Mathematical Theory and Modeling
ISSN 2224-5804 (Paper) ISSN 2225-0522 (Online)
Vol.7, No.3, 2017

Modelling Calcium Signal Intensity Difference Between Cells

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Abstract

Cell signaling involves the transmission of a signal from a sending cell to a receiving cell. Calcium ions (Ca^{2+}) are a widely used type of messenger. In this study the evolution over time of calcium signal intensity and how these evolutions depend on the four groups of cells of subjects with different health condition was investigated. A longitudinal data analysis based on 110 subjects was used and to account non-linearity and correlated nature of the data, non-linear mixed model was used. Based on the exploratory data analysis result supported with CurveExpert professional software the model used has sigmoid structure. From the result, the rate of change of average signal intensity was nearly 0.033 and the time at which the rate of change of average calcium signal intensity reaches its maximum (i.e. the inflection point) was nearly 198 seconds. Furthermore, there were statistically significant differences in average calcium signal intensity between the groups. It is also observed that significant differences between mild hyperplasia and benign tumor patient's cells and also between malignant tumor and healthy subject's cells.

Keywords: Calcium, Cell Signaling, Non Linear Mixed Model, Random Effect, Signal Intensity

1. INTRODUCTION

Cell response involves the transmission of a signal from a sending cell to a receiving cell. Although cells can respond to a range of signals, including light and touch, the most commonly used signals in cell-cell communication are chemical. These chemical signals, which are proteins or other molecules produced by a sending cell, are often secreted from the cell and released into the extracellular space. Response molecules produced in one cell can travel through the gap junction to the neighbor cell, triggering a response. Small particles, such as calcium ions (Ca^{2+}) can move easily through the channels between cells, but larger molecules like proteins may be unable to fit without special assistance. Calcium ions (Ca^{2+}) are a widely used type of messenger. In most cells, the concentration of calcium ions in the cytosol is very low, as ion pumps in the plasma membrane continually export calcium from the cell. Calcium ions from the cytosol may also be pumped into the endoplasmic reticulum, which serves as a sort of "holding tank" to be used during signaling (Kahan Academy, 2016).

The resting concentration of Ca^{2+} in the cytoplasm is normally maintained around 100 nM, variously reported as 20,000- to 100,000-fold lower than typical extracellular concentration (Clapham, 2007; Demaurex and Nunes, 2016). To maintain this low concentration, Ca^{2+} is actively pumped from the cytosol to the extracellular space, the endoplasmic reticulum (ER), and sometimes into the mitochondria. Certain

proteins of the cytoplasm and organelles act as buffers by binding Ca^{2+}. Cell response occurs when the cell is stimulated to release calcium ions (Ca^{2+}) from intracellular stores, and/or when calcium enters the cell through plasma membrane ion channels (Clapham, 2007).

In all eukaryotic cells, the endoplasmic reticulum (ER) and the mitochondria establish a tight interplay, which is structurally and functionally modulated through a proteinaceous tether formed at specific sub-domains of the ER membrane, designated mitochondria-associated membranes (MAMs). The tethering function of the MAMs allows the regulation of lipid synthesis and rapid transmission of calcium (Ca^{2+}) signals between the ER and mitochondria, which is crucial to shape intracellular Ca^{2+} signaling and regulate mitochondrial bioenergetics (van Vliet *et al.*, 2014). In some studies, it has been shown that the initial velocities of energy-dependent Ca^{2+} uptake were measured by stopped-flow and dual wavelength techniques in mitochondria isolated from hearts of rats. The first rate of Ca^{2+} uptake shows that the initial velocity ofCa^{2+} uptake was slow at low concentrations of Ca^{2+} and increased sigmoidally to 10 nM Ca^{2+} /s/mg protein at 300 ìM Ca^{2+}. Similar results were obtained by the employment of mitochondria subjected of a wide range of mitochondrial protein in the medium (0.5-10 mg/ml), when these organelles were oxidizing glutamate-malate and when acetate was replacing phosphate as a permanent anion (Patergnani *et al.*, 2011).

A role for MAMs in cancer disease has not been thoroughly investigated yet. In spite of this there are a number of indications suggesting that MAMs may have a bright future in cancer research. A clear link between MAMs and cancer comes in the form of promyelocytic leukemia (PML) and Akt, a proto-oncogene frequently found upregulated in cancer (van Vliet *et al.*, 2014).

This study aimed at investigating the evolution over time of calcium signal intensity and how these evolutions depend on the four groups of cells of subjects with different health condition, based on data from the repeated measures of calcium signal intensity. Furthermore, thorough investigation of a model based on this data will be done and the model which fit the data very well will be proposed for similar data.

1. METHODOLOGY

In this section, the data and various statistical techniques that can be used on this data set described. To explore the data and get some insight about the model different graphical methods were employed. In addition, non-linear mixed model was used to fit the data well.

1.1. Data and Variables

Our research objective was investigated by using longitudinal data obtained from 110 subjects and the outcome of this study was calcium signal intensity. The study subjects were sampled from those who have no disease, mild hyperplasia, benign brain tumor, and malignant brain tumor. Measures of calcium signal intensity of each subject were taken at each second for 10 minutes. Cells from the study subject are categorized in four groups as, healthy, mild hyperplasia, benign brain tumor, and malignant brain tumor.

For each subject the identification number and the time since they enter the study were observed.

1.2. Exploratory Data Analysis

Exploratory data analyses were performed to get insight of the data that may help us to make the decision on model building. By means of graphical techniques, the individual profile plot and the average profile of the subjects were explored to see how calcium signaling intensity evolves over time.

1.3. Non-Linear Mixed Models

Statistical models provide us with the opportunity to articulate and test our hypotheses against empirical data. At the same time, however, they offer only approximate renderings of our ideas about how and why individuals develop and change over time. Longitudinal data provide analysis opportunities and requirements that are different from those offered by other kinds of data (Ram and Grimm, 2007). Statistical models in which both fixed and random effects enter nonlinearly are becoming increasingly popular due to its wide variety of applications. The outcome variable which assumed to have linear average evolution over time had been fitted with linear mixed model and generalized linear mixed model. However, in many applications, particularly in the biological sciences, the time course of a continuous response for an individual may be characterized by a function that is non-linear in one or more parameters. A model has a non-linear mean function if the derivative of the mean function with respect to the parameters depends on at least one other parameter. In non-linear mixed models it is generally assumed that the conditional distribution of the response variable Y_{ij} given the random effect b_i belongs to the exponential family, encompassing both normally distributed and non-normal outcomes. In agreement with generalized linear mixed models, it is customary to assume normally distributed random effects with mean zero and covariance matrix $\boldsymbol{D}$, even though other distributions are possible in principle as well. The non-linear function may be chosen on empirical grounds for its ability to represent faithfully the apparent individual specific response-time relationship (Molenberghs and Verbeke, 2005).

1.4. Software

All analyses were performed using R version 3.2 (R Development Core Team, 2014), SAS® version 9.4 (Sas Institute Inc., 2013) and CurveExpert Professional. In addition, all tests were performed at a 5% level of significance.

2. RESULTS

In this section, the methods described in previous section applied to the calcium signal intensity data. In first section explore the data and assess the statistical modeling implications. In addition, based on the result from exploratory analysis and its model implication, further analyzed the data using the non-linear mixed model.

2.1. Exploratory Data Analysis

The individual profile plot of five randomly selected subjects and the average evolution over time of calcium signal intensity were presented (Figure 1). From the individual profile plot we can observe that there is large within and between variability of calcium signal intensity measurements suggesting random effect model is need to be fitted. The figure (i.e. Figure 1) also show that, generally, the calcium signal intensity measurements of subject increase sigmoidally. The average evolution over time of calcium signal intensity plot also shows that similar sigmoidally increasing trend of average calcium signal intensity measures. In addition, similar trend also observed from the average evolution over time of calcium intensity plot of each groups (Appendix A).

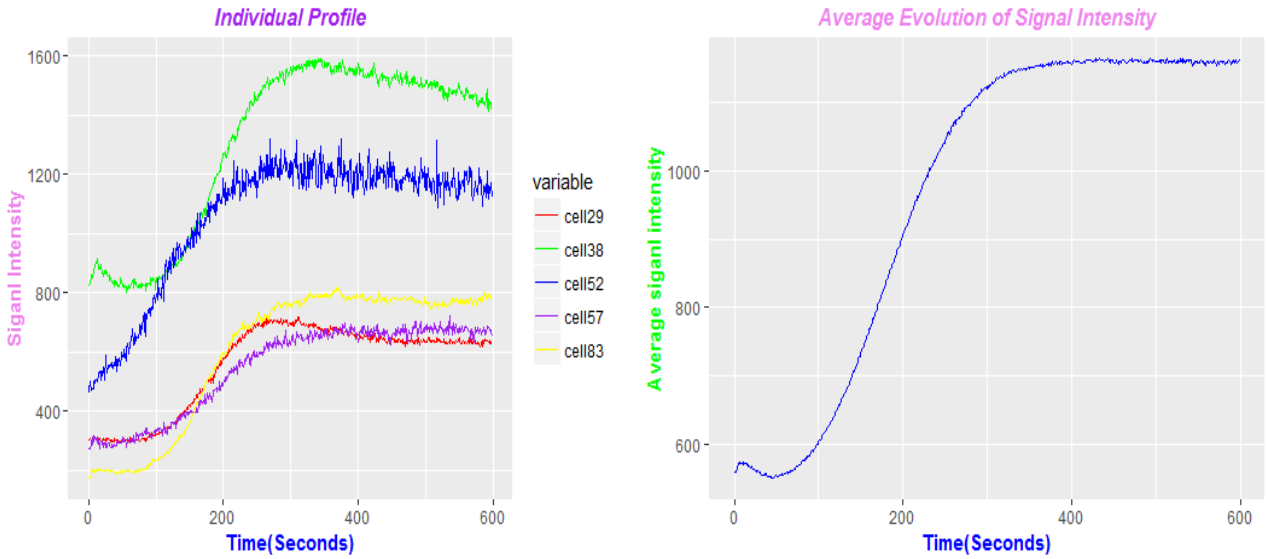

Figure 1: (a) Individual profile (sample subjects), (b) average evolution of calcium signal intensity.

2.1.1. Model Implication

From the results of exploratory data analysis, both the individual profiles as well as the average evolution structure depict linearity did not seem as a correct assumption hence a non-linear model was employed. Moreover, the individual profile indicates that there seems to be both between and within variability present which suggests the need of random effects model. Therefore, for this data a non-linear mixed model with sigmoidal structure was suggested.

2.2. Model Formulation

Individual change may be characterized by accelerations and decelerations of a particular form. Learning and population growth, for instance, often consist of multiple "phases," an initial period of adjustment where little growth occurs, a rapid growth phase, and a slowdown as ability or population approaches task or environmental capacity limits (Thieme, 2003). Such patterns of growth can be described by sigmoid curves that generally look like an elongated S. Sigmoid curves have a long history of use in many areas of study, including biology, physiology, and economics (Westerfeld, 1956; Winsor, 1932), where they have

been used to describe change processes ranging from bacterial growth to product innovation to early life increases in brain size. As we have seen in the exploratory data analysis, both the individual profile plot and the average evolution over time has sigmoidal shape. Hence, combining these results with the CurveExpert Professional and literature review results the following sigmoidal model was proposed at the initial stage.

$$Y_{ij} = \beta_0 + \frac{\beta}{1+exp-(time-\lambda)\alpha} + \varepsilon_{ij} \quad (1)$$

Physical interpretation of all the parameters of the proposed model presented (Figure 2). In the above equation (1), α denotes a rate of change and λ denotes the time at which the rate of change of calcium signal intensity reaches its maximum, the inflection point. When α is positive, growth proceeds from $\beta_0$an individual specific lower asymptote, to β_0+ β, an individual specific upper asymptote (and vice versa when α is negative) (Grimm and Ram, 2009).

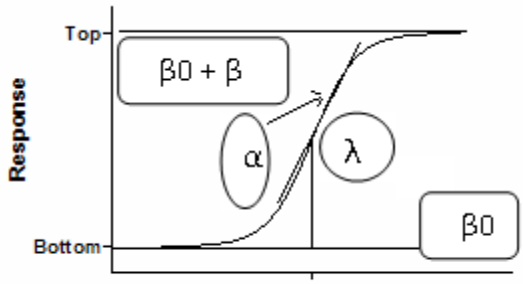

Figure 2: Physical interpretation of parameters of the proposed model

To see the effect of cell group on the calcium signal intensity, we introduced the covariate "group" in the proposed model as shown below.

$$Y_{ij} = (\beta_0 + \beta_1 G1 + \beta_2 G2 + \beta_3 G3 + b_{1j}) + \frac{\beta_4+\beta_5 G1+\beta_6 G2+\beta_7 G3+b_{2j}}{1+exp-(time-\lambda)\alpha} + \varepsilon_{ij} \quad (2)$$

where each terms defined as: G1 corresponds to patients with a malignant brain tumor, G2 corresponds to patients with a benign brain tumor, G3 corresponds to patients with mild hyperplasia and the reference group (G4) corresponds to healthy subjects. Further, $b_{1i}$and b_{2i} are the random effects with $\boldsymbol{b_i}$**~N(0, D)**, and the measurement errors $\boldsymbol{\varepsilon_i}$ **~ N(0,Σ)**

2.3. Non-Linear Mixed Model

In order to answer the research question, the non-linear mixed model specified in (2) was fitted using the NLMIXED SAS procedure. The result from fitting the model based on adaptive Gaussian quadrature approximation methods for the integral in the likelihood function with three quadrature points is presented (Table 1). In the analysis, other quadrature points also considered in order to assure stability and numerical convergence of the estimate and almost the same results were observed. A likelihood ratio test using a mixture of two chi-square distributions is used to check if a random effect is needed. This is done by using likelihood ratio test which follows approximately a mixture of two chi-square distributions. From the result

presented in (Table 1), the p-value is less the than level of significance 0.05 as indicated by '*M' and hence there is no sufficient evidence to remove the second random effect from the model. Similarly, the test for the first random effect was done by comparing a model having one random effect with the model which do not have the random effect and resulted with similar conclusion. Furthermore, the covariate "group" found to have statistically significant effect on calcium signal intensity of subjects, since all the estimated coefficient of the group parameter are significant at 0.05 level of significance.

Table 1: Parameter estimates and standard errors obtained from Non-Linear Mixed Model

Parameter	Estimate	Std Error
β_0	536.51	5.3233*
β_1	82.69	6.8126*
β_2	87.69	7.0239*
β_3	98.24	7.8057*
β_4	600.02	5.8273*
β_5	99.33	7.4577*
β_6	77.19	7.6968*
β_7	85.89	8.5587*
λ	197.54	0.0900*
α	0.03	0.0001*
Var(b_{1i})	638.72	2.9213*M*
Var(b_{2i})	760.80	3.9601*M*
Cov(b_{1i}, b_{2i})	0.00	2.6988
Var(ε_{ij})	1708.90	3.1245

**M* *p*-value from mixture chi-square less than 0.05

* *p*-value less than 0.05

From the predicted plot of average evolution over time of calcium signal intensity both the overall average evolution presented in (Figure 3(a)) and the average evolution by group presented in (Figure 3(b)) it was observed that the evolution of calcium signal intensity grows with sigmoid nature like the observed data. The predicted and observed average calcium signal intensity curves for both overall and by group are very close to each other as presented in (Figure 3(a) and 3(b)), which implies the model fitted the data very well.

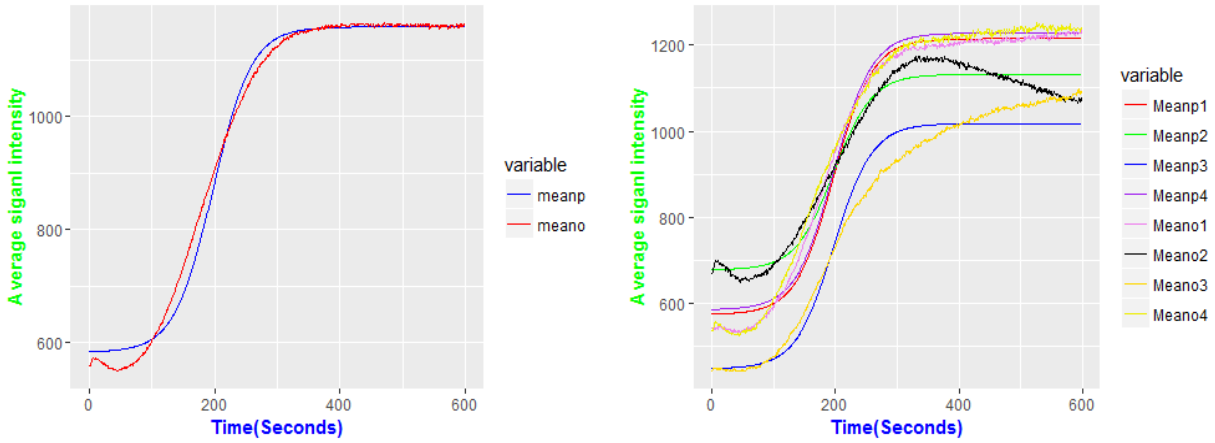

Figure 3: Predicted (Meanp) and observed (Meano) average evolution over time of calcium signal intensity: (a) for all subjects and (b) by cell groups.

3. DISCUSSION AND CONCLUSIONS

The objective of this study was to model the evolution of calcium signal intensity and investigate whether or not it depends on the "group". The outcome of interest is calcium signal intensity. A total of 110 subjects were considered and from each subject the calcium signal intensity measurements were taken at each second for 10 minutes. Since these measurements were taken from each subject over time, the statistical models which can take in to account correlation of measurements were considered. This was done by using a non-linear mixed model.

To find out a non-linear model which fit the data well, the average evolution plots for all subjects and for each group was observed. In addition, the individual profile plot also observed and supported these results with the CurveExpert professional software and finally found a non-linear model with sigmoid structure. The defining feature of the model considered is that the growth of calcium signal intensity is distributed equally before and after the inflection point.

From the fitted non-linear mixed model result, there were significant differences in average signal intensity among groups. The result also shows that there is statistically significant difference between mild hyperplasia and benign tumor patient. Similarly, significant difference also observed between malignant tumor and healthy subject's cells. In fact, calcium signaling depend on the health functioning of cells the difference observed from the result is not surprising. Moreover, the common rate of change of average signal intensity was nearly 0.033 with the p-value less than 0.05 and hence statistically significant. The time at which the rate of change of average calcium signal intensity reaches its maximum (i.e. the inflection point) was nearly 198 seconds which is also statistically significant as its p-value is less than 0.05. Finally, from the result above the proposed non-linear model with sigmoidal shape fitted the data very well. Therefore, to model the cell signaling data the model used above or similar model can be a good choice.

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APPENDIX

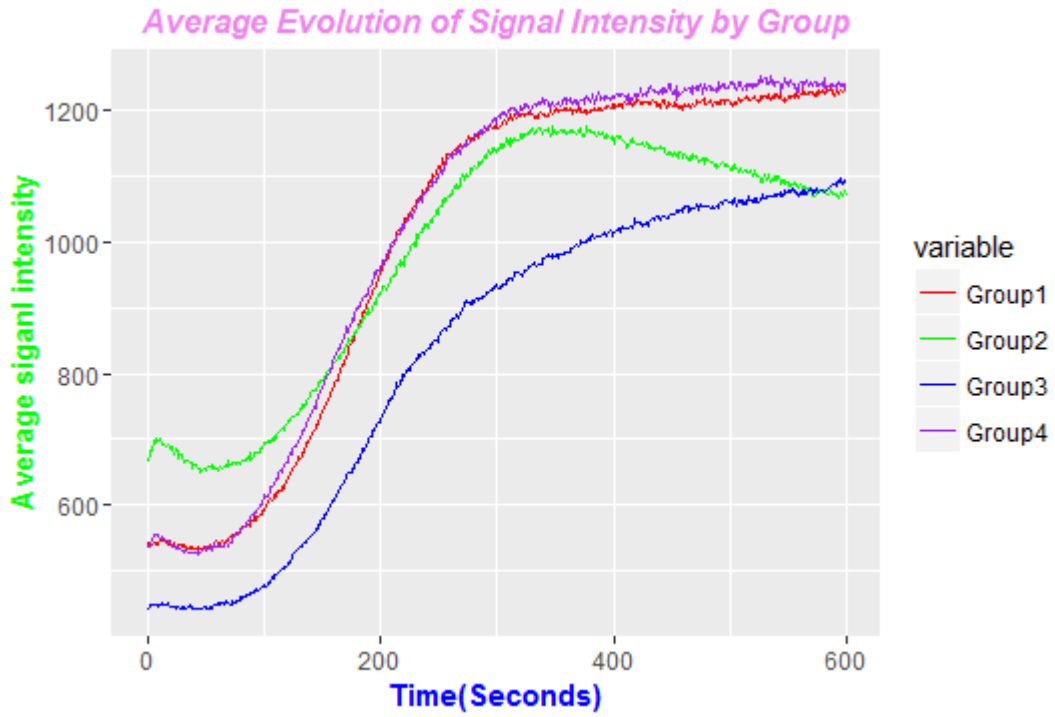

Figure 4: Average evolution over time of calcium signal intensity by cell group.