OPINION

Regulation of ionic flow in neuronal subcompartments: ~~the new nanophysiology~~

David Holcman and Rafael Yuste

Abstract | Cable theory and the Goldman–Hodgkin–Huxley–Katz models for the propagation of ions and voltage within a neuron have provided a theoretical foundation for electrophysiology and been responsible for many cornerstone advances in neuroscience. However, these theories break down when they are applied to small neuronal compartments, such as dendritic spines, synaptic terminals or small neuronal processes, because they assume spatial and ionic homogeneity. Here we discuss a broader theory that ~~incorporates~~ the Poisson–Nernst–Planck (PNP) approximation and electrodiffusion to more accurately model the constraints that neuronal nanostructures place on electrical current flow. This theory could advance our understanding of the physiology of neuronal nanocompartments.

How does an electrical signal propagate in a neuron? Traditional cable theory, which was refined by Goldman, Hodgkin and Katz[1,2], answered this question using a model that was based on the opening and closing of channels that are located on the surface of a thin cylinder. This model served as the foundation of our understanding of several fundamental aspects of neuronal physiology, such as local depolarizations, action potential generation and propagation and synaptic integration. This model can be safely applied to macroscopic structures, such as the squid giant axon. However, it assumes a lack of interaction between the local electric field and diffusional flux, an assumption that cannot be made for small neuronal microcompartments, in which electrodiffusion is likely to be important. Moreover, local geometry at the submicron level has significant effects on the motion and distribution of charged species within the cell. These local constraints are starting to be experimentally addressed through new imaging methods that have opened a new frontier in electrophysiology, allowing ~~us to measure~~ electrical phenomena within the microcompartments or nanocompartments of a neuron.

Here, we argue that traditional theoretical methods to analyse current flow that are based on the propagation of electrical current in macroscopic cables, such as cable theory and classical diffusion, are insufficient when examining small (nanoscale) compartments of the cell. We examine the limitations of traditional cable theory and use the dendritic spine as an example of a structure with nanoscale dimensions, although it is important to note that a similar argument can be made for any neuronal structure of similar dimensions. This may include small neuronal compartments (such as presynaptic vesicles or olfactory or hair-cell cilia), narrow astrocytic processes, thin axons and dendrites and the small neuronal processes found in invertebrate nervous systems. We discuss the need for novel theoretical and experimental frameworks that extend cable theory to understand and quantify current modulation in nanostructures and review of the use of the Poisson–Nernst–Planck (PNP) equation, electrodiffusion and spatial modelling to quantitatively understand the biophysical processes taking place in neuronal subcompartments.

Cable theory and its assumptions

Classical cable theory was originally developed in the nineteenth century by Lord Kelvin to explain the flow of electricity in submarine cables and was adapted to describe nerve fibre conduction between the 1920s and the 1940s by Cole, Goldman, Hodgkin and Katz[1–4]. In cable theory, the resistances and capacitances of the cell membrane and the properties of the electrolytes that surround it are used to create a one-dimensional (1D) diffusion model equation that describes the propagation of an electric field in a conducting nerve fibre[1]. The solution of this model equation provides a precise estimate for how the electric field decays away from its initiation site (the site of a synaptic input or of an electrode current injection), showing that relay channels along the neuronal membrane are needed to regenerate the field and extend the propagation to greater distances[5]. Importantly, this framework was based on the assumption that there is electro-neutrality within the neuron: that is, that there is a local equilibrium between negatively charged particles and positively charged particles [Au:OK] ( ) that their density is equal at each po[illegible].

This initial model was later expanded to take the influence of ion channels into account[6]. Various versions of the classical Hodgkin and Huxley (HH) model were created, using estimated ionic dynamics that did not necessarily reflect the real biophysical motion of ions, to study the electrical conduction and excitability of neurons. These theoretical approaches were later exploited to characterize the functional properties of dendrites[7], and transformed versions of HH models have often been used to study the excitability of neural networks[8]. It is thus fair to say that cable theory and modified versions of this theory have, over the decades, provided the theoretical foundations of cellular neurophysiology and given neuroscientists a means to understand the nervous system at different functional levels, from the excitability of ion channels to the description of neuronal ensembles.

[Au: definition OK? Could you briefly define 'dielectric constant']

Glossary

Back-propagating action potential
The wave propagation of an action potential that is due to the opening and closing of ionic channels, moving in the direction of the soma.

Debye length
The length after which an electric charge is screened from the effects of an electric field by water or other polar molecules [Au:OK?]

Dielectric medium
A media in which charged particles can become polarized, the properties of which are characterized by a dielectric constant (ε).

Diffusional coupling
Coupling of two compartments that is due to the exchange of diffusing particles, such as ions or molecules.

Diffusional flux
The number of particles per unit of time entering through a surface.

Electrodiffusion
The combination of diffusion and electrostatic forces that are applied to a charged particle. The particle motion results from the sum of these two forces.

Ficks's diffusion law
A macroscopic law that assumes that the diffusion flux is proportional to the gradient of concentration.

Monte Carlo simulations
Numerical simulations in which each particle (molecules or ions) is assumed to move through Brownian motion. This simulation allows all particle trajectories to be monitored at any moment of time.

Nanostructures
Complex geometrical domains with a clear identified electrophysiological function and with a characteristic length in a range from tens to hundreds of nanometres. Examples include dendritic spines, cilia, synapses, parts of sensory cells, protrusions and the endoplasmic reticulum.

Neuronal ensembles
Sets of neurons connected by synapses. A neuronal ensemble can sustain a network activity such as synchronization, oscillation or rhythm.

Steady-state regime
A system state described by stationary parameters that are by definition independent of time.

Transient regime
Period of time during which the parameters describing the state of a system vary and converge toward the steady-state regime.

Nanophysiology: dendritic spines

Dendritic spines have a crucial role in neuronal communication because they enable synaptic connections to be made with nanometre precision. In both pre- and postsynaptic structures of a synapse, the thousands of molecules that together generate and regulate synaptic currents, and the orchestrated behaviour of which is integral for cellular function, are precisely organized over spaces of less than one cubic micron[9]. Over the past 20 years, it has become clear that the properties and behaviour of individual molecules within spines are important: synaptic plasticity is due, at least partly, to changes in the number of postsynaptic receptors[10] and, in some cases, functional differences between synapses can be associated with the presence or absence of a few synaptic molecules[11].

Moreover, the recent introduction of high-resolution imaging techniques has revealed that, in living neurons *in vitro* and *in vivo*, spines are actually morphologically very plastic[12–14]. Recent findings suggest that synaptic currents, and probably also molecular trafficking, can also be modulated by the spine geometry[15–19]. Superresolution imaging techniques now enable measurements of individual molecules at spines with an accuracy of tens of nanometres[20]. These technical advances have provided a fascinating view of the cellular physiology of neurons — which are traditionally studied via somatic recordings with electrodes — and it will soon be possible to probe the function of nanocompartments such as dendritic spines one molecule at a time. The era of nanophysiology has arrived.

Limitations of existing theories

As outlined above, traditional cable theory was designed to describe macroscopic structures, such as copper wires or squid giant axons. During the electrical excitation of such structures, global changes in the concentration of electrolytes are negligible and spatial constraints are essentially homogeneous. This leads to the passive movement of electrical charges, driven mostly by classical diffusion.

However, the propagation of an electrical current in an axon or dendrite is different from that in an electrical wire. In neurons, current flows as a result of ions moving through a dielectric medium, whereas in a wire electrons are the charged particles moving throughout a metallic conductor. In spite of this, the HH model — based on the opening and closing of channels in a cylinder[2] and approximated by a succession of resistances and capacitances — has successfully provided a framework for studying neuronal conduction for the past 60 years and has accurately explained experimental results obtained with ~~classical~~ microelectrodes[4]. However, as neuroscientists start to have access to functional measurements from smaller cellular structures, one wonders whether cable theory (or HH models) can still provide the accuracy and robustness that are required to study the electrical properties of small compartments in a neuron. Do the smaller geometrical or electrical features of these structures have any functional impact?

In the case of dendritic spines, it seems that traditional models ~~are grossly~~ inadequate. Because of their geometry, dendritic spines cannot simply be approximated by cable geometries: even when simplified, they consist of a spherical head connected by cylindrical neck to the dendritic shaft. In reality, of course, spines are morphologically highly complex and exhibit enormous geometrical variability (FIG. 1a,b). In such submicron spaces, the variation in geometry between the two different spatial regions (head and neck) has a dramatic effect. For example, using modelling and simulations[21], it has been shown that the decay time of the diffusion of particles (such as molecules or ions) from the spine head into the neck [Au:OK?] is dominated by diffusional coupling between the spine neck and head[22,23]. This coupling takes into account the many possible ways in which an ion can leave the spine given that it may return a countable infinite number of times to the head before escaping. Diffusional coupling, a key contributor to the regulation of ionic concentrations in a spine, is an intrinsic consequence of the particular geometry of the dendritic spine and arises owing to the presence of the small radius of the neck at the entrance of the spine. Moreover, the convoluted and often constricted nature of the spine neck (FIG. 1c) makes it possible that there might be significant differences in the diffusional coupling in different dendritic spines.

In addition to the constraints that arise from their peculiar geometry, the small size of spines could also significantly alter current flow within the spine. For example, during synaptic stimulation or the opening of ion channels in the spine as a result of a back-propagating action potential, the number of ions entering the spine is large (estimated from ~~NMDA, AMPAR or voltage~~ calcium channel currents to be between a few to tens of thousands of ions[6]) when compared with the small volume of the spine. This will lead to a marked electrical effect that must be taken into account: the entering ions, by dramatically changing intracellular ion concentrations, alter the electric field at the narrow constriction at the spine neck–head junction (FIG. 1c). This large spatial change in the electric field cannot be captured by the linear cable theory (in which the spine is approximated as a wire and the spatial scale is not small enough to take into account small holes such as the spine–neck

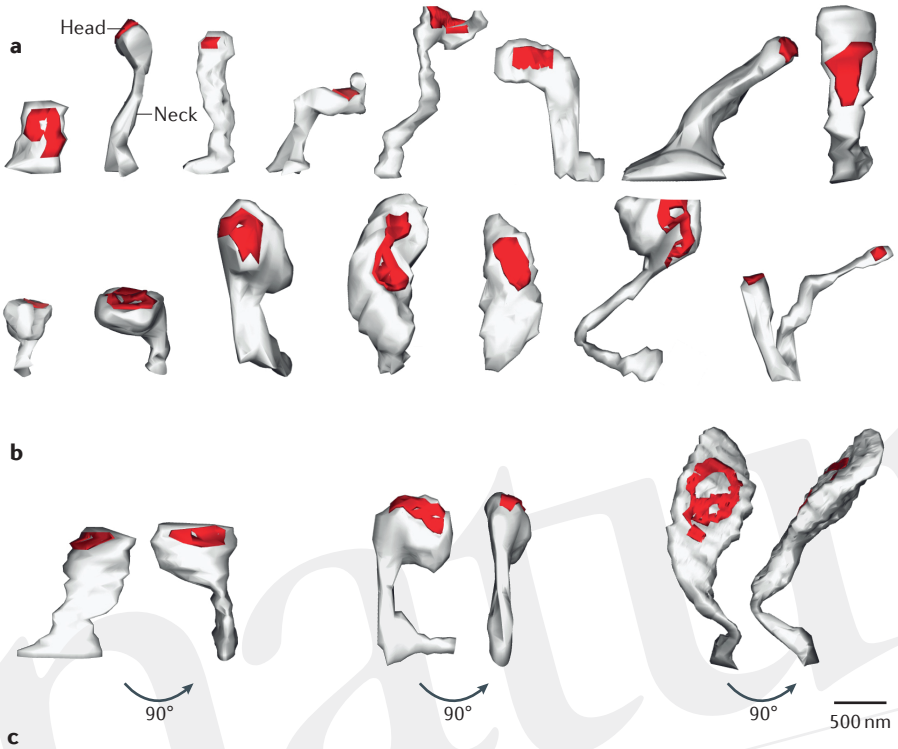


Figure 1 | **Geometric and morphological complexity of dendritic spines.** **a** | Three-dimensional ultrastructural reconstructions of spines from several layer 2/3 pyramidal cells in the mouse visual cortex. The large variability in the morphology of their heads and necks is apparent. Red areas mark the position of postsynaptic densities (PSDs). **b** | Rotational views of three spines, illustrating their morphological complexity and showing how the spine neck can become physically pinched. **c** | Physical obstruction of spine neck. The images show consecutive 4 nm thick serial sections cut through a dendritic spine from the mouse primary somantosensory cortex. The head of the spine is in the lower part of the images, and an asymmetric synapse can be seen (asterisk). The spine neck is open in the left section (arrow) but is obstructed by an intracellular electrodense organelle in the right section (red circle). Image size: 1500 × 1500 nm. Parts **a** and **b** are reproduced from REF. 51, Frontiers. Part **c** is courtesy of A. Merchan and J. De Felipe, Cajal Institute, Madrid, Spain.

junction or narrow passages). This large change in the electric field can markedly influence the flux of ions between the spine head and its neck and constitutes another breakdown of the classical cable theory for spine function.

It is also important to consider that, when cable theory is used as a general framework to calculate the electrical fields, current and other quantities of interest during the transient input, it is necessary to make certain assumptions, such as a non-zero current density at the channel boundary and often a rate of current change that is lower than the rate of ion relaxation-to-neutrality, a phenomenon that is not captured by cable theory. These assumptions are yet to be tested experimentally.

Taken together, these limitations make it clear that the geometry of the spine cannot be simply approximated by an ensemble of explicit capacitance and local known resistance. Molecular-level equations that account for the interaction between ion concentration and electric fields (an interaction that is ignored in traditional cable equations) are needed.

Modelling dendritic spines

With the advent of new imaging methods, it is now possible to record voltage responses in dendrites and spines during action potentials (FIG. 2). This opens new avenues for studying how changes in voltage propagate in these nanoscale neuronal compartments and means that better models will be required to describe such changes. What theoretical framework can be used to study the electrical properties of nanocompartments such as dendritic spines? One can use Monte Carlo simulations to explore the dynamics of the concentration of diffusing ions such as calcium in spines[24–26]; however, as described above, there are no simple theoretical frameworks that capture the physical dependencies between ion concentration and electric fields.

Electrodiffusion and the PNP equation. The diffusion of charged particles, such as ions, in an electric field (electrodiffusion) can be studied using the Fokker–Planck equation (equation 1), which uses Ficks's diffusion law to describe a situation in which the diffusing particles are under the influence of both an ionic concentration gradient and an electric field. In the Fokker–Planck equation (equation 1), the first term represents the concentration gradient and the second term the force generated by the electric field:

$$\frac{\partial p}{\partial t} = D(\Delta p + \frac{ze}{kT}\nabla(pU)) \quad (1)$$

in which p is the concentration of the species, D its diffusion coefficient, ~~∇~~ is the Laplacian operator, z is the valence, e is the elementary charge, k is the Boltzmann constant, T is the temperature, ~~Δp~~ is the gradient and U is the electric field **[Au:OK?]**.

This basic equation is further developed as the mean-field Poisson–Nernst–Planck (PNP) approximation (BOX 1), which consists of two coupled equations: the Fokker–Planck equation, which describes the density of charge for any ion (such as sodium, potassium and calcium) inside any microdomain; and the classical Poisson equation of electrostatics (~~written originally by Maxwell),~~ which describes the electric potential generated by the moving charges[4,27]. These two coupled partial differential equations can be resolved only algebraically for some simple cases, otherwise

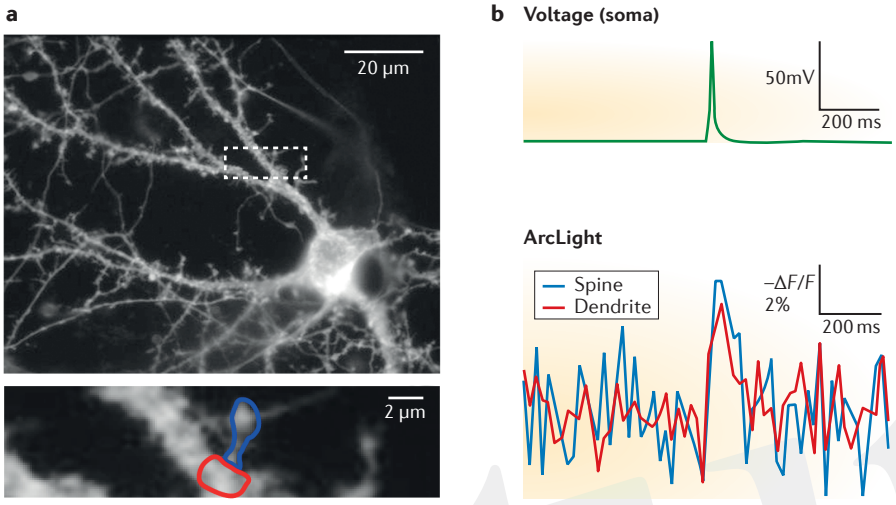


Figure 2 | **Imaging local ~~voltage changes~~ in a dendritic spine.** **a** | The top panel is a fluorescent image of a cultured mouse hippocampal neuron expressing the genetically encoded voltage indicator ArcLight. The bottom panel provides a higher magnification view of the dendrite (blue) and spine (red) in the boxed region. **b** | ~~The average optical waveforms of~~ the ArcLight ~~responses~~ in the dendrite and spine, and simultaneous somatic electrophysiological recording of action potentials (green) induced by a 20 ms current injection. This experiment illustrates the use of optical measurements of membrane potential from small neuronal subcompartments such as dendritic spines and dendritic shafts. These data could be used to quantitatively explore and model the role of electrodiffusion and the influence of the local geometry of nanocompartments in the propagation of electrical signals. ~~Figure is courtesy of~~ M. Sakamoto, ~~Columbia University, New York, USA.~~ **[Au: Please define ΔF/F]**

numerical simulations are necessary. The PNP approximation has recently been successfully applied to model glutamate diffusion in a two-dimensional (2D) synaptic cleft[28], providing predictions that were supported by experimental evidence[29,30]. These equations have also been used to study, for example, the ionic fluxes through the complex potential wells generated in an ion channel pore as well as ion channel selectivity in various conditions in which the channels were approximated as nanotubes[4,31–34]. Indeed, simulations using PNP equations could predict the experimental results in a certain number of channels[35].

Applying the PNP equation to dendritic spines. Although spines are much larger than ion channels, pioneering work developed a 1D electrodiffusion approach to model the spine's electrical properties, demonstrating the limitations of cable theory[36]. By approximating Poisson's equation for the electric field using a membrane capacitance and under the assumption that the membrane voltage is proportional to the ionic concentration, numerical simulations of ionic motion in the different compartments (spine head, neck and shaft) were possible on a timescale of milliseconds.

These results showed that spine morphology can regulate electrical propagation and electrodiffusion. However, the membrane voltage simplification did not allow for spatial changes in the electrical field. For example, at the entrance of the narrow spine neck, large changes in the electric field can be predicted that prevent charges from returning to the head once they enter the neck (which would not be the case if diffusion alone was operating)[37]. Some other measurements and theories of spine neck resistance[22,38] make the same implicit assumption that a diffusing ion, as it enters the neck, can return to the spine head an infinite number of times[21,23]. However, the neck diffusion time constant (τ) **[Au:OK?]** is given by formula shown in equation 2 (REF. 23), in which L is the spine neck length, a the radius, V the volume of the spine head and D the diffusion coefficient, and thus this diffusion time constant cannot be used to define the electrical resistance of the neck, because it reflects diffusion within the entire spine, including the head.

$$\tau = \frac{LV}{\pi a^2 D} + \frac{V}{4aD} + \frac{L^2}{2D} \qquad (2)$$

To properly understand how ions diffuse through the spine neck, it is necessary to consider the effect of the electric field. Traditional diffusion theory predicts that the distribution at equilibrium of ions in a bounded three-dimensional (3D) region, such as an isolated spine head, should be uniform (FIG. 3). However, if one uses an electrodiffusion framework that does not make an assumption of local electroneutrality, adding an electric field should alter the distribution of ions, which now become concentrated near the membrane surface (FIG. 3e,f), owing to the sum of repulsive forces between ions. Thus, as a particle driven by electrodiffusion crosses a 3D structure in high concentration of ions, its motion will preferentially occur along the ~~surface~~ membrane. This leads to different situation to that of pure diffusion, in which the motion occurs across the entire volume. In addition to the differences in ionic concentration, the capacitance of a dielectric ball (BOX 2) does not necessarily change in a linear manner with the total number of electric charges contained in the ball. This represents another significant deviation from the classical elementary electrostatic theory for conductors in which the charge is controlled by voltage through the capacitance. Finally, the movement of large proteins carrying a charge that are located in the membrane layers and are disturbed by a local ion current, such as that generated by the entry of ions from voltage calcium channels, could be substantial near the thin dendritic spine neck membrane, where the electric field is the largest in a dendritic spine[39].

In summary, narrow passages or small openings between microcompartments can affect the electrical field strength. Moreover, inside a nanocompartment, the change in ionic mobility **[Au:OK?]** could transiently create an excess of p… tive charge in the electrolyte that affects the motion and distribution of the charges and any travelling transient current. It may be crucial to take into account all of these effects to understand the refined electrical properties of dendritic spines.

Geometry matters. The diffusion of ions in small compartments is not only altered by the electric field; the exact shapes and geometries of the compartments are also crucial. For example, for most spines, the spine neck does not really resemble a simple tube, but is a tortuous passage that can expand and contract, creating obstructions due either to 'pinching' (the apposition of plasma membranes) or to 'plugging' by organelles such as the spine apparatus or endoplasmic reticulum[40] and other intracellular membranous structures (FIG. 1c). In these situations, ionic flow in and out of the spine could be significantly compromised or altered. Moreover, nanodomains of membrane lipids

Box 1 | **Poisson–Nernst–Planck equations for a dendritic spine**

To model the voltage change in a dendritic spine when a steady current (*I*) is injected at the spine head, we start with the stationary Poisson–Nernst–Planck (PNP) equation for the spine domain (Ω). This model describes the motion of charged ions in a dendritic spine. The electrical properties of the boundary (∂Ω) of the equation are modelled as a dielectric of permittivity (ε, in which ε_0 is the vacuum permittivity). At the boundary, there is no flux of the electric field nor flow of ions. The remaining part of the boundary, which is assumed to be a circular disk of radius, is grounded and absorbs ions. Thus, the coupled system for the electric potential and concentration of single positive ion species consists of the Poisson equation (equation 3) and the Nernst–(Fokker)–Planck equation (NPE; equation 4):

$$\Delta\phi = \frac{zep}{\varepsilon\varepsilon_0} \text{ in } \Omega, \quad \left.\frac{\partial\phi}{\partial n}\right|_{\partial\Omega_r} = 0, \quad \phi|_{\partial\Omega_a} = 0 \tag{3}$$

$$\frac{\partial p}{\partial t} = D\left(\Delta p + \frac{ze}{kT}\nabla(p\nabla\phi)\right) \text{ in } \Omega, \quad \left.\frac{\partial p}{\partial n} + \frac{ze}{kT}p\frac{\partial\phi}{\partial n}\right|_{\partial\Omega_r} = 0, \quad p|_{\partial\Omega a} = 0, \tag{4}$$

$$\left.\frac{\partial p}{\partial n} + \frac{ze}{kT}p\frac{\partial\phi}{\partial n}\right|_{\partial\Omega_{PSD}} = \frac{I}{\pi a^2}$$

in which *D* is the diffusion coefficient, *k* is the Boltzmann constant, *z* is the charge number, e is the electron charge, n is the normal unit vector at the boundary, ∇ is the Laplacian operator, *T* is the temperature, θ is **[Au: please add definition]** and Δ*p* **[Au:OK?]** is the concentration gradient **[Au:OK?]**; the last boundary condition is that current *I* is injected at the postsynaptic density (PSD), which is of size *a* **[Au:OK?]**. When several positive ions are considered, the set of equations becomes more complicated.

or proteins, particularly those that have an overall electrical charge, could alter the flow of electrical currents. At these nanometre scales, the proximity of membranes creates what is essentially a 2D or even a 1D physical system, the characteristics of which may not always be consistent with a macroscopic 3D view of the spines. A similar situation was found in the field of nanoscience, in which exploration of nanotubes and other physical systems with ~~small~~ dimensions revealed a new set of laws and rules[41].

Finally, as described above, the electroneutrality assumption that is inherent in classical theories allows many model equations, such as the cable theory, to be simplified. However, it is not clear that this assumption is satisfied in a situation in which a transient current is carried by charged molecules or ions or during a steady-state regime in which a current is continuously injected. Indeed, if this assumption is not made it can be shown that there can be long-range electrostatic interactions over distances much larger than the Debye length and thus a molecule's motion can be approximated as Brownian and insensitive to any electrical field~~).~~ These interactions can generate heterogeneity in the electric field and ionic gradient distribution. This situation could be particularly important in dendritic spines in which the small diameter and large length imposes large constraints on ionic distributions.

Extending cable theory

As was the case for traditional cable theory, solving the PNP equations for electrodiffusion could lead to the description of ion dynamics in nanostructures in steady-state and transient regimes. For example, using cable theory, the electrotonic length of a dendrite was derived as a direct function of basic parameters such as the membrane and intracellular resistance[6]. Using these analytical solutions, Rall derived the 3:2 power law of branching, which described the distribution of currents at a node at which a dendrite divides into three branches and has been influential in morphological and physiological studies of dendrites[42]. Exploring the parameter space of a model such as this by analytical or numerical simulation approaches is possible and allows the study of different regimes. A similar approach could be taken to explore PNP approximations at the nano- or micrometre scale level.

The main difficulty in extending cable theory to nanostructures is the fact that the PNP equations are nonlinear and much harder to solve analytically than the traditional, linear macroscopic cable theory. For example, in the case of a small opening in a sphere (as in spines) one can apply the 'narrow escape' theory[37] (which describes how small openings control diffusion fluxes in complex domains) to the PNP equations. This shows that, in contrast to the predictions of classical electrostatic theories of conductors, when the total charge (*Q*, equal to the number of charged particles present **[Au:OK?]** is enclosed in a ball (BOX 2) and we ~~do~~ not assume electro-neutrality below hundreds of nanometres (that is, the charges in the ball are not screened after few nanometres), the difference of voltage between the centre and the membrane of the ball increases in proportion to log *Q* and not linearly, as predicted by the classical law $U = Q/C$, where *U* is **[Au:please add definition]** and *C* is the capacitance. Interestingly, for this simple configuration the distribution of ions (which can be estimated mathematically) is not uniform at the steady state but shows an accumulation at the membrane (FIG. 3). However, the distribution of ions in a transient regime remains too difficult to be derived exactly. In the case of a transient regime, numerical simulations and design coarse-grained simulations can be used to estimate the voltage potential and distribution of charged particles in compartments with various geometries.

When PNP equations are applied to dendritic spines (with no ionic flux and no electric field on the membrane boundary outside the entrance of the neck and the spine channels) new predictions can be made about the effect of geometry on the regulation of electrical current flow. First, PNP equations predict that the voltage inside the spine (between the centre of the spine and the entrance of the neck) should saturate as the injected current increases. Second, PNP equations predict that ions will be concentrated at the surface of the spine when the spine head is isolated and the current leak through the neck is slow. Finally, a large electric field at the spine neck–head junction is predicted. When it becomes possible to test these predictions experimentally, we will find out whether a change in the geometry (neck length or diameter) affects the drop in voltage that occurs between the spine head and the dendritic shaft. Indeed, changing the spine geometry ~~would~~ lead to different charge distributions and local voltage changes that can affect the entire spine. Such a change in the voltage drop can be compared to the effects of adding or removing receptors from the synapse during synaptic plasticity. For example, when glutamatergic receptors are removed from the postsynaptic density, the electric current and the change in voltage in the dendritic shaft following a synaptic input will decay. We predict that a similar decay can be achieved by modulating the spine geometry; the exact changes should be estimated by the PNP equations. We suggest that changing the

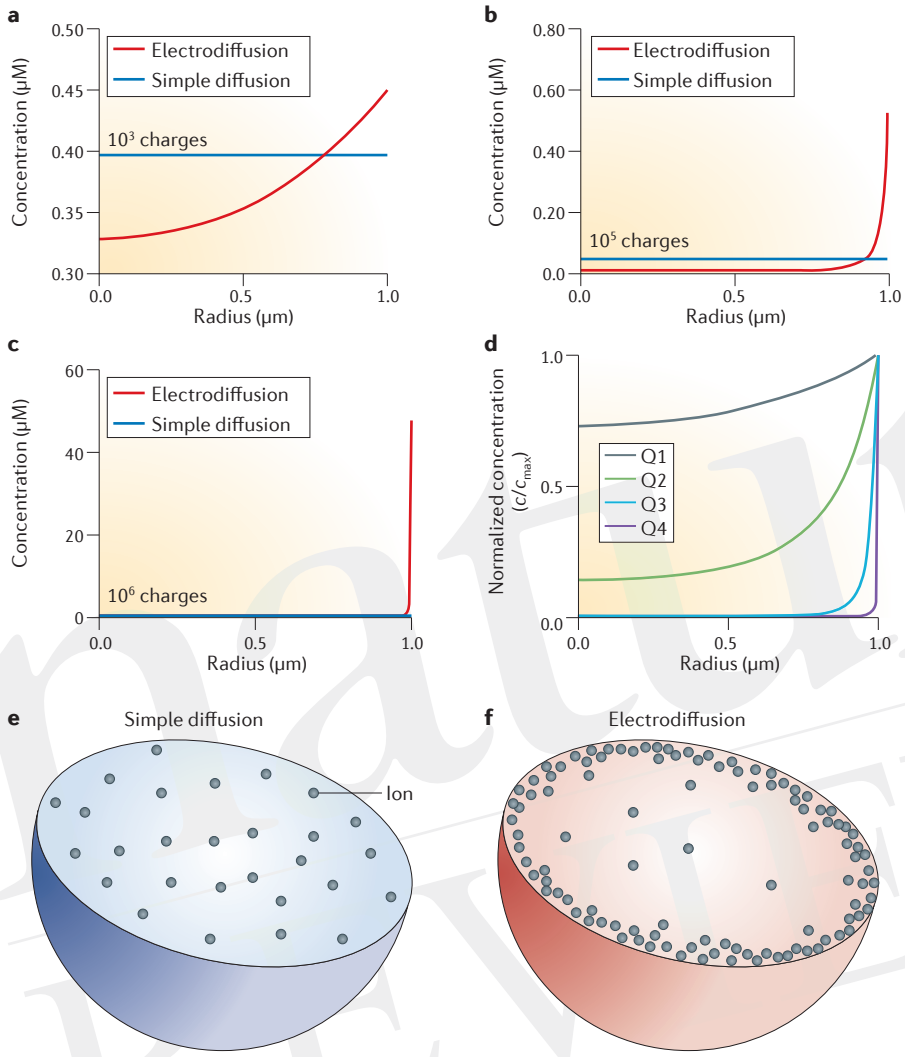


Figure 3 | **Comparison of simple diffusion and ~~electro-diffusion~~ theories.** Traditional diffusion theories and electrodiffusion theories make very different predictions about the distribution of ions within a three-dimensional structure such as a dendritic spine head. **a–d** | The change in the distribution of electrical charges with different total concentrations of ions at equilibrium, predicted by solving the Poisson–Nernst–Planck (PNP) equation for a sphere of 1 μm radius (red lines). The graphs show the concentration of ions at each position at the sphere's radius. As the total charge injected into the sphere increases from 10^3 in part **a** to 10^6 in part **c**, the charge progressively accumulates at the boundary. Part **d** summarizes this change: Q1 = 10^3 charges, Q2 = 10^4, Q3 = 10^5, Q4 = 10^6. This is in contrast with the predictions of the diffusion model (blue lines), in which the concentration of the diffusing particle is uniform throughout the sphere. **e–f** | Schematic illustration of these differences. In part **f**, the source of the electric field is an ensemble of steady-state charges (see BOX 2). **[Au: please define c/cmax]**

spine geometry may provide a mechanism for regulating synaptic plasticity without changing the number of receptors.

Despite the advances that may be made using this model, we note that the PNP equation-based theory that we discuss here is still a simplification. A full 3D model would also need to account for the interaction of water with all ionic species and the effect of local charges carried by ions. This more complete model and the simulations that it would enable could become numerically plausible as we develop new methods of modelling and algorithms that can consider molecular dynamics at a micrometre scale.

Applications: spines and beyond

If neuroscientists had access to a cable theory that incorporated electrodiffusion, they could explore many aspects of the physiology of neuronal compartments in quantitative detail. For example, a better understanding of the electrical properties of dendritic spines could have important repercussions for our understanding of synaptic plasticity, as it could help to explain how changes in spine geometry affect their electrical properties and modulate synaptic efficacy[17,19,38,43]. Even if changes in the size of postsynaptic potentials are mostly explained by a change in the number or location of postsynaptic receptors, they could still be modulated by altering the spine neck length and radius or the connection between the head and the neck. A theoretical understanding of these properties will help to clarify the relationship between the structure and function of dendritic spines.

Similarly, a 'new' cable theory that incorporates electrodiffusion and the PNP equation could be used to understand the electrochemical coupling that occurs in presynaptic terminals. These structures are similar in size to dendritic spines, and also have peculiar geometrical constraints, such as the small synaptic surface onto which the synaptic vesicle can dock[44]. It is likely that the large changes in the electric field that are associated with action potentials, and the existence of charged molecules bound to the vesicles, could influence, for example, the diffusion of calcium ions from the plasma membrane as they reach the vesicle, bind to synaptotagmin and result in vesicle fusion and neurotransmitter release.

Although most neurophysiologists measure electrical signals from the somata and large primary dendrites, axons and secondary or tertiary dendrites in most mammalian neurons are very thin, with diameters that can be smaller than 1 micron[45]. This makes it likely that geometrical constrains and electrodiffusion could matter.

In addition to these neuronal compartments, ~~astroglia~~ seem to carry out most of their interesting functions using their narrow processes, which are in close apposition to neuronal membranes and can even enter synaptic clefts[46]. These processes can have diameters of less than a hundred nanometres and are starting to be explored using modern imaging techniques.

Another type of structure in which local geometry makes it necessary to consider electrodiffusion are the thin cilia present in olfactory receptor neurons or in cochlear hair cells[47]. These cilia have diameters of less than a hundred nanometres and dimensions approximating thin spine necks. Their physiological roles rely on their electrical properties, as they regulate sensory transduction through chemoelectrical or mechanoelectrical coupling.

Box 2 | **Non-classical behaviour of membrane capacitance in a spherical nanocompartment [Au:Please reduce heading to fit on a single line]**

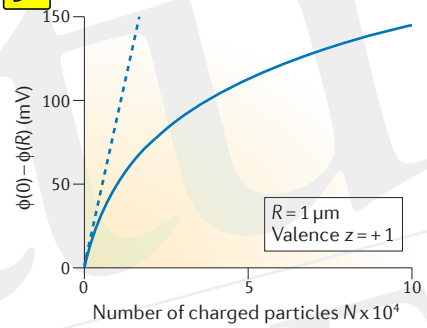
The classical electrical capacitance law states that $Q = CV$, in which Q is the total charge (sum of all charge carried by ions), C is the capacitance and V the voltage. This law is valid for classical electrostatic media but breaks down when applied to a dielectric ball. In such a situation, when there are large numbers of charge particles the relationship between total charge and voltage is not linear, but instead follows the law $V = \log Q$. This result is obtained by solving the nonlinear Poisson–Nernst–Planck (PNP) equation (see BOX 1) at steady state.

This results in a Poisson equation for the electric field that is the solution of the Laplace equation with an additional exponential term and a second equation that is the boundary condition at the membrane of the ball (equation 5).

$$\Delta\phi = -\frac{Q}{4\pi\varepsilon\varepsilon_0}\, exp(-\phi) \quad \text{in } \Omega, \quad \left.\frac{\partial\phi}{\partial n}\right|_{\partial\Omega r} = \frac{Q}{4\pi\varepsilon\varepsilon_0} \qquad (5)$$

in which ε is the dielectric ~~of~~ permittivity **[Au:OK?]**, ε_0 is the vacuum permittivity, φ is **[Au:please add definition]** and Ω is the spine domain. **[Au:OK?]**

The electrical properties of a dielectric medium in a structure such as a spine head cannot be properly derived using traditional models of electrical membranes (see the figure). In the PNP model, no electric currents flows through the dielectric sphere: the x-axis is the difference of potential $\varphi(0) - \varphi(R)$, and the y-axis is the number of charged particles N such that $Q = eN$ (where e is the charge of an electron. The continuous line in the figure shows the solution of equation 5, and the dashed line is shows the classical relationship, $Q = CV$. R, **[Au: please add definition for R]**; z, valence **[Au: OK?]**.



Finally, an even stronger reason to extend cable theory to the nanoscale comes from invertebrate neuroscience[48]. In many invertebrate preparations, such as those of *Caenorhabditis elegans*, *Drosophila melanogaster* and *Hydra vulgaris*, most neurites have diameters of a few hundred nanometres at most. In this case, one could argue that, to understand how activity propagates across the neural network, traditional cable theory cannot be applied at all, ~~as already suggested[36].~~

A new nanophysiology ~~framework~~

The introduction of new imaging methods, such as two-photon and superresolution microscopies, are enabling experimental scientists to measure the physiological behaviour of small neuronal compartments in living preparations, in some cases with single molecular precision. For example, recent superresolution stimulated emission depletion (STED) microscopy has shown that the spine neck diameter can be resolved in living cells[49,50] and can change in response to activity[18]. These data have confirmed the functional importance of these biological nanostructures, and are starting to reveal a rich and often unexpected phenomenology. At the same time, a theoretical framework to tackle the neuronal nanoscale needs to be developed and will be necessary for a proper quantitative understanding of these systems, which have critical functions such as synaptic transmission and plasticity. Exploring broader theoretical formulations, such as the PNP equations for electrodiffusion, may allow neuroscientists to make predictions on the importance of particular molecules, design experiments to test key variables and eventually understand how form and function are intimately linked in biological systems at the nanoscale.

David Holcman is at the Computational Biology and Applied Mathematics (IBENS), Ecole Normale Superieure, 75005 Paris, Franc

Rafael Yuste is at the Neurotechnology Center, Departments of Biological Sciences and Neuroscience, Columbia University, New York, New York 10027, USA.

Correspondence to D.H.
e-mail: david.holcman@ens.fr

doi:10.1038/nrn4022
Published online XXXX

1. Goldman, D. E. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**, 37–60 (1943).
2. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544 (1952).
3. Bart, D. & Bart, J. Sir William Thomson, on the 150th Anniversary of the Atlantic Cable. *Antique Wireless Association Rev.* **21**, (2008).**[Au:Please provide a page range.]**
4. Hille, B. *Ionic Channels in Excitable Membranes* 2nd edn (Sinauer, 1992).
5. Tuckwell, H. C. *Introduction to Theoretical Neurobiology* (Cambridge Univ. Press, 1988).
6. Koch, C. *Biophysics of Computation: Information Processing in Single Neurons* (Oxford Univ. Press, 2004).
7. Stuart, G., Spruston, N. & Hausser, M. *Dendrites* (Oxford Univ. Press, 1999).
8. Butera, R. J., Rinzel, J. & Smith, J. C. Models of respiratory rhythm generation in the pre-Bötzinger complex. I. Bursting pacemaker neurons. *J. Neurophysiol.* **82**, 382–397 (1999).
9. Kennedy, M. B., Beale, H. C., Carlisle, H. J. & Washburn, L. R. Integration of biochemical signalling in spines. *Nat. Rev. Neurosci.* **6**, 423–434 (2005).
10. Malinow, R. & Malenka, R. C. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 (2002).
11. Sabatini, B. L. & Svoboda, K. Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* **408**, 589–593 (2000).
12. Fischer, M., Kaech, S., Knutti, D. & Matus, A. Rapid actin-based plasticity in dendritic spine. *Neuron* **20**, 847–854 (1998).
13. Dunaevsky, A., Tashiro, A., Majewska, A., Mason, C. A. & Yuste, R. Developmental regulation of spine motility in mammalian CNS. *Proc. Natl Acad. Sci. USA* **96**, 13438–13443 (1999).
14. Lendvai, B., Stern, E., Chen, B. & Svoboda, K. Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex *in vivo*. *Nature* **404**, 876–881 (2000).
15. Hoze, N. & Holcman, D. Residence times of receptors in dendritic spines analyzed by stochastic simulations in empirical domains. *Biophys. J.* **107**, 3008–3017 (2014).
16. Hoze, N. *et al.* Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging. *Proc. Natl Acad. Sci. USA* **109**, 17052–17057 (2012).
17. Araya, R., Jiang, J., Eisenthal, K. B. & Yuste, R. The spine neck filters membrane potentials. *Proc. Natl Acad. Sci. USA* **103**, 17961–17966 (2006).
18. Tonnesen, J., Katona, G., Rozsa, B. & Nagerl, U. V. Spine neck plasticity regulates compartmentalization of synapses. *Nat. Neurosci.* **17**, 678–685 (2014).
19. Araya, R., Vogels, T. P. & Yuste, R. Activity-dependent dendritic spine neck changes are correlated with synaptic strength. *Proc. Natl Acad. Sci. USA* **111**, E2895–E2904 (2014).
20. Rust, M. J., Bates, M. & Zhuang, X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat. Methods* **3**, 793–795 (2006).
21. Biess, A., Korkotian, E. & Holcman, D. Diffusion in a dendritic spine: the role of geometry. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **76**, 021922 (2007).
22. Svoboda, K., Tank, D. W. & Denk, W. Direct measurement of coupling between dendritic spines and shafts. *Science* **272**, 716–719 (1996).
23. Holcman, D. & Schuss, Z. Diffusion laws in dendritic spines. *J. Math. Neurosci.* **1**, 10 (2011).
24. Franks, K. M., Bartol, T. M. Jr & Sejnowski, T. J. A Monte Carlo model reveals independent signaling at central glutamatergic synapses. *Biophys. J.* **83**, 2333–2348 (2002).
25. Franks, K. M. & Sejnowski, T. J. Complexity of calcium signaling in synaptic spines. *BioEssays* **24**, 1130–1144 (2002).
26. Holcman, D., Schuss, Z. & Korkotian, E. Calcium dynamics in dendritic spines and spine motility. *Biophys. J.* **87**, 81–91 (2004).
27. Jackson, J. D. *Classical Electrodynamics* 3rd edn (Wiley, 1998).
28. Savtchenko, L. P., Kulahin, N., Korogod, S. M. & Rusakov, D. A. Electric fields of synaptic currents could influence diffusion of charged neurotransmitter molecules. *Synapse* **51**, 270–278 (2004).
29. Sylantyev, S. *et al.* Electric fields due to synaptic currents sharpen excitatory transmission. *Science* **319**, 1845–1849 (2008).
30. Sylantyev, S., Savtchenko, L. P., Ermolyuk, Y., Michaluk, P. & Rusakov, D. A. Spike-driven glutamate electrodiffusion triggers synaptic potentiation via a homer-dependent mGluR–NMDAR link. *Neuron* **77**, 528–541 (2013).
31. Corry, B., Kuyucak, S. & Chung, S. H. Dielectric self-energy in Poisson–Boltzmann and Poisson–Nernst–Planck models of ion channels. *Biophys. J.* **84**, 3594–3606 (2003).

32. Mamonov, A. B., Coalson, R. D., Nitzan, A. & Kurnikova, M. G. The role of the dielectric barrier in narrow biological channels: a novel composite approach to modeling single-channel currents. *Biophys. J.* **84**, 3646–3661 (2003).
33. Eisenberg, R. S. From structure to function in open ionic channels. *J. Membr. Biol.* **171**, 1–24 (1999).
34. Gillespie, D. *et al.* A physical mechanism for large-ion selectivity of ion channels. *Phys. Chem. Chem. Phys.* **4**, 4763–4769 (2002).
35. Blunck, R., Chanda, B. & Bezanilla, F. Nano to micro — fluorescence measurements of electric fields in molecules and genetically specified neurons. *J. Membr. Biol.* **208**, 91–102 (2005).
36. Qian, N. & Sejnowski, T. J. An electro-diffusion model for computing membrane potentials and ionic concentrations in branching dendrites, spines and axons. *Biol. Cybern.* **62**, 1–15 (1989).
37. Holcman, D. & Schuss, Z. Control of flux by narrow passages and hidden targets in cellular biology. Reports on progress in physics. *Phys. Soc.* **76**, 074601 (2013).
38. Bloodgood, B. L. & Sabatini, B. L. Neuronal activity regulates diffusion across the neck of dendritic spines. *Science* **310**, 866–869 (2005).
39. McLaughlin, S. & Poo, M. M. The role of electro-osmosis in the electric-field-induced movement of charged macromolecules on the surfaces of cells. *Biophys. J.* **34**, 85–93 (1981).
40. Korkotian, E. & Segal, M. Synaptopodin regulates release of calcium from stores in dendritic spines of cultured hippocampal neurons. *J. Physiol.* **589**, 5987–5995 (2011).
41. Alivisatos, A. P. Less is more in medicine. *Sci. Am.* **285**, 66–73 (2001).
42. Rall, W. Branching dendritic trees and motoneuron membrane resistivity. *Exp. Neurol.* **1**, 491–527 (1959).
43. Harnett, M. T., Makara, J. K., Spruston, N., Kath, W. L. & Magee, J. C. Synaptic amplification by dendritic spines enhances input cooperativity. *Nature* **491**, 599–602 (2012).
44. Schikorski, T. & Stevens, C. F. Morphological correlates of functionally defined synaptic vesicle populations. *Nat. Neurosci.* **4**, 391–395 (2001).
45. Karube, F., Kubota, Y. & Kawaguchi, Y. Axon branching and synaptic bouton phenotypes in GABAergic nonpyramidal cell subtypes. *J. Neurosci.* **24**, 2853–2865 (2004).
46. Pannasch, U. *et al.* Connexin 30 sets synaptic strength by controlling astroglial synapse invasion. *Nat. Neurosci.* **17**, 549–558 (2014).
47. Peters, A. & Paley, S. L. & Webster, H. D. *Fine Structure of the Nervous System* (Saunders, 1976).
48. North, G. & Greenspan, R. J. *Invertebrate Neurobiology* (Cold Spring Harbor Press, 2008).
49. Nagerl, U. V., Willig, K. I., Hein, B., Hell, S. W. & Bonhoeffer, T. Live-cell imaging of dendritic spines by STED microscopy. *Proc. Natl Acad. Sci. USA* **105**, 18982–18987 (2008).
50. Ding, J. B., Takasaki, K. T. & Sabatini, B. L. Supraresolution imaging in brain slices using stimulated-emission depletion two-photon laser scanning microscopy. *Neuron* **63**, 429–437 (2009).
51. Arellano, J. I., Benavides-Piccione, R., Defelipe, J. & Yuste, R. Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies. *Front. Neurosci.* **1**, 131–143 (2007).

Acknowledgements

The authors thank members of both laboratories for their comments, and R.Y. thanks the Holcman group and the Ecole Normale Superieure for hosting him. R.Y. is supported by grants MH101218 and MH100561. This material is based upon work supported by, or in part by, the US Army Research Laboratory and the US Army Research Office under contract number W911NF-12-1-0594 (MURI). Research in D.H.'s laboratory is supported by the generosity of N. Rouach. The authors also thank J. Cartailler from the D.H. laboratory **[Au:OK?]**.

Competing interests statement

The authors declare no competing interests.

Author biographies **[Au: would you like to add links to personal or laboratory homepages?]**

David Holcman is a professor of computational biology and applied mathematics at Ecole Normale Superieure in Paris, France. He received a Ph.D. in Mathematics from Pierre et Marie Curie University in Paris, France. He pioneered the narrow escape theory, modelling receptor trafficking, diffusion laws in dendritic spines and stochastic methods for extracting features from super-resolution data. His domain of interest concerns biophysical multiscale modelling, analysis of stochastic and partial differential equations, statistical treatment of large data sets such as single-particle trajectories and the design of numerical simulations in neurophysiology.

Rafael Yuste is ~~a~~ professor of biological sciences and neuroscience at Columbia University, New York, USA, director of Columbia's Neurotechnology Center and co-director of the Kavli Institute for Brain Science ~~at Columbia University.~~ He obtained his M.D. at the Universidad Autónoma in Madrid, Spain, and his Ph.D. at Rockefeller University, New York, USA. He pioneered ~~several~~ optical imaging techniques in neuroscience and led the ~~researcher~~ team who proposed the ~~brain activity map~~, sponsored by the White House's BRAIN initiative, a decade-long effort to build tools to measure and modulate the activity of brain circuits. He is interested in ~~understanding~~ emergent properties of cortical circuits and the biophysics of dendritic spines.

ToC blurb

000

Regulation of ionic flow in neuronal subcompartments: the new nanophysiology

David Holcman and Rafael Yuste

Classical theories, such as cable theory, can successfully model signal propagation in neurons on a macroscopic scale. Holcman and Yuste argue that, as the functional importance of neuronal compartments such as dendritic spines becomes apparent, it is important to develop models that can account for the effects of their size and geometry on electrical current flow **[Au:OK?]**.

Subject categories

Biological sciences / Neuroscience / Computational neuroscience / Biophysical models
[URI /631/378/116/2392]
Biological sciences / Neuroscience / Spine regulation and structure / Spine structure
[URI /631/378/2597/2600]
Biological sciences / Neuroscience / Neuronal physiology
[URI /631/378/1697]