Sztretye et al., http://www.jgp.org/cgi/content/full/jgp.201010592/DC1

Example of a signal with high dynamic range

Fig. S1 A has the FRET ratio derived (as described in Materials and methods) in a Casq1-null cell subjected to the pulse protocol indicated at the top. The pipette solution was EGTA. Note that R went from 1.465 at rest to 0.530 at the end of the second pulse, for $\Delta R/R_{\mathrm{min}} = 1.86$. Note also that the final R level is consistent with essentially full depletion, as it corresponds to $[\mathrm{Ca}^{2+}]_{\mathrm{SR}} = 4$ µM.

Errors in an estimation of evacuability

This section compares the conclusions of the present paper with diverging conclusions arrived at earlier, through a study of the evacuability (Royer et al., 2008). The first estimate of Ca^{2+} release permeability was introduced by Schneider et al. (1987) in the form of the depletion-corrected release flux:

$$\dot{Rel}_C \equiv \frac{\dot{Rel}}{\mathrm{Ca}_{\mathrm{SR}}(0) - R(t)}. \tag{S1}$$

Royer et al. (2008) demonstrated that the depletion-corrected flux depends not just on the permeability but also on the buffering power of the SR for Ca. Specifically,

$$\dot{Rel}_C = \varphi \frac{P}{B} \equiv E, \tag{S2}$$

where P is permeability (defined as Ca^{2+} flux per unit area and unit Ca^{2+} concentration gradient), B is Ca buffering power of the SR, and φ is the area to volume ratio of the SR. Royer et al. (2008) also showed that an index named the *NFRC*, calculated from the time course of the release flux, has the following relationship with E:

$$NFRC \equiv \frac{d\dot{Rel}/dt}{Rel_{net}} = E - \frac{\dot{Rel}}{Rel_{net}} \frac{d\ln(\varphi P/B)}{dt}. \tag{S3}$$

Under conditions that make the second term in the right-hand side of this equation small, the *NFRC* becomes an estimator of E; Royer et al. (2008) proposed that such conditions apply for most of the time when release flux decays during a long depolarization. Crucially, one of the conditions is that E not be small. The present work now shows that this condition is not met. Therefore, the main observation is that P decreases, and unless B decreases even faster, E will also decrease. As shown in Fig. S2 for the experimental example of Fig. 2 (a WT cell perfused by the EGTA solution), the error term, calculated under the hypothesis that B remains constant, becomes very large. Therefore, the estimate afforded by the *NFRC* leads to the conclusion that E increases, when it probably does the opposite.

REFERENCES

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Schneider, M.F., B.J. Simon, and G. Szucs. 1987. Depletion of calcium from the sarcoplasmic reticulum during calcium release in frog skeletal muscle. *J. Physiol.* 392:167–192.

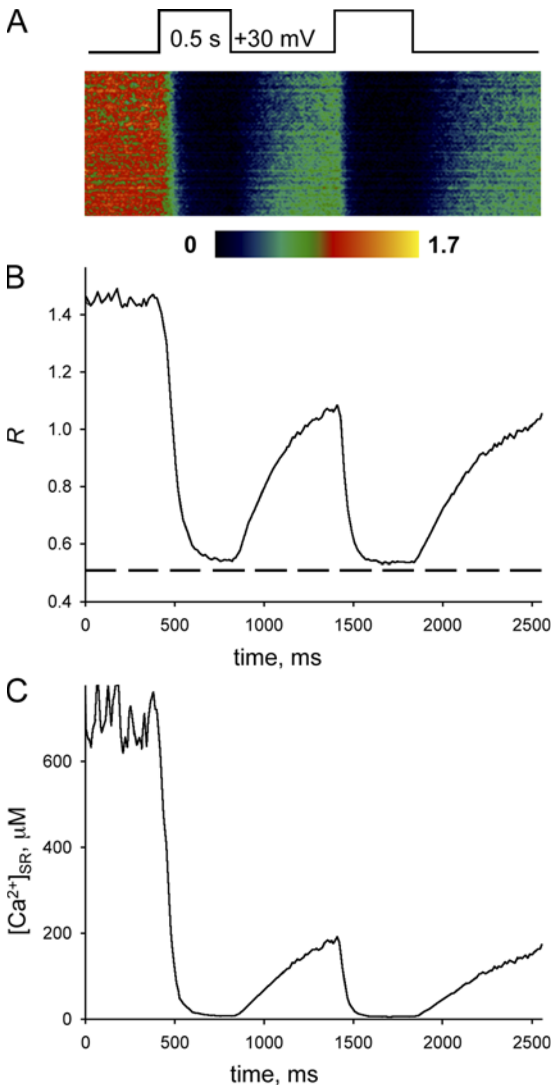

Figure S1. Example of a signal with high dynamic range. (A) $R = F_2(x,t)/F_1(x,t)$ of simultaneously recorded line scans of fluorescence of the biosensor in a Casq-null cell perfused with EGTA solution subjected to the pulse protocol indicated schematically at the top. (B) Spatially averaged ratio, $R(t)$. (C) $[Ca^{2+}]_{SR}$. Note also that the final R level is consistent with essentially full depletion, as it corresponds to $[Ca^{2+}]_{SR}$ = 4 µM. ID: 082009a, average of images 46–48.

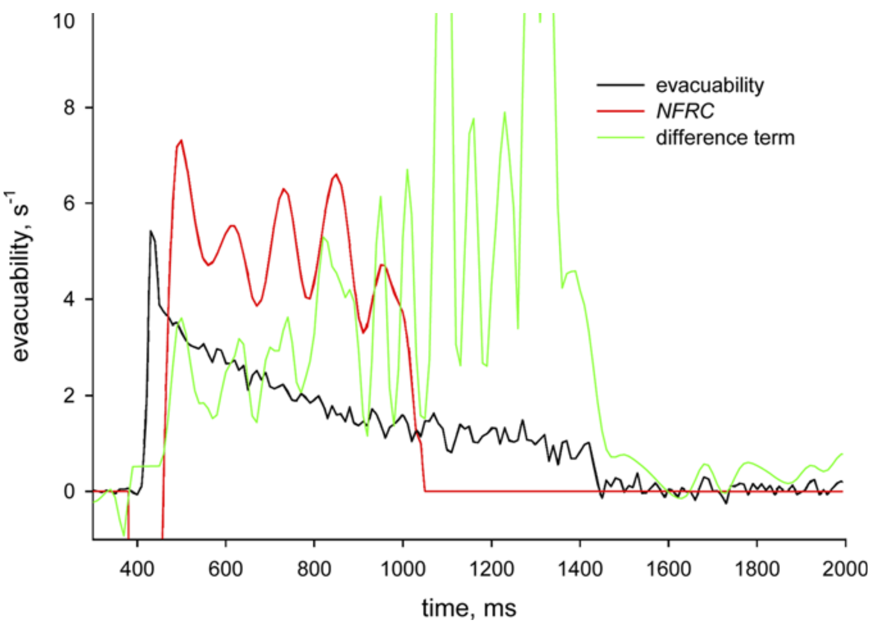

Figure S2. An error in the estimation of evacuability. The black trace shows evacuability, calculated from its definition (Eq. S1), for the line scans in Fig. 2. *E* decreases with time, which is consistent with a decreasing *P*. The red trace shows the *NFRC*, calculated from the release flux and its definition (Eq. S2). Note that *E* and *NFRC* evolve differently. The green trace shows the error term, which is the difference between *NFRC* and *E* (Eq. S3). The error term is large and grows larger with time. An assumption in Royer et al. (2008), required for the error to be small, was that *E* should not be decreasing. A decreasing *E* makes the logarithmic term in Eq. S3 large and negative, explaining the increasing error.