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Localized GABAergic inhibition of dendritic Ca^{2+} signalling

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Abstract | Neuronal circuits are defined by synaptic connections between their cellular constituents. In this article, I highlight several 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 have a key role in the localized regulation of neuronal Ca^{2+} signalling. 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 research[1]. 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 activity[2]. Indeed, the balance between excitation and inhibition is critical for normal brain function, and dysregulation of this balance is implicated in several neuropsychiatric disorders, including schizophrenia and autism[3,4]. Recent studies have broadened our understanding of the precision by which GABAergic interneurons innervate and regulate their target cells. That is, different types of interneurons 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. By exerting complex effects on both electrical and biochemical signalling, dendritic inhibition has important implications for synaptic plasticity.

Diversity of GABAergic inhibition

Neuronal activity in the neocortex and hippocampus is shaped by the interplay between excitatory glutamatergic pyramidal neurons and inhibitory GABAergic interneurons. Pyramidal neurons receive excitatory inputs onto small (~1 micrometre) membrane protrusions called dendritic spines, which function to compartmentalize biochemical and electrical signals[5,6]. Activation of AMPA-type glutamate receptors (AMPARs) and NMDA-type glutamate receptors (NMDARs) causes membrane depolarization and local Ca^{2+} influx[7] (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 signalling are less well understood.

GABAergic inhibition is mediated by two classes of receptors that are expressed ubiquitously throughout the nervous system[8] (FIG. 1). Type A GABA receptors ($GABA_A$Rs) are ionotropic channels (which are permeable to Cl^- 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 (BOX 2). $GABA_B$Rs are G protein-coupled receptors and activation leads to downregulation of cyclic AMP production, activation of inwardly rectifying K^+ channels that hyperpolarize the membrane potential and inhibition of voltage-gated Ca^{2+} channels (VGCCs)[9]. In the cortex and hippocampus, GABA is synthesized and released by inhibitory interneurons. A major challenge in understanding the contribution of GABAergic signalling to brain activity is the wide diversity of these cells. Interneurons are comprised of approximately 20–30% of all cortical and hippocampal neurons and can be subdivided into numerous classes with distinct physiology, synaptic specializations and molecular markers[10–12]. Recent research suggests that there are three principal groups of interneurons: first, cells expressing the Ca^{2+}-binding protein parvalbumin; second, cells expressing 5-hydroxytryptamine receptor 3A ($5\text{-}HT_{3A}$); and third, cells expressing the peptide transmitter somatostatin[11]. Each group has 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 pyramidal neurons, 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, feedforward inhibition mediated by these interneurons rapidly truncates afferent excitation of the pyramidal neuron, limiting the temporal window during which action potentials can be generated[15–17].

By contrast, interneurons expressing $5\text{-}HT_{3A}$ have only recently been 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-express vasointestinal peptide (VIP) seem to predominantly contact other interneurons[12]. Recent work suggests that VIP-expressing cells are specifically excited by long-range intracortical projections, such as those projecting from the motor to somatosensory cortex[20] (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 interneurons, largely consists of cells with pial-projecting axons that form contacts along pyramidal neuron dendritic

Box 1 | **Sources of dendritic Ca^{2+}**

Dendritic Ca^{2+} sources that are sensitive to GABAergic inhibition include two general categories: glutamate receptors and voltage-gated Ca^{2+} channels (VGCCs). The specific contributions made by each of these depend on the brain structure, cell class and subcellular compartment[7]. NMDA-type glutamate receptors (NMDARs) contribute a substantial fraction of synaptic Ca^{2+} influx to pyramidal cells of the neocortex and hippocampus[60]. The conductance of cations, including Ca^{2+}, through NMDARs is strongly regulated by membrane potential owing to pore blockade by extracellular Mg^{2+}.

Most non-NMDA-type glutamate receptors, including AMPA-type receptors (AMPARs), exhibit minimal Ca^{2+} permeability in pyramidal neurons. However, AMPARs lacking a GluA2 subunit are permeable to Ca^{2+} and have been primarily described in GABAergic interneurons[61]. AMPARs also contribute to Ca^{2+} signalling by depolarizing the membrane, which activates VGCCs and relieves Mg^{2+} block from NMDARs[62].

Another important contributor to dendritic Ca^{2+} signalling is the diverse group of VGCCs, which comprises a broad class of membrane channels with a wide range of voltage-dependence, activation and inactivation properties[63]. VGCCs in dendrites and dendritic spines open in response to strong synaptic depolarization. Indeed, co-activation of many synapses can induce a dendritic spike, a VGCC-dependent regenerative event that causes widespread Ca^{2+} influx and can influence somatic spike generation[34]. 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 arbor[34].

Each of these sources is potentially influenced by GABAergic inhibition. First, membrane hyperpolarization (via activation of either type A GABA receptors ($GABA_A$Rs) or type B GABA receptors ($GABA_B$Rs)) or shunting of synaptic depolarization (predominantly via $GABA_A$Rs) reduces the open probability of both NMDARs and VGCCs[21,41,42]. Second, $GABA_B$Rs are coupled to biochemical signalling pathways that reduce Ca^{2+} influx through NMDARs and VGCCs[47,48] (see main text).

arbors near the sites of glutamatergic inputs[13,21,22] (FIG. 1). The most characteristic of these is the Martinotti cell, which has an axon that ramifies extensively in layer 1 of the neocortex, contacting the apical tufts of pyramidal neurons[22]. Similar cells exist in the hippocampus, where their cell bodies are located in stratum oriens and their projections target distal tufts of CA1 pyramidal neurons in stratum lacunosum moleculare[23]. Notably, somatostatin-expressing interneurons receive facilitating feedback excitation from neighbouring pyramidal cells, which renders them sensitive to local network activity[24,25]. In summary, the existence of such diverse populations of GABAergic interneurons suggests that distinct functional roles for various forms of inhibition may be determined, in part, by the precise postsynaptic targeting of inhibitory synaptic contacts.

GABAergic targeting of dendritic spines

Seminal anatomical studies revealed that the majority of GABAergic presynaptic axon terminals form contacts on dendrites, including dendritic spines, where they converge with individual glutamatergic afferents[26,27] (FIG. 1). GABARs have also been identified within dendritic shafts and spines[28–30], but the precise arrangement of postsynaptic densities and active zones of co-localized glutamatergic and GABAergic synapses remains largely unknown.

Until recently, little was known about the density and distribution of GABAergic synapses along the length of individual dendritic branches. However, recent efforts using two-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 an average density of approximately 0.2 synapses per micrometre, which is roughly half that of excitatory contacts (estimated by counting dendritic spines)[31]. However, the distribution of synapses in the dendritic arbor was not uniform. Approximately 14% of spines located within 125 micrometres of the soma bore a GABAergic synapse, but this proportion was twofold higher at more distal spines in the most superficial cortical layers. Each dendritic spine receiving a GABAergic input also received a glutamatergic contact, indicating dual innervation that is consistent with a functional role of inhibition in the regulation of excitatory transmission[31]. Interestingly, dually innervated spines are targeted by glutamatergic terminals enriched with vesicular glutamate transporter 2 (VGluT2), a protein thought to be specific for thalamocortical rather than corticocortical synapses[32,33]. This finding suggests that inhibition of these spines may specifically regulate sensory information that is transmitted by ascending thalamic inputs. Both anatomical and physiological studies further indicate that at least some GABAergic inputs to dendritic spines originate from somatostatin-expressing interneurons[21,22]. This observation suggests that these interneurons are in a key position to influence postsynaptic activity by directly controlling excitatory transmission and dendritic signalling.

Inhibitory regulation of dendritic spikes

The transformation of input to output in single neurons is governed by the summation of synaptic potentials and the subsequent generation of somatic action potentials, a process now known to be heavily influenced by GABAergic inhibition. 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 in REF. 34). Indeed, several studies have shown that distal apical dendrites of pyramidal neurons in both neocortical layer 5 and hippocampal CA1 have electrogenic properties that can be influenced by inhibitory transmission[35–40]. For example, whereas Na^+-based action potentials originate near the cell body in the axon initial segment, a second initiation zone for broad Ca^{2+}-based action potentials called dendritic spikes is located in these distal dendritic compartments. Dendritic spikes are driven by the activity of 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.

Early studies showed that the retrograde invasion of somatic action potentials into distal dendrites, as well as the generation of dendritic spikes, is under the control of GABAergic inhibition[35,41,42]. More recently, Murayama and colleagues[37] found that the magnitude of dendritic spikes in the neocortex could encode the strength of a somatosensory stimulus in both awake and anaesthetized rats, thereby contributing to information representation. The slope of the relationship between sensory input and dendritic activity was strongly influenced by the activity of deep-layer interneurons. By using paired recordings in brain slices, they showed that disynaptic inhibition between pyramidal neurons, which was potentially mediated by somatostatin-expressing Martinotti-type interneurons[24,25], could block the

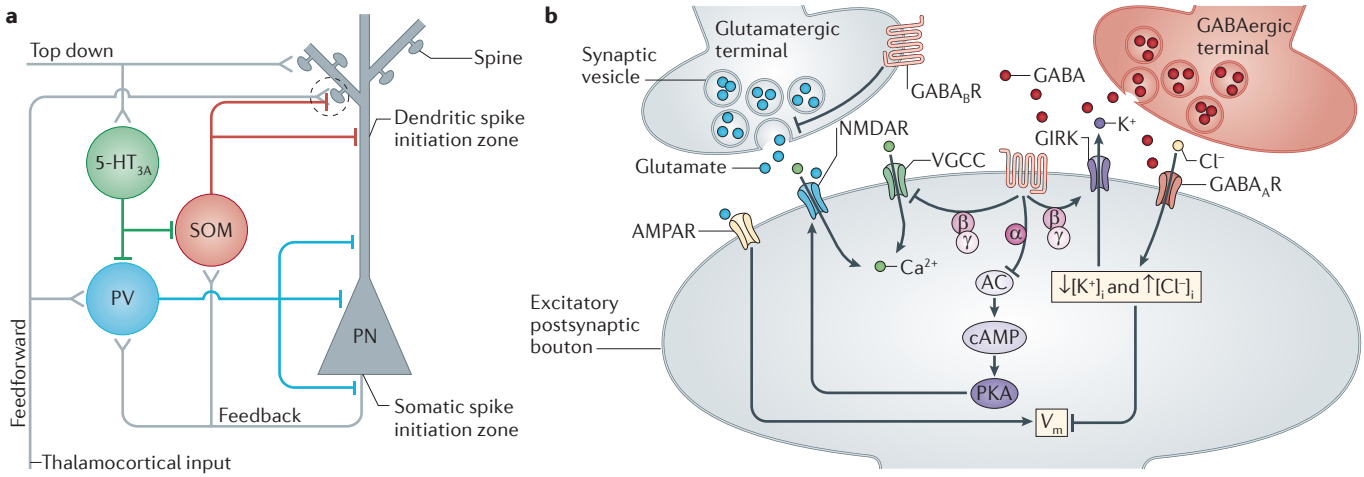

Figure 1 | **GABAergic interneurons and the targets of inhibition in cortical circuits. a** | Schematic illustration of the three major inhibitory circuits in the neocortex. Excitatory and inhibitory synapses are shown. Perisomatic-targeting interneurons that express parvalbumin (PV) are activated by feedforward and feedback excitation and sharply curtail the 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 (PNs). 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 5-hydroxytryptamine receptor 3A (5-HT_{3A}) 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 (dashed circle in part **a**). Ionotropic type A GABA receptors ($GABA_A$Rs) and metabotropic $GABA_B$Rs influence both spine Ca^{2+} signals and membrane potential (V_m) through regulation of glutamate receptors (AMPA receptors (AMPARs) and NMDA receptors (NMDARs)), voltage-gated Ca^{2+} channels (VGCCs) and G protein-coupled inwardly rectifying K^+ channels (GIRKs). AC, adenylyl cyclase; cAMP, cyclic AMP; PKA, protein kinase A; $[K^+]_i$, intracellular K^+ concentration.

initiation of dendritic spikes[37]. A similar mechanism seems to regulate the input–output transformation of pyramidal neurons in the CA1 region of the hippocampus. Lovett-Barron and colleagues[43] showed that dendritic inhibition mediated by somatostatin-expressing interneurons could gate dendritic spike generation and subsequent somatic burst firing, regulating the gain of cellular responsiveness to network activity. Together, these findings suggest that dendritic inhibition has a specific functional role in cortical and hippocampal circuits.

$GABA_A$Rs regulate dendritic spine Ca^{2+}

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, presynaptic GABAergic inputs often converge with individual excitatory terminals on dendritic spines. Synaptic excitation activates both glutamate receptors and VGCCs, producing membrane depolarization and Ca^{2+} influx that can be regulated by GABAergic inhibition. However, theoretical studies initially questioned whether dendritic spines are capable of supporting $GABA_A$R-mediated inhibition, as Cl^- influx into such a small compartment might rapidly diminish the inhibitory synaptic driving force[44]. However, in the mouse prefrontal cortex, stimulation of somatostatin-expressing interneurons and the resulting activation of $GABA_A$Rs were found to selectively inhibit VGCC- and NMDAR-mediated Ca^{2+} transients in single spines[21] (FIG. 2). Similar inhibition of action potential-evoked Ca^{2+} signals was recently described for hippocampal neurons[45]. Notably, the magnitude of Ca^{2+} inhibition was uncorrelated between adjacent spines, suggesting that GABAergic inhibition independently controls single excitatory inputs[21] (FIG. 2). Interestingly, NMDAR-dependent summation of synchronous excitatory inputs to spines that are directly contacted by a GABAergic synapse was reduced, indicating that synaptic integration crucially depends on the precise relationship between 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 NMDARs[21]. Notably, the high electrical resistance of the spine neck isolates the shunt in one spine from its neighbours. This conclusion was supported by recent theoretical work from Gidon and Segev[46], who showed that shunting inhibition can spread across large-calibre dendrites but is restricted by high-resistance structures such as fine dendritic branches and dendritic spine necks. Thus, the degree of compartmentalization of $GABA_A$R-mediated inhibition within the dendritic arbor is heavily dependent on the structural elements (for example, spines or dendrites) that are innervated by presynaptic GABAergic interneurons.

Modulation of dendritic Ca^{2+} by $GABA_B$Rs

Dendritic inhibition is not limited to ionotropic signalling, as two recent studies by Chalifoux and colleagues[47,48] demonstrated that $GABA_B$Rs regulate Ca^{2+} influx into dendritic spines by multiple parallel mechanisms (FIG. 1). First, they showed that presynaptic $GABA_B$Rs 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 $GABA_B$Rs decrease Ca^{2+} influx through NMDARs into single dendritic spines. Activation of the $GABA_B$R and its associated G protein α_i subunit inhibits adenylyl cyclase, leading to reduced cAMP production and downregulation of the cAMP-dependent kinase protein kinase A (PKA). PKA-mediated phosphorylation of the NR2B (also known as GluN2B) subunit normally enhances Ca^{2+} permeability through the NMDAR[49,50], a process that is reversed by the activation of $GABA_B$Rs

Box 2 | **Hyperpolarizing versus shunting inhibition**

An inhibitory synapse reduces the targeted cell's likelihood of generating action potentials. Two non-mutually exclusive modes of inhibition exist: hyperpolarization and shunting. Hyperpolarizing inhibition is typified by the actions of type B GABA receptors ($GABA_B$Rs), whose activation leads to the release of the βγ subunit from the receptor's associated G protein and subsequent opening of G protein-coupled inwardly rectifying K^+ 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:

$$V_{rev} = \frac{RT}{F} ln \left(\frac{\Sigma_i^N P_{C_i^+} [C_i^+]_{out} + \Sigma_j^M P_{A_j^-} [A_j^-]_{in}}{\Sigma_i^N P_{C_i^+} [C_i^+]_{in} + \Sigma_j^M P_{A_j^-} [A_j^-]_{out}} \right) \quad (1)$$

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 (I_{syn}) is then given by the equation:

$$I_{syn} = G_{syn} \times (V_m - V_{rev}) \quad (2)$$

where G_{syn} is the synaptic conductance. In the case of GIRKs, V_{rev} (~−90 mV) is more negative than the resting potential (~−70 mV). Upon opening, K^+ flows out of the cell, hyperpolarizing the membrane potential away from the spike threshold (~−45 mV).

Shunting inhibition is typified by the actions of $GABA_A$Rs, which are permeable to Cl^- and bicarbonate and have a V_{rev} of ~−70 mV, close to the cell's resting potential. Upon channel opening, there is little net current across the membrane ($I_{syn} \approx 0$). However, the $GABA_A$R-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, short-circuiting other synaptic inputs. Thus, without changing the resting membrane potential, the $GABA_A$R-mediated shunt has made it less likely that the cell will fire an action potential in response to excitatory input.

and other $G\alpha_i$-coupled receptors[47,51]. In a subsequent study, the authors showed that $GABA_B$Rs directly inhibit spine Ca^{2+} influx through VGCCs through a PKA-independent mechanism that may involve direct channel modulation by the G protein βγ subunit[48]. In addition to direct modulation of dendritic Ca^{2+} sources, $GABA_B$Rs can also produce hyperpolarizing inhibition of pyramidal neurons through their activation of G protein-coupled inwardly rectifying K^+ channels[52] that may deactivate voltage-dependent NMDARs and VGCCs. Thus, in combination with the above data on $GABA_A$R-mediated Ca^{2+} inhibition, these results suggest independent but parallel GABAergic control over electrical and biochemical signalling in dendritic spines.

Inhibition regulates synaptic plasticity

One clear hypothesis emerging from these findings is that GABAergic inhibition probably regulates the strength and direction of Ca^{2+}-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 activity[53–56]. In a recent report, when an 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 occurred[55] (FIG. 2). This result seemed to be a consequence of activating $GABA_A$Rs and a subsequent reduction in postsynaptic Ca^{2+} influx. Residual Ca^{2+} influx through NMDARs was still necessary for the induction of plasticity, indicating that dendritic inhibition does not 'veto' individual synaptic contacts but instead more subtly modulates transmission by adjusting overall Ca^{2+} levels[21,55]. Indeed, reduction (but not elimination) of synaptic Ca^{2+} by inhibition seemed to bias intra-spine biochemical signalling away from activation of Ca^{2+}/calmodulin-dependent protein kinase IIa (CaMKIIa), which is linked to spine enlargement, towards activation of the phosphatase calcineurin, which is linked to spine shrinkage[55] (FIG. 2c). Notably, the effect of GABA uncaging on plasticity was limited to within 15 micrometres of the targeted spine, corresponding to ~5–10 additional spines and further demonstrating that functional consequences of inhibition are highly localized in the dendritic arbor[55].

Interactions between excitatory and inhibitory synapses also seem to influence plasticity *in vivo*. By analysing the appearance and disappearance of dendritic spines and green fluorescent protein-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. Monocular visual deprivation is often used to investigate the effect of sensory input on the development of visual circuits. Four days of monocular visual deprivation was sufficient to induce a dramatic loss of GABAergic contacts on both dendritic shafts and spines that did not recover after re-opening the deprived eye[32]. 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 micrometres of each other, suggesting that there are mechanistic links between excitatory and inhibitory plasticity[31]. This hypothesis is supported by a recent study showing that GABAergic synaptic plasticity in a single mouse visual cortex neuron modifies the induction of glutamatergic plasticity in the same cell[56].

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, crucially depend 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 (for example, 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 dependent on activity. Ca^{2+} influx through glutamate receptors is coupled to both the 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

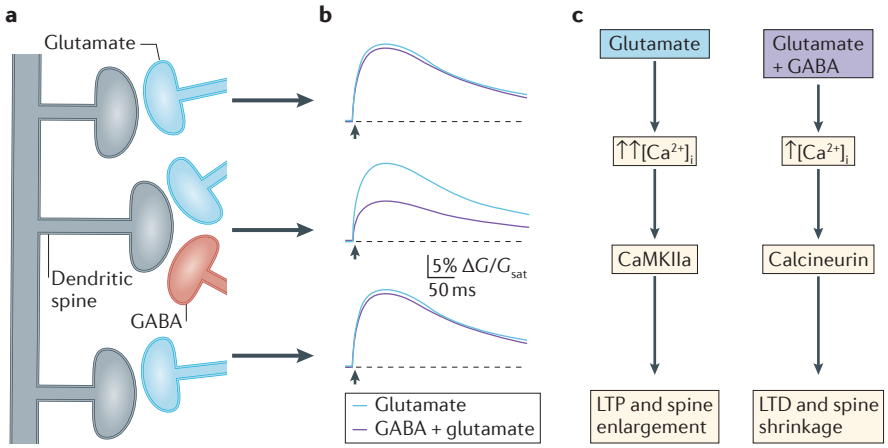

Figure 2 | **Inhibition regulates dendritic spine Ca^{2+} signalling and synaptic plasticity.** **a** | Schematic illustration of inhibitory control of dendritic spine Ca^{2+} signalling. Spines receive either glutamatergic or both glutamatergic and GABAergic inputs. **b** | Simulated Ca^{2+} transients from the indicated spines in response to glutamatergic input alone (blue traces) or paired glutamatergic and GABAergic input (purple traces). Inhibition only reduces ΔCa^{2+} for the spine directly targeted by the GABAergic synapse[21]. **c** | Glutamatergic excitation can produce a large Ca^{2+} influx into single spines, leading to the activation of Ca^{2+}/calmodulin-dependent protein kinase IIa (CaMKIIa), which in turn contributes to long-term potentiation (LTP). Pairing glutamatergic excitation with GABAergic inhibition produces lower bulk Ca^{2+} influx, leading to the activation of the phosphatase calcineurin and subsequent long-term synaptic depression (LTD)[55]. $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration.

a GABAergic synapse seem to be targeted by excitatory terminals expressing the thalamocortical synaptic marker VGluT2 (REFS 32,33). The mechanisms underlying this convergent targeting remain unknown, although studies in the cerebellum have begun to uncover molecular signals, such as ankyrin and neurofascin, that govern the subcellular localization of GABAergic synapses[59].

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 behavioural deficits.

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Competing interests statement
The author declares no competing interests.