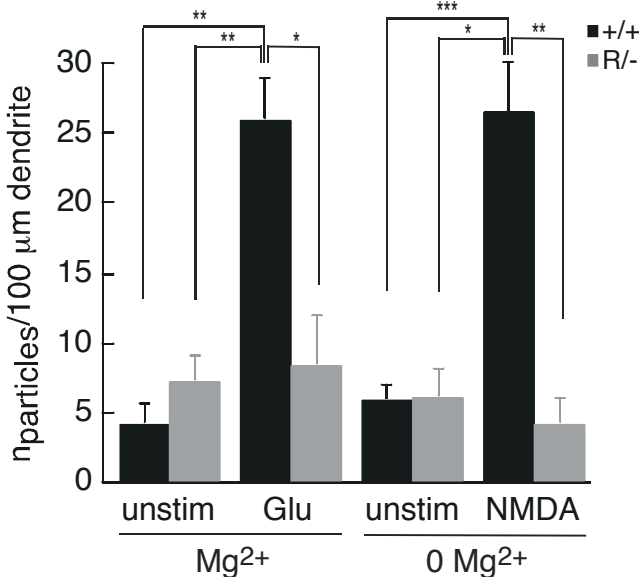


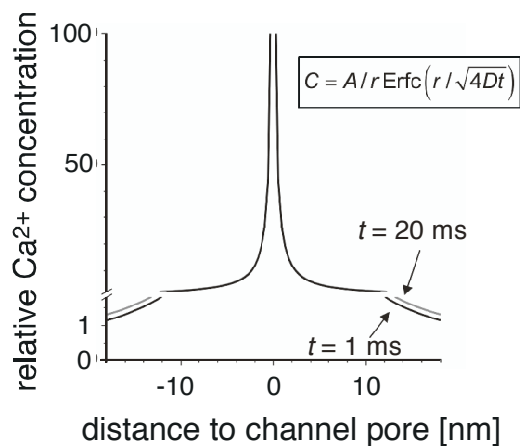
**Figure S7. Application of Kainate (100  $\mu$ M) does not translocate CaMKII clones.**

Experimental conditions and analysis of data as in Fig. 4; n (left to right)= 7, 4, 8, 4, 7, 4; mean $\pm$ SEM.



**Figure S8. Alternative analysis of CaMKII puncta by use of cell filler mRFP.**

CaMKII $\alpha$  translocation in cells expressing wildtype (wt) and calcium impermeable (R/-) NMDARs, respectively. Neurons were transfected with equal amounts of plasmid DNA of expression constructs for EGFP-tagged CaMKII $\alpha$  and plain mRFP. Translocation experiments and image acquisition were performed as described in Methods section. Confocal stacks were Z-projected for each channel individually. A dendritic region of approx. 50-300  $\mu$ m combined dendritic length was selected for correlation diagram analysis (ImageJ plugin 'colocalization finder'; <http://rsb.info.nih.gov/ij/>), where pixel were selected via a rectangular thresholding that allowed only for intensity values in the green channel exceeding the intensity values in the red channel by at least 25%. Particles in the obtained binary image that were of reasonable area (0.5-1.5  $\mu$ m diameter) were counted automatically. n (left to right)=5, 4, 6, 3, 9, 3, 8, 4; mean $\pm$ SEM; (\*) p<0.05, (\*\*) p<0.01, (\*\*\*) p<0.001.



**Figure S9. An estimated sub-membrane Ca<sup>2+</sup> concentration profile (nano-domain) in the proximity of the open NMDA receptor channel mouth.**

Because the NMDA receptor opening kinetics is slow (characteristic time scale 20-30 ms) compared to the nm-scale diffusion equilibration time, the profiles were obtained using solutions for the continuous diffusion source (Crank, 1975), equation see inset, where  $A$  is the dimensionality factor,  $C$  is concentration, Erfc denotes complementary error function,  $t$  is time;  $D \approx D_e/60$  is Ca<sup>2+</sup> diffusivity in conditions of heavy endogenous buffering inside the spine ( $D_e=0.25 \mu\text{m}^2/\text{ms}$  is the effective Ca<sup>2+</sup> intracellular diffusion coefficient) (Sabatini et al., 2001).

Crank, J. (1975) *The Mathematics of Diffusion*. Oxford University Press, Oxford, UK.

Sabatini, B.L., Maravall, M. and Svoboda, K. (2001) Ca<sup>2+</sup> signaling in dendritic spines. *Curr Opin Neurobiol*, **11**, 349-356.