Supplementary Information S1 (Box) **Modelling the effects of buffers in realistic coupling regimes.**

A lot of insight into the mechanisms of coupling between Ca^{2+} channels and Ca^{2+} sensors can be obtained by modelling the diffusion of Ca^{2+} and its reaction with buffers. As in other fields of Neuroscience, the Hopfield quote "build it, and you understand it" perfectly applies. How can one model the Ca^{2+} transient?

In a simple scenario, the steady-state solution to the linearized reaction-diffusion problem is obtained analytically[1,2]. In this framework, the Ca^{2+} concentration ($[Ca^{2+}]$) can be described by a simple equation, which is comprised of a 1 / r term (representing diffusion) and an exponential term (representing buffering):

$$[Ca^{2+}] = \frac{i_{Ca}}{4\pi F D_{Ca}} \; 1/r \;\; \exp(-r/\lambda) \qquad \text{(Eq. 1)}$$

with $\lambda = \sqrt{D_{Ca} / (k_{on} [B])}$,

where i_{Ca} is the Ca^{2+} current, F is the Faraday constant, D_{Ca} is the diffusion coefficient of Ca^{2+}, r is radial distance from a source, λ is the length constant, k_{on} is the Ca^{2+}-binding rate of the buffer, and [B] is the concentration of the buffer[1].

Although the linear approach represents a useful approximation for short distances from the source, it does not account for the time course of the Ca^{2+} transient, the phenomenon of buffer saturation, and the presence of fixed and mobile buffers[1].

The limitations can be overcome by obtaining the time-dependent solution to the full reaction-diffusion equations[3-7]. This can be done by numerically solving a set of partial differential equations, containing the Ca^{2+} and buffer concentrations as a function of space and time, as well as several partial derivatives.

Everything starts from Fick's first and second law of diffusion[8]. Fick's first law relates the diffusive flux to the concentration field. In the simplest possible form in one spatial dimension, the first law is

$$J = D_{Ca} \frac{\partial [Ca^{2+}]}{\partial x}, \qquad \text{(Eq. 2)}$$

where J is the flux in units mol s^{-1} m^{-2}. From the law of mass conservation and Fick's first law, Fick's second law can be derived[2].

$$\frac{\partial[Ca^{2+}]}{\partial t} = \frac{\partial J}{\partial x} = \frac{\partial}{\partial x}\left(D_{Ca}\,\frac{\partial[Ca^{2+}]}{\partial x}\right) \qquad \text{(Eq. 3)}$$

Equation 3 gives the partial differential equation that has to be solved. Equation 2 gives the boundary condition near the source. In addition, a second boundary condition has to be implemented remote from the source. This is usually a reflective boundary condition, which is given as $\partial[Ca^{2+}] / \partial x = 0$ for $x \to x_{max}$. As there is no gradient at this distance, Ca^{2+} cannot escape beyond this point. Furthermore, initial conditions have to be appropriately chosen. For example, $[Ca^{2+}]$ at t = 0 is set to the resting value. The partial differential equations can be solved numerically, e.g. using NDSolve of Mathematica[3,4,9].

Finally, the effect of the Ca^{2+} transient on transmitter release has to be simulated, using models of transmitter release derived from Ca^{2+} uncaging experiments[10-14]. Based on a 6- to 8-state reaction scheme, a set of ordinary differential equations can be formulated, which can be solved numerically.

The cookbook recipe (Eq. 1 – 3) describes the backbone of the simulations, defining the Ca^{2+} transients from a point source in the absence of buffers. For a more realistic simulation, several extensions have to be made. In the presence of buffers, the right hand side of equation 3 has to be extended by the sum of reaction terms. To simulate Ca^{2+} transients originating from Ca^{2+} channel clusters or other distributed sources, the one-dimensional simulations have to be extended into two or three dimensions[5-7].

Early studies have used several different approximations, such as the steady-state excess buffer approximation (EBA; buffer concentration is so high that it changes little during Ca^{2+} inflow) and rapid buffer approximation (RBA; buffers are so fast that they are in chemical equilibrium with Ca^{2+} at every point in time and space[7]). As computer power has increased, these approximations have become unnecessary.

1. Neher, E. Usefulness and limitations of linear approximations to the understanding of Ca^{++} signals. *Cell Calcium* **24**, 345-357 (1998).
2. Crank, J. *The Mathematics of Diffusion* (Clarendon Press, Oxford, 1975).

3. Bucurenciu, I., Kulik, A., Schwaller, B., Frotscher, M. & Jonas, P. Nanodomain coupling between Ca^{2+} channels and Ca^{2+} sensors promotes fast and efficient transmitter release at a cortical GABAergic synapse. *Neuron* **57**, 536–545 (2008).
4. Bucurenciu, I., Bischofberger, J. & Jonas, P. A small number of open Ca^{2+} channels trigger transmitter release at a central GABAergic synapse. *Nature Neurosci.* **13**, 19-21 (2010).
5. Matveev, V., Zucker, R. S. & Sherman, A. Facilitation through buffer saturation: constraints on endogenous buffering properties. *Biophys. J.* **86**, 2691-2709 (2004).
6. Klingauf, J. & Neher, E. Modeling buffered Ca^{2+} diffusion near the membrane: implications for secretion in neuroendocrine cells. *Biophys. J.* **72**, 674-690 (1997).
7. Smith, G. D. *Modelling local and global calcium signals using reaction-diffusion equations.* In Computational Neuroscience, E. de Schutter, ed. (Boca Raton, FL, CRC Press 2001), pp. 49 – 85.
8. Fick, A. Über Diffusion. *Ann. Physik* **170**, 59-86 (1855).
9. Trott, M. *The Mathematica guidebook for numerics* (Springer, New York, 2006).
10. Schneggenburger, R. & Neher, E. Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature* **406**, 889-893 (2000).
11. Bollmann, J.H., Sakmann, B. & Borst J.G.G. Calcium sensitivity of glutamate release in a calyx-type terminal. *Science* **289**, 953-957 (2000).
12. Lou, X., Scheuss, V. & Schneggenburger, R. Allosteric modulation of the presynaptic Ca^{2+} sensor for vesicle fusion. *Nature* **435**, 497-501 (2005).
13. Sun, J. *et al*. A dual-Ca^{2+} -sensor model for neurotransmitter release in a central synapse. *Nature* **450**, 676-682 (2007).
14. Sakaba, T. Two Ca^{2+}-dependent steps controlling synaptic vesicle fusion and replenishment at the cerebellar basket cell terminal. *Neuron* **57**, 406-419 (2008).