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A generalised method to estimate the kinetics of fast Ca^{2+} currents from Ca^{2+} imaging experiments

Karima Ait Ouares[a,b], Nadia Jaafari[a,b], Marco Canepari[a,b,c,*]

[a] Laboratory for Interdisciplinary Physics, UMR 5588, Université Grenoble Alpes and CNRS, 38402 Saint Martin d'Hères, France
[b] Laboratories of Excellence, Ion Channel Science and Therapeutics, France
[c] Institut National de la Santé et Recherche Médicale (INSERM), France

HIGHLIGHTS

- Imaging Ca^{2+} fluorescence at high temporal resolution.
- Estimate the kinetics of Ca^{2+} currents.
- Study the physiological function of neuronal Ca^{2+} channels.

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ABSTRACT

Background: Fast Ca^{2+} imaging using low-affinity fluorescent indicators allows tracking Ca^{2+} neuronal influx at high temporal resolution. In some systems, where the Ca^{2+}-bound indicator is linear with Ca^{2+} entering the cell, the Ca^{2+} current has same kinetics of the fluorescence time derivative. In other systems, like cerebellar Purkinje neuron dendrites, the time derivative strategy fails since fluorescence kinetics is affected by Ca^{2+} binding proteins sequestering Ca^{2+} from the indicator.
New method: Our novel method estimates the kinetics of the Ca^{2+} current in cells where the time course of fluorescence is not linear with Ca^{2+} influx. The method is based on a two-buffer and two-indicator model, with three free parameters, where Ca^{2+} sequestration from the indicator is mimicked by Ca^{2+}-binding to the slower buffer. We developed a semi-automatic protocol to optimise the free parameters and the kinetics of the input current to match the experimental fluorescence change with the simulated curve of the Ca^{2+}-bound indicator.
Results: We show that the optimised input current is a good estimate of the real Ca^{2+} current by validating the method both using computer simulations and data from real neurons. We report the first estimates of Ca^{2+} currents associated with climbing fibre excitatory postsynaptic potentials in Purkinje neurons.
Comparison with existing methods: The present method extends the possibility of studying Ca^{2+} currents in systems where the existing time derivative approach fails.
Conclusions: The information available from our technique allows investigating the physiological behaviour of Ca^{2+} channels under all possible conditions.

1. Introduction

The possibility of measuring the kinetics of Ca^{2+} currents from fluorescence transients of a Ca^{2+} indicator allows studying the local activation and de-activation of Ca^{2+} channels during physiological activity. The founding principle of this measurement is that the fractional change of Ca^{2+} fluorescence ($\Delta F/F_0$) following a transmembrane Ca^{2+} influx is proportional to the Ca^{2+} bound to the indicator if the dye-Ca^{2+} binding reaction has reached its equilibrium and if the indicator is not saturated (Sabatini and Regehr, 1998). Under this condition, the $\Delta F/F_0$ signal will be proportional to the integral of the Ca^{2+} current if two further conditions are met: (*A*) that the dye-Ca^{2+} binding reaction equilibration is faster than the kinetics of the Ca^{2+} current; and (*B*) that the Ca^{2+} binding reactions with the endogenous molecules of the cell are either also faster than the kinetics of the Ca^{2+} current or slower but negligible with

* Corresponding author at: Laboratoire Interdisciplinaire de Physique (UMR 5588), Bat. E45, 140 Avenue de la Physique, Domaine Univ., 38402 St. Martin d'Hères Cedex, France.
E-mail address: marco.canepari@univ-grenoble-alpes.fr (M. Canepari).

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respect to the dye-Ca^{2+} binding reaction during the time-window of the measurement.

We have previously demonstrated that both conditions are fulfilled by the Ca^{2+} indicator Oregon Green BAPTA-5N (OG5N) in the case of dendritic Ca^{2+} currents associated with back-propagating action potentials in CA1 hippocampal pyramidal neurons (Jaafari et al., 2014, 2015). OG5N is a low-affinity indicator (K_D = 35 μM, Canepari and Odgen, 2006) that equilibrates in <200 μs according to the Kao and Tsien theoretical estimate of the relaxation time (Kao and Tsien, 1988). Its ability to track the kinetics of voltage-gated Ca^{2+} channels (VGCCs) can be therefore considered universal in all cellular systems. The second condition, i.e. the linearity with the endogenous Ca^{2+} binding reactions, is however not universal. In the dendrites of CA1 hippocampal pyramidal neurons, where the endogenous buffer capacity (Canepari et al., 2008) is relatively low (~100, Helmchen et al., 1996; Maravall et al., 2000), the Ca^{2+}-$\Delta F/F_0$ signal associated with an action potential is essentially constant for the first 10 ms after the peak and decays only later in ~100 ms. Fig. 1A shows a representative example of dendritic Ca^{2+} transient associated with a back-propagating action potential from a cell filled with 2 mM OG5N. The $\Delta F/F_0$ signal starts rising near the peak of the action potential reaching a plateau for a few milliseconds after the end of the action potential. The flat behaviour of the $\Delta F/F_0$ signal after the end of the current indicates that not only the dye-Ca^{2+} binding, but also the Ca^{2+} binding to the endogenous buffer, have reached the equilibrium during this short time window (Jaafari et al., 2014). Hence, the time derivative of the $\Delta F/F_0$ signal has the same kinetics of the Ca^{2+} current. The CA1 hippocampal pyramidal neuron of Fig. 1B was filled with 2 mM OG5N and 100 μM BAPTA, a high-affinity Ca^{2+} buffer (K_D = 160 nM, Pethig et al., 1989). In contrast to the OG5N-Ca^{2+} binding reaction, the BAPTA-Ca^{2+} binding reaction equilibrates in the millisecond range. As BAPTA sequestrates Ca^{2+} from OG5N, the slope of the $\Delta F/F_0$ signal becomes negative after the end of the current deviating from the linear behaviour with the Ca^{2+} influx. In this case, the time derivative of the OG5N-$\Delta F/F_0$ signal does not match the kinetics of the Ca^{2+} current. Fig. 1C shows an example of dendritic Ca^{2+} transient associated with a climbing fibre excitatory postsynaptic potential (EPSP) from a cerebellar Purkinje neuron (PN) filled with 2 mM OG5N. In this cell type, where the endogenous buffer capacity is high (~2000, Fierro and Llano, 1996), the dendritic Ca^{2+}-$\Delta F/F_0$ signal associated with climbing fibre EPSPs decays more rapidly (Miyakawa et al., 1992) following the Ca^{2+} binding to slow endogenous buffers. Thus, the OG5N-$\Delta F/F_0$ time derivative in Fig. 1C has a negative component and does not reproduce the kinetics of the Ca^{2+} current. Since the theoretical ability of Ca^{2+} imaging experiments for tracking a fast Ca^{2+} influx depends exclusively on the equilibration time of the indicator, a way to extract the kinetics of the Ca^{2+} current can be still developed if the kinetics of Ca^{2+} sequestration is taken into account. This is clearly a difficult task since the kinetics of Ca^{2+} binding proteins, measured with flash photolysis techniques (Faas and Mody, 2012), is very complex and depends on many parameters that vary from cell to cell. In contrast, a useful method should utilise a simple model with only a small number of degrees of freedom, allowing the development of a semi-automatic procedure to standardise the extraction of the Ca^{2+} current kinetics. In this report, we present a novel method to estimate the kinetics of the Ca^{2+} current based on this principle. The method applies a simple two-buffer and two-indicator model to the hypothetical current input to match the experimental time course of the OG5N-$\Delta F/F_0$ signal. The parameters of the model are set, initially, using the rising phase of the OG5N-$\Delta F/F_0$ time derivative as initial approximation of the current. The full kinetics of the current is then extrapolated by maximising the similarity between the experimental OG5N-$\Delta F/F_0$ signal and the equivalent curve obtained by computer simulation.

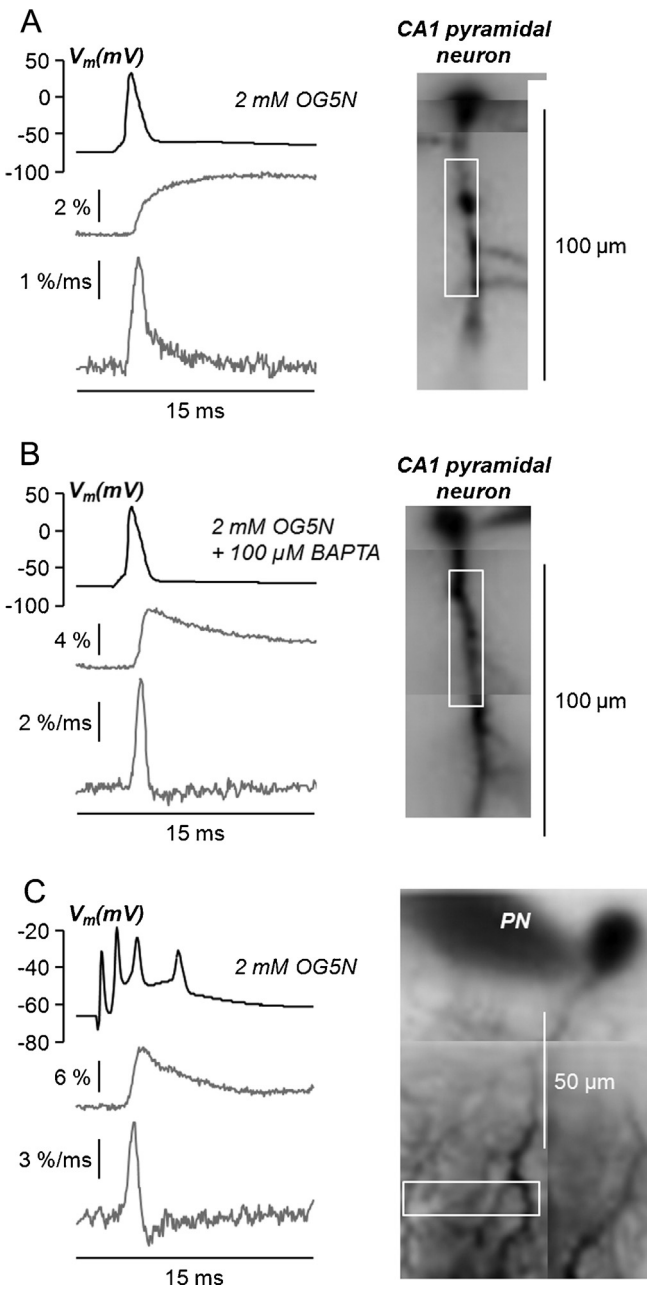

Fig. 1. OG5N-$\Delta F/F_0$ signals in CA1 hippocampal pyramidal neurons and in PNs. (A) OG5N-$\Delta F/F_0$ signal (2nd trace from the top) and its time derivative (3rd trace from the top) associated with an action potential (top trace) from the dendritic region of a CA1 hippocampal pyramidal neuron indicated on the right. The cell was filled with 2 mM OG5N. (B) Same as A but in another CA1 hippocampal pyramidal neuron filled with 2 mM OG5N and 100 μM BAPTA. (C) OG5N-$\Delta F/F_0$ signal (2nd trace from the top) and its time derivative (3rd trace from the top) associated with a climbing fibre EPSP (top trace) from the dendritic region of a PN indicated on the right. The cell was filled with 2 mM OG5N. All data, recorded at 20 kHz, were from averages of 16 trials.

To validate the method, we used combined fluorescence measurements from OG5N and Fura-2. In particular, since the time scale of Fura-2 equilibration is similar to that of endogenous Ca^{2+} binding proteins (Kao and Tsien, 1988), the measurement of Fura-2 $\Delta F/F_0$ (Fura-$\Delta F/F_0$) signal provided a direct estimate of Ca^{2+} sequestration. The usefulness and limitations of the estimate of the Ca^{2+} current kinetics, obtained with this approach, are discussed at the end of the paper.