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Automated detection and analysis of depolarization events in human cardiomyocytes using MaDEC

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ABSTRACT

Optical imaging-based methods for assessing the membrane electrophysiology of *in vitro* human cardiac cells allow for non-invasive temporal assessment of the effect of drugs and other stimuli. Automated methods for detecting and analyzing the depolarization events (DEs) in image-based data allow quantitative assessment of these different treatments. In this study, we use 2-photon microscopy of fluorescent voltage-sensitive dyes (VSDs) to capture the membrane voltage of actively beating human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs). We built a custom and freely available Matlab software, called MaDEC, to detect, quantify, and compare DEs of hiPS-CMs treated with the β-adrenergic drugs, propranolol and isoproterenol. The efficacy of our software is quantified by comparing detection results against manual DE detection by expert analysts, and comparing DE analysis results to known drug-induced electrophysiological effects. The software accurately detected DEs with true positive rates of 98–100% and false positive rates of 1–2%, at signal-to-noise ratios (SNRs) of 5 and above. The MaDEC software was also able to distinguish control DEs from drug-treated DEs both immediately as well as 10 min after drug administration.

1. Introduction

The derivation of human induced pluripotent stem cells (hiPSs) from somatic human cells has opened broad opportunities in the study of human cardiac cells. Previously limited by their minimal proliferation, human cardiomyocytes were difficult to obtain in significant number to allow widespread study. hiPS cells can be expanded into the required quantities and then differentiated into cardiomyocytes (hiPS-CMs) as a new, and seemingly endless, source of cardiomyocytes [18,30]. Accompanying this expanded availability, there has been an acceleration of the development of new methods for assessing the electrophysiological effects of drug compounds. Image-based tools for assessing excitable cells, such as cardiomyocytes, have particularly come to the fore [3,5,14,22,25,27]. With these new data acquisition methods comes a need for automated, user friendly, analysis methods of these image-based data.

Voltage-sensitive dyes (VSDs) are one such method for acquiring membrane voltage data. These dyes associate with cellular membranes and exhibit a characteristic increase in fluorescence intensity proportional to an increase in voltage across the membrane. This allows for non-invasive, non-destructive, and longitudinal assessment of hiPS-CM electrophysiology [11,29]. VSDs have previously been used in neuronal [8,21] and cardiac [7,10,15] cells and tissues. A wide range of VSD compounds and properties have been synthesized [29]. This study utilizes di-4-ANE(F)PPTEA, a hemicyanine class dye, to acquire a temporal fluorescent signal that corresponds to the changing voltage of hiPS-CM membranes.

To quantitatively assess these data acquired using VSDs in hiPS-CM, we built a custom Matlab software (MaDEC), capable of both detecting and analyzing individual VSD depolarization waveforms or depolarization events (DEs). Our previous work has analyzed hiPS-CM electrophysiology by performing supervised machine learning on pre-defined DE parameters [11] of already detected DEs. Here, detection is performed using a generalized matched filter for the entire waveform instead. This allows for non-biased event selection, as well as more reliable event selection in low-SNR environments. This detection method was previously shown to be successful for neuronal action potential detection in extracellularly recorded micro-electrode data [23], as well as neuronal calcium transient wave detection in calcium imaging data [24]. The detected DEs are subsequently compared, using a KS-test, across treatments and time points. The chronotropic drugs propranolol and isoproterenol were selected to validate this VSD-

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based approach and the corresponding analysis software. This method of analysis allows for quantitative assessment of the heterogeneity of DEs at a precise location on the membrane of an actively beating cardiomyocyte. Furthermore, this method allows quantitative assessment of how a given drug affects the DE shape of an actively beating cardiomyocyte.

In this study, we use 2-photon microscopy of fluorescent VSD to assess the depolarization of the cell membrane voltage of actively beating hiPS-CMs. Using MaDEC, we detect, quantify, and compare DEs of hiPS-CMs treated with common β-adrenergic drugs. The efficacy of our software is quantified by comparing detection results against manual DE detection by expert analysts, and comparing DE analysis results to known drug induced electrophysiological effects.

2. Methods

2.1. Human induced pluripotent stem cell-derived cardiomyocyte (hiPS-CM) culture and differentiation

hiPS-CMs were prepared for interrogation per the protocol previously described by Heylman et al. [11]. Briefly, wtc11 hiPS cells were differentiated into cardiomyocytes using a serum-free defined medium protocol [16]. Cells began spontaneously beating on approximately Days 12–15, and were stained with VSD and imaged on Day 33.

2.2. Voltage-sensitive dye staining and drug exposure

Culture medium was replaced with fresh medium containing 1 μM di-4-ANE(F)PPTEA (purchased from Leslie Loew, University of Connecticut) and incubated for 15 min at 37 °C. Cells were rinsed with RPMI/B-27 (+) insulin one time and then allowed to recover for at least 2 h prior to imaging. After staining with VSD, cells were qualitatively confirmed to still be spontaneously beating before addition of drugs. Medium was then replaced with fresh medium containing either 10^{-5} μM propranolol (SIGMA, P0884) or 10^{-7} μM isoproterenol (SIGMA, I6504). Data was collected immediately after addition of drugs (less than 60 s of exposure) and again 10 min or 15 min after addition to ensure complete exposure. Control images were captured from VSD stained cultures, not treated with either drug.

2.3. Two-photon microscopy

A Zeiss LSM 710 microscope (Carl Zeiss, Jena, Germany) with a 40X water immersion objective (C-Apochromat 40X/1.20 W Korr M27) was used for all measurements. VSD was excited by 850 nm light produced by a titanium:sapphire Mai Tai laser (Spectra-Physics, Mountain View, CA). Excitation light was separated from emission signal with a 760 nm dichroic. VSD fluorescence was collected in the 489–645 nm range. Line scan mode with 128 pixels per line and a 1.58 μs pixel dwell time was used to acquire temporal VSD depolarization data. Given a 1.67 kHz sampling rate, the total scan time per line was 600 μs. Each measurement consisted of 100,000 line scan repeats (total scan time 60 s). The Zen software package (Zeiss, Jena, Germany) was used to control all microscope components and acquisition processes. Brightfield images were used to identify clusters of spontaneously beating cardiomyocytes. The system was then switched to line scan mode with the parameters specified above. Line scan data were acquired along a line that was manually drawn across cell membranes. After completion of data acquisition, the system was switched back to brightfield mode to confirm that the cells were still spontaneously beating.

2.4. Data pre-processing

SimFCS commercial software developed in the Laboratory of Fluorescence Dynamics (LFD, University of California, Irvine) was used to analyze raw fluorescence data. A Gaussian tracking and correction algorithm (Supplemental Fig. 6) was used to compensate for motion artifacts resulting from the spontaneous beating of cell clusters. Fluorescence intensity along each corrected cell membrane trace was then extracted. Finally, a custom Matlab script that fit and subtracted a biexponential function from the resultant data was used to remove photobleaching artifacts (Supplemental Fig. 7).

2.5. Manual identification of depolarization events

Intensity traces, X, for all drug and control conditions, as described in Section 2.2, were derived as described in Section 2.4, and plotted in Matlab. Three trained human analysts then independently identified DE peak times from each trace.

2.6. DE detection and comparison analysis (MaDEC)

We have designed a custom Matlab software package to detect, quantify, and compare DEs of hiPS-CMs treated with common β-adrenergic drugs. The package consists of two components. First, the DEs are detected using a *Ma*tched-filter for *D*epolarization event (MaD) detection. The detected DEs are then quantitatively compared using our DE Comparison tool (DEC). Combined, these two tools are referred to as MaDEC. MaDEC was implemented in Matlab and is freely available along with a graphical user interface at sites.uci.edu/aggies/downloads or from the corresponding author.

2.6.1. Matched-filter for Depolarization event detection (MaD)

The matched filter used here was first implemented in Szymanska et al. [24]. A few modifications were made to tailor the filter specifically to voltage-sensitive dye and cardiomyocyte data, resulting in the MaD detector. Briefly, given a fluorescence intensity signal $x \in \mathbb{R}^{1\times N}$, where N is the number of samples spanning a DE, and assuming Gaussian noise statistics, we can express a decision rule as

$$\begin{array}{l}\text{accept } H_0 \quad \text{if } S(x) < \gamma \\ \text{accept } H_1 \quad \text{if } S(x) > \gamma\end{array}, \text{ where } \quad S(x) = s\Sigma^{-1}x^T \tag{1}$$

is the test statistic and matched-filter output, $s \in \mathbb{R}^{1\times N}$ is the DE template, $\Sigma \in \mathbb{R}^{N\times N}$ is the noise covariance matrix, γ is the threshold, H_0 is the null hypothesis (the signal contains noise only), and H_1 is the alternative hypothesis (the signal contains both noise and a DE). To detect DEs in the entire line scan, the matched filter is convolved with the full line scan signal $X \in \mathbb{R}^{1\times T}$, where $T \gg N$, and DEs are identified as peaks of activity above the threshold, γ.

MaD detector training protocol. The MaD detector is completely data driven as both s and Σ from Eq. (1) are estimated from the data. This allows the detector to be very flexible in accommodating various DE sizes, shapes, and durations, depending on the drug treatment applied and the specific data collected. The detector was trained under three detection conditions. The first was no drug exposure (control); the second was isoproterenol exposure and included data immediately after and 10 min after addition of isoproterenol; the third was propranolol exposure and included data immediately after and 10 min after addition of propranolol. The appropriately trained detector was then used to extract DEs from the data.