Answer to B. Barbour fiction-blog about

A perspective article:

"The new nanophysiology: regulation of ionic flow in neuronal subcompartments"

Nat. Rev. Neurosci. 16/685–92. doi: 10.1038/nrn4022

D. Holcman and R. Yuste

INTRODUCTION:

In his blog-fiction the experimental physiologist B. Barbour expresses his opinion about various subjects from politics to some sciences. He decided to re-iterate some comments about a perspective that we published 3 years ago (Nat. Rev. Neurosci. 16/685–92. doi: 10.1038/nrn4022). The first fiction to notice is that the author introduces himself as an imaginary reviewer. In reality, this presentation is misleading and this is not how scientists conduct themselves. Indeed referring a manuscript is usually anonymous and authors are invited to write criticism when justified.

Moreover, in the past years, we have already answered several times the comments of Mr Barbour about our Perspective published in 2015, directly by long emails, in pubmed, and through lab meeting organized in our Institute for students. Unfortunately, many of the comments of Mr Barbour showed a clear lake of professional knowledge in modeling, applied mathematics, simulations and theory of biophysics.

The goal of our perspective was to stimulate and to present a novel molecular approach to study the Voltage-Current relation, which is crucially missing in modern physiology at the nanometer scale. The attacks of Barbour on our perspective are often misleading because, as we shall see below, some classical concepts are not properly understood and his terminology is not used with appropriate meaning. In other cases, they are expressed in terms of classical electrical engineering concepts, which represent a coarser scale approach to physiology.

Our goal in that perspective, was to show that (as expressed in the title) classical resistance and capacitance are insufficient to describe accurately the voltage-current relation at the nanoscale physiology.

We now give our point-by-point response, but before we must recall the scientific classical terminology.

Lexica:

Solution of a Partial differential equation: a **partial differential equation** (**PDE**) is a differential equation that contains unknown multivariable functions and their partial derivatives. PDEs are used to formulate problems involving functions of several variables, and are either solved by hand, or used to create a computer model. A solution is a function that satisfies the PDE and boundary conditions. **https://en.wikipedia.org/wiki/Partial_differential_equation**

Boundary condition: In mathematics, in the field of differential equations, a **boundary value problem** is a differential equation together with a set of additional constraints, called the **boundary conditions**. A solution to a boundary value problem is a solution to the differential equation which also satisfies the boundary conditions. https://en.wikipedia.org/wiki/Boundary_value_problem

Electroneutrality: "In most quantitative treatments of membrane potential, such as the derivation of Goldman equation, **electroneutrality** is assumed", it is not derived.

https://en.wikipedia.org/wiki/Resting_potential

Debye length: is a measure of a charge carrier's net electrostatic effect in solution and how far its electrostatic effect persists. It is derived under two assumptions:
1- systems that are electrically neutral at all spatial scale
2- The field is not too large (lineriation of the exponential).
There are no Debye length concept in non-electroneutral medium.

Insulator: "An **electrical insulator** is a material whose internal electric charges do not flow freely; very little electric current will flow through it under the influence of an electric field." Wiki

https://en.wikipedia.org/wiki/Insulator_(electricity)

Conductor:" In physics and electrical engineering, a **conductor** is an object or type of material that allows the flow of an electrical current in one or more directions. Materials made of metal are common electrical conductors. In order for current to flow, it is not necessary for one charged particle to travel from the machine producing the current to that consuming it. Instead, the charged particle simply needs to nudge its neighbor a finite amount who will nudge its neighbor and on and on until a particle is nudged into the consumer, thus powering the machine. electrons are the primary mover in metals" https://en.wikipedia.org/wiki/Electrical_conductor

Electrolyte: "An **electrolyte** is a substance that produces an electrically conducting solution when dissolved in a polar solvent, such as water. The dissolved electrolyte separates into cations and anions, which disperse uniformly through the solvent. Electrically, such a solution is neutral.**"**

https://en.wikipedia.org/wiki/Electrolyte

Curve fitting[1][2] is the process of constructing a curve, or mathematical function, that has the best fit to a series of data points,[3] possibly subject to constraints. https://en.wikipedia.org/wiki/Curve_fitting

Capacitance: is the ratio of the change in an electric charge in a system to the corresponding change in its electric potential.

The capacitance is a function only of the geometry of the design (e.g. area of the plates and the distance between them) and the permittivity of the dielectric material between the plates of the capacitor. For many dielectric materials, the permittivity and thus the capacitance, is independent of the potential difference between the conductors and the total charge on them.

The capacitance of the majority of capacitors used in electronic circuits is computed at surfaces.

PNP: Poisson-Nernst-Planck theory: it is coarse-grained model for describing ion transport, not necessarily at equilibrium or not necessarily assuming electroneutrality (developed by several group, including B. Eisenberg).

Blogs:

The central aim of the perspective is to suggest that revolutionary ionic and electrical behaviour will be identified and understood if we no longer apply the classical constraint of electroneutrality when modelling electrodiffusion in neurones. However, the voltages available in vivo (~100 mV maximum) make it impossible to generate significant deviations from electroneutrality, at least in structures of the scale of spines. For a sphere delimited by typical membrane (with specific capacitance 1 µF cm-2) and typical spine radius (0.25 µm), we can calculate the number of electronic charges transferred when charging by 100 mV (~5000) and compare it to the number of charges contained in the sphere with 300 mM ions (~12 million). The ratio of net/total charges is thus ~0.0004. Furthermore, most of those excess charges will be largely neutralised as part of the membrane capacitance. This shows why, for spines and related structures, electroneutrality remains a very accurate approximation. A consequence of the difficulty of driving deviations from electroneutrality is that the net charges of Fig. 3b and c would be unattainable in real life.

>ANSWER: **As already discussed 3 years ago, I am not sure that Mr Babour has understood Fig 3: We are not computing the capacitance of a sphere, but we are solving the PNP equation with one charge to explore the Voltage profile in 3 cases. This is a classical approach in modeling to explore a range of the parameter space to see how the solution of an equation behaves in physiological ranges, but also in extreme cases.**

Fig3a-c: illustrates the difference between solution of the diffusion equation (flat concentration) versus the solution of the PNP. These new computations are tedious, but have been made explicit in Cartaill et al, Physical D 2016.

In addition, we demonstrated recently that the present theory introduced in this perspective about PNP does apply to real data and that the classical cable theory cannot account for voltage-concentration changes during a current transient in a dendritic spine: see Cartaill et al, Neuron 2018 and also the recent paper of B. Ros.

More specifically, the notion of capacitance (see lexica) applies to surface (capacitance between two two-dimensional plates,etc..) but not to three-dimensional volume, thus we had to extend this concept to a ball, by solving the PNP equation (see Cartailler et al, Physica D 2016) . Thus the above discussion misses the point because it does not apply to

volume. We have in addition shown that the effect of adding surface capacitance (Figure in the SI 9 of Cartailler, neuron 2018) is negligible on voltage prediction.

We recall that the aim of the present perspective was to attract attention on the nanoscale from few to hundreds of nanometers, including channel-cytoplasm nanodomains, mitochondria, glia protrusions and many more. We think that the new nanophysiology is currently revealing novel mechanisms about biophysical processes in physiology. The group of D. Rusakov has published over the past 15 years several result about electro-diffusion in the synaptic cleft (see ref list), showing how the electric field influences the motion of neurotransmitters. In the 80s, M. Poo and S. Lauglin already demonstrated that an endogenous electric field could drive membrane proteins. There are many of these examples.

Both intracellular and extracellular solutions in mammals contain about 150 mM of both positive and negative charges.

>**ANSWER: This statement is unclear and misleading. Clearly the chloride concentration does not counter balance potassium+sodium+free calcium, so this statement is certainly not supported by ions, which are the main fast messengers for electrical conduction.**

Indeed, in mM we have

Na+ 18

K+ 135

Cl- 7

Ca++ 0.0001

The goal of our perspective was precisely to discuss this effect, that we may not have electroneutrality at the tens of nanometer scale, because negative proteins cannot counter balance the excess of positive charges at any length scale, because they move much slower than ions. So we have proposed in this perspective that the electro-neutrality HYPOTHESIS should be revisited.

The presence of such huge numbers of positive and negative charges would greatly influence the behaviour of the small numbers of net charges, but the "background" ions have simply been omitted from the simulations in the article.

>ANSWER: The assumption of this perspective is to explore the effect of many more positive ions than negative so the framework we are exploring is not what is summarized in this comment. We do have here in the background the same amount of positive and negative ions. This has been made explicit from the beginning of the perspective. The purpose is thus to express the consequences of an excess of positive charges. To insist, one more time, and contrary to the present statement, according to the physiological concentration, there are no negative ions that are counter balancing the positive ions.

What are they? so no electrodiffusion is expected at tens of nanometer as discussed in that perspective. Again, the solution of PNP can be found in Cartaill et al, Physica D, 2016 and more recent reference cited below.

The authors have in effect simulated a few charges moving within an insulator, instead of a conductor.

>ANSWER: We have recall above the definition of the following terminology, which is not used appropriately. There are no conductors outside metal and/or semi-conductor. Physiology deals with electrolytes: which are ions in water, the theory of which remains difficult (see also the recent review from Rusakov and M. Poo).

The applicability of the insulator to real life is zero. Looked at another way, the high ionic strength of physiological solutions induces strong electrostatic screening on the scale of the Debye length, which is less than 1 nm under physiological conditions. This screening is completely absent from the simulations here.

>ANSWER: This point has already been discussed above: we are promoting here the idea of non-electro-neutrality and expressed the consequences on the ideal example like a ball. Indeed, electroneurotrality assumes that at all scale the concentration of positive charge== concentration of negative charge. This fact is not supported by ions concentration, indeed [chloride] is not equal to sum of concentration of potassium, sodium and free calcium. We think, that negative charges comes from proteins that do not move at the speed of ions, so electroneutrality must be violated at the scale of nano to tens of nanometers, thus concept like Debye length (see lexica), based on small voltage and electroneutrality cannot be applied, see article given below.

The concentrations in Fig. 3 are obviously incorrect, at least in panels 3b (where the mean concentration should be 40 µM) and 3c (where the mean concentration should be 400 µM).

>ANSWER: We indeed noticed that there is an obviously typos, mu M should be milli M, that after investigation, has been introduced probably in the final step of the converting our manuscript into proofs (see attached, where our submitted manuscript contains the correct mM units).

Submitted figure:

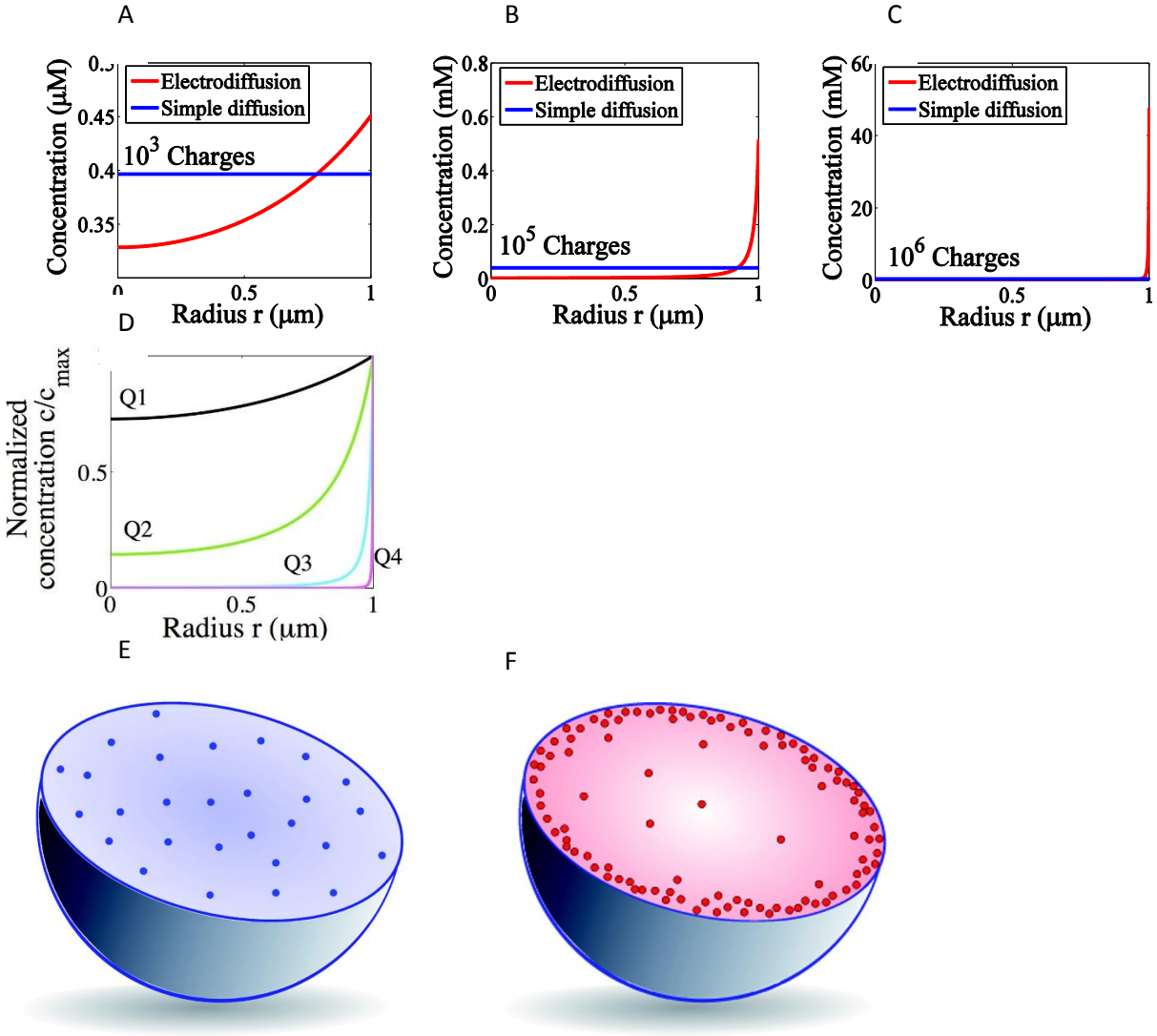

Figure 3: Comparison of simple diffusion and electro-diffusion theories. Traditional diffusion theories and electrodiffusion theories make very different predictions about the distribution of ions within a three dimensional structure such as a dendritic spine head **[Au:OK?]**. **A-D**|. The change in the distribution of electrical charges at equilibrium, predicted by solving the PNP equation for a sphere of 1 μm radius (red lines) **'Concentration' on the y-axis refers to the concentration of ions in the ball[Au: please clarify what 'Concentration' on the y axis refers to. Could 'Charges' be changed to 'charged particles' for clarity?OK]**. As the total charge injected into the sphere increases from 10^3 in panel A to 10^6 in panel C, the charge progressively accumulates at the boundary (Panel D summarizes this change: Q1=10^3 charges, Q2=10^4, Q3=10^5, Q4=10^6). This in contrast with the predictionsvof 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 F, the source of the electric field is an ensemble of steady state charges (see figure 4).

It is unclear how the red curves were calculated to fit these erroneous values.

> ANSWER: there is no fitting procedure here (see lexica for the definition of what is a fit): this red curve is the solution of the steady-state diffusion in a ball, which is the trivial line (the value of which has to be corrected for the typos introduced during the final stage of the proofs, see above).

The simulations of Fig. 3 were carried out exclusively for the perspective, but several aspects are not specified or are ambiguous. Bizarrely, the boundary conditions of Box 1 imply strict electroneutrality.

>ANSWER: this statement is incorrect. There cannot be electroneutrality (see lexica) with a single positive ion. At least two species are needed: one positive and one negative.

In Box 2, the boundary condition does imply a net charge (i.e. a deviation from electroneutrality), but appears to be incorrect. I believe it should contain R2 in the denominator (although the numerical value might be 1 µm, the units need to be compatible). The calculated voltage may therefore be incorrect.

>ANSWER: Box 2: the boundary condition comes from the compatibility condition (Gauss theory of electrostatic, see Feynmann's text book): integrating the charge inside the domain is the flux of the voltage. It does NOT imply a net flow of charge, see chapter 10 of our textbook:

Holcman, David, Schuss, Zeev, Asymptotics of Elliptic and Parabolic PDEs and their Applications in Statistical Physics, Computational Neuroscience, and Biophysics https://www.springer.com/fr/book/9783319768946

It is not clear what is incorrect. In our field, if a formula is incorrect, it should be said where, otherwise vague statement does leave a professional impression:

In general, the boundary condition (see lexica) involves 1/R^2 (where R is the radius of the ball), as mentioned in formula 10.7 of our book or 7 of Cartailler et al, J. Physisca D 2016,

But here R=1, so we do not need this term, because R^2=1.

About our incorrect computation, either Mr Barbour should tell us what is incorrect and provide or publish his computations otherwise, he should probably stop professing unjustified statement with no justification, because obviously, this is not how science is done in our community. The published articles in the math and physics literature are listed below and provide detailed explicit computations.

The ambiguity about the precise simulations being carried out in Fig. 3 and Box 2 should therefore be resolved. For completeness, the particle diffusion coefficient and the relative permittivity should be specified.

>ANSWER: As mentioned in our previous correspondences with Mr Barbour, **all parameters have already been summarized in table 1 of Cartailler et al, J. Physisca D 2016 and on page 360: table 10.1 of the textbook Holcman, David, Schuss, Zeev Asymptotics of Elliptic and Parabolic PDEs and their Applications in Statistical Physics, Computational Neuroscience, and Biophysics**

https://www.springer.com/fr/book/9783319768946

The simulations in Fig. 3 and Box 2 (apparently) contain no membrane, so the title of Box 2 confuses by purporting to investigate the membrane capacitance.

>ANSWER: The membrane in the simulation is modelled by a boundary value problem (lexica). It turns out that the effect of the membrane capacitance does not modify the result. This simulation was performed in SI figure 9 of Cartailler et al, Neuron, 2018 (ref below). We think that this subtitle is Ok.

In Box 2, the authors describe an apparently new and exciting result regarding nonlinearity of the membrane capacitance in a nanocompartment. As already stated, there is no membrane in the simulation.

> ANSWER: Mr Barbour keeps repeating himself: as mentioned already above, the effect of the membrane is incorporated in the boundary condition of the ball. The effect of membrane capacitance was discussed in SI figure 9 of Cartaill et Neuron, 2018.

Moreover, the behaviour is "non-classical" not because of the nanocompartment but because the authors have used a "non-classical" definition of the capacitance: measured from the centre of the sphere to its boundary, rather than to infinity.

> **ANSWER:yes: this is actually the only interesting point of this entire conversation: the notion of capacitance had to be re-defined or extended for an electrolyte in a limited volume, because we cannot use the notion of capacitance developed for a surface (see Feynmann's text book). This was also the goal of this perspective.**

It is of no practical application. For instance, were it to be applied in electrostatics, the classic isolated sphere would have zero capacitance.

>ANSWER: There is not necessarily an immediate measurement of a new concept, but we think that this approach should motivate the community to look at an experimental approach to measure this volume capacitance. A direct prediction is that membrane curvature creates voltage drop.

GENERAL CONCLUSION:

We hope that present didactic presentation would be helpful to alleviate the frustrations of Mr Barbour about imaginary mistakes we did or could have done in the last part of our perspective. We already answer similar comment publically 3 years ago. This review, written by a non-specialist in modelling, revealed a lack of understanding. In addition, obviously, Mr Barbour is not following the proper literature, where some of his questions were already answered few years ago. We invite him to read carefully the published research articles mentioned below. Finally, based on the present questions, we are little bit worry about the teaching activity of Mr Barbour especially about explaining electricity at the molecular scale.

Here, we agreed to play the game of answering these TV-style comments for the community, but Mr Barbour should adopt a proper scientific attitude. We do not think that this conversation is bringing anything substantial to improve the field of nanophysiology.

However, we need a serious effort to better understand how to study the I-V at tens of nanomerter resolution. In the past 3 years, we have not seen any constructive efforts from Mr Barbour to publish anything in that direction except his fiction blog. If capable, Mr. Barbour should contribute to this new area, like all of us by publishing research articles on that subject in the appropriate professional literature.

In addition, we have and are organizing meeting about electro-diffusion by gathering professional in this field such as Nanoscale mathematical modeling of synaptic transmission, calcium dynamics, transduction and cell sensing http://www.crm.sns.it/event/423/

and we welcome Mr Barbour to engage into a similar activity or to register to learn the basics.

We have now attached a list of peer reviewed publications about the understanding of I-V relation in nano- and microdomains, disseminated in the fields of neurobiology, chemistry, physics, biophysics and applied mathematics, which support the need of a new theory of nanophysiology, promoted in our perspective.

References:

J Cartailler, T Kwon, R Yuste, D Holcman, Deconvolution of Voltage Sensor Time Series and Electro-diffusion Modeling Reveal the Role of Spine Geometry in Controlling Synaptic Strength, Neuron 97 (5), 1126-1136. e10 2018

T Lagache, K Jayant, R. Yuste, Electrodiffusion model of synaptic potentials in dendritic spines, BioRxiv doi: https://doi.org/10.1101/274373 (revision Biophysical. J.) 2018

J Cartailler, D Holcman, Electrical transient laws in neuronal microdomains based on electro-diffusion, Physical Chemistry Chemical Physics, 32, 2018

J Cartailler, Z Schuss, D Holcman, Electrostatics of non-neutral biological microdomains, Scientific Reports 7 (1), 11269

Leonid P. Savtchenko, Mu Ming Poo & Dmitri A. Rusakov, Electrodiffusion phenomena in neuroscience: a neglected companion, *Nature Reviews Neuroscience* volume 18, pages 598–612 (2017).

J Cartailler, Z Schuss, D Holcman, Geometrical effects on nonlinear electrodiffusion in cell physiology, Journal of Nonlinear Science 27 (6), 1971-2000 2017

J Cartailler, D Holcman,Voltage laws for three-dimensional microdomains with cusp-shaped funnels derived from Poisson-Nernst-Planck equations arXiv preprint arXiv:1710.02423

J Cartailler, Z Schuss, D Holcman, Analysis of the Poisson–Nernst–Planck equation in a ball for modeling the Voltage–Current relation in neurobiological microdomains, Physica D: Nonlinear Phenomena 339, 39-48
2016

Finally, the mathematical modeling and analysis of the new theory have been summarized in a text book:

D Holcman, Z Schuss
Asymptotics of Elliptic and Parabolic PDEs: and their Applications in Statistical Physics, Computational Neuroscience, and Biophysics, Springer
Chapter 10.

https://www.springer.com/fr/book/9783319768946

see

https://www.springer.com/fr/book/9783319768946

Most of the curves presented in our perspective have been reproduced in that text book (ch. 10).