Supplementary Materials for

Compartmentalization of GABAergic Inhibition by Dendritic Spines

C. Q. Chiu[†], G. Lur[†], T. M. Morse, N. T. Carnevale, G. Ellis-Davies, M. J. Higley

[†]These authors contributed equally to this work.

correspondence to: m.higley@yale.edu

This PDF file includes:

Materials and Methods
Figs. S1 to S10

Materials and Methods

Slice Preparation

All animal handling was performed in accordance with guidelines approved by the Yale Institutional Animal Care and Use Committee and federal guidelines. Optogenetic ChR2 experiments were performed in acute prefrontal cortical slices taken from male and female SOM-Cre mice (postnatal day P26–45, kindly provided by Josh Huang, Cold Spring Harbor Laboratory) that were previously injected intracranially with a recombinant adeno-associated viral construct (see below). GABA uncaging experiments were conducted using slices from wild-type C57/Bl6 mice (P22-36). Under isoflurane anesthesia, mice were decapitated and coronal slices (300 µm thick) were cut in ice-cold external solution containing (in mM): 110 choline, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 20 glucose, 11.6 sodium ascorbate and 3.1 sodium pyruvate, bubbled with 95% O_2 and 5% CO_2. Slices containing the prelimbic-infralimbic regions of the PFC were then transferred to artificial cerebrospinal fluid (ACSF) containing (in mM): 127 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$ and 20 glucose bubbled with 95% O_2 and 5% CO_2. After an incubation period of 30 min at 34 °C, the slices were maintained at 20–22 °C for at least 20 min until use.

Electrophysiology and imaging

Experiments were conducted at room temperature (20–22°C), except where noted, in a submersion-type recording chamber. Whole-cell patch-clamp recordings were obtained from layer 2/3 pyramidal cells (200-300 µm from the pial surface) identified with video-infrared/differential interference contrast. For current-clamp recordings, glass electrodes (3.2–3.8 MΩ) were filled with internal solution containing (in mM): 135 $KMeSO_3$, 10 HEPES, 4 $MgCl_2$, 4 Na_2ATP, 0.4 NaGTP and 10 sodium creatine phosphate, adjusted to pH 7.3 with KOH. Red-fluorescent Alexa Fluor-594 (10 µM) and green-fluorescent Ca(2+)-sensitive Fluo-5F (300 µM) were included in the pipette solution to visualize cell morphology and changes of intracellular Ca(2+) concentration, respectively. Neurons were filled via the patch electrode for at least 15 min before imaging. In some cases, 0.2% Neurobiotin was included in the internal solution for post-hoc cell reconstruction and immunohistochemistry. To assess the mechanism of Ca(2+) inhibition, a high chloride internal solution was used in some experiments, containing (in mM): 80 $KMeSO_3$, 50 KCl, 10 HEPES, 4 $MgCl_2$, 4 Na_2ATP, 0.4 NaGTP and 10 sodium creatine phosphate. For consistency across experiments, the cell membrane potential was adjusted to -60 mV by injection of small amounts of depolarizing current through the recording pipette. For voltage-clamp recordings, cesium was substituted for potassium in the internal solution to improve space clamp. For perforated patch experiments, pipettes were backfilled with internal solution containing gramicidin (128 µg/ml) and (in mM): 130 CsCl, 10 HEPES, 4 $MgCl_2$, 0.5 $CaCl_2$, 4 Na_2ATP. For whole-cell voltage-clamp recordings, series resistance was 15-25 MΩ and uncompensated. For perforated patch recordings, series resistance was 10-30 MΩ using 50-70% series compensation. Recordings were discarded if series resistance changed >20% during the experiment. Electrophysiological recordings were made using a Multiclamp 700B amplifier, filtered at 4 kHz, and digitized at 10 kHz. Whole-cell recordings were adjusted post-hoc for liquid junction potentials of 7.7 mV and 6.5 mV for $KMeSO_3$ and $CsMeSO_3$,

respectively. Loose patch recordings of fluorescent SOM-INs were performed with potassium-based internal in current-clamp mode.

2-photon imaging was accomplished with a custom-built microscope, including components manufactured by Mike's Machine Company. Fluorophores were excited using 840 nm light from a pulsed titanium-sapphire laser. Emitted green and red photons were separated with appropriate dichroics and filters and collected by photomultiplier tubes. A mechanical shutter was placed in front of the collectors to prevent damage during blue light stimulation.

Imaged spines from the apical dendrite were located between 50 μm and ~200 μm (the pial surface) from the cell body. Action potentials were evoked using a brief depolarizing current pulse (1 ms, 2 nA) through the recording pipette. For Ca(2+) imaging, signals were collected during 500 Hz line scans across a spine and a neighboring dendrite. Reference frame scans were taken between each acquisition to correct for small spatial drift of the preparation over time. Ca(2+) signals were first quantified as increases in green fluorescence from baseline normalized to the average red fluorescence ($\Delta G/R$). To permit comparison of the imaging data across various microscope configurations, we expressed fluorescence changes as the fraction of the G/R ratio measured in saturating Ca(2+) ($\Delta G/G_{sat}$). To calculate G_{sat}, we imaged a 1:1 mixture of internal solution and 1 M $CaCl_2$ in a sealed recording pipette in the specimen plane under conditions identical to those used during recordings.

Data acquisition and analysis

Imaging and physiology data were acquired using custom software written in MATLAB. Off-line analysis was performed using custom routines written in MATLAB and IgorPro. IPSP/IPSC and EPSP amplitudes were calculated by finding the peak of the voltage/current traces and averaging the values within a 3 ms window. AP- and synaptic-evoked ΔCa(2+) was calculated as the average $\Delta G/G_{sat}$ over a 140 ms window, starting 5 ms after the stimulus.

AP-evoked Ca(2+) transients are inherently variable from trial to trial due to stochastic fluctuations in the opening of VGCCs (*38*). Therefore, we performed a bootstrap analysis to determine the minimal significant change in ΔCa(2+) detectable for a single spine. We first generated a dataset of control AP-evoked Ca(2+) transients (30+ trials per spine, n=5). For each spine, we then resampled 5, 10, or 20 transients with replacement, repeated 1000 times, to derive a pool of estimates for the population average (derived from the entire set of transients). This process allowed us to define a 95% confidence interval around the "true" average for the given number of samples. For a sample size of 5 transients (the minimum used per condition in our experiments), a reduction in ΔCa(2+) of more than 15% corresponds to a significant inhibition at a $p<0.05$ level (Fig. S3E-F).

Pharmacology

For all experiments, except where noted, the ACSF included 3 μM CGP-55845 to block $GABA_B$ receptors. For a subset of experiments (see text), the ACSF included (in μM): 50 picrotoxin, 3 nimodipine, 1 TTA-A2, 0.1 SNX 482, 10 mibefradil, 1 conotoxin ω-MVIIC, 40 cadmium, or 1 TTX. TTA-A2 was a gift from Bruce Bean, Harvard Medical School.

ChR2 expression and activation

To stimulate SOM-INs, SOM-Cre mice were injected at P12-15 into the prefrontal cortex with recombinant adeno-associated virus containing a plasmid coding for the conditional expression of a ChR2-EYFP fusion protein under the Ef1a promoter (AAV-DIO-Ef1a-ChR2-EYFP (*10, 39*). Virus was produced by the UNC Vector Core (Chapel Hill, NC). Mice were sacrificed 2-4 weeks post-injection for slice preparation as described above. To activate ChR2-positive fibers, we overfilled the back aperture of the microscope objective (60x, 1.0 NA) with collimated blue light from a fiber-coupled 473 nm laser. Spherical aberrations due to fiber-coupling resulted in a ~15-20 μm diameter disc of light at the focal plane centered on the field of view. A brief (3-5 ms) pulse of light (1-2 mW at the sample) reliably stimulated SOM-INs and evoked IPSPs/IPSCs in pyramidal neurons. Power was adjusted to the minimum value capable of evoking local inhibition. Trials including AP alone, IPSP alone, and IPSP-AP (15 ms interval) were interleaved with a 15 second inter-trial interval. For mapping experiments, we fixed the position of the microscope objective to activate the same population of ChR2-expressing fibers and imaged neighboring dendritic regions by adjusting the scan mirrors.

1-photon GABA uncaging

Visible light-evoked GABA uncaging was accomplished using the same light source and path as for ChR2 activation. RuBi-GABA (10.8 μM) was bath-applied in the ACSF. Due to the slightly slower kinetics of uncaging-evoked IPSPs (see Fig. S5), uncaging pulses (1-2 mW, 2 ms) were timed 40 ms prior to AP/$2PLU_{Glu}$ stimulation. Power was adjusted to the minimum value capable of evoking local inhibition. Interleaved trials were performed as for ChR2 experiments.

2-photon GABA and Glutamate uncaging

$2PLU_{GABA}$ and $2PLU_{Glu}$ were accomplished using a second pulsed titanium-sapphire laser tuned to 720 nm whose output was combined with the imaging path using polarization optics and directed to the scan mirrors. CDNI-GABA (1 mM) or CDNI-Glutamate (1 mM) was bath-applied (*14, 15*). For $2PLU_{GABA}$, a spine showing inhibition of AP-evoked ΔCa(2+) mediated by photoactivation of ChR2-expressing SOM-INs was first identified. Subsequently, uncaging pulses (20-30 mW, 1-2 ms) were directed to 1-4 locations around the spine head (~0.5 μm from the membrane) in order to determine a "hot spot" that produced similar Ca(2+) inhibition. For each spine, Ca(2+) inhibition was evaluated for this "hot spot" (notated position 1) and for the uncaging location on the opposite side of the spine (position 2). For all experiments, the interval between $2PLU_{GABA}$ and the somatic action potential was 10-15 ms.

For $2PLU_{Glu}$, a spine showing inhibition of AP-evoked ΔCa(2+) mediated by 1-photon GABA uncaging was first identified. Uncaging pulses (20-30 mW, 0.5-1.0 ms) were then directed around the spine head (~0.5 μm from the membrane) to identify an excitatory "hot spot" that produced the largest ΔCa(2+) in the spine head and somatic EPSP. $2PLU_{Glu}$ was paired with a preceding IPSP (40 ms interval) evoked by 1-photon GABA uncaging.

Post-hoc Cell Reconstruction and Immunohistochemistry

Brain slices were fixed after recording in 4% formaldehyde in phosphate buffer for 1 hour at 4°C. Slices were then embedded in 10% gelatin, post-fixed overnight at 4°C, and re-sectioned to 40 μm on a vibratome. Slices were incubated in buffer containing 10% normal goat serum, 1% bovine serum albumin, and 0.1% Triton X-100 for 4 hours. A primary antibody against either gephyrin (Synaptic Systems #147021, 1:200) or green fluorescent protein (Invitrogen #A6455, 1:500) was applied overnight at 4°C. Sections were then stained with Alexa 488-conjugated secondary antibody and Alexa 546-conjugated streptavidin for 4 hours, rinsed repeatedly with buffer, and mounted on microscope slides for confocal imaging at 63x. To estimate the percentage of spines receiving putative GABAergic synapses, we looked for co-localization of either a gephyrin punctum or a ChR2-YFP-expressing varicosity with dendritic spines using the ImageJ co-localization analysis plugin:

(http://www.uhnresearch.ca/facilities/wcif/imagej/colour_analysis.htm). Counts were averaged across data from three independent observers.

To visualize SOM-INs, we crossed SOM-Cre (+/-) mice with Ai9 reporter mice (*40*) (JAX) in which tdTomato expression is Cre-dependent. The resulting F1 offspring have tdTomato expression selectively in SOM-INs. To confirm this, lightly fixed (4 hours in 4% formaldehyde) brains were taken from SOM-Cre;Ai9 mice. Brain sections were prepared and immuno-stained for somatostatin (Millipore #MAB354, 1:200) using the methods described above.

Computational Modeling

We developed a multi-compartment dendritic model using NEURON v7.2 (*41*, ModelDB accession number 143604). The model consisted of a 600 μm length of dendrite (2 μm diameter cylinder) connected to a soma (isopotential cylinder, 7.4 μm length, 14.8 μm diameter) whose purpose was to generate an action potential. Three spines were located 90, 100, and 110 μm from the soma. Spines comprised a head (1 μm diameter) attached to the parent dendrite by a neck (1 μm length, 0.07 μm diameter). For default simulations, membrane specific capacitance was 0.75 μF/cm, membrane specific resistance (leak) was 40 kΩ cm^2, and cytoplasmic resistivity was 200 Ω cm^2. These default conditions yielded a spine neck resistance of 520 MΩ, similar to a recent experimentally determined value (*26*). In separate simulations, we examined the impact of varying spine neck resistance on local inhibition by adjusting the neck diameter (range 60-1020 MΩ, Fig. S9F). In diagnostic experiments, varying the location of the spines or sealing the dendritic shaft immediately distal to the spines, produced qualitatively similar results.

Action potentials were generated via fast sodium and delayed rectifier currents similar to those described previously and expressed in the soma and dendritic shaft (*42*, see ModelDB accession number 8210). The density of these channels was set to allow active AP propagation in the dendrite with minimal decrement over distance. Ca(2+) currents were produced via high-threshold channels similar to those found in cortical pyramidal neurons (Fig. S9E). Voltage-gated channel parameters correspond to 34^oC. A GABAergic synapse (utilizing $GABA_A$ receptors) was implemented with the NEURON Exp2Syn mechanism with a conductance specified by:

$G(t) = G_{max} * S_{norm} * (\exp(-t/\tau_2) - \exp(-t/\tau_1))$,

where G_{max} is the maximum inhibitory conductance (default value 0.4 nS) and S_{norm} serves to normalize the sum of exponentials. We set G_{max} to 0.4 nS as a biologically plausible default value. The time constants τ_1=5 ms, and τ_2 =74 ms were obtained by fitting the Exp2Syn current to ChR2-evoked IPSCs. The default chloride equilibrium potential was -70 mV. Resting membrane potential was -60 mV. The inhibitory synapse was placed either on the middle spine head or the neighboring dendritic shaft (see main text) and was activated 15 ms prior to generating an action potential in the soma. For simulations, the dendrite was discretized into compartments 1 μm long and data were generated using fixed time step integration (implicit Euler, dt = 0.025 ms).

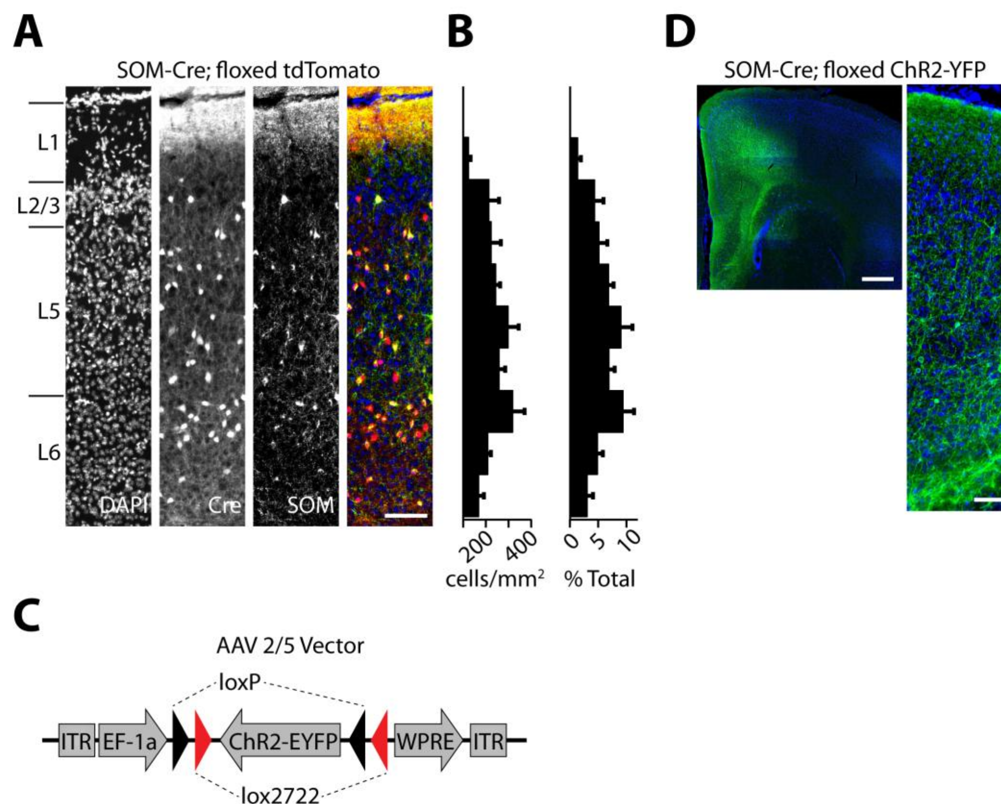

Fig. S1.

Conditional expression of ChR2 in SOM-INs of the prefrontal cortex. (**A**) Layer specific distribution of SOM-INs in the prefrontal cortex. To visualise SOM-INs, SOM-Cre +/- mice were crossed to the floxed dTomato-expressing Ai9 reporter line. Nuclei were labelled with DAPI (1st panel, blue merged). SOM-INs were labelled by the expression of cytosolic tdTomato (2nd panel, red merged). Somatostatin was visualized immunohistochemically (3rd panel, green merged). Scale bar: 100µm. (**B**) Density (*left*) and overall percentage (*right*) of tdTomato-expressing cells by layer. (**C**) Schematic of the Cre-inducible AAV_5-Ef1a-DIO-ChR2-EYFP viral vector. (**D**) *Left*, Fluorescence image of the virus injection site in the prefrontal cortex of SOM-Cre +/- mice. Scale bar: 500µm. *Right*, Higher magnification image of the medial PFC. Scale bar: 50µm.

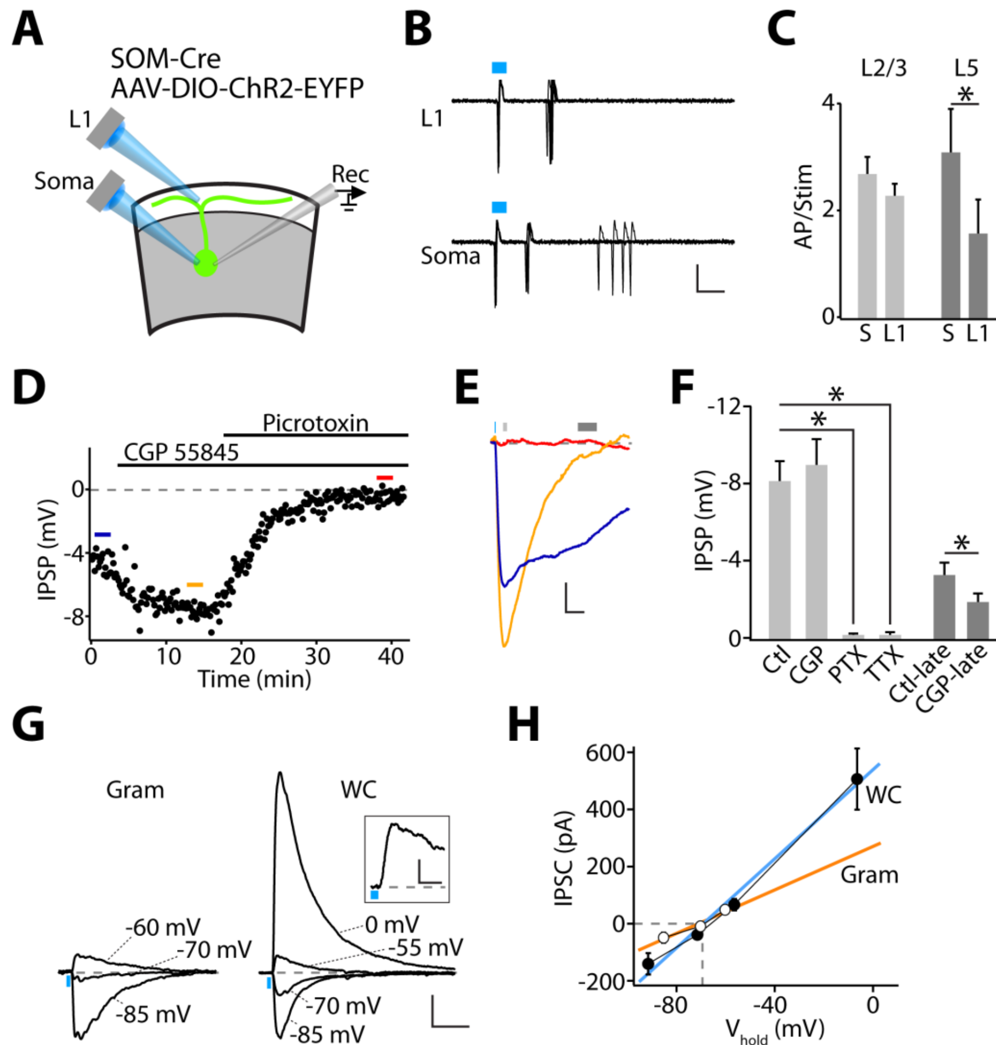

Fig. S2.

Optical stimulation of SOM-INs evokes GABAergic inhibition in pyramidal neurons. (**A**) Schematic drawing of the experimental configurations for recording ChR2-EYFP expressing SOM-INs. (**B**) Example loose patch recording from an ChR2-EYFP-labeled SOM-IN in layer 2/3. ChR2 was activated by a brief pulse of 473 nm laser light (blue bar) directed either to the region of dendritic synapses in layer 1 (top) or to the soma of the recorded cell (bottom). Scale bars: 20 mV, 10 ms. (**C**) Light-evoked spike counts (mean ± SEM) for ChR2-expressing SOM-INs located in either layer 2/3 (light gray) or layer 5 (dark gray). Light pulses were directed either to the soma ("S") or layer 1 ("L1"). (**D**) Representative time course showing the effect of the GABA receptor antagonists CGP 55845 ($GABA_B$) and picrotoxin ($GABA_A$) on the amplitude of light-evoked IPSPs in L2/3 pyramidal neurons. (**E**) Average IPSPs taken at the time points indicated in the experiment shown in (D). Blue: control, yellow: in CGP 55845, red: in CGP 55845 + picrotoxin. Scale bars: 1 mV, 100 ms. (**F**) IPSP amplitudes (mean ± SEM) in control, CGP 55845, CGP 55845 + picrotoxin at the two time points indicated in (E) measured at 50 ms (light gray) or 400 ms (dark grey) after the light pulse. (**G**) ChR2-evoked IPSCs in a L2/3 pyramidal cell voltage clamped at the indicated membrane potentials, obtained via

either gramicidin-based perforated patch (*left*) or whole cell (*right*) recordings. Scale bars: 100 pA, 50 ms. Inset shows the expanded whole cell IPSC at -55 mV. Scale bars: 20 pA, 10 ms. (**H**) Perforated patch (open circles, orange line, n=6) and whole-cell (closed circles, blue line, n=5) chloride reversal potentials calculated from experiments similar to (G). * indicates $p<0.05$ (paired Student's t-test).

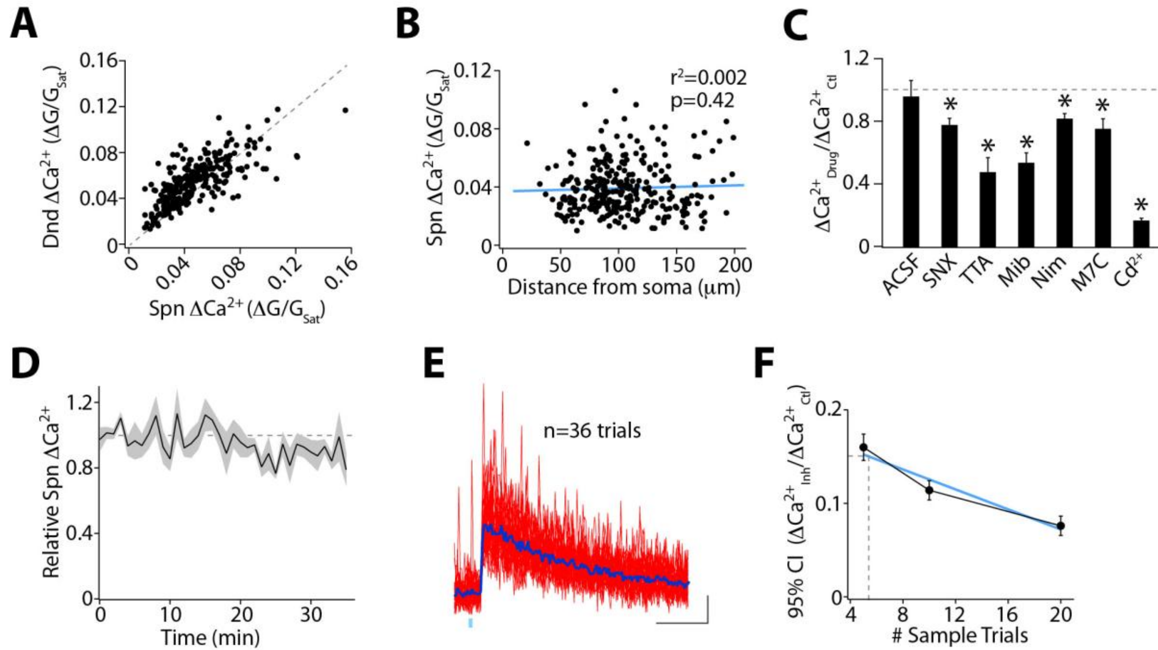

Fig. S3.

Back-propagation of action potentials opens voltage-gated Ca(2+) channels in dendritic spines and shafts. (**A**) Relationship between AP-evoked Ca(2+) in dendritic spines and in the neighboring dendritic shafts. (**B**) Amplitude of AP-evoked ΔCa(2+) in dendritic spines does not vary as a function of distance from the cell body. (**C**) AP-evoked ΔCa(2+) are mediated by voltage gated Ca(2+) channels. ACSF: artificial cerebrospinal fluid (control, n=8), SNX: SNX 482 (R-type blocker, n=8), TTA: TTA-A2 (T-type blocker, n=7), Mib: mibefradil (L-,R-,T-type blocker, n=10), Nim: nimodopine (L-type blocker, n=10), M7C: ω-conotoxin MVIIC (N,-P-,Q-type blocker, n=14), Cd(2+): cadmium (nonspecific VGCC blocker, n=10). (**D**) Average (n=10) magnitude of AP-evoked ΔCa(2+) (± SEM) is stable over long recordings. (**E**) Thirty individual AP-evoked ΔCa(2+) obtained in one spine. Average transient is shown in blue. Scale bars: 5% ΔG/Gsat, 50 ms. (**F**) Calculated 95% confidence intervals for changes in ΔCa(2+) as a function of the number of trials resampled. * indicates $p<0.05$ (paired Student's t-test).

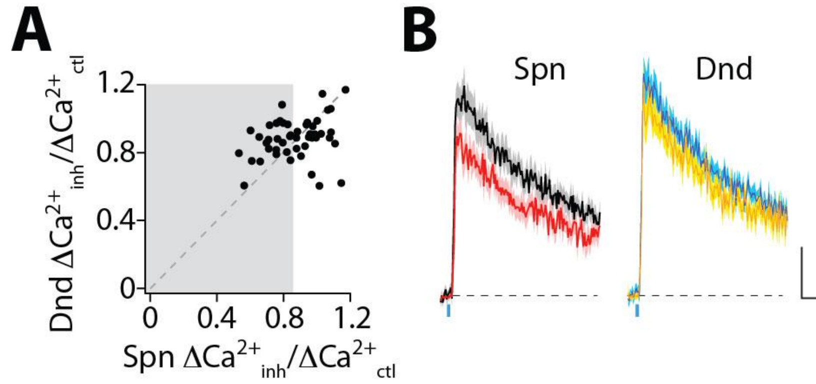

Fig. S4.

(**A**) Ca(2+) inhibition for shafts versus spines in basal dendrites. Gray region indicates significant spine Ca(2+) inhibition. (**B**) Average ΔCa(2+) (± SEM) in spines and dendritic shafts of basal dendrites evoked by AP (black, blue) or paired IPSP-AP (red, orange) for locations showing significant inhibition. Scale bars: 2% $\Delta G/G_{sat}$, 50 ms.

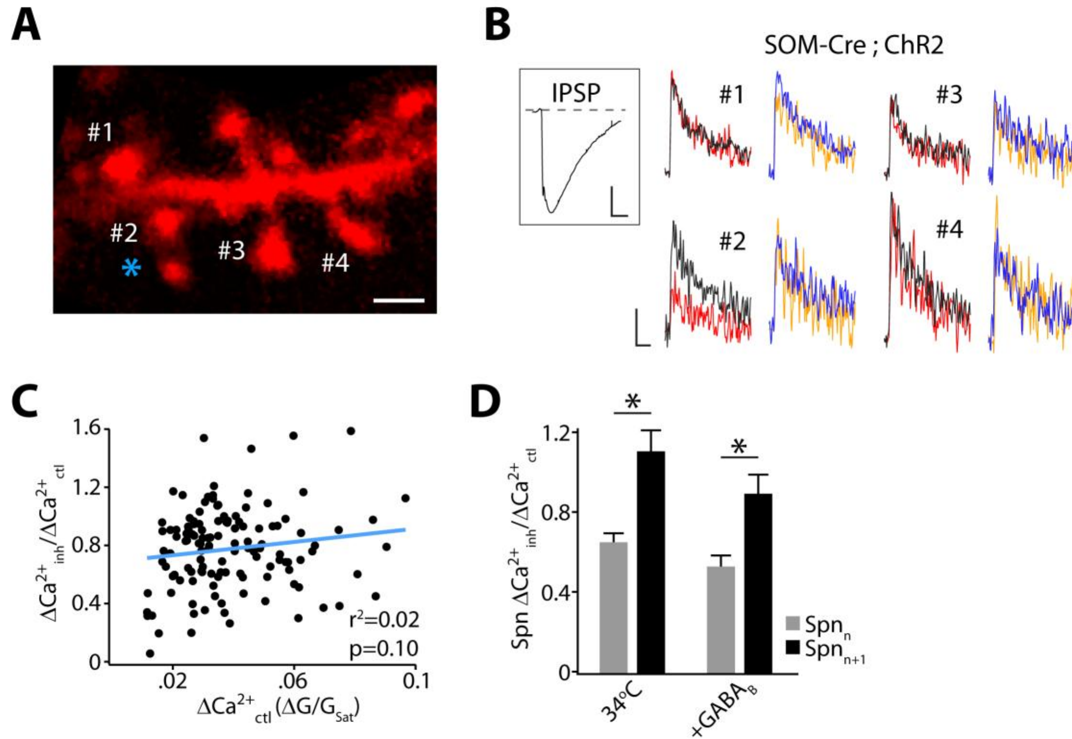

Fig. S5.

SOM-INs mediate compartmentalized inhibition of Ca(2+) signals in dendritic spines. (**A**) 2PLSM image of a dendritic segment used for inhibition mapping utilizing ChR2 stimulation of SOM-INs. Blue asterisk indicates light stimulus location. Scale bar: 1 µm. (**B**) ΔCa(2+) evoked by AP and IPSP-AP for spines (black and red, respectively) and dendritic shafts (blue and orange, respectively) indicated in (A). Scale bars: 2% $\Delta G/G_{sat}$, 50 ms. Inset: somatic IPSP. Scale bars: 2 mV, 100 ms. (**C**) Lack of correlation between the magnitude of Ca(2+) inhibition and the magnitude of the control ΔCa(2+). (**D**) Average magnitude of Ca(2+) inhibition for reference spine and adjacent neighbor observed at near-physiological temperature (34°C, n=5) and with $GABA_B$ receptors intact (n=7). * indicates significant difference (p<0.05, paired Student's t-test).

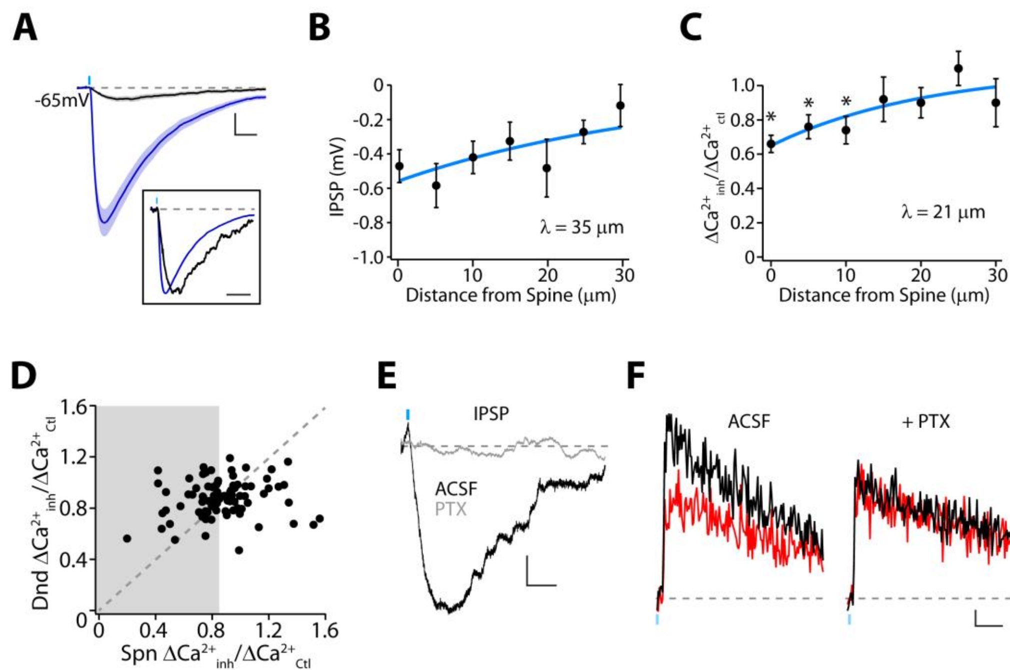

Fig. S6.

1-photon GABA uncaging evokes postsynaptic IPSPs and inhibits dendritic Ca(2+) signaling. (**A**) Average ChR2- (blue) and uncaging-evoked (black) IPSP (± SEM). Scale bars: 1 mV and 50 ms. Inset: average traces, normalized to illustrate similar kinetics. Scale bar: 100 ms. (**B**) Average (n=12) IPSP (± SEM) versus uncaging distance from the imaged spine head. Blue line indicates single exponential fit. (**C**) Average (n=12) Ca(2+) inhibition (± SEM) versus uncaging distance from the imaged spine head. Blue line indicates single exponential fit. * indicates less than 1.0 ($p<0.05$, paired Student's t-test). (**D**) Ca(2+) inhibition for dendritic shafts versus spines. Gray region indicates significant spine (Ca2+) inhibition. (**E**) Example uncaging-evoked IPSP before (black) and after (gray) application of picrotoxin. Scale bars: 0.25 mV, 50 ms. (**F**) Example spine ΔCa(2+) from experiment in (E) evoked by AP (black) and IPSP-AP (red) before and after application of picrotoxin. Scale bars: 2% $\Delta G/G_{sat}$, 50 ms.

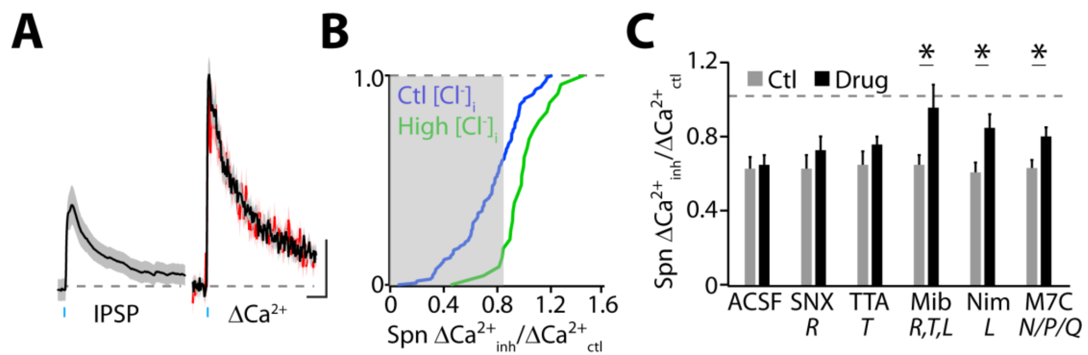

Fig. S7.

$GABA_A$ reversal potential and VGCC subtypes influence the magnitude of dendritic Ca(2+) inhibition. (**A**) Average (n=24) ChR2-evoked IPSP and ΔCa(2+) (± SEM) for AP (black) and IPSP-AP (red) obtained with high chloride internal solution. Scale bars: 2 mV, 5% $\Delta G/G_{sat}$, 50 ms. (**B**) Cumulative distribution of Ca(2+) inhibition in spines for control (blue) and high chloride (green) internal solutions. Gray region indicates significant difference from control. (**C**) Average (± SEM) Ca(2+) inhibition for control conditions (gray) and after flow-in of control ACSF (n=8) or VGCC blockers (black bars). SNX 482 (n=8); TTA-A2 (n=7); Mibefradil (n=10); Nimodipine (n=9); ω-conotoxin MVIIC (n=13). * indicates significant difference ($p<0.05$, paired Student's t-test).

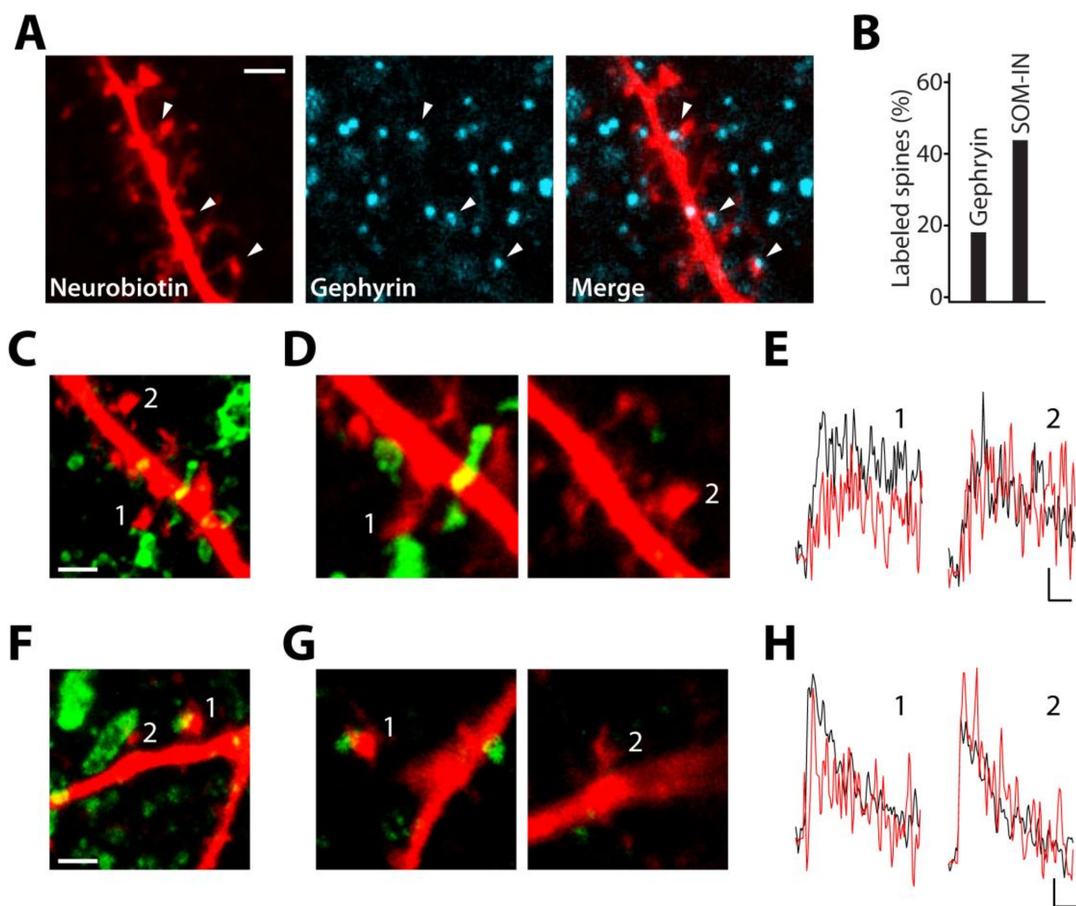

Fig. S8.

SOM-INs target dendritic spines. (**A**) Post-hoc confocal maximum projection of a dendrite (red, *left*), immunolabeled gephyrin puncta (cyan, *middle*), and merge (*right)*. Arrowheads indicate spines with putative GABAergic synapses. Scale bar: 1 μm. (**B**) Average percentage of spines positive for gephyrin or apposed to EYFP-positive SOM-IN boutons. (**C**) Maximum projection of dendrite (red) and putative SOM-IN terminals (green). Scale bar: 1 μm. (**D**) Single section images of spines indicated in (C). SOM-IN terminals are apposed to spines 1 and 2. (**E**) ΔCa(2+) measured in the spines from (D) for AP (black) and IPSP-AP (red). Scale bars: 1% $\Delta G/G_{sat}$, 50 ms. (**F-H**) As in (C-E) for another example dendrite.

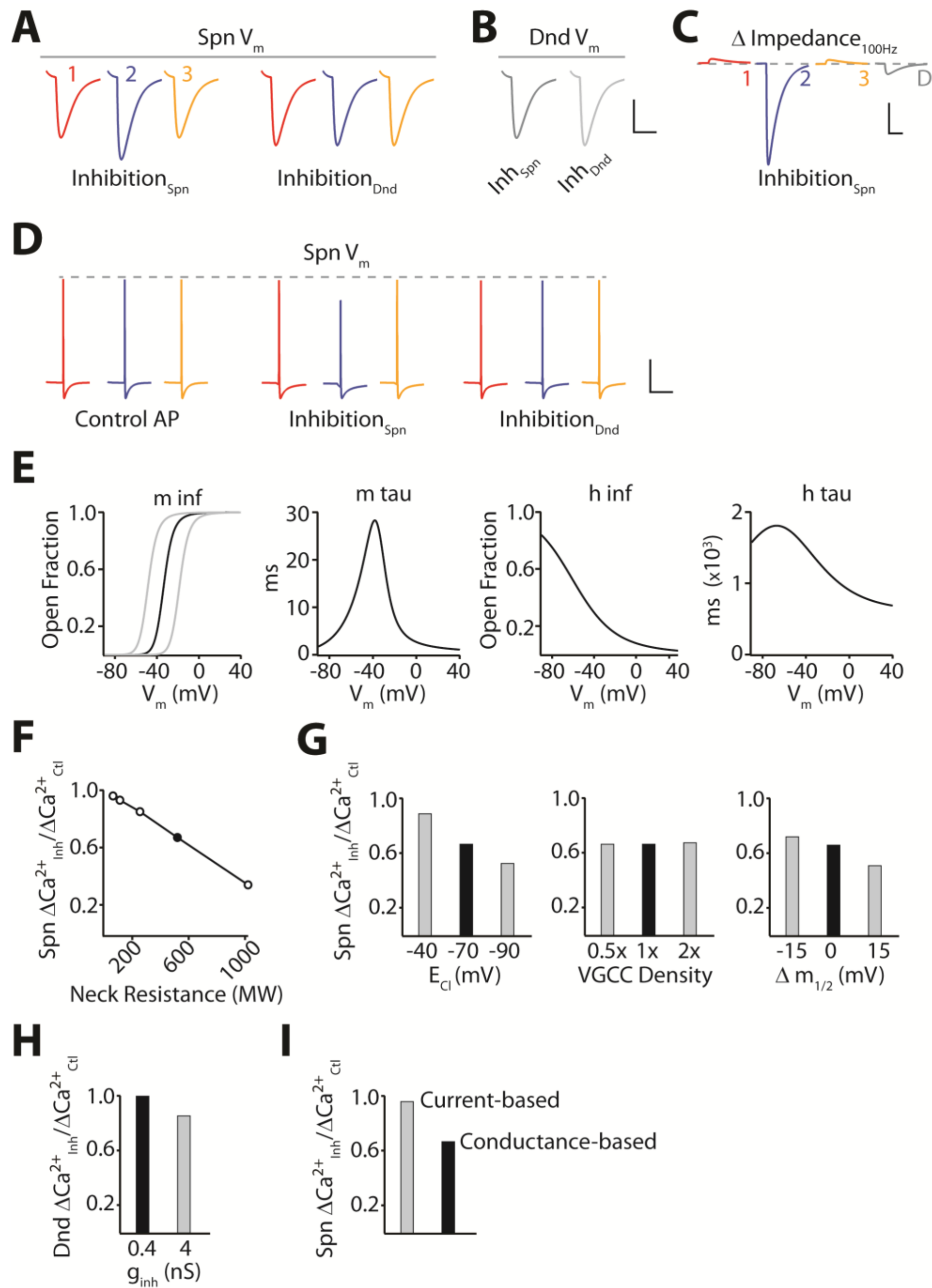

Fig. S9.

Computational modeling of GABAergic inhibition onto dendritic spines. (**A**) GABAergic synapse (0.4 nS) onto spine 2 (see Fig. 3H) evokes an IPSP in the targeted spine and smaller IPSPs in neighboring spines (V_m = -60 mV, E_{Cl} = -70 mV). GABAergic synapse onto the dendritic shaft evokes similar IPSPs in all spines. (**B**) GABAergic synapses onto either spine 2 or the dendritic shaft evoke similar IPSPs in the dendrite. Scale bars for A,B: 1 mV, 200 ms. (**C**) GABAergic synapse onto spine 2 evokes a large drop in impedance only in the targeted spine. Scale bars: 5% ΔImpedance,

200 ms. (**D**) Inhibition to spine #2 reduces the AP amplitude only in the targeted spine. Inhibition to the dendritic shaft does not alter the AP amplitude. Scale bars: 20 mV, 100 ms. (**E**) Voltage dependence of steady state open probability and gating kinetics for simulated high-threshold VGCCs. Channel opening ~ $m^2 \times h$. (**F**) Simulated Ca(2+) inhibition versus spine neck resistance. Default value (520 MΩ) shown by filled circle. (**G**) Relationship of simulated Ca(2+) inhibition to chloride reversal potential, VGCC density in the spine head, and voltage-dependence of channel opening (gray lines in (E)). Black bars indicate default values. (**H**) Simulated inhibition of dendritic Ca(2+) for default (black) and 10-fold increased (gray) GABAergic conductance placed on the dendritic shaft. (**I**) Simulated inhibition of spine Ca(2+) for current-based (gray) and default conductance-based (black) GABAergic synapse placed on the spine head.

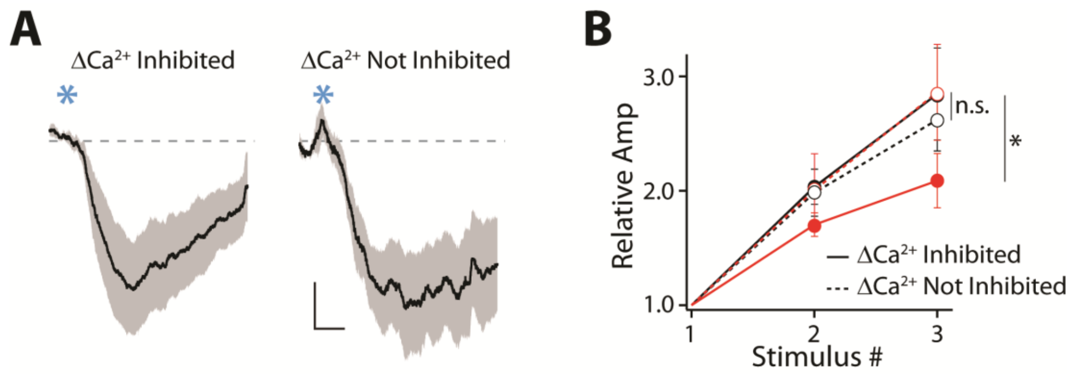

Fig. S10.

(**A**) Average voltage transients (± SEM) for IPSPs evoked by $1PLU_{GABA}$ for $2PLU_{Glu}$ summation experiments in which local spine ΔCa(2+) was either inhibited (*left*, n=11) or not inhibited (*right,* n=8) relative to control. Scale bars: 0.25 mV, 50 ms. (**B**) Relative summation for EPSPs (black) or IPSP-EPSPs (red), for experiments in which local spine ΔCa(2+) was inhibited (solid lines, data reproduced from Fig. 4H) or not inhibited (dashed lines). * indicates $p<0.05$ (Wilcoxon matched pairs test).