ACTION POTENTIAL PROPAGATION IN T-TUBULAR NETWORK

L. Sacconi\textsuperscript{1,2}, R. Coppini\textsuperscript{3}, C. Ferrantini\textsuperscript{4}, J. Lotti\textsuperscript{1}, C. Tesi\textsuperscript{4}, E. Cerbai\textsuperscript{3,5}, P. Yan\textsuperscript{6}, L. M. Loew\textsuperscript{6}, C. Poggesi\textsuperscript{4}, F. S. Pavone\textsuperscript{1,2}

\textsuperscript{1}European Laboratory for Nonlinear Spectroscopy (LENS), \textsuperscript{2}Department of Physics, \textsuperscript{3}Department of Pharmacology, \textsuperscript{4}Department of Physiology, \textsuperscript{5}Interuniversity Center of Molecular Medicine and Applied Biophysics (CIMMBA), University of Florence, Italy; \textsuperscript{6}Center for Cell Analysis and Modeling (CCAM), University of Connecticut Health Center, U.S.A.

E-mail: sacconi@lens.unifi.it

KEY WORDS: RAMP, two-photon, random access, optical recording, VSD, T-tubule, network, action potential, myocyte

ABSTRACT
In cardiac cells many membrane channels are heterogeneously distributed between Surface and T-tubule membranes. Simultaneous recording of membrane potential in the two sarcolemma domains can reveal potential peculiarities of T-tubule electrophysiology. This is not feasible with traditional electrophysiological techniques. Current optical techniques for recording membrane potential can potentially overcome the microelectrode limitation. However, most approaches to the optical recording of membrane potential events lack the spatial and temporal resolution needed for regional assessment of action potential (AP) profile. Here, we developed an ultrafast random access two-photon microscope capable of optically recording fast membrane potential transients in multiple positions of the cell membrane with \( \mu m \) spatial resolution. The random access microscope, in combination with a novel voltage sensitive dye, was used to simultaneously record AP in surface sarcolemma and T-tubules in isolated cardiac myocytes with sub-millisecond time resolution. We found that in myocytes, paced at 0.2 Hz, the AP in the T-tubule has identical amplitude and kinetics as in the surface sarcolemma, indicating that the tight electrical coupling between the two membrane domains prevails over the inhomogeneous distribution of membrane currents. Consistently, in myocytes that had been acutely detubulated by formamide-induced osmotic shock, T-tubule AP was absent, indicating a complete uncoupling from the surface sarcolemma. The electrophysiological properties of t-tubules may be altered in pathological conditions, when detubulation and T-tubule remodelling occur. To mimic a model of pathological detubulation, myocytes were cultured for 24-36 hours, thus obtaining a significant loss and disorganization of the T-tubular network. Membrane staining confirmed the loss and morphological alterations of T-tubules; however, the electrical activity in the remaining remodelled T-tubules was preserved, suggesting that remodelled T-tubules were still coupled to the surface sarcolemma.