# VARIATIONS ON AN INHIBITORY THEME: PHASIC AND TONIC ACTIVATION OF $GABA_A$ RECEPTORS

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Abstract | The proper functioning of the adult mammalian brain relies on the orchestrated regulation of neural activity by a diverse population of GABA (γ-aminobutyric acid)-releasing neurons. Until recently, our appreciation of GABA-mediated inhibition focused predominantly on the $GABA_A$ (GABA type A) receptors located at synaptic contacts, which are activated in a transient or 'phasic' manner by GABA that is released from synaptic vesicles. However, there is growing evidence that low concentrations of ambient GABA can persistently activate certain subtypes of $GABA_A$ receptor, which are often remote from synapses, to generate a 'tonic' conductance. In this review, we consider the distinct roles of synaptic and extrasynaptic GABA receptor subtypes in the control of neuronal excitability.

GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the adult mammalian CNS. Its principal action, which is mediated by ubiquitous ionotropic $GABA_A$ (GABA type A) receptors, is to increase membrane permeability to chloride and bicarbonate ions. In most mature neurons, this leads to a net inward flow of anions and a hyperpolarizing postsynaptic response — the inhibitory postsynaptic potential (IPSP) (BOX 1). This event occurs when postsynaptic $GABA_A$ receptors are activated following brief exposure to a high concentration of GABA, which is released from presynaptic vesicles. The resultant increase in membrane conductance underlies what is known as 'phasic' inhibition. The transient and specific point-to-point nature of this process has an important role in many aspects of GABA-mediated signalling.

In recent years, it has become evident that GABA receptor activation can also take place in a less spatially and temporally restricted manner. GABA escaping from the synaptic cleft can activate receptors on presynaptic terminals or at neighbouring synapses on the same or adjacent neurons (a phenomenon termed 'spillover'). In addition, low GABA concentrations in the extracellular space can result in the persistent or 'tonic' activation of $GABA_A$ receptors, in a manner that is temporally dissociated from phasic synaptic events. In this review, we examine the diversity of $GABA_A$ receptor-mediated signalling in the mammalian brain, emphasizing the different mechanisms that underlie tonic and phasic receptor activation. We discuss how factors such as $GABA_A$ receptor heterogeneity, receptor localization and the GABA concentration transient might interact to generate functionally distinct modes of neuronal inhibition.

## Modes of $GABA_A$ receptor activation

***Phasic receptor activation.*** $GABA_A$ receptor-mediated synaptic communication is tailored to allow the rapid and precise transmission of presynaptic activity into a postsynaptic signal. On the arrival of an action potential at the nerve terminal, a local calcium influx triggers the fusion of synaptic vesicles with the presynaptic membrane at the release site. Each vesicle is thought to liberate several thousand GABA molecules into the synaptic cleft, generating a peak GABA concentration in the millimolar range[1]. Clustered opposite the release site are a small number of receptors (from ten to a few hundred)[1–4]. These receptors experience a rapid increase in GABA concentration and, for a proportion of them, the binding of GABA triggers the near-synchronous opening of their ion channels.

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Box 1 | **Multiple actions of $GABA_A$ receptors**

**Responses generated by ionotropic receptors result from the dissipation of transmembrane ionic gradients, which are produced by ion pumps and carriers. $GABA_A$ (γ-aminobutyric acid type A) receptors are permeable to chloride and bicarbonate anions[179,180]. The functional outcome of receptor activation depends on the transmembrane distribution of these two anions and on the membrane potential of the cell. In most mature neurons, the activity of the chloride-extruding potassium–chloride co-transporter KCC2 (REF. 181) results in a chloride equilibrium potential that is more negative than the resting membrane potential ($V_m$). The equilibrium potential for bicarbonate is more positive than $V_m$, but bicarbonate is much less permeable than chloride. Therefore, $GABA_A$ receptor activation typically results in the net entry of anion, and the classically described hyperpolarizing inhibitory postsynaptic potential (IPSP). In this case, both the increase in conductance (that causes shunting of excitatory inputs) and the hyperpolarization (that sums with depolarizations) contribute to the 'inhibitory' effect of GABA, thereby reducing the probability that an action potential will be initiated.**

**However, this model is an oversimplification. A hyperpolarizing GABA response might not be inhibitory if it triggers hyperpolarization-activated excitatory conductances to produce rebound spikes[182,183]. Moreover, the response to GABA itself can be depolarizing. This is true for most immature neurons that lack KCC2 and instead accumulate chloride by way of the sodium- and potassium-coupled co-transporter NKCC1 (REFS 181,184), and is also true for some mature neurons[185,186]. Although inhibition can still occur owing to the shunting effect of the increase in conductance[78,187], the effect of the IPSP depends on its location and timing in relation to excitatory inputs, and the interplay between the respective conductance and voltage changes[188]. In immature neurons, the depolarization might be sufficient to trigger calcium influx, a phenomenon that is implicated in GABA-mediated modulation of neuronal proliferation, migration, growth and synapse formation[189–191]. It has recently been suggested that even when GABA-induced depolarization is insufficient to activate voltage-gated calcium channels, calcium entry might still occur following sustained receptor activation in response to the osmotic load that is induced by chloride entry[182].**

A defining feature of this phasic mode of receptor activation is the short duration of the GABA transient to which the postsynaptic receptors are exposed. Experiments using low-affinity competitive antagonists indicate that the synaptic GABA concentration decays with a time constant of <500 μs[5], and related studies with agents that slow down the binding of GABA to its receptors indicate a time constant of synaptic GABA clearance of ~100 μs[6,7]. The short dwell time of GABA within the cleft can be attributed to its rapid diffusion away from the release site[5]. Efficient gating of $GABA_A$ receptor ion channels requires the receptor to be occupied by two agonist molecules[8], and, for GABA, the binding rate is slow relative to diffusion[9]. Although the peak concentration of GABA might be higher than that required to produce maximal receptor activation at steady state, the short exposure time means that not all postsynaptic receptors will necessarily be fully occupied. Although postsynaptic receptor saturation (full occupancy) following the release of GABA from a single vesicle does occur at certain synapses, the degree of receptor occupancy often varies between synapses on different neurons, and can even vary between synapses on a single neuron[3,6,10–12].

The time course of the GABA transient in the synaptic cleft might be influenced by variations in vesicle size and content, the nature of vesicle fusion, the geometry of the synaptic cleft, and the number and spatial arrangement of GABA transporters and postsynaptic receptors in relation to the site of transmitter release. By governing the time course and peak concentration of GABA to which receptors are exposed, these variables will not only influence occupancy, but will also dictate the subsequent changes that lead to channel opening. To account for the elementary functional properties of $GABA_A$ receptors, various kinetic schemes of microscopic gating have been proposed on the basis of steady-state and/or transient receptor activation[13–18]. It is envisaged that conformational changes in GABA receptors result in transitions between various closed, open (ion-conducting) and desensitized (relatively long-lived, agonist-bound closed) states. The time spent in each of these states is determined by the intrinsic properties of the channels and the temporal profile of GABA exposure.

When recorded from compact cells, which afford voltage-clamp measurements of high temporal resolution, spontaneously occurring miniature inhibitory postsynaptic currents (mIPSCs), generated by GABA released from a single synaptic vesicle, have a rapid onset, with rise times of a few hundred microseconds[3,4,19]. This reflects the proximity of the receptors to the site of GABA release and the speed of the closed-to-open transition[15,18,20,21]. If the time course of the GABA concentration transient is brief, the decay of the IPSC is dominated by the ion channel closure that follows ligand removal, a macroscopic phenomenon known as deactivation. The speed of this process is determined by the microscopic kinetics of the receptors and reflects the various transitions — most notably, entry into and exit from agonist-bound desensitized states that effectively trap GABA on the receptor before the eventual unbinding step[15,17,22,23]. The expression of receptor subtypes that incorporate different subunits (BOX 2) is proposed to contribute to the differences observed in the decay of IPSCs at different stages of development[24,25] and in different cell types[26–28].

The above description of phasic receptor activation addresses only the most straightforward situation, in which a single vesicle is released from an active zone and the liberated transmitter activates only those receptors that are clustered in the underlying postsynaptic density (FIG. 1a). In reality, there are further levels of complexity, which might include the activation of receptors at adjacent postsynaptic densities within the same synaptic bouton, or interactions between multiple vesicles that are released from a single synaptic specialization at a short interval (≤1 ms), from several nearby synapses or following repeated synaptic activation. If an action potential triggers the release of multiple vesicles at a single active zone (multivesicular release), the postsynaptic receptors will be exposed to a different GABA concentration transient. The time course of the synaptic GABA concentration change will be modified substantially if this vesicle release is temporally dispersed (asynchronous release). Following diffusion from its release site(s), GABA might activate adjacent (perisynaptic) receptors, receptors at other postsynaptic densities made by the same bouton, more remote extrasynaptic receptors or receptors at

### Box 2 | Molecular heterogeneity of $GABA_A$ receptors

**Like other members of the cysteine-loop ligand-gated ion channel family, such as nicotinic acetylcholine, glycine and 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) receptors[192], $GABA_A$ (γ-aminobutyric acid type A) receptors are pentameric assemblies of subunits that form a central ion channel. Nineteen $GABA_A$ receptor subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3) have been cloned from the mammalian CNS, with further variation resulting from ALTERNATIVE SPLICING (for example, for the γ2 subunit[193]). The combinatorial co-assembly of these various subunit proteins allows a potentially enormous molecular heterogeneity of $GABA_A$ receptor subtypes.**

**Of the many subunit combinations that are theoretically possible, only a few dozen have been shown to exist, reflecting the differential distribution of subunit types among brain regions and neuronal populations[194–196], but also implying several basic 'rules' of assembly[106,197]. The most abundantly expressed receptor subtype in the brain is formed from α1, β2 and γ2 subunits[198–200]. The likely stoichiometry is two α, two β and one γ subunit[201,202], with the subunits arranged pseudo-symmetrically around the ion channel in the sequence γ–β–α–β–α, anti-clockwise when viewed from the synaptic cleft[196,203]. Other common assemblies also contain α, β and γ2 subunits (for example, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_x\gamma_2$, $\alpha_5\beta_3\gamma_2$ and $\alpha_6\beta_x\gamma_2$), whereas receptors in which the γ2 subunit is replaced by γ1, γ3, or δ are less abundant. Further variability arises from the fact that individual pentamers might contain two different α or two different β subunit isoforms (reviewed in REF. 198). In some cases, the γ subunit can be replaced by an ε, δ or π subunit, and the π and θ subunits might also be capable of co-assembling with α, β and γ subunits to form receptors that contain subunits from four families[204–206]. Finally, although the ρ1 subunits can form homomeric receptors that share certain properties with the $GABA_C$ (GABA type C) subfamily of ionotropic GABA receptors[207,208], there is evidence that they can also form receptors with γ2 subunits[209] or with both α1 and γ2 subunits[210]. This molecular heterogeneity has important functional consequences for $GABA_A$ receptor subtypes: subunit composition dictates not only the properties of the receptors, but also their cell surface distribution and dynamic regulation[106,198,211].**

nearby synapses. In this case, the GABA waveform to which any particular receptor is exposed will be determined by its location relative to the release site, the geometry and spatial arrangement of the neighbouring cellular elements, diffusional barriers and the proximity of GABA transporters in neurons and astroglia[5,29,30,31]. It is important to note that the currents that result from GABA spillover can still be considered phasic, in the sense that they are temporally related to the release event.

***Tonic receptor activation.*** Phasic activation of synaptic receptors is fundamental to information transfer in the brain. However, it is recognized that neurotransmitters that are traditionally considered to participate in rapid point-to-point communication through the activation of ionotropic receptors might also participate in slower forms of signalling[32]. At the extreme, this might include the tonic activation of receptors. Underlying this PARACRINE activity is the widespread presence of receptors in somatic, dendritic and axonal regions of neuronal membrane that are distant from sites of neurotransmitter release[33].

Tonic activation of $GABA_A$ receptors is evident in certain embryonic neurons before synapse formation has taken place[34–37]. In mature neurons that display IPSCs, the tonic activation of $GABA_A$ receptors, as opposed to the superimposition of high-frequency phasic events[38–40], was first identified in voltage-clamp recordings from rat cerebellar granule cells[41]. The $GABA_A$ receptor antagonists bicuculline and SR-95531 (gabazine) not only blocked spontaneously occurring IPSCs, but also decreased the 'holding' current that was required to clamp the cells at a given membrane potential. This reduction in the input conductance was associated with a reduction in current variance, consistent with a decrease in the number of open $GABA_A$ receptor channels[41–44]. Subsequent studies have indicated that GABA-mediated tonic conductances exist in granule cells of the dentate gyrus[45], thalamocortical relay neurons of the ventral basal complex[46], layer V pyramidal neurons in the somatosensory cortex[47], CA1 pyramidal cells[48] and certain inhibitory interneurons in the CA1 region of the hippocampus[49].

Identifying the GABA source, or sources, is important if we are to understand how tonic receptor activation is modulated. Although certain recombinant[23,50–52] and native[53] $GABA_A$ receptors have been shown to open spontaneously with low probability in the absence of agonists, most $GABA_A$ receptors require the binding of agonist molecules to promote entry into open states. Accordingly, the most parsimonious explanation for the presence of a tonic conductance is that GABA (or some other $GABA_A$ receptor agonist) must be present in the extracellular space at a sufficiently high concentration to cause persistent receptor activation. On the basis of theoretical considerations of GABA transporter stoichiometry[54], or solute recovery during *in vivo* microdialysis[55–58], estimates of the concentration of ambient GABA vary from tens of nanomolar to a few micromolar. This range probably reflects the uncertainties involved in different methods of estimation, but it also underscores the likelihood of genuine regional and temporal variations in extracellular GABA concentration.

In the postnatal brain, the origin of GABA has been investigated most extensively in cerebellar granule cells. In the juvenile animal, action potential-dependent vesicular release clearly underlies the maintenance of the ambient GABA concentration that is responsible for tonic receptor activation, and one factor that contributes to this process is the presence of many GABA-releasing Golgi cell axon terminals in the cerebellar GLOMERULUS[41–44]. For mature granule cells, the situation is less clear: vesicular release does have a role[59,60], but it has been suggested that there is also a non-vesicular source of GABA[44,61], although this has not yet been identified.

The concentration of GABA in the extracellular space reflects not only the number and 'activity' of GABA-releasing elements, but also the action of GABA transporters. These sodium and chloride symporters, which are normally responsible for removing GABA from the extracellular space, can also operate in the reverse direction, so, in certain circumstances, can themselves provide a source of GABA[54]. However, following pharmacological blockade of transport[44,45,49,61], and in transporter-deficient mice[62], the magnitude of the tonic current increases, which indicates that reversed transporter activity does not usually contribute to ambient GABA.

ALTERNATIVE SPLICING
During splicing, introns are excised from RNA after transcription and the cut ends are rejoined to form a continuous message. Alternative splicing allows the production of different messages from the same DNA molecule.

PARACRINE SIGNALLING
A signalling process that involves the secretion from a cell of molecules that act on other cells expressing appropriate receptors in the immediate neighbourhood, rather than acting on the same cell (autocrine signalling) or on remote cells (endocrine signalling).

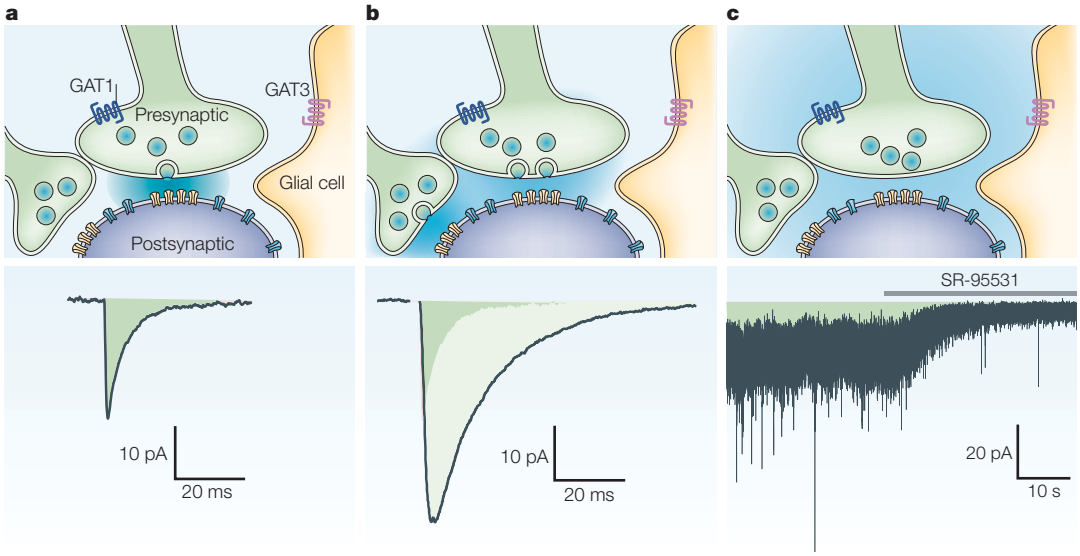


Figure 1 | **Modes of $GABA_A$ receptor activation. a** | The release of a single vesicle from a presynaptic terminal activates only those postsynaptic $GABA_A$ (γ-aminobutyric acid type A) receptors that are clustered in the membrane immediately beneath the release site (yellow). The diffuse blue shading indicates the spread of released GABA. The current record shows an averaged waveform of miniature inhibitory postsynaptic currents (mIPSCs) recorded in the presence of the sodium channel blocker tetrodotoxin. The area beneath the record is shaded to indicate the charge transfer. GAT, GABA transporter. **b** | Action potential-dependent release of multiple vesicles or evoked release from several terminals promotes GABA 'spillover', and activates both synaptic receptors and perisynaptic or extrasynaptic receptors (blue). The current record shows the larger and much slower averaged waveform of IPSCs evoked by electrical stimulation. The area of the mIPSC is superimposed for comparison. **c** | A low concentration of ambient GABA, which persists despite the activity of the neuronal and glial GABA transporters (GAT1 and GAT3), tonically activates high-affinity extrasynaptic receptors. The trace shows the 'noisy' tonic current that results from stochastic opening of these high-affinity $GABA_A$ receptors, with superimposed phasic currents (in this case, the synaptic events would be arising at sites not depicted in the schematic diagram). A high concentration (10 μM) of the $GABA_A$ antagonist gabazine (SR-95531) blocks the phasic IPSCs and tonic channel activity, causing a change in the 'holding' current and a reduction in current variance. The infrequent phasic events that remain in SR-95531 are glutamatergic excitatory postsynaptic currents. The shaded area beneath the current record before SR-95531 application represents the charge carried by tonically active $GABA_A$ receptors. The frequency of spontaneous IPSCs is relatively low and the tonic receptor activity generates a conductance several-fold larger than the averaged conductance that is carried by phasic IPSCs[42,61]. The current records are from whole-cell patch-clamp recordings of granule cells in acute cerebellar slices from adult mice. The recordings were made with symmetrical chloride concentrations at a holding voltage of –70mV and a temperature of 25°C. pA, pico amp. Traces in panels **a** and **b** courtesy of S. G. Brickley and M. F. Trace in panel **c** modified, with permission, from REF. 116 © (2001) Macmillan Magazines Ltd.

GLOMERULUS
Axon terminals end in various configurations within the neuropil. The most common is *en passant* or *de passage*, in which axons make simple synapses as they pass dendrites or cell bodies. By contrast, some axons end in — or produce strings of — enlargements that are often packed with synaptic vesicles. These glomerular-type endings might synapse with large numbers of dendrites. In the cerebellum, each large excitatory mossy fibre terminal contacts dendrites from many granule cells and, together with inhibitory Golgi cell axon terminals, forms a glomerular structure that is wrapped with glia.

THETA FREQUENCY NETWORK OSCILLATION
Rhythmic neural activity with a frequency of 4–8 Hz.

GAMMA FREQUENCY NETWORK OSCILLATIONS
Rhythmic neural activity with a frequency of 25–70 Hz.

## Functional roles of phasic and tonic inhibition

The main feature of phasic $GABA_A$ receptor-mediated inhibition is the rapid synchronous opening of a relatively small number of channels that are clustered at the synaptic junction, whereas tonic inhibition results from random, temporally dispersed activation of receptors that are distributed (albeit in a potentially non-uniform manner) over the neuronal surface. This distinction implies a profound difference in the control of neuronal network activity by phasic and tonic forms of inhibition.

*Functional roles of phasic inhibition.* Preventing over-excitation of neurons, and thereby avoiding the development of pathological states of network activity, is an essential task of GABA-releasing interneurons and $GABA_A$ receptors in the adult CNS. However, it is clear that interneurons have more complex roles than the provision of generalized inhibition, and depend crucially on synapse location and IPSC timing[63–68]. One important function of phasic inhibition, the effectiveness of which is determined by both of these variables, is the generation of rhythmic activities in neuronal networks. A notable example is provided by the action of the cortical and hippocampal basket cells that innervate the perisomatic regions of pyramidal cells. By phasing and synchronizing the activity of a large population of pyramidal cells, these interneurons have an essential role in generating and maintaining THETA and GAMMA FREQUENCY NETWORK OSCILLATIONS[65,68,69]. This action requires the mutual interconnection of interneurons by chemical and electrical synapses[69,70]. For $GABA_A$ receptor-mediated postsynaptic conductances, a rapid time course (~5 ms) is essential for synchronization at high frequencies (for example, gamma frequency[71,72]). A role for phasic inhibition in the generation or regulation of synchronous population activity has also been shown in several other brain regions, including the thalamus[73] and olfactory bulb[74].

The exact location of GABA-releasing synapses, and the temporal relationship between their activation and that of other synaptic or voltage-gated conductances, is also important in the control of regenerative electrical activity in dendrites[75,76]. Synapse location also affects the impact of phasic GABA-mediated input on synaptic integration. For example, the selective

activation of somatically terminating interneurons during feed-forward inhibition of hippocampal pyramidal cells produces a requirement for precise COINCIDENCE DETECTION of excitatory input at the soma, whereas dendrites can integrate synaptic input over longer time periods[77]. Finally, whether the depolarizing action of GABA that persists in mature cortical pyramidal cells is inhibitory or excitatory (BOX 1) depends on the location of synapses and the timing of their activity relative to excitatory inputs[78,79].

These examples show the importance of spatially restricted IPSPs in enabling certain neuronal behaviours. In cells that receive spatially segregated plastic inhibitory input from several sources, it is also important to appreciate that this input might be subject to exquisite modulation, either through changes in the activity of the parent interneurons, or by the regulation of transmitter release from their terminals. Cortical and hippocampal interneurons are known to express, in a cell type-specific manner, receptors for various neurotransmitters and neuromodulators, including GABA, glutamate, serotonin (5-hydroxytryptamine or 5-HT), opioids, monoamines, acetylcholine and endocannabinoids, and they respond uniquely to alterations in the levels of these neuromodulators with changes in their firing frequency or GABA release[80]. By changing the activity or output reliability of specific interneuron types, this modulation allows sophisticated control that is much more refined than a simple change in the frequency, amplitude or duration of all IPSCs in the cell. For example, if cortical axo–axonic cells were selectively silenced, there would be little change in the total IPSC frequency in postsynaptic target cells, but their output would be profoundly affected. Similarly, if basket cell-evoked perisomatic IPSCs were desynchronized, there would be no change in the total phasic inhibition, but rhythmic network activity would probably collapse. So, small spatially and/or temporally restricted alterations in interneuron activity that do not greatly affect total phasic inhibition might crucially alter the way in which subcortical information is conveyed to cortical networks.

***Functional roles of tonic inhibition.*** Compared with phasic $GABA_A$ receptor activation, we might expect tonic $GABA_A$ receptor activation to be much more limited in its capabilities and less susceptible to modulation. Tonic activation of $GABA_A$ receptors has one straightforward outcome: a persistent increase in the cell's input conductance. This affects the magnitude and duration of the voltage response to an injected current, and increases the decrement of voltage with distance. So, for a given excitatory input (excitatory postsynaptic current or EPSC), the size and duration of the excitatory postsynaptic potential (EPSP) will be reduced, and the temporal and spatial window over which signal integration can occur will be narrowed, making it less likely that an action potential will be generated.

How is asynchronous tonic receptor activation distinct from a high frequency of synaptic input? If we consider the postsynaptic conductance in isolation, then at some point the integrated response to many phasic events, when viewed from the soma, becomes indistinguishable from a tonic conductance. However, there are clear differences between the two phenomena. In the case of high-frequency phasic transmission, the signals that result from discrete vesicular release events are integrated in the postsynaptic cell. In the case of tonic inhibition, 'integration' takes place in the extracellular space, where GABA is pooled to achieve an averaged ambient concentration, albeit one that can still change over time. This has implications for the way in which the two processes reflect network activity, and might also have significant energetic considerations. If the contributing IPSCs occur at different synapses that are distributed on a complex dendritic tree, the input is discrete and phasic for each dendritic location and could still participate in temporally precise local processing. Moreover, for neurons with many inputs, it might be feasible to achieve a sustained conductance through the integration of IPSCs from many sources, whereas for neurons with few inputs, such as cerebellar granule cells, this might be achieved only by sharing a restricted number of GABA-releasing elements[42].

Several groups have investigated how tonic inhibition in cerebellar granule cells affects their excitability. If step current injections of increasing amplitude are used to evoke action potentials in granule cells, blockade of tonic inhibition with $GABA_A$ antagonists decreases the current that is required to achieve a given firing rate — the input–output relationship is shifted to the left[42,81,82]. The same reduction in firing rate is seen at all levels of excitation — equivalent to a subtractive mathematical operation (FIG. 2). Mitchell and Silver[83] used a dynamic clamp to restore tonic conductance to granule cells bathed in $GABA_A$ receptor antagonists, and their results were complementary to those seen with pharmacological blockade. However, they also showed that the effect of the tonic inhibitory conductance depended on the nature of the excitatory input. If, instead of a step excitation, random trains of synaptic conductances were used to excite the cells, shunting inhibition no longer simply shifted the input–output relationship to the right, but also decreased its slope, which corresponds to a change in gain (a divisive mathematical operation). The slope of the input–output relationship depends on the variability of the input conductance: with tonic inhibition, a higher frequency of synaptic excitatory input (with higher variance) is required to achieve a given output rate[83–85].

An increase in the slope of the input–output relationship on blockade of the tonic conductance was also seen when mossy fibres, which provide excitatory synaptic input to granule cells, were stimulated at high frequency[81]. Changes in tonic $GABA_A$ receptor activation, through changes in Golgi cell firing[42,59], will therefore modify the sensitivity of the granule cell to changes in the frequency of mossy fibre input, and contribute to the sparse coding of sensory input by granule cells that is thought to be necessary for effective motor control[86,87]. Network models of the cerebellar cortex indicate that tonic inhibition of granule cells might also limit the oscillatory behaviour that is entrained by phasic feedback inhibition from Golgi cells[88].

COINCIDENCE DETECTION
A situation in which two different subthreshold excitatory inputs are sufficiently closely timed that they summate to trigger the generation of an action potential.

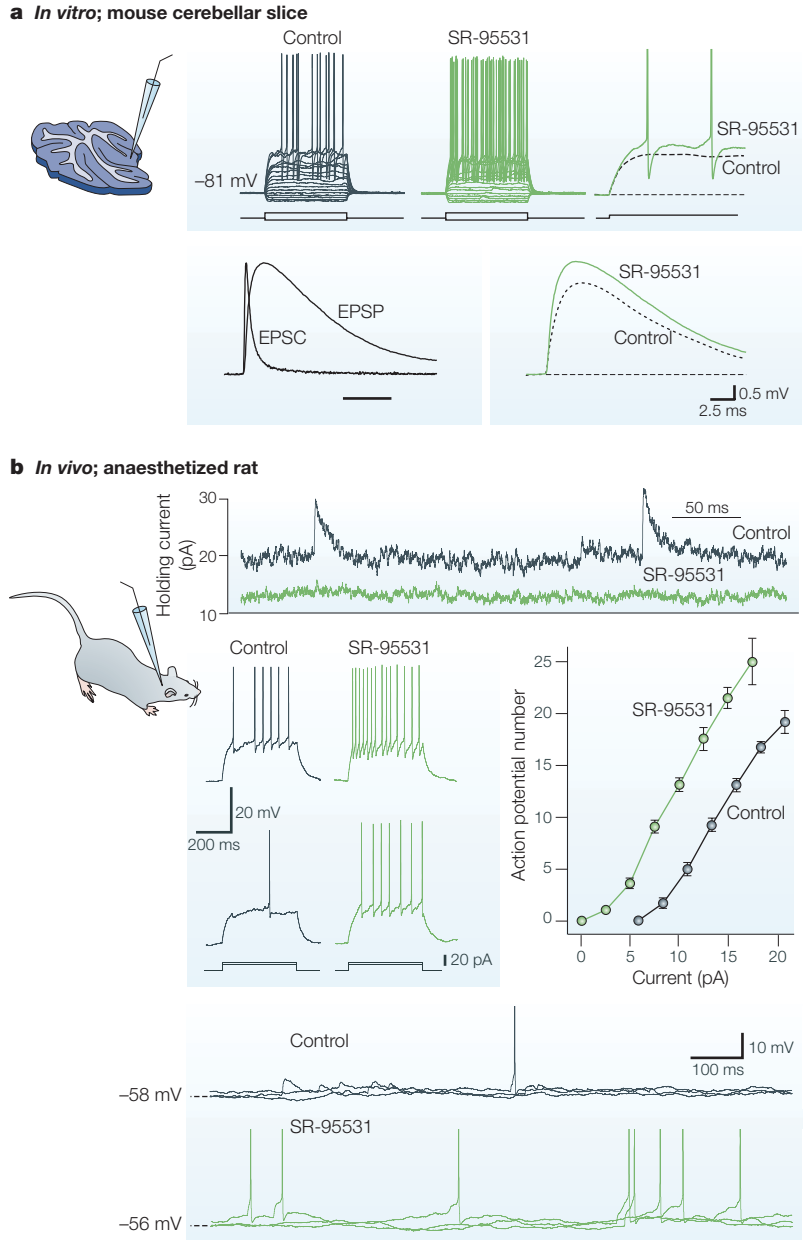


Figure 2 | **Effects of tonic inhibition on granule cell excitability. a** | Recordings from an acute cerebellar slice (35-day-old mouse). When current injection (–8 to +24 pico Amps (pA) in increments of 2 pA) was repeated in the presence of 10 μM SR-95531 (gabazine), there was a marked increase in the number of action potentials that were evoked. The top right trace shows voltage records from a different cell with threshold current injection, in the presence (solid green line) and absence (dashed line) of SR-95531. The bottom left trace shows superimposed normalized average excitatory postsynaptic potential (EPSP) and excitatory postsynaptic current (EPSC) waveforms, recorded from a single granule cell. Bottom right are average EPSP waveforms taken from a different cell showing that in the presence of SR-95531, the magnitude and duration of the EPSP is increased. **b** | Recordings from granule cells in the cerebellar cortex of anaesthetized, freely breathing 18–27-day-old Sprague–Dawley rats. The upper traces (voltage clamp at 0 mV) show that topical application of SR-95331 (0.5 mM) abolished IPSCs and reduced the tonic current. Below this, responses to current injection before and after SR-95531 application are shown; the increased excitability in SR-95531 is reflected in a leftward shift of the frequency–current relationship (graph). The bottom traces show how a low spontaneous firing rate is enforced by tonic inhibition *in vivo*. Three overlaid current-clamp traces from a granule cell at rest (black) and three traces recorded after $GABA_A$ receptor blockade with SR-95531 (green) show that $GABA_A$ receptor blockade results in an increase in spontaneous firing. Panel **a** modified, with permission, from REF. 116 © (2001) Macmillan Magazines Ltd. Panel **b** modified, with permission, from REF. 82 © (2004) Macmillan Magazines Ltd.

In the hippocampus, the contribution of some interneurons to the generation of network oscillations might also be affected by tonic inhibition[49]. Moreover, it has been suggested that differences in the tonic conductance of interneurons and pyramidal cells might contribute to the homeostatic regulation of phasic inhibition in pyramidal cells[49,85]. Although tonic $GABA_A$ receptor activity is present in developing pyramidal cells[37], it is not generally seen in brain slices from adult animals unless GABA uptake or degradation are blocked[89–91], or GABA receptor affinity is increased[92,93] (but see REF. 48). So, under normal conditions, pharmacological blockade of tonic inhibition selectively enhances the excitability of interneurons, leading to an increase in the frequency of IPSCs in CA1 pyramidal cells[49].

The question of whether axonal or presynaptic $GABA_A$ receptors are tonically activated has received less attention, so the possible functional consequences of such activation are poorly understood. Although axonal receptors are unlikely to influence integration at the somato–dendritic level, there is evidence that they might modulate action potential conduction and transmitter release[33].

## Properties of $GABA_A$ receptors

Next, we consider how the different modes of $GABA_A$ receptor activation might be determined by differences in the biophysical properties and subcellular location of receptor subtypes. The subunit composition of $GABA_A$ receptors is summarized in BOX 2.

*Receptor localization and subunit composition.* The rapid onset and rise time of $GABA_A$ receptor-mediated IPSCs that are evident in various CNS neurons indicates that there is a high density of receptors close to the transmitter release sites. In the late 1980s, studies using newly derived monoclonal antibodies against $GABA_A$ receptor subunits (BOX 2), in conjunction with electron microscopic (EM) immunoperoxidase reactions, revealed immunoreactivity for α1 and β2/3 subunits at non-synaptic (extrasynaptic) membranes[94–97]. However, owing to technical limitations, synaptic enrichment of these subunits could not be convincingly shown. Subsequent light-microscopic immunofluorescence and EM immunogold methods allowed more precise subcellular localization of $GABA_A$ receptors, and enrichment of the α1, α2, α3, α6, β2/3 and γ2 subunits within the postsynaptic specialization of GABA-containing synapses was shown in many brain regions, including the cerebellum, globus pallidus, hippocampus and neocortex[98–103] (FIG. 3). However, it should be noted that each of these receptor subunits was also found in extrasynaptic plasma membranes, and no $GABA_A$ receptor subunit type has yet been found to have an exclusively synaptic location. Even in the case of $\alpha_1\beta_{2/3}\gamma_2$ $GABA_A$ receptors, which are highly enriched in synapses, more receptors are found outside than inside synaptic junctions[98].

Some $GABA_A$ receptors do not seem to accumulate at synaptic junctions; for example, the δ subunit was shown to be present exclusively in the extrasynaptic somatic and dendritic membranes of cerebellar granule cells[99] (FIG.3),

and at extrasynaptic and perisynaptic locations in hippocampal dentate gyrus granule cells[104]. In the cerebellum, gold particles labelling the δ subunit were typically hundreds of nanometres from the edge of the nearest postsynaptic density, whereas in the hippocampus they were concentrated in a perisynaptic position, just outside the postsynaptic density (within 30 nm). The δ subunit forms receptors specifically with the α6 and β2/3 subunits ($\alpha_6\beta_{2/3}\delta$ and $\alpha_1\alpha_6\beta_{2/3}\delta$) in cerebellar granule cells and with the α4 and βx subunits ($\alpha_4\beta_x\delta$) in several areas of the forebrain, including the thalamus, neostriatum and dentate gyrus[105]. For each of these receptor subtypes, the lack of a γ subunit is probably responsible for their failure to be incorporated at the synapse.

The postsynaptic density of GABA-releasing synapses contains many proteins that have been postulated to have roles in the targeting and stabilization of $GABA_A$ receptors[106–108]. The precise molecular architecture remains to be established, but it is clear that the γ2 subunit has a central role in the clustering of synaptic $GABA_A$ receptors. Deletion of the *γ2* gene in mice leads to a profound reduction in the clustering of both $GABA_A$ receptors and the $GABA_A$ receptor-associated protein gephyrin, which is paralleled by a reduction in mIPSC frequency[109]. This requirement for the γ2 subunit exists not only in embryonic neurons that are undergoing synapse formation, but also in more mature neurons with existing synaptic contacts[110,111].

δ subunit-containing receptors seem to be purely extrasynaptic, but other subtypes might also be present predominantly, if not exclusively, outside synapses. In hippocampal pyramidal cells, the α5 subunit (which probably forms $\alpha_5\beta_3\gamma_2$ receptors) shows diffuse surface labelling at the light microscopic level without detectable synaptic clustering, as judged by the lack of co-localization with gephyrin[103,112] (FIG. 4). In this case, the presence of the α5 subunit seems to override the ability of the γ2 subunit to promote synaptic localization. Notably, in hippocampal slices from mice lacking the α5 subunit, the amplitudes of action potential-dependent or evoked IPSCs in CA1 pyramidal cells were reduced compared with those in wild-type mice[113]. This difference was not seen for mIPSCs in cultured CA1 neurons[90]. Although the reduction in evoked IPSC amplitude could reflect compensatory changes in either the probability of GABA release or the number of release sites without any change in quantal size, it is also consistent with the idea that phasic activation of α5-containing receptors might require synchronous multivesicular release and spillover of GABA onto receptors located beyond the synaptic cleft, as described for extrasynaptic or perisynaptic α6 and α4 subunit-containing receptors in granule cells of the cerebellum[114] and dentate gyrus[104]. This could be one of the mechanisms that contribute to the generation of slow dendritic IPSCs that are seen in CA1 neurons[115].

Overall, these findings indicate that receptors containing a γ2 subunit in association with α1, α2 or α3 subunits ($\alpha_1\beta_{2/3}\gamma_2$, $\alpha_2\beta_{2/3}\gamma_2$ and $\alpha_3\beta_{2/3}\gamma_2$) are the predominant receptor subtypes that mediate phasic synaptic inhibition. Receptors that contain α4, α5 or α6 subunits ($\alpha_6\beta_x\delta$, $\alpha_4\beta_x\delta$ and $\alpha_5\beta_x\gamma_2$) are predominantly or exclusively extrasynaptic. In cerebellar granule cells, the delayed development of the $GABA_A$ receptor-mediated tonic conductance was shown to mirror the delayed expression of α6 and δ subunits[42,44,99], and it was subsequently shown that this conductance was abolished after deletion of the α6 or δ subunits[89,116]. Similarly, deletion of the δ subunit (and the concomitant loss of α4 expression[117]) reduces tonic receptor activation in granule cells of the dentate gyrus[89] and relay neurons of the ventral basal thalamus[46], and deletion of the α5 subunit eliminates tonic conductance in cultured hippocampal neurons[90]. By contrast, ectopic overexpression of the α6 subunit in hippocampal pyramidal neurons results in an increased tonic conductance[92].

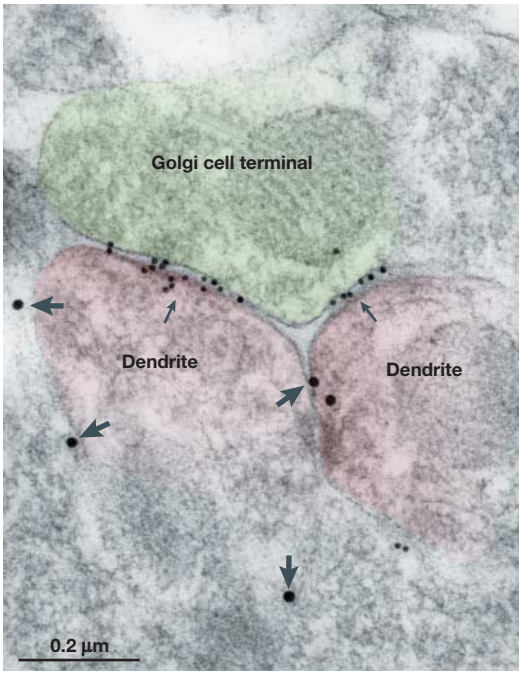


Figure 3 | **$GABA_A$ receptors in the mouse cerebellum.** Electron micrograph of mouse cerebellum showing synapses between a Golgi cell terminal (shaded green) and two granule cell dendrites (shaded red). The tissue is double labelled for $GABA_A$ (γ-aminobutyric acid type A) receptor β2/3 (10 nm gold particles) and δ (20 nm gold particles) subunits. Synapses (small arrows) made by the Golgi cell terminal with granule cell dendrites are not labelled for the δ subunit, although the enrichment of immunoparticles for the β2/3 subunits shows that receptor immunoreactivity is well preserved in these GABA-releasing synapses. Note the presence of immunoparticles for the δ subunit (large arrows) and β2/3 subunits (small arrows) at extrasynaptic dendritic membranes. Modified, with permission, from REF. 99 © (1998) Society for Neuroscience.

***Biophysical properties and subunit composition.*** Although variations in the subcellular locations of receptor subtypes undoubtedly contribute to their selective participation in tonic and phasic forms of activity, this distinction alone is not sufficient to account for their differential activation. Given the variations in

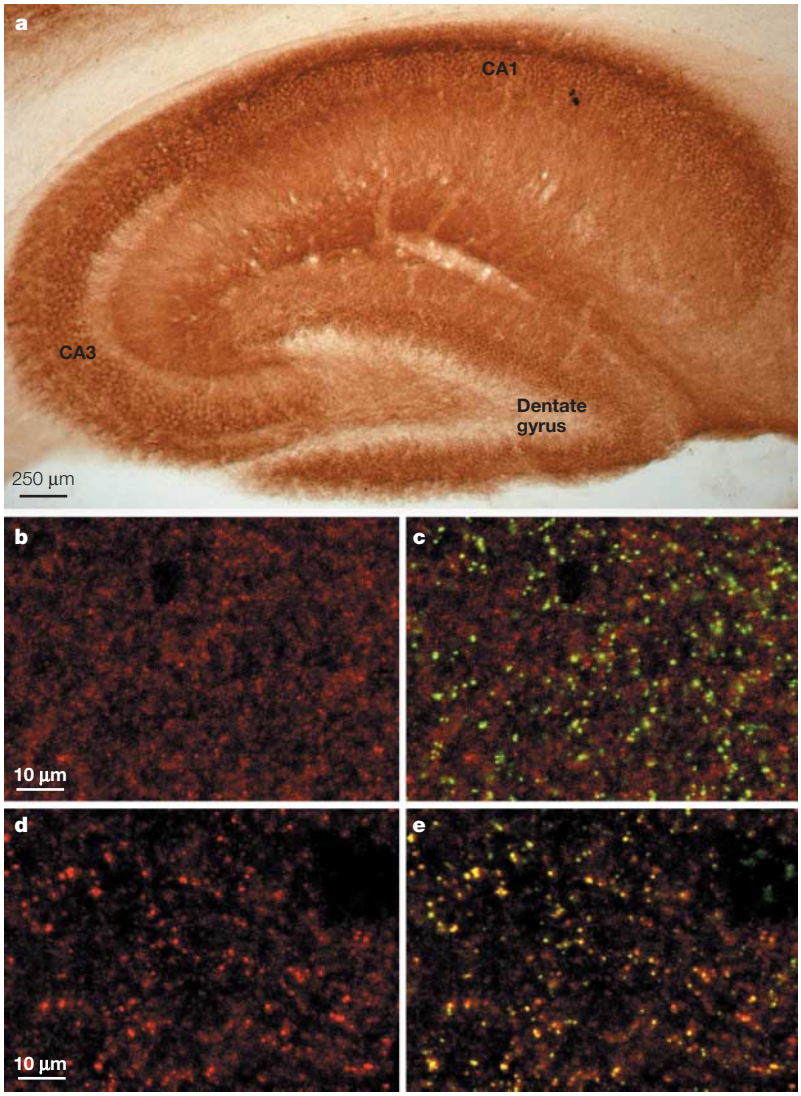


Figure 4 | **Expression of $GABA_A$ receptor subunits in the hippocampus.** **a** | Immunohistochemical localization of the $GABA_A$ (γ-aminobutyric acid type A) receptor α5 subunit in adult mouse hippocampus (peroxidase staining) reveals a layer-specific distribution in CA1, CA3 and the dentate gyrus. **b**,**c** | Double immunofluorescence labelling for the α5 subunit (red) and the $GABA_A$ receptor-associated protein gephyrin (green) in the adult mouse stratum radiatum. Gephyrin labels presumptive postsynaptic sites of GABA-releasing synapses. At high magnification we can see that the α5 subunit immunoreactivity is diffusely distributed in the neuropil. The double-labelled panel seems to show no evidence for α5 subunit clustering and co-localization with gephyrin. **d**,**e** | Double immunofluorescence labelling for the α2 subunit (red) and gephyrin (green) in the adult mouse stratum radiatum. In contrast to the α5 subunit, clusters of α2 subunit immunoreactivity co-localized with gephyrin are clearly visible (yellow), indicating α2 subunit aggregation at postsynaptic sites. Panel **a** courtesy of J.-M. Fritschy, Institute of Pharmacology and Toxicology, University of Zürich, Switzerland. Panels **b**–**e** reproduced, with permission, from REF. 112 © (2002) National Academy of Sciences USA.

GABA exposure that such receptors are likely to encounter, we might expect them to be endowed with distinct biophysical properties, particularly those associated with processes of binding (how the agonist interacts with the receptor) and gating (how the channel opens and closes in response).

Measurable macroscopic parameters include the concentration of ligand that gives the half-maximal response ($EC_{50}$), the rate of activation of the current following exposure to agonist, the rate and extent of desensitization of the current in the continued presence of the agonist and the deactivation of the current following agonist removal. These measurements reflect various microscopic parameters attributed to the receptors, which include the agonist binding and unbinding rates, and the rate constants of the transitions to and from open and desensitized states. Other measurable features that depend on the interplay of these various gating processes include the mean open and closed times and the mean burst durations of the channels, as well as the probability of channels being open when the receptors are fully occupied.

A key property of any ligand-gated ion channel is its sensitivity to endogenous agonist — that is, how much ligand is required to produce a given response. This reflects both the affinity of the receptor for its ligand (the equilibrium constant for the binding step) and the efficacy of the ligand (how effectively it promotes ion channel gating)[118]. For recombinant receptors that contain α, β and γ subunits, sensitivity to GABA is most strongly affected by the type of α subunit that is present, with α3 subunits conferring the highest and α6 subunits the lowest $EC_{50}$ values[119–123]. Across studies, the absolute $EC_{50}$ values for specific subunit combinations — and the relative differences between the various combinations — are variable, but, in studies in which α subunits have been compared, the rank order was shown to be α6<α1<α2<α4<α5<<α3 (REF. 121). Replacing the γ2 subunit in $\alpha_4\beta_3\gamma_2$ assemblies with a δ subunit decreases the $EC_{50}$ for GABA[124], but makes no difference to $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ assemblies[122]. Overall, $\alpha_6\beta_3\delta$ or $\alpha_4\beta_3\delta$ combinations have the lowest $EC_{50}$s for GABA (~0.3–0.7 µM), whereas for $\alpha_1\beta_3\gamma_2$ or $\alpha_2\beta_3\gamma_2$ subtypes they are an order of magnitude higher (~6–14 µM).

For receptors that become occupied by ligand and subsequently open, the conductance of their channels will influence the size of the response. The single-channel conductance of $GABA_A$ receptors depends on their subunit composition. Compared with changes in $EC_{50}$, the changes in conductance are more modest, and are essentially restricted to those that are produced by switching from dimeric αβ to ternary αβγ or αβδ assemblies. When recorded at room temperature in outside-out patches, αβ receptors (for example, $\alpha_1\beta_1$ or $\alpha_1\beta_3$) have a single-channel conductance of ~15 pS, whereas those incorporating a γ2 or a δ subunit (for example, $\alpha_1\beta_2\gamma_{2S}$ or $\alpha_1\beta_3\delta$) have a conductance of ~25–28 pS[4,120,125–127]. Changing the type of α or β subunit in the assembly has little or no effect on the conductance. Although recordings from immature neurons indicate the existence of native dimeric αβ receptors[4], most receptors in mature neurons probably contain either a γ or a δ subunit. So, we would expect that, in most mature neurons, $GABA_A$ receptors of roughly similar channel conductance underlie tonic and phasic inhibition.

The magnitude of the response is not determined solely by channel conductance: the time that the channels spend in the open state is equally important, and the kinetic properties that govern this phenomenon are also influenced by subunit composition. In the case of αβγ

and αβδ receptors, significant differences have been documented for both microscopic channel behaviours and macroscopic kinetic parameters. In $\alpha_1\beta_3\gamma_2$ receptors, replacing the γ2 with a δ subunit results in a roughly 5-fold reduction in both the mean open time and the duration of bursts of channel openings[120]. Single-channel data for the prevalent $\alpha_4\beta_x\delta$ combination[128] indicate that these receptors probably behave similarly, which is consistent with the idea that GABA has high affinity but low efficacy (GABA is a partial agonist) at δ subunit-containing receptors[124,129,130].

The activation, deactivation and desensitization of recombinant receptors are also greatly affected by their subunit composition. For $\alpha_x\beta_x\gamma_2$ receptors, direct comparison of receptors with different α subunits reveals up to 4-fold differences in the activation rate of currents evoked by rapid application of high concentrations of GABA, with rise times in the order α2<α1<α3 (REFS 21,131,132). The presence of a δ or γ2 subunit also influences activation, with rise times in the order $\alpha_1\beta_3\gamma_{2L}$«$\alpha_1\beta_3\delta\approx\alpha_1\beta_3$ (REF. 17). Insertion of a γ2 subunit (S but not L splice variant) into αβ receptors increases deactivation speed ~ 2-fold[133,134], as does replacing the γ2 with a δ subunit[17]. Moreover, for both αβγ and αβδ assemblies, the rate of deactivation depends on the type of α subunit present; for example, α1-containing $\alpha_x\beta_1\gamma_2$ receptors deactivate ~5-fold faster than those containing the α2 subunit[21], and α1-containing $\alpha_x\beta_3\delta$ receptors deactivate ~4-fold faster than those containing the α6 subunit[135].

The entry of $GABA_A$ receptors into desensitized states is thought to be important for shaping the time course of IPSCs[5,15,17,22,23,136]. Desensitization also affects the ability of postsynaptic receptors to respond to repetitive high-frequency activation[137] (but see REFS 138,139), and is of obvious importance with regard to the effect of a persistent low concentration of GABA. Ambient GABA can promote entry of receptors into partially bound, slowly desensitizing states[15,140,141], which potentially limits the magnitude of any tonic conductance, and also reduces the availability of synaptic receptors[140]. Consistent with the interrelation of deactivation and desensitization, the addition of a γ2 subunit to αβ receptors slows macroscopic desensitization[133,134]. Likewise, αβδ receptors desensitize more slowly and less extensively than αβγ receptors[17,137,142]. Again, for both αβγ and αβδ assemblies, the rate and extent of desensitization is influenced by the type of α subunit: receptors with an $\alpha_1\beta\gamma$ subunit composition desensitize more rapidly than those containing an α5 (REF. 90) or α6 subunit[143], whereas the opposite effect is observed for substitution of the α1 with an α6 subunit in αβδ receptors[135].

In summary, data from recombinant receptors show that all macroscopic and microscopic properties of $GABA_A$ receptors depend strongly on their subunit composition. The most important differences between receptors that mediate phasic inhibition and those that have been implicated in tonic inhibition are their affinities for GABA and the speed and extent of their desensitization. These different biophysical features, together with their differential cell surface distributions, are wholly consistent with their involvement in phasic and tonic signalling. However, this distinction does not preclude the possibility that under conditions of elevated extracellular GABA, or drug-induced increases in receptor affinity, other extrasynaptic and/or synaptic receptors might contribute to the generation of a tonic conductance. Notably, studies that have addressed the biophysical properties of recombinant receptors commonly investigate the effects of high concentrations of GABA that are relevant specifically to synaptic transmission. The use of lower GABA concentrations should provide a clearer view of the behaviour of receptor subtypes that are activated by ambient GABA[17,144,145].

## Modulation of phasic and tonic inhibition

The pattern of phasic inhibition that a neuron receives is obviously determined by the number, variety and activity of presynaptic GABA-releasing neurons, but whether tonic inhibition is similarly determined by neuronal activity is less clear. An ability to modulate the tonic conductance would seem to be essential if this form of inhibition is to reflect dynamic network activity, as opposed to simply providing a constant brake on excitability. Synaptic transmission relies on the interplay of many tightly regulated processes that together determine the timing, magnitude and kinetics of postsynaptic responses, and each of these processes might be subject to modulation. Tonic inhibition shows far fewer degrees of freedom, yet, owing in part to the low receptor occupancy, the dynamic range of modulation is potentially much larger. In theory, both phasic and tonic inhibition could be modulated by changes in GABA release or uptake and/or by changes in the number and properties of receptors.

***Modulation of GABA release and uptake.*** If the GABA that is released from synaptic vesicles contributes in any way to tonic inhibition, changes in presynaptic activity or release would be expected to modify the magnitude of the tonic conductance. This has been shown to be the case in the hippocampus, where stimulation of interneuron firing by the glutamate receptor agonist kainate increases the $GABA_A$ receptor-mediated tonic conductance in both pyramidal cells[146] and interneurons[147]. Furthermore, in cerebellar granule cells, facilitation of GABA release by acetylcholine causes a calcium-dependent, action potential-independent increase in the tonic conductance[61]. As the cerebellum receives cholinergic innervation, this latter mechanism could provide a physiologically relevant modulation of granule cell excitability. Blockade of action potential firing with TETRODOTOXIN has also been shown to reduce the tonic conductance in cultured neurons from the hippocampus[145] and cerebellum[148].

GABA transporters have well-documented effects on phasic inhibition, and they also have an important and dynamic influence on ambient GABA[149]. The extracellular GABA concentration at which they are at equilibrium, and consequently the magnitude of GABA flux, will vary depending on the membrane potential

TETRODOTOXIN
A potent marine neurotoxin that blocks voltage-gated sodium channels. Tetrodotoxin was originally isolated from the tetraodon pufferfish.

and the transmembrane gradients for the transported substrates (GABA, sodium and chloride). This also means that their ability to function as potential sources of GABA will be of greatest significance under pathological conditions or during exposure to drugs that increase the intracellular GABA concentration[149–152]. It is also recognized that transporters can undergo rapid redistribution between surface and intracellular compartments, and that their function can be altered by phosphorylation[153,154] or intermolecular interactions[155]. So, even in the face of unchanging GABA release, it is possible that ambient GABA concentration, and thereby tonic inhibition, could be modulated by changes in uptake.

***Physiological modulation of $GABA_A$ receptors.*** Many processes are known to modulate $GABA_A$ receptor number and function and these are likely to be relevant to both phasic and tonic inhibition. For example, the intracellular loops of β and γ subunits contain sites for phosphorylation by various protein kinases[156], and the intracellular loop of the γ2 subunit is a substrate for PALMITOYLATION by the thioacyltransferase GODZ[157]. These reversible, post-translational modifications have been shown to affect both the properties[158,159] and subcellular location[160,161] of the receptors. $GABA_A$ receptors can cycle rapidly between surface and intracellular domains, and probably move laterally within the membrane. In addition, through interaction with various cytosolic proteins, they can cluster at synaptic and non-synaptic sites[106,156,162]. This dynamic behaviour not only allows rapid changes in receptor number, but might also induce changes in receptor properties. For example, clustering of recombinant $GABA_A$ receptors by the receptor-associated protein GABARAP changes both their kinetic behaviour and single-channel conductance[163,164], and in cultured hippocampal neurons, cytoskeleton disruption reduces receptor clustering and alters the behaviour of both synaptic[165] and extrasynaptic[145] receptors. Finally, various pathological conditions (for example, epilepsy), hormonal fluctuations and chronic ethanol withdrawal have been shown to differentially affect the expression of subunits that have been implicated in tonic and phasic inhibition[166,167].

***Pharmacological modulation of tonic inhibition.*** Just as the biophysical properties of $GABA_A$ receptors are determined by their subunit composition, so are their pharmacological properties. The most frequently cited example is the role of the α subunits in defining their affinity for benzodiazepines (widely used $GABA_A$ receptor ALLOSTERIC modulators). Receptors formed from α1, α2, α3 or α5 subunits, together with two β and one γ2 subunit, have a high affinity for diazepam, a classic benzodiazepine agonist. Receptors containing the α1 subunit also have a high affinity for the imidazopyridine zolpidem (Ambien; Sanofi-Synthelabo). Changing the α subunit to α4 or α6 eliminates diazepam and zolpidem sensitivity, as does substitution of the γ2 subunit with a δ, ε or π subunit[105]. Subunit positioning within the pentamer is also crucial; for example, benzodiazepine sensitivity is affected by the type of α subunit that neighbours the γ2 subunit[123].

As might be expected, differences in subunit composition between synaptic and extra- or perisynaptic receptors are reflected in the differential modulation of phasic and tonic inhibition by benzodiazepine site ligands. In dentate gyrus granule cells, zolpidem prolongs the decay of IPSCs, but has no effect on the tonic conductance[45], which is thought to be mediated by benzodiazepine-insensitive $\alpha_4\beta\delta$ receptors. A similar differential effect is seen with diazepam in cerebellar granule cells[81], in which the tonic conductance is mediated by $\alpha_6\beta\delta$ receptors. In cultured hippocampal neurons, IPSCs are prolonged by both midazolam[48] (Versed; Roche) and zolpidem[90], whereas the tonic conductance that is seen in the presence of the GABA transaminase inhibitor vigabatrin (Sabril; Sanofi-Aventis) is enhanced only by midazolam[48,90,168], consistent with a tonic activation of $\alpha_5\beta_3\gamma_2$ receptors. By contrast, the tonic conductance in CA1 interneurons is enhanced by low concentrations of zolpidem[49] — a result that is inconsistent with the activation of α5 subunit-containing receptors in these cells. It should be noted that enhanced tonic conductance in the presence of a positive allosteric modulator is not as easy to interpret as a lack of effect, because any drug-induced increase in receptor affinity might recruit receptor populations (including synaptic receptors) that are not ordinarily activated by the low ambient GABA concentration.

For the experimental investigation of tonic and phasic inhibition, several competitive and non-competitive $GABA_A$ receptor antagonists have also been useful, not because of differences in their affinity for the receptors that underlie the tonic and phasic currents, but because their function depends on the affinity of the receptors for GABA and on the conditions of receptor activation. All $GABA_A$ receptor-mediated conductances are blocked by high concentrations of bicuculline, picrotoxin or SR-95531. However, a sub-micromolar concentration of SR-95531 selectively blocks phasic currents[48,49,91,168], consistent with the underlying receptors having a lower affinity for GABA than those that mediate the tonic current[91]. A differential block of phasic and tonic currents has also been observed with the open-channel blocker penicillin[168], and this has been proposed to reflect the low occupancy of the receptors that mediate the tonic conductance. Few $GABA_A$ receptor antagonists show clear subunit selectivity, but the diuretic furosemide (Lasix; Aventis) has ~100-fold selectivity for α6 over α1 subunit-containing receptors[123,169], and has been used to determine the role of synaptic and extrasynaptic α6-containing receptors in cerebellar granule cells[81,170,171]. The only agonist that shows a clearly different profile of action at receptors of synaptic or extrasynaptic subtype is 4,5,6,7-tetrahydroisothiazolo-[5,4-c]pyridin-3-ol (THIP (Gaboxadol; Lundbeck/Merck)). This compound is a partial agonist at $\alpha_4\beta_3\gamma_2$ receptors but behaves as a full or 'super' agonist at $\alpha_4\beta_3\delta$ receptors, producing a maximum response greater than that produced by GABA[124].

Tonic inhibition also seems to be highly sensitive to modulation by various clinically relevant agents, including endogenous neuroactive steroids (FIG. 5), intravenous and inhalation anaesthetics, certain NOOTROPIC agents

PALMITOYLATION
The covalent attachment of a palmitate (16-carbon, saturated fatty acid) to a cysteine residue through a thioester bond.

ALLOSTERIC
A term originally used to describe enzymes that have two or more receptor sites, one of which (the active site) binds the principal substrate, whereas the other(s) bind(s) effector molecules that can influence the enzyme's biological activity. More generally, it is used to describe the indirect coupling of distinct sites within a protein, mediated by conformational changes.

NOOTROPIC
Refers to agents that enhance memory or other cognitive functions.

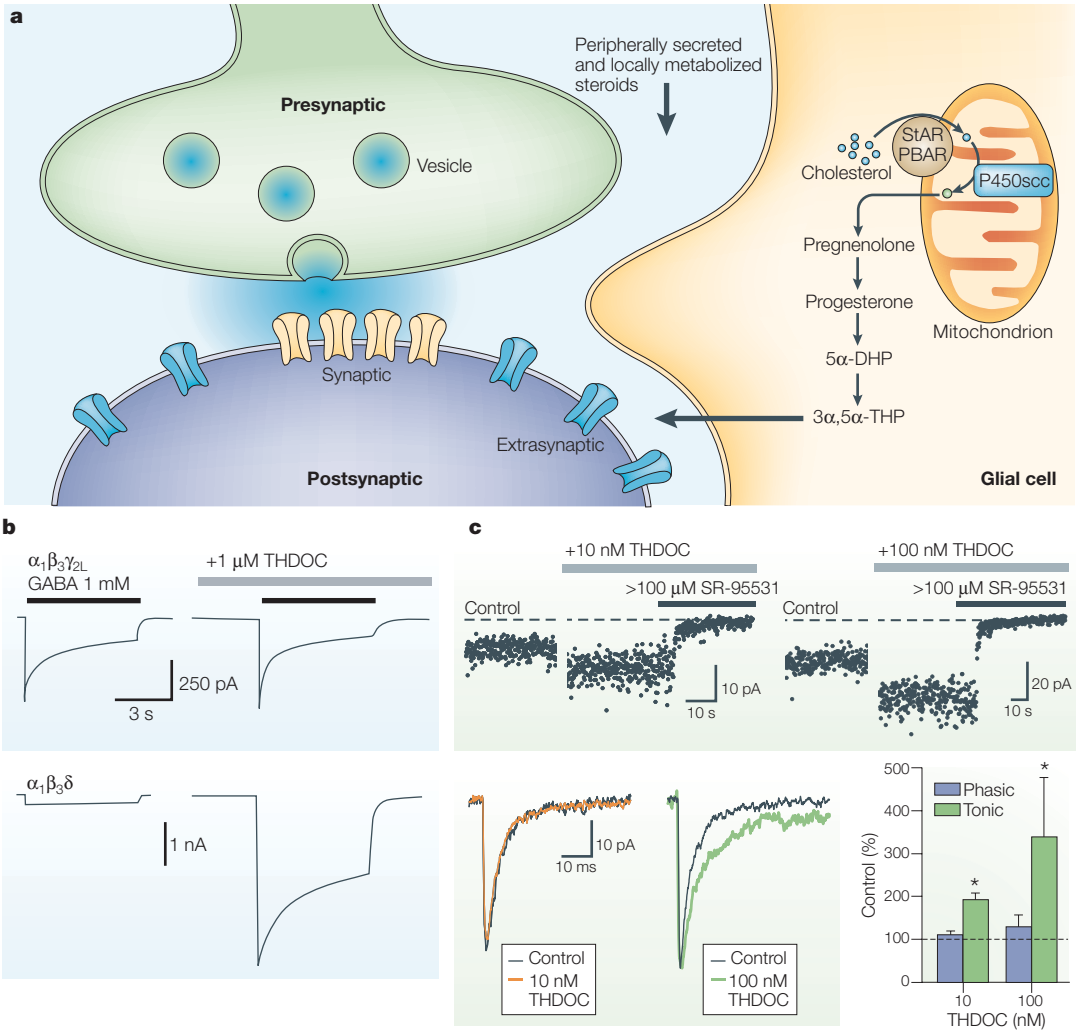


Figure 5 | **Modulation of δ subunit-containing $GABA_A$ receptors by neuroactive steroids. a** | Neuroactive steroids are formed *de novo* in neurons and glia, or generated by the metabolism of circulating precursors that originate in peripheral steroidogenic organs[212]. One pathway for glial neurosteroidogenesis is shown: cholesterol is taken into mitochondria by the action of the steroidogenic acute regulatory protein (StAR) and the peripheral benzodiazepine receptor (PBAR). Pregnenolone is formed by the action of the enzyme cytochome P450 side chain cleavage (P450scc). In this example, subsequent metabolism in the smooth endoplasmic reticulum through progesterone and 5α-dihydroprogesterone (5α-DHP) leads to the formation of 3α,5α-tetrahydroprogesterone (3α,5α-THP; allopregnanolone). Secretion from the glial cell is indicated by a bold arrow. **b** | The steroid 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone, THDOC) is secreted from the adrenal gland and is also formed in the brain from its peripherally secreted precursor, deoxycorticosterone. THDOC differentially modulates $\alpha_1\beta_3\gamma_{2L}$ and $\alpha_1\beta_3\delta$ receptors when they are expressed in human embryonic kidney 293T cells. Currents evoked from $\alpha_1\beta_3\gamma_{2L}$ receptors are minimally affected by THDOC (1 μM), but those from $\alpha_1\beta_3\delta$ receptors are markedly enhanced. **c** | Low concentrations of THDOC selectively enhance the magnitude of the tonic current that is mediated by extrasynaptic $\alpha_6\beta\delta$ receptors in cerebellar granule cells (blue receptors in **a**), with little effect on synaptic responses (yellow receptors in **a**). Current values were averaged over 10-ms epochs at 100-ms intervals. Horizontal bars indicate the application of THDOC (grey) and the $GABA_A$ (γ-aminobutyric acid type A) antagonist SR-95531 (gabazine) (black). The dashed line is the mean current observed after complete block of $GABA_A$ receptors, which was used to calculate the magnitude of the tonic $GABA_A$ receptor-mediated conductance. This conductance (top panel) is increased in the presence of both 10 and 100 nM THDOC, whereas spontaneous inhibitory postsynaptic potentials (IPSCs) (lower traces) are minimally affected. The histogram shows the effects of THDOC on the tonic conductance (green) and average charge transfer through phasic IPSCs (blue) expressed as a percentage of control values in the absence of THDOC (dashed line). Error bars denote standard error mean, and asterisks denote statistical significance. nA, nanoAmp; pA, picoAmp. Panel **b** modified, with permission, from REF. 130 © (2003) Society for Neuroscience. Panel **c** modified, with permission, from REF. 89 © (2003) National Academy of Sciences USA.

and alcohol. In line with the selective enhancement of the GABA responsiveness of δ subunit-containing receptors by neurosteroids[124,129,172,173], a low concentration of 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone or THDOC) significantly increases the tonic conductance in granule cells of the dentate gyrus and cerebellum without modifying phasic currents[89]. Similarly, the high sensitivity of $\alpha_4\beta\delta$ receptors to ethanol[174,175] is mirrored by the selective augmentation of tonic inhibition in granule cells of the dentate

ALCOHOL NON-TOLERANT RATS (ANT rats). A rat line that has been selectively bred to be highly sensitive to motor impairment after ethanol intake.

gyrus[176]. Recent evidence also indicates that $\alpha_6\beta_3\delta$ receptors, when formed from α6 subunits that have the same point mutation found in ALCOHOL NON-TOLERANT RATS, are particularly sensitive to enhancement by ethanol, and might underlie the motor impairment that is produced by alcohol consumption[177]. In the case of ethanol and the neurosteroids, it is a particular challenge to distinguish between the relatively slow changes induced by prolonged exposure to or withdrawal from these agents (see above) and their acute allosteric modulatory effects.

In cultured hippocampal neurons, the amnesic drugs propofol (Diprivan; Zeneca)[48,168] and isoflurane (Forane; Abbott)[178] preferentially enhance the $GABA_A$ receptor-mediated tonic conductance, whereas the nootropic α5-selective inverse agonist L-655,708 preferentially inhibits the tonic conductance[90], consistent with a proposed role for extrasynaptic α5 subunit-containing receptors in regulating learning and memory[113].

## Conclusions

In the decade since the tonic activation of $GABA_A$ receptors in mature CNS neurons was first identified, a considerable amount of experimental evidence has accumulated to show the presence of tonic inhibition in various brain regions. In contrast to the spatially and temporally discrete nature of phasic inhibition, tonic inhibition results from random, persistent activation of $GABA_A$ receptors. The receptor subtypes that mediate the two forms of inhibition have distinct biophysical and pharmacological properties, as well as different subcellular locations. For high-affinity extrasynaptic receptors that mediate tonic inhibition, their low occupancy, combined with the low efficacy of GABA, allows their regulation over a large dynamic range. Future work will surely consolidate our understanding of these fundamental differences, and facilitate the development of selective methods for modulating neuronal excitability under both physiological and pathological conditions.


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## Competing interests statement

The authors declare no competing financial interests.

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