Exploring the spread of excitation throughout the tubular network in mammalian skeletal muscle using superfast confocal microscopy

J.N. Edwards,[1] T.R. Cully,[1] P. Thorn,[1] D.F. Gilbert[2] and B.S. Launikonis,[1] [1]School of Biomedical Sciences, The University of Queensland, St Lucia, QLD 4072, Australia and [2]Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia.

In skeletal muscle, the rapid spread of excitation across the sarcolemma and throughout the tubular (t-) system is essential for uniform Ca^{2+} release and subsequent force production. The longitudinal spread of excitation within the t-system network has been reported in spontaneously active mechanically skinned fibres. In such a large cell, every transverse tubule may not be excited following depolarization at the cell surface. Any longitudinal spread of excitation between sarcomeres where transverse tubules fail to depolarize cannot be easily measured with conventional imaging techniques. By imaging Ca^{2+} transients with Oregon Green Bapta 5N at 15.5 μs $line^{-1}$ on a Zeiss 5 LIVE confocal system, we a) tracked the longitudinal spread of excitation along the t-system from the subsequently released Ca^{2+} and b) also resolved the Ca^{2+} release waveform with the highest temporal resolution to date. Following field stimulation of skinned fibres, we observed that in areas where transverse tubules failed to be excited by the initial stimulus, Ca^{2+} release propagated in from the adjacent regions at a rate of ~16μm ms^{-1}(n=6). The rise time of the Ca^{2+} transient showed two phases. It initially rose rapidly for 1ms and then continued at a slowing rate for a further 0.5ms until the peak of the transient. Nav1.4 immunostaining identified a complex subsarcolemmal t-system network which may help ensure the synchronous spread of excitation throughout the fibre from the surface membrane. However, uniform calcium release in skeletal muscle also requires longitudinal tubules deep within the t-system network to pass action potentials between excited and ’failing’ transverse tubules.