



Published in final edited form as:

Nat Rev Neurosci. 2014 September ; 15(9): 567–572. doi:10.1038/nrn3803.

Localized GABAergic inhibition of dendritic calcium signaling

Michael J. Higley

Program in Cellular Neuroscience, Neurodegeneration and Repair, Dept. of Neurobiology, Yale School of Medicine, New Haven, CT 06510

Abstract

Neuronal circuits are defined by synaptic connections between their cellular constituents. Here, I highlight a number of recent studies emphasizing the surprising level of precision exhibited by inhibitory GABAergic synapses within the neocortex and hippocampus. Specifically, GABAergic inputs to dendritic shafts and spines of pyramidal cells play a key role in the localized regulation of neuronal calcium signaling. These findings provide important new insights into the cellular mechanisms underlying the contributions of inhibitory transmission to both normal and abnormal brain activity.

Understanding the precision of neuronal connectivity is a major focus of current neuroscientific research¹. Although most of these efforts focus on excitatory glutamatergic circuits, there is a growing appreciation for the role of GABAergic inhibition in the regulation of cellular and network activity². Indeed, the balance between excitation and inhibition is critical for normal brain function, and dysregulation of this balance is implicated in a number of neuropsychiatric disorders, including schizophrenia and autism^{3,4}. Recent studies have broadened our understanding of the precision by which GABAergic interneurons (INs) innervate and regulate their target cells. That is, different types of INs form connections onto highly specific subregions of their target cell's somatodendritic arbor, enabling fine spatial control of postsynaptic activity. Here, I review several recent findings that support the hypothesis that, rather than serving as a simple brake on action potential output, GABAergic inhibition can sculpt neuronal activity at the subcellular level, exerting complex effects on both electrical and biochemical signaling.

Diversity of GABAergic inhibition in cortical and hippocampal circuits

Neuronal activity in the neocortex and hippocampus is shaped by the interplay of excitatory glutamatergic pyramidal neurons (PNs) and inhibitory GABAergic INs. PNs receive excitatory inputs onto small (~1 μm) membrane protrusions called dendritic spines, which serve to compartmentalize biochemical and electrical signals^{5,6}. Activation of AMPA- and NMDA-type glutamate receptors (AMPA- and NMDARs, respectively) causes membrane depolarization and local calcium (Ca^{2+}) influx⁷ (see Box 1) that contributes to the generation of somatic action potentials and influences long-term changes in synaptic strength. Dendritic integration of these excitatory signals is countered by the actions of GABAergic inhibition, although the subcellular targets and consequences of GABAergic signaling are less well understood.

GABAergic inhibition is mediated by two classes of receptors expressed ubiquitously throughout the nervous system (Fig. 1)⁸. Type-A receptors (GABA_ARs) are ionotropic channels, permeable to chloride and bicarbonate, that typically produce minimal direct change in membrane potential but generate a large conductance that shunts the impact of excitatory input-mediated depolarization (see Box 2). Type-B receptors (GABA_BRs) are G protein-coupled and lead to downregulation of cAMP production, activation of inwardly rectifying potassium channels that hyperpolarize the membrane potential, and inhibition of voltage-gated Ca²⁺ channels⁹. In the cortex and hippocampus, GABA is synthesized and released by inhibitory INs. A major challenge to understanding the contribution of GABAergic signaling to brain activity is the wide diversity of these cells. INs comprise approximately 20-30% of all cortical and hippocampal neurons and can be subdivided into numerous classes with distinct physiology, synaptic specializations, and molecular markers¹⁰⁻¹². Recent work suggests three principal groups: cells expressing (1) the Ca²⁺ binding protein parvalbumin, (2) the 5HT_{3a}-type serotonin receptor, or (3) the peptide transmitter somatostatin (SOM)¹¹, each of which plays a distinct role in local circuit function.

In the neocortex and hippocampus, parvalbumin-expressing basket cells and chandelier cells make strong inhibitory contacts onto the perisomatic regions of their target PNs, including the axon initial segment^{13,14} (Fig. 1). These powerful inputs exert well-documented control over the timing and magnitude of neuronal output. For example, feed-forward inhibition mediated by these INs rapidly truncates afferent excitation of the PN, limiting the temporal window during which action potentials can be generated¹⁵⁻¹⁷.

In contrast, INs expressing the 5HT_{3a} receptor were only recently described, and much less is known about their function. They are present throughout the cortex and hippocampus, particularly in more superficial layers^{18,19}. A subgroup of these neurons that co-expresses vasointestinal peptide (VIP) appears to predominantly contact other INs¹². Recent work suggests that VIP-expressing cells are specifically excited by long-range intracortical projections, such as those projecting from motor to somatosensory cortex²⁰ (Fig. 1). This long-range circuit may subserve top-down disinhibition of afferent responses, providing a mechanism for sensorimotor integration.

The third group, somatostatin-expressing INs, consists largely of cells whose pial-projecting axons form contacts along PN dendritic arbors, near the sites of glutamatergic inputs^{13,21,22} (Fig. 1). The most characteristic of these is the Martinotti cell, whose axon ramifies extensively in layer 1 of the neocortex, contacting the apical tufts of pyramidal neurons²². Similar cells exist in the hippocampus, where their cell bodies are located in stratum oriens and their projections target distal tufts of CA1 PNs in stratum lacunosum moleculare²³. Somatostatin-expressing INs receive facilitating feedback excitation from neighboring pyramidal cells, making them sensitive to local network activity^{24,25}.

GABAergic targeting of dendritic spines

Ultrastructural analyses of dye-injected neurons in the cat visual cortex revealed that the majority of GABAergic presynaptic contacts are formed onto dendrites, including dendritic

spines where they converge with individual glutamatergic afferents^{26,27} (Fig. 1). Immunogold labeling has also identified GABA receptors within dendritic shafts and spines²⁸⁻³⁰. However, the precise arrangement of postsynaptic densities and active zones of co-localized glutamatergic and GABAergic synapses remains largely unknown.

Until recently, there was little data on the density and distribution of GABAergic synapses along the length of individual dendritic branches. However, recent efforts utilizing 2-photon imaging have tracked the location of inhibitory synapses *in vivo*. In two independent studies, the authors expressed a fluorescently tagged version of gephyrin, a scaffolding protein unique to inhibitory synapses, in layer 2/3 pyramidal neurons of the mouse visual cortex^{31,32}. GABAergic inputs exhibited a density of approximately 0.2 synapses per micron, roughly half that of excitatory contacts estimated by counting dendritic spines³¹. Remarkably, of the imaged spines located within 125 microns of the soma, approximately 14% also bore a GABAergic synapse. This fraction was increased two-fold for more distal spines present in the most superficial cortical layers. Each dendritic spine receiving a GABAergic input also received a glutamatergic contact, indicating dual innervation³¹. Interestingly, spines receiving inhibitory synapses are targeted by glutamatergic inputs enriched for the thalamocortical synaptic marker Type 2 vesicular glutamate transporter (VGluT2)^{32,33}, suggesting that inhibition to dendritic spines may specifically regulate sensory information arising from ascending thalamic inputs. Both anatomical and physiological studies further indicate that GABAergic inputs to dendritic spines arise, at least in part, from somatostatin-expressing INs^{21,22}.

Somatostatin-expressing interneurons regulate dendritic spikes

As a consequence of their target specificity, a critical function of somatostatin-expressing INs appears to be the regulation of dendritic electrical activity in both the neocortex and hippocampus. Several decades of work have now shown that neuronal dendrites do not passively relay synaptic inputs to the cell body. Rather, an array of voltage-gated conductances shape the dynamics of synaptic integration across multiple subcellular compartments within the dendritic tree (recently reviewed in³⁴). Several studies have emphasized the electrogenic properties of distal apical dendrites of pyramidal neurons in both neocortical layer 5 and hippocampal CA1³⁵⁻⁴⁰. While sodium-based action potentials originate near the cell body in the axon initial segment, a second initiation zone for broad Ca²⁺-based action potentials called dendritic spikes occurs in these distal dendritic compartments, driven by the activity of voltage-gated calcium (Ca²⁺) channels (VGCCs) with important contributions from NMDARs. These dendritic spikes can be initiated by spatiotemporally convergent synaptic input and can spread to the soma to evoke bursts of spike output. Evidence from *in vivo* experiments suggest dendritic spikes shape receptive field properties in somatosensory cortex and participate in feedback control of neuronal activity by long-range intracortical projections³⁸⁻⁴⁰.

Early studies showed that the retrograde invasion of somatic action potentials into distal dendrites, as well as the generation of dendritic spikes, is under control of GABAergic inhibition^{35,41,42}. More recently, Murayama and colleagues found that the magnitude of dendritic spikes in the neocortex could encode the strength of a somatosensory stimulus in

both awake and anesthetized rats, thereby contributing to information representation³⁷. The slope of the relationship between sensory input and dendritic activity was strongly influenced by the activity of deep layer INs. By using paired recordings in brain slices, they showed that disynaptic inhibition between pyramidal neurons, potentially mediated by somatostatin-expressing Martinotti-type INs, could block initiation of dendritic spikes³⁷. A similar mechanism appears to regulate the input-output transformation of pyramidal neurons in the CA1 region of the hippocampus. Lovett-Barron and colleagues showed that dendritic inhibition mediated by somatostatin-expressing INs could gate dendritic spike generation and subsequent somatic burst firing, regulating the gain of cellular responsiveness to network activity⁴³. Together, these findings suggest that dendritic inhibition serves a specific functional role in cortical and hippocampal circuits.

Inhibition by GABA_ARs regulates Ca²⁺ signaling in dendritic spines

Until recently, most studies of inhibition have focused on the regulation of postsynaptic spiking, whether initiated in the dendrites or cell body. However, as noted above, GABAergic inputs often converge with individual excitatory synapses on dendritic spines. Synaptic excitation activates both glutamate receptors and voltage-gated Ca²⁺ channels, producing membrane depolarization and Ca²⁺ influx that should be sensitive to GABAergic inhibition. However, theoretical studies initially questioned whether dendritic spines are capable of supporting GABA_AR-mediated inhibition, as chloride influx into such a small compartment might rapidly diminish the inhibitory synaptic driving force⁴⁴. To address this question, we used cell-type specific expression of Channelrhodopsin2 in somatostatin-expressing INs of the mouse prefrontal cortex in combination with 2-photon Ca²⁺ imaging to monitor the consequences of dendritic inhibition²¹. We studied local Ca²⁺ influx mediated by either VGCCs (opened by retrogradely propagating action potentials) or by NMDARs (opened by synaptic stimulation) and found that activation of GABA_ARs following optical stimulation of somatostatin-expressing INs could selectively inhibit Ca²⁺ transients in single spines (Fig. 2)²¹. Similar inhibition of action potential-evoked Ca²⁺ signals was recently described for hippocampal neurons⁴⁵. Notably, the magnitude of Ca²⁺ inhibition was uncorrelated between adjacent spines, suggesting independent control of single excitatory inputs by GABAergic inhibition (Fig. 2)²¹. Interestingly, NMDAR-dependent summation of synchronous excitatory inputs to spines directly contacted by a GABAergic synapse was reduced, indicating that synaptic integration depends critically on the precise relationship of excitatory and inhibitory synapses in the dendritic arbor.

Pharmacological and computational studies showed that this local inhibition was mediated by a highly compartmentalized shunting conductance that reduces the membrane depolarization necessary for opening VGCCs and NMDARs²¹. Notably, the high electrical resistance of the spine neck isolates the shunt in one spine from its neighbors. This conclusion was supported by recent theoretical work from Gidon and Segev⁴⁶ who showed that shunting inhibition can spread across large-caliber dendrites but is restricted by high resistance structures such as fine dendritic branches and dendritic spine necks. Thus, the compartmentalization of GABA_AR-mediated inhibition within the dendritic arbor is heavily dependent on the structural elements innervated by presynaptic GABAergic INs.

GABA-B receptors also regulate dendritic Ca²⁺ signaling

Dendritic inhibition is not limited to ionotropic signaling, as two recent studies by Chalifoux and colleagues demonstrated that GABA_BRs regulate Ca²⁺ influx into dendritic spines by multiple parallel mechanisms^{47,48} (see Fig. 1). First, they showed that presynaptic GABA_BRs decrease glutamate release from presynaptic terminals in the mouse prefrontal cortex by reducing the number of vesicles released per action potential. Second, they found that postsynaptic GABA_BRs decrease Ca²⁺ influx through NMDARs into single dendritic spines. Activation of the GABA_BR and its associated G protein α_i subunit inhibits adenylate cyclase, leading to reduced cAMP production and downregulation of cAMP-dependent kinase (PKA). PKA-mediated phosphorylation of the NR2B subunit normally enhances Ca²⁺ permeability through the NMDAR^{49,50}, a process reversed by activation of GABA_BRs and other G α_i -coupled receptors^{47,51}. In a subsequent study, the authors showed that GABA_BRs directly inhibit spine Ca²⁺ influx through VGCCs via a PKA-independent mechanism that may involve direct channel modulation by the G protein $\beta\gamma$ subunit⁴⁸. In addition to direct modulation of dendritic Ca²⁺ sources, GABA_BRs can also produce hyperpolarizing inhibition of PNs via their activation of G protein-coupled inwardly rectifying potassium channels (GIRKs)⁵² that may deactivate voltage-dependent NMDARs and VGCCs. Thus, in combination with the above data on GABA_AR-mediated Ca²⁺ inhibition, these results suggest independent but parallel GABAergic control over electrical and biochemical signaling in dendritic spines.

GABAergic inhibition regulates synaptic plasticity

One clear hypothesis emerging from these findings is that GABAergic inhibition likely regulates the strength and direction of Ca²⁺-dependent synaptic plasticity. Indeed, multiple studies have suggested that long-term potentiation (LTP) of excitatory synaptic strength in both the cortex and hippocampus is modified by GABAergic activity⁵³⁻⁵⁶. In a recent report, when a LTP induction protocol that normally caused dendritic spine enlargement (a structural correlate of glutamatergic synaptic strengthening) was paired with local GABA uncaging, spine shrinkage corresponding to long-term depression occurred⁵⁵. This result appeared to be a consequence of a reduction in bulk Ca²⁺ mediated by GABAergic inhibition. Residual Ca²⁺ influx through NMDARs was still necessary for plasticity induction, indicating that dendritic inhibition does not “veto” individual synaptic contacts, but instead more subtly modulates transmission^{21,55}. Moreover, the effect of GABA uncaging on plasticity was limited to within 15 μm of the targeted spine, further demonstrating that functional consequences of inhibition are highly localized in the dendritic arbor⁵⁵.

Interactions between excitatory and inhibitory synapses also appear to influence plasticity *in vivo*. By analyzing the appearance and disappearance of dendritic spines and GFP-tagged GABAergic synapses, two recent studies estimated the stability of excitatory and inhibitory synapses. The dynamics of GABAergic inputs were found to depend on their location, with inhibitory spine contacts showing significantly greater turnover rates than those on dendritic shafts^{31,32}. Inhibitory synapses were also sensitive to sensory experience. Within the first four days of monocular visual deprivation, there was a dramatic loss of GABAergic contacts

to both dendritic shafts and spines that did not recover after re-opening the deprived eye³². Moreover, the turnover of excitatory and inhibitory synapses was spatially clustered, as dynamic (versus stable) excitatory and inhibitory inputs were likely to occur within 10 μm of each other, suggesting mechanistic links between excitatory and inhibitory plasticity³¹. This hypothesis is supported by a recent study showing that GABAergic synaptic plasticity in a single mouse visual cortex neuron modifies induction of glutamatergic plasticity in the same cell⁵⁶.

Conclusions and open questions

In recent years, a remarkable combination of methodological approaches has yielded data supporting a novel hypothesis for the role of GABAergic function in cortical circuits. The consequences of GABAergic transmission, like glutamatergic excitation, depend critically on the precise subcellular targeting of inhibitory synapses. Specifically, multiple studies demonstrate that GABAergic inhibition can be localized to small dendritic compartments, down to the level of individual dendritic spines. This localized inhibition has critical consequences for both electrical and biochemical (e.g., Ca^{2+}) activity in postsynaptic neurons, and may regulate both action potential generation and excitatory synaptic plasticity.

Importantly, these observations raise several questions regarding the mechanisms that control targeting of GABAergic synapses to pyramidal neuron dendrites. One possibility is that the recruitment of inhibitory contacts is activity dependent. Ca^{2+} influx through glutamate receptors is coupled to both potentiation and depression of GABAergic synapses depending on the relative activity of CaMKIIa and calcineurin^{57,58}. Thus, active excitatory inputs may specifically attract (or repel) an inhibitory bouton. A second possibility is that GABAergic inputs are recruited by the presence of specific glutamatergic afferents. As noted above, spines receiving a GABAergic synapse appear to be targeted by excitatory terminals expressing the thalamocortical synaptic marker VGlut2^{32,33}. The mechanisms underlying this convergent targeting remain unknown, though studies in the cerebellum have begun to uncover molecular signals, such as ankyrin and neurofascin, that govern the subcellular localization of GABAergic synapses⁵⁹.

Finally, the growing links between GABAergic dysfunction and neuropsychiatric disorders^{3,4} suggest that inhibitory control of Ca^{2+} may be a key factor in the maintenance of synaptic connections in the brain. Future studies must begin to address how alterations in GABAergic interneurons and inhibitory synapses may lead to widespread perturbations of neuronal circuits and behavioral deficits.

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Box 1**Sources of Dendritic Calcium**

Dendritic Ca²⁺ sources that are sensitive to GABAergic inhibition include two general categories: glutamate receptors and voltage-gated calcium (Ca²⁺) channels (VGCCs). The specific contributions made by each of these depend on the brain structure, cell class, and subcellular compartment⁷. NMDA-type glutamate receptors contribute a significant fraction of synaptic Ca²⁺ influx in pyramidal cells of the neocortex and hippocampus⁶⁰. The conductance of cations, including Ca²⁺, through NMDARs is strongly regulated by membrane potential due to pore blockade by extracellular magnesium ions.

Most non-NMDA-type glutamate receptors, including AMPA-type receptors (AMPA receptors), exhibit minimal Ca²⁺ permeability in pyramidal neurons. However, AMPARs lacking a GluA2 subunit are Ca²⁺ permeable and have been primarily described in GABAergic interneurons⁶¹. AMPARs also contribute to Ca²⁺ signaling by providing membrane depolarization that activates VGCCs and relieves magnesium block from NMDARs⁶².

Another significant contributor to dendritic Ca²⁺ signaling is the diverse group of VGCCs, which comprise a broad class of membrane channels with a wide variety of voltage-dependence, activation, and inactivation properties⁶³. VGCCs in dendrites and dendritic spines open following strong synaptic depolarization. Indeed, co-activation of many synapses can induce a dendritic spike, a VGCC-dependent regenerative event that causes widespread Ca²⁺ influx and can influence somatic spike generation³⁴. Sufficient depolarization for VGCC opening can also be provided by the antidromic propagation of somatically-generated action potentials through at least the proximal portions of the dendritic arbor³⁴.

Each of these sources is potentially influenced by GABAergic inhibition. First, membrane hyperpolarization (via activation of either GABA_A or GABA_B receptors) or shunting of synaptic depolarization (predominantly via GABA_ARs) reduces the open probability of both NMDARs and VGCCs^{21,41,42}. Second, GABA_BRs are coupled to biochemical signaling pathways that reduce Ca²⁺ influx through NMDARs and VGCCs^{47,48} (see main text).

Box 2**Hyperpolarizing Versus Shunting Inhibition**

An inhibitory synapse is one whose activity reduces the targeted cell's likelihood of crossing the threshold for action potential generation. Within this definition, two non-mutually exclusive modes of inhibition exist: hyperpolarization and shunting. Both types can be mediated by GABAergic signaling, but they produce distinct consequences for the postsynaptic cell.

Hyperpolarizing inhibition is relatively straightforward and is typified by the actions of GABA_BRs. Activation of GABA_BRs leads to release of the βγ subunit from the receptor's associated G protein. The βγ subunit then triggers the opening G protein-coupled inwardly rectifying potassium channels (GIRKs). The direction of current flow through any open channel is determined by the relationship between the cell's resting membrane potential (V_m) and the reversal potential of the channel (V_{rev}) as defined by the Goldman-Hodgkin-Katz equation:

$$V_{Rev} = \frac{RT}{F} \ln \left(\frac{\sum_i^N P_{C_i^+} [C_i^+]_{out} + \sum_j^M P_{A_j^-} [A_j^-]_{in}}{\sum_i^N P_{C_i^+} [C_i^+]_{in} + \sum_j^M P_{A_j^-} [A_j^-]_{out}} \right)$$

where R is the ideal gas constant, T is temperature, F is Faraday's constant, $[C^+]$ and $[A^-]$ are the concentrations of cationic and anionic species and P_C and P_A are their permeabilities, respectively. Synaptic current is then given by the equation:

$$I_{Syn} = G_{Syn} \times (V_M - V_{Rev})$$

where I_{Syn} is the current through the channel and G_{Syn} is the channel conductance. In the case of potassium, V_{Rev} in pyramidal neurons is approximately -90 mV and is more negative than the resting potential of approximately -70 mV, leading to a positive (outward) synaptic current. Thus, upon GIRK channel opening, potassium flows out of the cell, hyperpolarizing the membrane potential. As this moves V_m further from spike threshold (~-45 mV), the result is to inhibit the cell's activity.

Shunting inhibition is somewhat more complex, and is typified by inhibition mediated by GABA_ARs. GABA_ARs are primarily permeable to the anions chloride and bicarbonate, and exhibit a reversal potential of approximately -70 mV, very close to the cell's resting potential. Thus, upon GABA binding and channel opening, there is very little net current across the membrane ($I_{Syn} \approx 0$). However, the GABA_AR-mediated synaptic conductance is quite large, leading to an increase in the overall conductance of the cell's membrane during the time that the channels are open. Imagine another excitatory synaptic channel (e.g., an AMPA-type glutamate receptor) opening at the same time. Inward current through the glutamate receptor would typically depolarize the cell's membrane potential. However, this depolarizing current is short-circuited by the GABAergic conductance

rather than charging the membrane. Thus, without changing the resting membrane potential, the GABA_AR-mediated shunt has made it less likely that the cell will fire an action potential in response to excitatory input.

The temporal dynamics of these two forms of inhibition are also distinct. In the case of hyperpolarizing inhibition, capacitive filtering of synaptic currents by the cell produces a change in the membrane potential that outlasts the underlying conductance. In contrast, shunting inhibition only occurs for the duration of the inhibitory conductance.

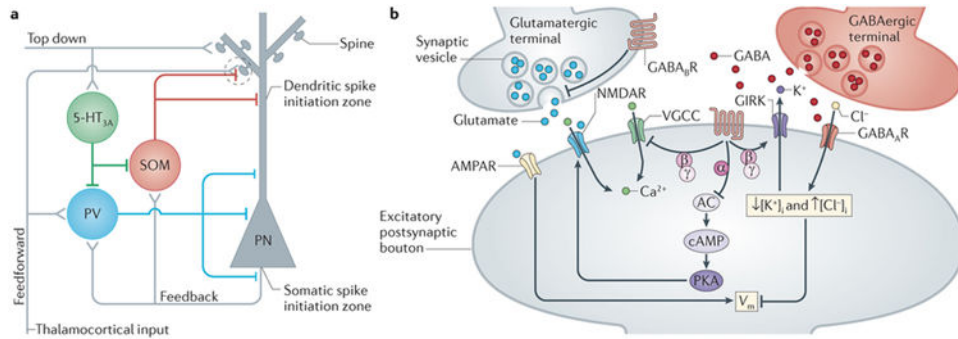


Figure 1. GABAergic interneurons and the targets of inhibition in cortical circuits

A, Schematic illustration of the three major inhibitory circuits in the neocortex. Excitatory (+) and inhibitory (-) synapses are shown. Perisomatic-targeting interneurons that express parvalbumin (PV) are activated by feedforward and feedback excitation and sharply curtail generation of somatic action potentials in response to afferent inputs. Dendrite-targeting interneurons that express somatostatin (SOM) are strongly engaged by feedback excitation originating from local cortical pyramidal neurons. They form synapses on both dendritic shafts and spines that converge with excitatory inputs (dashed circle) to regulate synaptic integration and dendritic spike initiation. Interneurons expressing the serotonergic 5HT_{3a} receptor target other interneurons and receive excitatory inputs from top-down intracortical projections. B, Schematic of the local actions of GABAergic inhibition in dendritic spines receiving both excitatory and inhibitory inputs. Ionotropic GABA_A receptors and metabotropic GABA_B receptors influence both spine Ca²⁺ signals and membrane potential (V_m) through regulation of glutamate receptors (AMPA and NMDARs), voltage-gated Ca²⁺ channels (VGCCs), and G protein-coupled potassium channels (GIRKs). Other abbreviations: AC (adenylate cyclase), cAMP (cyclic adenosine monophosphate), Cl⁻ (chloride), K⁺ (potassium), PKA (cAMP-sensitive protein kinase), V_m (membrane potential).

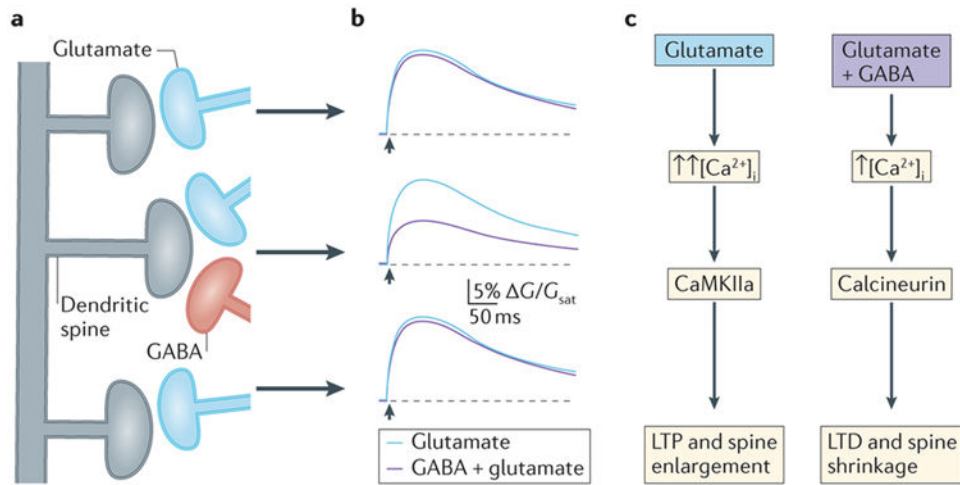


Figure 2. Inhibition regulates dendritic spine Ca^{2+} and synaptic integration

A, Inhibition of responses evoked by 2-photon uncaging of glutamate (yellow arrowheads) using one-photon GABA uncaging (large blue circle). Scale bar, 1 μ m. B, Ca^{2+} transients and excitatory postsynaptic potentials (EPSP) for glutamatergic responses alone (black) or paired with preceding GABAergic stimulation (red), for spines indicated in (A). Scale bars, 4% F/F_{max} , 50 ms (top) or 0.1 mV, 5 ms (bottom). C, Integration of responses evoked by 2-photon glutamate uncaging (yellow arrowheads). Scale bar, 1 μ m. D, Average EPSPs (\pm SEM) for glutamatergic response alone (black) and when paired with preceding inhibition (red) evoked on three neighboring spines, recorded in control saline or with NMDARs blocked by CPP. Scale bars, 0.25 mV, 20 ms. E, Relative summation of responses recorded in control saline or with CPP. * $P < 0.05$ (Wilcoxon matched pairs test); n.s., not significant. Modified with permission from ²¹.