Supplementary data 4. Neuronal CaM Concentration

Our simulation used a [CaM] of 100 μM. If CaM is reduced in the simulation from 100 μM to 50, 25 or 0 μM the κ of the system with 30 μM CB reduces from 19 to 10, 5 or 1, respectively. If instead CB is taken out of the simulation, the κ of the system with 100 μM CaM reduces to 12. These results indicate that the fast buffering property of the whole system is directly dependent on CaM while the slower CB can only add to κ when a fast component is in place. This indicates that CaM is essential for the fast buffering in this system and not merely the dominant fast buffer because of its high concentration. The measured fast buffer capacity of spines is ~20[4,5] which can be well accounted for by 100 μM CaM. Thus, if 100 μM CaM is an overestimate of the [CaM] in a spine, there must be an additional molecular species contributing to fast buffering. Thus far it has been challenging to directly measure the intracellular CaM concentration. However, many studies have measured the soluble fraction of CaM from brain homogenates relative to the total protein content (supplementary Table 1), which varies based on the specific brain region and species. Based on values from human, rat and bovine for CaM (μg/mg protein) and the total protein content (~10%[6,7,8]), we estimated the range of intracellular [CaM] (CaM (μM), supplementary Table 1) assuming that the soluble fraction should be dissolved in the water content of the brain (~77%). This value could be further refined by the assumption that all soluble CaM came from intracellular content (~80%). This volume corrected [CaM] (vol. corrected CaM (μM), supplementary Table 1) varied between 31 and 337 μM. The large variation is mainly caused by the outliers for the reported CaM content in brain homogenates. We also calculated the [CaM] based on the median reported CaM content per brain region (green numbers, supplementary Table 1) and found the [CaM] varied from ~65 μM for the cerebellum to 152 μM for the caudate nucleus. Therefore, in our simulation of a spine in the hippocampus, a [CaM] of 100 μM might actually be an underestimate, especially when one considers that CaM could be enriched in specific cellular compartments[9,10]. Although these considerations are important in determining the role of CaM as the fast buffer, the absolute [CaM] is not essential to our discussion considering the activation of CaM or its individual lobes in a nano-domain. There we discussed the probability of a single CaM molecule being activated independent of the overall [CaM].

Supplementary Table 1. Calculation of neuronal CaM concentration

	Water (w/w %)	Protein (w/w %)	soluble CaM (µg/mg protein)	soluble CaM[a] (w/w %)	CaM[b] (µM)	extracell. vol.[11] (%)	CaM vol. corrected[c] (µM)
Whole Brain	77–78[6] 77.8[12] 76–78[13]	8[6] 8–13[7] 9–10[8]					
Cerebrum/ Cerebral cortex			15.9[14] 16.9–17.8[15] 14–15[16] 13[17] 14[18] 13.7–13.8[19] 20.9[20]	0.104–0.272	80–214 112[e]	18–20	97–268 141[f]
Cerebellum			11.2[15] 4[16] 9.5[21] 6[18] 7.5–7.7[19] 18.1[20]	0.032–0.235	25–185 52[e]	21–31	31–268 65[f]
Hippocampus			14.3[14] 15.2[15] 12[16]	0.096–0.198	74–156 111[e]	22	94–200 139[f]
Caudate nucl.			15.2[14] 16.2[15]	0.122–0.211	94–166 122[e]	21	119–210 152[f]
Striatum			15[16] 8[17] 26.4[20]	0.064–0.343	49–270 117[e]		61–337[d] 146[f]
Amygdala			13[16]	0.104–0.169	80–133 101[e]		100–166[d] 126[f]

[a]) $\text{soluble CaM (w/w \%)} = \frac{\text{soluble CaM } (\mu\text{g/mg protein})/10}{100} \times \text{Protein (w/w \%)}$ (eq. S4)

[b]) $\text{CaM } (\mu\text{M}) = \frac{\text{soluble CaM } (\mu\text{g/mg protein}) \times 10}{16.7\text{kDa} \times \left(\text{water (w/w \%)}/100\right)}$ (eq. S5)

[c]) $\text{vol. corrected CaM } (\mu\text{M}) = \frac{\text{CaM } (\mu\text{M})}{1 - \left(\text{extracell. vol.(w/w \%)}/100\right)}$ (eq. S6). An overall brain density of 1 g/ml was assumed, based on published volume and weight data[6].

[d]) For these calculations 20% extracellular was used, estimated from the values of the other regions.

[e]) Value calculated using 77% water (w/w %), 10% protein (w/w %) and the median value for CaM (µg/mg protein).

[f]) Values calculated using values from e) and a extracellular volume of 20%.