Scientific answer to B. Barbour blog-fiction

about

"Deconvolution of Voltage Sensor Time Series and Electro-diffusion Modeling Reveal the Role of Spine Geometry in Controlling Synaptic Strength"

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J. Cartailler and D. Holcman

This is third episode of the Barbour-fiction-paranoia saga. Usually, if a colleague from the ninth floor wants to ask questions with a colleague from the sixth floor, he has to go down three floors. Following the new trends of writing blogs instead of direct communication, we have recently discovered unprofessional statements about our work and will now answer them in a scientific and up-to-date responses.

The first episode started 3 years ago in a public discussion (pubmed), following the intellectual frustrations of Mr Barbour to accept a novel vision about nanophysiology, published as a perspective. In the second act, he reformulated some previous questions and the most substantial one, he noticed, an editorial misprint from mM to microM in the y-axis of fig3b-3c, which are usually undergrad student remarks.

In the present case, probably, without consulting a professional in signal processing, statistical physics, biophysics or applied mathematics, Mr Barbour decided to offer his public view in his pseudo-scientific-political blog of how to apply electrical engineering concepts to nanophysiology of dendritic spines, in the fiction form where he present himself as a fake hypothetical reviewer, however a picture of himself allowed us to recognize him.

Unfortunately, the comments below show several confusions, lack of knowledge and understanding in elementary mathematics, physics and modeling, a field where Mr Barbour had no publication records, but insisted on obtaining answers by contacting directly editors of journals to try to destabilize them and to create hypothetical concerns and suspicion.

We first note that Mr Barbour is not following the adequate literature where many of his comments have already been answered. The vision projected in this blog is based on classical electrical engineering approach, where electricity in conductors is studied by a combination of the resistance, capacitance, etc..., which are the building blocks of classical electrical circuits and formulate in terms of linear algebra or linear differential equations. However, electrophysiology medium is modeled differently, as an electrolyte is not a conductor nor an insulator (we presented a lexica in the previous answer), modeled by nonlinear partial differential equations, a field that is not similar to linear algebra. Thus the classical electrical engineering approach is now largely insufficient to describe the physiology at a nanoscale (as reviewed recently in Savtchenko et al, Nat Rev Neur 2017). Because questions such as what is the intrinsic resistance of an electrolyte? What is the role

of the shape? etc... remained to that day unanswered. The interplay between ions and voltage is not easy to model and simulate, but can be computed from the molecular level by considering the fundamental equations of physics (Poisson-Nernst-Planck). Investigating electrical and chemical properties of nanometric biological units is a new challenge and new techniques, methods and ideas have been developed in past years (nanopipettes, voltage sensitive dyes, PNP theory, asymptotics, mixed boundary value simulation).
Any new results in that direction should be published in the peer-reviewed literature. The present blogs does not contain any new information or relevant comments that move the field in the right direction.

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: It is not clear that our manuscript has been read carefully and understood: the goal of our paper was to study the I-V relation in a dendritic spine and we have shown that the resistance approximation is largely insufficient: we proposed at the end of the manuscript that a non-linear diode is actually a much better idealization of a dendritic spine. This summary made here is not reflecting the goal of our manuscript.

In addition, to connect the present modeling, numerical simulations to data we also developed a novel procedure to extract an effective resistance of a spine and we also give a range from our simulations of resistance, that depends on the range of the smallest passage inside the spine neck. These results have even been confirmed by analytical derivation (see reference 1 below). The errors that are made here are not contained in our paper, but in the comments.

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.

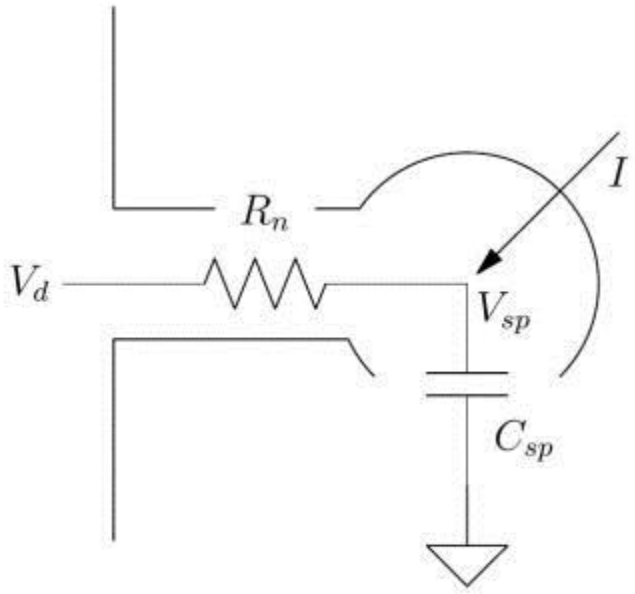

Fig. 1. Equivalent electrical circuit of a spine, with a spherical head and a cylindrical neck. The membrane resistance and neck capacitance have already been neglected, and we'll see that the head capacitance C_{sp} can be, too. Only the neck resistance R_n has any influence on single-spine biophysics. 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: Mr Barbour assumes what he needs, this is not how to proceed in the hard sciences. Because biophysical properties should be derived from equations and theory. We should also start with a correct model: a spine head is not a surface but a three dimensional electrolyte ball. Making computation using a two-dimensional model instead of a three-dimensional one is misleading.

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): $4\pi r^2 \approx 1$ µm^2. Given the specific membrane capacitance of 1 µF cm^{-2}, we obtain an estimate of the spine capacitance of ~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: The concept of capacitance does apply for a conducting sphere in vacuum or in a dielectric, not for 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 assumed, as an empirical law. In the reference below, we found by using the fundamental relation equation (Ref 7) that this relation cannot be applied here in a three dim ball or in electrolyte.

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: The RC-approximation is not appropriate for electrolyte or where there are an imbalance of charges to estimate the time scale of relaxation (see Ref 7, where new relations were derived).

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: This is an assumption not a derivation from first physical or mathematical principles. Thus a conclusion based on incorrect assumptions should be rejected.

(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.)

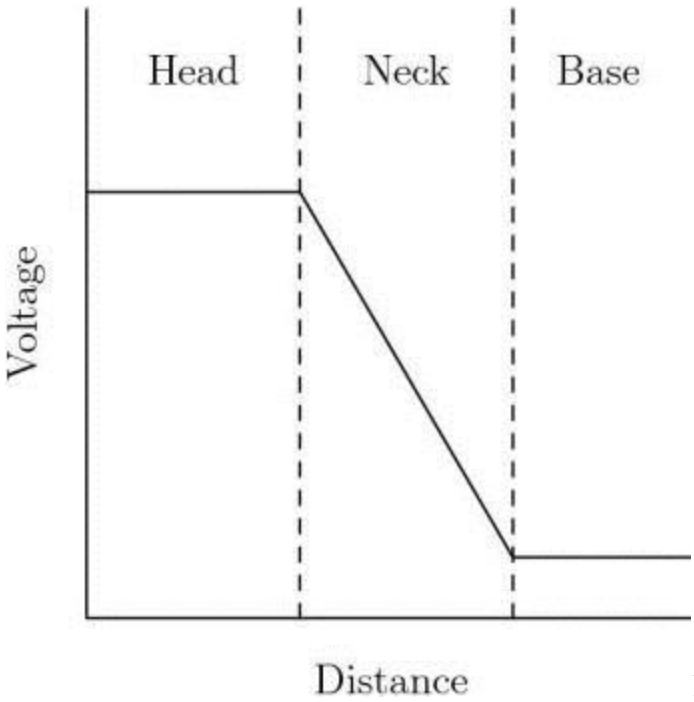

Fig. 2. When a synaptic current flows, the approximate voltage profile expected in a spine is uniform across the head and linear down the neck.Armed with the uniformity of the head voltage and the fact that the neck resistance is the only significant electrical component of the spine,

>ANSWER: Again, the present discussions consist on spelling out assumptions, that cannot be justified. For example, there is no reason for the voltage to be uniformed in the spine head, when a current is flowing. This assumption is actually incorrect wrong, as we have shown in our manuscript.

a very usable approximation for the voltage profile in the spine during the synaptic current is shown in Fig. 2:

ANSWER: This figure 2 is a cartoon, not derived by solving any equation or measurements and has no quantitative value. We have derived the correct profile in our manuscript in fig. 3D.

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: This is a cartoon description, not an experiment nor theory. We could not find in the past 9 years on that subject any linear behavior. The decay of the voltage in the head can be approximated by a linear voltage drop, but in general it is not (see formula, 5 of Ref 1).

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.

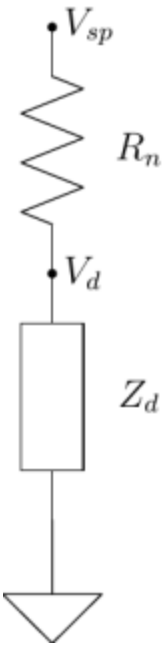

Fig. 3. The spine, reduced to its neck resistance, is of course attached to a dendrite. The absolute voltages in the spine head will depend upon the dendritic impedance Z_d. In the paper being analysed, the complex dendritic impedance is assumed to be purely resistive.

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).

ANSWER: This is an incorrect distinction, which we explained page 4 and SIe7.

Data and processing

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: This is an incorrect understanding of this associated pre-print, which deals with a different question about reducing the PNP approach into two non-linear differential equation.

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: the debate about finding a procedure "complex" is not very informative. Todays computer power allows resolving in no time this deconvolution. If the blogger has a better and relevant approach, he should publish it in the professional literature.

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. 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: The statement about time constant has already been explained in our manuscript, where we compared the electrophysiological time-course in the soma vs the voltage dye response. This leads to the deconvolution kernel that we applied to dendritic spine. The deconvolution method is new and robust (see below) as shown on 6 examples.

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. 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.

>ANSWER: thinking about using 2 equations to resolve two unknown is the classical standard in algebra. However, it is possible to find two unknown, from fitting a continuous curve, but this requires a different approach.

PNP based optimization procedure is not straightforward. The steps are:

- **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 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 $[t_i\ t_f] = [0, 20ms]$ 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.

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…

>ANSWER: The value estimated should be considered as the one proposed by electro-diffusion. The new effort here is to focus on computing the I-V relation, determined by the spine geometry. We are not sure that the controversy about the spine neck resistance is that relevant anymore.

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.

>ANSWER: In a small compartment, in the presence of non equilibrium of charges, even a small fluctuations of charges can dramatically change the concentration and thus affect the I-V relation.

The optimisation procedure by which the authors claim to extract the resistance while knowing only the voltage (i.e. not the current)

>ANSWER: Obviously the procedure we have described cannot understood in the context of linear algebra. We computed the current by optimization but this is no equivalent of

solving 2 equations to find two unknown. This procedure is only valid in some RLC-circuit electrical approximation, which is not relevant here.

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.

>ANWER: This summary is not accurate as it is actually missing the most crucial point: see summary above.

If the optimisation converges,

>ANWER: There is no convergence condition (see wikipedia for a definition) here, because we are iterating a procedure and then take the minimum.

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

>ANSWER: The word converging refer to a sequence, not a parameter (see wiki for a definition).

(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!**

>ANWER: The optimization is in the fit of the computed curve with an iterative value of the resistance to the data. We chose at the end the value for which the mean square difference between the two curves is minimal. This is a classical procedure in optimization

The value of 100 MΩ in the abstract **was not "extracted"**, but chosen a priori.

>ANSWER: this is a wrong conclusion: this is an average value (with a variance of 35) computed over 5 spines, described in the SI8.

Thus, unsurprisingly, the authors have not managed to determine two unknowns from a single equation.

>ANSWER: To dissipate another confusion, we recall that the intrinsic 1/G and the effective resistance are different. The intrinsic resistance appears in the expression $of\ the$ current $I(t) = G\,V_{head}(t) + C\frac{dV_{head}(t)}{dt}$. 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: Indeed, we recently derived that I-V relation in a neck, and we found that

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

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.

>ANSWER: We used 200 $\mu m^2/s$.

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: The value for the effective diffusion coefficient can be considered between 20-200 $\mu m^2/s$, as used in most of our articles. The effect of heavy tortuosity, crowding, ER, etc… should be taken into account (Chen & Nicholson and many others such as Biess et al, Plos CB 2011). This reduction is more than classically used by experts.

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 $I(t) = G\,V_{head}(t) + C\frac{dV_{head}(t)}{dt}$ 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 head. In this end, it does not contribute.

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

almost constant in the head gives a zero electrical field. This assumption is supported by 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.

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, 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.

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.

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

>ANSWER: Mixed boundary value problems are routinely solved by numerical method (finite elements or spectral methods) thus to predict PDE solutions. When electroneutrality is used, the analytically PNP show the exact dependency of the voltage, which is actually in log, as obtained in the case of non-electroneutrality (ref 5,7)

What use is electrodiffusion?

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 $0.11\mu m^2$, 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.

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: We are happy that Mr Barbour has some findings and we suggest him to publish his result in peer review journals.

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: This is indeed entirely discussed in the discussion section, where we have done time-dependent simulations.

2. The possible diffusion coefficient error may affect these gradients.

   >ANSWER: The error is not ours.

3. 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.

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.

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

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

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

is a trivial inequality, but it clarifies the relationship between both 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?

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: The cable theory is indeed very bad and it is not used by anybody to model the neck, as depicted in the first figure above. Indeed, by reducing the spine neck to a circuit resistance.

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.

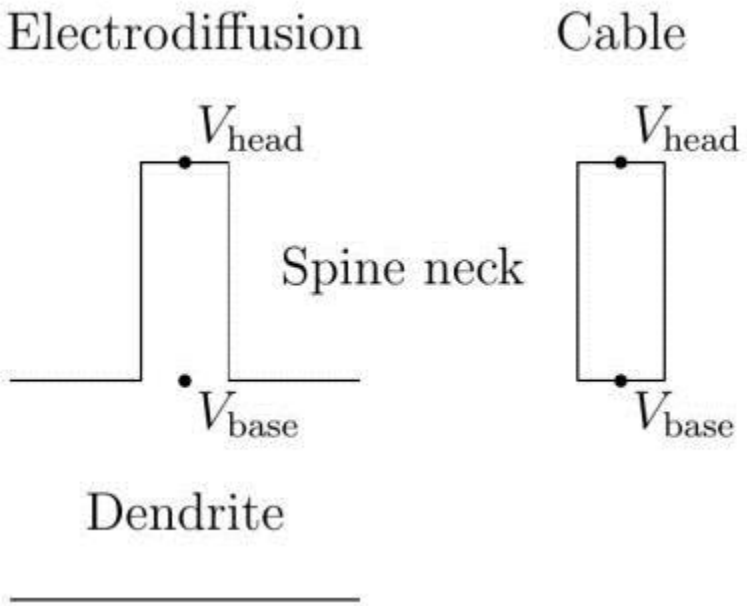

Fig. 4. In comparing their electrodiffusion model and a cable model of voltage attenuation down the spine neck, the authors mistakenly compare two quite different configurations. The cable (right) is not attached to a dendrite.

ANSWER: It is very hard to guess from a rough drawing what the boundary conditions Mr Barbour had in mind. A little effort would be needed here to move on from advanced drawing to elementary 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

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.

ANSWER: This statement is in correct. The present resistance shown originally with a mean and variance had been explained in the manuscript.

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.

ANSWER: This statement is incorrect as discuss above.

In the electrodiffusion modelling, an error appears to have been introduced while converting the units of the diffusion coefficient.

ANSWER: This statement is incorrect and D~200 $\mu m^2/s$ is very acceptable and the value of this parameter is OK. The error is in the conclusion.

The authors use boundary conditions that are inconsistent with the biophysical model, with unknown effects on the results.

ANSWER: This statement is incorrect: our boundary conditions are consistent.

Unrealistic parameter choices are likely to have exaggerated the reported effects, particularly regarding voltage non-uniformity in the spine head.

ANSWER: This is not a conclusion but an assertion with no justification.

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

ANSWER: As everybody agree and this is not new, you cannot use cable equation here.

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.)

I welcome discussion, either below or on PubPeer.

ANSWER: This is not the format of a discussion and we first suggest the author to learn about electro-diffusion starting with the classical physics of electrolyte to be able and to look carefully to the following list of research articles:

References:

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

2-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

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

4-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).

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

6-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

7-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).
