Answer to B. Barbour fictional review-blog about

A perspective article:

“The new nanophysiology: regulation of ionic flow in neuronal subcompartments”

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INTRODUCTION:

Dr Barbour recently (July 2018) posted comments on his blog (The electroneutrality liberation front, referee3) criticising a perspective that we published in 2015 (Nat. Rev. Neurosci. 2015 16/685–92. doi: 10.1038/nrn4022). He had in fact previously posted similar comments in PubMed Commons on 12/25/2016. Despite Dr Barbour’s claim, we did not ignore his comments but answered them in Pubmed on 12/26/2016 and also through direct interactions with him, through long email chains and lab meeting organized in our Institute for students. Unfortunately, Dr Barbour judged all these interactions fruitless and we thus respond here again to his criticisms.

The goal of our perspective was to stimulate and to present a novel theoretical approach to study membrane biophysics, which, we think, is crucially missing in modern physiology at the nanometer scale. We appreciate the concerns of Dr. Barbour and thank him for his frankness and for the time that he has spent on our work. But we alert the reader that our publication was not a proper experimental nor theoretical study, but an opinion piece with a very limited set of data, which were added strictly to illustrate the main points, and did not represent a comprehensive or final analysis of electrodiffusion in spines, something which has started to unfold in our publications since them (see references). Our review should be viewed precisely as what it is, a "Perspective", highlighting new and potentially controversial topics. The role of electrodiffusion in nanophysiology has been traditionally ignored by cable theory because of the difficulty to analytically solve the corresponding joint equations and to connect with experimental data at this scale. Moreover, the interaction between electric fields and diffusion and geometry are not easy to dissect because they are highly nonlinear and sometimes counter intuitive and consequently difficult to discuss without adequate computational simulations. We, and others, are exploring this potentially important phenomenon with simulations and theoretical studies, some of which have already been published (see references), and which will hopefully address all the concerns of Dr. Barbour. We encourage the reader not to jump to quick dismissive conclusions but to be patient and wait for further works as this field unfolds. In the past three years, many news evidences coming from experiments using Voltage dyes and theoretical approaches have confirmed clear deviations between classical diffusion and electro-diffusion.

Also, we would highlight that the comments of Dr. Barbour on our perspective are often misleading because most of his reasoning is expressed in terms of classical electrical engineering concepts, which represent a coarse grain scale approach to physiology than our own approached, focused at the nanoscale. Our goal in that perspective, was to show, as expressed in the title, that classical resistance and capacitance are insufficient to describe accurately current-voltage relations in cellular nano-compartments. Because of this, and the sometimes lax use of the terminology by Dr. Barbour, we have defined it precisely at the end of the piece, to avoid confusion.

Response to "The electroneutrality liberation front referee3" Blog:

Barbour: 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.

Answer: *The main goal of our perspective was actually not to assume electro-neutrality but instead to explore the possibility that complete electro-diffusion equations rather than simplified cable theory might be more suitable to model ion dynamics and voltage inside cellular nano-domains. In this theoretical treatment, we questioned not one but three main assumptions of cable theory which are*

- ***the cylindrical geometry*** *which is far from obvious for many compartments such as dendritic spines*
- ***the constant and uniform concentration of ions****. Indeed, large ion influx in femto-liter compartments, such as synaptic activation on dendritic spines, should lead to important transient changes in ion concentration and their spatial distribution. This hypothesis, which was already questioned in the previous theoretical paper of Qian & Sejnowski (Biological Cybernetics, 1989), has been recently demonstrated with simulations (Cartallier et al. Neuron 2018) and with experimental measures using fluorescent sodium indicators (Miyazaki & Ross, J. Neuroscience 2017)*
- *and finally,* ***the electro-neutrality*** *assumption which might arise from the different mobilities of ions and that we extensively discuss hereafter. We also would argue that there is very little direct experimental confirmation of electroneutrality at the nanoscale, so we should not assume it dogmatically.*

Barbour: 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.

Answer: *This calculation is misleading as it neglects ion dynamics. Indeed, the chloride concentration does not counter balance potassium+sodium+free calcium. So, 300 mM ions should rather be decomposed into ~ 150 mM positive, mobile ions*

(Na+ ~ 18 mM, K+ ~ 135 mM and Ca++ ~ 0.0001 mM), ~ 7 mM chloride ions and mostly negative charges located in membranes and almost immobile macro-molecules. These differences in ion motility might result in important junction potentials (i.e. local depletions in specific ion species), especially during synaptic activation and important influx of positive charges. 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 charge at any length scale, because they move much slowler than ions. So we have proposed in this perspective that electro-neutrality should be revisited.

Barbour: 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.

Answer: *Again, due to steric effects, mobile positive ions might not be able to undefinitely pile up on membrane and macro-molecules. Thus, a part of exceeding positive ions (without negative counterpart) might be freely diffusing. This excess would be even more pronounced during excitatory response and positive ions influx.*

Barbour: 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 with Dr. Barbour in the past 3 years, we are not computing the capacitance of a sphere in Fig. 3, but we are solving the PNP equation with only positive charges to explore the voltage profile in an extreme case of non electro-neutrality. This is classical approach in modeling to explore a large range parameter space and see how the solution of an equation behave in physiological range but also in extreme cases.*

Fig3 a-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 Cartallier et al, Physical D 2016. More specifically, the notion of capacitance (see lexica below) 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 Cartallier et al, Physica D 2016). Thus the above discussion misses the point because it does not apply to volume.

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. Laughlin already demonstrated that an endogenous electric field could drive membrane proteins. There are many of these examples.

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

***Answer:** As discussed previously, 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.*

Barbour: 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 goal of this perspective was 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. To insist, one more time, and contrary to Dr. Barbour statement, according to the physiological concentration, there are no negative ions that counter balance the positive ions, so electro-neutrality might not hold locally.*

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

***Answer:** We have recalled below (**Terminology list below**) the definition of the following terminology, which is not used appropriately. There are no conductors outside metals 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).*

Barbour: 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:** We are promoting here the idea of non-electro-neutrality and expressed the consequences on the ideal example like a ball. Indeed, electroneutrality assumes that at all scale the concentration of positive charge is equal to the 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 (**Terminology**), based on small voltage (compared to energy fluctuations) and electroneutrality cannot be applied.*

Barbour: 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**: That was a typo (mu M should milli M) which was introduced during one of the stages of the manuscript's proofs (see figure attached with correct units)*

Original Submitted figure:

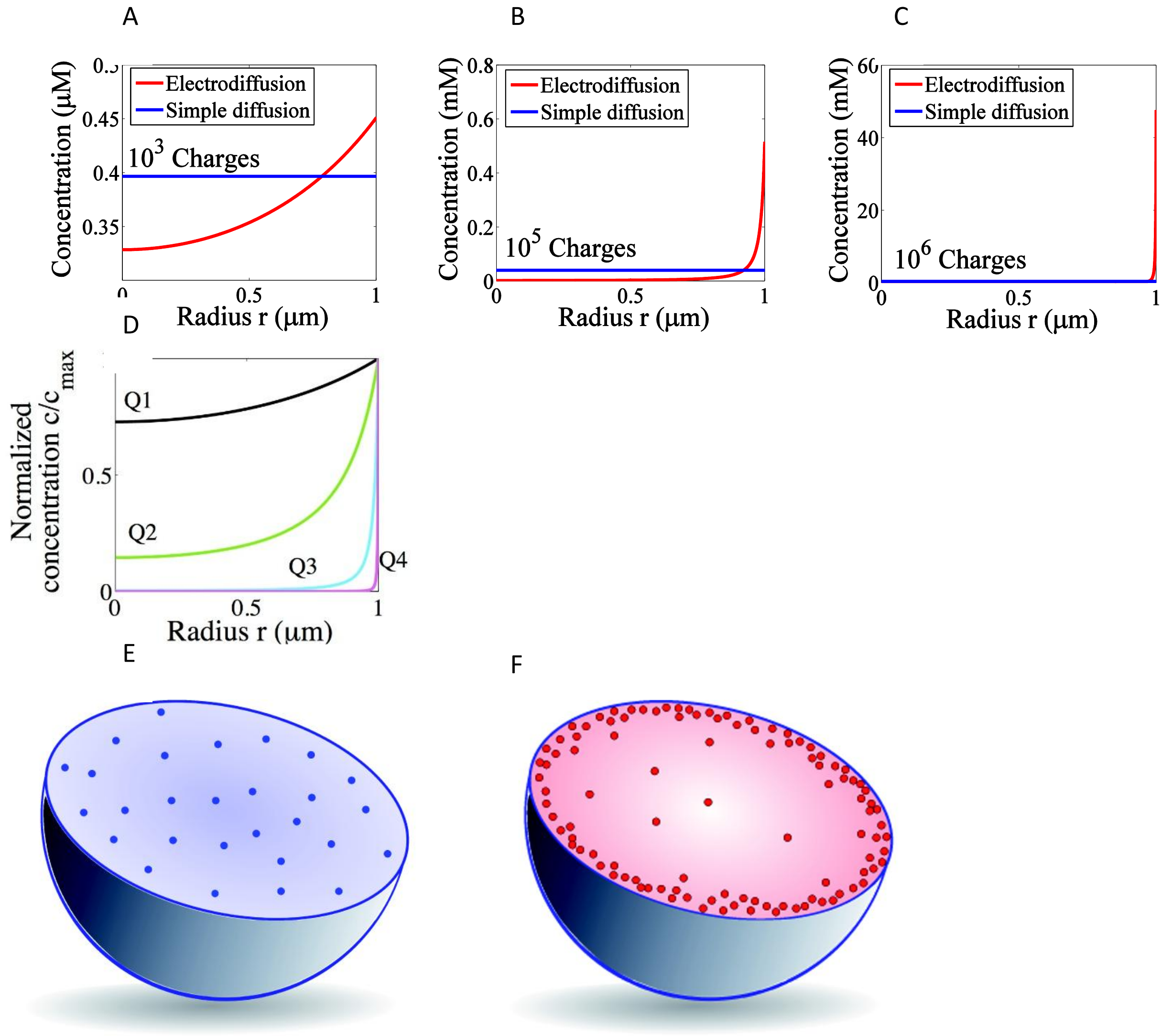

Figure 3: Comparison of simple diffusion and electro-diffusion theories. *Traditional diffusion theories and electrodiffusion theories make very different predictions about the distribution of ions within a three dimensional structure such as a dendritic spine head.* ***A-D****. The change in the distribution 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. 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).*

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

Answer: *there is no fitting procedure here: 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).*

Barbour: 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 (****Terminology****) with a single positive ion. At least two species are needed: one positive and one negative (here for simplicity, there is no absorption condition at the spine neck and no flux through the PSD, that we studied later on. Fig. 3 shows a simple Steady-State for a fixed number of charges that equilibrate in a ball).*

Barbour: 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: *We voluntarily omitted the R^2 in the denominator as it is equal to 1. Complete formula is given by* ***formula (10.7) of our book or (7) of*** *in Cartailler et al, J. Physica D 2016. or Holcman-schuss, Textbook Springer 2018.*

Barbour: 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 Dr Barbour, all parameters have been summarized in table 1 of Cartailler et al, J. Physisca D 2016 and also 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

Barbour: 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. 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: *The membrane in the simulation is modelled by a boundary value problem (****Terminology****): Boundary condition is given at the right-hand side of equation 5, and results from integration of the Poisson equation in a ball (spine head).*

Barbour: 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: *Indeed, 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 Lectures in Physics). This was also the goal of this perspective.*

Barbour: 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.*

Conclusion:

We hope that we have now answered Dr. Barbour's concerns. We would like to emphasize that the main goal of this perspective was to sensitize the neuroscience community to the importance of using first-principles physics and complete electro-diffusion equations when dealing with ionic fluxes in nano-compartments. Indeed, most of the classical physiology has been developed for larger compartments (especially for the giant squid axon used by Hodgkin & Huxley in the 60's) where local ion flux do not change significantly ion concentrations, and where only global electro-neutrality matters. These simplifications, necessary back then since researchers lacked proper tools for large numerical stimulations, are not pertinent nowadays.

We believe that our 2015 review was timely and stimulating regarding the number of recent theoretical studies, and new experimental observations in dendritic spines that show the important changes in ion concentration (Miyazaki & Ross, J. Neuroscience 2017) and voltage (Jayant et al. Nature Nano. 2017) during spontaneous and evoked synaptic activity. 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.

Finally, since Dr. Barbour appears quite interested in these topics, we encourage him to pursue them giving them proper research treatment, and, as we do, present to the community his ideas, supported by proper research, and publish them in peer-reviewed, scientific publications, rather than blogging in his own web page. As another venue for discussion, we have organized a meeting this fall on electro-diffusion "Nanoscale mathematical modeling of synaptic transmission, calcium dynamics, transduction and cell sensing" http://www.crm.sns.it/event/423/ and we encourage Dr Barbour to engage in similar activities for the open discussion of these ideas.

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).

Terminology used

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 (linearization 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

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 in the context of physiology by several groups, including B. Eisenberg, B. Roux, Z. Schuss, A. Singer,etc...).