Reply to 'Only negligible deviations from electroneutrality are expected in dendritic spines'

David Holcman and Rafael Yuste

Our original Opinion piece described our intuitions about novel experimental and theoretical directions to study voltage at a nanoscale from a few to hundreds of nanometres in cellular nanodomains to microdomains (The new nanophysiology: regulation of ionic flow in neuronal subcompartments. *Nat. Rev. Neurosci.* **16**, 685–692 (2015))[1]. We proposed that a new 'nanophysiology' is needed to interpret data that will be recorded in the future for channel–cytoplasm nanodomains, dendritic spines, axonal terminals, mitochondria, glia protrusions, neck–head junctions and other nanostructures with large fluctuations in membrane curvature. As we pointed out, traditional cable theory assumes that concentration changes associated with ionic currents are negligible and, therefore, ignores electrodiffusion (that is, the interaction between electrical fields and ionic diffusion). This assumption, although true for large neuronal compartments such as the original squid giant axon, could be incorrect when applied to femtolitre-sized structures such as dendritic spines. In his correspondence (Only negligible deviations from electroneutrality are expected in dendritic spines. *Nat. Rev. Neurosci.* https://doi.org/10.1038/s41583-019-0238-x (2019))[2], Barbour ignores the wider scope of our argument and the recently published literature and narrowly reduces the focus of our Opinion piece[1] to our discussion of electroneutrality, claiming that it is confusing and not relevant. However, the main goal of our article was not to deny electroneutrality but, instead, to explore the possibility that a complete set of electrodiffusion equations might be more suitable to model ion dynamics and voltage inside cellular nanodomains than traditional cable theory. Below, we respond to Barbour's main criticisms. Note that we have already addressed most of these points in detail in a response that is available online[3]. Moreover, our recent modelling based on data from voltage-sensitive dyes and nanopipette recordings actually confirms our intuitions about significant concentration effects in dendritic spines[4,5], demonstrating the need for electrodiffusional models.

Electrodiffusion and electroneutrality

Electrodiffusion is a theory that combines the Poisson (P) equation, which relates the local electrical field to the concentrations of ions, and the Nernst–Planck (NP) equation, which models the motion of ions with respect to the electrical field and concentration gradients. PNP equations thus form a coupled system of equations that can account for multiple ions, with different concentrations and motility, and can predict the local and transient changes in their concentrations. For simple geometries, the PNP model has been extensively used to model ionic fluxes inside channels, and the computation of their steady-state solution in reduced 1D geometries led to the well-known Goldman–Hodgkin–Katz formula of the reversal potential[6]. But for more complex neuronal morphologies, this coupled set of partial differential equations is unsolvable, and because of this, to untangle them, traditional cable theory assumes an infinite volume, with no changes in ionic concentration owing to electrical field changes.

Electroneutrality is the assumption that, at all spatial scales, the concentration of positive charges equals the concentration of negative charges. This assumption has never been measured and calls for meticulous experiments. In the idealized case described by Barbour, in which positive and negative ions are highly mobile, electroneutrality should be preserved except in a thin boundary layer (known as the Debye layer) near the membrane. We emphasize that we are not arguing against electroneutrality in these conditions. Indeed, we have actually observed it numerically[4,5] and studied it mathematically[7]. That said, even in electroneutral conditions, PNP equations have revealed that a large ion influx in femtolitre compartments, such as occurs following synaptic activation on dendritic spines, should nevertheless lead to important transient changes in ion concentration[4,5,7]. In fact, in many biological nanodomains, such as inside dendritic spines, the concentration of mobile chloride ions may not counterbalance the mobile positive ions (essentially potassium, sodium and free calcium ions). The 300 mM ions described by Barbour should be decomposed into ~150 mM positive, mobile ions (~18 mM Na^+, ~135 mM K^+ and ~0.0001 mM Ca^{2+}) and ~7 mM Cl^- ions and mostly negative charges located in membranes and almost immobile macromolecules. These differences in ion motility might result in important junction potentials (that is, local depletions in specific ion species), especially during transient synaptic activation, following an important influx of positive charges through AMPA-type glutamate receptors. In fact, even by using the same assumption as that used by Barbour (that is, there are equal amount of mobile positive and negative charges (~150 mM each)), we have demonstrated, both numerically[4,5] and analytically[7], that electrodiffusion effects are important except in the Debye layer. We emphasized these effects in our Opinion article and suggested that electroneutrality may break down at the tens of nanometre scale[1], as we later explored in several studies[8–12].

Boundary conditions matter

We are afraid that there is a misunderstanding by Barbour as Boxes 1 and 2 do not model the same biophysical situation, as they assume different boundary conditions. In Box 1, we modelled an influx of positive ions into a ball, representing ion flow after the opening of receptors in a dendritic spine head, where charges would be reflected except at a small absorbing window that would model the entry of the ions into the spine neck[4,13]. In Box 2, presented solely for pedagogical purposes, we instead described a simplified ideal case of a closed ball containing one species of ion, as a model of a local excess of a positive charge in water, the results of which were confirmed by further simulations for the ball and other geometries[8–13].

For Box 2, the distribution of charge can be directly computed by solving the PNP equations at steady-state; that is, the Poisson–Boltzmann equation. The solution is influenced by the boundary. Interestingly, the charges accumulate at the boundary, and their distribution decays with distance with a log profile[4–12]. Moreover, we have found that this situation should hold even when a small fraction of negative charges is added (see Appendix of REF.[10]). Thus, Box 1 focuses on a transient situation in which the boundary is divided into an absorbing part $\partial\Omega_a$, where charges can escape, and a reflecting part $\partial\Omega_r$, where the flux of charges is zero. By contrast, Box 2 reflects a steady-state situation where the charges are trapped and the boundary is purely reflecting. Concerning the electrical potential, in Box 1, we grounded the absorbing part of the boundary $\varphi_{|\partial\Omega_a} = 0$, which would approximate a very short

neck with negligible resistance[4], and set the electrical field to zero on the reflecting part $\frac{\partial\varphi}{\partial n}\Big|_{\partial\Omega_r} = 0$. The latter condition models an ideal capacitor where the permittivity of the membrane bilayer would be zero[2]. In Box 2, as there is no absorbing boundary, the Gauss compatibility condition imposes that the electrical field at the reflecting boundary is non-zero $\int_{\partial\Omega_r}\frac{\partial\varphi}{\partial n}dS = -\frac{Q}{\varepsilon_0}$. We highlight that the boundary condition in Box 2 cannot be obtained by reducing the absorbing boundary surface to zero in Box 1, because the problem becomes singular[12,13].

Redefining capacitance

In idealized electrolytes as described by Barbour, with equal ionic concentrations and equal mobility, charges in excess accumulate close to the membrane boundary, and except in that thin boundary layer, the bulk solution is isopotential and electroneutral[4]. In that case, the electrical potential inside the ball is nearly constant and increases linearly with the number of charges $\varphi(r) \approx \varphi = \frac{Q}{C_m}$, with C_m the total membrane capacitance. In our model in Box 2, where negative charges would be immobile at the membrane, the ball is no longer isopotential[8–11]. As a matter of fact, there is no longer a simple linear relationship between the total number of charges and the electrical potential, which classically subtends the definition of the membrane capacitance. Therefore, we did not compute the membrane capacitance but the difference in potential between the centre of the ball and the surface $\varphi(r=0) - \varphi(r=R)$, which increases nonlinearly with respect to the number of charges. This is in contrast to classical surface capacitance. This effect is interesting and we found it in other cases, such as fluctuation of the membrane of a dendrite, in which there were further specific effects on the difference of potential between two points, depending on the local curvature[9].

Nanophysiology is happening

Four years after our original Opinion piece[1], it seems to us, and to others[19], that 'nanophysiology' is not only a thought experiment but also on its way to revealing novel biophysical properties, such as voltage distribution in small neuronal nanocompartments. Indeed, since our article was published, a series of follow-up publications and reviews have confirmed the importance of expanding cable theory by taking into account electrodiffusional effects when modelling dendritic spines and other small cellular compartments[5,7–11,14–19], leading to new predictions, such as the existence of postsynaptic facilitation due to ionic accumulation during excitatory postsynaptic potentials[5], which could be tested experimentally.

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Competing interests

The authors declare no competing interests.