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Elementary calcium release events in muscle studied with a new automated detection and analysis algorithm

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The function of ryanodine receptor (RyR) calcium release channels in the membrane of the sarcoplasmic reticulum of skeletal muscle can be studied with high spatial and temporal resolution using confocal microscopy and fluorescent Ca^{2+} dyes. The synchronized opening of groups of clustered RyRs produces fast local elevations of the intracellular Ca^{2+} concentration termed elementary calcium release events (ECRE). The morphology of these events provides insight in the channel kinetics of RyRs *in situ* and alterations are known to be associated with important pathophysiological conditions.
In this thesis, a novel automated computer program for the automated detection and analysis of ECRE is developed. Key steps of the method are based on the discrete wavelet transform (DWT), a modern mathematical tool for digital signal processing. The resulting algorithm includes image background normalization followed by DWT based noise reduction and ECRE detection procedures. Detected ECRE are analyzed automatically and the morphological parameters are computed. The algorithm is designed, tested and its statistical detection properties are evaluated quantitatively with model data sets. The algorithm simultaneously improves detection sensitivity and reliability compared with conventional methods. This effect is more pronounced at high background noise levels. Using data sets that are simulated in accordance with the “off-center” sampling effect of confocal linescan images, it is shown that morphological parameters of ECRE are measured with high accuracy. A modified algorithm is developed for the analysis of image series obtained with multifocal multiphoton microscopy. Using this approach it is shown that in contrast to conventional confocal microscopy, multifocal multiphoton microscopy can be used for dynamical ECRE imaging in two spatial dimensions. With the modified algorithm, a better analysis of the spatial dynamics of ECRE is achieved.
The novel algorithm is used to analyze the action of the anaesthetic drug thiopental on ECRE in mammalian skeletal muscle acquired with conventional confocal microscopy. Focal application of the drug shows that thiopental acts as an agonist of the RyR1 isoform and leads to a reversible increase in ECRE frequency in a dose-dependent manner. Thiopental elicited ECRE show a modal amplitude distribution and an increased mean peak amplitude, spatial width and duration. Furthermore, a repetitive mode of elementary Ca^{2+} release is identified and analyzed in terms of cumulative Ca^{2+} release flux and inter-event intervals. It is found that repetitive events can show a decaying cumulative Ca^{2+} release flux and provides some new insights in the dynamics of RyR clusters *in situ*.