Supplemental Information



Supp. Fig. 1. Ca²⁺ kinetics in PV^{-/-} and PV/CB^{-/-} mice confirm predictions of the model

A. Ca^{2+} transients from a spine and the adjacent dendrite of a parvalbumin deficient (PV^{-/-}) PN (black lines) recorded under conditions identical to those described in Fig. 1. The grey lines represent double exponential fits to the decays of the transients. B. Median decays (black lines) in spines and dendrites (large and small amplitude transient, respectively, n = 140). As in Fig. 1D, the coloured lines represent decays simulated with a kinetic model that excluded (red lines, *left*) or included (green lines, *right*) diffusional coupling between spine and dendrite. Parameters were kept as in the wild-type (WT), except that PV has been omitted from the simulation. The insets show the integrated differences between spineous data and simulations. Note that the model failed to describe the spineous decay in the absence of diffusional coupling, which is consistent with the minor contribution of PV to buffered Ca^{2+} diffusion observed in WT. C. as in A but from a mouse line that lacked both PV and calbindin ($PV/CB^{-/-}$). *D*, as in *B* but for the $PV/CB^{-/-}$ data (n = 134). All parameters were kept as in the $PV^{-/-}$ except for the removal of CB from the simulation, the alter spine morphology (Ref. 10), and an approximately two-fold increased maximum pump velocity. Note that in PV/CB^{-/-} recordings, the contribution of buffered diffusion was strongly reduced for the following reasons: first, PV and CB, which accounted for ~40% of the transport in the wild type, were absent: second. PV/CB^{-/-} spines show a drastically altered morphology (including longer spine necks, see Supp. Table 1); third, due to the absence of the relatively slow CaBPs CB and PV, the Ca²⁺ transients show a rapid decay that reduces the effective time window for diffusional exchange between spine and dendrite.



Supp. Fig. 2. Simulation of Ca²⁺ kinetics recorded at a low indicator concentration

A, Median decay kinetics in spines recorded at a dye concentration of ~40 μ M (black; n = 40). The green line represents a decay simulated with the kinetic model with diffusional coupling between spine and dendrite. All parameters but the dye concentration were kept as in the WT simulation. Note that although the overlap between fit and data is not as good as in Fig. 1 and Supp. Fig. 1, characteristic features are well reproduced: the decay is more rapid than with high dye concentration while the amplitude remained almost constant. *B*, Same as in *A* but for the dendrite. *C*, Diffusional flux of free and buffer-bound Ca²⁺ across the spine neck for the simulations in *A* and *B*. *D*, Temporal integral of the diffusion flux.

Supplemental Methods

The kinetic, two-compartment model

The Ca²⁺ current (I_{Ca}) during the CF-mediated complex spike (Fig. 1 and 2*A*) and during VOCC/iGluR-mediated spine responses (Fig. 2*B*,*C*, 4*B*, S1 and S2) was represented by a Gaussian function adjusted to the time course of the measured complex spike.

$$I_{Ca} = I_0 \, 10^{-\left(\frac{t-t_0}{\sigma}\right)^2}$$
(3)
(Note that Eqs. 1 and 2 are to be found in the main text)

 I_0 denotes the maximal amplitude of the current which is reached at t_0 and σ is the width of the function. The mGluR-mediated spineous Ca²⁺ increase (Fig. 3 and 4) was represented by an alpha-function that allowed for separate adjustment of the time constants τ of the rising and the decay phase.

$$I_{Ca} = I_0 \left(e^{-\left(\frac{t \cdot t_0}{\tau_{decay}}\right)} - e^{-\left(\frac{t \cdot t_0}{\tau_{rise}}\right)} \right)$$
(4)

The increase in $[Ca^{2+}]_i$ caused by the Ca^{2+} influx is given by

$$\left(\frac{d[Ca^{2+}]}{dt}\right)_{influx} = \frac{A I_{Ca}}{2 F V}$$
(5)

where A is the surface of the spine or dendrite, respectively, F is Faraday's constant, and V the volume of the compartment. Ca^{2+} binding to OGB, CaM, CB, and PV was simulated assuming second order kinetics for all reactions. The four binding sites of CB and the two binding sites of PV were simulated as individual reaction partners, neglecting possible cooperativity. For Ca^{2+} binding to CaM only the rate-limiting binding of the first Ca^{2+} ion was considered. Under these assumptions, the rate of change in $[Ca^{2+}]_i$ due to binding of Ca^{2+} to the j-th binding site (BS_j) is given by

$$\left(\frac{d[Ca^{2+}]}{dt}\right)_{buffer,j} = -k_{on,j}[Ca^{2+}][BS_{j}] + k_{off,j}[CaBS_{j}] , \qquad \begin{array}{l} j = 1 \text{ for OGB} \\ j = 2 - 5 \text{ for CB} \\ j = 6,7 \text{ for PV} \\ j = 8 \text{ for CaM} \end{array}$$
(6)

For OGB, CB, and CaM the following relationship between free ([BS]_j) and Ca²⁺-bound binding sites ([CaBS]_j) applies:

$$\left(\frac{d[BS_j]}{dt}\right) = -\left(\frac{d[CaBS_j]}{dt}\right) , \qquad j = 1 - 5, 8 \qquad (7)$$

For PV the situation is complicated by its medium affinity for Mg^{2+} which significantly affects its Ca^{2+} -binding kinetics. The change in the occupancy of PV's binding sites is given by

$$\left(\frac{d[BS_j]}{dt}\right) = -\left(\frac{d[CaBS_j]}{dt}\right) - \left(\frac{d[MgBS_j]}{dt}\right) \quad , \qquad j = 6,7 \tag{8}$$

and

$$\left(\frac{d[MgBS_j]}{dt}\right) = k_{on,j,Mg}[Mg^{2+}][BS_j] - k_{off,j,Mg}[MgBS_j] , \qquad j = 6,7$$
(9)

The intracellular Mg^{2+} concentration was kept constant at 590 μ M, a value calculated for our pipette solution using WinMaxC 2.1 software (http://www.stanford.edu/~cpatton/maxc.html). The starting conditions were calculated from chemical equilibrium at $[Ca^{2+}]_{rest}$ of 45 nM. A single surface-based Ca^{2+} extrusion mechanism was simulated assuming Michaelis-Menten kinetics (1):

$$\left(\frac{\mathrm{d}[\mathrm{Ca}^{2^{+}}]}{\mathrm{dt}}\right)_{\mathrm{pump}} = -v_{\mathrm{max}} \frac{\mathrm{A}}{\mathrm{V}} \left(\frac{[\mathrm{Ca}^{2^{+}}]}{[\mathrm{Ca}^{2^{+}}] + K_{\mathrm{M}}}\right)$$
(10)

where $K_{\rm M}$ is the Michaelis-Menten constant and $v_{\rm max}$ the maximal pump velocity. In order to establish the resting $[{\rm Ca}^{2+}]_{i}$, the ${\rm Ca}^{2+}$ clearance was balanced by a leak current:

$$\left(\frac{d[Ca^{2+}]}{dt}\right)_{leak} = v_{max} \frac{A}{V} \left(\frac{[Ca^{2+}]_{rest}}{[Ca^{2+}]_{rest} + K_{M}}\right)$$
(11)

For a given molecular species X, the diffusional current J_X across the spine neck was simulated as

$$J_{X} = D_{X} \frac{\pi r_{\text{neck}}^{2}}{l_{\text{neck}}} (C_{X,\text{spine}} - C_{X,\text{dendrite}})$$
(12)

where D_X and C_X are the diffusion coefficient and the concentration of the diffusing species, respectively, and r_{neck} and l_{neck} are the radius and the length of the spine neck. The rate of change in the concentration of X due to diffusion follows as

$$\left(\frac{d[X]}{dt}\right)_{\text{diffusion}} = -\frac{J_X}{V_{\text{spine}}}$$
(13a)

for the spine and

$$\left(\frac{d[X]}{dt}\right)_{\text{diffusion}} = \frac{J_X}{V_{\text{dendrite}}}$$
(13b)

for the dendrite. The simulation included independent diffusion of the free binding sites of OGB, CB, PV, and CaM, of the corresponding Ca^{2+} -bound binding sites, of the Mg²⁺-bound binding sites of PV, and of free Ca^{2+} . The time course of the total change in $[Ca^{2+}]_i$ is given by the sum of Eqs. 5 - 11 in the absence of diffusion. Diffusional coupling was implemented by adding Eqs. 12 and 13a or b.

| $ \begin{bmatrix} Ca^{2^{+}}]_{bet} & 45 \text{ nM} & \text{ref. (2)} \\ [Mg^{2^{+}}]_{1} & 590 \mu M & \text{calculated, held constant} \\ \end{bmatrix} \\ Total Ca^{2^{+}} influx & CF signal & -4,700 ions & Dendrite & -35,000 ions & VOCC/iGluR-mediated signal & -4,700 ions & mGluR-mediated signal & spine & -4,700 ions & mGluR-mediated signal & spine & -4,700 ions & Michaelis-Menten constant, K_{M} & 3 \mu M & Maximal pump velocity, v_{max} & 30-300 & pmol cm^{-2}s^{-1} & \\ \end{bmatrix} \\ Coregon Green BAPTA-1 & Effective concentration & 160 or 40 \mu M & 80\% of pipette conc. & K_{D} & 325 nM & ref. (3) & k_{orr} & 140 s^{-1} & ref. (4) and a^{-1} & \\ K_{0} & 325 nM & ref. (3) & k_{orr} & 430 \mu M^{-1}s^{-1} & \\ Effective concentration & 100 \mu M & ref. (5) and b^{-1} & \\ K_{0} & K_{0} & K^{-1} & K^{-1} & K^{-1} & \\ Binding sites (non-cooperative) & 4 & ref. (6) & \\ Ratio of high- to & medium-aff. & 55.8 s^{-1} & ditto & \\ K_{0} & medium aff. & 55.8 \mu M^{-1}s^{-1} & ditto & \\ K_{0} & medium aff. & 55.8 \mu M^{-1}s^{-1} & ditto & \\ K_{0} & medium aff. & 55.8 \mu M^{-1}s^{-1} & ditto & \\ K_{0} & medium aff. & 43.5 \mu M^{-1}s^{-1} & ditto & \\ K_{0} & medium aff. & 822 nM & calculated & \\ \\ Parvalbumin & Effective concentration & 75 \mu M & ref. (5) & \\ After correction for wash-out & 40 \mu M & ref. (7) & \\ K_{0} & K_{0} & M^{-1} & M^{-1} & \\ K_{0} & M_{0} & M^{-1} & M^{-1} & \\ K_{0} & M_{0} & M^{-1} & M^{-1} & \\ K_{0} & M_{0} & M^{-1} & M^{-1} & \\ K_{0} & M_{0} & M^{-1} & M^{-1} & \\ K_{0} & M_{0} & M^{-1} & M^{-1} & \\ K_{0} & K_{0} & M^{-1} & M^{-1} & \\ K_{0} & K_{0} & M^{-1} & M^{-1} & \\ K_{0} & K_{0} & M^{-1} & M^{-1} & \\ K_{0} & K_{0} & M^{-1} & M^{-1} & \\ K_{0} & K_{0} & K^{-1} & \\ K_{0} & K_{0} & K^{-1$ | Parameter | V | alue | Notes |
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| $\begin{array}{llllllllllllllllllllllllllllllllllll$ | Maximal pump velocity, v_{max} | 30-300 | pmol cm ⁻² s ⁻¹ | |
| Oregon Green BAPTA-1Effective concentration160 or 40 μ M80% of pipette conc. K_D 325 nMref. (3)koff140 s ⁻¹ ref. (4) and akon430 μ M ⁻¹ s ⁻¹ calculatedCalbindin D _{28k} Effective concentration100 μ Mref. (5) and bAfter correction for wash-out50 μ McBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-aff.35.8 s ⁻¹ dittokoff, medium aff.2.6 s ⁻¹ koff, medium aff.2.6 s ⁻¹ dittokon, medium aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, medium aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.474 nMcalculatedParvalbuminEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7)After correction for wash-out40 μ McKoff, Ca0.95 s ⁻¹ ref. (8)Koff, Mg25 s ⁻¹ dittoKoff, Mg31 μ Mref. (7)kon, Kag31 μ Mref. (7)kon, Ca107 μ M ⁻¹ s ⁻¹ calculated | Oragon Croos DADTA 1 | | | |
| Effective concentration100 of 40 μ M80% of pipette conc. K_{D} 325 nMref. (3) k_{off} 140 s ⁻¹ ref. (4) and a k_{on} 430 μ M ⁻¹ s ⁻¹ calculatedCalbindin D _{28k} Effective concentration100 μ Mref. (5) and bAfter correction for wash-out50 μ McBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2.2koff, medium aff.35.8 s ⁻¹ dittokoff, high aff.2.6 s ⁻¹ dittokon, medium aff.5.5 μ M ⁻¹ s ⁻¹ dittokon, medium aff.5.5 μ M ⁻¹ s ⁻¹ dittokon, medium aff.822 nMcalculatedKo, medium aff.822 nMcalculatedKo, high aff.474 nMcalculatedKo, high aff.2ref. (7)koff, high aff.0.95 s ⁻¹ ref. (8)koff, Ca0.95 s ⁻¹ dittoKoff, Ca9 nMdittoKoff, Mg31 μ Mref. (7)koff, Mg31 μ Mref. (7)kon, Ca107 μ M ⁻¹ s ⁻¹ calculated | Effective concentration | 160 cm 10 | uМ | 800/ of pinotte cone |
| Ap32.5iffiffiff k_{off} 140 s^{-1} ref. (3) k_{off} 140 s^{-1} ref. (4) and a k_{on} 430 $\mu M^{-1}s^{-1}$ calculatedCalbindin D _{28k} Effective concentration100 μM ref. (5) and bAfter correction for wash-out50 μM cBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2:2dittokoff, ingh aff.2.6koff, high aff.2.6s^{-1}dittokoff, high aff.5.5 $\mu M^{-1}s^{-1}$ dittokon, nedium aff.43.5 $\mu M^{-1}s^{-1}$ kon, high aff.5.5 $\mu M^{-1}s^{-1}$ Kon, high aff.6.5 μM calculatedKD, nedium aff.474RD affer correction for wash-out40 μM effective concentration75 μM ref. (5)After correction for wash-out40 μM Binding sites (non-cooperative)2ref. (7)koff, Ca0.95 s^{-1} ref. (8)koff, Mg25 s^{-1} dittoKDCa9nMdittoKon, Ca107 $\mu M^{-1}s^{-1}$ calculated | | 225 | μM | 80% of pipette conc. |
| kott kon140 s140 s161 (4) and cCalbindin D28kEffective concentration100 μ Mref. (5) and bAfter correction for wash-out50 μ McBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2:2dittokoff, medium aff.35.8 s ⁻¹ koff, high aff.2.6 s ⁻¹ dittokon, medium aff.5.5 μ M ⁻¹ s ⁻¹ ditto and dkon, medium aff.5.5 μ M ⁻¹ s ⁻¹ ditto and dkon, high aff.5.5 μ M ⁻¹ s ⁻¹ ditto and dKon, high aff.5.5 μ M ⁻¹ s ⁻¹ ditto and dKon, high aff.75 μ McalculatedParvalbuminEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7)koff, Mg25 s ⁻¹ ref. (8)koff, Mg31 μ Mref. (7)koff, Mg31 μ Mref. (7)kon, Ca0.8 μ M ⁻¹ s ⁻¹ calculated | Λ _D k m | 140 | s ⁻¹ | ref (4) and a |
| K_{on} 4.50 µM scalculatedCalbindin D_{28k} Effective concentration100 µMref. (5) and bAfter correction for wash-out50 µMcBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2:2dittokoff, medium aff.35.8 s ⁻¹ kon, medium aff.2.6 s ⁻¹ dittokon, medium aff.2.6 s ⁻¹ dittokon, medium aff.5.5 µM ⁻¹ s ⁻¹ ditto and dkon, high aff.5.5 µM ⁻¹ s ⁻¹ dittoKon, medium aff.822 nMcalculatedKon, high aff.4.74 nMcalculatedParvalbuminEffective concentration75 µMref. (5)After correction for wash-out40 µMcBinding sites (non-cooperative)2ref. (7)koff.Mg2.5 s ⁻¹ ref. (8)koff.Mg2.5 s ⁻¹ dittoKoff.Mg31 µMref. (7)kon, Ca0.8 µM ⁻¹ s ⁻¹ calculated | K _{off} レ | /30 | $M^{-1}e^{-1}$ | calculated |
| $\begin{array}{cccc} \mbox{Calbindin } D_{28k} & \mbox{ref. (5) and }^b & \mbox{ref. (5) and }^b & \mbox{c}^c & \mbox{c}^c & \mbox{Binding sites (non-cooperative)} & 4 & \mbox{ref. (6)} & \mbox{Ratio of high- to} & \mbox{medium-affinity binding sites} & 2:2 & \mbox{ditto} & \mbox{korf, medium aff.} & 35.8 & \mbox{s}^{-1} & \mbox{ditto} & \mbox{korf, high aff.} & 2.6 & \mbox{s}^{-1} & \mbox{ditto} & \mbox{korf, high aff.} & \mbox{2.5 } & \mbox{medium aff.} & \mbox{ditto} & \mbox{korf, high aff.} & \mbox{2.5 } & \mbox{medium aff.} & \mbox{ditto} & \mbox{korf, high aff.} & \mbox{2.5 } & \mbox{medium aff.} & \mbox{ditto} & \mbox{ditto} & \mbox{korf, high aff.} & \mbox{2.5 } & \mbox{medium aff.} & \mbox{ditto} & \mbox{calculated} & \mbox{Korf, high aff.} & \mbox{2.5 } & \mbox{medium aff.} & \mbox{ditto} & \mbox{calculated} & \mbox{Korf, high aff.} & \mbox{ditto} & \mbox{calculated} & \mbox{Correction for wash-out} & \mbox{40 } \mbox{\muM} & \mbox{c}^c & \mbox{c} $ | K _{on} | 450 | μινι s | calculated |
| Effective concentration100 μ Mref. (5) and bAfter correction for wash-out50 μ McBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2:2dittokoff, medium aff.35.8 s ⁻¹ dittokoff, high aff.2.6 s ⁻¹ dittokon, medium aff.43.5 μ M ⁻¹ s ⁻¹ ditto and dkon, high aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.75 μ McalculatedKon, high aff.474 nMcalculatedParvalbuminccEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7)koff,Ca0.95 s ⁻¹ ref. (8)koff,Mg25 s ⁻¹ dittoKoff,Mg31 μ Mref. (7)kon,Ca107 μ M ⁻¹ s ⁻¹ calculated | Calbindin D _{28k} | | | |
| After correction for wash-out50 μ McBinding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2:2dittokoff, medium aff.35.8koff, medium aff.2.6s^{-1}kon, medium aff.43.5 μ M ⁻¹ s ⁻¹ kon, high aff.5.5 μ M ⁻¹ s ⁻¹ kon, high aff.5.5 μ M ⁻¹ s ⁻¹ kon, high aff.822nMcalculatedKD, medium aff.822KD, medium aff.822nMcalculatedCalculatedKD, high aff.75 μ Meffective concentration75 μ Ckonf.Mg <td< td=""><td>Effective concentration</td><td>100</td><td>μΜ</td><td>ref. (5) and ^b</td></td<> | Effective concentration | 100 | μΜ | ref. (5) and ^b |
| Binding sites (non-cooperative)4ref. (6)Ratio of high- tomedium-affinity binding sites2:2dittokoff, medium aff.35.8 s ⁻¹ dittokoff, high aff.2.6 s ⁻¹ dittokon, medium aff.43.5 μ M ⁻¹ s ⁻¹ ditto and ^d kon, high aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.822 nMcalculatedKon, high aff.474 nMcalculatedKon, high aff.94ParvalbumineeEffective concentration75 μ Mref. (5)After correction for wash-out40 μ MeBinding sites (non-cooperative)2ref. (7)koff,Ca0.95 s ⁻¹ ref. (8)koff,Mg25 s ⁻¹ dittoKoff,Ca9 nMdittoKon,Ca107 μ M ⁻¹ s ⁻¹ calculatedkon,Ca0.8 μ M ⁻¹ s ⁻¹ calculated | After correction for wash-out | 50 | μM | c |
| Ratio of high- to medium-affinity binding sites2:2dittokoff, medium aff.35.8s ⁻¹ dittokoff, high aff.2.6s ⁻¹ dittokon, medium aff.43.5 μ M ⁻¹ s ⁻¹ ditto and ^d kon, high aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.5.5 μ M ⁻¹ s ⁻¹ dittoKon, high aff.822nMcalculatedKon, high aff.474nMcalculatedParvalbuminccEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7)koff,Ca0.95s ⁻¹ ref. (8)koff,Mg25s ⁻¹ dittoKD,Ng31 μ Mref. (7)kon,Ca107 μ M ⁻¹ s ⁻¹ calculated | Binding sites (non-cooperative) | 4 | | ref. (6) |
| medium-affinity binding sites2:2ditto $k_{off, medium aff.}$ 35.8 s ⁻¹ ditto $k_{off, high aff.}$ 2.6 s ⁻¹ ditto $k_{on, medium aff.}$ 43.5 $\mu M^{-1}s^{-1}$ ditto and ^d $k_{on, high aff.}$ 5.5 $\mu M^{-1}s^{-1}$ ditto $K_{D, medium aff.}$ 822 nMcalculated $K_{D, high aff.}$ 474 nMcalculated $K_{D, high aff.}$ 474 nMcalculatedParvalbuminccEffective concentration75 μM ref. (5)After correction for wash-out40 μM cBinding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μM ref. (7) $k_{on,Ca}$ 0.8 $\mu M^{-1}s^{-1}$ calculated | Ratio of high- to | | | |
| koff, medium aff. 35.8 s^{-1} dittokoff, high aff. 2.6 s^{-1} dittokon, medium aff. $43.5 \mu M^{-1} \text{s}^{-1}$ ditto and ^d kon, high aff. $5.5 \mu M^{-1} \text{s}^{-1}$ dittoKD, medium aff. 822 nM calculatedKD, high aff. 474 nM calculatedParvalbumin c calculatedEffective concentration $75 \mu M$ ref. (5)After correction for wash-out $40 \mu M$ c Binding sites (non-cooperative) 2 ref. (7) $k_{off,Ca}$ 0.95 s^{-1} ref. (8) $k_{off,Mg}$ 25 s^{-1} ditto $K_{D,Ca}$ 9 nM ditto $K_{D,Mg}$ $31 \mu M$ ref. (7) $k_{on,Ca}$ $107 \mu M^{-1} \text{s}^{-1}$ calculated | medium-affinity binding sites | 2:2 | | ditto |
| koff, high aff.2.6 s^{-1} dittokon, medium aff.43.5 $\mu M^{-1}s^{-1}$ ditto and dkon, high aff.5.5 $\mu M^{-1}s^{-1}$ dittoKD, medium aff.822nMcalculatedKD, high aff.474nMcalculatedParvalbuminEffective concentration75 μM ref. (5)After correction for wash-out40 μM cBinding sites (non-cooperative)2ref. (7)koff,Ca0.95 s^{-1} ref. (8)koff,Mg25 s^{-1} dittoKD,Ng31 μM ref. (7)kon,Ca107 $\mu M^{-1}s^{-1}$ calculated | ${ m k}_{ m off,\ medium\ aff.}$ | 35.8 | s ⁻¹ | ditto |
| $k_{on, medium aff.}43.5 \mu M^{-1} s^{-1}ditto and dk_{on, high aff.}5.5 \mu M^{-1} s^{-1}dittoK_{D, medium aff.}822 nMcalculatedK_{D, high aff.}474 nMcalculatedParvalbuminEffective concentration75 \mu Mref. (5)After correction for wash-out40 \mu McBinding sites (non-cooperative)2ref. (7)k_{off,Ca}0.95 s^{-1}ref. (8)k_{off,Mg}25 s^{-1}dittoK_{D,Ca}9 nMdittoK_{D,Mg}31 \mu Mref. (7)k_{on,Ca}107 \mu M^{-1} s^{-1}calculated$ | k _{off, high aff.} | 2.6 | S ⁻¹ | ditto |
| $k_{on, high aff.}$ 5.5 $\mu M^{-1} s^{-1}$ ditto $K_{D, medium aff.}$ 822 nMcalculated $K_{D, high aff.}$ 474 nMcalculatedParvalbumineffective concentration75 μM ref. (5)After correction for wash-out40 μM eBinding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μM ref. (7) $k_{on,Ca}$ 107 $\mu M^{-1} s^{-1}$ calculated | kon, medium aff. | 43.5 | $\mu M^{-1} s^{-1}$ | ditto and ^a |
| $K_{D, medium aff.}$ 822 nMcalculated $K_{D, high aff.}$ 474 nMcalculatedParvalbuminEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | k _{on, high aff.} | 5.5 | $\mu M^{-1}s^{-1}$ | ditto |
| $K_{D, high aff.}$ 474 nMcalculatedParvalbuminEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | $K_{\rm D, medium aff.}$ | 822 | nM | calculated |
| ParvalbuminEffective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | $K_{ m D,\ high\ aff.}$ | 474 | nM | calculated |
| Effective concentration75 μ Mref. (5)After correction for wash-out40 μ McBinding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | Parvalbumin | | | |
| After correction for wash-out40 μ Mref. (7)Binding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | Effective concentration | 75 | μM | ref. (5) |
| Binding sites (non-cooperative)2ref. (7) $k_{off,Ca}$ 0.95 s ⁻¹ ref. (8) $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | After correction for wash-out | 40 | иM | c |
| $k_{off,Ca}$ 0.95 s^{-1} ref. (8) $k_{off,Mg}$ 25 s^{-1} ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ $31 \ \mu\text{M}$ ref. (7) $k_{on,Ca}$ $107 \ \mu\text{M}^{-1}\text{s}^{-1}$ calculated $k_{on,Mg}$ $0.8 \ \mu\text{M}^{-1}\text{s}^{-1}$ calculated | Binding sites (non-cooperative) | 2 | F | ref. (7) |
| $k_{off,Mg}$ 25 s ⁻¹ ditto $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | k _{off Ca} | 0.95 | s ⁻¹ | ref. (8) |
| $K_{D,Ca}$ 9 nMditto $K_{D,Mg}$ 31 μ Mref. (7) $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculatedkon Mg0.8 μ M ⁻¹ s ⁻¹ calculated | k _{off Ma} | 25 | s ⁻¹ | ditto |
| $\begin{array}{cccc} & 31 & \mu M & \text{ref. (7)} \\ & k_{\text{on,Ca}} & 107 & \mu M^{-1} \text{s}^{-1} & \text{calculated} \\ & k_{\text{on Mg}} & 0.8 & \mu M^{-1} \text{s}^{-1} & \text{calculated} \\ \end{array}$ | $K_{\rm D Ca}$ | | nM | ditto |
| $k_{on,Ca}$ 107 μ M ⁻¹ s ⁻¹ calculated $k_{on,Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | | 31 | μM | ref. (7) |
| $k_{on Mg}$ 0.8 μ M ⁻¹ s ⁻¹ calculated | k _{on.Ca} | 107 | $\mu M^{-1} s^{-1}$ | calculated |
| | k _{on,Mg} | 0.8 | $\mu M^{-1} s^{-1}$ | calculated |

Supplemental Table 1. Values and Parameters of the Simulation

| Supplemental Table 1, continued | | | |
|---------------------------------|-------|--------------------|-------------------------------|
| Parameter | Value | | Notes |
| Calmodulin | | | |
| Effective concentration | 10 | μM | estimated from ref. (3) |
| Binding sites | 1 | | |
| k_{off} | 2,200 | s ⁻¹ | e |
| K _D | 55 | μM | ditto |
| k _{on} | 40 | $\mu M^{-1}s^{-1}$ | ditto |
| Geometry | | | |
| Wild type and PV ^{-/-} | | | |
| Volume of spine head | 0.083 | μm ³ | ref. (9) |
| Surface area of spine head | 0.9 | μm^2 | ditto |
| Radius of spine neck | 0.09 | μm | ditto |
| Length of spine neck | 0.66 | μm | ditto |
| Spine density | 3.4 | μm ⁻¹ | ref. (10) |
| Radius of dendritic segment | 1 | μm | |
| Length of dendritic segment | 0.3 | μm | calculated from spine density |
| PV/CB ^{-/-} | | 2 | |
| Volume of spine head | 0.2 | μm ³ | estimated ^t |
| Surface area of spine head | 1.8 | μm^2 | ditto |
| Radius of spine neck | 0.1 | μm | ditto |
| Length of spine neck | 1.3 | μm | ditto |
| Spine density | 5 | μm ⁻¹ | ref. (10) |
| Radius of dendritic segment | 1 | μm | |
| Length of dendritic segment | 0.2 | μm | calculated from spine density |
| Diffusional mobility | | | |
| D_{Ca} | 223 | $\mu m^2 s^{-1}$ | ref. (11) |
| $D_{ m OGB}$ | 15 | $\mu m^2 s^{-1}$ | ref. (12) and ^a |
| $D_{ m PV}$ | 43 | $\mu m^2 s^{-1}$ | ref. (13) |
| $D_{ m CB}$ | 20 | $\mu m^2 s^{-1}$ | ref. (14) |
| D_{CaM} | 21 | $\mu m^2 s^{-1}$ | this study |
| Fraction of immobile CB | 0.2 | | ref. (14) |
| Fraction of immobile CaM | 0.2 | | this study |

^a Data for Calcium Green 1, an indicator dye closely related to OGB; ^b assuming that 33% of CB is occupied by Mg^{2+} ; ^c assuming a ~50% washout during our whole-cell recordings; ^d assuming two-fold slower kinetics in the cytosol compared to the *in vitro* data (15); ^e Faas *et al.* (2004), Soc. Neurosci. Abstr. No 165.5; ^f calculated from the wild-type EM data based on the observation of a ~2 fold increase in volume, surface, and length in spines of PV/CB^{-/-} mice (10).

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