Curve-Fitting the Intracellular Calcium Dynamics

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"No one believes modeling results except the one who performed the calculation; ...everyone believes the experimental results except the one who did the measurements."

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Curve-fitting is a process of fitting mathematical functions (or models) to the observed data points. This exercise can produce additional insights, especially when the fitted mathematical function can characterize the underlying physical processes that produced the data. An excellent example is the brilliant work by Hodgkin and Huxley[1] who used curve fitting to extract the dynamics of "gating particles" in ion channels in squid giant axon. However, a dilemma often exists among experimenters as to how to find the best fit to the results without incurring unnecessary complexity. Given that biological functions usually involve intricately connected multiple processes operating in parallel with the outcome, which are heavily influenced by intrinsic and extrinsic factors, the rationale for curve fitting a single function to a physiological observation becomes difficult to establish.

Intracellular calcium (Ca_i) cycling plays a critical role in cardiac excitability and contractility. The sequence of the underlying dynamical events in the excitation-contraction coupling at the cellular level has been well-documented.[2] At the beginning of an action potential, calcium influx occurs mainly through the L-type calcium channel, and to a minor degree through sodium-calcium exchange (NCX). The calcium entry initiates a unique process called calcium-induced calcium release (CICR) that triggers massive calcium release from the sarcoplasmic reticulum (SR). The intracellular calcium concentration increases dramatically, which allows calcium binding to the myofilament protein troponin C, resulting in cardiac contraction. Subsequently, the removal of intracellular calcium is facilitated by SR calcium ATPase, sarcolemmal NCX, sarcolemmal calcium-ATPase and the mitochondrial calcium uniporter. This leads to the dissociation of calcium from myofilament and cardiac relaxation. These orchestrated events are tightly linked and mediated by many mediators. The machinery of calcium regulation in cardiomyocytes is complex and subtle, being co-operated by multiple proteins. Thus, these seemingly sequential events also involve many parallel and mutually dependent processes whose function cannot be independently characterized.

The increased understanding of intracellular calcium cycling can be attributed to the development of calcium indicators that are nowadays widely available commercially. The optical method to measure intracellular calcium has been developed for over two decades and has become quite popular and mature.[3] However, the interpretation of the acquired optical signal presents a major challenge to the experimenters. The optical intensity can fluctuate due to experiment conditions such as illumination, temperature, dye injection and retention, single-cell or the use of electromechanical uncoupler, etc. With the relatively noise-free optical signal, roughly speaking, the ascending phase with a rise in the Ca_i can be related to calcium release from the SR, and the descending phase with a fall in Ca_i suggests re-uptake by the SR. Due to the many components and processes in calcium cycling, the time course and morphology of the intracellular calcium recording do not provide information directly linked to the individual intracellular or transmembrane process. One way to interpret the results is to build detailed cellular models[4] to account for

Received: April 18, 2013 Accepted: May 3, 2013
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as many intracellular and transmembrane processes as possible, and attempt to numerically reconstruct the recorded curve. This approach is painfully laborious and involves substantial trial-and-error. Alternatively, curve fitting to a single mathematical function may produce a generalized macroscopic representation of the calcium transient, much like the use of diffractive index to characterize optical materials.

The study by Mizuno et al.[5] in this issue of the Journal extended their previous method to assess and quantify calcium transient. They used aequorin, a photoprotein sensitive to calcium, to record calcium transient in isolated mouse papillary muscle, in which the muscle contraction was inhibited by 2, 3-butanedione monoxime (BDM). The calcium transient recording was divided into four phases, and the second half of the ascending phase of the calcium transient was curve-fitted with both the half-logistic (h-L) and mono-exponential (m-E) functions. They showed that the h-L function could more accurately fit calcium transient than the m-E function. When the fitting results are improved, this is good news, especially considering that both functions have three parameters; therefore, fitting with either function does not incur additional complexity. However, when it comes to data analysis, the tools are only as good as the data. The experiment setup using aequorin in a multicellular preparation treated with BDM clearly limits the scope of the finding. The kinetics of aequorin are relatively slow when compared with other calcium-sensitive dyes; the multicellular recording could smooth out the cellular heterogeneity in calcium cycling, and BDM has been shown to alter calcium transients.[6] Consequently, the data used in their study may be too specific for developing a generalized approach for curve fitting, and the parameters obtained by fitting to this small data set are not informative. It is necessary to demonstrate that the h-L curve-fitting can be more generally applied to calcium transients obtained under different experiment conditions using normal and diseased tissue from different animal species. Without this effort, the marginally improved curve-fitting may only be a mathematical exercise, and the gain in physiological knowledge is meager in comparison to the fun of playing with mathematics.

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