Cardiac E-C Coupling from electrical excitation to Ca^{2+} signaling
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The rate of Ca diffusion within the sarcoplasmic reticulum has important functional implications for release. However, experimental studies differ in their derived diffusion coefficient (D_{SR}) [1–3]. Higher free SR [Ca] ($[Ca]_{SR}$) increases ryanodine receptor open probability [4–7]. Slow intra-SR Ca diffusion would promote nonuniform distribution of $[Ca]_{SR}$ and produce regions that are more likely to release Ca spontaneously or in response to a Ca trigger. Fast intra-SR Ca diffusion, on the other hand, would tend to reduce Ca gradients and would promote movement from Ca uptake to Ca release sites within the SR, thus limiting cardiac alternans [8].

A study was conducted in order to address this issue which combined experimental results with mathematical modeling [9]. Local SR Ca depletions were measured in isolated rabbit cardiomyocytes in which the organelle was loaded with the Ca-sensitive dye Fluo-5N. Confocal microscopy of field stimulated myocardial cells showed no discernible Ca gradients between junctional and non-junctional regions of the SR. A mathematical model consisting of a half sarcomere of 20 cytosolic and 20 SR compartments (2 junctional, 18 non-junctional) was constructed in order to evaluate the data. Ca release was modeled from the junctional SR using a predefined wave form [10]. Calcium was allowed to freely diffuse within the cytosol from the release sites to the non-junctional compartments where uptake sites were located. It was then transported into the SR where it diffused back into the junction. Easily detectable Ca gradients were generated when D_{SR} dropped below 30 □m^2/s indicating that the diffusion must be rapid. The model was expanded to include a SR network of inter-connected junctions and a "Ca blink" [11] was simulated as release from a single central junction (with all others inactive). The model predicted significant SR Ca gradients at even very rapid D_{SR}. Blinks measured in permeabilized myocytes confirmed this prediction with a junctional □ of Ca recovery of 200 ms and moderate Ca gradients between the junctional SR and the non-junctional SR at the mid-sarcomere. However, computational simulation at even very slow D_{SR} produced a very rapid rate of Ca recovery with Ca gradients much larger than the data. Only when a late component was added to the release waveform (as in Brochet, et al, 2011 [12]) was the data reproduced at a D_{SR} consistent with the global release data above (60 □m^2/s). The data supports the hypothesis that this late release component may represent an important aspect of local Ca signaling. How this local release may be regulated in an intact cell could be an important direction for future research.

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In addition to intrinsic factors such as SR Ca, Ca release from the RyR is also regulated by a variety of external biochemical and mechanical signals. A large number of studies have focused on the biochemical pathways involving phosphorylation [1] or redox modification [2] of RyR. However, less is known the sensitivity of RyR to mechanical stretch. By stretching myocytes glued to glass rods, Iribe et al. demonstrated that stretch induces a rapid yet transient burst of Ca sparks [3]. These mechano-sensitive Ca sparks required intact microtubules, which were found to be closely associated with the T-tubule-SR membrane complex [3].

Prosser *et al.* further investigated the molecular mechanisms linking stretch, microtubules, and Ca sparks [4]. Mechanical stretch induced reactive oxygen species in concert with Ca spark frequency, and both effects were prevented by microtubule depolymerization. Using small molecule, peptide and genetic perturbations they discovered that the mechanosensitive ROS and Ca sparks were mediated by NADPH oxidase 2 (NOX2). Further, *mdx* myocytes (a mouse model of Duchenne muscular dystrophy) exhibited enhanced mechanosensitivty of Ca sparks and waves which were suppressed by NOX2 inhibition [4].

This thought-provoking study triggered a number of questions. First, to what extent does this NOX2 mechanotransduction pathway contribute to the Frank-Starling response of the heart? The stretch levels studied (8%) indicate that this pathway may already be activated at normal diastole. Response to greater or cyclic stretch would be of interest. Second, what redox modifications of RyR are involved? The effect was rapidly reversible, which would seem to rule out direct oxidation [2]. Nitric oxide has been shown to mediate mechanosensitivity of Ca sparks over longer durations [5], but the acute responses shown in these studies were insensitive to NOS inhibition [3]. Another possibility is RyR S-glutathionylation, which has been shown to enhance Ca release in response to tachycardia and NOX activation [6]. Finally, questions were raised about the rates and sensitivity of Ca sparks in intact muscle. While it has been difficult to study Ca sparks in vivo, Ca sparks and waves similar to those in single cells have been imaged in isolated trabeculae [7].

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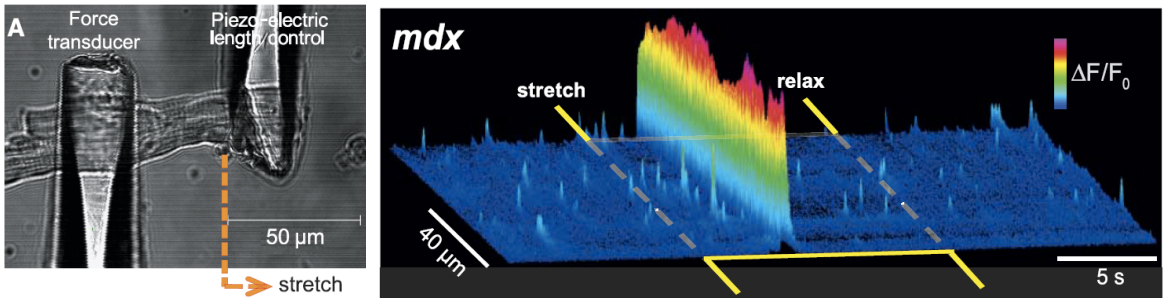

Potential Figure: A) Single adult ventricular myocytes are attached to glass rods using MyoTak and stretched by controlled amounts. B) Myocytes from *mdx* mice, a mouse model of Duchennne muscular dystrophy, exhibit enhanced Ca sensitivity to stretch, inducing enhanced Ca sparks and waves. (Prosser et al Science 2011).