Cell Calcium 54 (2013) 71–85

Contents lists available at SciVerse ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Estimating background-subtracted fluorescence transients in calcium imaging experiments: A quantitative approach

Sébastien Joucla[a,*], Romain Franconville[a], Andreas Pippow[b,c], Peter Kloppenburg[b,c], Christophe Pouzat[d]

[a] Cerebral Physiology Laboratory, Université Paris-Descartes, CNRS, UMR 8118, 45 rue des Saints-Pères, 75006 Paris, France
[b] Institute for Zoology, Cologne Biocenter, University of Cologne, 50674 Cologne, Germany
[c] Center for Molecular Medicine Cologne (CMMC) and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50674 Cologne, Germany
[d] MAP5 – Mathématiques Appliquées à Paris 5, CNRS UMR 8145, 45 rue des Saints-Pères, 75006 Paris, France

ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 14 April 2013
Accepted 15 April 2013
Available online 18 June 2013

Keywords:
Quantitative imaging
Fluorescence model
Maximum likelihood estimate
Confidence intervals
Goodness-of-fit tests
Mouse hypothalamus neurons

ABSTRACT

Calcium imaging has become a routine technique in neuroscience for subcellular to network level investigations. The fast progresses in the development of new indicators and imaging techniques call for dedicated reliable analysis methods. In particular, efficient and quantitative background fluorescence subtraction routines would be beneficial to most of the calcium imaging research field. A background-subtracted fluorescence transients estimation method that does not require any independent background measurement is therefore developed. This method is based on a fluorescence model fitted to single-trial data using a classical nonlinear regression approach. The model includes an appropriate probabilistic description of the acquisition system's noise leading to accurate confidence intervals on all quantities of interest (background fluorescence, normalized background-subtracted fluorescence time course) when background fluorescence is homogeneous. An automatic procedure detecting background inhomogeneities inside the region of interest is also developed and is shown to be efficient on simulated data. The implementation and performances of the proposed method on experimental recordings from the mouse hypothalamus are presented in details. This method, which applies to both single-cell and bulk-stained tissues recordings, should help improving the statistical comparison of fluorescence calcium signals between experiments and studies.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Using fluorescent indicators to dynamically image physiological parameters of single neurons or neuronal populations has become a routine technique in neuroscience, starting with the use of aequorin, a bioluminescent protein from jellyfish [1–3]. Beginning in the 80s with the development of quin2, fura-2 or indo-1 [4,5], a full battery of chemical calcium indicators covering a large range of affinities has been synthesized, among which the Fluo [6], Fura and Oregon-green families are the most commonly used. These fluorophores are introduced in the cytoplasm of the cell of interest using a patch-clamp pipette, or by loading a population of neurons through bath application of the acetoxymethyl ester (AM) forms of the dye. The development of genetically encoded GFP-based calcium indicators, which started in the 90s with proteins such as Cameleon [7], is still a very active field, and the latest indicators, such as GCamp [8,9] or TN-XXL [10], allow relatively non invasive and non cytotoxic labeling of targeted cell populations, while showing dynamical fluorescent properties that start approaching those of synthetic probes.

The development of these various chromophores and fluorescent proteins has been paralleled by the rapid evolution of optical microscopy and advances in laser and detector technologies. Those progresses allowed extended studies of calcium signaling at the cellular level with a particular emphasis for neurons on presynaptic transients and local dendritic dynamics (for review, see [11]). Calcium imaging is also used as a proxy for neuronal firing. Combined with two-photon laser scanning microscopy [12], allowing the dynamical observation of thick samples, it is now widely used as an alternative to extracellular recordings to study neuronal population responses in relatively intact preparations (see [13] for review).

Those techniques are growing in versatility and become available to a larger community. They also tend to generate large data

* Corresponding author at: Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, (INCIA), CNRS UMR 5287, Bâtiment B2 – Biologie Animale – 4ème étage, Avenue des Facultés, 33405 Talence Cedex, France. Tel.: +33 5 40 00 25 70.
E-mail addresses: sebastien.joucla@u-bordeaux1.fr, joucla_sebastien@yahoo.fr (S. Joucla).

0143-4160/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ceca.2013.04.005

sets, and the underlying cellular processes are usually complex. As such, it appears important that efficient analysis tools are developed [14,15]. Ideally, those methods should provide measurements of the reliability of the extracted information [16,17], so that results can be meaningfully interpreted and compared between studies from different laboratories, and eventually shared among the community.

A mandatory step before fluorescence data analysis is the estimation and/or subtraction of the background fluorescence corrupting the images at the location of the Region(s) Of Interest (ROI). This is especially the case for the estimation and comparison of fluorescence amplitudes or calcium concentration. An inaccurate knowledge of the background fluorescence may lead to improper estimation of the basal fluorescence values (F_0) and then on relative fluorescence values or fluorescence variations (F/F_0 or $\Delta F/F_0$, respectively), resulting in biases in the estimation of calcium concentrations. In other words, the amplitude of calcium transients cannot be compared one to the other without a good estimation of the background fluorescence. In this framework, a standard approach consists in measuring the fluorescence intensity in "unstained" regions in the vicinity of the cell. This assumes that the background fluorescence is locally homogeneous in the image. However, there are many instances where this hypothesis does not hold, and results obtained with such method should thus be treated cautiously. Another approach, that we used in a previous work [18], consists in measuring the fluorescence before loading the cell of interest with the dye and verifying that the fluorescence outside the cell did not vary much during the recordings. Unfortunately, this technique is only suited for single cell loading experiments and not for tissues bulk-stained with an AM-dye or expressing a genetically encoded indicator, since we do not know in advance the localization in the tissue at which cell(s) will be recorded. In such cases, the previous technique is also not usable, since the fluorophore stains most of the tissue, making the determination of a "background region" impossible. It is worth noticing that in *in vivo* studies using two-photon microscopy, authors often discard any background subtraction step in their analysis, given the absence of acceptable background estimation routines and the lower background fluorescence expected in 2-photon microscopy compared to full-field fluorescence imaging.

To overcome these drawbacks, Chen et al. developed "a novel image-processing algorithm that estimates the background of a given ROI by exploiting the information contained in the intensity dynamics of its individual pixels" [19]. Their approach was based on the following model: In a given Region of Interest, all pixels share a common background (due to the local autofluorescence) and the same variations in calcium concentration. The amount of dye excited by the incoming light is, however, variable from pixel to pixel: In a soma, the optical section is much thinner at the periphery than it is at the center of the cell. One can then describe the fluorescence intensity time course of the ith pixel of the ROI (noted $y_i(t)$) as:

$$y_i(t) = u_i \cdot f(t) + b + n_i(t), \tag{1}$$

where u_i is a pixel-specific scaling factor related to the amount of dye excited in pixel i, $f(t)$ is the normalized time course common to all pixels of the ROI, and b is the value of the common background fluorescence. This model can be fitted to all the individual pixel traces: As soon as the recording has more than two time points and the ROI more than two pixels with different u_is, such a fit will theoretically converge: In other terms, a single combination of b, u_is and $f(t)$ will fit to the data. In the Chen et al. model, the noise value at time t ($n_i(t)$ in Eq. (1)) is assumed to follow a Gaussian distribution with zero mean and a variance identical for all pixels of the ROI. The authors then developed a maximum-likelihood-based algorithm to estimate an optimal value of b, of all u_is and all $f(t_j)$ (the value of $f(t)$ at the j^{th} recording time). Using numerical simulations, they showed that this algorithm was unbiased and quantified a global precision on the estimation of b as a function of the number of time samples and pixels, the signal-to-noise ratio and the coefficient of variation of the u_i within the ROI. Finally, they proposed an iterative procedure to detect background inhomogeneities in the ROI and exclude the pixels showing a large background variation compared to the common value.

The Chen et al. method has the advantage to be usable in several types of experiments, including those with bulk-stained tissues, displaying heterogeneous distributions of background fluorescence. However, even if an expression of the global precision on the estimation of b was given, no confidence interval on b can be constructed based on a single data set (*i.e.* from a single ROI), which is crucial for the quantification of the data. The iterative procedure dealing with background inhomogeneities has the same drawbacks. Finally, a major assumption of the model, which should lead to the construction of relevant confidence intervals, stems from the noise model, assumed to be Gaussian with a space- and time-independent variance. However, as noted in a previous article [20], this is clearly not the case, since the fluorescence intensities measured with a CCD camera follow instead a statistics derived from the Poisson distribution. In any light detector used in microscopy, like the photomultiplier tubes, widely used in confocal and 2-photon microscopy, the recording noise follows a statistics that differs from the Gaussian model with variance independent on the signal intensity. Regarding the importance of confidence intervals for a correct comparison of results between experiments and studies, it appears essential to use a correct noise model, thus allowing a quantitative estimation of the background fluorescence.

The aim of the present work is thus to provide a novel way to estimate background-subtracted fluorescence transients in a quantitative way, that is to estimate both optimal values and precision on these values from single data sets. For that purpose, we use a non-linear regression approach to fit the data with a model of fluorescence that includes the background fluorescence as a parameter. First, in the case of homogeneous-background data, we show that this approach leads to the construction of meaningful confidence intervals for all values of interest (common background fluorescence, normalized true fluorescence time course and amplitude factor for each pixel) for a correct description of the noise model. Then, we present a non-homogeneous background model and evaluate an iterative procedure for the detection of background inhomogeneities inside the ROI. Finally, we illustrate the contribution of our method on experimental recordings from proopiomelanocortin (POMC)-expressing neurons in the mouse hypothalamus. This method, which works both on single-cell and bulk-stained tissues recordings, will help improving the statistical comparison of fluorescence calcium signals between experiments and studies.

2. Methods

2.1. Homogeneous background

2.1.1. Fluorescence model

A Region of Interest (ROI) is defined as a set of pixels in which the calcium concentration (and, thus, the normalized fluorescence time course $f(t)$) is homogeneous, but where the total quantity of dye excited can vary from pixel to pixel. In this section, we consider a fluorescence model where the background fluorescence is uniform in the ROI. Thus, we can write the ideal (noise-free) fluorescence time course $F_i(t)$ in pixel i as:

$$F_i(t) = \phi_i \cdot f(t) + b, \quad i = 1 \ldots I, \tag{2}$$

Download English Version:

https://daneshyari.com/en/article/10926337

Download Persian Version:

https://daneshyari.com/article/10926337

Daneshyari.com