Answer to Matters Arising "One equation, two unknowns,

J. Cartailler and D. Holcman

History:

We already answered in November 2018 in our website a similar text entitled "The electroneutrality liberation front, referee 3" posted as a fiction-Blog by Dr. B. Barbour https://referee3.org/2018/08/24/one-equation-two-unknowns/ , about our manuscript "Deconvolution of Voltage Sensor Time Series and Electro-diffusion Modeling Reveal the Role of Spine Geometry in Controlling Synaptic Strength" Neuron. 2018 Mar 7;97(5):1126-1136.e10. doi: 10.1016/j.neuron.2018.01.034. Epub 2018 Feb 8 by J. Cartailler, T. Know, R. Yuste and D. Holcman.

Summary:

We would like to emphasize the importance of electro diffusion when analyzing the electrical properties of electrolytes in femto-liter compartments such as dendritic spines. In a classical electrical engineering approach, electricity in conductors is studied by a combination of terms like resistance, capacitance, etc..., which are the building blocks of classical electrical circuits and formulated with linear algebra or linear differential equations.

However, electrophysiological media are electrolyte-based, neither conductors nor insulators, and are better modeled by nonlinear partial differential equations, not with linear algebra. Thus the classical electrical engineering approach becomes limiting to describe the current flow at a nanoscale (Holcman & Yuste Nat. Rev. Neuro. 2015; Savtchenko et al, Nat. Rev. Neuro. 2017). Important questions such as the intrinsic resistance of an electrolyte, or the role of the shape etc... remain, only partially answered using traditional linear formulations, which assume macroscopic volumes where concentration effects are negligible.

But, although the two-way interplay between ionic concentration and voltage is not easy to model and simulate solving systems of coupled nonlinear partial differential equations, it can be still computed at the molecular level using two fundamental equations of physics, the Poisson & Nernst-Planck equations, which capture the effect of ionic diffusion on the electric field and viceversa. Moreover, investigation of electrical and chemical properties of nanometric biological units is also an experimental challenge that has driven many technical innovations in the past few years, such as the super-resolution microscopy and the development of voltage sensitive dyes and nano-fluidics tools. If necessary, we present at the end a lexica that defines all the used terms, in our previous answer to Dr. Barbour's comments.

Dr. Barbour: **One equation, two unknowns**

A paper from the group of David Holcman, Cartailler et al., (2018) [1], investigates the biophysics of dendritic spines by analysing fluorescence measurements of voltage-sensitive dyes during focal uncaging of glutamate and by electrodiffusion modelling. Complex analysis and optimisation procedures are reportedly used to extract an estimate of the spine neck resistance. However, examination of the procedures reveals that the resistance value is wholly determined by fixed parameter values: there is no extraction. The results are also potentially affected by errors in the modelling and unrealistic parameter choices. Finally, the paper highlights a potential dilemma for authors who share data—should they sign the resulting paper if they disagree with it?

ANSWER: We will detail hereafter our point-by-point response to Dr. Barbour's concerns but we would like to emphasize that our manuscript aimed at understanding the dynamic electrical response of a dendritic spine with first-principles physics. As the electrical response does depend on fluctuating ion concentration in these femto-liter compartments, we had to design a complex yet robust mathematical method to extract physical parameters such as the neck resistance or the ion current. We emphasize, again, that simplified and linear electrical circuits cannot account for the complex interplay between ion motion and voltage dynamics in complex-shaped dendritic spines. We actually proposed at the end of the manuscript that a non-linear diode is a much better idealization of a dendritic spine.

Dr. ***Barbour***: Before digging into the paper, it will be helpful to justify an approximate and very simple model of the spine, in which it is reduced to just the neck resistance. Spine experts can skip to the next section.

> ANSWER: We think that on the contrary, spine experts from modeling or experiments should look at this section carefully because it contains many assumptions that should not be made but questioned.

Dr. ***Barbour***:

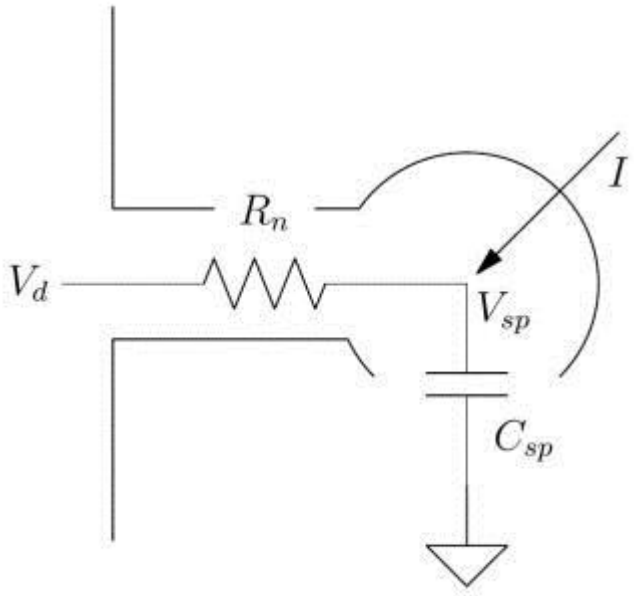


Fig. 1. Equivalent electrical circuit of a spine, with a spherical head and a cylindrical neck. The membrane resistance and neck capacitance have already been neglected, and we'll see that the head capacitance C_{sp} can be, too. Only the neck resistance R_n has any influence on single-spine biophysics. I, synaptic current, V_{sp} spine head voltage, V_d dendritic voltage.

Although spines display a degree of variability, we'll consider as typical a spine with a head of radius 0.3 µm, a neck of diameter 0.1 µm and length 1 µm. The key to attaining a useful intuitive understanding of spine behaviour is to clarify which electrical elements can be neglected. It is generally assumed that a spherical conductor can be well approximated as isopotential, unless particularly concentrated currents flow. So for now we'll consider the voltage throughout the spine head to be uniform (we'll re-examine this assumption below, in the light of the authors' results).

> ANSWER: Due to the submicron size of dendritic spines and the experimental difficulties to measure their electrical properties, the role of the dendritic spine geometry in filtering synaptic

potentials remains controversial. There are published articles arguing for and against this topic for at least 4 decades. Even if approximating the problem with a linear electrical circuit might account for (some) observed phenomena, our goal was to deduce electrical properties of spines with first-principles physics and simulations, and thus without "general assumptions". Conversely, the classical approach of Dr Barbour, that consists of reducing the electrophysiology problem to an equivalent electrical circuit is based on assumptions whose validity can only be asserted with either measurements (that are difficult and often require cumbersome deconvolution) or physics (our approach). In particular, his approach ignores the local changes in ion concentration or the important role of geometrical interfaces such as the PSD or the head-to-neck junction, which is exactly what we are interested in investigating. Actually, simulations of our model demonstrated that some of the hypothesis of Dr. Barbour's electrical circuit were (almost) valid (e.g. spine head is indeed a nearly ideal capacitor with almost uniform voltage except close to the PSD and the head-neck junction), and others were erroneous (e.g. neck resistance is actually dynamic and depends on ion current and concentration inside the spine head). Thus Fig 1 in essence is misleading and is not appropriate to model a spine as an electrolyte. In particular the figure do not distinguish the resistance of the head from neck or from the input resistance of the current, represented by an oblique arrow.

Dr. ***Barbour***: The narrowness of the neck means that most of the membrane is found in the head, so let's begin by calculating its surface area (ignoring the neck attachment): $4\pi r^2 \approx 1\ \mu m^2$. Given the specific membrane capacitance of 1 $\mu F\ cm^{-2}$, we obtain an estimate of the spine capacitance of $\sim 10^{-14}$ F. This is small and, as we shall see, can be neglected from most points of view. (Similar arguments also lead us to neglect the membrane resistance, which isn't shown, but not the synaptic conductance of the spine.) How much charge would be required to change the voltage of the spine head capacitance? For a round maximum of 100 mV, Q = CV gives 10^{-15} C, equivalent to 1 pA flowing for 1 ms. In other words, the current flowing through about one AMPA receptor channel is sufficient to charge the spine capacitance; typically there are tens to hundreds of receptors in a spine.

>ANSWER: We do not think that this computation makes much sense. First, the concept of capacitance as explained above in our response does apply for a conducting sphere (two-dimensions) in vacuum or in a dielectric, but not for an electrolyte in three dimensions. We recall that the empirical relation Q=CV works for a surface but not a 3d-ball. This relation is actually not a fundamental physical relation, but it is postulated (empirical), as an empirical law. It breaks down in many cases. We invite the interested reader to look at ref. 7 below, where we found that the relation Q=CV (which is used for two dimensional plates) cannot be applied here in a three dimensional ball or in electrolyte. Thus it is not clear what the meaning or interpretation of " spine capacitance" as it is an ideal representation: does that mean that the internal membrane surface of the spine retain ions? and why it would do so? why the ions are not flowing inside the spine volume. Thus it is not clear that capacitance plays any role in the bulk.

Dr. ***Barbour***: The highest estimates of spine neck resistance so far reported are about 1 GΩ. This would give an RC time constant of 10 μs. Thus, if the voltage in the parent dendrite changed, the spine would follow with this time constant. Conversely, if a constant synaptic current flows across the spine neck to the dendrite, the spine voltage will equilibrate to its new value with the same time constant. These relaxations are all quite fast, close to negligibly fast, on biologically relevant time scales.

> ANWER: Dr Barbour proposes to use the RC-approximation to estimate the time constant of equilibration of voltage. This is however a very coarse description because it assumes that voltage propagates along the surface, which is again an assumption insufficient to describe voltage propagation, which is carried by sodium ion propagation (see reference [8] for experimental data and clear difference in the propagation of sodium versus calcium). The time scale of sodium ions (carrying voltage changes) arriving in the dendrite is much longer than 10 µs, as proposed in the computation carried above under the RC-assumption.

Dr. ***Barbour***: From this we can conclude that the only electrical parameter of any significance to spine behaviour is the neck resistance. In the absence of a synaptic current, the spine voltage follows the dendritic voltage. When there is a synaptic current, the voltage across the neck resistance is determined by Ohm's law.

> ANWER: first, as we shown in the present manuscript a huge resistance drop is present at the entrance of the head due to local boundary effect, which is neglected in the RC-theory, thus it does not appear in the conclusion of Dr Barbour. However we found from simulations of electro-diffusion a large resistance drop at the entrance of the synaptic current. Second, this statement about assuming Ohm's law inside the head depends whether we consider or not constriction in the head, which is true for spine that contains a spine apparatus (see also [1]).

To conclude, if it is true that the charging time constant of spine head capacitor as a surface should be around tens of microseconds, which is small compared to the other time constants of the synaptic transmission (around milliseconds), the spine head geometry does shape incoming synaptic potentials and cannot be reduced to a plate capacitor. The time scale of voltage propagation inside the head remains an open question. Indeed, head volume determines the amplitude of fluctuations in ion concentration (and neck resistance!). Moreover, the geometry of the head-to-neck junction should be an important determinant of ions' exchange rate between the spine head and the neck (as discussed in Holcman & Schuss, Diffusion laws in dendritic spines, Journal of Mathematical Neurosciences, 1(1) 10 (2011)).

Dr. ***Barbour***: (It should be noted, however, that the collective contribution of spine capacitance to dendritic and cellular capacitance can be very significant; for instance, spines contribute about 80% of the total capacitance of cerebellar Purkinje cells.)

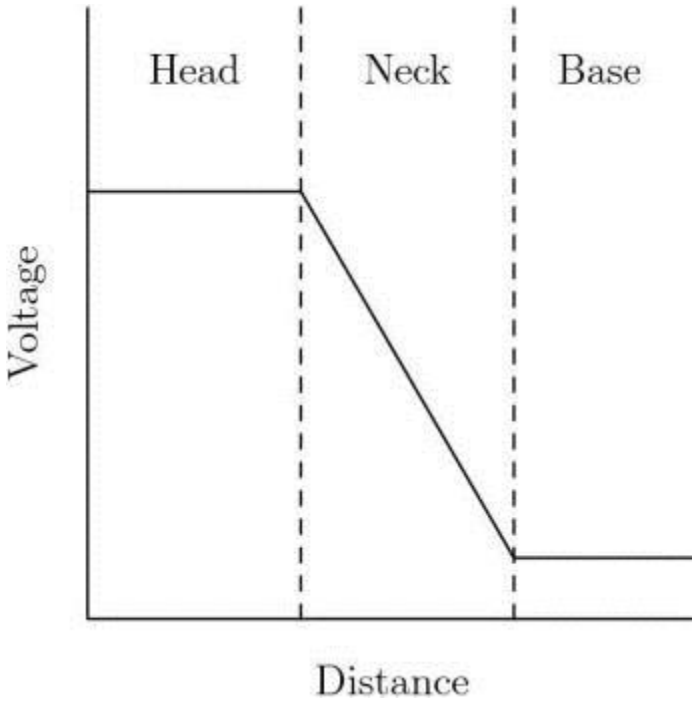


Fig. 2. When a synaptic current flows, the approximate voltage profile expected in a spine is uniform across the head and linear down the neck.

Armed with the uniformity of the head voltage and the fact that the neck resistance is the only significant electrical component of the spine,

>ANSWER: This assumption of uniform distribution of voltage of the head is only correct in the bulk far away from the current entrance and the neck-head junction, which are actually critical location for I-V relation.

Indeed, it well known that injecting a current in an electrolyte is facing an input resistance due to the migration of charges toward the current. As we have shown in our article this create a significant drop penetrating to 50-100nm inside the bulk.

To conclude, the " uniformity of the head voltage **"assumption should be rejected for the reasons mentioned above.**

Dr. ***Barbour***:: a very usable approximation for the voltage profile in the spine during the synaptic current is shown in Fig. 2: the head will be at a uniform voltage, more depolarised than the dendrite, and there will be a linear decline of voltage between head and base.

ANSWER: fig. 2 is a drawing representation and does not come from any measurements or electrolyte modeling and thus cannot be taken for granted. We have derived the profile from PNP equation in our manuscript in fig. 3D. For example, a huge difference is shown in the head. Linear decrease of voltage along the neck is a common assumption in linear electrical circuit representation, but one that actually does not hold when ion concentration varies (which is the case here, in femto-liter dendritic spines!). Indeed, cytoplasmic conductivity is proportional to ion concentration and thus, while concentration decrease along a neck with constant cross-section is linear (Fig. 4D), the voltage decrease is in turn rather logarithmic (Fig. 3D).

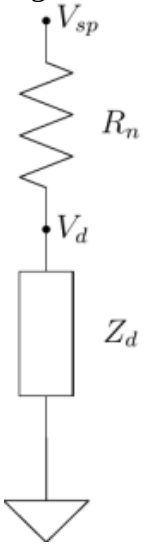
Moreover, as ion concentration increases with the amplitude of the synaptic current, we computed that I-V relation is not linear (Fig. 4E) and is actually given by (see analytical computation in Reference 1)

$$\mathbf{R(I) = U/I = \frac{k_B T}{I e} \ln\left(1 + \frac{IL}{2C\pi r_0^2 D_p F}\right).}$$

where C is the ion concentration in the dendritic reservoir, D_p is the diffusion coefficient of sodium ions, r_0 is the radius of the neck and L its length. k_B, T, F, and e are respectively the Boltzmann constant, temperature, Faraday constant and elementary charge.

Dr. ***Barbour*** It is often necessary to take the voltage change in the dendrite into account. We shall therefore consider the voltage divider formed by the neck resistance and input impedance of the dendrite.

Fig. 3. The spine, reduced to its neck resistance, is of course attached to a dendrite. The absolute voltages in the spine head will depend upon the dendritic impedance Z_d.



In the paper being analysed, the complex dendritic impedance is assumed to be purely resistive. With rather unnecessary complexity, the authors call R_n the *effective neck resistance* and $R_n + Z_d$ the *intrinsic resistance* (they neglect the dendritic and cell capacitances).

Data and processing

Dr. ***Barbour***: The data comes from the Yuste lab, but, notably, the author contribution statement carefully limits their involvement to supplying this data; they had no other involvement in this paper. The data have already been published once by the Yuste lab and a spine neck resistance of 90–100 MΩ reported [7]. It is also worth pointing to a theoretical preprint from the Yuste lab that covers much of the same modelling ground as the present paper.

ANSWER: The recent preprint from the Yuste lab does not simulate full PNP equations in 3D spine, but rather proposes a coarse-grain reduction of PNP equations into two non-linear and coupled differential equations for the dynamic of ion concentration and voltage inside the spine head. The mentioned preprint then analyzes the coupled dynamics of voltage and ion concentration at different time scales following a synaptic input.

To conclude the preprint of Lagache is complementary to the present work and does not cover the same topics as described here. It is actually a dual publication of ref [1]. The preprint appeared in August 1, 2018.(https://www.biorxiv.org/content/10.1101/274373v2.article-info), 3 months after the present manuscript was already published. We encourage Dr. Barbour to contact the authors of that preprint if he has any questions.

Dr. ***Barbour***: The data are voltage dye ("ArcLight") fluorescence measurements of the simultaneous voltages in spine heads and parent dendrites during focal uncaging of glutamate or backpropagating action potentials. As a general comment, the fluorescence signals are unavoidably small, noisy and slow. A very complex deconvolution procedure is applied to work back to the original voltages: filtering, fitting with constrained waveforms, deconvolution.

>ANSWER: Our deconvolution procedure is not "complex" but quite standard. The detailed steps are actually a bit different from Dr Barbour summary: 1-filtering, 2-fitting, 3-approximation by analytical equations 4-Laplace inversion to resolve the causal deconvolution. The result is implemented in a fast algorithm. Note that if we omit the analytical step in the summary, some instability can appear in the Laplace inversion. Of course, we welcome any simpler procedure because we were not able to find a simpler one.

Dr. ***Barbour***: The deconvolution appears to work for somatic signals, but anybody who has tried signal deconvolution will retain a healthy scepticism about the robustness of the procedure as applied to the very noisy spine signals.

ANSWER: The robustness comes from the analytical step that Dr. Barbour omitted to mention in the summary " filtering, fitting with constrained waveforms, deconvolution. **". The deconvolution is actually quite robust due to the analytical form that we obtained.**

All the deconvolved signals are still slow—the response to uncaging lasts 100 ms (perhaps calling into question the synaptic specificity), while backpropagating action potentials are an eye-catching 100 ms in duration (it turns out that some of the current-clamp recordings were made using a Cs-based internal solution). The authors thus have at their disposal time courses of deconvolved voltages at the head and base of the spine during uncaging. Referring to Fig. 3, they have estimates of V_{sp} and V_d.

>ANSWER: Part of the controversy about the electrical role of dendritic spines is coming from the difficult and cumbersome signal deconvolution of fluorescent voltage sensors, which, we would argue, are still inadequate to the difficult task of measuring spine voltage. Nevertheless, measurements with current voltage sensors are reproducible and should be reported and carefully analyzed. The ArcLight data published in Kwon et al., had low signal to noise ratio and a slow change of conformations (fluorescent vs. silent state of the voltage actuator), compared to voltage dynamics. Thus, robust (and unfortunately "complex") procedures, that combine time-deconvolution of the ArcLight signal (exponential deconvolution here) and denoising (standard Savitsky-Golay polynomial filter here) are necessary. For a sake of clarity and reproducibility, we have already detailed our step-by-step procedure in the SI of our manuscript. We encourage Dr. Barbour to contact the authors of Kwon et al. if he has any technical questions of the exact experiments reported.

Dr. ***Barbour***: **One equation, two unknowns**

We now see the benefit of our initial analysis simplifying the spine to its neck resistance. The voltage across the spine neck is given by the following relation:

$(V_{sp} - V_d) = I_{syn}R_n$.

This is of course Ohm's law, although the resistance may not be perfectly Ohmic. The authors have a problem. There is only one equation, with two unknowns: the desired resistance and the synaptic current induced by the focal uncaging of glutamate.

>ANSWER: Contrary to the claim of Dr Barbour "The authors have a problem**": we do not have a problem, because we have actually not one, as claims in this comment, but three equations: the Poisson and two Fokker-Planck equations that have to be solved at the same times. The direct measurement allows us to estimated** V_{sp} **and** V_d **and we are using the coupled system of equations to recover the current from the time response, which give us many discreet equations to constrain the model. This approach requires to think outside Ohm's law and linear framework.**

Dr. ***Barbour***: There is no way of splitting $I_{syn}R_n$ without additional information. Although the authors don't present the problem in this way, the additional complexity of their formulation does nothing to get around the underlying biophysics or fact that they do not know the current at any point in time.There are a few methods in the literature for resolving this problem. In one elegant recent method, Popovic et al (2015) [2] integrate the voltage difference to obtain QR_n and estimate the synaptic charge Q from a simultaneous somatic recording, which, despite filtering, is able to recover much of the synaptic charge (and the loss can be estimated for greater accuracy). The previous Yuste lab analysis of the present data estimated Z_d, allowing them to estimate the current and then the resistance [7]. Various other groups have monitored the activation of calcium entry in spines via voltage-dependent channels or NMDA receptors to determine the spine voltage indirectly [3, 4, 5]. Here, the authors do none of these things, instead they use electrodiffusion modelling…

Spine models

The authors employ a number of models. One is equivalent to the capacitor and resistor of Fig. 1 (although we know that the capacitance should be neglected), attached when necessary to a dendritic resistance (Fig. 2). They also examine more geometrically detailed models. Finally, they sometimes use full electrodiffusion models, in which the concentrations and fluxes of ionic species are represented explicitly. These can be particularly useful to track changes of the ionic concentrations, but are often unnecessarily complex if only electrical behaviour is of interest. The optimisation procedure by which the authors claim to extract the resistance while knowing only the voltage (i.e. not the current) is particularly complicated. It combines the simple RC spine model *and* an electrodiffusion model. No rationale is given for this combination, although one consequence is that the procedure would have appeared mightily complex to referees. A summary of the method is as follows.

1. The authors initialise the neck conductance G in the simple model (we'll ignore the capacitance C for now).
2. From the voltage data, they generate a current trace from the simple model.

3. They feed this current trace into the electrodiffusion model to generate a voltage trace.
4. By comparing this voltage trace with the data, they adjust the neck conductance G. Return to 2.

If the optimisation converges, a conductance value for the simple model should have been obtained such that the voltage output from the electrodiffusion model matches the data. However, no part of the electrodiffusion model is altered in the optimisation, which means that G in the simple model should converge (approximately) to the conductance set by the fixed parameters of the electrodiffusion model (these are the geometry, ionic concentrations and diffusion coefficients). In other words, **there is no optimization of the neck conductance!** The value of 100 MΩ in the abstract **was not "extracted"**, but chosen a priori. Thus, unsurprisingly, the authors have not managed to determine two unknowns from a single equation.

Answer: **To estimate the current I(t) and "effective" neck resistance $R_{neck}(t)$ (we named it "effective" as it actually depends on intensity I(t), see Fig. 4E), we have more unknowns than data, and thus, we cannot solve directly a system of linear equations with a given number of unknowns as usually done with electrical circuits and suggested by Dr. Barbour. As neck resistance depends (non trivially!) on both the neck geometry (i.e. its length (measured) and its radius (fixed to r_0 = 100 nm)) and intensity I(t) of the current, we have designed and implemented a complex yet robust optimisation procedure to estimate both I(t) and $R_{neck}(t)$ from the measured head $V_{head}(t)$ and dendrite $V_{dend}(t)$ voltages.**

For a sake of clarity let us recapitulate ***What we know*** *(=inputs of the optimiztaion procedure),* ***What we want to estimate*** *(outputs of the procedure) and* ***What we fix*** *(parameters of the model):*

> ***What we know (inputs of the optimization procedure)****: The head voltage $V_{head}(t)$ and the dendrite voltage $V_{dend}(t)$, the length L of the spine neck.*
>
> ***What we want to estimate*** *The current I(t), the "effective neck" resistance $R_{neck}(t)$, the "intrinsic" impedance G (corresponding to the sum of the neck and dendritic impedances) and capacitance C.*
>
> ***What we fix (input parameters of the model):*** *the sodium ion diffusion coefficient $D_p=200\mu m^2/s$ and initial concentration c_0 = 150 mM, and the radius of the neck r_0=100 nm.*

We also remind that neck resistance depends non-trivially on neck geometry and current intensity, a relation that we recently computed (see reference 1) but didn't know when this paper was published.

Main steps of optimization procedure then consist of:

- **Coarse-grained the spine geometry, to 1D neck, where the head and the local dendritic are 0D.**
- **Model the current in the head as an output of a linear system, where the deconvolved voltage V_{head} is the input. At this stage, there are two unknown parameters that we called G and C.**
- **Estimate C and G using an optimization method computed from PNP: start with an initial guess, then from the measured V_{head}, estimate the input current I in the neck coming from**

the head, (that will minimize the difference between the computed and measured voltage). This current is a boundary condition for solving PNP.

- **From the previous step, generate a solution of PNP, then compute the error between simulated and measured voltages and, update C and G to reduce the error at the next iteration.**
  Here, we assumed that G and C are time-independent. We show that this hypothesis is correct and robust by extracting parameters on a small interval of time $[\mathrm{t_i}\ \mathrm{t_f}] = [0, 20\mathrm{ms}]$ then we confirm the matching between measurements and simulation on the entire trace of 400ms(Fig. S4).
  Finally, we computed the ratio $\Delta V/I$ and the optimization procedure allows estimating the current and then to deduce the effective resistance.

The intrinsic 1/G and the effective resistance are different. The intrinsic resistance appears in the expression of the current from the head to the neck $I(t) = G\,V_{head}(t) + C\frac{dV_{head}(t)}{dt}$. This equation means that $I(t)$ is the output of a system where $V_{head}(t)$ represents the input, G and C are the parameters of this system (linear and time invariant). Although $1/G$ is in Ohms, it does not explain how the spine converts a current into voltage which is the *effective* spine resistance R_{spine}. We distinguish R_{spine} from $1/G$ by calling the latter an *intrinsic resistance.*

We also insist that the constant resistance approximation from Ohm's law does not hold in general: Indeed, we recently derived that the I-V relation in a neck:

$$R(I) = U/I = \frac{k_B T}{I\,e}\ln\left(1 + \frac{IL}{2C\pi r_0^2 D_p F}\right).$$

To conclude, as neck resistance depends nonlinearly on the current intensity and neck geometry, it cannot be deduced from the resolution of a linear system of equations with the right number of unknowns as usually done in electrical engineering. We have thus designed a robust optimization procedure with few fixed parameters. We agree that these fixed parameters (ion initial concentration c0, diffusion coefficient Dp and neck radius r0) actually constrain neck resistance, as we recently computed (reference 1), but don't fully determine it as it also depends on current intensity and neck length. Our optimization procedure is thus a straightforward method to extract important parameters from measured voltage traces. With this optimization procedure, we finally obtained a mean neck resistance of 100 MΩ (with a variance of 35) computed over 5 spines, described in the SI8.

Dr. ***Barbour***: And thus depends on measured voltage. The diffusion coefficient D_p is that for potassium ions taken from Chen & Nicholson (2000). There, it is given as 2.2 x 10^{-5} cm^2/s. That is equivalent to ~2200 $\mu m^2/s$, not the 200 $\mu m^2/s$ given in Table 2, an 11-fold difference. What happened there? An error while converting units (as well as reasonable rounding)? It might be worth checking which value was employed in the modelling and why.

>**ANSWER: We used 200 $\mu m^2/s$. The value for the effective diffusion coefficient can be considered in the rage of 200-400 $\mu m^2/s$, it is not completely settled down today. Indeed, using the notion of tortuosity, crowding, presence of an ER, etc (see Chen & Nicholson and many others such as Biess et al, Plos CB 2011) reduces the diffusion coefficient of the ion in water. We used here a factor 10 reduction, as was shown experimentally (using patch pipette experiment) and theoretically [Biess et al, Plos CB 2011].**

Dr. ***Barbour***: The capacitance values obtained through the optimisation (Table 1) are complete nonsense for 2/5 recordings. 18 pF is about 1000-fold greater than the approximate real capacitance calculated above. In reality, that trivial calculation could have shown the authors that the spine capacitance would be completely negligible and undetectable in their recording situation.

>ANSWER: Similarly to the intrinsic resistance $1/G$, the *intrinsic capacitance* C appears in the current from the head to the neck $I(t) = G\,V_{head}(t) + C\frac{dV_{head}(t)}{dt}$ (that was fit to the voltage dyes) and is one of the parameters in the linear system where $V_{head}(t)$ and $I(t)$ are the input and the output respectively. Consequently, this intrinsic capacitance is not the spine *membrane capacitance* but a parameter with Farad units and specific to the system formed by the three dimensional head. In this end, G is negligible does not contribute to the current. But it is a conclusion of the optimization procedure.

Dr. ***Barbour***: The electrodiffusion models appear to have boundary conditions that are inconsistent with the biophysics under investigation. Thus, Eq. 39 has $\partial V/\partial x = 0$, whereas any current flow through a resistor would give a non-zero voltage gradient (Ohm's law again).

>ANSWER: The boundary condition $\partial V/\partial x = 0$ appears in the coarse grained approximation where the head is reduced to a point with no geometry (0D model). Assuming that the voltage is almost constant in the head gives a zero electrical field. Thus this assumption is correct and is well supported by our simulations in a 3D spine (Fig.3D) where the electrical field in the head is indeed small such that the coarse-grained approximation $\partial V/\partial x = 0$ holds.

Dr. ***Barbour***: Additionally, the $\partial C_m/\partial x = 0$ condition is probably intended to reflect the fact that the synaptic current is purely cationic. However, the anions are not independent of the cations. If there is a synaptic flux of cations that tends to establish a concentration gradient (as the authors will suggest), then electroneutrality will impose a corresponding anion gradient, including at the boundary.

>ANSWER: It is explicit said that there is a concentration gradient, thus the Poisson's equation needs to be solved (chapter 10 of D. Holcman-Schuss, AMS, Springer 2018). Imposing an ANIONIC flux boundary condition, would be equivalent of saying that anions are passing through a cation selective membrane, which is not correct.

Dr. ***Barbour***: Similarly inconsistent boundary conditions are applied in the full 3d model of the spine head and neck (Eqs. 58; the injection boundary is Ω_i). In apparent contradiction with the condition of zero voltage gradient, we can see a very strong voltage gradient at the site of current injection in Fig. 3. In Fig. S7 there is an analogous gradient for C_p at the site of injection, which by electroneutrality must be mirrored by a non-zero C_m gradient, which would also contradict a boundary condition.

>ANSWER: This statement is not really clear: the classical physics (see Bazant school) of cation selective membranes shows that a build-up of positive charges develops near a cation source and is neutralized inside the domain. Moreover, electroneutrality is always assumed not derived from Maxwell equations. This is something to keep in mind.

Dr. ***Barbour***: Quite how the solution has been affected by these inconsistent boundary conditions is difficult to predict.

>ANSWER: Contrary to Dr Barbour's statement, our boundary conditions are compatible with the charge dynamic at the boundary. The solution we obtained here should be considered as an approximation of the voltage in the spine and as prediction for future studies. Mixed boundary value problems are routinely solved by numerical method (finite elements or spectral methods) to predict PDE solutions. When electroneutrality is assumed, the analytically PNP shows the exact dependency of the voltage, which is actually in log, as obtained in the case of non-electroneutrality (ref 5,7 below).

What use is electrodiffusion?

Dr. ***Barbour***: Putting aside for now the above doubts about the accuracy of the electrodiffusion modelling, what new biophysical behaviour have the authors discovered? If we compare the intuitive prediction for the voltage profile (Fig. 3) with the authors' Fig. 3B,D, we see that the main deviation is a strong voltage gradient near the site of current injection. Beyond that, there are less striking deviations from voltage uniformity across the head and from a linear decline of voltage down the neck. The relation between current and voltage across the neck also becomes nonlinear.

The voltage gradient at the site of injection is probably strongly exaggerated, for at least two reasons:

1. The currents are modelled as entering the spine head through a postsynaptic density (PSD) of radius 10 nm. Ref [6] allows calculation of a mean spine PSD area of 0.11μm^2, which yields a radius of 0.18 μm if a circular shape is assumed. It can be shown that the peak voltage is approximately inversely proportional to the PSD radius, so this parameter choice alone accounts for a factor of 15–20.

>ANSWER: the statement that "It can be shown that the peak voltage is approximately inversely proportional to the PSD radius**" is misleading. First it not clear by whom this result about the peak voltage has been shown. Is it an experimental result? In fact, it is very hard to link the voltage dynamics with the area where the current has been injected. We think that it is even harder to say something about the voltage peak. Thus we are not sure that the number provided by Dr Barbour have any foundations. Here we injected the current over a radius of 10nm. In general it can spread**

among few receptors but not the entire PSD, as claim here. Note however that the concentration drop is actually weakly dependent of the radius a of the surface where the current is injected. It is proportional locally by int0^infty e^{-sz} J_0(as) J_0(rs)ds/s, in coordinate (r,z), where J_0 is Bessel.

2. If an error of the diffusion coefficient is confirmed, the intracellular resistivity and therefore the peak voltage may have been overestimated by an additional factor.

It is therefore likely that under more realistic conditions there is no meaningful deviation from voltage uniformity across the head in the spine, including under the PSD. The peak sub-PSD voltage caused by the synaptic current can also be estimated directly by modelling a circular disk current source in a semi-infinite medium. With a radius of 180 nm, a 100 pA current and an intracellular resistivity of 150 Ωcm, I calculate a peak voltage deviation of 0.26 mV, which is much smaller than the deviations predicted by the authors.

>ANSWER: Considering that all the PSD is conducting sodium ions and that the radius of the source is around 180 nm seems quite unrealistic. In that "worst-case" scenario, AMPA receptors would be uniformly distributed within the PSD. The voltage response near such combined punctual sources of current is difficult to predict without full PNP simulations, but should be much more significant than the 0.26 mV computed by Dr. Barbour. If these computations are correct, Dr. Barbour should publish them. To our knowledge, such computations in an electrolyte medium were never made because there are generalizing the electrified disk framework of Weber (mixed boundary value problem) to PNP and are today very hard. But our computations in the diffusion approximations and the present simulation shows that the voltage has no peak but is a decreasing function of the entrance location (Fig 3).

Dr. ***Barbour***: The deviations from Ohmic linearity in the neck result from another mechanism. The authors point out that, as positive ions enter, their concentration at the point of entry increases, attracting anions. Over time a spatial concentration gradient is established (Fig. S7). The concentration gradient causes a gradient of resistivity and thus a nonlinear voltage gradient. This proposed mechanism seems sound, but the magnitude of the effect is uncertain, for several reasons:

1. The effect is evaluated in the steady state, which allows ionic gradients to accumulate. Conversely, synaptic currents are brief, especially at physiological temperature.

>ANSWER: We discuss ionic gradient **in the discussion section, where we have done time-dependent simulations (Fig 4):**

"For slower electrical events, ionic concentrations should follow the changes of the local voltage and there is a significant gradient of charges of the order of 150 mM in a spatial scale of 1 μm, although the average concentration is stable around 100 mM. However, this effect does persist for a transient current lasting 100 ms, where the concentration gradient at time to peak is of the order of 30 mM. Most likely, fast oscillations or voltage fluctuations due to the opening and closing of the channels will not lead to an extended concentration gradient and, in that case, the electro-diffusion could be neglected. »

2. Dr. ***Barbour***: The possible diffusion coefficient error may affect these gradients.

>ANSWER: There is no today a final value for the effective diffusion of ions inside neurons and inside the spine, where crowding can largely diminish the diffusion constant.

Dr. ***Barbour***: The modelling includes very mobile anions. Most anions inside cells are somewhat larger, less mobile molecules. This reduced mobility will impede the accumulation of anions and, through electroneutrality, oppose accumulation of cations also. This will reduce all of the effects somewhat. An extreme example of this was reported by Qian & Sejnowski (1989), who simply ignored anions in their modelling, in essence assuming they were all immobile. In consequence, they predicted only the tiniest variations of total ion concentration.

>ANSWER: Motility of anions is an important parameter of electro-diffusion models, that we have extensively discussed in our previous perspective (Holcman & Yuste, The new nanophysiology: regulation of ionic flow in neuronal subcompartments, Nature Review Neurosciences, 16 (11), 685 (2015)). For the sake of illustrating the point, in that perspective we explored the admittedly extreme scenario where only positive charges are motile and negative charges accumulate at membranes and non-motile organelles (it is worth noting that at that time, Dr. Barbour published a blog article on the website pubpeer https://pubpeer.com/publications/1569DF613F954511466AD49CF363B6, where he refused the hypothesis of non motile negative charges and claimed that "The applicability of the insulator to real life is zero". He seems to have, fortunately, changed his mind and now agrees to explore different model hypothesis).

With these simplified assumptions, in that NRN perspective we used PNP formalisms and computed that positive cations would then interact at long distance and accumulate near the spine membrane. These non-linear effects determine the flux of ions through the neck and the electrical response of a spine. It is worth noting that such non-linear effects cannot be captured with a simplified, linear electrical circuit.

Dr. ***Barbour***: I would expect more careful parameter choices (and, if required, a corrected model) to show that the electrical approximation of (Fig.3) remains adequate for most uses. The Yuste lab preprint estimates that the maximum reduction of resistance during a synaptic current is about 20%, and that reduction will only be attained sometime after the peak of the synaptic current. Certainly not a totally negligible effect, but maybe not of great physiological significance nor easy to measure with today's techniques.

>ANSWER: 20% was reached during spontaneous activity with given spine geometry and set of physiological parameters (diffusion constant of ions, concentration etc...). This value exemplifies that neck resistance should vary significantly during synaptic activity, and might be actually higher. Moreover, we emphasize that the generation of an action potential is a highly non-linear event and thus, even a small variance of synaptic conductivity might change drastically the overall response of the neuron.

On a positive note, I did find it interesting to realise that a typical synaptic current could transiently replace quite a significant fraction of the potassium ions in the spine with sodium ions (Qian & Sejnowski, 1989). We can calculate that a spine contains about 10 million charges, so about 5 million potassium ions. A 100 pA x 1 ms synaptic current injects 100 fC which is equivalent to about 0.5 million sodium ions.

a + b > a

Dr. ***Barbour***: The authors' complex neologisms "intrinsic conductance" and "effective neck resistance" were explained with respect to Fig. 2. The supplementary information contains a section to show that $R_n < R_n + Z_d$, where the dendritic impedance is assumed to be purely resistive. In other words, after 4 lines of equations, we discover that the sum of two strictly positive numbers (a, b) is greater than one of them: a + b > a.

>ANSWER: Expression

$$\boldsymbol{R_{neck} = \frac{1}{G}\left(1 - \frac{V_2}{V_1}\right) < \frac{1}{G}},$$

is a trivial inequality, but we wrote it to insist on the relationship between the effective R_{neck} and intrinsic 1/G resistances. In our study, we define $R_{neck} = \frac{V_1 - V_2}{I}$ (Ohm's law), then using the $I(t)$ expression for C negligible we obtain the expression above.

Limitations of the cable equation?

Dr. ***Barbour***: Throughout the manuscript the authors inflate the importance of electrodiffusion modelling. The whipping boy is the old-fashioned cable theory. Amongst the hype, there is an absolute brain fart towards the end of the supplementary information. In the section entitled "Limitation of the cable theory", the authors compare the ability of electrodiffusion and cable models of the spine neck to reproduce the attenuation of voltage from spine head to base. The results are shown in Fig. S6. For the electrodiffusion model there is a head-to-base voltage attenuation of about 50%. For the cable model, there is essentially none (the head and base traces superimpose). In order to recover the observed attenuation in the cable model, it proved necessary to increase the intracellular resistivity by a factor of greater than 10^5! Who knew the cable equation was that bad?

>ANSWER: We disagree with the mischaracterization of our position by Dr. Barbour, who is setting up a conflict between both formalism, whereas in reality they are complementary. In fact, cable equations corresponds to the special case of a PNP formalism without change in ion concentration, but PNP extends cable theory and enables explore the effect of ionic concentration in electric field, which, we would argue, are likely to occur in nanocompartments. Now, Dr. Barbour may argue the opposite, that there are not significant changes in ionic concentration in nanocompartments. At this point, since accurate measurements of ionic concentrations are difficult, it remains essentially an experimentally open question. However, the analytical tools we provide enable the theoretical exploration of these questions and the design of experiments to test these hypotheses.

Dr. ***Barbour***: Inspection of the actual equations offers an alternative explanation. The boundary condition of Eq. 61 implies no current flow. This is a cable with a closed end that is not terminated by a dendritic impedance. This is illustrated graphically in Fig. 4. It seems not to have crossed the authors' minds that if the standard approaches really were in error by a factor of 10^5, somebody might just have had the wit to notice before.

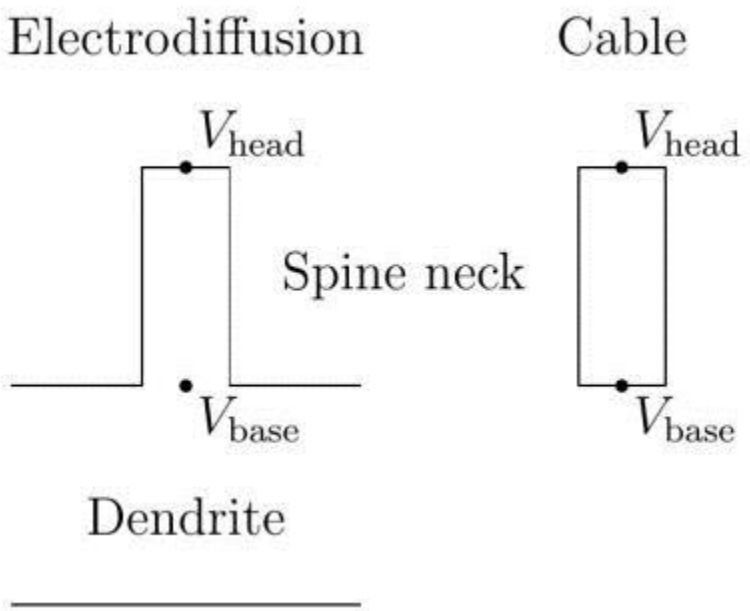


Fig. 4. In comparing their electrodiffusion model and a cable model of voltage attenuation down the spine neck, the authors mistakenly compare two quite different configurations. The cable (right) is not attached to a dendrite.

ANSWER: It is hard to guess from a rough drawing what the boundary conditions Dr Barbour had in mind. Some more would be needed here to move the argument from drawing to mathematics. In practice, we used here $V = V_1$ (measured voltage dye in the head) and dV/dn=0 at the interface spine dendrite, which has been justified by our previous simulations, looking at the interface head-neck. We used this boundary condition to predict the voltage at the spine-dendrite interface and compared it with the measured one.

Conclusion

Dr. ***Barbour***: The headline figure of 100 MΩ for the spine neck resistance was selected in specifying the electrodiffusion model, not extracted from the experimental data as reported.To have done as they claimed, the authors would have had to determine two unknowns from a single equation in which only their product appears.In the electrodiffusion modelling, an error appears to have been introduced while converting the units of the diffusion coefficient.: The authors use boundary conditions that are inconsistent with the biophysical model, with unknown effects on the results. Unrealistic parameter choices are likely to have exaggerated the reported effects, particularly regarding voltage non-uniformity in the spine head.

ANSWER: We hope that we have now convinced the reader (and maybe Dr. Barbour too) that indeed, neck resistance depends on physiological parameters (ion concentration and diffusion coefficient) and neck geometry (we only fixed neck radius to r_0=100 nm, length was extracted from

experimental data), but, as resistance also depends on synaptic current, its computation requires a robust (and somehow complex) estimation procedure. Moreover, we emphasize that we extensively explored how neck resistance varies with neck geometry and current in Fig. 4.

Concerning boundary conditions and parameters, we have justified each of our choices with reasonable hypothesis or derivations, and the "inconsistency" claimed by Dr. Barbour seems to be rather an arbitrary claim rather than a conclusion from biophysical modeling.

Dr. ***Barbour***: Finally, criticism of the cable equation is wildly misplaced, the result of another screw-up involving boundary conditions.

ANSWER: We think this dismissive criticism is inappropriate and not particularly collegial and borders harassment. As we explain above, PNP formalisms can extend cable theory to the regimes where changes of concentrations could be significant, such is nanocompartments.

Dr. ***Barbour***: This paper also raises an interesting question of principle. These days, authors are encouraged, indeed obliged, to share data. I don't think it is unreasonable for them to receive credit for that in the form of authorship, as long as the author contributions are specific, as they are in this case. However, what should they do if they do not agree with the conclusions drawn from their data? (I don't know how Kwon and Yuste view this paper.)

ANSWER: We encourage Dr. Barbour to contact Dr. Kwon and Yuste directly.

I welcome discussion, either below or on PubPeer.

ANSWER: We hope that we have now answered the technical concerns of Dr. Barbour about the consistency of our procedure to compute the neck resistance from electro-diffusion theory. Our manuscript aims at demonstrating that, due to the femto-liter size and complex shape of dendritic spines, synaptic potentials induce important variations of ion concentration and non linear I-V relations that cannot be properly modeled with standard electrical circuit simplification. Many geometrical and physiological parameters such as the molecular crowding inside spines or the exact ionic motilities remain unknown, and electro-diffusion is, in our minds, a potential framework that will allow to integrate future measurements and explore how varying these parameters influence the global integration of individual spine signals by the neuron.

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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...).