconducting (to the main order of approximation) walls of microcapillaries. These charges generate their own secondary electric field $\boldsymbol{E}^s$ which alters the

primary macroscopic brain stimulation field $\boldsymbol{E}^i$ (due to a TMS coil for example) and thus changes expected activating threshold of the neurons.

Our study is subject to several substantial restrictions and limitations. First, we consider the smallest (but asymmetric and severely cut out) IARPA Phase I L2/L3 sample [5],[6],[7]. Second, we only obtain a solution for the extracellular "homogeneous" space assuming non-conducting membranes of the neurons at the end of initial polarization [34] or at any time later if the effect of transmembrane currents due to changes (changes in the local field) is small compared to the primary stimulation field. We also assume non-conducting walls of blood capillaries which is valid as a first approximation [35],[36].

In this approximation, the extracellular solution becomes fully decoupled from the intracellular solution. For a finite brain sample subject to an impressed field $\boldsymbol{E}^i$, the extracellular solution is a unique solution of the exterior Neumann problem [37],[38]. Its mean value is still $\boldsymbol{E}^i$. At the same time, its variations on the cellular level result in an "electric field spatial noise" with a *mean value of zero*. In contrast to temporal noise studies [39],[40], such noise is strong and is not additive but is rather multiplicative since microscopic field perturbations are directly proportional to the local values of the impressed field $\boldsymbol{E}^i$ itself.

As a result, a map of distributions of the extracellular electric field within the entire brain sample has been created. The electric field computations alone required approximately 3 months of continuous CPU time, utilizing 7 modern terminal servers simultaneously as well as multiple nodes of a high-performance computing cluster.

Next, we averaged the computed total extracellular field over membrane cross-sections and obtained a microscopically altered 1D activating function [41],[42],[43] at every cross-section of a neuronal process, in the approximation of the 1D cable equation. Thus, we did not model the volumetric intracellular space and did not employ the true bidomain approach [21],[44]. This is because the length-to-diameter ratio for an axonal arbor (most sensitive to activation) of a realistic cortical neuron may be as high as 60,000 [7],[8], which justifies the use of the 1D cable model.

The extracellular electric field noise defined above alters the activating function, causing local membrane depolarization and inward transmembrane current injection at its positive peaks, while nothing flows at the hyperpolarization peaks—the noise effectively is being rectified. Our hypothesis, verified by computer codes for simple "classic" examples in Supplements A, B, and C, is therefore that the microscopic extracellular noise eases neuron activation as compared to microscopically homogeneous brain modeling.

The logical question we would like to answer is: by how much is neuron activation modified? To do so, a standard biophysical analysis with Neuron software [45] is carried out based on neuron morphologies precisely linked to the physical neurons and known ion channel models for rodents [46],[10],[11],[12]. The brain sample under study is considered as it stands, i.e., no cloning, elongation, or scaling of neuronal processes/capillaries of any kind are made. Our result is a reduction of the stimulation field strength necessary for neuron activation by approximately a factor of 0.7 (or by 30%) on average due to the microscopic perturbations of the extracellular electric field.

This result is sample specific. Its generalization to neurons in a full brain requires further investigation. This is especially valid for directional sensitivity observations reported in this study. These might be affected by both sample anisotropy and the geometrical asymmetry of the sample.

The study is organized as follows. Section 2 Materials and Methods introduces the concept of the electric field spatial noise (further verified in Supplements A, B, and C), describes assembly of the computational brain sample model (further detailed in Supplement D), describes the computational workflow for the extracellular field (further verified in Supplement E) as well as the biophysical modeling setup. Section 3 Results presents major computational findings with other results reported in Supplement D. Section 4 Discussion summarizes findings of the study and states limitations of our approach. Section 5 Conclusions concludes the paper.

2. Materials and Methods

2.1 Why and how do microscopic field perturbations lower activation thresholds?

Consider a simple numerical experiment with a Hodgkin-Huxley axon originally studied by Rattay [43]. A cathodic stimulation of the 10 mm long axon by a point electrode located at the origin is shown in Fig. 1a. The axonal radius is 5 µm, the length constant λ is 1.5 mm, the time constant τ is 3.3 ms, the resting potential is – 65 mV. A monophasic electric pulse ($T = 0.1\ \mathrm{ms}$) is applied. Field perturbers are 2D cylinders with non-conducting walls located perpendicular to the figure plane and with a large radius of 200 µm, chosen for better visualization. Charge deposition on the cylinder walls perturbs the electrode field from Fig. 1a as shown in Fig. 1b where the total field is displayed.

As a result, an electric field “spatial noise” with a mean value of *zero* appears for the extracellular collinear electric field along the axon, as shown in Fig. 1c in blue. In contrast to temporal noise studies [39],[40], this noise is not additive but is rather multiplicative since field perturbations are proportional to the local applied field in a spatial domain of interest. The spatial noise amplitude can be large; it can approach or exceed the applied field itself when the scatterers are in close proximity to the axon (cf. basic electromagnetic solutions for current conduction, for example [47]).

When $t < T$ (Fig. 1e,f), the transmembrane potential closely follows the activating function (derivative of the collinear electric field) with sharp positive peaks of the latter indicating membrane depolarization [43]. The inward (negative in Fig. 1) ionic current flows within the depolarization peaks (Fig. 1f) while nothing flows within the hyperpolarization peaks – the noise is being "rectified". This process is strongly nonlinear – cf. Fig. 1f,h. There, one local inward current injection starts to dominate other inward injections while all outward ionic currents are still negligibly small.

Despite the following low pass filtering as in Fig. 1i,j, this local current injection appears to be strong enough to support further action potential generation (Fig. 1k,l,m,n) when the stimulation cathodic current is only 0.94 mA. This contrasts with the effect of the unperturbed field from Fig. 1a which would generate action potential if the cathode current is 1.34 mA or higher. Thus, the threshold reduction factor due to the spatial noise is 0.94/1.34=0.70 in the present case.

Different cases have been tested with a similar effect – reduction of the activating threshold due to local nonlinear inward current injections from certain portions of noise. Supplement A presents

(i) computer code for the setup from Fig. 1 with arbitrarily varying geometry and parameters;
(ii) computer code for a similar setup but with a *uniform* stimulation electric field and terminal axonal excitation that is particularly important for TMS and;
(iii) computer code to study the effect of generic spatial electric field noise with mean value zero on the Hodgkin-Huxley axon.

Although fast, the demonstration setup from Fig. 1 uses a basic analytical solution for the field of an infinite cylinder with non-conducting walls (cf. [34]). It also does not take into account interactions between individual cylindrical scatterers. To show that these limitations do not significantly change the result, a concurrent full-wave computer code using the boundary element fast multipole method and with finite-length cylinders has been constructed and made available in Supplement B. This solution also replaces the point current electrode by a spherical electrode with the radius of 50 μm. As a result, for the finite-length interacting cylinders with a length-to-diameter ratio equal to five, the threshold reduction factor becomes 0.77.

The full-wave setup of Supplement B also predicts a change in the cathodic electrode voltage (and thus in the electrode power) caused by the present multi-cylinder load. Such a change is less than 1% (-5.03 V versus -4.99 V). This result states that the extracellular field non-uniformity indeed plays the major role in reducing the threshold, not the overall increase of the electric field strength due to the load, even without principal separation between the extracellular and intracellular space (below).

2.2 Modeling approach: decoupling extracellular solution for insulating membranes

Our electromagnetic modeling setup only computes the extracellular electric field in a finite brain sample. It is partially based on two relevant yet smaller-scale studies [32],[33]. We assume that the membrane is non-conducting (insulating), as at the end of initial polarization [34],[55],[56]. The same condition applies to the walls of the blood capillaries. The approximation of the insulating membrane to be discussed later in the text makes it possible to *fully* decouple the extracellular solution from the intracellular one in the form of a classic uniquely solved exterior Neumann problem with the Neumann (zero normal current density) boundary conditions at the outer surface of the membrane (walls of the blood vessels).

In response to an arbitrary impressed or primary field $\boldsymbol{E}^i$ with the (local) potential φ^i applied to a brain sample, induced surface charges will accumulate on the insulting membrane surfaces when considered as the exterior Neumann problem. The secondary field of these charges, $\boldsymbol{E}_{\mathrm{e}}^s$ (subscript *e* stands for extracellular), will alter the impressed electric field in the extracellular domain. The total extracellular field in the brain sample will become $\boldsymbol{E}_{\mathrm{e}} = \boldsymbol{E}_{\mathrm{e}}^i + \boldsymbol{E}_{\mathrm{e}}^s$ and the total extracellular potential will become $\varphi_{\mathrm{e}} = \varphi_{\mathrm{e}}^i + \varphi_{\mathrm{e}}^s$. The goal of the electromagnetic part of the modeling pipeline is to find this secondary extracellular field and potential. For simplicity, we apply a uniform primary electric field $\boldsymbol{E}^i$ with the amplitude of $E^i = 100\ \mathrm{V/m}$, for each independent field polarization (x, y, z).

Fig. 2a illustrates the concept of the above setup. There, the grey circles indicate the intracellular space, the dashed rectangle is the brain sample with extracellular conductivity σ_0, which is equal to the conductivity of the surrounding space, and the red arrows denote the primary field. Fig. 2b illustrates the expected extracellular solution. There, the local charge accumulation (membrane polarization for the exterior Neumann problem) is schematically shown. The red arrows within the rectangle illustrate the total (perturbed) electric field in the extracellular space. On average, these perturbations must cancel out. In other words, the total averaged extracellular field within the sample *must approach* the primary field when the sample size is much larger than the characteristic size of inhomogeneities.

As an example, Supplement C presents parameters of the extracellular field in an artificial specimen mimicking a finite sample of a convoluted neuronal arbor and the corresponding full wave computer code. These results show that the mean extracellular field approaches the constant impressed field with a deviation below 3%. Fluctuations close to the boundary are also rather minor.

2.3 Comparison with "invisible" cells in a microscopically homogeneous brain

The present solution is not intended to be valid and is indeed not valid in intracellular space. The intracellular electric field is zero at the end of initial polarization with a high degree of accuracy while the intracellular potential becomes equal to the resting potential [34]. After that, the intracellular field follows changes in the physiological state governed by the membrane dynamics [34].

To compare the suggested solution with the prior solutions for invisible cells in the microscopically homogeneous brain [10],[11],[12], an attempt might be made to set the entire intracellular space as non-conducting. Thus, the finite brain sample with cells from Fig. 2a would be replaced by a homogeneous sample with a smaller overall macroscopic conductivity and with a higher total electric field due to obvious sample polarization as shown in Fig. 2c.

This approach is physically incorrect. It explicitly utilizes much higher electric fields inside the "nonconducting" cells, which contradicts the biophysical theory. The total electric field inside cells is zero after initial polarization ([34], cf. also [51],[52],[53]). Furthermore, this approach would lead us to unusually small macroscopic conductivity values of the cortical grey matter with densely packed cells having a fill factor up to 70% (cf. estimates [48],[49],[50] and Fig. 1 b), which contradicts measurements (cf. [54]).

Yes, the insulating cell membrane makes the extracellular electromagnetic field fully decoupled from the intracellular solution and uniquely solvable. However, the intracellular space remains highly conducting and behaves accordingly. Since the intracellular space does not physically exist (has zero volume) in the model of the homogeneous brain and invisible cells [10],[11],[12], the primary field $\boldsymbol{E}_{\mathrm{e}}^{i}$ in a homogeneous medium with the same ambient conductivity σ_0 should be used there for comparison purposes. It should be compared with the effect of the total extracellular field of the microscopic solution, which has the same mean value as the primary field $\boldsymbol{E}_{\mathrm{e}}^{i}$ but is subject to spatial noise with mean zero (cf. Supplement C). The absolute value of σ_0 does not matter for comparison purposes.

2.4 Brain sample – MICrONS L2/L3 dataset of mouse visual cortex

The brain sample is a semi-automated reconstruction of the L2/3 P36 male mouse primary visual cortex volume of approximately 250×140×90 μm (Fig. 3) from electron microscopic images with a resolution of 3.6×3.6×40 nm (Fig. 3a,b). This reconstruction includes physical (not skeletonized) pyramidal and non-pyramidal neurons, astrocytes, microglia, oligodendrocytes and precursors, pericytes, vasculature, nuclei, mitochondria, and synapses [5],[7]. In total, 364 excitatory (Fig. 3e) and 32 inhibitory (Fig. 3f) neurons with cell bodies within the volume (Fig. 3c) were segmented, along with some microvasculature (Fig. 3d) [7]. For 301 neurons, the labeled neuronal centerlines

(skeletons) in the common `swc` format were also made available [7]. This provides a unique opportunity to estimate the effect of the local field perturbations using biophysical analysis [45].

2.5 Construction of computational cell meshes

Only the neurons themselves and the vasculature (with a few astrocytes) – the largest perturbers of the impressed or primary electric field $\boldsymbol{E}_{\mathrm{e}}^{i}$ – were included into the present numerical analysis. Numerous neuroglia cells seen in Fig. 3a,b were not included in the analysis due to their small size, which made them impossible to model, even with advanced numerical simulation tools. The volume occupied by these microstructures (extracellular space) was considered approximately homogeneous. The volumetric fraction of the neuronal cells within the brain volume was estimated in Table D1 of Suppl. D as 30±5% by computing the volume of each neuron and taking into account neuronal mesh intersections (below). This is significantly less than the previously reported values on the order of 70% [48],[49],[50] (Suppl. A). The reason is that all neurons with somas outside of this relatively small sample were omitted and their dendritic/axonal processes, which are still within the sample, were ignored during the segmentation.

Supplement D describes the post-processing procedure for the original sample and its results. Based on this data, Table D1 of Supplement D lists major topological and biophysical parameter of the created computational testbed, including estimated sample anisotropy. Both the facet size for membrane discretization (Fig. 4a) and the skeleton's spatial resolution (Fig. 4b,c) for the biophysical analysis were kept at approximately 100 nm to resolve large yet very localized variations of the impressed field due to multiple nearfield interactions of the neuronal processes as well as multiple intersections of neuronal arbors of different neurons (below).

2.6 Neuronal mesh intersections (synaptic interconnections) and their resolution

The neuronal and vasculature surface meshes so constructed were found to heavily intersect with each other at multiple locations (cf. Fig. D2 of Suppl. D). These intersections reflect true synaptic interconnections between different neurons with a list of approximately 2,000 synaptic clefts quantified in [6] for the same sample. At the same time, many intersections are likely due to the imprecise segmentation and subsequent, not entirely identical, remeshing. Fig. 4d illustrates the most common intersections – interconnections – including the dendrite to dendrite and axon to dendrite intersections. Two different colors correspond to the two different neurons. Fig. 4e illustrates the less common intersections – dendrite to soma and axon to axon, respectively. Additionally, Supplement D demonstrates an intersection map between neighboring excitatory neurons #238 and #290, respectively (Fig. D2). Fig. D2 also presents a mesh intersection roster of excitatory neuron #238 with all other neurons of the sample. It is observed that neuron #238

intersects with almost all neurons of the sample. Similar results were observed for other larger neurons within the sample.

To resolve the mesh intersections numerically, we used the method illustrated in Fig. 4f,g,h. For two intersecting processes from Fig. 3f, all facets belonging to either process and having at least one node within another process (mesh) have been removed. This operation does not exactly join the two meshes but leaves a narrow gap (one the order of triangle size or ~100 nm) in between as seen in Fig. 4h. When the gap size is small compared to the intersection domain itself, which is achieved by a present fine mesh resolution, the gap has a minor influence on the results as demonstrated by our refined mesh computations (Supplement D).

2.7 Computing extracellular electric field and extracellular electric potential

For the computation of the extracellular electric field, the approach developed in two relevant yet smaller-scale studies [32],[33] has been used. It assumes that the membrane is non-conducting (insulating) at the end of initial polarization [34],[55],[56]. The same condition applies to the walls of the blood capillaries. The condition of the insulating membrane makes it possible to fully decouple the extracellular solution in the form of a classic uniquely solved exterior problem with the simple Neumann (zero normal current density) boundary conditions at the outer surface of the membrane. In response to an impressed or primary extracellular field $\boldsymbol{E}_{\mathrm{e}}^{i}$ with the (local) potential φ_{e}^{i}, induced surface charges will accumulate on the membrane surfaces. The secondary field of these charges, $\boldsymbol{E}_{\mathrm{e}}^{s}$, will alter the extracellular electric field. The total field in the brain will become $\boldsymbol{E}_{\mathrm{e}} = \boldsymbol{E}_{\mathrm{e}}^{i} + \boldsymbol{E}_{\mathrm{e}}^{s}$ and the total extracellular potential will become $\varphi_{\mathrm{e}} = \varphi_{\mathrm{e}}^{i} + \varphi_{\mathrm{e}}^{s}$. The goal of the electromagnetic part of the modeling pipeline is to find the secondary field and potential. We have applied a uniform electric field $\boldsymbol{E}_{\mathrm{e}}^{i}$ with the amplitude of 100 V/m for each independent field polarization (x, y, z).

2.8 Application of BEM-FMM and nested iterative algorithm

The single-layer charge formulation for the exterior Neumann problem allows for direct application of the charge-based boundary element fast multipole method (BEM-FMM) [30],[31],[32],[33]. The BEM-FMM is ideal for this type of problem since it does not require construction of an explicit system matrix and does not require a volumetric mesh for the complicated interior/exterior domains. For the present case, however, the iterative BEM-FMM was only capable of computing the field of ~100 interacting neurons (with ca 0.15 billion facets) simultaneously. For larger samples, it required a prohibitively large RAM more than 1 TB and was experiencing slower convergence of the underlying GMRES algorithm [57].

Therefore, a nested iterative method has been developed and is illustrated in Fig. 5a. At initial step 1, each neuron is solved in isolation and is not affected by charges deposited on other neurons. The membrane charge deposition in response to the impressed field $\boldsymbol{E}_{\mathrm{e}}^{i}$ (or the self-interaction) is found via the standard iterative BEM-FMM solution for every neuron in homogeneous space, with other neurons still not existing. Local charge density variations are solely due to the realistic irregular soma shapes clearly seen in Fig. 4a, which are quite different from simplified spherical/ellipsoidal approximations frequently used.

At step 2, the primary field $\boldsymbol{E}_{\mathrm{e}}^{i}$ is modified by the field of charges deposited on other neurons found in step 1. The membrane charge deposition for every neuron is recomputed with this modified primary field. The process repeats itself until a convergence criterion is met. In Fig. 5, the convergence criterion used required the relative 2-norm induced surface charge density error to be below 3% on average and the relative 2-norm centerline electric field error to be below 1% on average.

The corresponding convergence curves (Fig. 5b,c) indicate a sub-percent error for the collinear electric field at the neuronal centerlines after 6 iterations. An additional test of solution accuracy has been performed by comparing with the global BEM-FMM solution for 11 neighbor neurons treated as a single network. This test is described in Supplement E. The relative average charge density error for every neuron versus the global iterative solution for that single network after six iterations was approximately 1%.

Emphasize that the neuron-to-neuron interactions are taken into account at every step of the nested iterative solution except the trivial first iteration (Fig. 5a). In its turn, the nested iterative algorithm used in this study is a full-wave solution; it rapidly converges to the exact result when the number of iterations becomes sufficient (as shown Supplement E).

This method is potentially capable of modeling an unlimited number of cells, although CPU time now increases as $O(N^2)$ where N is the total number of cells. Electromagnetic computational results of this study required approximately 3 months of continuous CPU time when simultaneously utilizing 7 terminal servers and multiple nodes of a high-performance computing cluster.

2.8 Extracellular potential computed at centerlines/skeletons of neuronal processes

The activating function of the one-dimensional cable equation [41],[42],[43] needs extracellular potential or the collinear extracellular electric field at the centerlines of neuronal processes in the form of infinitesimally thin, transparent “wires”. For finite-thickness neuronal process, these values are extracellular fields averaged over the cross-section. In the extracellular domain (*and only there*), the field solution obtained previously is equivalent to the solution for the entirely non-conducting

interior. Therefore, the averaged values are most accurately obtained when the field and the potential are directly computed at the centerlines using the same extracellular solution which simultaneously holds for the entirely non-conducting interior [32],[33].

An appealing result of this approach is that both the transmembrane potential and the double layer density at the end of initial polarization are obtained automatically. Once the extracellular potential φ_{e} is known, we subtract its value from the constant resting potential value inside the cell and obtain the transmembrane potential φ_{m}. Its product with the electrical permittivity of the membrane is the density of the double layer of charges across the cell membrane.

2.9 Biophysical modeling

We implemented rodent neuron models developed in Refs. [46],[10],[11],[12] with 6 active channels for the soma, 8 active channels for the axons, 1 active channel for the basal dendrites, and 3 active channels for the apical dendrites initially developed and used by Aberra et al. [10]. The NEURON Software [45] with fine spatial and temporal resolutions has been employed to study neural activation in response to the extracellular potential/electric field computed previously at the centerlines of the neuronal processes. The neuron morphology for 267 available neuron models (including soma, axons, apical and basal dendrites) was loaded into NEURON by its Import3D tool. The implementation for the most important axonal channels was additionally verified against in-house MATLAB-based biophysical software. Every neuron was modeled independently, following the approach of Refs. [10],[11],[12]. Intercellular communications were not yet considered.

We used four stimulation pulse forms (Fig. D3 of Supplement D): (i) a monophasic positive/negative rectangular pulse starting at $t = 0$ and with a duration of $0.1\ \mathrm{ms}$ and; (ii) an experimentally measured positive/negative biphasic TMS pulse with an overall duration of $0.3\ \mathrm{ms}$. The pulse amplitude gradually increased until the neuron activating criterion (the transmembrane voltage rises above zero anywhere in the arbor) was met. After that, the amplitude was decreased with a smaller step to pass the activating threshold in the opposite direction. This automated search procedure – the bisection method – requires approximately 15 steps and about 15-20 min per neuron on average. The overall simulation time for all neurons with x, y, and z polarizations, for all pulse forms, and for both primary and total extracellular fields/potentials did not exceed two CPU months. Other details of the biophysical modeling setup are described in Supplement D.

The L2/L3 dataset does not provide information about axon myelination. However, the rodent axons are partially myelinated [10],[11],[12]. To account for this, we also modeled the case of artificial myelination. All axonal branches were assigned a myelin sheath with a thickness of 0.2

times the local radius. The nodes of Ranvier were placed at bifurcations, terminations, and along the segmental length, 20 µm apart from each other.

3. Results

3.1 Solution accuracy estimated via self-convergence of the numerical solution

Fig. 5b,c shows solution convergence results (y-polarization of the primary field) for both the induced surface charge density (Fig. 5b) and the collinear electric field projected onto the centerlines of the neuronal processes (Fig. 5c). For every neuron labeled versus its height and every blood vessel appended at the end of the list, Fig. 5b gives a relative 2-norm error for the induced surface charge density between two subsequent iterations as well as the average relative error for all compartments. Fig. 5c provides similar results for the collinear electric field at the neuronal centerlines. Six iterations are fully sufficient to obtain a sub-percent relative error.

An additional test of solution accuracy has been performed by comparing with the global BEM-FMM solution for 11 neighbor neurons treated as a single network. This test is described in Supplement E. The relative average charge density error for every neuron versus the global iterative solution for that single network after six iterations was approximately 1%.

3.2 Membrane charge deposition and induced surface charge density

Fig. 6 demonstrates the Induced surface charge density for neuron #321 and its 200 nearest neighbors in sagittal and coronal planes. Fig. 6a,b is the induced surface charge density for neuron #321. Fig. 6a is the induced charge density when only neuron #321 is present (iteration 1 or self-interaction). Fig. 6b is the Induced charge density when all neurons and microcapillaries of the sample are present (iteration 6). Fig. 6c,d is the induced surface charge density for 200 nearest neighbors of neuron #321. Fig. 6c gives the data for the complete network of 200 nearest neighbors. Fig. 6d is a zoomed in area (x33) of the network in Fig. 6c with some intersynaptic interconnections clearly visible. We were unable to simultaneously plot electromagnetic data for more than 200 neurons simultaneously due to the limitations of the graphical software used.

3.3 Collinear field at centerlines of neuronal processes for biophysical modeling

Fig. 7 illustrates the resulting effect of membrane charge deposition on the collinear electric field projected onto the centerlines of neuronal processes for the same neuron #321. The sagittal plane view is shown corresponding to Fig. 6a,b. Fig. 7a is the electric field projection in homogeneous space, when the neuronal membrane is not present per se and the same unform impressed electric field of 100 V/m is applied along the y axis (from dorsal to ventral). Fig. 7b is the electric field projection when all neuron membranes are present, and the primary field is distorted by the field of the induced charges. One observes that perturbations of the collinear electric field are quite

significant. Moreover, they are constructively or “in phase” accumulated in space so that a positive perturbation is added to the originally positive field projection and vice versa.

3.4 Solution of cable equation. Reduction of neuronal activating thresholds

Fig. 8 illustrates computed activating threshold reduction factors. We choose the first 90 neurons with the longest non-cut axonal arbors in excess of 0.5 mm (Supplement D). The neurons have been renumerated versus the axonal arbor length in descending order.

The action potentials were mostly initiated at the axonal terminations following previously published observations [10],[11],[12]. For some neurons – primarily where the neuronal processes have been substantially cut out – it was also located at the axon hillock.

The threshold reduction results in Fig. 8b are given for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue) and for the $0.1\ \mathrm{ms}$ rectangular negative stimulating pulse. The reduction factor is defined as the ratio of the activating threshold of a physical neuron within the brain (with the total field $\boldsymbol{E}_{\mathrm{e}}$ and the total potential φ_{e}) to the activating threshold of the neuron skeleton in the homogeneous space (subject to the field $\boldsymbol{E}_{\mathrm{e}}^{i}$ and the potential φ_{e}^{i}). Fig. 8c gives the absolute activating thresholds for the corresponding 90 neuron skeletons in homogeneous space. Fig. 8d gives the absolute activating thresholds for the same 90 physical neurons within the sample. The units in Fig. 8c,d are in terms of the field strength of 100 V/m; one unit corresponds to 100 V/m. Figs. D4-D10 of Supplement D report the same data for the remaining pulse forms and for myelinated axons. The last figure there was again computed for the rectangular negative $0.1\ \mathrm{ms}$ long pulse. Average threshold reduction factors for all pulse forms/polarizations of $\boldsymbol{E}_{\mathrm{e}}^{i}$ are given in Table 1 (non-bold entries).

In Fig. 8 and Figs. D4-D10 of Supplement D, one observes substantially different threshold reduction factors for three different polarizations (directions) of the primary field. First, this observation correlates with the sample dimensions (cf. Fig. 3). Simultaneously, it also correlates with a moderate anisotropy of the brain sample. We can characterize the anisotropy as an equivalent straight fiber density distribution of the sample (Supplement D). Fig. 8a schematically illustrates the equivalent straight fiber density distribution of the brain sample under study. A more detailed analysis is provided in the Discussion section.

3.5 Deviation of activating thresholds for different polarizations of primary field

Table 2 summarizes normalized standard deviation of the average neuronal activating thresholds for three different polarizations of the primary electric field. The first 90 neurons with the longest axonal arbor have again been processed. One observes that the normalized standard deviation between average thresholds for different directions of the applied primary field is generally smaller

for the brain sample as compared to individual neuron skeletons in homogeneous space; the cell membrane interactions tend to make brain stimulation more “equal” in all directions.

3.6 Strength-duration results

Fig. 9a illustrates a very weak dependency of the threshold reduction factor on the duration of the applied stimulation pulse. A case in point is neuron #348 within the brain when the negative rectangular pulse is applied. At the same time, the absolute values of the activating threshold indeed follow the classic strength duration dependency [58] with a high degree of accuracy (cf. inset in Fig. 9a, note that a log-log scale has been used).

3.7 Dynamics of threshold reduction factor

Fig. 9b,c,d illustrates the threshold reduction factor dynamics as a function of the number of neighboring cells included in the computations. For each of three representative neuron cells (#238, 321, 348, all having different lengths of axonal arbor), we start adding its nearest neighbors (0, 5, 10 neighbors, etc.) from the entire brain sample and then repeat both electromagnetic and biophysical computations to find new activating thresholds. The x-axis is the corresponding relative volumetric fraction of neuronal mass computed using known volumes of each cell. The first point of every curve is the threshold reduction factor without neighbors, which is due to self-interaction of the neuron itself; the last point is the result for complete brain sample.

3.8 Extrapolation of threshold reduction factor to more realistic neuronal density

The volumetric fraction of the neuronal cells within the volume was estimated in Table D1 (Supplement D) as ~30%. This is less than the known value of ~60-70% [48],[49],[50] in rats and mice since all neurons with somas outside the brain sample were omitted and their dendritic/axonal processes, which are still within the sample, were ignored. The threshold reduction factor curves in Fig. 9b,c,d could in principle be extrapolated (as shown by dashed lines in Fig. 9b,c,d) to estimate the threshold reduction factor for the likely more realistic volumetric neuronal fill factor of 70% (shown by a red vertical line in Fig. 9b,c,d). Average extrapolated threshold reduction factors for all pulse forms and all polarizations of $\boldsymbol{E}_{\mathrm{e}}^{i}$ are also collected in Table 1 (bold font). These data were obtained by averaging extrapolated results for 5 neurons (#238, 261, 321, 333, and 348) with different lengths of axonal arbor and applying the corresponding slopes to all previously obtained average threshold reduction factors from Table 1 (non-bold entries).

4. Discussion

4.1 Limitation of this study: a small sample size makes absolute thresholds too high

The small size of the L2/L3 brain sample leads to cutting out a large portion of the neuronal processes, most notably axon collaterals. As an example, the average length of the existing axonal arbor per neuron is ~0.5 mm for the present L2/L3 sample, ~2.5 mm (with the maximum length of 36 mm!) for the 1mm^3 sample from the Allen Inst. [7],[8], ~6 mm for Markram's *et al* elongated data in L2/L3, and ~7 mm for Markram's *et al* data in L5 [46].

Our computations show that the absolute activating threshold values are severely affected by the total axonal length. Additionally, the arguably most sensitive excitatory neurons of L5 [11] are not included into the sample. This is perhaps why the absolute threshold values in Fig. 8 and Figs. D4-D7 (Supplement D) overestimate the experimentally measured value of ~90 V/m in mouse primary motor cortex (Supplement D) very significantly. Even in the most favorable case of myelinated axons, there are only nine neurons with the activating threshold below 200 V/m (Fig. D8, Supplement D) and none of them has the activating threshold below 100 V/m.

4.2 Size of brain sample might still be sufficient to correctly predict relative threshold reduction factors

A simple numerical experiment with 20 neurons having the longest axonal arbors and the average arbor length of 2.5 mm versus 90 neurons having the longest axonal arbors and the average arbor length of 1.4 mm shows that the *relative* threshold reduction factors remain nearly the same in both cases, to within 3%. This is valid for all polarizations and all pulse forms considered. While the average axonal length reduces by 44% in this example, the threshold reduction factors change by less than 3%. Thus, the relative threshold reduction factors weakly depend on the retained axonal arbor length.

4.3 Limitation of this study: non-cubic sample size may affect directional results

Fig. 8, Figs. D4-D7 of Supplement D, and Table 1 predict different activating threshold reduction factors for different directions of the impressed electric field in the mouse visual cortex. The average reduction factor (after extrapolation to the realistic neuronal density) is: strongest (0.55) in the anterior-posterior direction with the sparsest equivalent straight fiber density of $0.9e5\ \mathrm{mm}^{-2}$; medium (0.76) in the dorsal-ventral directions with the medium equivalent straight fiber density of $1.0e5\ \mathrm{mm}^{-2}$; and lowest (0.85) in the medial-lateral direction with the densest equivalent straight fiber density of $1.3e5\ \mathrm{mm}^{-2}$.

On one hand, this fact could be straightforwardly explained by sample anisotropy. Consider most sensitive near-field interactions or "intersections" with the equivalent perpendicular fibers – cf.

Fig. 8a – which lower the activating threshold. For fibers in the anterior-posterior direction, the number of such interactions per unit length in Fig. 8a is the *highest* and is proportional to the fiber density in the two other directions, i.e. to $1.0e5 \times 1.3e5$. For fibers in the dorsal-ventral direction, the number of such interactions per unit length in Fig. 8a is *medium*; it is again proportional to the fiber density in two remaining directions, i.e. to $0.9e5 \times 1.3e5$. Finally, for fibers in the medial-lateral direction, the number of such interactions per unit length in Fig. 8a is the *lowest* and is proportional to the fiber density in two other directions, i.e. to $0.9e5 \times 1.0e5$. These three numbers of interactions per unit length relate as 1.0, 0.90, and 0.70 when normalized to their largest value. Simultaneously, the inverse normalized threshold reduction factors for the three polarizations established above relate as 1.0, 0.72, and 0.65 when normalized to the largest value. Qualitive agreement between the two sets of numbers becomes obvious (with a Pearson correlation of 0.87).

On the other hand, the respective normalized sample dimensions relate as 1, 0.56, and 0.36, which gives us the Pearson correlation of 0.99. It is therefore also possible that the higher absolute activating thresholds, when polarized along the shortest sample dimension, are more severely affected by the electric field spatial noise. This might likely be a nonlinear effect. A further study of a larger cubic sample such as a 1 mm^3 mouse brain sample [7],[8] or a cubic human 1 mm^3 sample [9] might answer this question. Emphasize that this effect has nothing in common with the overall sample polarization which should have a negligibly small influence on the extracellular electric field when the sample size is much greater than the arbor diameter (Supplement C).

Table 2 also predicts the much lower variance in thresholds for the negative vs. positive monophasic rectangular pulse – compare Fig. 8d (for the negative pulse) and Fig. D4 of Supplement D (for the positive pulse). This is due to the larger difference in the dorsal-ventral direction due to alignment with somatodendritic axes.

4.4 Reduction of the effect of brain sample anisotropy

A further observation as best seen in Fig. 8d, Fig. D7, and Fig. D8 of Supplement D is that the microscopic field interactions tend to equate neuron activating thresholds for different directions of the applied stimulation field (cf. also Table 2). The reasoning of the previous section could again be used to explain this effect. A further study of a cubic sample might help to determine whether it is a true anisotropic effect or a consequence of non-equal sample dimensions.

4.5 Effect of blood vessels

Separate electromagnetic and biophysical computations with and without blood vessels have been performed for the y-polarization of the primary impressed field and for the rectangular negative stimulation pulse with the duration of $0.1\ ms$. It was found that the microcapillaries are responsible

for changing the average activating threshold reduction factor from 0.79 (no blood network) to 0.76 (Table 1) when blood vessels are present.

Note that segmenting the brain blood supply network was not the major priority of the L2/L3 MICrONS IARPA Phase I project [5],[6],[7]. As such, we may expect a higher influence when all microcapillaries and multiple astrocytes are carefully included within the much larger 1mm^3 MICrONS IARPA Phase III mouse brain sample [59],[60] – cf. Fig. 10.

4.6 Other limitations of this study

Our biophysical modeling treats all neuronal cells independently, following previous work [10],[11],[12] to demonstrate the impact of realistic microscopic structures on direct neuron activation threshold. On the other hand, the L2/L3 brain sample is already supplied with a list of ~2,000 synapses between 334 excitatory pyramidal cells [7] obtained in [6] allowing future extension of our approach to simulating network/synaptic modulation [61].

Though used ubiquitously, there are inherent limitations of the one-dimensional cable equation, especially close to the large somas. Modern bidomain models (cf. [44]) could possibly resolve this issue.

Another limitation of this paper is the lack of 3D modeling of intracellular space. However, the true bidomain modeling approach, either using FEM [21] or BEM [44], is very cumbersome and is therefore presently limited to simple cylindrical or spherical structures or, perhaps, some simplified neuron models. On the other hand, the classic and proven 1D cable model used here seems to be naturally suited for studying very long and thin axonal processes in the cortex, which play a major role in neuron activation during brain stimulation [10],[11],[12]. For example, the total axonal length of a cortical neuron from IARPA Phase III 1mm^3 sample [7],[8] may reach or exceed 30 mm (including axon collaterals) while the axonal diameter is generally less than 0.5 µm. This gives us a length-to-diameter ratio of 60,000 (cf. Fig. 10), which justifies the use of the cable model. A meaningful future application of the bidomain approach would perhaps best target large somas of the neuronal cells.

The electric field spatial noise – the major subject of this paper – could partially explain the common issue of thresholds in biophysical neuron simulations being generally higher than in vivo measurements [10][11],[12],[32]. However, other factors such as lack of ongoing activity and potential inaccuracies in simulated membrane dynamics could also play a role. Moreover, connectome-based communications between individual neurons and the synaptic cleft assembly [6],[61] may significantly contribute as well. These factors are not explored in this study.

The next logical yet extremely challenging step is to analyze the much larger 1mm^3 MICrONS mouse brain sample, which includes a considerably better-developed axonal arbor and

~75,000 neuronal cells, and another very recently published human brain sample [9]. This would require further improvements and modifications to the computational method.

Our results should be extrapolated with caution to humans. It is reasonable to expect that the same trends would be observed if performing analogous computational analysis for a human tissue sample, however given different sizes and packing densities of cells (as well as the size of the tissue block itself), the exact numerical factors might differ. Notwithstanding these limitations, the present study provides the methodology for addressing all these issues.

5. Conclusions

A method is proposed to model perturbations of an impressed extracellular electric field within a microscopically realistic brain, with many tightly spaced neuronal cells and blood microcapillaries. This impressed field could be due either to brain stimulation or to a firing cluster of neurons themselves. For the first time, we have been able to model the relatively sizable fully anatomical mouse brain sample containing ~400 detailed neuronal cells and blood microcapillaries in detail.

Electric field modeling was coupled with biophysical modeling that is based on neuron morphologies precisely linked to the physical neurons and known ion channel models for rodents. The method was applied to predict changes in neuron activating thresholds caused by the surrounding microenvironment – the electric field spatial noise with a mean value of zero on the cellular and microcapillary level.

We observed and justified activating threshold reduction factors (reduction in the stimulation field strength) of 0.85–0.55 (0.7 on average) for different directions of the impressed field and different representative stimulation pulse forms. The obtained results are clearly explainable and are consistent across the wide range of brain stimulation conditions. The application of the presented multi-scale modeling framework spans all forms of electromagnetic brain stimulation.

All results of this study are made available online through BossDB, a volumetric open-source database for 3D and 4D neuroscience data [62]. The supporting computer codes (Supplements A, B, and C) may be found in [63],[64],[65].

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Sundaram of Massachusetts General Hospital for useful discussions. Special thanks are extended to Dr. Ermal Toto and Dr. James Kingsley of Academic and Research Computing at Worcester Polytechnic Institute for their great support in assembling and executing highly demanding parallel computational tasks. This study has received support from the NIMH grant R01MH130490 and NIBIB grant R01EB035484 (SNM), NIDCD grant R01DC020891 (ARN), NINDS grant U24NS120053 (BD) as well as the Intramural Research Program of NIH, NIMH ZIAMH002955 (Z-DD), and Intramural Research Program of NIH, NIDA ZIADA000638 (HL).

Tables and Figures

Table 1. Averaged relative reduction factor of the neuronal activating thresholds for different polarizations of the impressed (primary) electric field and different pulse forms (Suppl. C) between a neuronal skeleton in a homogeneous medium and the microscopically realistic brain, respectively. The first 90 neurons with the longest (uncut) axonal arbors have been processed. Since data for myelinated axons are quite similar (Supplement C), only one such dataset has been included.

Modeling setup	Medial-lateral polarization of $\boldsymbol{E}_{\mathrm{e}}^{i}$	Dorsal-ventral polarization of $\boldsymbol{E}_{\mathrm{e}}^{i}$	Anterior-posterior polarization of $\boldsymbol{E}_{\mathrm{e}}^{i}$
A Monophasic rect. pulse, 100 µs, negative	0.92	0.81	0.67
B Monophasic rect. pulse, 100 µs, positive	0.90	0.85	0.68
C Biphasic pulse (TMS), 300 µs, negative	0.90	0.84	0.68
D Biphasic pulse (TMS), 300 µs, positive	0.89	0.85	0.68
E Same as A but with all axons myelinated	0.91	0.81	0.65
A Extrapolated to realistic neuronal density	**0.86**	**0.76**	**0.54**
B Extrapolated to realistic neuronal density	**0.85**	**0.72**	**0.55**
C Extrapolated to realistic neuronal density	**0.82**	**0.78**	**0.57**
D Extrapolated to realistic neuronal density	**0.81**	**0.78**	**0.54**
E Extrapolated to realistic neuronal density	**0.85**	**0.76**	**0.52**
Average	**0.85**	**0.76**	**0.55**

Table 2. Normalized standard deviation of the neuronal activating thresholds for three different polarizations of the primary electric field (Suppl. C). The first 90 neurons with the longest (uncut) axonal arbors have been processed.

Modeling setup	Normalized STD, Homogeneous medium	Normalized STD, Microscopically realistic brain
A Monophasic rect. pulse, 100 µs, negative	0.18	0.04
B Monophasic rect. pulse, 100 µs, positive	0.35	0.23
C Biphasic pulse (TMS), 300 µs, negative	0.19	0.15
D Biphasic pulse (TMS), 300 µs, positive	0.15	0.14
E Same as A but with all axons myelinated	0.25	0.07

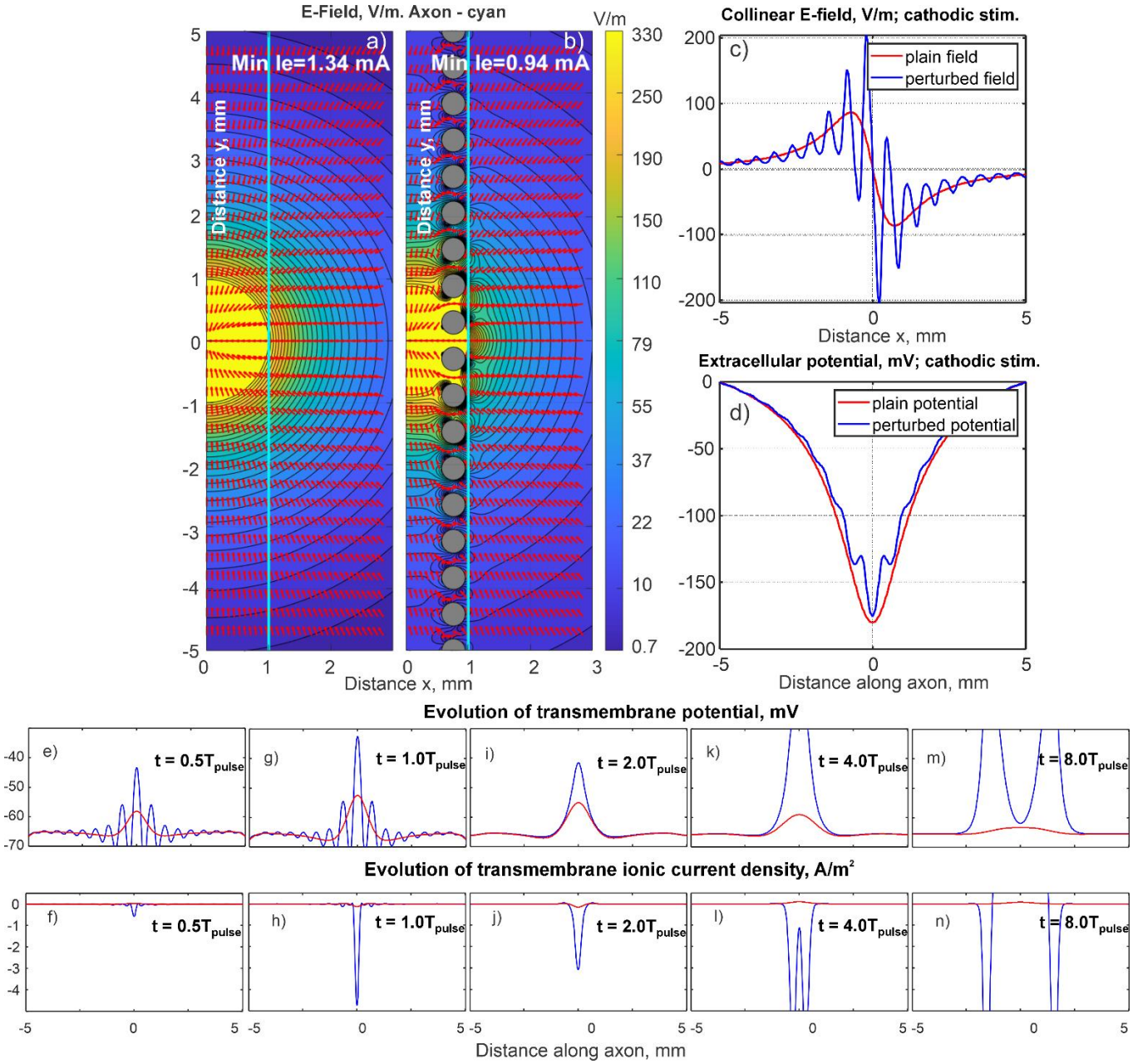

Fig. 1. Concept of the electric field spatial noise. a) Straight HH axon from Ref. [43] and the electric field of a cathodic stimulation. b) The same case as in a) but with artificial field perturbers – infinite cylinders. c,d) Collinear electric field and extracellular potential with (blue) and without (red) field perturbations. In both cases, the electrode current is 0.94 mA. e-n) Resulting evolution of transmembrane potential and transmembrane ionic current density for both cases at different times. Red curves are those without field perturbations; blue curves correspond to the perturbed extracellular field.

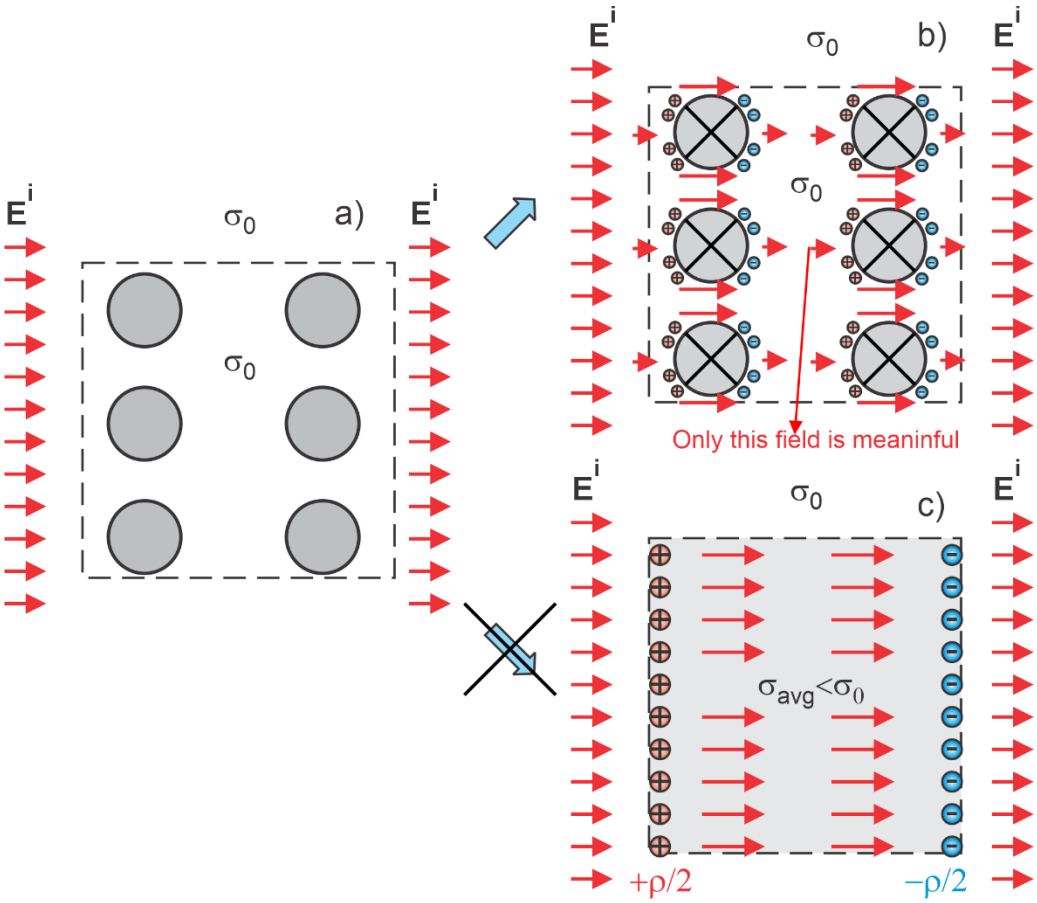

Fig. 2. Concept of the present modeling setup. a) Brain sample (dashed rectangle) is embedded into homogeneous space with the same extracellular conductivity. Grey circles indicate conducting intracellular space with non-conducting membranes; red arrows indicate the primary (impressed) field. b) Cartoon of expected extracellular solution with red arrows indicating total extracellular field. Its deviation from the primary field has a mean value of zero (cf., for example, Supplement C). Local charge accumulation (membrane polarization for exterior Neumann problem) is schematically shown. c) Incorrect averaging simplification of the problem assigning non-conducting intracellular space with a higher electric field.

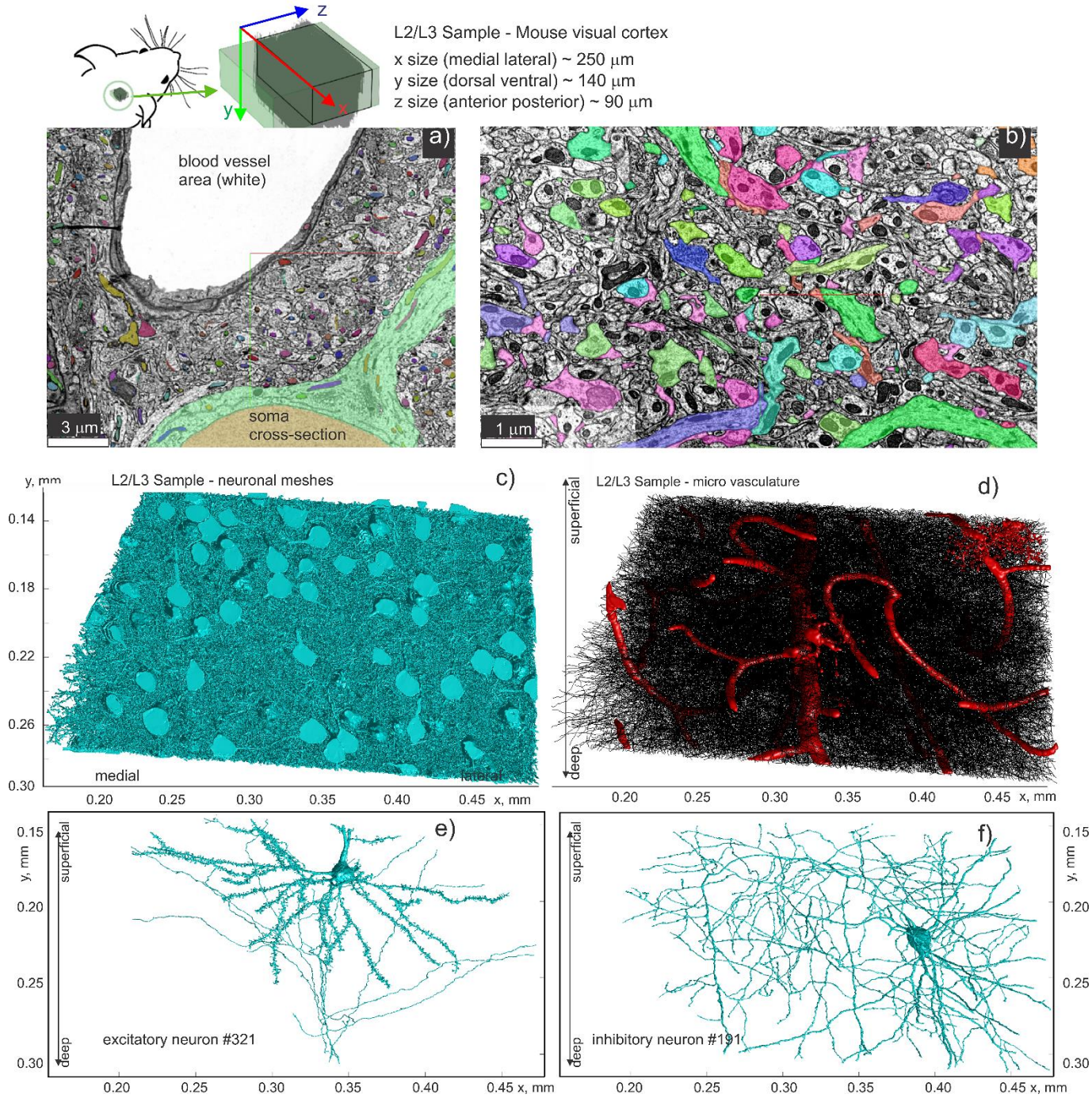

Fig. 3. Structure of L2L3 sample under study. a) Snapshot of relative position of a neuron (soma) vs. a nearby blood capillary (white area) within the sample [7], [5]. b) Snapshot of different neuronal processes labeled with different colors at a higher resolution. c) Segmented neuronal bodies of the entire sample. d) Centerlines/skeletons of individual neurons and segmented blood vessels in red (with an astrocyte at top right). e,f) Excitatory (pyramidal) and inhibitory (basket) neurons within the sample volume to scale. The axes differ from the mouse-centric coordinate system [5].

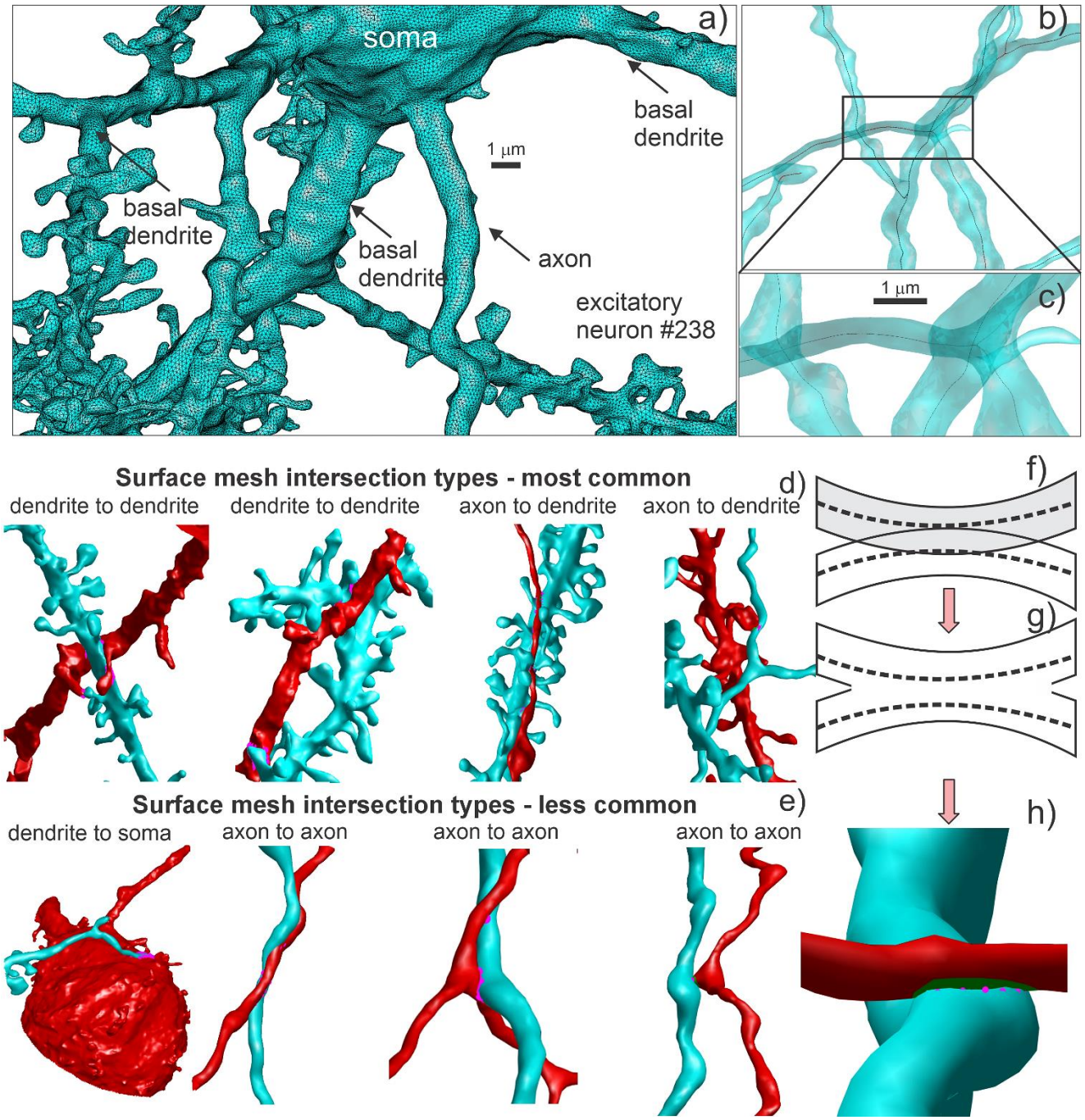

Fig. 4. a) Final high-quality membrane surface triangular mesh for one neuron (#238) which is located approximately in the middle of the sample. The membrane mesh has 2 million facets in total and a 100 nm resolution. b,c) Healed centerlines of a neuron. d) Most common intersections observed – dendrite to dendrite and axon to dendrite intersections. Two different colors correspond to the two different neurons. e) Less common intersections – dendrite to soma and axon to axon, respectively. f,g) Mesh intersection resolution via removal of inner triangles. h) Result of this operation for an axon-to-dendrite intersection. Removed triangular facets are marked dark green.

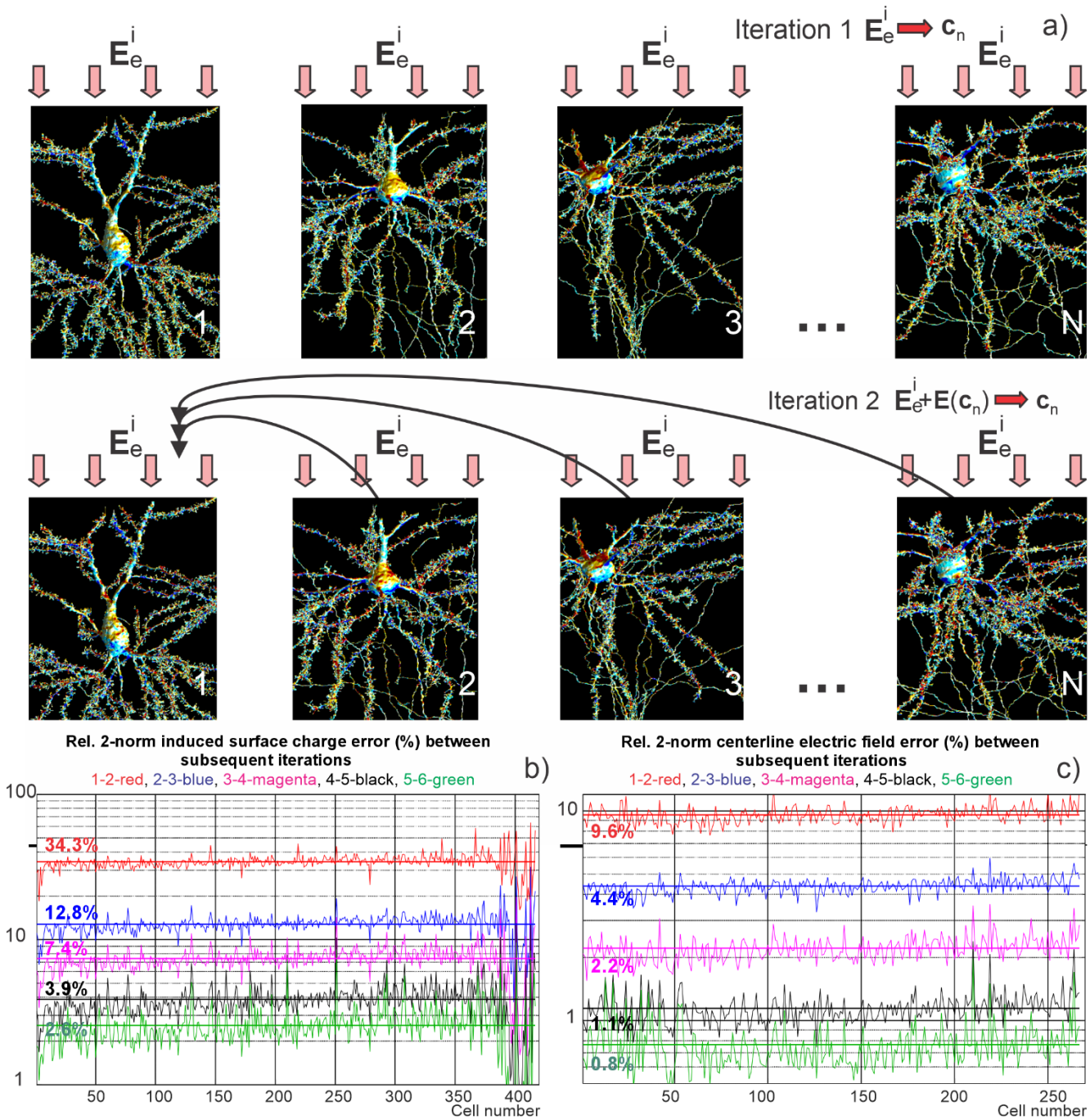

Fig. 5. a) Nested iterative solution method. At step 1, every neuron is considered independent. Membrane charge deposition in response to impressed or primary field $\boldsymbol{E}_{\mathrm{e}}^{i}$ is found via an iterative BEM-FMM solution. At step 2, the primary field $\boldsymbol{E}_{\mathrm{e}}^{i}$ is modified by the field of charges deposited on all other neurons. Membrane charge deposition for every neuron in response to this modified field is found next. The process further repeats itself until a convergence criterion is met. b) Convergence curves for the relative 2-norm surface charge density error. c) Convergence curves for the relative 2-norm error for the collinear electric field projected onto centerlines of neuronal processes.

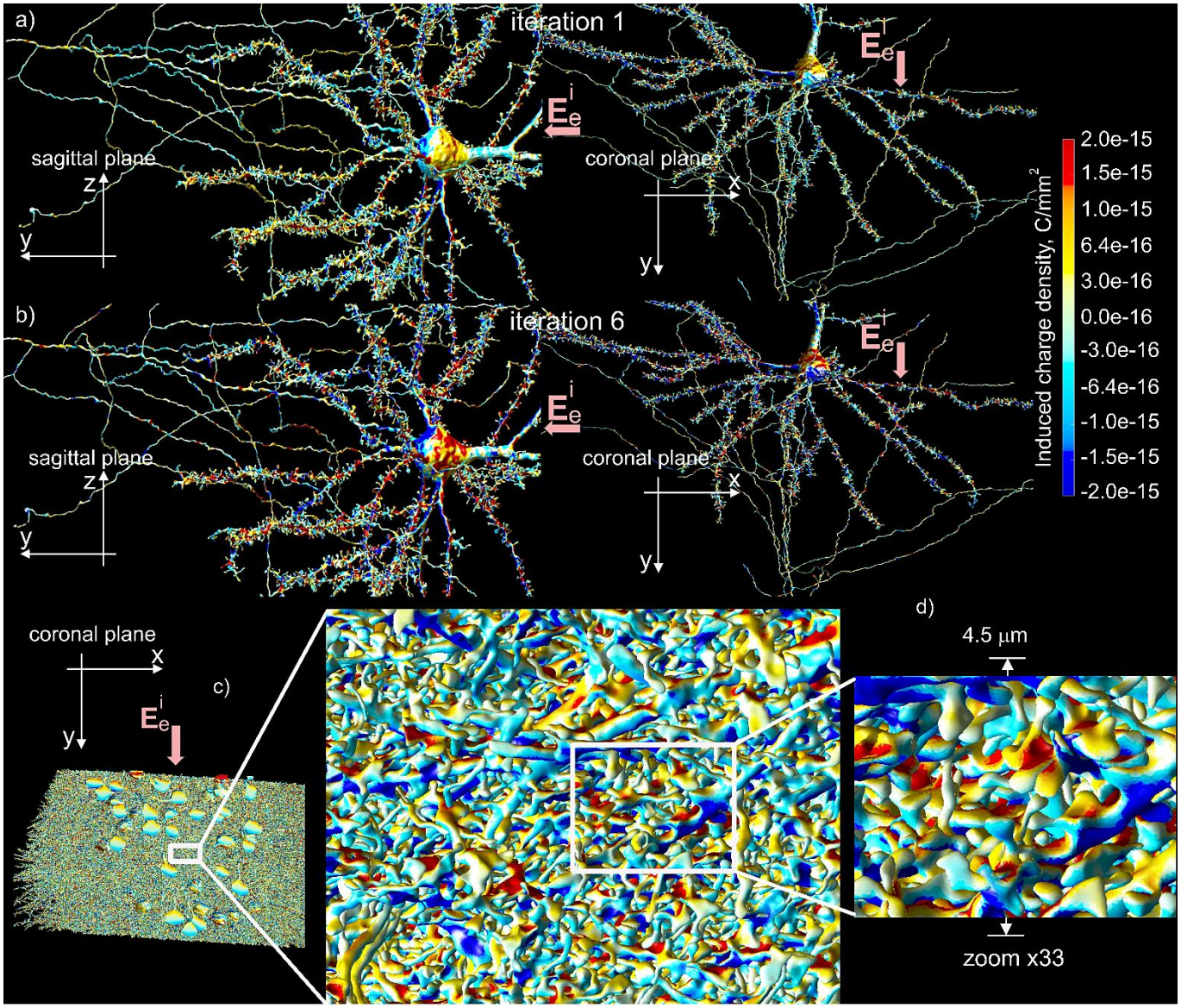

Fig. 6. a,b) Induced surface charge density for neuron #321. a) Induced charge density when only neuron #321 is present (iteration 1 or self-interaction). b) Induced charge density when all neurons and microcapillaries of the sample are present (iteration 6). c,d) Induced surface charge density for 200 nearest neighbors of neuron #321. c) Complete network of 200 nearest neighbors. d) Zoomed in area (x33) of the network in c). We were unable to simultaneously plot electromagnetic data for more than 200 neurons due to the limitations of the graphical software used.

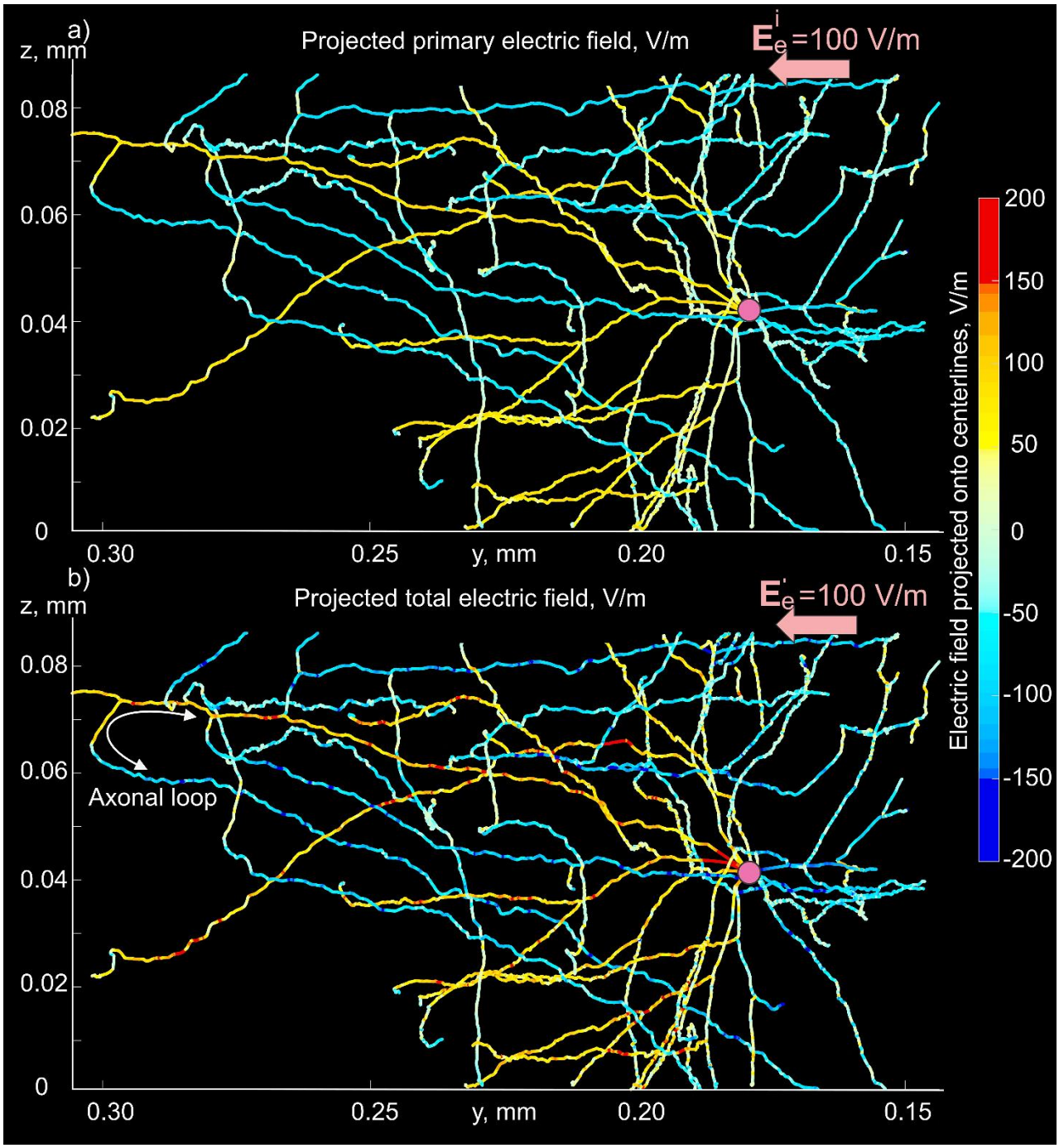

Fig. 7. Effect of membrane charge deposition on the collinear electric field projected onto the centerlines of neuronal processes for neuron #321. The sagittal plane view is shown corresponding to Fig. 6a,c,e. a) Electric field projection in homogeneous space, when the neuronal arbor is not present and a uniform impressed electric field of 100 V/m is applied along the y axis (from dorsal to ventral). b) Electric field projection when all neurons are present, and the primary field is distorted by the field of all induced charges.

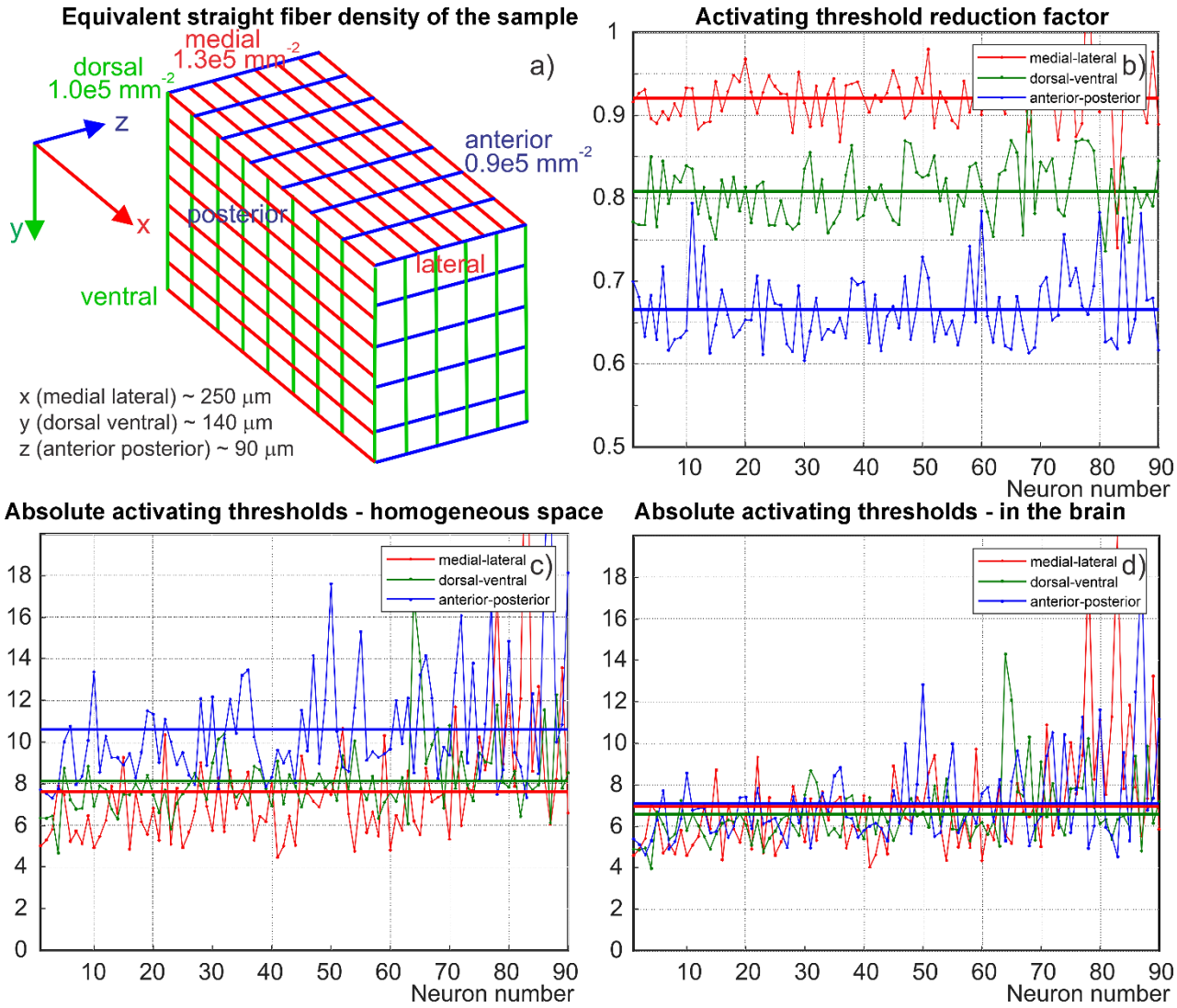

Fig. 8. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbors (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the unmyelinated axonal arbor and a negative rectangular activation monophasic pulse with the duration of 0.1 ms. Results for all other cases are given in Suppl. C.

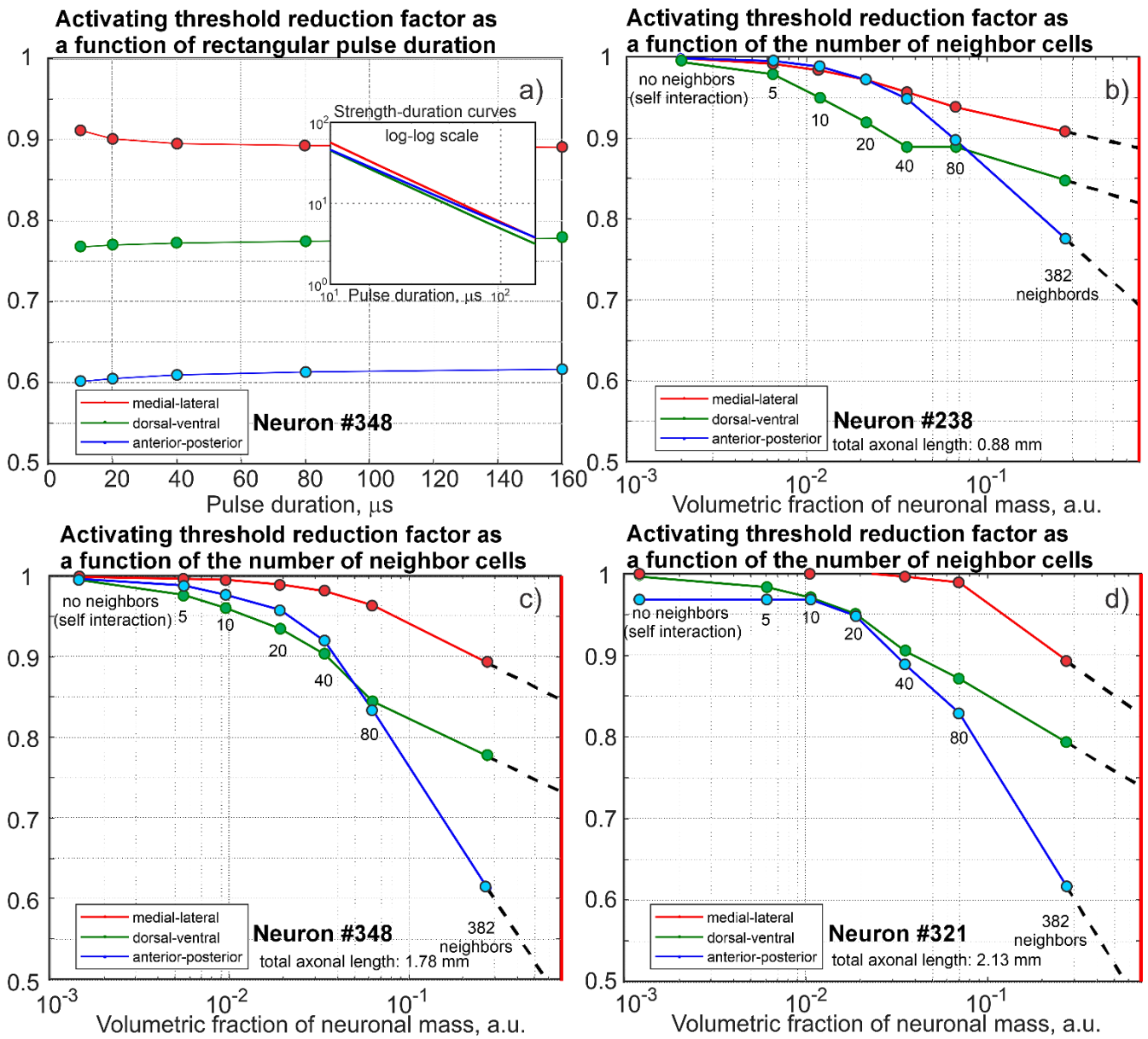

Fig. 9. a) Activating threshold reduction factor as function of rectangular negative pulse duration. The inset shows the corresponding strength-duration curves for neuron #348 within the brain sample. Inset units in are in terms of the initial field strength of 100 V/m; one unit corresponds to 100 V/m. b,c,d) Activating threshold reduction factors as function of the number of neighboring cells for three representative excitatory neurons with different lengths of the axonal arbor (for the rectangular negative pulse). Abbreviation a.u. stands for “arbitrary units” – dimensionless. All three neurons belong to the set of 90 neurons with the longest axonal arbor. The x-axis is the corresponding volumetric fraction of neuronal mass computed separately for each neuron when sequentially adding its neighboring cells. Red lines show the expected fraction of 70% [50]; dashed lines show expected linear extrapolation results.

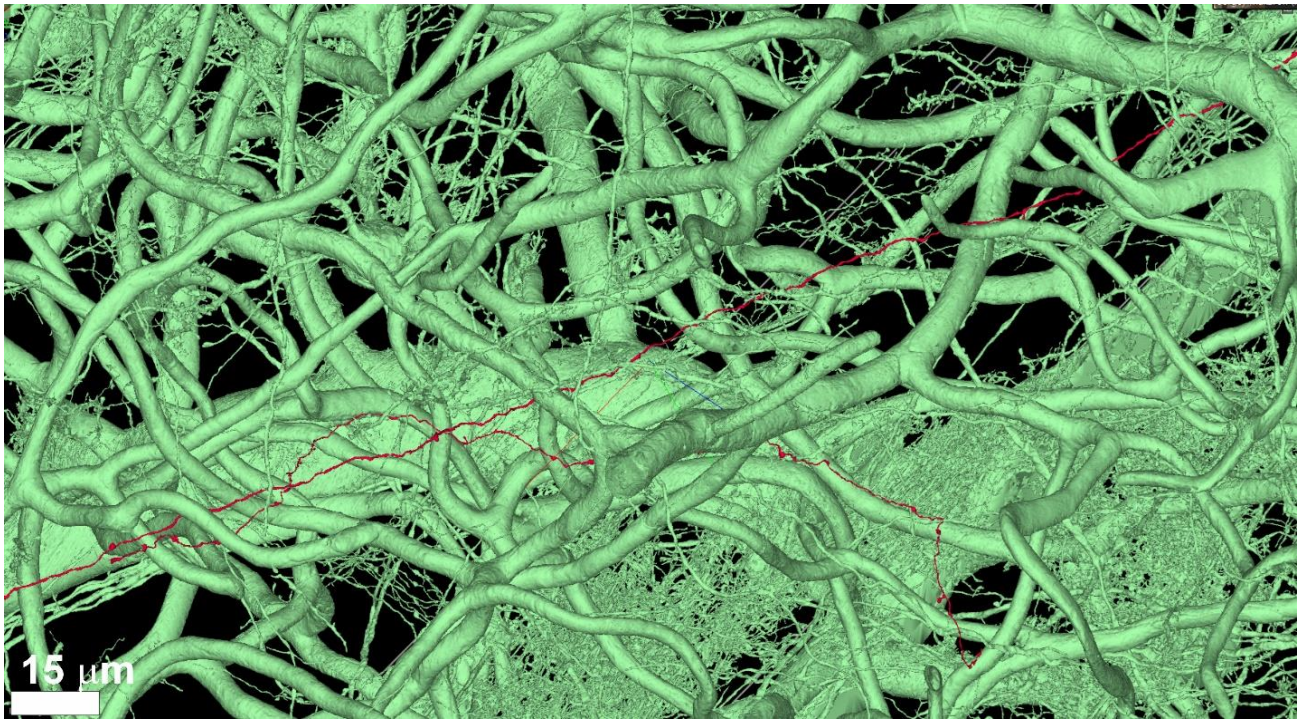

Fig. 10. Illustration of the usefulness of the 1D cable model: two very thin axons (red) traveling through the 1mm^3 vascular network of IARPA Phase III mouse brain sample (green) [7],[8]. Trace interruptions are due to screening by microcapillaries from direct light. Using a bidomain 3D approach instead of the 1D description would be less appealing for these common thin long axons.

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[65]. Computer code to Supplement C. 2024. Dropbox Online.

Supplement A

to manuscript “Enabling Electric Field Model of Microscopically Realistic Brain”

Contents

A1. Computer code for cathodic stimulation + noise with arbitrarily varying parameters

A2. Computer code for stimulation with uniform electric field + noise (terminal effects)

A3. Computer code to study generic spatial electric field noise with mean value zero (on the base of terminal activation)

A4. Code availability

A1. Computer code for cathodic stimulation + noise with arbitrarily varying parameters

This code supports Section 2.1 of the Materials and Methods Section of the main text. It is written within the MATLAB platform and should run on Windows, Linux, and MacOS machines.

A simple numerical experiment with a Hodgkin-Huxley axon originally studied by Rattay [1] is considered. A cathodic stimulation of the 10 mm long axon by a point electrode located at the origin is modeled. The default axonal radius is 5 μm, the length constant λ is 1.5 mm, the time constant τ is 3.3 ms, the resting potential is – 65 mV. A monophasic electric pulse (T=0.1 ms) is applied. Field perturbers are 2D cylinders with non-conducting walls located perpendicular to the axon and with a large radius of 200 μm, chosen for better visualization. An analytical solution for every infinite 2D cylinder secondary field is employed (cf. [2]).

First, the MATLAB script "script01_define_axon_HH_model.m" should be executed. There, the major biophysical and computational parameters are defined. All units are SI units. Extensive comments are provided for every quantity of interest.

Second, the MATLAB script "script02_obtain_axon_solution.m" should be executed. This script runs biophysical computations for the transmembrane potential and the ionic transmembrane current density. If necessary, the total transmembrane current density could be plotted as well.

Two biophysical solutions are executed simultaneously: the first one is for the homogeneous medium (red color) and the second one is for the perturbed extracellular field. Fig. A1 shows the graphical output of the MATLAB script "script02_obtain_axon_solution.m," which is updated at every time moment.

Solution parameters (electrode current, axon data, time course, spatial and temporal resolutions, cylinder radii, their spacing and positions) as well as the output format can be modified using both executable MATLAB scripts. Other MATLAB functions should not be changed.

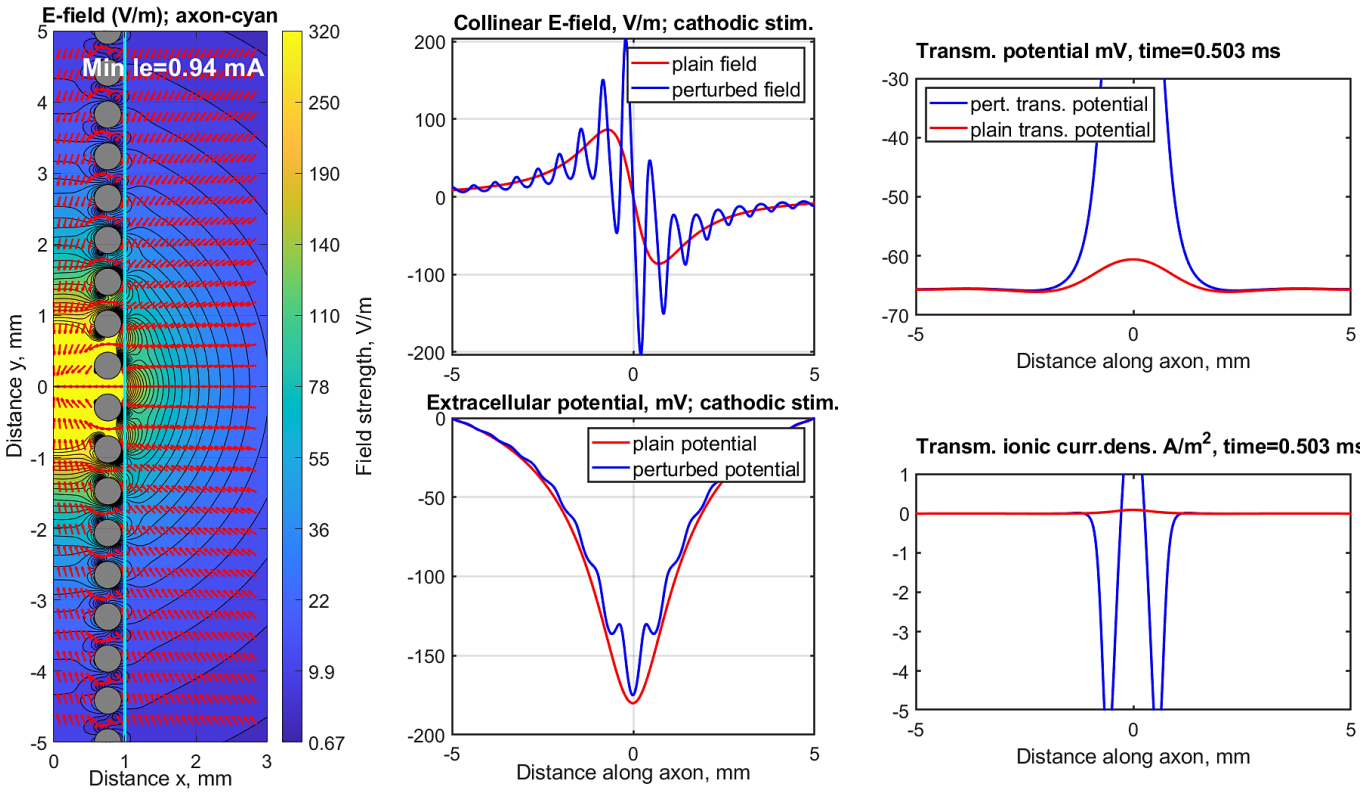

Fig. A1. Interactive graphical output of the computational code – cf. Fig. 1 of the main text.

A2. Computer code for stimulation with uniform electric field + noise (terminal effects)

This code again supports Section 2.1 of the Materials and Methods Section of the main text. It is written within the MATLAB platform and should run on Windows, Linux, and MacOS machines.

A simple numerical experiment with a Hodgkin-Huxley axon originally studied by Rattay [1] is considered. This time, a uniform electric field along the axonal process is applied. The default axonal radius is again 5 μm, the length constant λ is 1.5 mm, the time constant τ is 3.3 ms, the resting potential is – 65 mV. A monophasic electric pulse (T=0.1 ms) is applied. Field perturbers are 2D cylinders with non-conducting walls located perpendicular to the axon and with a large radius of 200 μm, chosen for better visualization. An analytical solution for every infinite 2D cylinder secondary field is employed (cf. [2]).

First, the MATLAB script “script01_define_axon_HH_model.m” should be executed. There, the major biophysical and computational parameters are defined. All units are SI units. Extensive comments are provided for every quantity of interest.

Second, the MATLAB script “script02_obtain_axon_solution.m” should be executed. This script runs biophysical computations for the transmembrane potential and the ionic transmembrane current density. If necessary, the total transmembrane current density could be plotted as well.

Two biophysical solutions are executed simultaneously: the first one is for the homogeneous medium (red color) and the second one is for the perturbed extracellular field. Fig. A2 shows the graphical output of the MATLAB script “script02_obtain_axon_solution.m,” which is updated at every time moment.

Solution parameters (electrode current, axon data, time course, spatial and temporal resolutions, cylinder radii, their spacing and positions) as well as the output format can be modified using both executable MATLAB scripts. Other MATLAB functions should not be changed.

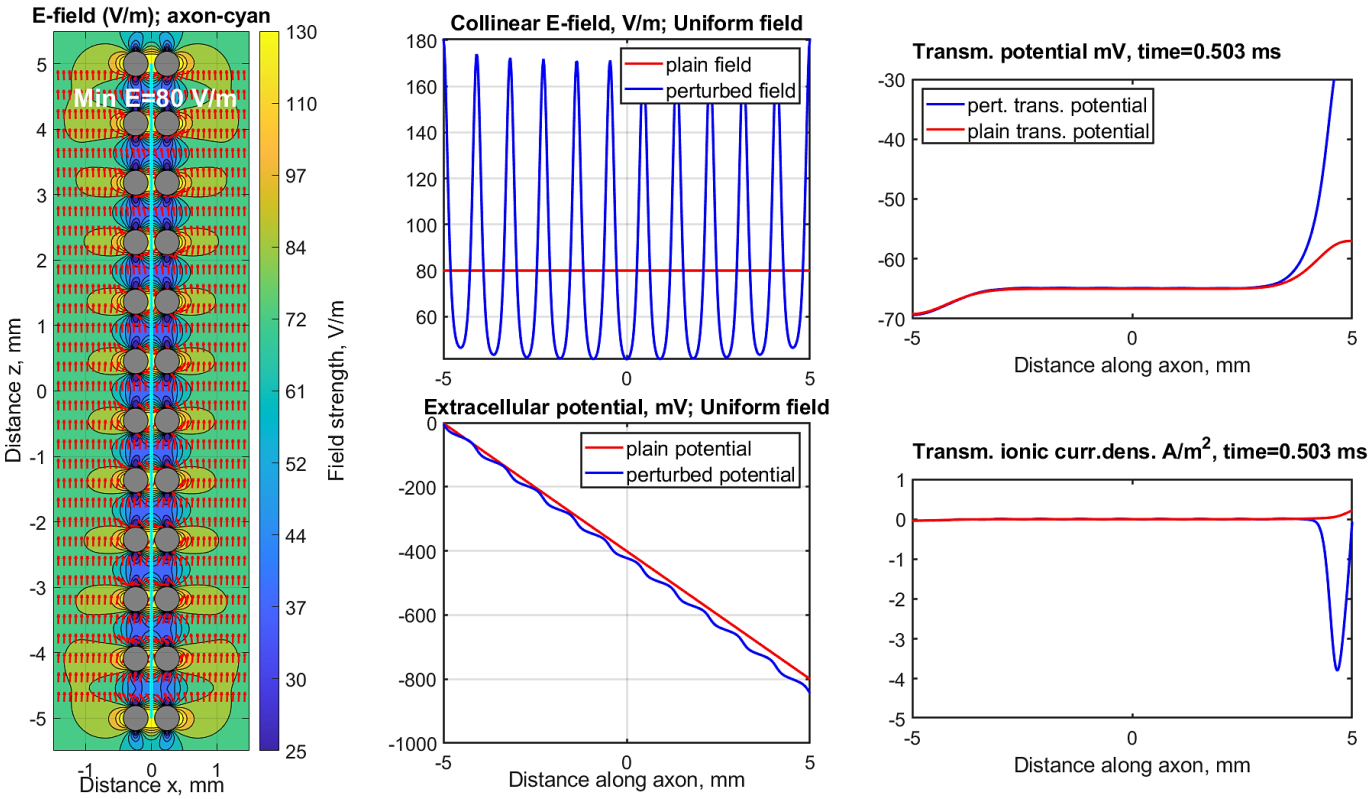

Fig. A2. Interactive graphical output of the computational code for the uniform electric field and terminal excitation.

A3. Computer code to study generic spatial electric field noise with mean value zero (on the base of terminal activation)

This code again supports Section 2.1 of the Materials and Methods Section of the main text. It is written within the MATLAB platform and should run on Windows, Linux, and MacOS machines.

A simple numerical experiment with a Hodgkin-Huxley axon originally studied by Rattay [1] is considered. This time, a uniform electric field along the axonal process is applied. An electric field noise with a mean value of zero is added to the collinear field.

The default axonal radius is again 5 μm, the length constant λ is 1.5 mm, the time constant τ is 3.3 ms, the resting potential is – 65 mV. A monophasic electric pulse (T=0.1 ms) is applied.

First, the MATLAB script "script01_define_axon_HH_model.m" should be executed. There, the major biophysical and computational parameters are defined. All units are SI units. Extensive comments are provided for every quantity of interest.

Second, the MATLAB script "script02_obtain_axon_solution.m" should be executed. This script runs biophysical computations for the transmembrane potential and the ionic transmembrane current density. If necessary, the total transmembrane current density could be plotted as well.

Two biophysical solutions are executed simultaneously: the first one is for the homogeneous medium (red color) and the second one is for the perturbed extracellular field. Fig. A3 shows the graphical output of the MATLAB script "script02_obtain_axon_solution.m" that is updated at every time moment.

Since the noise is a random combination of harmonic function, the code may or may not generate action potential(s) for the perturbed solution.

Solution parameters (electrode current, axon data, time course, spatial and temporal resolutions, cylinder radii, their spacing and positions) as well as the output format can be modified using both executable MATLAB scripts. Other MATLAB functions should not be changed.

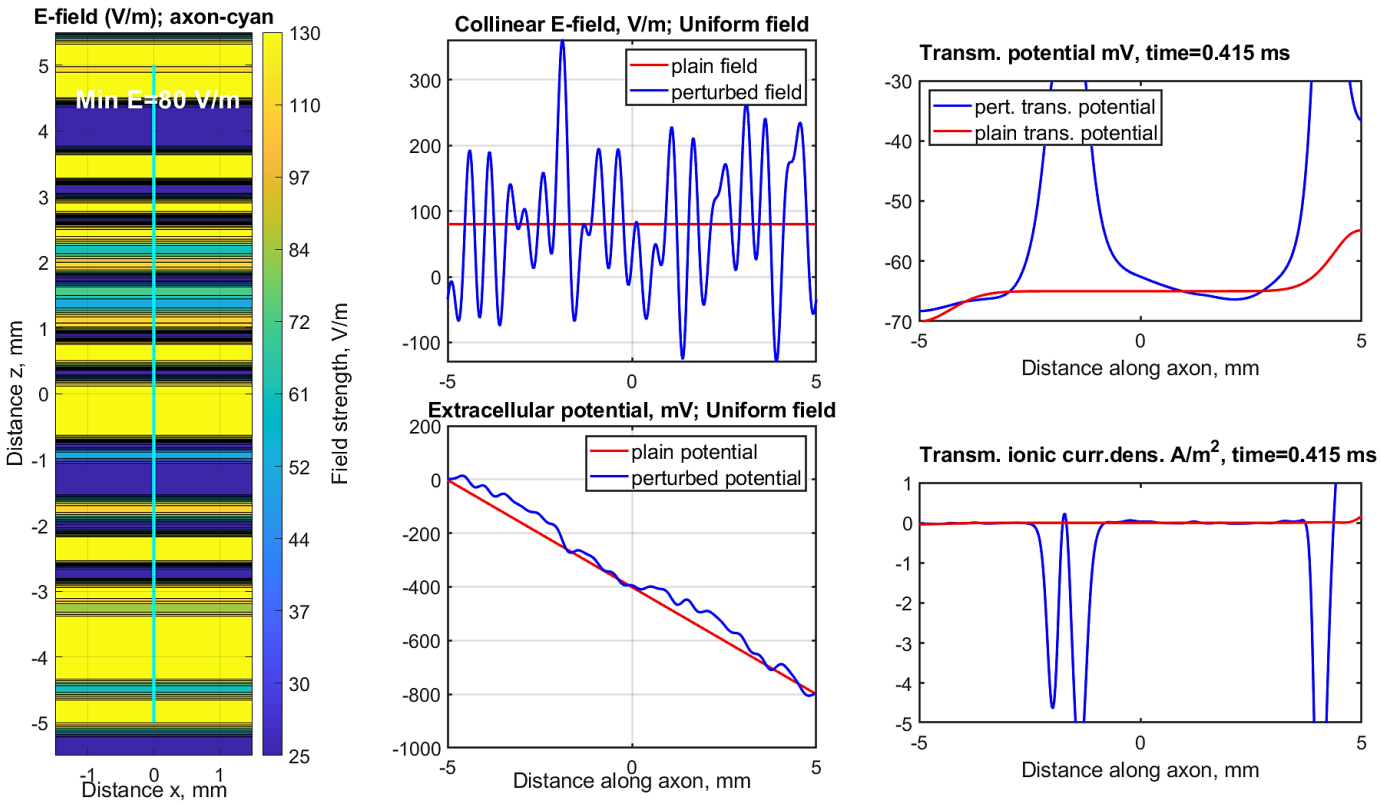

Fig. A3. Interactive graphical output of the computational code for the uniform electric field and noise excitation.

A4. Code availability

The Dropbox link [3] contains a fully executable computational module built within the MATLAB platform under Windows and a short guide that replicates the above example. This enables both testing of different polarization directions as well as extraction of collinear fields along any desired axonal path.

References – Supplement A

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[2]. Noetscher GM, Tang D, Nummenmaa AR, Bingham CS, McIntyre CC, Makaroff SN. Estimations of Charge Deposition onto Convoluted Axon Surfaces within Extracellular Electric Fields. *IEEE Trans Biomed Eng*. 2023 Aug 3; doi: 10.1109/TBME.2023.3299734.

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Supplement B

to manuscript “Enabling Electric Field Model of Microscopically Realistic Brain”

Full-wave computer code using the boundary element fast multipole method and with finite-length cylinders for cathodic stimulation

B1. Full-wave computer for cathodic stimulation + noise with arbitrarily varying parameters

This code supports Section 2.1 of the Materials and Methods Section of the main text. It is written within the MATLAB platform and should run on Windows machines.

A simple numerical experiment with a Hodgkin-Huxley axon originally studied by Rattay [1] is considered. A cathodic current stimulation of a 10 mm long axon *by a finite spherical electrode with the radius of 50 µm* located at the origin is modeled. The default axonal radius is 5 µm, the length constant λ is 1.5 mm, the time constant τ is 3.3 ms, the resting potential is – 65 mV. A monophasic electric pulse (T=0.1 ms) is applied.

Field perturbers are finite cylinders with non-conducting walls located perpendicular to the axon and with a large radius of 200 µm, chosen for better visualization. A full-wave boundary element fast multipole method is employed (cf. [2]).

First, the MATLAB script “model01_model_creator.m” from subfolder Model should be executed. This script creates the model. If necessary, model parameters can be changed using the same script. All units are SI units. Second, the MATLAB script “model02_nintegral_generator.m” should be executed from subfolder Model. This script creates neighbor integrals necessary for BEM-FMM. It runs in about 20-30 seconds.

Next, the MATLAB scripts “bem0_em_constants.m”, “bem1_define_planes.m”, “bem2_solver_full.m” from the main folder should be executed sequentially. The last script defines the electrode current as 1.054 mA. After that, the induced surface charge is displayed by the script “bem3_surface_field_c.m,” as shown in Fig. B1. The surface electric potential is displayed by the following script “bem3_surface_field_potential.m,” as shown in Fig. B2.

The last script simultaneously outputs the electrode potential variation caused by the multi-cylinder load. Such a change is less than 1% (-5.03 V versus -4.99 V). This result shows that the extracellular field non-uniformity indeed plays the major role in reducing the threshold, not the overall increase of the electric field strength due to the load, even without principal separation between the extracellular and intracellular space.

Additionally, the MATLAB scripts “bem4_plane_field_e.m” and “bem5_axon_field_e.m” can be executed to display electric field distribution in the axial plane (Fig. B3) and the collinear electric field along the axonal axis (Fig. B4), respectively.

Finally, the biophysical modeling is performed, very similar to that from Supplement A and using the scripts “script01_define_axon_HH_model.m” and “script02_obtain_axon_solution.m”. The result is shown in Fig. B5.

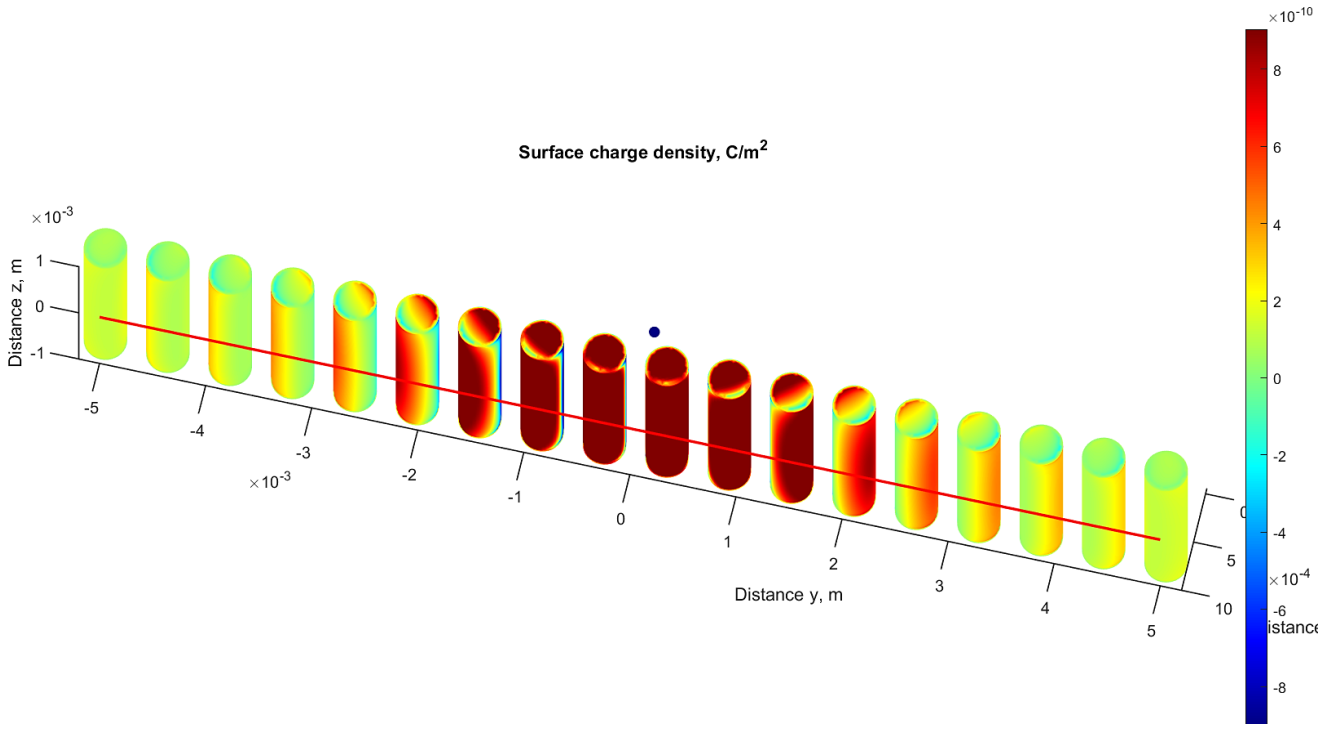

Fig. B1. Surface charge density in C/m^2 due to a current electrode (cathode, a small blue sphere) field.

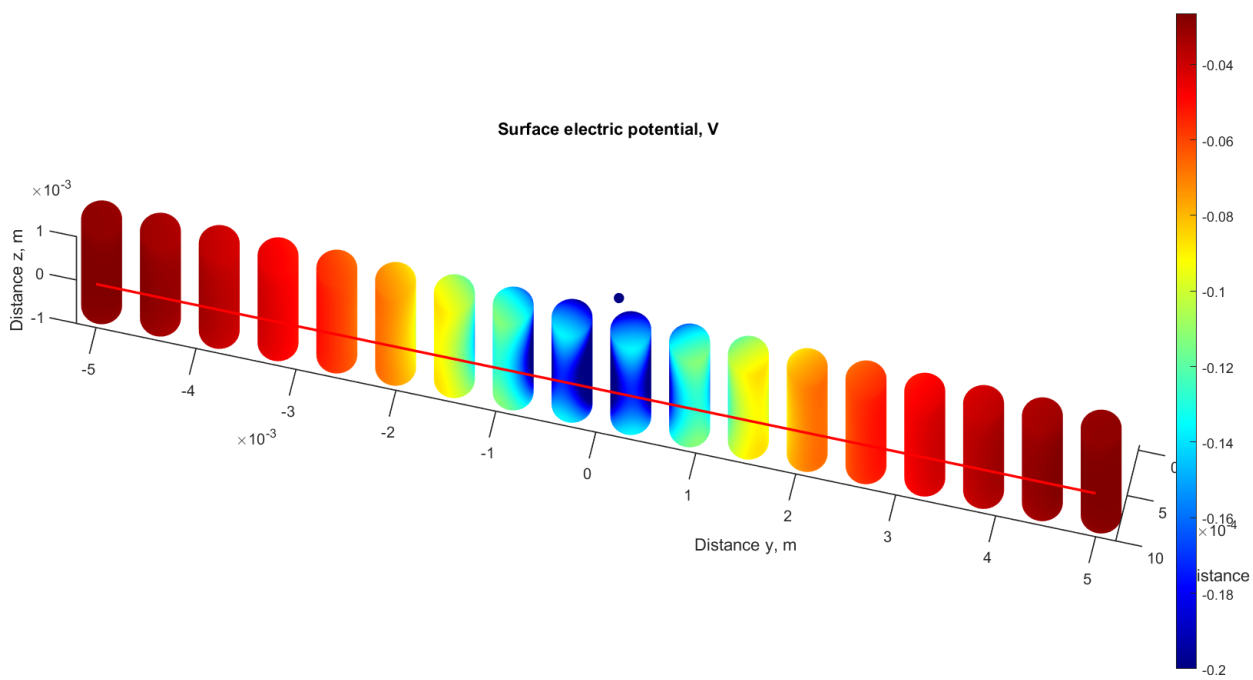

Fig. B2. Surface electric potential distribution in V due to a current electrode (cathode, a small blue sphere) field.

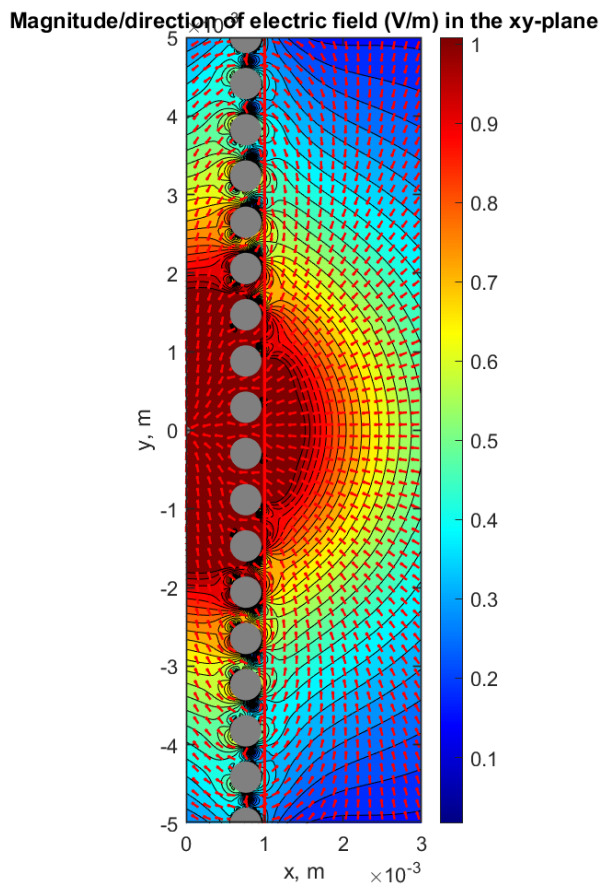

Fig. B3. Electric field distribution in the axial plane in V/m due to a current electrode (cathode, a small blue sphere) field.

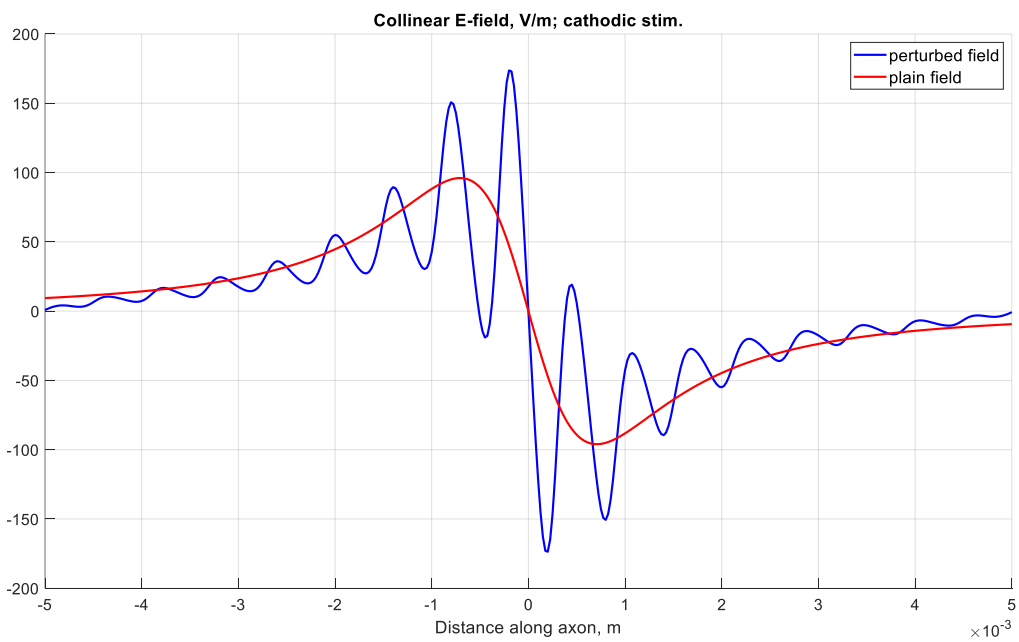

Fig. B4. Collinear electric field distribution along the axon due to a current electrode.

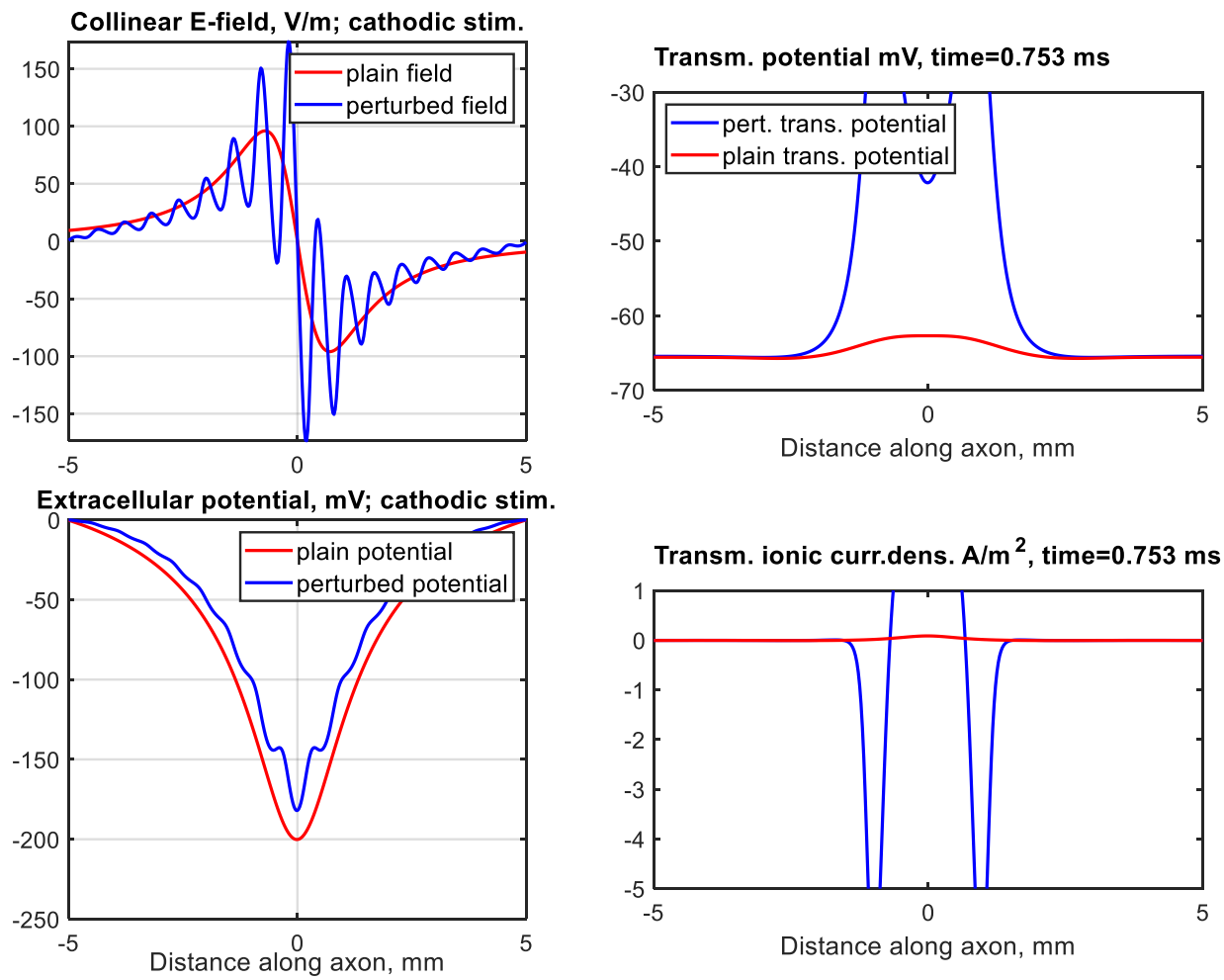

Fig. B5. Interactive graphical output of the biophysical modeling part.

B2. Code availability

The Dropbox link [3] contains a fully executable computational module built within the MATLAB platform under Windows and a short guide that replicates the above example. This enables both testing of different polarization directions as well as extraction of collinear fields along any desired axonal path.

References – Supplement B

[1]. Rattay F. Analysis of models for extracellular fiber stimulation. IEEE Trans Biomed Eng. 1989 Jul;36(7):676-82. doi: 10.1109/10.32099.

[2]. Makarov SN, Golestanirad L, Wartman WA, Nguyen BT, Noetscher GM, Ahveninen JP, Fujimoto K, Weise K, Nummenmaa AR. Boundary element fast multipole method for modeling electrical brain stimulation with voltage and current electrodes. J Neural Eng. 2021 Aug 19;18(4):10.1088/1741-2552/ac17d7. doi: 10.1088/1741-2552/ac17d7.

[3]. Computer code to Supplement B. 2024. Dropbox Online.

Supplement C

to manuscript "Enabling Electric Field Model of Microscopically Realistic Brain"

Characteristics of the extracellular field in an artificial specimen mimicking a finite sample of convoluted neuronal arbor

C1. Sample under study

The specimen under study is shown in Fig. C1. It includes 271 straight finite cylinders approximately uniformly distributed along three dimensions and forming a 3D lattice. The cylinders are located within a cube of 600×600×600 μm (indicated by yellow color in Fig. B1) and have a radius of 12 μm each. The inner cylinder volume (the "intracellular space") constitutes 33% of the total volume; the "extracellular space" comprises the remaining 67%. The specimen mesh is 1.00 M facets.

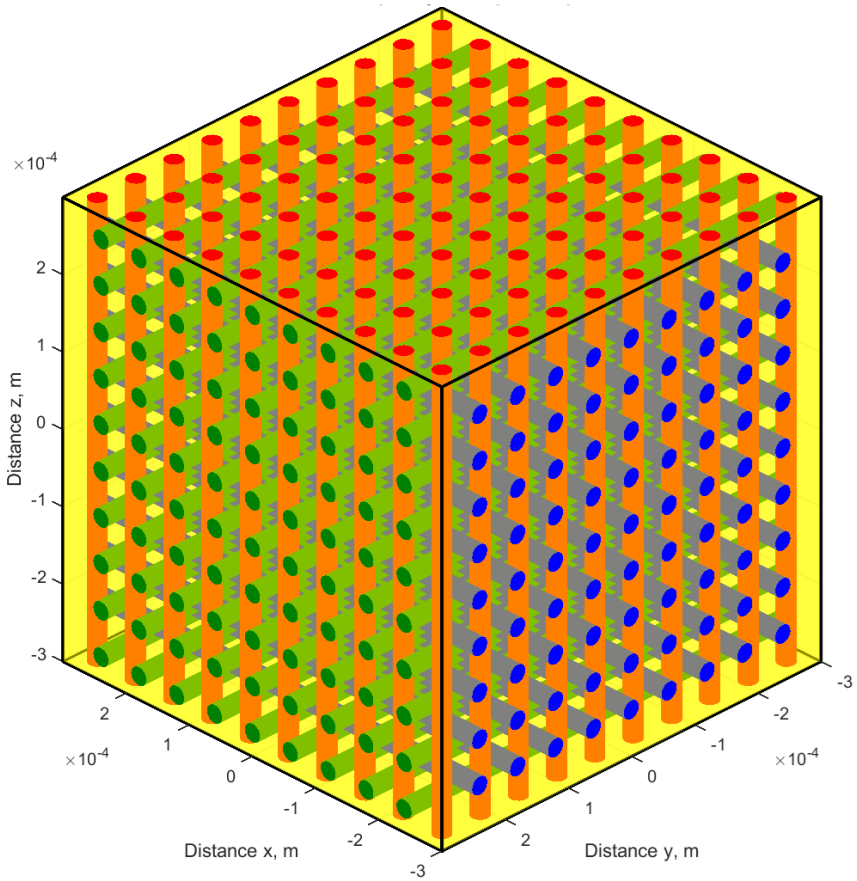

Fig. C1. Artificial specimen mimicking a finite sample of convoluted neuronal arbor.

The specimen is embedded in a conducting medium. An impressed or primary electric field of 100 V/m is applied to the specimen along the positive x-axis. If cylinder walls ("cell membranes") are assumed to be non-conducting for the extracellular solution, the particular value of medium conductivity (chosen here as 0.3 S/m) does not affect the results for the secondary and total electric field.

The BEM-FMM method (which achieves a relative residual of 1e-6 after 11 iterations) discussed in the main text is employed to compute deposited surface charges and their secondary electric field for the extracellular solution. The sum of the impressed and the secondary electric field gives us the total extracellular field in the sample. Fig. C2 shows the magnitude of the total extracellular field in the xy plane located at the center of the specimen. The intracellular space, where the present solution is invalid, is marked gray.

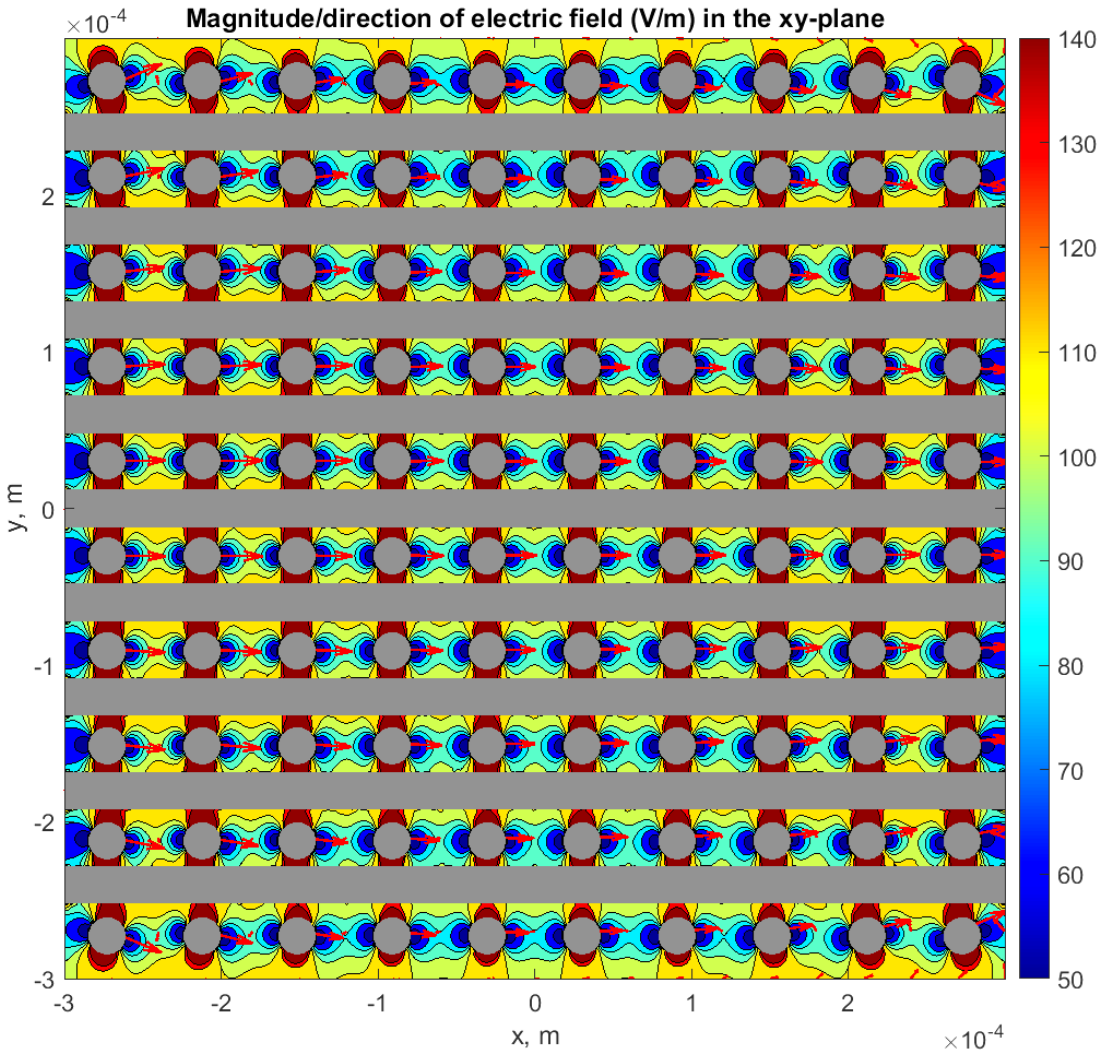

Fig. C2. Magnitude of the extracellular electric field in the xy-plane.

To characterize the mean value of the total extracellular field within the sample versus the impressed field of 100 V/m, as well as its standard deviation and variance, we introduce a uniform 3D grid of 1 M observation points within the cube and compute the total vector field at all extracellular points (outside cylinder bodies). Table C1 shows the corresponding results for the x-polarized primary field. One observes that the mean field value of the dominant polarization approaches exactly the impressed field value, with some minor variation of 2.6%. Similar results have been obtained for other polarization directions.

Table C1. Mean value, standard deviation, and variance for three components of the extracellular total electric field within the specimen. The co-polar (dominant) x-component of the extracellular field is marked bold.

	Mean	STD	Variance
Ex	**97.4 V/m**	**28.9 V/m**	**837.3 V/m**
Ey	0.0003 V/m	26.3 V/m	690.8 V/m
Ez	-0.0001 V/m	23.6 V/m	555.8 V/m

C2. Code availability and execution

The Dropbox link [1] contains a fully executable computational module built within the MATLAB platform under Windows and a short guide that replicates the above example. This enables both testing of different polarization directions as well as extraction of collinear fields along any desired axonal path.

To run the code (MATLAB platform under Windows), the MATLAB scripts from subfolder Model “model01_model_loader.m” and “model02_nintegral_generator.m” should be executed first. The first script loads the pre-created cylinder lattice (it can be modified if necessary in subfolder Combined_Mesh_Creator) while the second script computes neighbor integrals necessary for running BEM-FMM.

After that, all MATLAB scripts of the main folder should be executed *sequentially*. The MATLAB script “bem4_volume_e.m” is most time consuming; it computes the extracellular field within the sample at 1 million observation points.

References – Supplement C

[1]. Computer code to Supplement C. 2024. Dropbox Online.

Supplement D

to the manuscript "Enabling Electric Field Model of Microscopically Realistic Brain"

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D1. Post-processing segmentation and skeletonization of L2/L3 sample

1.1. Preparing neuronal surface meshes for electromagnetic modeling

All 396 neurons were initially numbered following the y-coordinate (dorsal-to-ventral direction) of their center of mass. The lowest numbers correspond to the deepest neurons where the bulk of the axonal arbor was cut out (see below). Neurons with the highest numbers have a (relatively) well developed axonal arbor (Fig. 3 of the main text).

Initial computational surface triangular meshes for the neurons downloaded from the MICrONS database [1] were not entirely suitable for accurate numerical analysis. Automated CGAL alpha wrapping C++ library [2],[3] was used for all neurons to re-mesh the data and convert them to the strictly 2 manifold high-quality (min triangle quality is 0.1) high-resolution individual surface objects. A relative α (vs 1e-3*longest diagonal; parameter α limits the maximum triangle size) of 1000 and a relative offset (vs 1e-3*longest diagonal, parameter offset limits the tightness of the result around the input mesh) of 4000 were used in the CGAL program. We were unable to properly heal 13 cells. These cells were eliminated from the analysis which reduced the total cell number from 396 to 383.

Fig. 4 of the main text illustrates the final membrane mesh for neuron #238. A typical membrane mesh has approximately 1-2 million triangles on average, with an average facet size kept at 100 nm. This is necessary to keep a proper spatial accuracy for intersection resolution (Section Materials and Methods of the main text) and also resolve microscopic, localized variations of the impressed electric field. The total number of facets of the sample approaches 0.5 billion. The average facet size is 100 nm. An identical procedure also was applied to the microcapillaries (cf. Fig. 3d of the main text).

1.2. Volumetric density of neuronal cells computed from surface meshes and its comparison with literature values

The volumetric fraction of all neuronal cells within the SEM (scanning electron microscopy) volume was estimated as 30%±5% by computing the volume of each neuron using the CGAL tools and also taking into account neuronal mesh intersections (Section Materials and Methods of the main text).

This is less than the known estimates in rats and mice [4],[5],[6] because all neurons with somas outside the brain sample were omitted and their dendritic/axonal processes, which are still within the sample, were ignored.

In general, the brain tissue is composed of extracellular space and intracellular space, i.e. neurons and glia cells (intracellular space) do not take up all the volume of brain tissue. Oxygen molecules and nutrients (e.g. glucose molecules) diffuse from capillary through extracellular space to neurons and glia and are taken up through transporters. According to [4] (rats), neuronal elements occupy about 74% and glial elements about 8% of the total volume. According to [5] (rats), the extracellular space takes up about 20% of total cortex volume in adult rats. According to [6] (Table 5), the fraction of neuronal mass in mice cerebral cortex is 70%. Based on these data and also on some data from Ref. [7], we estimate the realistic volume fraction of neuronal mass in mice neocortex as approximately 70%.

1.3. Healing and refining centerlines of neuronal processes – neuron skeletons

The original neuronal skeletons or morphological reconstructions (301 in total) from the MICrONS database [1] didn't remain entirely in the center of the corresponding neuronal processes represented by the relevant surface mesh. They also had a rather sparse discretization. The skeleton branches or centerlines have been therefore refined, smoothed, and corrected via an automated in-house routine that finds every point's distance from the enclosing membrane walls in the up, down, left, right, in, and out directions and averages them for every cross-section of the relevant triangular mesh. Fig. 3b,c of the main text shows the centerlines of the neuronal processes healed in such a way. We attempted to maintain a nearly uniform spatial resolution of 100 nm for the centerline discretization (observation) points, which should be sufficient for catching fast field variations over the length on the order of 1 μm or so. We were unable to properly heal 34 centerlines. These centerlines were eliminated from the subsequent analysis which reduced their total number from 301 to 267.

We also estimated the average axonal length per neuron, with the maximum length of 8.3 mm for inhibitory neuron #191 and the minimum length of 0 mm (for 39 other neurons in total). The latter case is non-physical; it is a deficiency of the centerline extraction method.

1.4. Estimating sample anisotropy from neuron morphology

To estimate the degree of anisotropy, we introduced an equivalent straight–fiber density measure. It is obtained when centerlines for all neuronal processes including both axons and dendrites are projected onto three Cartesian axes (Fig. D1b,c,d below). This density was estimated by finding the total projection length and then dividing this value by the product of three sample dimensions (the sample volume). The following values of the equivalent straight fiber density have been obtained: $1.3e5\ \mathrm{mm}^{-2}$ for the medial-lateral or x direction, $1.0e5\ \mathrm{mm}^{-2}$ for the dorsal-ventral or y direction and $0.9e5\ \mathrm{mm}^{-2}$ for the anterior-posterior or z direction.

1.5. Selecting proper neurons with the longest retained axonal arbors for biophysical modeling

The lengths of all segments of the neuronal processes (axons and dendrites) were computed using the available `*.swc` files from the MICrONS database. Fig. D1a shows the length of axonal arbor per neuron, now sorted in descending order. It is seen that neurons with numbers greater than 90-100 have a vanishingly short axonal arbor, which was cut out of this sample almost entirely. Therefore, they were eliminated from the final results of biophysical analysis (otherwise non-physical, large values of the activating threshold could be obtained) but were still kept for the electromagnetic analysis.

Thus, only the neurons with the overall axonal length of 0.5 mm or greater (with the sorted numbers from 1 to 90, Fig. D1a below) were included into the relevant biophysical modeling outcomes – see Fig. 8 of the main text, Figs. D4 through D7 of the present Supplement, and Tables 1 and 2 of the main text.

Fig. D1b additionally shows the total length of axonal arbor per neuron projected onto three Cartesian axes (x, y, z). The neurons have been sorted as in Fig. D1a. Similarly, Fig. D1c demonstrates projected (onto three Cartesian axes) length of dendritic arbor per neuron. The neurons have again been sorted as in Fig. D1a. Finally, Fig. D1d demonstrates the projected length of all neuronal processes (axonal plus dendritic) per neuron. The neurons have been sorted as in Fig. D1a. Again, one clearly observes in Fig. D1b that the neurons with numbers greater than approximately 90 have vanishingly small axonal arbor, which was cut out of the brain sample almost entirely.

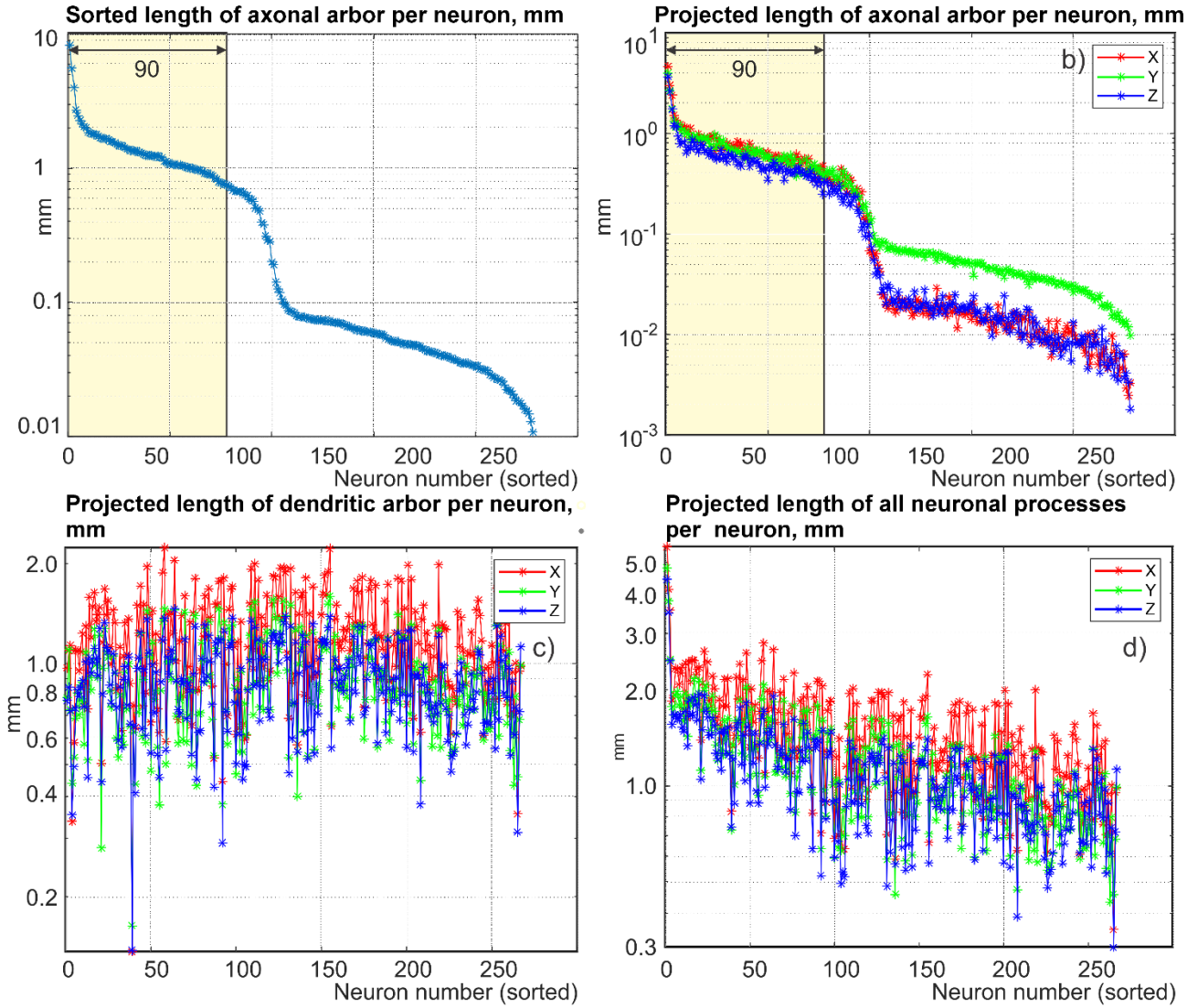

Fig. D1. a) Sorted (in descending order) length of axonal arbor per neuron. Neurons with numbers greater than approximately 90 have vanishingly small axonal arbor, which was cut out of the sample almost entirely. b) Projected (onto three Cartesian axes) length of axonal arbor per neuron. The neurons have been sorted as in a). c) Projected (onto three Cartesian axes) length of dendritic arbor per neuron. The neurons have been sorted as in a). c,d) Projected (onto three Cartesian axes) length of all neuronal processes (axonal plus dendritic) for all neurons with the healed morphological reconstructions. The neurons have been sorted as in a).

1.6. Summary of post-processing parameters

Table D1 of this Supplement lists major topological and biophysical parameters of the created computational testbed (electromagnetic and biophysical) including estimated sample anisotropy. Emphasize again that both the facet size for membrane discretization and the skeleton's spatial resolution for the biophysical analysis were kept at approximately 100 nm to resolve large yet very localized variations of the impressed field due to multiple nearfield interactions of the neuronal processes as well as to resolve multiple intersections of neuronal arbors of different neurons (Fig. 4 of the main text).

Table D1. Post-processing parameters of the L2/L3 mouse brain sample.383 Neurons were kept for electromagnetic analysis. Data for 90 neurons with the longest axonal arbor were included in the final biophysical outcomes.

Parameter	Value
Neuronal meshes for EM modeling	396 – initial; 383 – final
Vascular meshes for EM modeling	34, partially disconnected
Total mesh size/triangular facet size/triangular facet quality	~0.5 billion facets/100 nm/0.1 min
Volumetric fraction of neuronal mass within the sample	~30±5%
Neuron skeletons	301 initial/267 final
Spatial discretization of healed skeletons	100 nm
Largest length of axonal arbor per neuron	8.3 mm for inhibitory neuron #191 and 2.3 mm for excitatory neuron #333
Equivalent straight-fiber density of neuronal processes in three orthogonal directions (sample anisotropy)	Fiber density of $1.3e5\ \mathrm{mm}^{-2}$ for the medial-lateral or x direction, $1.0e5\ \mathrm{mm}^{-2}$ for the dorsal-ventral or y direction and $0.9e5\ \mathrm{mm}^{-2}$ for the anterior-posterior or z direction
Neuron skeletons with developed axonal arbor suitable for biophysical modeling	90

D2. Membrane mesh intersection maps

2.1. Mesh intersection maps

Fig. D2a demonstrates a typical surface mesh intersection map. Two neighboring excitatory neurons – #238 and #290 – have been chosen.

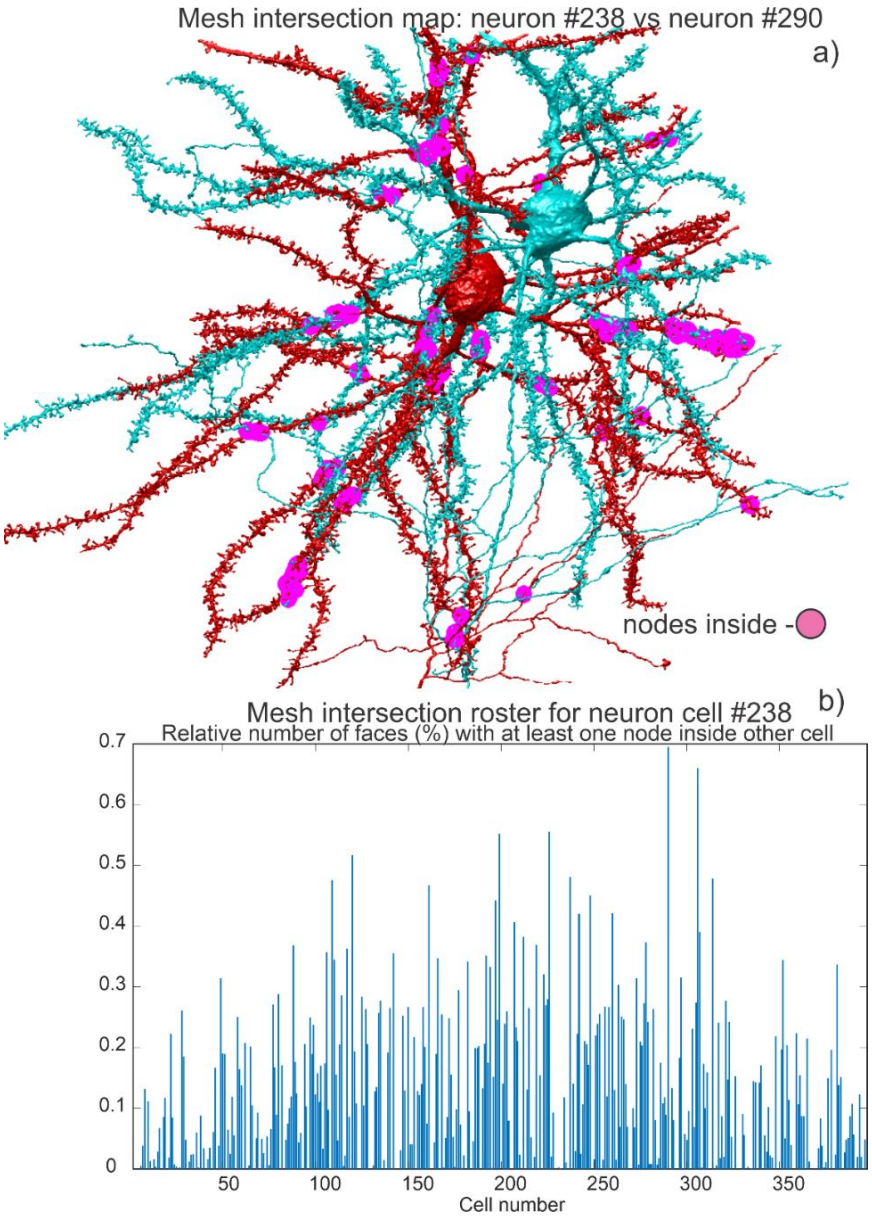

Fig. D2. a) Surface mesh intersection map between neighboring excitatory neurons #238 and #290, respectively. Intersection domains are marked by magenta color. b) Mesh intersection roster of neuron #238 with all other neurons of the sample. The y axis gives the relative number of facets (% versus the total number of faces for neuron #238) with at least one facet node contained within another neuronal shell. The x axis is the neuron number.

In Fig. D2a, the mesh intersection domains are marked by magenta color. There are approximately 30 such independent intersection domains.

Fig. D2b also presents a mesh intersection roster of excitatory neuron #238 with all other neurons of the sample. It is observed in Fig. D2b that neuron #238 intersects with almost all neurons of the sample. Similar, and even more dramatic results were observed for other larger neurons of the sample. To identify the mesh intersections for large yet manifold meshes, we used vectorized function `inpolyhedron` written by Sven Holcombe in MATLAB [8]. Still, this has been a very time-consuming operation.

2.2. Test of intersection resolution method

The intersection resolution method described in the Materials and Methods section was tested as follows. We selected a smaller set of 22 neurons (set #1). We further refined all surface meshes of this set in proportion 1:4. This comprises set #2. We analyzed set#1 and computed collinear electric fields at the skeleton centerlines. The same operation was done with set #2. We computed the average normalized 2-norm difference between two sets of projected fields and found that this error is within 6%.

D3. Biophysical modeling

3.1. Summary of NEURON modeling parameters

Standard NEURON software [9] has been used to study neural activation in response to the impressed extracellular potential/electric field computed previously at the centerlines of the neuronal processes. The post-processed neuron morphologies (described above in this supplement) for 267 available neuron models (in `swc` format, including soma, axons, apical and basal dendrites) were loaded into NEURON by its `Import3D` tool. Following references [10],[11],[12] we applied the existing ion channel models for rodents (rats) which have been used starting with Aberra et al. [10]. We used 6 active channels for the soma, 8 active channels for the axons, 1 active channel for the basal dendrites, and 3 active channels for the apical dendrites. The implementation for the most important axonal channels was tested and verified against in-house MATLAB-based software.

The centerlines of the neuronal processes were discretized in space with the step $d_\lambda = L/\left[n_{seg}\lambda_w(f = 100\ Hz)\right]$, where L is the section length, n_{seg} is the number of segments in this section, and $\lambda_w = \sqrt{D/(4\pi f R_a c_m)}$ is the length constant, with D being the neuron diameter; f is a frequency that is high enough for transmembrane current to be primarily capacitive, but is still within the frequency range relevant to neuronal function. Here, R_a is the resistivity of axoplasm and c_m is the membrane capacity per unit area. In the computations, we tested several d_λ values between $d_\lambda = 0.01$ and $d_\lambda = 0.0005$, respectively, to address expected rapid field variations at microscale. We compared the results for a representative number (6 in total) of large neurons with many local extracellular field variations. The difference in the activating thresholds was found to be negligibly small between the two extreme values. Therefore, the value $d_\lambda = 0.01$ or 0.005 has been routinely used everywhere after test runs. The time step size was chosen as $1\ \mu s$.

3.2. Pulse forms of the stimulation field

As the simple base form, we used the monophasic rectangular pulse form for the primary or impressed field starting at $t = 0$ and with a duration of $0.1\ \mathrm{ms}$ – cf. Fig. D3a,b). The positive and negative pulses (positive and negative electric field directions) have been modeled separately. Along with this, we used a realistic biphasic TMS (transcranial magnetic stimulation) pulse form (Fig. D3c) from a B65 coil of MagVenture, Denmark measured in the Noninvasive Neuromodulation Unit, Experimental Therapeutics & Pathophysiology Branch, NIMH. Both positive and negative pulse forms have been investigated. Some computations have also been done for a monophasic TMS pulse.

The pulse magnitude was gradually increased until the neuron activating criterion (the transmembrane voltage goes above zero anywhere in the arbor [10]) was met. After that, the amplitude was decreased with a smaller step to pass the activating threshold in the opposite direction. This automated search procedure – the bisection method – requires approximately 15 steps and about 15-25 min per neuron on average. The total simulation time for all neurons with x, y, and z polarizations for both primary and total extracellular fields/potentials did not exceed one week per polarization.

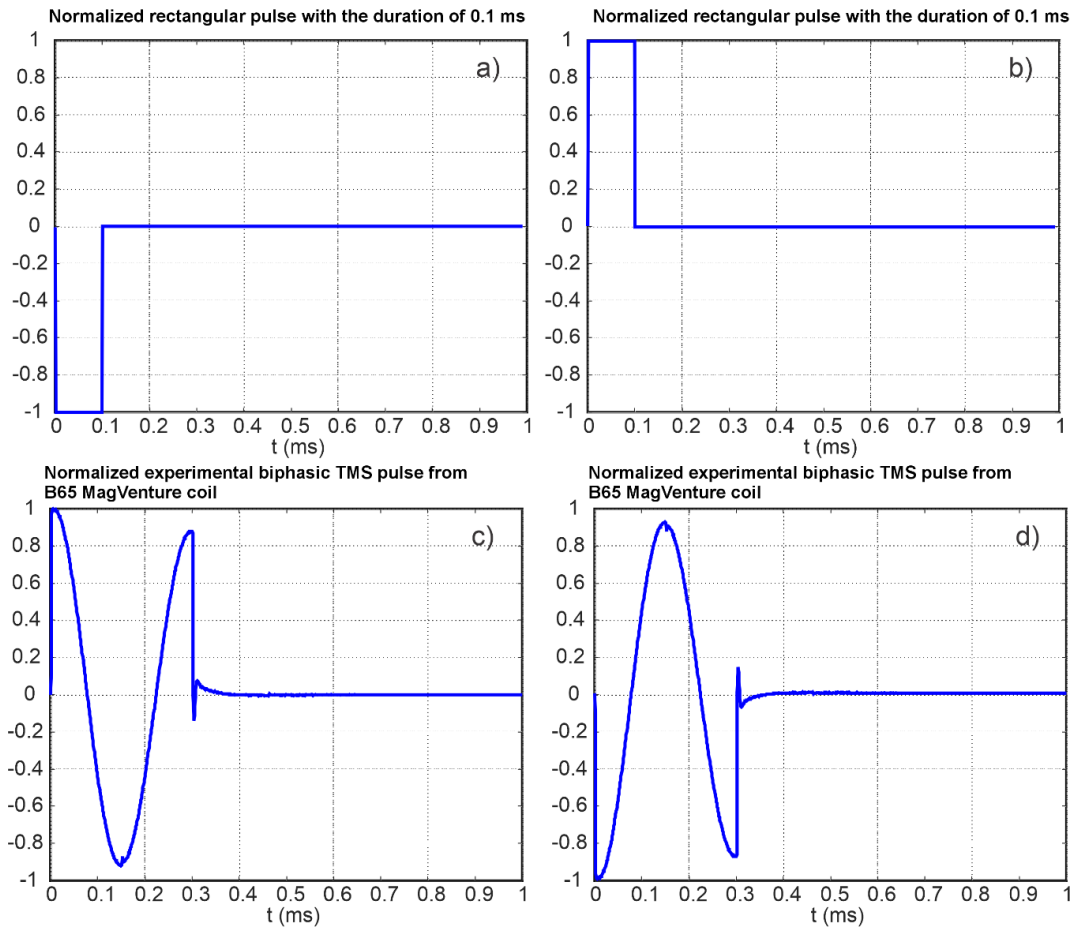

Fig. D3. Four pulse forms for the primary impressed field used for biophysical modeling.

3.3. Reduction of neuronal activating thresholds for different pulse forms and myelinated axons

Figs. D4 through D10 given below replicate Fig. 8 of the main text for different pulse forms and with considering axonal myelination. They have been used to fill out Tables 1 and 2 of the main text.

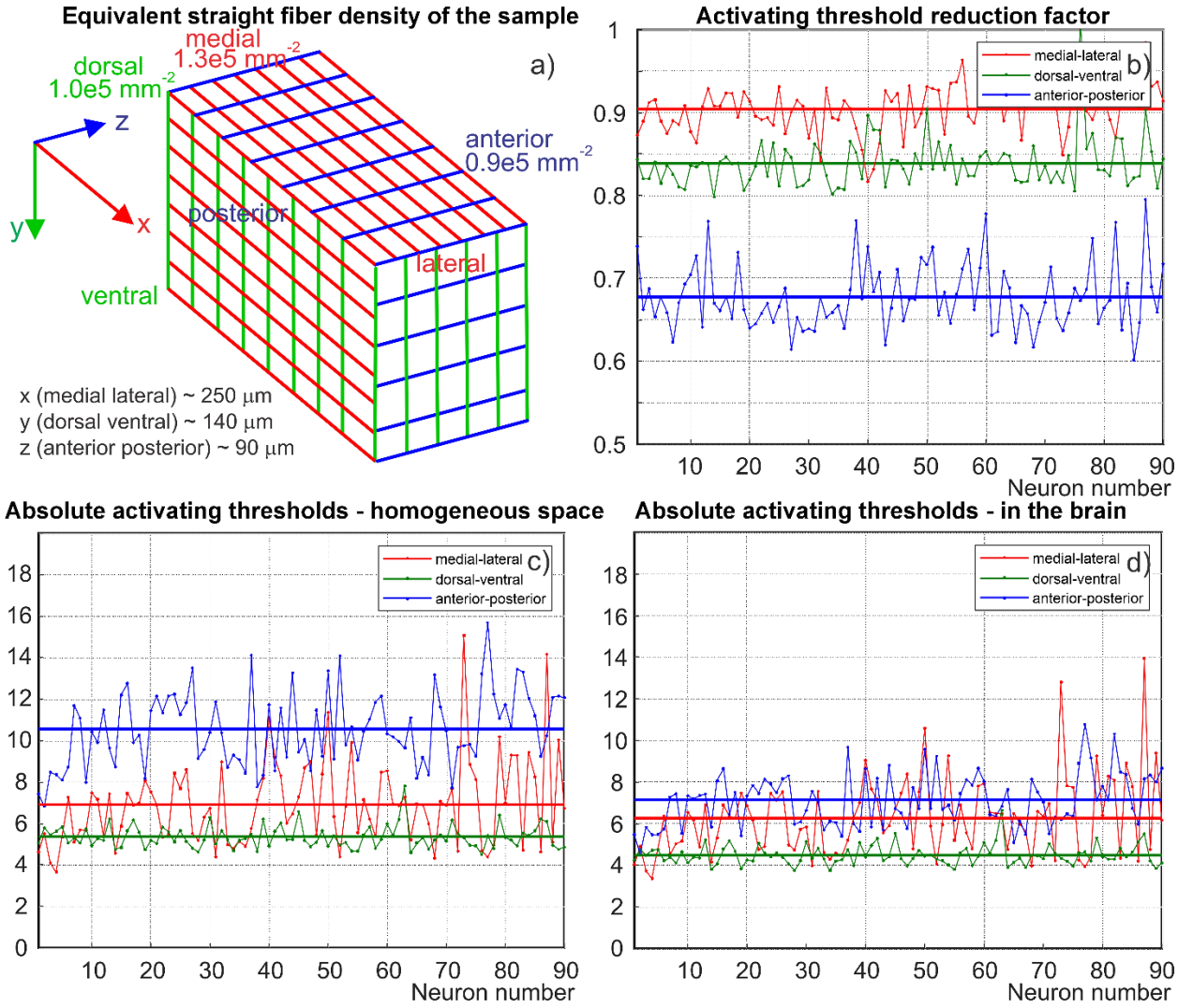

Fig. D4. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the unmyelinated axonal arbor and a positive rectangular activation monophasic pulse with the duration of 0.1 ms.

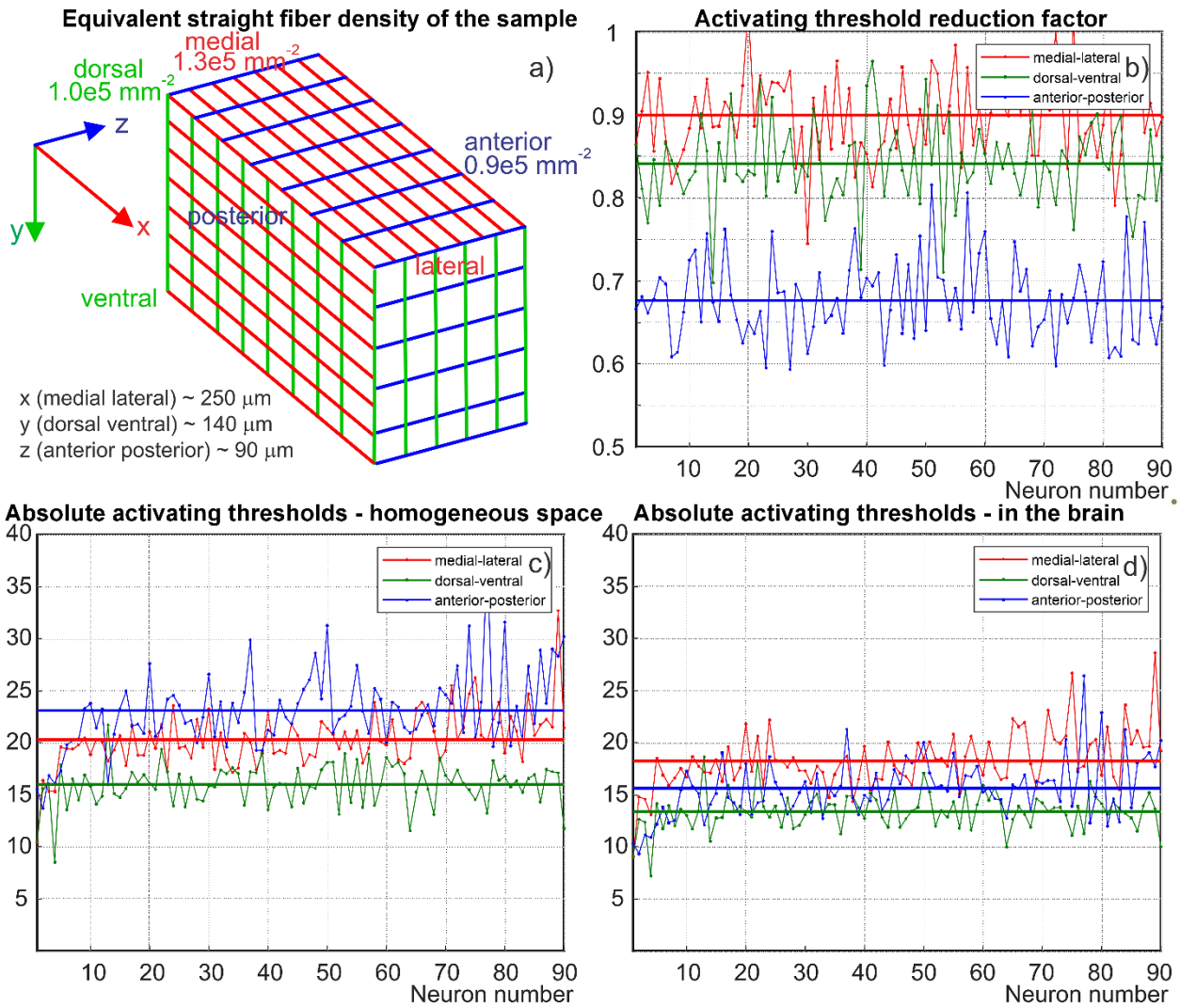

Fig. D5. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the unmyelinated axonal arbor and a negative biphasic activation pulse with the duration of 0.3 ms (Fig. D3).

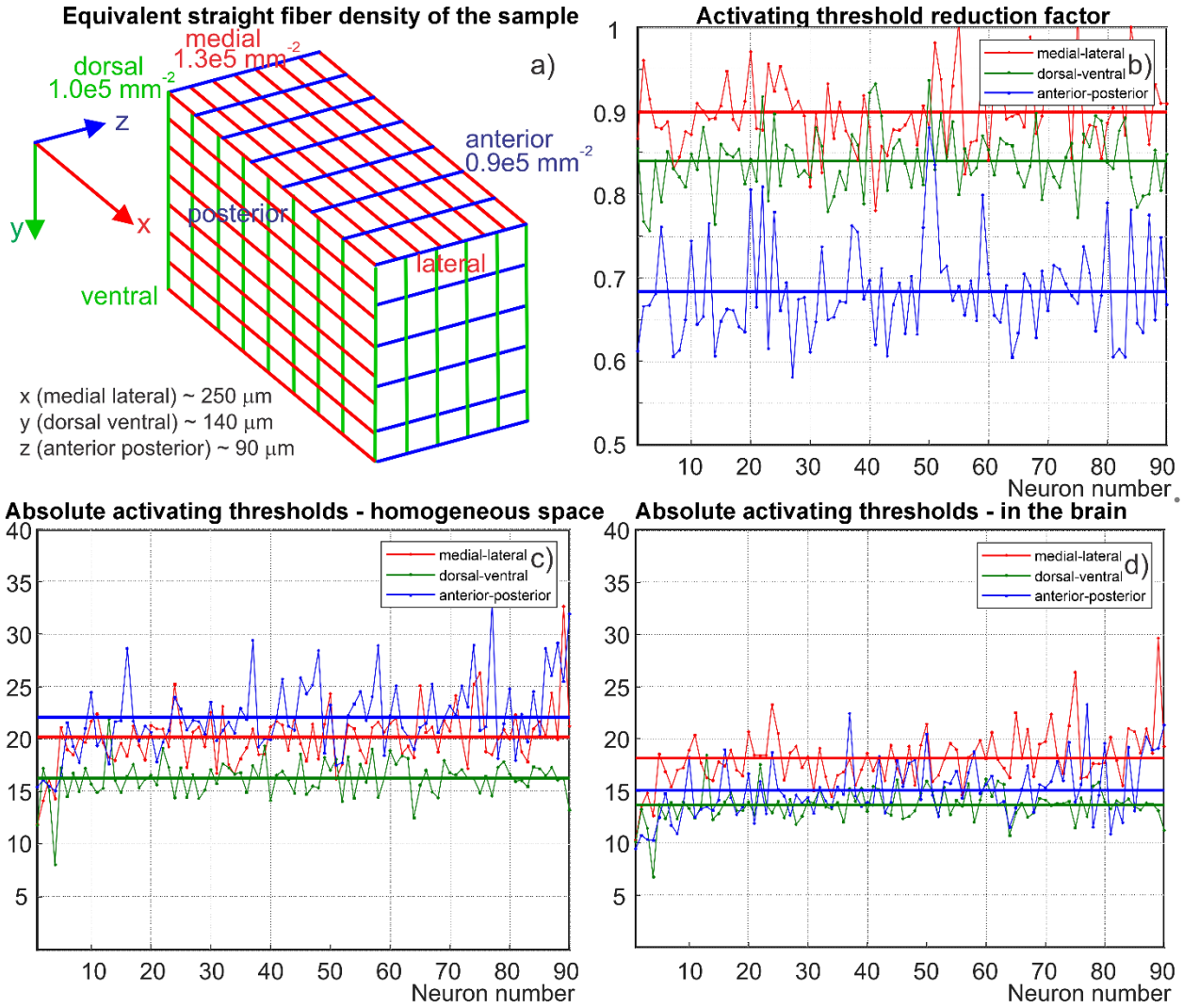

Fig. D6. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the unmyelinated axonal arbor and a positive biphasic activation pulse with the duration of 0.3 ms (Fig. D3).

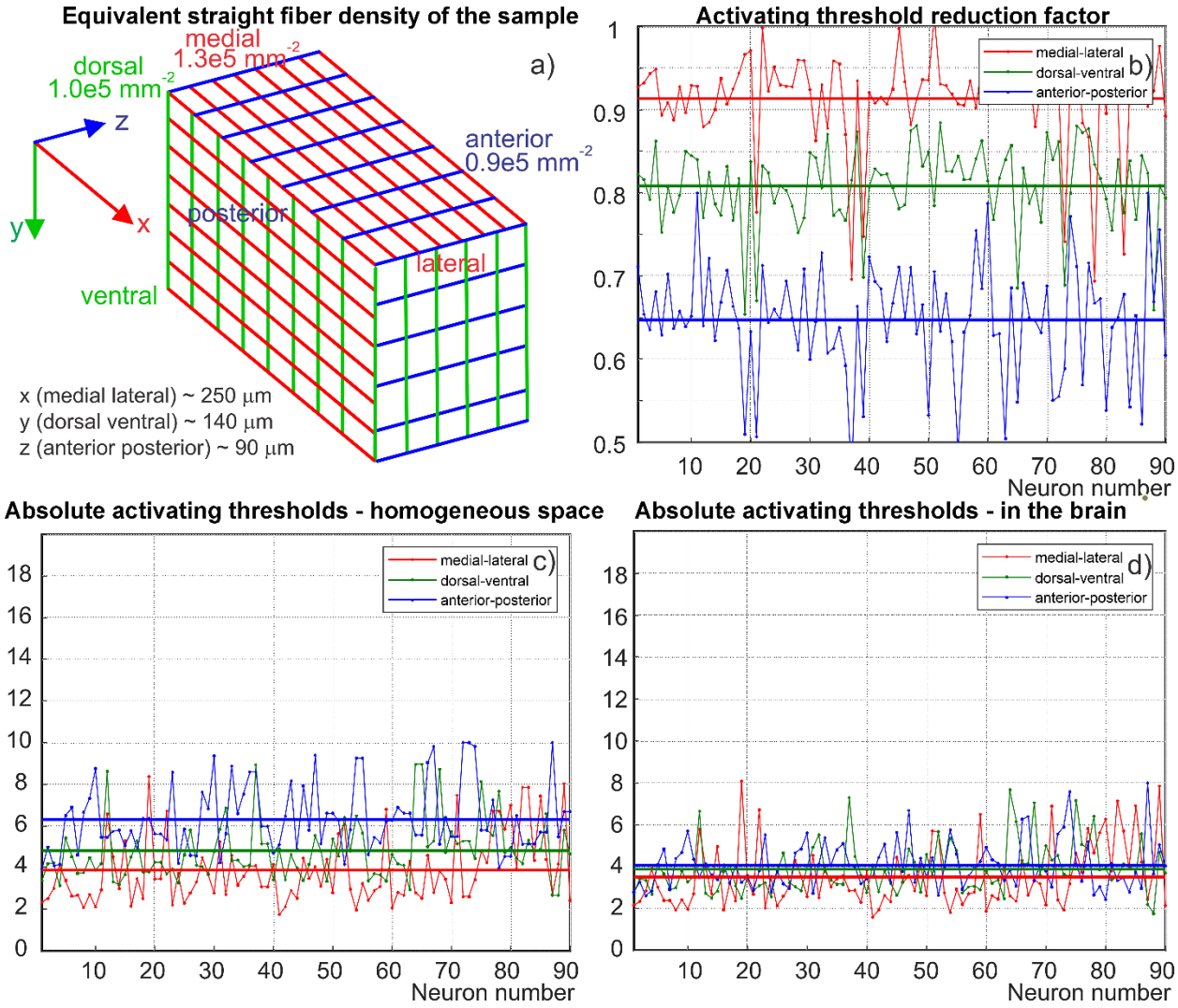

Fig. D7. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the myelinated axonal arbor and a negative rectangular activation monophasic pulse with the duration of 0.1 ms.

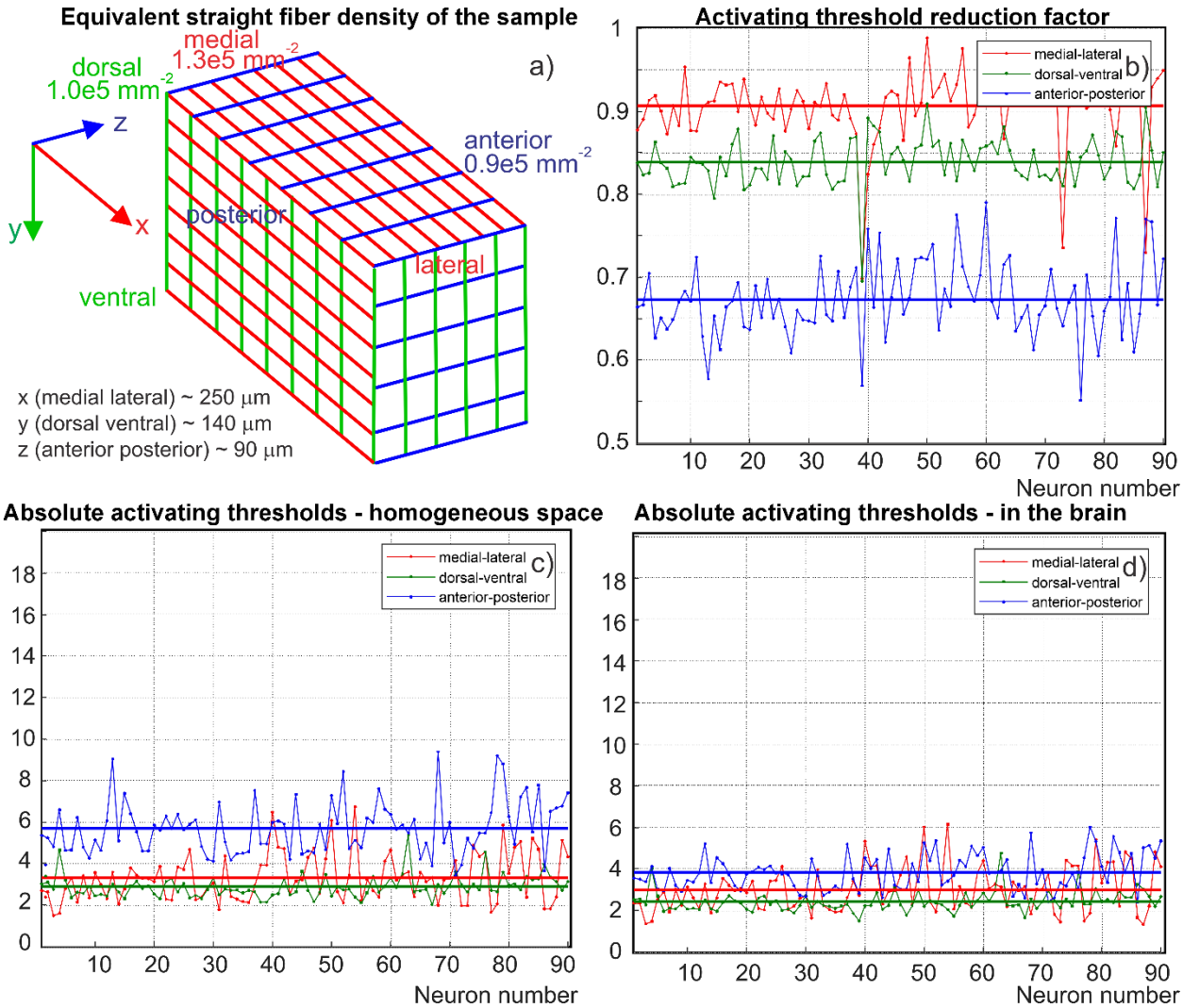

Fig. D8. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the myelinated axonal arbor and a positive rectangular activation monophasic pulse with the duration of 0.1 ms.

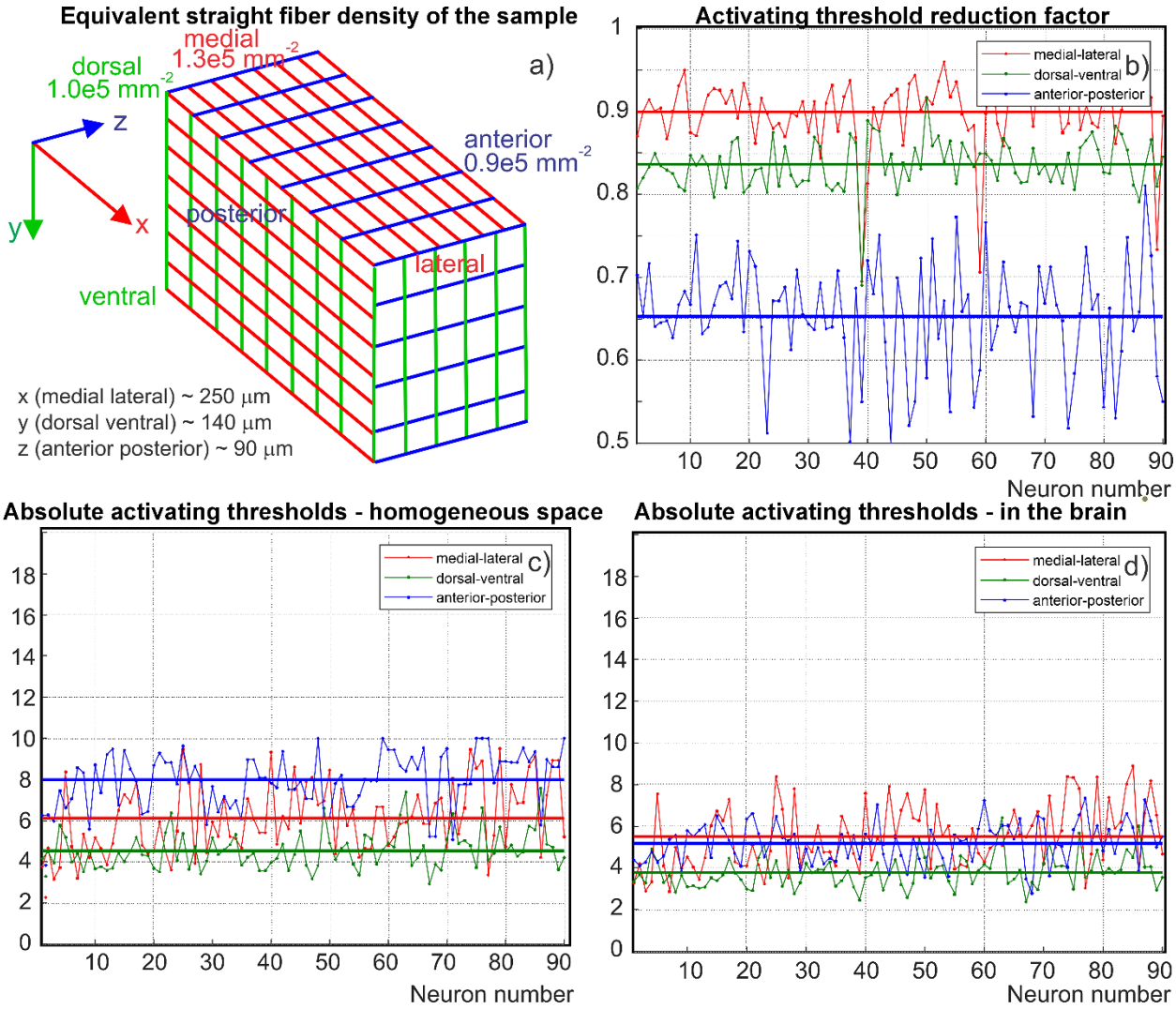

Fig. D9. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the myelinated axonal arbor and a negative biphasic activation pulse with the duration of 0.3 ms (Fig. D3).

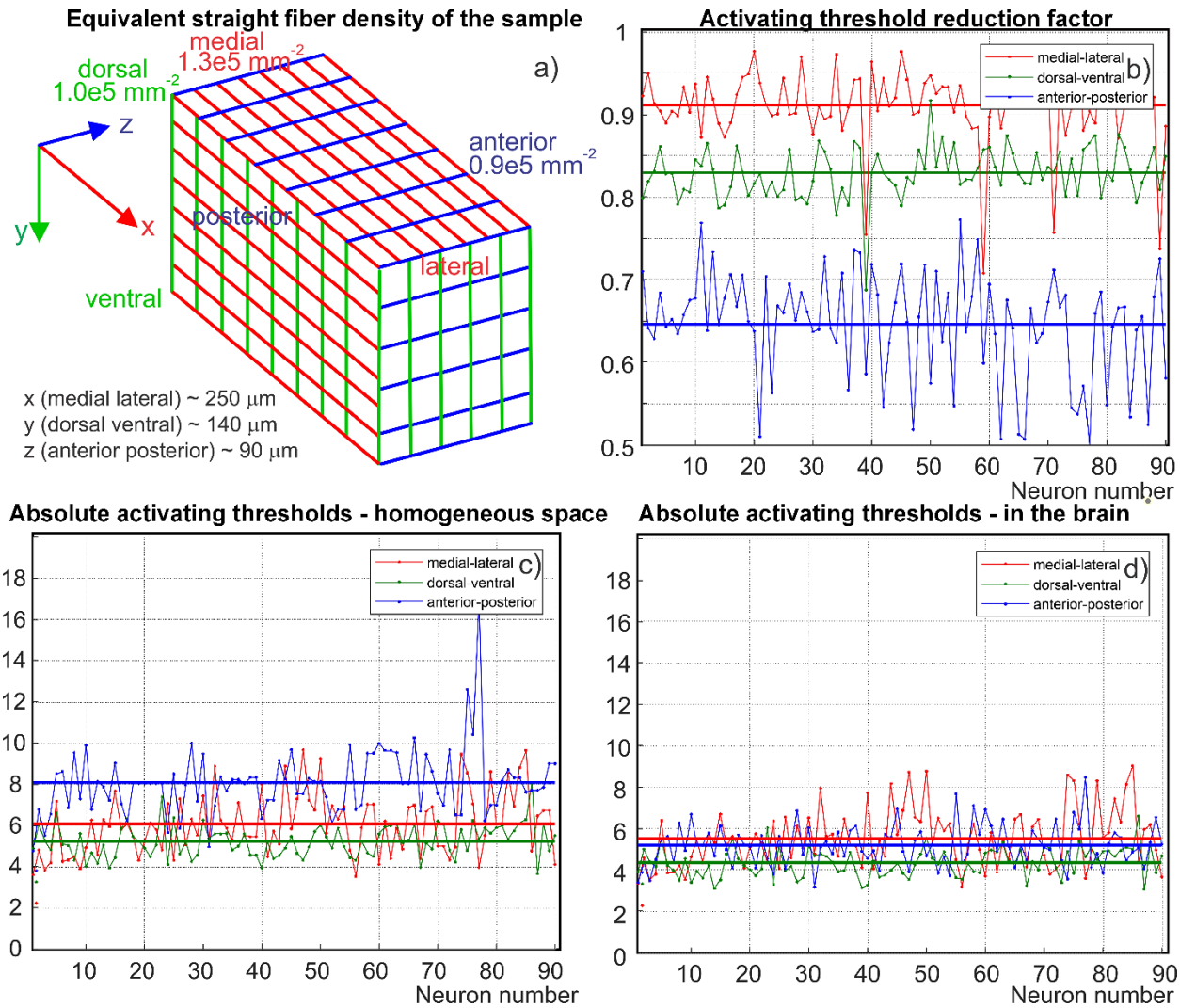

Fig. D10. a) Equivalent straight fiber density distribution of the sample characterizing its anisotropy. b) Activating threshold reduction factor for the first 90 neurons with the longest axonal arbor (sorted) for three polarizations of the primary electric field: medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue). c) Absolute activating thresholds for the same 90 neurons in homogeneous space. d) Absolute activating thresholds for the same 90 neurons within the sample. The units in c,d) are given in terms of the base field strength of 100 V/m; one unit corresponds to 100 V/m. These results are given for the case of the myelinated axonal arbor and a positive biphasic activation pulse with the duration of 0.3 ms (Fig. D3).

D4. Estimates of absolute TMS activating threshold of mouse primary motor cortex from experimental data

4.1. Experimental procedure

In vivo, TMS experiments were performed on male C57BL/6J mice (n¼6) using a custom rodent TMS coil with a magnetic core (Fig. D11c). Animals were anesthetized with sodium pentobarbital intraperitoneally (50 mg/kg), MEP (motor evoked potential) was recorded using a similar approach reported by Rotenberg et al. [13]. All procedures were approved by National Institute on Drug Abuse/NIH (NIDA) animal care and use committee. The coil was mounted to a customized three-axis micromanipulator.

The focal electrical field point was carefully aligned to the targeted mouse motor cortex. Fine adjustments of the coil were made to induce limb twitch on the contralateral hindlimb, but not the ipsilateral hindlimb, any of the forelimbs or any other body part. More detailed description of TMS experiments is given in [14]. Detailed description of a similar custom focal TMS rodent coil was previously given in Ref. [15].

4.2. Modeling setup

Detailed brain model [16] (Fig. D11a) along with the model of the custom TMS rodent coil with a magnetic core (Fig. D11c) has been used to simulate the electric field distribution both in three principal planes (Fig. D11b) as well as just inside the brain compartments (Fig. D11d). The skin shell conductivity was chosen as 0.3 S/m, the skull conductivity – 0.01 S/m. All brain compartments were assigned the conductivity of 0.3 S/m. Some conductivity variations were studied. They did not substantially alter the computational results. In general, the small mouse body quite significantly (by a factor of ~2 or so) blocks the primary applied solenoidal electric field of the TMS coil.

4.3. Estimating electric field strength within mouse brain from measured motor evoked potentials

Simulations were performed for two experimental parameters – the coil current of ~3,000 A and dI/dt of 94 A/μs – corresponding to the induced MEPs. Fig. D11d shows the resulting magnitude of the total electric field just inside the brain compartments. The circle approximately labels the area of the primary motor cortex. One observes that the field strength there approaches 90 V/m. Conductivity variations of the head compartments lead to the field strength changes within the M1 area (white circle in Fig. D11d) to within ±15% about this value. Similar results have been obtained

for the total field magnitude distributions in three principal planes shown in Fig. D11e. Another mouse CAD model [17] has also been tested; it also generated very similar results.

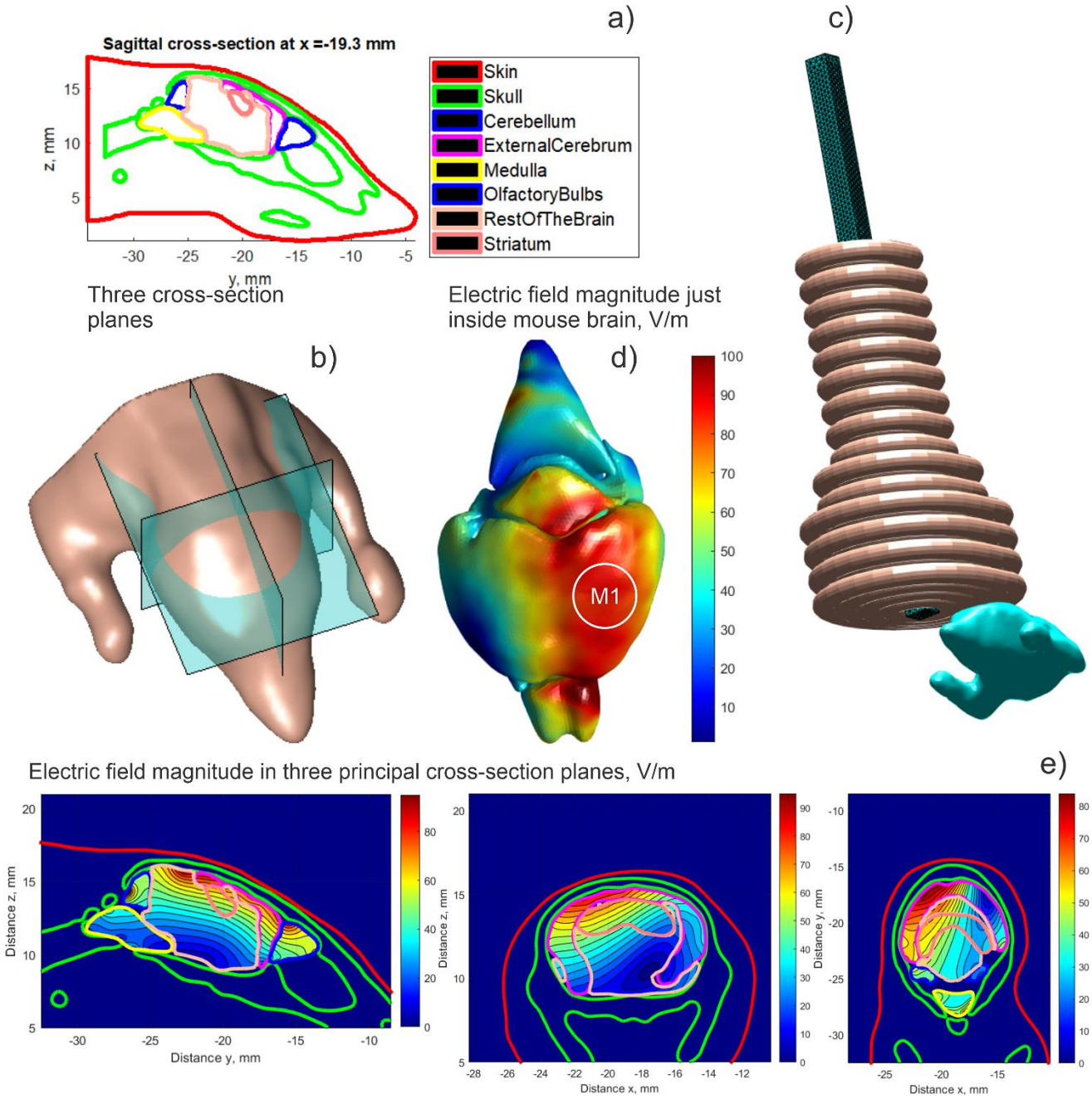

Fig. D11. a) CAD Mouse model [16]. b) Three principal planes for field output. c) Shape and position of the custom TMS coil. d) Electric field magnitude distribution just inside mouse brain. Circle approximately indicates the primary motor cortex.

4.4. Comparison with previously reported computed data for mouse primary motor cortex

In Ref. [17], the maximum fields strengths of 81.3 V/m,113.4 V/m, and 86.5 V/m in the brain have been computed for three different coil positions of a 25 mm figure-8 coil for the coil's dI/dt of 100 A/μs In Ref. [18], it was concluded from numerical experiments that, for 100 A/μs, the predicted fields in mouse brain are on the order of 120 V/m.

Our experimental data agree with these modeling predictions. They suggest that 90 V/m ± 15% is a reasonable MEP estimate in a mouse brain.

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Supplement E

to manuscript "Enabling Electric Field Model of Microscopically Realistic Brain"

Verification of nested iterative algorithm

E1. Test sample under study

For comparison purposes, ten nearest neighbors of neuron #192 have been selected as shown in Fig. E1. The entire membrane mesh of the sample includes 16 million facets. After that, two methods have been applied:

i. Global iterative BEM-FMM algorithm with the relative residual of 1e-4;

ii. Nested iterative BEM-FMM algorithm of the present study with the total number of 6 neuron-to-neuron outer iterations (as shown in Fig. 5 of the main text) and with the relative residual of 1e-4 for the inner iterative solution.

The y-polarized primary electric field of 100 V/m (corresponding to a vertical direction in Fig. E1) has been applied in both cases. The global algorithm converges after 15 iterations, in ~1,000 seconds, when using a 3 GHz workstation.

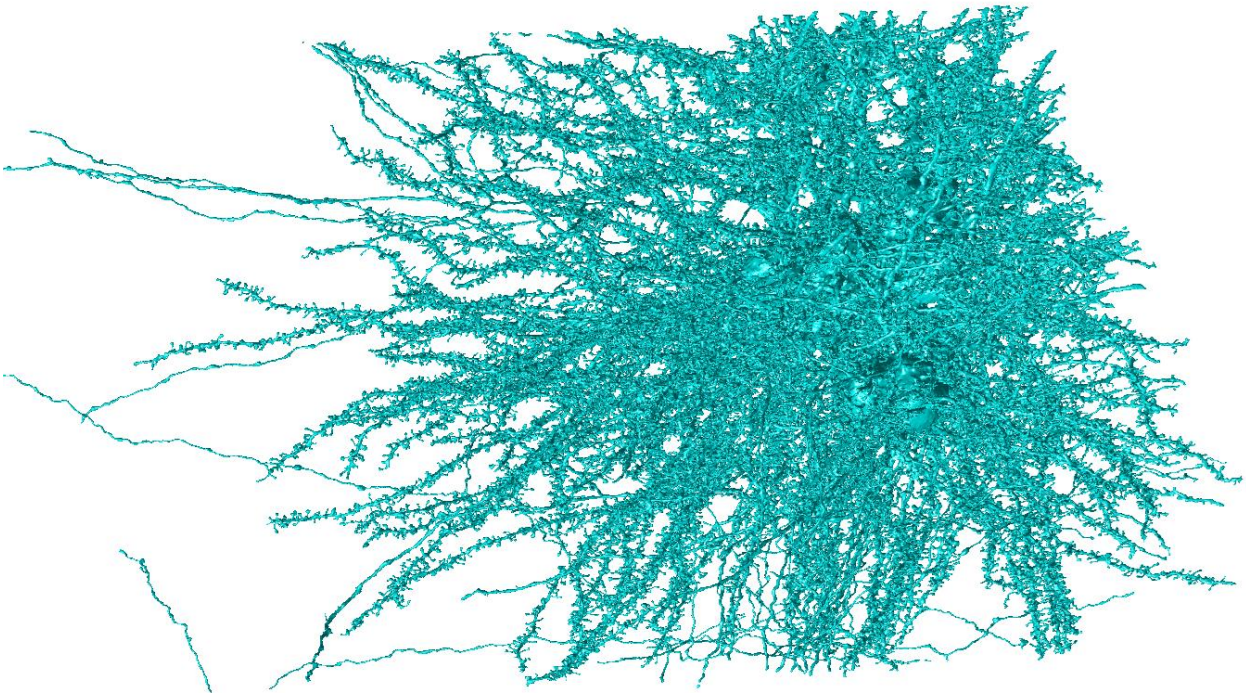

Fig. E1. Truncated sample mesh of 11 neighbors of neuron #192.

E2. Comparison results

The two solutions have been compared to each other regarding the induced membrane charge densities. Fig. E2 shows the relative 2-norm error in the local charge density for every neuron (11 in total) separately. One observes that this error does not exceed 1% on average.

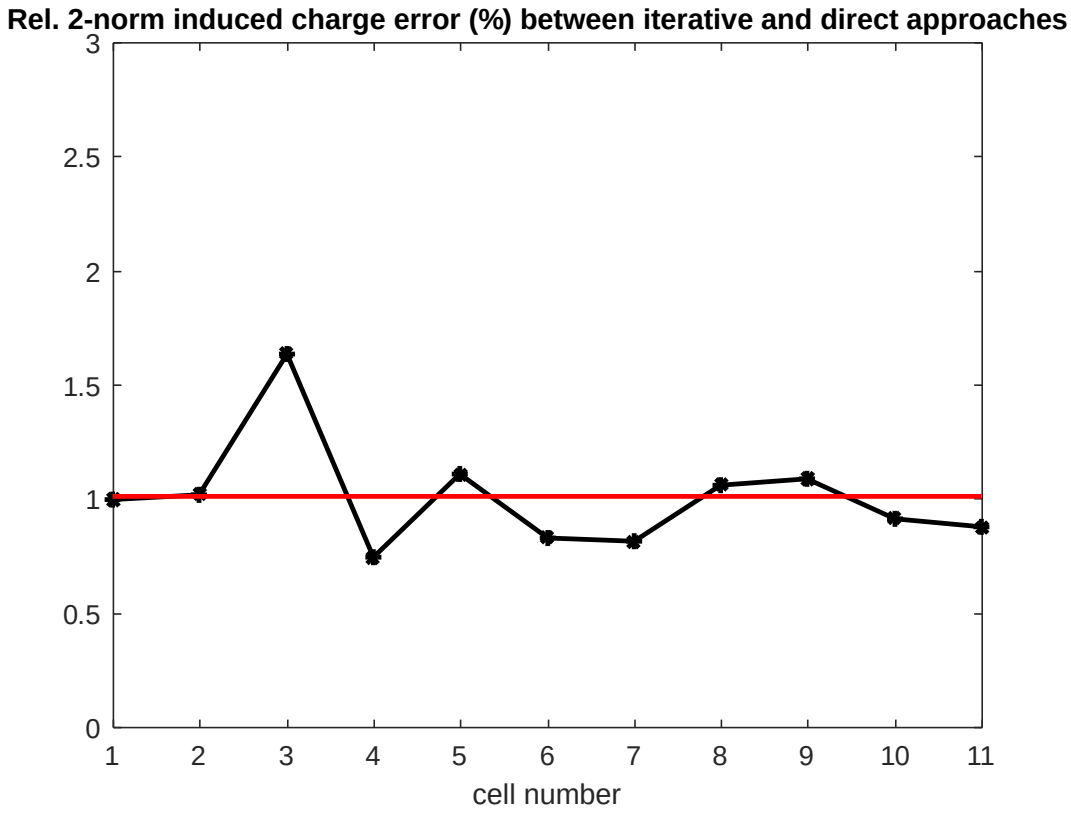

Fig. E2. Relative 2-norm error for the induced charge density between the two solutions for every neuron of the sample.