Supplementary figure legend. A more detailed description of figure 2 in the main text

In figure 2 a single neuronal compartment was simulated with a buffer composition as expected in CA1 pyramidal cells. In this compartment with 100 μM CaM and 30 μM CB the $[Ca^{2+}]$ was very rapidly (τ=10 μs) increased by 50 μM ($\Delta[Ca^{2+}]_{total}$). At 20 μs (the maximum time resolution in the figure is 20 μs) $\Delta[Ca^{2+}]_{free}$ peaks at 8.8 μM. As the total $[Ca^{2+}]$ increases, the bulk of the $\Delta[Ca^{2+}]_{total}$ is rapidly bound to the N-terminus of CaM. At ~40 μs, 36.4 μM Ca^{2+} (73% of $\Delta[Ca^{2+}]_{total}$) is bound to the N-terminus ($\Delta[Ca^{2+}]_{N}$), while at that moment 5.2 μM (10% of $\Delta[Ca^{2+}]_{total}$) and 1.7 μM (3.3% of $\Delta[Ca^{2+}]_{total}$) are bound to the C-terminus ($\Delta[Ca^{2+}]_{C}$) and CB ($\Delta[Ca^{2+}]_{CB}$) respectively. After this, the $\Delta[Ca^{2+}]_{N}$ drops with two τ's of 0.38 ms (64%) and 2.1 ms (36%) to 0.54 μM (1.1% of $\Delta[Ca^{2+}]_{total}$) at 30 ms. Around 0.9 ms the $\Delta[Ca^{2+}]_{C}$ reaches its maximum of 21.4 μM (43% of $\Delta[Ca^{2+}]_{total}$) while at that moment there is 11.0 μM $\Delta[Ca^{2+}]_{N}$ (22% C and 14.9 μM $\Delta[Ca^{2+}]_{CB}$ (30% of ($\Delta[Ca^{2+}]_{total}$). After this peak the $\Delta[Ca^{2+}]_{C}$ drops with a τ of 3.7 ms to 9.8 μM (19.7% of $\Delta[Ca^{2+}]_{total}$) at 30 ms. Over the whole experimental period, the $[Ca^{2+}]_{CB}$ steadily increases with τ's of 0.63 ms (27%) and 3.1 ms (73%) to 39.3 μM (78.6% of $\Delta[Ca^{2+}]_{total}$) at 30 ms. With 99.3% $\Delta[Ca^{2+}]_{total}$ bound to either the N-terminus, C-terminus or CB 0.35 μM of $\Delta[Ca^{2+}]_{total}$ (i.e., 0.7%) remains unbound after 30 ms.

b) To better understand how Ca^{2+} moves through the system composed of 3 buffers, the amount of Ca^{2+} flowing between the four states, free ($\Delta[Ca^{2+}]_{free}$), bound to the N-terminus ($\Delta[Ca^{2+}]_{N}$), bound to the C-terminus ($\Delta[Ca^{2+}]_{C}$) and bound to CB ($\Delta[Ca^{2+}]_{CB}$) were calculated for 3 epochs. The concentrations of Ca^{2+} in the different states is represented by the area covered by the different circles where the grey area represents the concentration at the beginning of the epoch, while the colored circles represent the concentration at the end of the epoch. The numbers in the figure indicate percent of $\Delta[Ca^{2+}]_{total}$. During the first epoch (red), which runs from the start of the simulation (t=0 s) to when $[Ca^{2+}]_{N}$ peaks (t=40 μs) 86.6% of the 50 μM $\Delta[Ca^{2+}]_{total}$ (top grey circle) is directly bound by the 3 buffers (78.7% $[Ca^{2+}]_{N}$, 6.0% $[Ca^{2+}]_{C}$ and 1.9% $[Ca^{2+}]_{CB}$). Small amounts of buffered Ca^{2+} are already redistributed from $[Ca^{2+}]_{N}$ to $[Ca^{2+}]_{N}$ (4.4%) and $[Ca^{2+}]_{CB}$ (1.5%). During the second epoch (green), which runs from 40 μs to when $[Ca^{2+}]_{C}$ peaks (t=900 μs), and the last epoch (green, 0.9–30 ms) a further total of 11.7% of free Ca^{2+} is directly bound by the N-terminus whereas the amounts of free Ca^{2+} directly binding to either C-terminus (0.8%) or CB (0.2%) are less than 1% and for clarity are not shown. After the first 40 μs the main 'flow' of Ca^{2+} is the redistribution between the 3 buffering components. During the second epoch 33.0% moves from $[Ca^{2+}]_{N}$ to $[Ca^{2+}]_{C}$, 25.3% from $[Ca^{2+}]_{N}$ to $[Ca^{2+}]_{CB}$ and 1.0% from $[Ca^{2+}]_{C}$ to $[Ca^{2+}]_{CB.}$ Remarkably, during the third epoch 19.3% moves back from $[Ca^{2+}]_{C}$ to $[Ca^{2+}]_{N}$ which is then directly redistributed to the C-terminus as part of the 44.4% that moves from $[Ca^{2+}]_{N}$ to $[Ca^{2+}]_{CB}$. Also a further 4.2% moves from $[Ca^{2+}]_{C}$ to $[Ca^{2+}]_{CB}$. All the net redistributions that take place during the 30 ms of the simulation are shown in the diagram with the black arrows where the black circles correlate with the end concentration and the white circles indicate the maxima reached during the experiment. Over the whole period more than 89% of the $\Delta[Ca^{2+}]_{total}$ is first bound by the fast

N-terminus which is later bound to the slower C-terminus and CB which eventually outcompete the N-terminus based on their higher affinity for Ca^{2+}.

References to Supplementary Information

1. Berggard, T. *et al.* Calbindin D28k exhibits properties characteristic of a Ca^{2+} sensor. *J.Biol.Chem.* **277**, 16662–16672 (2002).
2. Nagerl, U. V., Novo, D., Mody, I. & Vergara, J. L. Binding kinetics of calbindin-D(28k) determined by flash photolysis of caged $Ca(^{2+})$. *Biophys J* **79**, 3009–3018, doi:S0006-3495(00)76537–4 [pii] 10.1016/S0006-3495(00)76537–4 (2000).
3. Faas, G. C., Karacs, K., Vergara, J. L. & Mody, I. Kinetic properties of DM-nitrophen binding to calcium and magnesium. *Biophys J* **88**, 4421–4433, doi:S0006-3495(05)73489–5 [pii] 10.1529/biophysj.104.057745 (2005).
4. Sabatini, B. L., Oertner, T. G. & Svoboda, K. The life cycle of $Ca(^{2+})$ ions in dendritic spines. *Neuron* **33**, 439–452, doi:S0896627302005731 [pii] (2002).
5. Cornelisse, L. N., van Elburg, R. A., Meredith, R. M., Yuste, R. & Mansvelder, H. D. High speed two-photon imaging of calcium dynamics in dendritic spines: consequences for spine calcium kinetics and buffer capacity. *PLoS ONE* **2**, e1073, doi:10.1371/journal.pone.0001073 (2007).
6. McIlwain, H. & Bachelard, H. S. *Biochemistry and the central nervous system*. 5th edn, (Churchill Livingstone, 1985).
7. Banay-Schwartz, M., Kenessey, A., DeGuzman, T., Lajtha, A. & Palkovits, M. Protein content of various regions of rat brain and adult aging human brain. *Age* **15**, 51–54 (1992).
8. Pond, W. G. *et al.* Severe protein deficiency and repletion alter body and brain composition and organ weights in infant pigs. *J Nutr* **126**, 290–302 (1996).
9. Mori, M. X., Erickson, M. G. & Yue, D. T. Functional stoichiometry and local enrichment of calmodulin interacting with Ca^{2+} channels. *Science* **304**, 432–435 (2004).
10. Kubota, Y., Putkey, J. A. & Waxham, M. N. Neurogranin controls the spatiotemporal pattern of postsynaptic Ca^{2+}/CaM signaling. *Biophys J* **93**, 3848–3859, doi:S0006-3495(07)71639–9 [pii] 10.1529/biophysj.107.106849 (2007).
11. Nicholson, C. & Sykova, E. Extracellular space structure revealed by diffusion analysis. *Trends Neurosci* **21**, 207–215, doi:S0166-2236(98)01261–2 [pii] (1998).
12. Reinoso, R. F., Telfer, B. A. & Rowland, M. Tissue water content in rats measured by desiccation. *J Pharmacol Toxicol Methods* **38**, 87–92, doi:S1056-8719(97)00053–1 [pii] (1997).
13. Altman, P. L. & Katz, D. D. *Biology data book*. 2d edn, (Federation of American Societies for Experimental Biology, 1972).
14. Klee, C. B. & Vanaman, T. C. Calmodulin. *Adv Protein Chem* **35**, 213–321 (1982).
15. Kakiuchi, S. *et al.* Quantitative determinations of calmodulin in the supernatant and particulate fractions of mammalian tissues. *J Biochem* **92**, 1041–1048 (1982).
16. Biber, A., Schmid, G. & Hempel, K. Calmodulin content in specific brain areas. *Exp Brain Res* **56**, 323–326 (1984).
17. Vargas, F. & Guidotti, A. Calmodulin in brain of schizophrenics. *Neurochem Res* **5**, 673–681 (1980).
18. Sano, M. & Kitajima, S. Ontogeny of calmodulin and calmodulin-dependent adenylate cyclase in rat brain. *Brain Res* **283**, 215–220 (1983).
19. Kitajima, S., Seto-Ohshima, A., Sano, M. & Kato, K. Production of antibodies to calmodulin in rabbits and enzyme immunoassays for calmodulin and anti-calmodulin. *J Biochem* **94**, 559–564 (1983).
20. Teolato, S., Calderini, G., Bonetti, A. C. & Toffano, G. Calmodulin content in different brain areas of aging rats. *Neurosci Lett* **38**, 57–60, doi:0304-3940(83)90110–6 [pii] (1983).
21. Lin, C. T., Dedman, J. R., Brinkley, B. R. & Means, A. R. Localization of calmodulin in rat cerebellum by immunoelectron microscopy. *J Cell Biol* **85**, 473–480 (1980).
22. Neher, E. & Augustine, G. J. Calcium gradients and buffers in bovine chromaffin cells. *J.Physiol* **450**, 273–301 (1992).
23. Helmchen, F., Imoto, K. & Sakmann, B. Ca^{2+} buffering and action potential-evoked Ca^{2+} signaling in dendrites of pyramidal neurons. *Biophys J* **70**, 1069–1081, doi:S0006-3495(96)79653–4 [pii] 10.1016/S0006-3495(96)79653–4 (1996).
24. Lee, S. H., Rosenmund, C., Schwaller, B. & Neher, E. Differences in Ca^{2+} buffering properties between excitatory and inhibitory hippocampal neurons from the rat. *J Physiol* **525 Pt 2**, 405–418, doi:PHY_9975 [pii] (2000).
25. Sabatini, B. L., Oertner, T. G. & Svoboda, K. The life cycle of Ca^{2+} ions in dendritic spines. *Neuron* **33**, 439–452 (2002).
26. Einstein, A. The motion of elements suspended in static liquids as claimed in the molecular kinetic theory of heat. *Ann. Phys.-Berlin* **17**, 549–560 (1905).
27. Smoluchowski, M. Theory of the Brownian movements. *Bulletin de l'Academie des Sciences de Cracovie|Bulletin de l'Academie des Sciences de Cracovie|Annalen der Physik*, 577–602|756–780 (1906).
28. Meinrenken, C. J., Borst, J. G. & Sakmann, B. Calcium secretion coupling at calyx of held governed by nonuniform channel-vesicle topography. *J Neurosci* **22**, 1648–1667, doi:22/5/1648 [pii] (2002).
29. Azab, H. A., Orabi, A. S. & El-Salam, E. T. A. Role of biologically important zwitterionic buffer secondary ligands on the stability of the mixed-ligand complexes of divalent metal ions and adenosine 5 '-mono-, 5 '-di-, and 5 '-triphosphate. *Journal of Chemical and Engineering Data* **46**, 346–354, doi:Doi 10.1021/Je0001779 (2001).

[30] Borst, J. G., Helmchen, F. & Sakmann, B. Pre- and postsynaptic whole-cell recordings in the medial nucleus of the trapezoid body of the rat. *J Physiol* **489 (Pt 3)**, 825–840 (1995).
[31] Ainscow, E. K., Mirshamsi, S., Tang, T., Ashford, M. L. & Rutter, G. A. Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP-independent control of ATP-sensitive K(+) channels. *J Physiol* **544**, 429–445, doi:PHY_022434 [pii] (2002).
[32] Brocard, J. B., Rajdev, S. & Reynolds, I. J. Glutamate-induced increases in intracellular free Mg^{2+} in cultured cortical neurons. *Neuron* **11**, 751–757, doi:0896-6273(93)90084–5 [pii] (1993).
[33] Grubbs, R. D. Intracellular magnesium and magnesium buffering. *Biometals* **15**, 251–259 (2002).
[34] Keller, D. X., Franks, K. M., Bartol, T. M., Jr. & Sejnowski, T. J. Calmodulin activation by calcium transients in the postsynaptic density of dendritic spines. *PLoS ONE* **3**, e2045, doi:10.1371/journal.pone.0002045 (2008).
[35] Faas, G. C., Schwaller, B., Vergara, J. L. & Mody, I. Resolving the fast kinetics of cooperative binding: Ca^{2+} buffering by calretinin. *PLoS Biol* **5**, e311, doi:07-PLBI-RA-1024 [pii] 10.1371/journal.pbio.0050311 (2007).