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Comment to the Editor

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Is the Extracellular Impedance High and Non-resistive in Cerebral Cortex?

ABSTRACT A recent commentary to *Biophysical Journal* criticized a previous study published in the same journal by Gomes et al. in 2016, and an alternative interpretation of the measurements was proposed. We reply here to these criticisms and provide some additional clarification, in particular, about a possible misinterpretation of the electrical circuit corresponding to these experiments. We suggest that, indeed, the extracellular impedance in cerebral cortex could be high and non-resistive, and we propose further experiments to settle this issue.

In a paper published in *Biophysical Journal* in 2016, Gomes et al. (1) provided measurements of the extracellular impedance in cerebral cortex and basal ganglia, in vivo and in vitro, and found an unexpected frequency dependence suggesting that the extracellular medium is more complex than the simple resistance ("resistive medium") usually assumed. The measured frequency dependence conformed to a diffusive impedance in agreement with previous theoretical studies, which predicted this impedance (2). It was also in agreement with indirect measurements of the extracellular impedance from local-field potential (LFP), electroencephalogram (EEG), or magneto-encephalogram (MEG) measurements (3,4), which also predicted such frequency dependence (see ref. (5) for a recent review of these measurements). There exists contradictory evidence from impedance measurements in neural tissue, some of which suggests resistive or weakly frequency-dependent media (6–9), while others measured a strong frequency dependence of the extracellular conductivity (1,10,11).

In a Comment to the Editor, Barbour (12) criticized some aspects of the Gomes et al. (1) study. He proposed another interpretation of these measurements based on cable filtering, as proposed previously by Pettersen and Einevoll (13). One main argument of Barbour was that the electrode was not passive as claimed and that the impedance was measured anomalously high because the Gomes et al. estimate neglected the contribution of dendrites. Thus, he proposes that the experiments can be explained by a purely resistive model, with no need to consider the extracellular medium as frequency dependent.

The first clarification that we would like to make here is that there is a misinterpretation of the electrical circuit corresponding to the experiments. Barbour's statement that "any current circulating in the recording electrode must also traverse the reference electrode, so it would not be passive" is false. The current was collected by a third electrode in the bath, which was put to the ground. The second micropipette ("reference") is therefore completely passive, because it just records the extacellular potential between the two other electrodes. The measurements published in Gomes et al. (1) were all taking this second micropipette as reference, and thus the potential indicated is the difference between the two micropipettes: one pipette injects currents intracellularly, and the other pipette acts as a passive reference.

Altough this was mentioned in the paper, we provide here a more explicit circuit of the experiments, where the three electrodes (including the bath electrode) are shown (Fig. 1). This circuit takes into account the current flow through the soma and the dendrites. In particular, it is clear that, in this setting, the current flows from the dendrite to the ground.

A second important issue is cable filtering. As explained in the paper, we took into account the presence of dendrites explicitly (Fig. 6 in ref. (1)). The presence of dendrites and the associated cable filtering influence the scaling of the impedance, we certainly agree with this fact. Indeed, with dendrites, the scaling of the impedance modulus approaches the measurements, but the phase of the impedance is not correct. In particular, the phase converges in a stable fashion toward -45° (see Fig. 6 in ref. (1)), while it converges to zero in a resistive model. This convergence to a zero phase in the resistive model is due to the fact that, at high frequencies, the modulus of the membrane impedance will necessarily become lower than the (constant) modulus of the extracellular medium impedance (region a in Fig. 1).

This shows that, when measuring impedances, it is important to take the phase into account. The behavior of the phase at high frequencies (typically greater than 100 Hz) permits to distinguish between different models and formally identify if the system is resistive. Future measurements should

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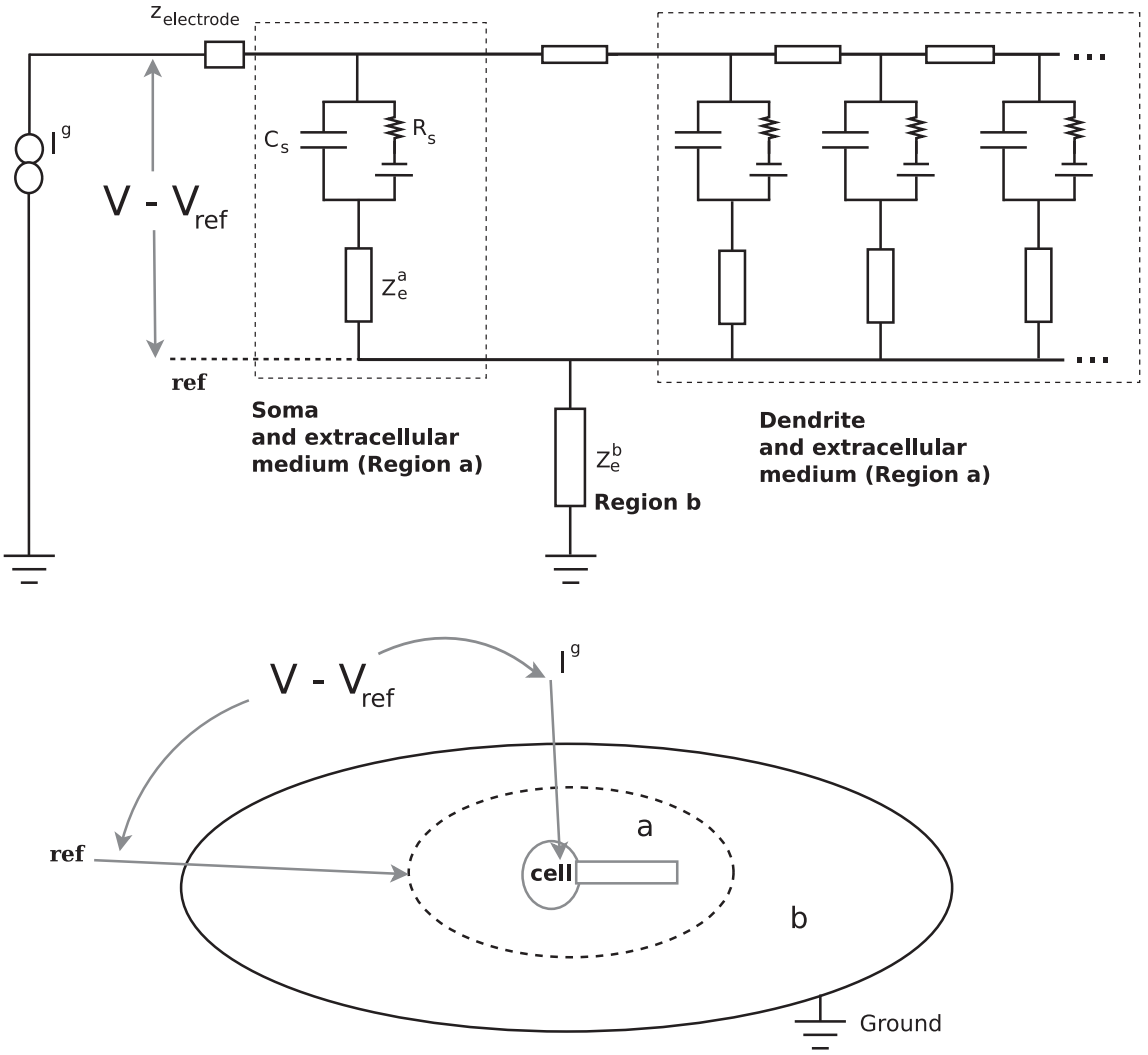

FIGURE 1 Equivalent circuit for the impedance measurements of Gomes et al. (1). A first electrode injects current intracellularly, and the current flows to an electrode in the bath. Another electrode (micropipette) measures the extracellular potential passively and was used as a reference to calculate the impedance. The current flows through the soma membrane as well as the dendritic membrane, as indicated (see (17)).

focus on the high-frequency profile of the impedance to definitively settle this issue.

Another main point in Barbour's Comment to the Editor is that the impedance measured is very high compared to previous estimates. To make this estimate, Barbour estimated the resistance R of the extracellular space from the expression $R = 1/4\pi r\sigma$, where σ is the extracellular conductivity of the extracellular space, and used this value to compare resistive and diffusive impedances. However, there is a conceptual error here, because it assumes that this expression is always valid, while it is only valid for a resistive medium. Thus it cannot be used to make any claim about non-resistive media. This is why one must use a generalized formalism that includes resistive and non-resistive media as particular cases, as we did in Gomes et al. (1). Furthermore, Barbour compares absolute values of the potential (in Volts) to values taken in Fourier space (in Volts/Hz) without making an inverse Fourier transform, which is also erroneous.

The argument that there will be a huge measurement error because of a 10,000 factor in magnitude is also likely wrong, because this would predict a huge cell-to-cell variability in the measurement, which was not the case—all cells robustly showed the same frequency profile. Similarly, the argument of overfitting or low sampling density is not plausible either, because it would also predict a huge cell to cell variability, whereas all of our fits converged to a unique solution that was consistent from cell to cell. We add here the precision that our Monte-Carlo fitting method was tested by running 100 different fits, each using 10,000 different sets of parameters, and all fits converged to the same solution, suggesting that the convergence was robust.

Although Barbour's reasoning contains errors, it is true that the modulus of the impedance is larger than previously

thought. In some of the previous measurements, it is impossible to know what fraction of the current goes though the extracellular medium and what fraction goes elsewhere. This issue was discussed in detail in a recent review paper (5), where an experiment was proposed to force all the current to flow into the medium, which should settle the issue of the impedance amplitude.

On this issue, Barbour further states "…the measurements of the Bedard and Destexhe group are likely to have quite similar tissue damage, as two electrodes are still inserted into the tissue. The mechanism therefore cannot explain the difference between their and previous measurements". This is again an error. It is true that the mechanical damage will also occur in our experiments, but it is not a problem here—there is no current leak to the cerebro-spinal fluid in our experiments because we used whole-cell recordings, where the electrode is tightly sealed to the membrane. This is precisely why whole-cell recordings were used! On the other hand, there will be a current leak if the electrode is directly inserted into the extracellular space. This current leak will necessarily be of resistive nature, and it will necessarily bias the measurement toward a purely resistive system. The whole point of the measurments of Gomes et al. is that it is the only type of impedance measurement that does not suffer from this potential problem.

If it is true that the extracellular impedance is higher than expected, it means that, in a traditional metal-electrode measurement, a large fraction of the current may flow through this resistive leak, while very little current would flow in the extracellular medium. There is a serious possibility of contamination of metal-electrode measurements and should be checked with appropriate experiments, as discussed in (5).

Finally, we would like to point out that the Gomes et al. measurements are not isolated; there is other evidence that the medium is more complex than a resistance, such as Gabriel et al. (10) measurements. As pointed out before, there is indirect evidence from other brain signals, such as LFP, EEG, and MEG (3–5), that can be accounted for by diffusive impedances. Furthermore, resistivity measurements point to a very inhomogeneous structure in cerebral cortex (14,15), and theoretical work has established that such inhomogeneity will lead to frequency filtering (16). The response of neural tissue to the electric field was also found to be very slow (B. Rebollo et al., 2016, Soc. Neurosci., abstract), which implies a strong frequency dependence. Thus, a frequency-independent impedance would be contradictory to such measurements (unless they are all considered invalid?). It is difficult to imagine that none of these potential filters will play any role. Finally, it must be pointed out that, while diffusive impedances can explain these experimental observations (5), a purely resistive theory cannot explain these measurements, and we must necessarily postulate that they are all erroneous, with no further explanation, which does not seem very plausible.

In conclusion, we hope we have clarified some of the issues related to the measurements provided in Gomes et al. (1). We agree that these measurements trigger a number of questions, some of which are valid and others invalid, but, so far, we see no valid resistive explanation to these measurements. It is important to note that a plausible reason was provided to explain why resistive media are sometimes observed (5), while no plausible reason has been proposed for why non-resistive measurements would all be wrong. As theoreticians, we feel more satisfied with the theory that provides explanations for all experimental observations. We made efforts to find a framework where all data can be explained coherently (5); of course, this framework may be incomplete and probably needs to be improved, but at least it provides a constructive basis to make further experiments.

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