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On the validity of the two-cells model in the analysis of passive electrical properties of gap-junction connected cells

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Abstract It is a rather extended practice to derive electrophysiological data (membrane and contact conductances) from experimental data for gap-junction tissues assuming that electrical connections are reduced to cell pairs. It is here shown that, if the length constant is sufficiently large, the mentioned procedure can lead to qualitatively incorrect results.

Keywords Electrical coupling · Two-cells model · Computational models

Introduction

Knowledge of cell membrane biophysical properties was greatly improved after the introduction of the patch-clamp technique (Sakmann and Neher 1983). This method, and the associated conceptual tools, allowed very powerful approaches to the characterization of the membrane ionic conductance k_m and the definitive experimental confirmation of the ion channel-based theory for excitability, originally formulated by Hodgkin and Huxley (1952). Nevertheless, most of the information on ionic channels was obtained from isolated individual cells in culture. The obvious next step was to understand the role of ionic conductances in cell collectives (tissues). There are two immediate hypotheses: (1) the behaviour of cell collectives is the linear sum of the individual components; (2) non-linearities emerge in the collective behaviour. Some experimental evidence (Andreu et al. 1997, 2000) indicates that the second proposal seems to be more reasonable. This evidence shows that the elements that allow electrical connections (gap junctions) are not fixed and that the properties of the individuals are determined by the tissue architecture. It follows, then, that the extrapolation of individual behaviour to collectives is far more doubtful than could be anticipated. An additional problem came to introduce further confusion into this scenario: the ionic conductances can be directly measured in isolated cells and apparently measured in tissues (provided there is no contamination in the measurements by the current spreading through the gap junction). Unfortunately, there is only one case where an accurate measurement of gap-junction conductance k_c can be attained: when the experimental preparation consists of an aggregate of two cells. In all other cases, spreading of the current to neighbouring cells introduces uncertainties in the measurements. The assumption of a minor contribution of this current spreading has allowed measuremnt of well-accepted estimates of k_c through the use of the so-called two-cells model (Bennett 1966) that obviates the complexity added by the tissue architecture. It seemed to us necessary to probe deeper into the building of a theoretical framework in order to explain the deviations from linearity of the cellular collective behaviour.

The effects of cell coupling have been studied using basically the following experimental approach. Current is injected into a given cell of the tissue and the subsequent voltage deflections at that site, and sites at increasing distances from it, are measured (Hille 1991; Andreu et al. 1997). These data give the input conductance $k_0 = I/V_0$, where I is the injected current and V_0 the input voltage, and, combined with a measurement of the

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intercell distance, the length constant λ. From these two values one can in principle derive the membrane k_m and the contact k_c conductances. This procedure is, however, hindered by the complex effects of tissue morphology (Lamb 1976; Lamb and Simon 1976; Umino et al. 1994; Petersen and Ueda 1997; Andreu et al. 2000). Bennett (1966) suggested that an alternative way to derive k_c and k_m consists of measuring the electrical properties of freshly isolated cell pairs of a given tissue. This method, commonly known as the two-cells (2C) model, allows a straightforward determination of k_m and k_c and has been extensively used (Perez-Armendariz et al. 1991; Qian et al. 1993). The method is based upon the assumption that the aforementioned conductances in the multiply connected tissue are similar to those in cell pairs.

Owing to its overwhelming simplicity and, in some cases, to the lack of information on the tissue morphology, some authors actually analyse experimental data for multiply connected tissues assuming that the connections are reduced to cell pairs (Petersen and Ueda 1976; Iwatsuki and Petersen 1978; Kettenmann and Ransom 1988; Monti-Bloch et al. 1993; Andreu et al. 1997). The underlying assumption is that electric signals do not spread much and, therefore, the 2C model applied to the tissue should at least give a qualitatively correct estimation of the membrane and contact conductances. It is the purpose of the present work to investigate the validity of such an approach. Our results suggest that using the 2C model to analyse the electrical properties of gap-junction tissues may lead to qualitatively incorrect results.

Model and methods

We consider arrangements of cells with membrane conductance k_m, coupled to their nearest-neighbour cells through connections of conductance k_c (a schematic view is shown in Fig. 1). The key parameter of the system, as far as spreading of the signal is concerned, is $\alpha = k_m/k_c$. In order to obtain the input voltage (or input conductance), one has to solve the system of linear equations derived from Ohm's law for a given cell arrangement (two cells, linear chain, square lattice, Bethe lattice, etc.). As the procedure has been thoroughly discussed by several authors (Lamb and Simon 1976; Poznanski and Umino 1997; Andreu et al. 2000), here we shall only quote the exact results for the membrane conductance k_m versus α and the input conductance, in the lattices of models pertinent to the present discussion. In the 2C model, k_m is given by:

$$k_m = \frac{1+\alpha}{2+\alpha} k_0 \tag{1}$$

while for a Bethe lattice (a lattice without rings; see Andreu et al. 2000) of coordination $p > 2$ the result is:

$$k_m = \frac{k_0 \alpha}{\alpha + \frac{p}{2(p-1)}\left[p - 2 - \alpha + \sqrt{(p+\alpha)^2 - 4(p-1)}\right]} \tag{2}$$

In the case of coordination $p = 2$, the result corresponds to the well-known case of the linear chain:

$$k_m = \frac{k_0 \alpha}{e^{\sqrt{\alpha}} - e^{-\sqrt{\alpha}}} \tag{3}$$

Finally, the membrane conductance in a square array is:

$$k_m = \frac{2k_0 \alpha}{\pi(4+\alpha)} K\left(\frac{4}{4+\alpha}\right) \tag{4}$$

where K is the complete elliptic integral of the first kind.

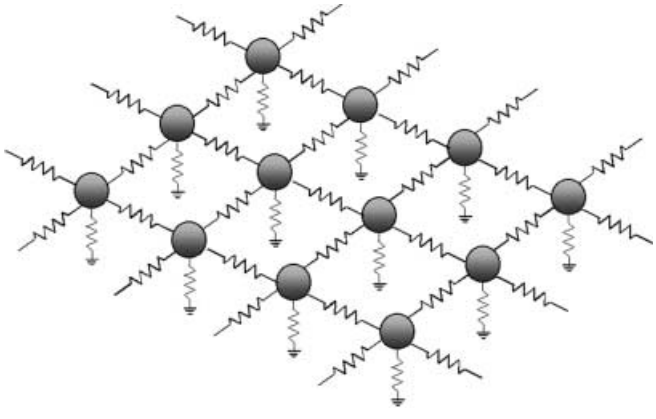

Fig. 1 Sketch of the model used in this work. In the figure the cell network is represented by a square lattice

Results and discussion

We concentrate on the case in which the spreading of the signal is large, or, alternatively, the length constant λ is much larger than the intercellular spacing a. The parameter which controls signal spreading is the ratio $\alpha = k_m/k_c$. As α decreases, the signal spreading increases. The limit of small α (or large signal spreading) is, on the other hand, the relevant case in many tissues such as mammalian retina (Piccolino et al. 1984). For small α the 2C model gives the following result for the membrane conductance:

$$k_m = \frac{k_0}{2} \tag{5}$$

as can be easily derived from Eq. (1). This means that all changes in the input voltage are directly translated into changes in the membrane conductance, the latter being independent of α, or the length constant.

The result changes qualitatively for Bethe or periodic lattices with coordination p greater than 2 (Andreu et al. 2000). In this case, Eq. (2) gives:

$$k_m = \frac{p-1}{p(p-2)} k_0 \alpha \tag{6}$$

while for the infinite square lattice an expansion of the complete elliptic integral in Eq. (4) leads to:

$$k_m = \frac{k_0}{4\pi} \alpha \ln \frac{32}{\alpha} \tag{7}$$

Now, as $\alpha = k_m/k_c$, it is the contact conductance, the one given by the input voltage, that is scarcely affected by changes in the length constant (note that the

logarithm is a slowly varying function; see also Andreu et al. 2000). A similar result is obtained for other two- or three-dimensional lattices.

The case of a linear chain is intermediate between that of the 2C model and periodic or Bethe lattices. In particular, the result obtained from Eq. (3) is:

$$k_{\mathrm{m}} = \frac{\sqrt{\alpha}}{2} k_0 \tag{8}$$

These results clearly illustrate to what extent the electrical properties may depend on tissue morphology.

Of course, the 2C model is exact when applied to isolated cells pairs (within the limits of the RC model used to describe the electrical properties of the cell membrane and the gap junctions). As the above results indicate, it will, however, fail, even at a qualitative level, when applied to tissues having a large number of interconnected cells. Then the electrical behaviour of a tissue having a finite number of connected cells will continuously change from that of a pair of cells to that of an infinitely large cell arrangement as the number of connected cells in the tissues increases. The key question is how the crossover from the 2C behaviour to the multiply connected tissue behaviour occurs. This can be addressed by investigating how the input conductance varies as the number of interconnected cells increases. We have carried out numerical calculations in $L \times L$ clusters of the square lattice with periodic boundary conditions. Numerical results for k_{m}/k_0 are illustrated in Fig. 2. The results over a given range of α and a fixed cluster size L were fitted by:

$$\frac{k_{\mathrm{m}}}{k_0} = \alpha + b\alpha + c\alpha^2 \tag{9}$$

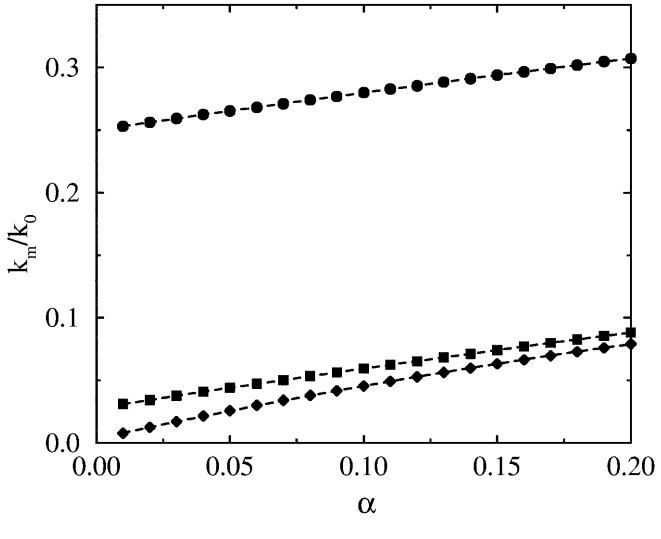

Fig. 2 Numerical results for the ratio k_{m}/k_0 versus the parameter $\alpha = k_{\mathrm{m}}/k_{\mathrm{c}}$ (in the small α range) for different clusters $L \times L$ of the square lattice; $L = 2$ (*circles*), $L = 6$ (*squares*) and $L = 20$ (*diamonds*). The results were fitted by means of $k_m/k_0 = a + b\alpha + c\alpha^2$ with $a = 0.25$, $b = 0.311$ and $c = -0.122$ ($L = 2$); $a = 0.027$, $b = 0.33$ and $c = -0.144$ ($L = 6$); and $a = 0.0035$, $b = 0.46$ and $c = -0.432$ ($L = 20$)

This equation is nothing but an expansion of k_{m} in powers of α that, in the small α limit, can be safely truncated. As shown in Fig. 2, the fits are very satisfactory. Note that while in the 2C model $a = 0.5$ (see Eq. 5), it should vanish as the limit of infinite L is approached, as the results of Eqs. (6) and (7) indicate. The latter is clearly seen in Fig. 3, where results for a and b versus L obtained in two ranges of the parameter α are shown. For $L = 10$, constant a has already decreased by two orders of magnitude (see vertical arrows in Fig. 3); while the fitted parameters depend on the range of α, the qualitative behaviour is unchanged as long as α is sufficiently small (see below).

As an example of the important implications of the present study, we consider the effects of dopamine on the length constant of horizontal cells of turtle retina investigated by Piccolino et al. (1984). These authors observed that dopamine decreased the length constant from 8.4 to 4.7 (in units of the intercellular distance) and, after an analysis of the results by means of different models, they concluded that this decrease in the length constant was due to a reduction of the contact conductance. However, as discussed hereafter, the conclusion can be qualitatively different. Among the results reported by Piccolino et al. (1984) there is one particularly significant, namely, that dopamine decreased only slightly the input conductance (from 1 to 0.9 μS). The experimental data and the present results are summarized in Table 1. As shown in the table, if these data are analysed by means of the 2C model, the effect of dopamine is to decrease the contact conductance by

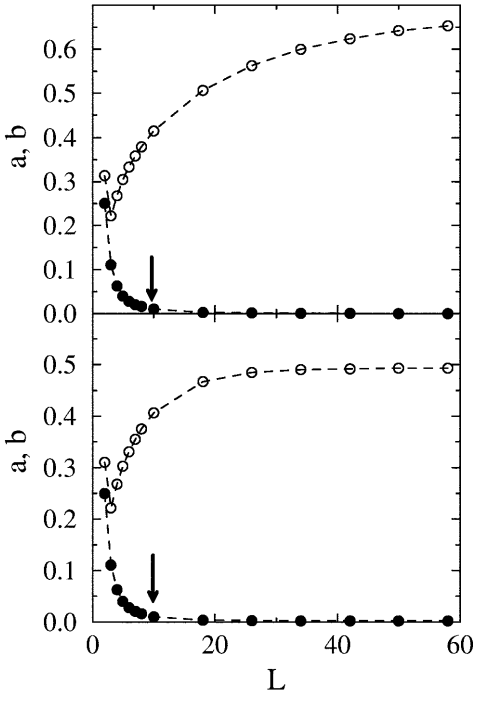

Fig. 3 Parameters fitted in Fig. 2 versus cluster size (*a*, *filled circles*; *b*, *empty circles*). Fittings were carried out for α in the ranges: *top*, 0.001–0.02; *bottom*, 0.01–0.2

Table 1 Experimental data (Piccolino et al. 1984) for the input conductance k_0 (in nS) and the length constant λ (in μm) of horizontal cells in turtle retina with and without dopamine. The intercellular distance in this tissue is $a = 45$ μm (Piccolino et al. 1984). Values of the membrane k_m and contact k_c conductances derived from those experimental data using the two-cells model (2C) and the square lattice (SL)

Dopamine	λ	k_0	k_m (2C)	k_c (2C)	k_m (SL)	k_c (SL)
No	380	1000	531	4210	3.5	696
Yes	210	900	498	2080	11.5	521

around 50% and the membrane conductance by less than 10% (in line with the Piccolino et al. analysis). On the other hand, if the square lattice is used in the analysis of the data, the contact conductance is decreased upon dopamine additions by less than 25% while the membrane conductance increases by almost a factor of 4. As it is likely that the tissue can be better described by means of a network, these results illustrate the failure of the 2C model in the case of large signal spreading.

Concluding remarks

In conclusion, our analysis indicates that when the spreading of the electrical signal is large, using the 2C model to derive the membrane and contact conductance from electrophysiological data obtained in tissues may lead to qualitatively incorrect results. In particular, the 2C model gives results for k_m and k_c opposite to those given by models based on periodic lattices. Our results indicate that for α in the range 0.001–0.2 the crossover from the 2C behaviour to the periodic lattice behaviour occurs for clusters of linear size $L \approx 10$ (see Fig. 3), which means around 100 connected cells.

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