SUPPLEMENTARY METHODS

Analytical Model of synapticall- evoked Ca transients

The spatiotemporal profile of $[\text{Ca}^{2+}]$ within a cell following a stimulus is determined by the diffusion coefficient of free $\text{Ca}^{2+}$, the diffusion coefficients of the $\text{Ca}^{2+}$ buffers, the $\text{Ca}^{2+}$ binding capacity of each $\text{Ca}^{2+}$ buffer, and the rate of extrusion of $\text{Ca}^{2+}$ from the cytoplasm. Here we calculate or derive each of these parameters and formulate an analytical model of $\text{Ca}^{2+}$ diffusion within stellate cell dendrites that accurately describes the data presented in Figures 4-7. The model assumes the presence of two $\text{Ca}^{2+}$ buffers within the cell, an endogenous $\text{Ca}^{2+}$-binding protein of low mobility and the exogenous $\text{Ca}^{2+}$ indicator. The analytical model is used to estimate $\text{Ca}^{2+}$ mobility in stellate cells in their native, unperturbed state (i.e. without the addition fluorescent $\text{Ca}^{2+}$ indicator).

The spatial spread and the kinetics of synaptically-evoked $\text{Ca}^{2+}$ signals are affected by the concentration and affinity of the $\text{Ca}^{2+}$-sensitive fluorophore used to monitor them. In a manner completely analogous to the effects of proton buffers on pH, the $\text{Ca}^{2+}$ indicators bind or release $\text{Ca}^{2+}$ to counteract changes in intracellular $\text{Ca}^{2+}$ concentration (reviewed). Thus, $\text{Ca}^{2+}$ indicators decrease the amplitude of evoked $\text{Ca}^{2+}$ transients and slow the extrusion of $\text{Ca}^{2+}$ from the cytoplasm. Furthermore, the $\text{Ca}^{2+}$ indicators used here are small, water-soluble molecules that accelerate the diffusion of $\text{Ca}^{2+}$ within the cell by circumventing diffusion barriers posed by low-mobility $\text{Ca}^{2+}$-binding proteins. This occurs by binding $\text{Ca}^{2+}$ in regions of elevated $[\text{Ca}^{2+}]$, diffusing to distant sites of lower $[\text{Ca}^{2+}]$, and, in the process of re-equilibrating with local $\text{Ca}^{2+}$ levels, releasing $\text{Ca}^{2+}$.

A theoretical and quantitative framework exists for understanding the effects of $\text{Ca}^{2+}$ buffers on $\text{Ca}^{2+}$ transients. The analytical solution for the spatiotemporal profile of $\text{Ca}^{2+}$ increases in an infinitely long, 1-dimensional cylinder assuming an instantaneous $\text{Ca}^{2+}$ increase occurring at $x=0$, $t=0$ is given by
\[ \Delta Ca(x, t) \mu \frac{1}{\sqrt{D_{app} t}} e^{\frac{t}{\tau_{eff}}} e^{-\frac{x^2}{4D_{app} t}} \]  

(eq 1)

where \( D_{app} \) is the apparent \( Ca^{2+} \) diffusion coefficient in the cytoplasm and \( \tau_{eff} \) is the time constant of \( Ca^{2+} \) extrusion from the dendrite. Both \( \tau_{eff} \) and \( D_{app} \) are affected by the properties of the \( Ca^{2+} \) buffers present within the cell.

Increasing the concentration of a \( Ca^{2+} \) buffer or increasing the affinity of a buffer for \( Ca^{2+} \) increases the efficiency of the buffer. More precisely, the strength of a \( Ca^{2+} \) buffer \( X \) is the number of \( Ca^{2+} \) ions that bind to the buffer for every ion that is left free, or in differential form \( \kappa = \frac{\delta [CaX]}{\delta [Ca]} \). Experimentally the differential buffer capacity, \( \kappa \), which for a particular \( Ca^{2+} \)-binding molecule \( X \) is calculated from

\[ \kappa_X = \frac{X_T K_D}{(K_D + [Ca^{2+}]_{rest})(K_D + [Ca^{2+}]_{rest} + \Delta [Ca^{2+}])} \]  

(eq 2)

where \( K_D \) is the \( Ca^{2+} \) affinity of the buffer, \( X_T \) is the total concentration of the buffer, \( Ca^{2+}_{rest} \) is pre-stimulus \( Ca^{2+} \) level, and \([Ca^{2+}] \) is the increase in free \( Ca^{2+} \) resulting from the stimulus. \( \kappa \) determines the time constant of \( Ca^{2+} \) extrusion (\( \tau_{eff} \)) and the effective mobility of \( Ca^{2+} \) in the cytoplasm (\( D_{app} \)) as follows:

\[ \tau_{eff} = \tau_0 \frac{1 + \kappa_E + \kappa_B}{1 + \kappa_E} \]  

(eq 3)

and

\[ D_{app} = \frac{D_{Ca} + \kappa_E D_E + \kappa_I D_I}{1 + \kappa_E + \kappa_I} \]  

(eq 4)

where \( \tau_0 \) is the time constant of \( Ca^{2+} \) extrusion in the absence of exogenous \( Ca^{2+} \) buffers and \( D_{Ca} \) is the diffusion coefficient of \( Ca^{2+} \) in the cytoplasm in the absence of buffers. \( D_{app} \) is the apparent diffusion coefficient of \( Ca^{2+} \) given interactions with endogenous buffer (E)
and the exogenous Ca\(^{2+}\) indicator (I) with respective buffer capacities (\(\kappa_x\)) and free diffusion coefficients (\(D_x\)). Since typically \(D_I > D_E\), increasing indicator concentration or affinity (increasing \(\kappa_I\)) will result in faster apparent diffusion of Ca\(^{2+}\) (higher \(D_{app}\)). Because \(\kappa_x\) are typically large, \(D_{Ca}\) in the numerator and 1 in the denominator can be ignored.

In order to model our Ca\(^{2+}\) diffusion data using equation 1, values of \(\kappa_I\), \(\kappa_E\), \(D_I\), \(D_E\), and \(\tau_0\) need to be determined. For 300 M of the Ca\(^{2+}\) indicator Fluo5F, we use our measurements of \(G/R_{max}\), \(G/R_{rest}\), and \(G/R_{sync}\) (see methods) and the published value of \(K_D\) (1.6 M) to plug into equation 2 and obtain \(\kappa_{300\text{Fluo5F}} \approx 130\). When \([\text{Ca}^{2+}] \ll K_D\), the indicator is in its linear range such that fluorescence changes are directly proportional to changes in [Ca\(^{2+}\)] and the equation 2 simplifies to \(\kappa_x = X_T / K_D\). This approximation is valid here for Fluo4FF because it is a low-affinity indicator used at high concentrations. Using published values of \(K_D\) (10.6 M), we find \(\kappa_{300\text{Fluo4F}} \approx 30\) and \(\kappa_{600\text{Fluo4F}} \approx 60\).

Based on the known properties of parvalbumin, the dominant Ca\(^{2+}\) buffer in stellate cells, removing intracellular Mg\(^{2+}\) dramatically increases its affinity Ca\(^{2+}\) such that \([\text{Ca}^{2+}]_{rest} \gg K_D\). Therefore, \(\kappa_E \rightarrow 0\) (eq 3) and \(D_{app} \sim D_I\) (eq 4). Thus, from measurements of \(D_{app}\) in 0 Mg intracellular (Figure 6), we obtain \(D_I \sim 25\) m\(^2\)/sec, similar to the value of 24 m\(^2\)/sec measured for HPTS (Figure 7).

Using these values of \(\kappa_E\), experimental measurements of \(D_{app}\), and equation 3, \(\text{Ca}(x,t)\) (equation 1) is reduced to a function of 2 unknowns, \(\tau_0\) and \(\kappa_E\). Since the Ca\(^{2+}\) indicators employed here are used within their linear range (see Methods), the amplitude of the fluorescence transients is linearly related to intracellular Ca\(^{2+}\) levels such that \(\text{Ca}(x,t) \sim G/R(x,t)\) and can be used to derive these parameters. Because of the lateral blurring introduced by the point-spread function of the microscope, measurements of
$G/R(x,t)$ reflect the averaged fluorescence over a spatial extent of the dendrite. Furthermore, because of improved signal to noise in the signal to be fit, it is more practical to derive $\tau_0$ and $\kappa_E$ from fits to the spatially averaged Ca$^{2+}$ signal $C(\Delta x, t) = \frac{1}{2\Delta x} \int_{-\Delta x}^{\Delta x} \Delta C a(x, t) dx$ calculated over a given spatial extent ($x$) of dendrite. We measured $\int_{-\Delta x}^{\Delta x} \Delta G / R(x, t) dx$ over stretches of $\pm 0.25$, $0.5$, $1$, $2$, $3$ microns of dendrite centered about the peak of the synaptically-evoked fluorescence transient in each of the 3 added buffer conditions (300 M Fluo5F, 300 M Fluo4FF, and 600 M Fluo5F) (Sup Figure 3A-C). Given the lateral resolution of $\sim 0.5$ m of our 2-photon microscope, we assume that the true spatial extent of integration is increased by $\pm 0.5$ m (i.e. true integration over $\pm 0.75$, $1.0$, $1.5$, $2.5$, $3.5$ m). In this manner, for each buffer condition we obtained 5 fluorescence transients proportional to $C(\Delta x, t)$. The best fit to these 15 fluorescence transients was obtained with $\tau_0 \sim 300$ ms and $\kappa_E = 170$ (Sup. Fig. 3a-c). Lastly, using this value of $\kappa_E$ and equation 4, we obtain $D_E \sim 1$ m$^2$/sec.

How well does equation 1 with the calculated and derived parameters (Sup. Table 1) describe our experimental data? Calculation of $C(\Delta x, t)$ for the five integration windows and 3 buffer conditions and comparison to the measured data yields residual errors of less $< 20\%$ (Sup. Fig. 3a-c). Furthermore, calculation of $D_{app}$ in each buffer condition yields good fits to the measured relationship between $(\sigma^2/2)$ and $t$ taken from Figure 6 (Sup. Fig 3d). Lastly, no information from the repetitive stimulation data was used to derive the parameters of the model. Nevertheless, using equation 1 to calculate the time course of Ca$^{2+}$ diffusion during the stimulation train yields an excellent fit to the measurements (Sup. Fig. 3e).
Using equations 1-4 with $\kappa = 0$ we calculated the spread of Ca$_{2+}$ in the absence of added Ca$_{2+}$ indicator (Sup. Fig. 3f). We find that the lateral spread of Ca$_{2+}$ following a single synaptic stimulus is small such that the maximal $[\text{Ca}_{2+}]$ reached 500 nm from the site of Ca$_{2+}$ influx is only 14% of the peak value reached at the source. During repetitive stimulation, $[\text{Ca}_{2+}]$ at a distance of 1 m from the Ca$_{2+}$ source reaches a maximum of 22% of the peak at the source. Thus, in the unperturbed stellate cells, Ca$_{2+}$ is highly localized to small stretches of dendrite near the active synapse even during prolonged, repetition stimulation.