Comments by Boris Barbour, Tony Gardner-Medwin and Alain Destexhe on the paper:

Intracellular Impedance Measurements Reveal Non-ohmic Properties of the Extracellular Medium around Neurons

Biophysical Journal (2016)
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Boris Barbour commented 3 years ago [27/12/16]

In this and previous papers, the authors report that the extracellular impedance is high and non-resistive, compared to many previous measurements that have found it to be much lower and essentially resistive. I argue in this arXiv preprint

https://arxiv.org/abs/1612.08457

that the authors' estimate was probably confounded by an inaccurate representation and extraction of the series neuronal impedance in their measurement. In consequence, there is no compelling evidence to abandon the well established consensus that the extracellular impedance is low and essentially resistive in the frequency range of interest for biological signals.

Tony Gardner-Medwin commented 3 years ago [8/2/2017]

This paper (Gomes et al., 2016) raises a dilemma for readers. If well founded, it clearly merits much work to understand it and its implications. But the conclusions conflict so strongly with conventional wisdom that it is tempting to dismiss it as probably somehow incorrect. All credit therefore to Barbour (2016) for critiquing it and pinpointing questions that clearly need answering. Hopefully, both the authors and others with their own perspectives may contribute to clarify the situation.

Broadly I concur with the points Barbour raises. I would add however what seems possibly a key oversight in the papers from Gomes' group. This is in the argument that observed filter characteristics and cell input impedances that fall off with the square root of frequency at high frequencies are indicative of diffusion processes, rather than R-C elements as in conventional modelling. Cable equations for the input impedance of even the simplest dendritic model (with uniform characteristics and a length of many space constants: $V'' = \Lambda^{-2}(1+j\omega\tau)V$, $I = -V'/R$, $Z = R\Lambda / \sqrt{(1+j\omega\tau)}$, $|Z| = R\Lambda\,(1+\omega^2\tau^2)^{-0.25}$) predict just such a relation (Rall & Rinzel, 1973: see equations A13, A15 for the more general solution with dendrites of any length). So the argument of Gomes et al. that the data implicate diffusion processes (which can also lead to square root relationships) seems to collapse.

Though the external pathway for current generated by neurons is usually regarded as largely within interstitial space, it is not exclusively so, even at low frequencies or DC.

Around 6% of DC current passed through rat cortex is accounted for by K^+ flux (Gardner-Medwin, 1983). Since current in interstitial space would only account for a K^+ flux of 1.2%, the difference is presumably due to trans-cellular passage of at least 5% of long distance current flow, probably largely through the astrocytic syncytium. This is a small adjustment to the notion that low frequency currents are largely extracellular, but it does represent a 5-fold enhancement of K^+ flux driven by an electro-chemical gradient, which when applied to chemical (concentration) gradients implies greatly enhanced K^+ dispersal around regions of build up in interstitial space, compared with diffusion alone - the so-called 'spatial buffer' mechanism for K^+ .

An additional, larger, component of macroscopic cortical conductance appears to arise from extracellular but not interstitial pathways, possibly via perivascular tissue. This may not have been studied in detail, but is indicated by the fact that measured cortical impedance is in at least some circumstances only around half what would be expected on the basis of measurements of the volume and tortuosity of local interstitial space around a microelectrode (Gardner-Medwin, 1980; Nicholson & Phillips, 1981). Barbour (2016) points out that these two ways of approaching impedance both give an order of magnitude hugely below that of Gomes et al. (2016). Taking account of interstitial tortuosity shows, however, that they do differ by a factor of about 2.

Barbour B. (2016) Analysis of claims that the brain extracellular impedance is high and non-resistive. https://arxiv.org/abs/1612.08457

Gardner-Medwin A.R. (1980) Membrane transport and solute migration affecting the brain cell microenvironment. Neurosci. Res. Progr. Bull. 18:208-226

Gardner-Medwin A.R. (1983) A study of the mechanisms by which potassium moves through brain tissue in the rat. J Physiol 335:353-374

Gomes J.-M., C. Bédard, S. Valtcheva, M. Nelson, V. Khokhlova, P. Pouget, L. Venance, T. Bal, and A. Destexhe (2016) Intracellular impedance measurements reveal non-ohmic properties of the extracellular medium around neurons. Biophysical Journal, 110(1):234-246

Nicholson C. & Phillips J.M. (1981) Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. J. Physiol. 321:225-257

Rall W. & Rinzel J. (1973) Branch input resistance and steady attenuation for input to one branch of a dendritic neuron model. Biophysical Journal 13(7):648-688

Alain Destexhe commented 3 years ago [8/2/2017]

Dr Barbour casts doubts on our analysis, but makes several confusions: first he confuses measurements in Fourier frequency space, which cannot be extrapolated so simply to the temporal domain (for example translate mV/Hz into a LFP in mV is not so straightforward). Consequently, he obtains an aberrant LFP amplitude of 20 mV, which our measurements do not predict at all. Barbour makes a second confusion by making an analysis that assumes that the medium is resistive, while our measurements did not make this assumption, so his analysis also predicts aberrant values for this reason.

We have to say that it is very harmful to allow such non peer-reviewed criticisms, which spread wrong statements and cause harm to published papers, because uninformed readers will believe we were not careful in our analysis. Our papers, measurements and

analyses were all peer reviewed, in journals such as Physical Review or The Biophysical Journal. It was thus seen by reviewers (mostly physicists) specialists in electromagnetism theory, electrophysiology or biophysics. We suggest that, instead of spreading non-reviewed and wrong critisms, it is more scientific to make himself biophysical measurements and gets his work published in peer-reviewed journals (ie, not just words), and in journals of the same standard as Physical Review or Biophysical Journal (ie, not just arXiv).

Finally, we fully understand that our measurements are against the current belief, and it is of course much easier and reassuring to try find arguments against them. In our recent review paper https://arxiv.org/abs/1611.10047 , we suggested an experiment which will determine if the evidence for resistive medium was contaminated by shunt currents. So instead of exchanging non peer reviewed statements, we suggest to make this experiment, which should definitively solve the issue.

Boris Barbour commented 3 years ago [18/2/2017]

Tony Gardner-Medwin shows how even a simple cable model with a low-resistance extracellular space comes remarkably close to generating the somatic impedance spectra reported in Gomes et al. This cable property was described no later than 1973.

Destexhe calls for more experiments, but both both prior and recent work on pyramidal cells have clearly demonstrated that the ~1/sqrt(f) impedance can be fully accounted for by the cellular impedance without any need to invoke a high or reactive extracellular impedance, eliminating the only supporting (and very indirect) argument Destexhe has so far advanced. A prior paper was

Yaron-Jakoubovitch, Jacobson, Koch, Segev and Yarom (2008) A paradoxical isopotentiality: a spatially uniform noise spectrum in neocortical pyramidal cells Front. Cell. Neurosci., https://doi.org/10.3389/neuro.03.003.2008

It shows that the somatic impedance (i.e. the impedance measured at the soma) of juvenile rat pyramidal cells exhibits a slightly steeper than 1/sqrt(f) decrease with frequency, just like in Gomes et al (although the curves are shifted somewhat): see the red somatic impedance trace in Fig. 2A of Yaron-Jakoubovitch et al. A very similar impedance spectrum emerges from a simulation with a reconstructed pyramidal cell: see red simulated somatic impedance in Fig. 4A of Yaron-Jakoubovitch et al. Thus, impedance spectra of the form observed by Gomes et al. can be quite precisely accounted for by the electrical morphology of the pyramidal cell, without invoking an implausible reactive and elevated extracellular impedance. This has been known for at least 9 years.

A recent paper that directly addresses the results of Gomes et al.:

Miceli, Ness, Einevoll and Schubert (2017) Impedance spectrum in cortical tissue: Implications for propagation of LFP signals on the microscopic level. eNeuro, https://doi.org/10.1523/ENEURO.0291-16.2016

The authors reproduce a ~1/sqrt(f) impedance in the presence of a low and resistive extracellular space (see their Fig. 4D). Furthermore, that low, resistive impedance was verified directly, yet again. Miceli et al conclude

"... the overall evidence points to an essentially real (Ohmic) extracellular conductivity with negligible effects from ionic diffusion in the frequency range between 5 and 500Hz."

Destexhe questions my suggestion that his proposed high extracellular impedance would be associated with gigantic extracellular action potential signals. My argument does contain several implicit approximations. However, because the proposed extracellular impedance is of the same order of magnitude as the membrane impedance, I still believe that membrane and extracellular voltage responses to a given current would be predicted to have quite comparable amplitudes. In other words, those in the extracellular space would indeed be gigantic.

The shunt-current mechanism proposed by Destexhe in the recent ArXiv was already addressed and ruled out in my preprint (the tissue fraction occupied by the electrode is too small to make a big difference).

The majority of the points I raised in my preprint regarding the experimental design and analysis strategy of Gomes et al. have not been addressed at all, except by appeal to authority. In particular, the assertion by Destexhe to have measured the extracellular impedance is precisely the point in question. I maintain they have simply measured the intracellular impedance and are unable to draw from it any conclusions regarding the extracellular impedance. This point of view is in full agreement with the results of the Segev/Yarom and Einevoll/Schubert groups, as well as the arguments of Gardner-Medwin drawing on the work of Rall and Rinzel. In summary, Gomes et al. provide no good reason to prefer their conclusions over those of the many researchers who have previously measured the extracellular impedance directly, including Ranck, Nicholson and Logothetis.

Boris Barbour commented 3 years ago [5/3/2017]

See also this recent paper measuring brain extracellular impedance in humans and finding no evidence for a frequency dependence.

Ranta R, 2017

Alain Destexhe commented 3 years ago [8/3/2017]

The discussion becomes interesting... we are very happy that our paper triggers so much comments. To the last Barbour's reply, it contains several simplifications and errors about the 1/f scaling, in particular the claim that cable filtering explains the 1/f scaling, while it only partially explains it. Barbour focuses on the evidence for non-resistive media from impedance measurements, and we agree that those are subject to controversy and to multiple interpretations (but all of them, not only ours...) A more detailed reply is obviously needed here - we are preparing this and it will take some time.

The evidence for non-resistive media goes well beyond impedance measurements, and because Barbour does not mention these evidences, while they are important for the discussion, we are summarizing them here. We hope that it will be apparent that our view is coherent, perhaps even more coherent than the traditional view.

(1) The first point is te observation that LFP and EEG can scale as 1/f. This 1/f is only seen in the so-called "desynchronized EEG" states, with no slow-waves (with slow-waves it scales as 1/f2). In 2006, in a paper in PRL, by relating LFPs with unit activity, we suggested for the first time that there is a 1/f filter somewhere. Three explanations were

proposed for this: first, a random arrangement of capacitive and resistive elements (like in extracellular space) is known to create a 1/f filter; second, the cable filtering (Pettersen and Einevoll, 2008), and third, the influence of ionic diffusion (Bedard et al. Biophys J, 2009). All generate 1/f, but cable filtering generates 1/f only at high frequencies (the kernel is flat at low frequencies), and this is also true for the very recent measurements discussed by Barbour. On the other hand, ionic diffusion predicts 1/f over the whole frequency spectrum, and thus accounts better for the LFP and EEG, which also scale as 1/f at low frequencies (the cable filtering is unable to explain this). This is already a first indication that cable filtering is not entirely satisfactory.

(2) A second point is that if one relates the power spectra of intracellular and LFP recordings in vivo, one cannot fit the transfer function obtained using a resistive medium, but it better fits the prediction from ionic diffusion (Bedard et al., JCNS 2010). In the same paper, we showed that the cable filtering only works in a silent neuron (in vitro conditions), but this effect vanishes in the presence of synaptic bombardment (in vivo conditions), so cable filtering is unlikely to provide a satisfactory explanation for these in vivo data as well. The fact that ionic diffusion fits better is of course not any kind of proof, but only an indication that the spectral structure of these signals is consistent with it.

(3) A third evidence comes from the scaling exponent of EEG and MEG signals. If the media traversed by the fields are resistive, electric potential and magnetic field should scale the same, and they are not (Dehghani et al., 2010). This is true for all locations on the scalp, and when the exponents were similar, it is when the SNR was low, so there seems not to be a single location in the brain which behaves as a resistive medium according to that analysis.

(4) The inhomogeneity of the impedances is also important. In 2004, we made a theoretical study showing that if there are impedance inhomogeneities (like a succession of fluids and membranes), then the electric potential is strongly filtered (Bedard et al., Biophys J. 2004). Note that this model was resistive, but with spatial variations in resistivity (which is actually known to exist in cortex; see conductivity measurements of Herreras lab). Later, a study by Nelson et al. (J Neurosci 2013) showed that the neuropil in cerebral cortex is also inhomogeneous at smaller scales. This does not show anything in favor or disfavor of a resistive medium, but it shows that the inhomogeneous electric structure of the medium necessarily predicts a filtering effect on the extracellular potential.

(5) The Gomes et al. measurements (Biophys J 2016, discussed here) also show a filtering filtering consistent with ionic diffusion. However, the present discussion is about whether the same result can also be explained by cable filtering rather than an effect of the extracellular medium. It remains to be shown (1) if the match of cable filtering is as good as claimed for biophysically realistic conditions; (2) whether it also works in vivo, where we see the same effect, while cable filtering is supposed to be much attenuated. This is the discussion we have at the moment also with Tony Gardner-Medwin, and I hope we can reach an agreement at least on that one.

(6) Finally, we suggested a framework where all these data can be explained, in a recent review paper (Bedard et al., J Integrative Neurosci, 2017). We showed that all the above data can be explained by a diffusive medium, although we agree that this is a theory, nothing has been demonstrated about diffusion, there may be something else scaling as 1/sqrt(omega). We also suggested that there is a current shunt in the water column that surrounds electrodes, which could explain measurements of resistivity. We also sketched an experiment to test it.

On the other hand, if we understand well, Barbour's "theory" postulates that 1,2,3,4,5,6 are all wrong. Of course this is also a possibility, but frankly, we find it not really satisfying and not constructive, since no experiment is proposed to test it. This looks like a tentative to close the discussion, while our approach is the opposite, our paper tries to open the debate.

Moreover, as theoreticians, we are more satisfied with the diffusive theory because it builds on a much wider set of experimental observations and analyses. Building only on impedance measures is dangerous, because we do not know at 100% the accuracy of these measures (and indeed they are controverted, the proof is all this discussion we have here...) In any case, we made efforts to find a framework where all data can be explained. Of course this framework may be incomplete and probably needs to be improved, but at least it provides a solid basis to make further experiments.

So in the future, one should examine the respective contributions of cable filtering, impedance inhomogeneity, ionic diffusion (also recently investigated by the group of Einevoll), and possibly other unknown factors, to yield a precise biophysical picture of the genesis of extracellular potentials and magnetic fields. In our mind, it is clear that all these factors contribute, and considering the medium as just a resistance is an dangerous oversimplification.

Boris Barbour commented 2 years ago [4/10/2017]

My preprint has been published as a letter to the editor

http://www.cell.com/biophysj/fulltext/S0006-3495(17)30913-X

Bédard and Destexhe reply

http://www.cell.com/biophysj/fulltext/S0006-3495(17)30914-1

I have since found a metaphor that might offer a useful intuition to newcomers to the subject. Imagine that the extracellular impedance is represented by wallpaper and the membrane impedance by a castle wall. You wish to measure the thickness of the wallpaper (extracellular impedance). You must choose one of the following measurement methods. 1) Measure the wallpaper thickness directly, before sticking it to the wall. 2) Measure the thickness of the paper AND the castle wall to which it is stuck. Obviously it is much, much more difficult to extract an accurate estimate of the wallpaper thickness if it is measured together with that of the wall. Yet this is essentially the approach that Gomes et al have chosen by placing one of their electrodes in the intracellular compartment and measuring membrane and extracellular impedances in series. The paper contains no independent validation or justification of their ability to perform the separation of membrane and extracellular components of the impedance to the required accuracy.

Boris Barbour commented 2 years ago [25/1/2018]

Alain Destexhe is an author on a recently posted preprint using a low and resistive value for the extracellular impedance.

https://www.biorxiv.org/content/early/2018/01/05/243808

This is completely inconsistent with the position he puts forward here. If he has changed his opinion, it would be helpful to readers to close this discussion by making that clear.

Alain Destexhe commented 2 years ago [26/1/18]

Not at all - this paper uses NEURON so we can't include any complex extracellular impedance. As we said previously, we are waiting for appropriate experiments to settle this issue of extracellular impedance, evaluate the importance of the short-cut and determine which model should be considered in which circumstance. The previous discussion has shown that each party has its own logic and supporting data, so there is no point of continuing the debate in the absence of these new experiments. So no, the discussion is not "closed" at all, but it is "on hold" waiting for new experiments.

Tony Gardner-Medwin commented 2 years ago [26/1/2018]

I think for most people with some knowledge of biophysics it will be fairly clear already that the paper Gomes et al. 2016 is quite inadequate to provide any sort of challenge to conventional electrophysiological modelling.

. I had an extensive and constructive correspondence with Destexhe last year. It was clear to me (and at least to some extent to Destexhe) that conventional theory can closely and quite simply fit the data of Figs 2,8 in Gomes et al.. At first Destexhe and colleagues argued that such a model was too simplistic, leaving out certain factors. When these were included and more complete fits could be made, there have been no clear suggestions from them that critical parameters were actually wrong or implausible (for example, over-compensated negative capacitance to explain phase data in Fig. 8). I had hoped to publish these fits jointly with Destexhe and colleagues, without getting involved in the more detailed disputes over Barbour's comments. Destexhe didn't agree to this, and I have not so far felt it worth publishing on my own what might be seen as a rather unnecessary attack on an already somewhat discredited paper.

. Since the issues seem still continuing in a manner reminiscent of tweets, I have put up the model that I developed in the correspondence with Destexhe at http://tmedwin.net/docs/Gomes_fits_v4.xlsx and encourage anyone interested to play around with it. I have a more sophisticated version (in Labview) that takes account of the distributed e-c field potentials generated by dendritic current in the model - but the differences are pretty negligible in their effect on what Gomes et al. measured. Comments and queries are of course welcome, either here or to me at ucgbarg@ucl.ac.uk .

. Tony Gardner-Medwin

Alain Destexhe commented 2 years ago [26/1/2018]

Thanks Tony for sharing your model, which indeed reproduces part of our measurements. As pointed in our discussions, this match concerns the impedance amplitude (modulus), which can be reproduced by different resistive models. However, this is not true for the phase, no resistive model can account for the phase converging to -45 degrees that we observed. These points were detailed in our commentary in Biophys. J:

http://www.cell.com/biophysj/fulltext/S0006-3495(17)30914-1

So to your question, can resistive models fit our data, our answer is no.

As we said before, there is no point in discussing this any further now. The only way we can agree is to do the right experiment that everybody will fully accept.

Tony Gardner-Medwin commented 2 years ago [31/1/2018]

Destexhe suggests here (and in his published response to Barbour) that conventional modelling of their data must predict a resistive impedance at high frequencies, which they did not see. The argument is that a capacitative membrane impedance must become negligible at sufficiently high frequencies compared with a series extracellular resistance. This is an incomplete argument and misleading, as I thought was clear to Destexhe early in our correspondence.

The conclusion would indeed be true (though only evident at frequencies orders of magnitude higher than the 1kHz maximum in the data) if it were possible to directly measure an impedance between cytoplasm and an extra-cellular site. However, real measurements require electrodes with resistance and capacitance that need to be included in a model. Gomes et al. used a single patch electrode for both current passage and voltage measurement, which further limits the interpretation. When capacitance of electrode walls and amplifier input are included, impedance phase tends towards -90deg at moderately high frequencies, while compensation in the amplifier using negative capacitance can push it towards +90deg, as may explain the steeply increasing phase in Fig. 8. These effects can be explored in the model I provide, and I thought they were acknowledged by Destexhe many months ago.

My model of course makes simplifying assumptions. The parameters set in the download are only examples of sets that can fit well to the data of Figs 2,8 in the paper. Indeed they rather arbitrarily use equal time constants for soma and dendrite membranes since Destexhe thought for some unstated reason that this should be a constraint. It is not at all clear that implausible parameters are needed to fit the data using conventional electrophysiological analysis, though of course there are many unknown details about the preparations used. Destexhe coments (26 Jan) that more experiments could settle matters. It is indeed possible of course that new experiments could overturn conventional electrophysiological understanding. But the point at issue at present is not whether conventional understanding is wrong, but whether the paper of Gomes et al. (2016) shows that it is wrong, as claimed in the title.

Gomes J.-M. et al. (2016) Intracellular impedance measurements reveal non-ohmic properties of the extracellular medium around neurons. Biophysical Journal, 110(1):234-246

Alain Destexhe commented 2 years ago [1/2/2018]

I see your points but I have no idea whether they are valid or not. Why don't you try to publish your model? At the moment, you have a model drawn on the back of the envelope, and for which we don't know if it is valid or flawed. You use this model to criticize a series of papers published in journals like Biophysical Journal or Physical Review, so having been reviewed by biophysicists and physicists. I suggest you do the same to give more credibility to your criticism, otherwise it seems a bit easy...

About the issue of ohmic or non-ohmic, as said above, this paper is not alone, it is part of a series of papers which bring evidence based on other signals, such as intracellular recordings, LFP, EEG, MEG... This is the impedance-measurement part of the story. You can of course say - in non peer-reviewed media - that these peer-reviewed papers are all wrong, but frankly it is not very credible.

(and also not very constructive - as you do not propose anything to go forward)

In you read our review paper published in J. Integrative Neurosci (preprint copy at https://arxiv.org/abs/1611.10047), we review all these elements, and propose new experiments to go forward. In my opinion, this is the only way to go.

Tony Gardner-Medwin commented 2 years ago [19/2/2018]

Perhaps I should write a paper showing how the data of Gomes et al. (2016) is consistent with conventional cable theory. This is hard to do without collaboration however, because one is always open to the possibility that the experimenters may come up with a detail differing from assumptions made in a model. Conspicuous at present is the absence of any information in the Gomes paper or in response to discussion, about how capacitances of the input and the electrodes were or were not compensated in the experiments. The model I presented (fitting phase as well as modulus of impedance - contrary to what Destexhe implies above) makes what seem to me plausible assumptions that involve under-compensation in Fig. 2 and over-compensation in Fig. 8. It scarcely requires as much as the back of an envelope to see that Destexhe's claim that measured impedance must become resistive (zero phase) at high frequencies can only be true if capacitance compensation is perfect, and it's not clear that there would be any way in these experiments of ensuring that that is the case. The lack of discussion of this kind of issue in the paper unfortunately seems indicative of rather cursory refereeing. It would be nice if publication in a respected peer-reviewed journal was always a guarantee of soundness, but sadly that isn't always true. Hence the value of this kind of discussion.

I have chosen to look closely at this paper rather than others from Destexhe and colleagues claiming to challenge conventional electrophysiological analysis because, unlike the others, the Gomes data seemed completely open to conventional modelling. I don't claim that these other papers are unsound, and I can't claim particular expertise in their domains, but I suspect that they are even more susceptible than the present paper to physiological assumptions that cannot be fully verified. Given the title of Gomes et al. 2016, the onus seems on them to show that well established conventional interpretations will not work. If they could do this, I would indeed be very interested in experiments to test some alternatively formulated hypothesis. At present, I can't see the point.

Since it seems that PubMed Commons may be discontinued (https://ncbiinsights.ncbi.nlm.nih.gov/2018/02/01/pubmed-commons-to-be-discontinued/), I suggest that further discussion should continue on the Biophysical Journal's (possibly new) comment site, at http://www.cell.com/biophysj/comments/S0006-3495(15)01176-5 .