Preserving the balance: diverse forms of long-term GABAergic synaptic plasticity

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Abstract | Cellular mechanisms that regulate the interplay of synaptic excitation and inhibition are thought to be central to the functional stability of healthy neuronal circuits. A growing body of literature demonstrates the capacity for inhibitory GABAergic synapses to exhibit long-term plasticity in response to changes in neuronal activity. Here, we review this expanding field of research, focusing on the diversity of mechanisms that link glutamatergic signalling, postsynaptic action potentials and inhibitory synaptic strength. Several lines of evidence indicate that multiple, parallel forms of plasticity serve to regulate activity at both the input and output domains of individual neurons. Overall, these varied phenomena serve to promote both stability and flexibility over the life of the organism.

The ability of an organism to successfully interact with its environment requires that the nervous system exhibit sufficient functional malleability to adapt to changing contingencies. Learning, in various forms, is thought to rely on the plasticity of chemical synapses formed between neurons. Studies of synaptic plasticity have largely focused on changes to excitatory glutamatergic connections, which exhibit a wide range of modifications over many timescales, including both long-term depression and potentiation[1,2]. By contrast, considerably less is known about the plasticity of inhibitory GABAergic synapses in the mammalian brain. However, a growing body of literature demonstrates that these connections also exhibit varying forms of long-term plasticity that may play critical roles in establishing the proper dynamic operating range of neural circuits, particularly in areas such as the neocortex, hippocampus and cerebellum[3–5].

Excitation and inhibition are often said to be in balance with each other, allowing for both directionally and recurrently wired networks that promote information processing while preventing runaway activity[6]. Moreover, disruption of this balance has been linked to a number of neuropsychiatric disorders, including schizophrenia, autism and epilepsy[6–8]. However, the lack of precise definitions for balance presents challenges to fully appreciating the interactions between glutamatergic and GABAergic signalling. For example, over short timescales (for example, seconds), the average excitatory and inhibitory inputs received by a neuron are approximately but not exactly equal, driving fast fluctuations in the membrane potential that allow precisely timed generation of action potentials[9–12]. Over longer timescales (for example, hours to days), excitation and inhibition appear to track each other, with alterations in the magnitude of one leading to similar alterations in the magnitude of the other, potentially leading to stable modification of the average firing rate of a neuron[13,14]. This latter view suggests a level of homeostasis, whereby cellular mechanisms exist to regulate the interplay of excitation, inhibition and neuronal firing[13]. Together, these perspectives suggest that slower mechanisms of synaptic plasticity may provide the fine tuning necessary for preservation of fast network dynamics across varying environmental constraints. In addition to the temporal relationship between excitation and inhibition, variable spatial distributions along the somatodendritic axis of individual neurons may also play a key role in shaping cellular signalling[15–17].

In the present Review, we focus on the long-term modification of inhibitory GABAergic synapses as a mechanism for controlling neuronal activity. A number of recent studies have shown that changes in both neuronal output (that is, action potential generation) and input (that is, glutamatergic excitation) can drive functionally compensatory alterations of inhibitory inputs. These relationships may be mediated by plasticity at synapses formed by distinct populations of GABAergic interneurons (INs) in a number of brain regions and involving a wide array of molecular signalling pathways. This diversity provides a rich and robust framework by which inhibitory plasticity acts as a key player in establishing balanced network activity in the brain.

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Diverse sources of GABAergic inhibition

A major challenge to establishing a unified view of inhibitory synaptic plasticity and its role in maintaining normal levels and patterns of neuronal activity is the great diversity of GABAergic INs present throughout the brain. For example, in the cortex, INs comprise ~20% of all neurons and can be subdivided into numerous classes with distinct electrophysiology, morphology, synaptic specializations and molecular markers[18,19]. Recent work in the neocortex suggests three principal groups: cells co-expressing the calcium-binding protein parvalbumin (PV-INs), the peptide co-transmitter somatostatin (SOM-INs) or the ionotropic serotonin receptor 5-HT_{3A}. In general, PV-INs are represented by basket cells and chandelier cells, which target the perisomatic and axon initial segment regions of postsynaptic glutamatergic pyramidal neurons (PNs), respectively. PV-INs exhibit fast firing characteristics and exert potent influence over the magnitude and timing of spike output from their targeted PNs[20,21]. For example, PV-INs are thought to provide the strong feedforward inhibition necessary for generating brief temporal windows during which action potentials can occur[21]. Moreover, they play a strong role in driving patterned, oscillatory activity (for example, the ~40 Hz gamma band) in cortical networks[20,22,23]. In the hippocampus (and also the neocortex), another class of perisomatic-targeting INs expresses the peptide cholecystokinin (CCK-INs). CCK-INs exhibit regular firing patterns and are thought to provide finely tuned control over postsynaptic activity[24]. For example, in contrast to PV-INs, CCK-IN terminals express the type 1 cannabinoid receptor (CB1R), which modulates GABA release as a result of retrograde signalling from target PNs[25,26] (see below). Furthermore, PV-containing basket cells have short membrane time constants that allow them to follow repetitive stimulation at high rates, whereas CCK-INs have long membrane time constants that favour integration of synaptic activity over broad time windows[27]. In addition to cortical structures, basket cells also exist in the cerebellum, where they generate large perisomatic inhibitory inputs that regulate the output of Purkinje cells[28,29].

In contrast to these perisomatic-targeting INs, SOM-INs in the neocortex are commonly classified into bitufted or Martinotti cells, which target the dendrites of PNs, forming contacts on both dendritic shafts and spines[30]. Indeed, the observation that spines (the traditional target of excitatory glutamatergic inputs) can also receive GABAergic inhibition has opened up new investigations into the development, plasticity and function of target-specific synaptic subpopulations (BOX 1). Similar dendrite-targeting neurons exist in the hippocampus, termed oriens-lacunosum-moleculare cells[31], and in the striatum[32]. In both the neocortex and hippocampus, SOM-INs restrict the temporal and spatial spread of both electrical and biochemical signals in PN dendrites[16,33]. This form of dendritic inhibition can also influence glutamatergic plasticity through the regulation of dendritic calcium activity, converting long-term excitatory potentiation into depression[34]. SOM-INs can also influence network dynamics, potentially by inhibiting dendritic spikes that drive bursts of somatic action potentials[35] and contributing to other forms of patterned circuit activity[36]. In the cerebellum, a similar role may be played by stellate cells that innervate Purkinje cell distal dendrites[29].

Finally, in the neocortex, 5-HT_{3A}-expressing INs form a heterogeneous group of cells largely present in more superficial layers. Neurogliaform cells inhibit PN dendrites, exerting powerful suppressive actions on network activity[37–39]. By contrast, cells expressing vasoactive intestinal peptide (VIP) largely target other IN populations to drive network disinhibition[19,40], though they also form inhibitory connections with PN dendrites with unclear functional consequences[41,42].

Overall, this work leads to two general motifs: perisomatic versus dendritic inhibition. In the following sections, we review recent work suggesting that these two classes of inhibitory inputs exhibit long-term plasticity that is generally, though not exclusively, coupled to somatic spiking and glutamatergic signalling, respectively. This functional differentiation leads to separate regulatory mechanisms for controlling cellular input and output (FIG. 1).

Box 1 | **Convergence of glutamatergic and GABAergic synapses onto dendritic spines**

The majority of GABAergic contacts are formed onto postsynaptic dendrites, including spines, where they converge with glutamatergic afferents[136,137]. Type A GABA receptors have also been identified within dendritic shafts and spines[138–140], but the precise arrangement of glutamatergic and GABAergic colocalization is largely unknown. In two independent studies, the authors imaged fluorescently tagged gephyrin, a scaffolding protein unique to inhibitory synapses, in layer 2 and/or layer 3 PNs of the mouse visual cortex in vivo[15,133]. GABAergic inputs exhibited an average density of approximately 0.2 synapses per micrometre, roughly half that of excitatory contacts estimated by counting dendritic spines[15]. However, the distribution of synapses in the dendritic arbour was not uniform. While most contacts targeted dendritic shafts, approximately 14% of spines located within 125 micrometres of the soma bore a GABAergic synapse, and this proportion was twofold higher at more distal spines present in the most superficial cortical layers. Shaft synapses were largely stable, turning over at a rate of ~5% over 8 days, while ~18% of spine synapses turned over during the same interval[15]. The reorganization of inhibitory innervation was strongly enhanced by changes in visual experience. Monocular deprivation produced a dramatic loss of both shaft and spine synapses that persisted for more than 1 week[15,133]. Interestingly, inhibitory synapses were found to disappear and reappear at the same dendritic location, suggesting a mechanism for reversibly gating postsynaptic signalling[134]. GABAergic inputs to dendritic spines arise, at least in part, from somatostatin-expressing interneurons (SOM-INs)[16,141], suggesting that these cells are in a position to influence excitatory transmission and dendritic signalling. Indeed, stimulation of SOM-INs can inhibit calcium influx through NMDA-type glutamate receptors and voltage-gated calcium channels in dendritic branches and single spines[16,142]. Local dendritic inhibition is likely mediated by a highly compartmentalized shunting conductance[16], enabling the high electrical resistance of the spine neck to spatially isolate inhibition[143].

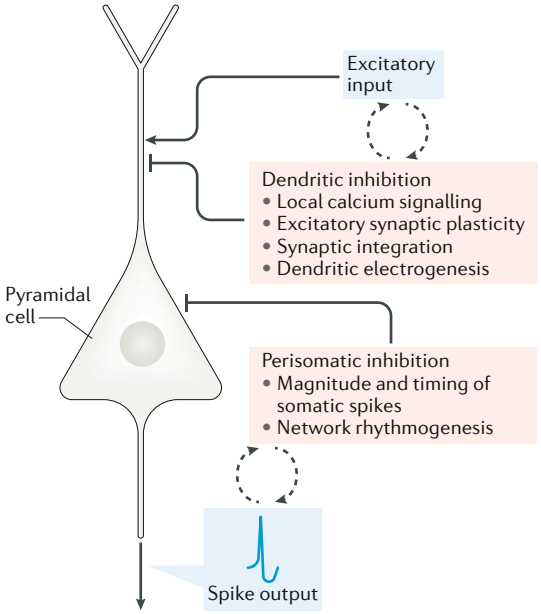

Fig. 1 | **Schematic view of inhibitory inputs targeting different regions of a postsynaptic neuron.** Perisomatic inhibition largely regulates neuronal spike output and exhibits forms of plasticity coupled to postsynaptic action potentials. Dendritic inhibition regulates local electrical and biochemical signalling and can undergo plasticity coupled to excitatory glutamatergic activity. This model suggests two parallel systems for establishing a punctuated, dynamic balance between excitation and inhibition in neuronal circuits.

GABAergic synaptic organization

As with excitatory glutamatergic connections, inhibitory synapses consist of a variety of structural elements that provide stability alongside the ability to be dynamically regulated (FIG. 2). Functionally, fast synaptic inhibition relies on the enrichment of type A GABA receptors ($GABA_A$Rs) at postsynaptic sites and requires the interaction of these receptors with postsynaptic scaffold proteins. $GABA_A$Rs are pentameric, comprising a mixture of subunits (for example, α and β, along with γ or δ) that convey distinct functional properties and pharmacological sensitivity[43,44]. Functionally, they act as ligand-gated ion channels, mediating inhibitory currents carried by permeant chloride and bicarbonate ions[43].

The inhibitory postsynaptic density includes a variety of components that shape the localization, stability and regulation of GABAergic signalling. Gephyrin has been identified as a major component of the type A GABAergic scaffold, directly interacting with α1–α3 subunits and mediating the anchoring of $GABA_A$Rs. Although gephyrin loss can have a strong impact on inhibitory synaptic innervation[45–49] (BOX 2), in some cases, the functional clustering of $GABA_A$Rs can be achieved in the absence of gephyrin, indicating that synaptic stabilization may also involve other structural components[49–51]. For instance, it has been proposed that α1-containing and α2-containing receptors may cluster via binding to different scaffolding elements, although a comprehensive model for the subtype-dependence of the synaptic organization of $GABA_A$Rs is still lacking[52,53].

The guanine nucleotide exchange factor collybistin (CB) directly binds both gephyrin and the α2 subunit of the $GABA_A$R, thus leading to the formation of trimeric CB–gephyrin–$GABA_A$R complexes[54,55]. In addition, CB activates CDC42, a small GTPase involved in both gephyrin clustering and actin cytoskeleton remodelling[56,57]. Deletion of CB results in loss of GABAergic synaptic clusters and weakened synaptic inhibition in the hippocampus[58].

Neuroligin 2 (NL2), a postsynaptic adhesion protein that binds to presynaptic partner neurexins, also plays a central role in the molecular organization of inhibitory synapses[47,59]. In one model, NL2 binds gephyrin and activates CB, inducing the recruitment of gephyrin and $GABA_A$Rs at postsynaptic sites[60]. This molecular interaction was originally thought to be restricted to perisomatic synapses but has been recently shown to similarly occur in perisomatic and dendritic compartments in the hippocampal CA1 pyramidal layer and the stratum radiatum, respectively[53]. Moreover, the NL2 interaction with the adhesion protein ST3 (also known as SLITRK3) through an unconventional binding between the NL2 and ST3 extracellular domains also regulates the formation and the stabilization of inhibitory synapses[61,62]. Interestingly, the disruption of the NL2–ST3 interaction induces the alteration of gamma oscillations in the CA3 region of the hippocampus[62]. In addition, another adhesion protein, IgSF9b, indirectly interacts with NL2 through the synaptic scaffolding molecule S-SCAM, participating in the formation and the stabilization of inhibitory synapses selectively located on hippocampal INs[63].

The dystrophin–glycoprotein complex (DGC) is another important structural element for a subset of somatic GABAergic synapses and is composed of an extracellular domain (α-dystroglycan) binding presynaptic neurexins, a transmembrane domain (β-dystroglycan) and an intracellular domain (dystrophin) interacting with the cytoskeleton[64–66]. The DGC is present at inhibitory synapses in different brain areas such as the neocortex, hippocampus and cerebellum. In the hippocampus, the DGC plays an essential role in the formation and maintenance of synapses formed by CCK-INs onto CA1 PNs[66]. In addition, similarly to IgSF9b, β-dystroglycan indirectly interacts with NL2 through S-SCAM[67].

Recently, a member of the lipoma HMGIC fusion partner-like family, LHFPL4 (also known as $GABA_A$R-regulatory LHFPL4 (GARLH4)), has been identified as an important player for the stabilization of $GABA_A$Rs at inhibitory synapses[68,69]. LHFPL4 tightly binds $GABA_A$Rs and interacts with NL2, acting as a $GABA_A$R transmembrane accessory protein[68,69]. LHFPL4 deletion causes a reduction in $GABA_A$Rs and gephyrin at synaptic sites that is paralleled by the severe loss of inhibitory miniature postsynaptic currents[68]. Importantly, these LHFPL4 effects appear to be cell type-specific, as they were selectively observed in hippocampal PNs but not in INs[68].

While our understanding of the inhibitory postsynaptic molecular organization remains largely incomplete, the aforementioned body of work indicates that adhesion

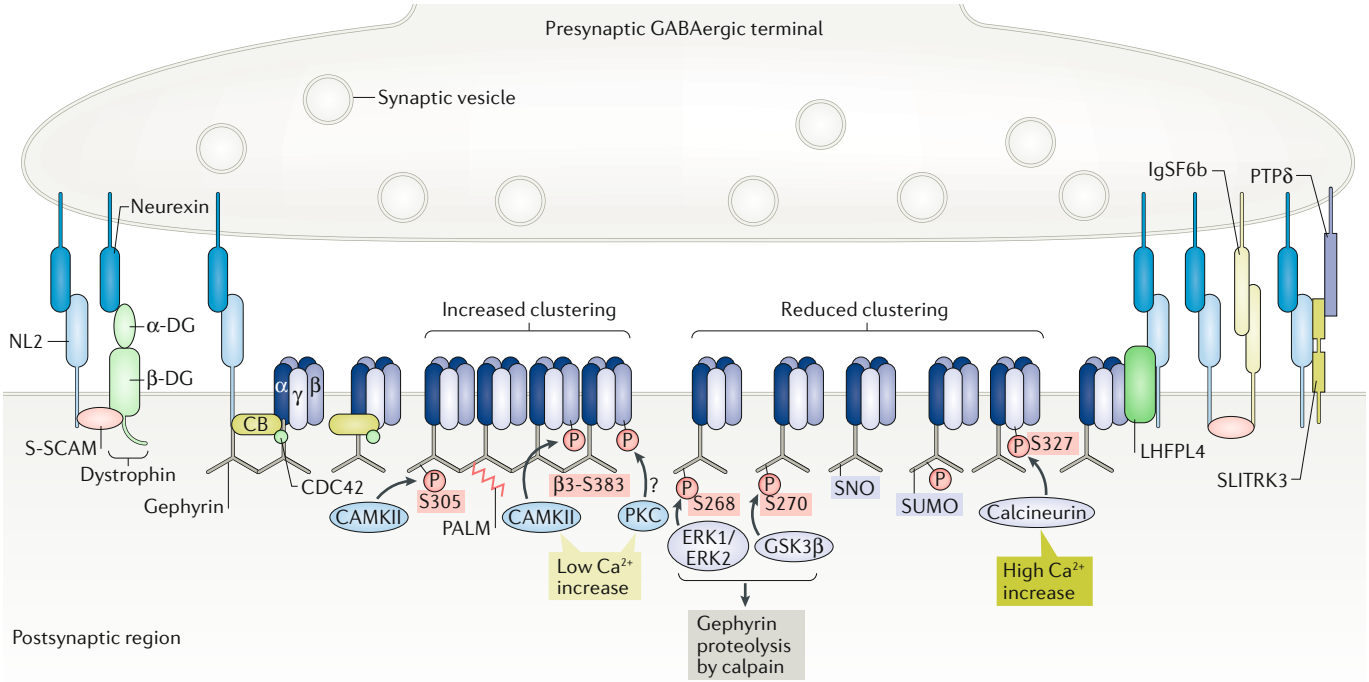

Fig. 2 | **Schematic representation of the molecular components involved in the structure and regulation of ionotropic GABAergic synapses.** The adhesion molecule neuroligin 2 (NL2) interacts with presynaptic neurexins to bridge the synaptic cleft. Gephyrin, collybistin (CB), CDC42, lipoma HMGIC fusion partner-like 4 (LHFPL4), NL2 and pentameric $GABA_A$Rs form complexes critical to synaptic clustering and function. Several other molecular interactions share a direct or indirect binding with NL2, including other adhesion proteins (SLITRK3, IgSF6b and PTPδ) and the dystroglycan (DG) complex (α-DG, β-DG and dystrophin). Post-translational modifications, including phosphorylation, palmitoylation (PALM), S-nitrosylation (SNO) and sumoylation (SUMO), of both $GABA_A$Rs and gephyrin influence their aggregation state. The phosphorylation of $GABA_A$Rs (β3 subunit S383) and gephyrin (S305) by calcium/calmodulin-dependent kinase (CAMKII) (activated by low levels of Ca^{2+} influx) and PKC promotes $GABA_A$R clustering. Synaptic clustering is also increased by gephyrin PALM. By contrast, gephyrin phosphorylation by ERK1/ERK2 and GSK3β at S268 and S270, respectively, reduces $GABA_A$R aggregation and promotes gephyrin proteolysis by calpain. Similarly, gephyrin SNO and SUMO destabilize synaptic clustering. Dephosphorylation of $GABA_A$Rs (γ2 subunit S383) by calcineurin (activated by high levels of Ca^{2+} influx) also increases lateral mobility, thus inducing the dispersion of receptors from synaptic sites.

proteins like NL2 may be placed in a higher hierarchical order with respect to other scaffold proteins, suggesting that pre–post appositions are a necessary first step in the formation of GABAergic synapses, followed by functional clustering of other components. In addition, the molecular mechanisms of postsynaptic clustering show remarkable cell type specificity and synapse specificity, suggesting a substantial diversity in the function and plasticity of GABAergic synapses formed onto different locations of the somatodendritic arbor.

Plasticity linked to postsynaptic spiking

Given that a major role of GABAergic inhibition is to shape the magnitude and timing of postsynaptic spike output, it is unsurprising that these processes should be functionally coupled to preserve neuronal activity. Indeed, this homeostatic view of inhibitory plasticity was strongly supported by work in neuronal culture systems, where chronic pharmacological perturbation of neural activity modified both glutamatergic and GABAergic signalling to bring cellular output back to baseline levels[70]. In this setting, the mechanisms underlying GABAergic plasticity were attributed to presynaptic[71,72], postsynaptic[73] or mixed presynaptic and postsynaptic[74,75] causes. A recent study carefully investigating the time course of inhibitory plasticity suggested an initial accumulation of postsynaptic $GABA_A$Rs followed by an increase in presynaptic GABA release[74]. Nevertheless, the precise molecular signalling pathways leading to these forms of plasticity remain elusive. Moreover, the presynaptic interneurons forming the modified synapses were unidentified.

Presynaptic control of inhibition. Recently, genetic modification of neuronal activity in vivo was shown to modify GABAergic inhibition[14]. Specifically, upregulating or downregulating activity in layer 2 and/or layer 3 PNs of the mouse visual cortex via overexpression of sodium or potassium channels, respectively, led to a concomitant increase or decrease in perisomatic inhibition from PV-INs[14]. Notably, dendritic inhibition from SOM-INs was unaffected by these manipulations. While the molecular mechanism of this plasticity was not explored, additional evidence suggests it might involve presynaptic alterations in release probability. Somatic depolarization of cortical PNs in layer 5 was sufficient to trigger calcium-dependent mobilization of the retrograde signalling molecule nitric oxide (NO),

which diffuses to nearby inhibitory axon terminals to persistently increase GABA release from PV-INs but not SOM-INs[76]. Importantly, distal inhibitory synapses are insensitive to exogenous application of a NO donor, supporting the idea that this selective form of inhibitory plasticity arises from a preferential expression of NO-sensitive guanylyl cyclases at PV-IN terminals[77] and not due to an inability of somatic spiking to sufficiently depolarize distal dendritic sites.

Postsynaptic spiking may also drive retrograde signalling via brain-derived neurotrophic factor (BDNF). Genetic deletion of BDNF in the mouse barrel cortex results in loss of perisomatic inhibition arising from PV-INs[78]. This form of plasticity may involve the BDNF target tyrosine receptor kinase B (TRKB), which is preferentially expressed at axonal terminals of PV-INs to modulate calcium-dependent GABA release[79]. Somatic action potentials directly lead to BDNF release from cultured PNs, leading to enhanced presynaptic GABA release, suggesting a coupling between spiking and retrograde signalling[80]. A similar mechanism may drive long-term potentiation of GABA release in the developing visual cortex following high-frequency stimulation[81].

As mentioned above, retrograde endocannabinoid signalling can also powerfully suppress GABA release from presynaptic terminals[25,82,83]. For example, depolarization-induced suppression of inhibition (DSI) occurs when trains of postsynaptic action potentials drive dendritic calcium influx through the opening of voltage-gated channels, leading to synthesis of endogenous cannabinoids (2-AG and anandamide) followed by their diffusion across the synaptic cleft. Activation of presynaptic CB1Rs then leads to short-term (seconds) modulation of terminal calcium influx and reduced release probability. This process has been well characterized in the hippocampus, where CCK-INs robustly exhibit DSI that is thought to modulate circuit function[25,84,85], but is also prominent in the cerebellum[83] and neocortex[86]. Interestingly, action-potential-dependent generation of endocannabinoids may also unconventionally potentiate GABA release in immature layer 4 neurons of the visual cortex[87].

Postsynaptic control of inhibition. In contrast to these presynaptic modifications, neuronal firing may also induce postsynaptic changes, as high-frequency action potentials in cortical layer 5 PNs can drive potentiation of perisomatic inhibition via calcium entry through R-type voltage-gated calcium channels[88]. Surprisingly, similar manipulations at hyperpolarized membrane potentials triggered synaptic depression that required calcium influx through L-type channels. In both cases, the likelihood of inducing plasticity was reduced following the addition of botulinum toxin into the intracellular patch pipette (which blocks vesicular exocytosis and insertion of new receptors into the membrane), suggesting a change in postsynaptic $GABA_AR$ number[88,89]. These findings are similar to results in the cerebellum, where Purkinje neurons show long-term potentiation of GABAergic input following short bursts of postsynaptic firing[90]. As with neocortical plasticity, postsynaptic firing is both necessary and sufficient to trigger this rebound potentiation, which occurs selectively at perisomatic basket cell synapses[29]. Interestingly, this form of plasticity requires $GABA_ARs$ that contain the β2 subunit, which is directly phosphorylated by calcium/calmodulin-dependent kinase (CAMKII), leading to increased trafficking of receptors to the synapse[29].

Presynaptic and postsynaptic coordination. In many cases, plasticity of GABAergic synapses requires coordination between postsynaptic spiking and presynaptic activity. In paired recordings between fast-spiking PV-INs and layer 2 and/or layer 3 PNs, short timing differences between presynaptic and postsynaptic firing led to long-term depression, while longer delays produced potentiation[91]. This difference may reflect protocol-dependent variation in the magnitude of postsynaptic calcium signals, which reflect the dynamics of voltage-gated channels[92], consistent with spike-timing-dependent excitatory synaptic plasticity[93]. Inhibitory synaptic potentiation was also triggered by coordinated activity of PV-INs and layer 4 PNs in the developing visual cortex, where potentiation of perisomatic GABAergic transmission was triggered by pairing interneuron firing with subthreshold postsynaptic depolarization[94]. In the hippocampus, coordinated presynaptic and postsynaptic spiking of mossy fibre to CA3 connections leads to an upregulation of GABA release that requires endogenous BDNF and L-type calcium channels[95]. In addition, coincident presynaptic and postsynaptic spiking in hippocampal cultures can modify GABAergic synaptic function via a reduction

Box 2 | **Gephyrin and GABAergic plasticity**

Gephyrin is a key molecule in the postsynaptic density of inhibitory synapses[45–47], and GABAergic synaptic aggregation relies on the concerted interaction of gephyrin with type A GABA receptors ($GABA_ARs$) and other postsynaptic scaffold and regulatory proteins[52,60,65,144]. Moreover, gephyrin underlies several forms of inhibitory plasticity. Gephyrin phosphorylation of residues S268 and S270 by ERK1/ERK2 and GSK3β, respectively, modulates gephyrin aggregation and the amplitude-inhibitory synaptic currents[100,145,146]. Phosphorylation of the same S270 residue by CDK5 also regulates gephyrin clustering and the abundance of γ2-subunit-containing $GABA_ARs$ at synapses[147]. Interestingly, phosphorylation of S268 and S270 also promotes gephyrin proteolysis by the calcium-activated protease calpain, suggesting convergence of different regulatory pathways[145,146,148]. Gephyrin clusters and inhibitory transmission are also enhanced following NMDA-type glutamate receptor (NMDAR)-dependent phosphorylation of gephyrin S305 by calcium/calmodulin-dependent kinase (CAMKII)[101]. NMDAR activation also promotes gephyrin clustering and GABAergic transmission following $GABA_AR$ phosphorylation by CAMKII through unclear molecular events[102]. By contrast, sustained network activity reduces gephyrin clusters in association with decreased $GABA_AR$ trapping at synapses following activation of the calcium-dependent phosphatase calcineurin[110,111]. Gephyrin also undergoes other post-translational modifications. Palmitoylation and de-sumoylation promote gephyrin aggregation and potentiate GABAergic synaptic currents, whereas *S*-nitrosylation negatively modulates gephyrin clustering[149–151]. Supraresolution microscopy studies showed that gephyrin clusters contain a few hundred molecules that are non-homogeneously distributed within the cluster[103,152], while GABAergic potentiation increases the fraction of gephyrin organized in nanodomains[103]. Gephyrin phosphorylation controls not only nanodomain organization but also the density of gephyrin molecules at synaptic clusters[100]. These results emphasize that the rearrangement of synaptic gephyrin organization at the nanoscale level is a major determinant for gephyrin function at synapses and for the expression of inhibitory synaptic plasticity.

in postsynaptic expression of the chloride transporter KCC2, leading to alterations in the local chloride driving force[96].

Glutamate drives GABAergic plasticity

Presynaptic and postsynaptic mechanisms. In addition to links between postsynaptic firing and inhibitory synaptic strength, a number of studies have also demonstrated that glutamatergic signalling can directly regulate GABAergic transmission, leading to either long-term depression or potentiation of inhibition. As noted above, endocannabinoids are potent short-term regulators of presynaptic vesicle release. However, in the hippocampus, stimulation of Schaffer collaterals can activate postsynaptic group 1 metabotropic glutamate receptors and drive production of retrograde endocannabinoid signalling, leading to a persistent presynaptic reduction in GABA release from dendrite-targeting INs[97]. Altered release probability also requires presynaptic cAMP–PKA and calcineurin signalling and the presence of the active zone protein RIM1α[98,99].

Contrasting with presynaptic long-term plasticity, glutamatergic activity can also lead to postsynaptic long-term changes in GABAergic synaptic transmission. We recently demonstrated that calcium influx through postsynaptic NMDA-type glutamate receptors (NMDARs) in layer 2 and/or layer 3 PNs of the mouse prefrontal cortex could produce a persistent strengthening of dendritic inhibitory synapses formed by SOM-INs[41]. This plasticity could also be evoked by optogenetic stimulation of thalamocortical afferents that also target PN dendrites, suggesting a need for close spatial proximity to induce potentiation. Notably, synaptic strengthening was not observed for perisomatic synapses formed by PV-INs. This form of GABAergic potentiation required CAMKII activity and involved postsynaptic insertion of additional $GABA_A$Rs into the membrane. As with CAMKII-dependent inhibitory plasticity in the cerebellum[29], dendritic potentiation required expression of the β2 subunit of the $GABA_A$R, as deletion of this protein abolished plasticity. Pharmacological assays demonstrated the presence of the β2 subunit at synapses formed by SOM-INs but not PV-INs, providing a potential explanation for selective potentiation of the former inputs. Finally, we demonstrated that in vivo deletion of the obligatory GluN1 subunit of the NMDAR resulted in loss of SOM-IN inputs and a surprising potentiation of PV-IN inputs to target PNs. We hypothesized that the gain in perisomatic inhibition may reflect compensatory changes caused by elevated firing rates in the face of weakened dendritic inhibition, as discussed above.

Biochemical mechanisms of plasticity. Similar forms of glutamate-induced inhibitory long-term potentiation have been observed for hippocampal neurons, in both dissociated and organotypic cultures, enabling additional examination of the molecular mechanisms involved in GABAergic plasticity. For example, the NMDAR–CAMKII signalling pathway has been reported to induce inhibitory plasticity via multiple molecular targets[44,100–108]. $GABA_A$Rs are substrates of CAMKII, and their phosphorylation modulates receptor function[44,106]. Studies performed in both heterologous systems and hippocampal neurons showed that phosphorylation of the $GABA_A$R β3 subunit at S383 increased the surface expression of $GABA_A$Rs, leading to potentiated inhibitory currents[107–109]. Moreover, brief NMDA application promoted the CAMKII-dependent exocytosis of $GABA_A$Rs at the surface of hippocampal cultured neurons and enhanced GABAergic transmission in both hippocampal cultured neurons and CA1 PNs from acute hippocampal slices[104]. The same chemical protocol was used to demonstrate that CAMKII-dependent phosphorylation of S383 increased the trapping and the clustering of receptors at synapses, a result that also required the local accumulation of the inhibitory synaptic scaffolding protein gephyrin[102]. Importantly, the precise role of gephyrin recruitment in synaptic structure and plasticity remains unclear (BOX 2). Both chemical and electrical plasticity-inducing protocols can result in gephyrin phosphorylation at S305, positively modulating gephyrin aggregation in CA1 PNs of organotypic cultures[101]. Interestingly, a similar set of experiments utilizing direct application of glutamate to hippocampal neurons resulted in the dispersal of $GABA_A$Rs, presumably associated with synaptic depression, via a mechanism that required the activation of the phosphatase calcineurin through NMDAR-mediated calcium influx and subsequent dephosphorylation of S327 of the $GABA_A$R γ2 subunit[105,110,111] (BOX 2). The determination of depression versus potentiation was later suggested to rely on the magnitude of calcium influx and a balance between activation of CAMKIIa and activation of calcineurin at single synapses[112].

The control exerted by NMDAR–CAMKII signalling on inhibitory synaptic strength implies functional interplay between excitatory and inhibitory plasticity. First, excitatory synapses must be engaged to induce heterosynaptic plasticity at GABAergic synapses. Second, CAMKII signalling has been widely demonstrated to be one of the main mechanisms of postsynaptic plasticity at glutamatergic synapses[2], indicating that inhibitory and excitatory inputs share similar regulatory pathways. This finding raises the possibility that plasticity of glutamatergic and GABAergic synapses may be locally coordinated. CAMKII is selectively translocated from excitatory to inhibitory synapses in postsynaptic dendrites following the aforementioned brief NMDA applications, leading to mild levels of calcium entry, a protocol that concomitantly induces the depression of excitatory transmission[102,112,113]. These observations suggest that plasticity at excitatory and inhibitory synapses may, in some situations, follow opposite calcium-dependent rules. This paradigm has been further supported by a recent study showing that low glutamate concentrations promote $GABA_A$R clustering through a PKC-dependent mechanism[114].

As an additional consideration, $GABA_A$Rs can laterally diffuse in the neuronal membrane, being only transiently trapped at synapses by dynamic interactions with scaffold proteins, possibly representing an efficient mechanism for tuning postsynaptic strength across multiple temporal scales[115,116]. Recent work from our group using single particle tracking has shown that $GABA_A$Rs

Organotypic cultures
Cell culture systems prepared from slices of neonatal or early postnatal brain, in which the general synaptic and circuit architecture is preserved.

diffuse between two adjacent synapses over a temporal range similar to the lifetime of $GABA_AR$ desensitization (from hundreds of milliseconds to seconds)[117,118]. Thus, after desensitization of receptors at a given synapse, the lateral diffusion of desensitized receptors can transiently reduce the amplitude of evoked currents at neighbouring locations. These studies reveal an intriguing interplay between lateral mobility, microdomain compartmentalization and gating properties of neurotransmitter receptors in the modulation of synaptic function. Further studies will be needed to understand the concerted regulation of glutamatergic signalling and GABAergic receptor trafficking, synaptic clustering and functional plasticity in both space and time.

Plasticity of tonic inhibition

In addition to mediating fast synaptic inhibition, $GABA_ARs$ can also be located at extrasynaptic sites, where their activity can produce slow regulation of the membrane conductance and potential[43]. Extrasynaptic $GABA_ARs$ mediating this tonic inhibition show higher affinity for GABA, which is conferred by their differential molecular composition that typically includes α4–α6 and δ subunits. While the structural mechanisms shaping the localization of extrasynaptic GABAergic receptors are not well understood, several studies have shown that tonic inhibition can exhibit distinct forms of plasticity, often in response to glutamatergic signalling. In the hippocampus, activation of kainate-type glutamate receptors can potentiate or depress tonic versus phasic inhibition, respectively[119]. Additionally, genetic deletion of NMDARs in immature neurons leads to augmentation of tonic inhibition[120]. Similar findings in vivo showed that stroke-induced activation of NMDARs decreased expression of extrasynaptic δ-subunit-containing $GABA_ARs$ and reduced tonic GABAergic currents[121].

Other neuromodulators also influence tonic GABAergic inhibition. In the neocortex, activation of CB1Rs directly enhances the expression of extrasynaptic $GABA_ARs$[122,123], and NO may directly act on $GABA_ARs$ to suppress tonic GABAergic currents in hippocampal neurons[124]. Finally, activation of muscarinic acetylcholine receptors has been shown to enhance the function of both synaptic and extrasynaptic $GABA_ARs$[125]. Overall, these findings suggest that diverse forms of ionotropic GABAergic signalling are regulated via an array of mechanisms to promote circuit development and function.

Plasticity of metabotropic inhibition

In contrast to fast inhibition mediated by ionotropic $GABA_ARs$, metabotropic signalling via $GABA_BRs$ engages a variety of downstream biochemical signalling cascades often resulting in slow synaptic inhibition via subsequent activation of G protein-coupled inwardly rectifying potassium (GIRK) channels[126,127]. However, despite their well-characterized roles in regulating neuronal activity, the capacity of these receptors to exhibit plasticity is not well understood. Unlike other G protein-coupled receptors, $GABA_BRs$ may not undergo typical activity-dependent and β-arrestin-dependent internalization, though they are targets for a variety of kinases that may influence membrane stabilization and degradation[127,128].

In mice, aversive foot shock has been linked to reduced $GABA_BR$-mediated currents in the lateral habenula via a process that also requires dopaminergic and glucocorticoid signalling, though the cellular and molecular mechanisms remain elusive[129]. In addition, prolonged seizure activity was shown to produce a loss of $GABA_BR$ signalling in the hippocampus, again by an unclear signalling cascade[130]. In general, these studies suggest that plasticity of metabotropic GABAergic transmission may occur, but considerable work remains to elaborate both its underlying mechanisms and functional consequences.

Conclusions and future directions

As discussed, GABAergic signalling plays a central role in shaping neuronal activity at the circuit, cellular and subcellular scales. Thus, it is unsurprising that a variety of mechanisms exist to functionally couple the strength of GABAergic inhibition with both glutamatergic excitation and action potential generation. Indeed, the question is no longer whether inhibitory synapses are plastic but rather what are the circumstances under which the various forms of synaptic regulation are engaged. For example, which plasticity mechanisms are engaged during normal development, and which persist into adulthood? What forms of learning or memory might be mediated or shaped by inhibitory plasticity? How might perturbations of these phenomena at the molecular level lead to maladaptive changes possibly linked to neurodevelopmental disorders such as epilepsy, autism and schizophrenia? These inquiries are at the frontier of our current knowledge and will demand converging efforts across multiple levels of analysis to generate satisfactory answers.

While theories linking GABAergic plasticity to overall nervous system function are likely to be premature, here we provide evidence for the general hypothesis that synapses formed by dendrite-targeting INs are most likely influenced by glutamatergic signalling, in part owing to the close physical proximity of these two types of input. By contrast, synapses formed by perisomatic-targeting INs are most likely to be influenced by postsynaptic firing. This organization provides for parallel but independent control at the input and output stages of neuronal processing (FIG. 1). The generalizability of this hypothesis will rest on future experiments utilizing novel tools to probe circuit-specific synapses at targeted locations along the somatodendritic axis. For example, optogenetics now enables the selective manipulation of specific subsets of GABAergic INs, while focal GABA uncaging can be combined with fluorescent labelling of inhibitory synapses to target specific postsynaptic locations[16,41,131,132]. Additionally, in vivo imaging enables monitoring of the structural plasticity of GABAergic synapses over multiple days[15,133,134], potentially opening up new directions for directly linking learning and memory to alterations in inhibitory function. Likewise, the study of the nanoscopic structure of GABAergic synapses and the analysis of the dynamic interactions

Tonic inhibition
An inhibitory signal that is thought to utilize extrasynaptic receptors (not directly apposed to presynaptic release sites), may be decoupled from presynaptic spiking and occurs over long (minutes or more) periods.

Phasic inhibition
An inhibitory signal that relies on postsynaptic receptors closely apposed to presynaptic release sites, is typically coupled to presynaptic action potentials and is brief (tens to hundreds of milliseconds) in duration.

between synapses will enable clarification of the relationship between neuronal activity and diverse forms of inhibitory synaptic plasticity.

Another critical avenue for future studies is the molecular diversity of GABAergic synapses across the somatodendritic arbor. As noted above, inhibitory plasticity rests heavily on the molecular constituency of individual synapses, with receptor subunit composition and the phosphorylation state of various scaffolding molecules playing key roles in establishing permissive substrates for synaptic modification[29,41,102]. Meanwhile, new molecular members of inhibitory synapses continue to be identified[69,135]. The principles and mechanisms responsible for differential trafficking of distinct components to various subcellular targets remain poorly understood but are likely to be central to a complete model of inhibitory plasticity and, indeed, GABAergic function.

In conclusion, we are currently in a golden age of exploration into the mechanisms that regulate GABAergic synaptic function. However, it will be vital to not lose sight of the fact that, in the intact brain, excitation and inhibition are inextricably linked across time and space. Thus, manipulations of one will almost certainly lead to perturbations of the other in both subtle and obvious ways. In this sense, the notion of balance between these two opposing forces may be better thought of as a punctuated and dynamic equilibrium that serves to promote both stability and flexibility across the life of the organism.

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