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Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone

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$GABA_A$ receptors not only respond to the local release of GABA from presynaptic terminals, but can also mediate a persistent 'tonic current'. This reflects the activation of high-affinity $GABA_A$ receptors by ambient GABA concentrations. Tonic $GABA_A$-receptor-mediated signalling occurs in different brain regions, shows cell-type-specific differences in magnitude and pharmacology, and changes during brain development. Some clues to the adaptive significance of this phenomenon are beginning to emerge: in cerebellar granule cells, it alters the gain of transmission of rate-coded sensory information; in the hippocampus, it acts in a cell-type-specific manner to regulate the excitability of the network. Because tonic conductances can be modulated by changes in GABA release and uptake, and by modulators of high-affinity $GABA_A$ receptors including neurosteroids, this phenomenon provides a potentially important new window onto neuronal information processing and pathological states such as epilepsy.

$GABA_A$ receptors are essential for information processing and are involved in many pathological processes such as epilepsy, pain and anxiety. Individual inhibitory postsynaptic currents (IPSCs), which arise from synaptic contacts, transiently inhibit neurons for 10–100 ms. This 'phasic' inhibitory action is important for timing-based signalling, setting the temporal window for synaptic integration [1] and synchronizing networks of neurons [2]. Recent studies suggest that $GABA_A$-receptor-mediated signalling also operates on much slower time scales. Some slow $GABA_A$-receptor-mediated IPSCs probably arise from spillover of neurotransmitter from synapses that are formed on neighbouring cells [3]. An extreme form of this phenomenon is the continuous activation of $GABA_A$ receptors by ambient neurotransmitter, which has now been demonstrated in several areas of the CNS. Although the underlying mechanisms of tonic $GABA_A$-receptor-mediated conductance have been explored extensively, much less is known about its physiological and/or pathological significance. This article reviews recent advances in our understanding of this intriguing phenomenon, its potential function and areas for future research.

Where are tonic $GABA_A$-receptor-mediated currents found?

Tonic $GABA_A$-receptor-mediated currents were first described in cerebellar granule cells in voltage-clamp experiments, where application of $GABA_A$ receptor antagonists was found to reduce the current required to keep the cell at a fixed potential (the 'holding current'). This was accompanied by a decrease in the background noise, consistent with block of stochastic ion channel openings [4–6] (Figure 1a). At about the same time, tonic $GABA_A$ receptor activation was also described in embryonic neocortical cells *in situ* in the ventricular zone [7]. A similar phenomenon was subsequently reported in granule cells of the dentate gyrus [8–10] (Figure 1b) and inhibitory interneurons in the CA1 region of the hippocampus [11] (Figure 1c). However, a significant tonic $GABA_A$ receptor current is absent in adult hippocampal pyramidal cells and can be detected only in early development [12] or in specific circumstances. These include blockade of GABA uptake [13], ectopic expression of α_6 $GABA_A$ receptor subunits [14], kainate application (as will be discussed) [15] or during GABA perfusion [16]. In granule cells and interneurons, the current carried by the tonic $GABA_A$-receptor-mediated conductance can be larger than the time-averaged spontaneous IPSCs. It is therefore likely to be a major determinant of neuronal excitability.

Do specific $GABA_A$ receptor subtypes mediate the tonic current?

Detection of the low extracellular GABA concentrations that persist in the presence of GABA uptake requires high-affinity non-desensitizing receptors. The finding that the tonic conductance can be increased by manipulations that elevate the extracellular GABA concentration implies that the receptors involved are not saturated by ambient neurotransmitter. It is likely, however, that the tonic current is mediated by a heterogeneous population of receptors, with differing GABA affinities. Increasing the GABA concentration alters the relative contribution of specific $GABA_A$ receptors to the tonic current as different receptor populations are recruited [11]. It is also possible that a component of the current is independent of GABA concentration because some receptors can open spontaneously in the absence of agonist [17]. Spontaneously

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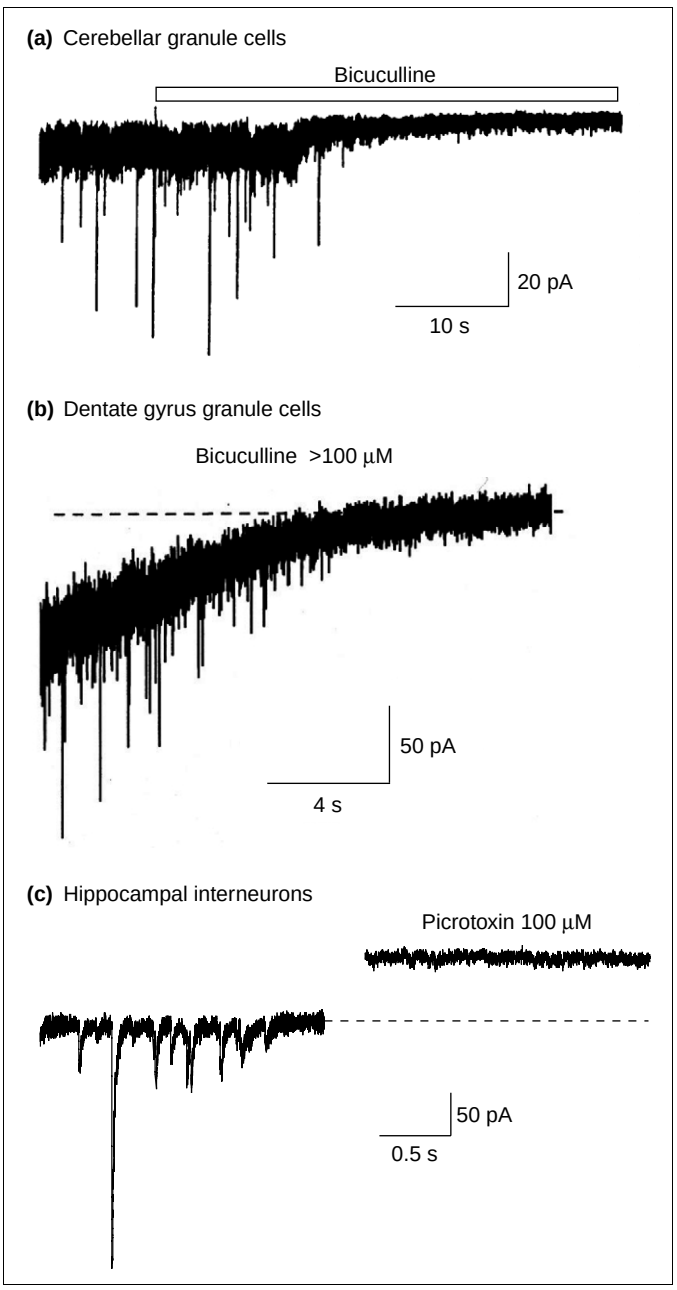

Figure 1. Tonic $GABA_A$-receptor-mediated current in different cell types in the brain. **(a)** Application of bicuculline (10 μM), a $GABA_A$ receptor antagonist, to cerebellar granular cells from young (postnatal-day 14) rats (holding potential −70 mV) decreased not only spontaneous inhibitory postsynaptic currents (sIPSCs) but also the background noise, indicating a block of stochastic ion channel openings. This is consistent with inhibition of tonically active $GABA_A$ receptors. Reproduced, with permission, from Ref. [5]. **(b)** Addition of bicuculline to the perfusate (final concentrations of 100–150 μM) abolished sIPSCs and reduced the holding current in dentate granule cells; the shift in the holding current indicates inhibition of the tonic $GABA_A$-receptor-mediated current. Reproduced, with permission, from Ref. [8]. **(c)** Picrotoxin (another $GABA_A$ receptor antagonist) at 100 μM blocked sIPSCs and reduced the holding current in hippocampal interneurons, illustrating the presence of a tonic $GABA_A$-receptor-mediated current in these cells. Reproduced, with permission, from Ref. [11] © Nature Publishing Group (http://www.nature.com/).

open receptors cannot, however, be the sole contributor to the tonic current, because enzymatic degradation of ambient GABA reduces the tonic current in granule cells [6].

Although the receptors mediating the tonic current are likely to have a high affinity for GABA, a variety of $GABA_A$ receptor subtypes are involved in specific neuronal populations (Box 1). Benzodiazepine-insensitive

Box 1. Which subunits are responsible for tonic activation of $GABA_A$ receptors?

Cerebellar granule cells

Involvement of $GABA_A$ receptors containing α_6 and δ subunits (Figure I of this box) in the tonic conductance seen in cerebellar granule cells is supported by the following observations. First, $GABA_A$ receptors containing α_6 and δ subunits show little desensitization and high sensitivity to GABA [60]. Second, these subunits are expressed with a similar developmental profile to the tonic current [5,6,61,62]. Third, the α_6-selective antagonist furosemide selectively blocks the tonic component [21]. Similarly, the sensitivity of the tonic conductance to the neurosteroid allotetrahydrodeoxycorticosterone (THDOC) [16,21] is consistent with the pharmacological profile of $\alpha_6\delta$-containing receptors [63,64]. Fourth, the tonic conductance mediated by $GABA_A$ receptors is absent from α_6-knockout [65] and δ-knockout [16] mice. Finally, immunohistochemical studies have established that δ-subunit-containing receptors are located extrasynaptically in granule cells [18] and thus can only be activated by ambient GABA or spillover of GABA from synapses.

Dentate gyrus granule cells

Benzodiazepine-insensitive δ-containing $GABA_A$ receptors have also been implicated in generating tonic $GABA_A$-receptor-mediated currents recorded in hippocampal dentate granule cells [8–10]. Consistent with this observation, tonic $GABA_A$-receptor-mediated currents recorded in granule cells, either in the cerebellar cortex or in the dentate gyrus, have been reported to be unaffected by benzodiazepine agonists. The recent finding that a tonic current can be detected in dentate granule cells from δ-knockout mice, albeit reduced in amplitude, implies that a heterogeneous population of $GABA_A$ receptors is responsible for tonic conductance in these cells [16] (Figure I of this box), unless there is a compensatory upregulation of other subunits.

Hippocampal interneurons

Hippocampal interneurons have been reported to express δ subunits at low level [66]. This observation provides a candidate high-affinity receptor to mediate the tonic GABA signal in these cells. However, the tonic conductance is robustly increased by the benzodiazepine agonist zolpidem [11], which is relatively ineffective at δ-containing receptors studied in heterologous expression. The zolpidem effect points instead to the involvement of γ-subunit-containing receptors (Figure I of this box). Possibly relevant to this finding is that very low concentrations of GABA can activate benzodiazepine-sensitive $GABA_A$ receptors in cultured hippocampal neurons [20,67].

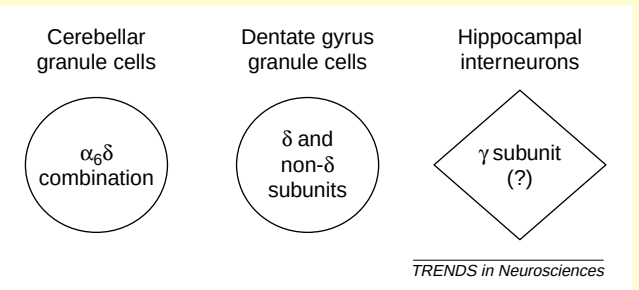

Figure I. Subunits contributing to tonically active $GABA_A$ receptors in different cells. The tonic current in cerebellar granule cells is mediated by $GABA_A$ receptors containing δ and α_6 subunits. In dentate granule cells, the tonic current only partially depends on $GABA_A$ receptors containing the δ subunit. In hippocampal interneurons (and pyramidal cells when ambient GABA levels are elevated), at least part of the tonic current is mediated by γ-subunit-containing receptors.

δ-subunit-containing receptors are probably the major contributors to the tonic current in cerebellar and dentate granule cells [8,18], but the other subunits that make up the high-affinity $GABA_A$ receptors are different in these two cell types. In interneurons, the tonic current is mediated by benzodiazepine-sensitive (and therefore probably γ-containing) receptors [11]. Because γ subunits also contribute to the synaptic targeting of $GABA_A$ receptors [19], it is possible that synaptic receptors can both detect local exocytosis and respond to the tonic extracellular GABA concentration. Two observations nevertheless suggest that different receptors mediate phasic and tonic signalling in interneurons, pyramidal cells and granule cells. Low concentrations of the antagonist SR95531 (gabazine) completely abolish IPSCs while having little or no effect on tonic $GABA_A$-receptor-mediated signalling in hippocampal neurons [10,11,13,20], and furosemide (frusemide), a selective blocker of α_6-containing receptors, selectively blocks the tonic current in cerebellar cells [21]. This does not, however, exclude the possibility that singly and doubly bound $GABA_A$ receptors mediate tonic and phasic signalling respectively, or that different bound states contribute to the distinct pharmacologies [22]. In the hippocampus proper, blocking GABA uptake reveals a tonic current that is partially sensitive to low concentrations of SR95531 in both interneurons and pyramidal cells [11]. This suggests that at higher GABA concentrations, the tonic current can be mediated by $GABA_A$ receptors that are pharmacologically similar to those that mediate IPSCs.

The role of regional extracellular GABA regulation

The properties of the $GABA_A$ receptors, although important, are not the only mechanism that could underlie the cell-type specificity of tonic $GABA_A$ receptor conductances. The magnitude of the tonic current depends crucially on the concentration of GABA, as demonstrated by the enhancement of tonic $GABA_A$-receptor-mediated currents by increasing the GABA concentration in the slice perfusate or by inhibiting GABA metabolism or uptake. Local differences in the ambient extracellular GABA concentration could thus also play a crucial role in the regional specificity of tonic inhibition.

Ambient GABA can originate from various sources (Box 2). Once released, GABA diffuses throughout the neuropil before being taken up by transporters. The local morphology is therefore likely to influence the lifetime of GABA molecules in the extracellular space and how effectively they activate GABA receptors. Synapses onto cerebellar granule cells are made within glomeruli, which comprise excitatory and inhibitory terminals and dendritic processes all ensheathed within a glial coat. This synaptic compartment is thought to act as a diffusion barrier, trapping GABA molecules before their slow clearance by transporters. In contrast to this unusual morphological arrangement, neurotransmitter fluxes in the cerebral cortex are conventionally described by release from synaptic varicosities into a relatively porous neuropil [23]. Thus, diffusion dominates the early escape of GABA from the synaptic cleft, with uptake by transporters mainly contributing to later clearance of GABA from the extracellular space.

There is also evidence of regional differences in GABA uptake and release in different strata of the hippocampus [24,25]. Thus, the GABAergic synaptic density is high within the pyramidal cell layer, which also shows a relatively low extracellular volume fraction [26]. Because the GABA transporter GAT-1 is abundant in GABAergic terminals, it is likely that uptake has a larger role to play in this stratum (and diffusion a relatively lesser role) than in stratum oriens or stratum radiatum. The cellular location of the $GABA_A$ receptors mediating the tonic current is unknown, so we cannot be certain of the influence of these findings on the expression of tonic signalling in interneurons and pyramidal cells. Nonetheless, the principle of regional variation of extracellular GABA levels has important implications for tonic $GABA_A$-receptor-mediated signalling, and its cell-type specificity.

Cell-type-specific development of the tonic current

Tonic $GABA_A$ receptor currents are expressed in neuronal progenitor cells, where they might play a part in neural development [7,27]. What about the role and variation of the tonic current during development in differentiated neurons?

In cerebellar granule cells, the tonic $GABA_A$-receptor-mediated conductance increases with maturation [5,6], while IPSCs become smaller and faster [5,28]. The tonic conductance becomes comparable to the peak amplitude of the phasic component in granule cells in the adult [5]. When charge transfer is considered, the tonic component dominates, conveying the vast majority of the inhibitory charge [21]. Increases in the tonic component are paralleled by morphological changes to the cerebellar glomerulus, including increased numbers of Golgi cell terminals, and a more complete ensheathing by glial cells [29]. It is likely that these changes lead to greater GABA release and entrapment in the glomerulus, and thus to the increased tonic conductance seen in older animals [6]. However, it is also possible that some of the increased tonic current arises from an increase in GABA release via a non-vesicular Ca^{2+}-independent mechanism [30] (Box 2), and/or an increase in the expression of $GABA_A$ receptors.

Hippocampal pyramidal cells early in development appear to show evidence of a robust tonic $GABA_A$-receptor-mediated conductance [12] that is not present in adult tissue. This suggests that the phenomenon shows an opposite developmental progression to that observed in cerebellar granule cells.

These developmental changes are accompanied by changes in the reversal potential for $GABA_A$ receptors, which is at least partly explained by increased expression of the Cl^- transporter KCC2 [31]. This results in depolarizing GABA responses in neonatal animals and hyperpolarizing responses in juvenile rats [32]. The excitatory actions of GABA might play a role in cell maturation and regulating DNA synthesis by stimulating Ca^{2+} entry [5,7], whereas in juveniles and adults the tonic $GABA_A$-receptor-mediated conductance has a mainly inhibitory function.

Box 2. Sources of GABA contributing to ambient concentrations

Spillover

GABA escaping the synaptic cleft can potentially reach receptors located on extrasynaptic membranes, neighbouring presynaptic terminals, and receptors present in neighbouring synapses [11,68–71] (Figure I of this box). Transmitter spillover is thought to underlie both slow-rising $GABA_A$-receptor-mediated inhibitory postsynaptic currents (IPSCs) [3,21] and part of the tonic $GABA_A$ currents in cerebellar granule cells [5].

Non-synaptic release

Several action-potential-independent mechanisms have been proposed to mediate amino acid neurotransmitter release, including reversed transport, stretch-activated anion channels and $P2X_7$-receptor-triggered release that depends on Cl^- and HCO_3^- [72–74] (Figure I of this box). However, a hyperosmotic solution, which reduced the mean diameter of cerebellar granule cells by 21%, had no effect on the tonic current, arguing against a major role for stretch-activated channels [30]. Reversed transport is thermodynamically more feasible for GABA than for glutamate because two rather than three Na^+ are cotransported [35]. However, in adult cerebellar granule cells, block of the GABA transporter GAT-1 has no effect on the tonic current, whereas block of GAT-3 increases the current [30]. These results suggest that action-potential-independent GABA release does not occur by reversed transport, at least in cerebellum. Instead, GABA could arise either from Golgi cell terminals or astrocytes from a non-vesicular Ca^{2+}-independent mechanism [30]. Such a mechanism might be analogous to a form of Ca^{2+}-independent and soluble-NSF-attachment-protein receptor (SNARE)-independent release of GABA observed before synapse formation in the developing hippocampus [12].

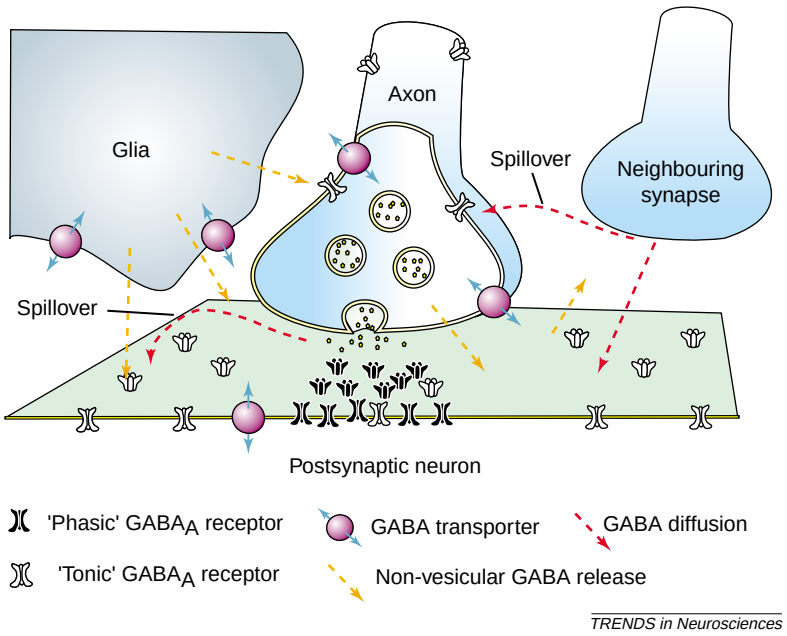

Figure I. Sources and targets of extrasynaptic GABA. Extracellular GABA is regulated by release, diffusion and uptake mechanisms. GABA can escape from synaptic cleft (GABA spillover) and can be released via non-vesicular mechanism by neurons and glia. Once released, GABA can reach target receptors on presynaptic terminals, axon and somatodendritic compartments.

Plasticity and modulation

Several recent studies have shown that changes in GABA release can modulate tonic $GABA_A$-receptor-mediated currents. During periods of intense synaptic activity, extracellular GABA concentrations rise [33], potentially increasing the magnitude of the tonic current. This is illustrated by the effect of kainate application, which produces robust firing of interneurons (and thus GABA release), resulting in an increase in tonic $GABA_A$-receptor-mediated currents both in hippocampal pyramidal cells [15] and in interneurons [34]. In mature cerebellar granule cells, where GABA arises partly from action-potential-independent sources, tonic inhibition can be modulated via a different mechanism: application of ACh produces a fourfold increase in the tonic conductance via a GABA release mechanism that depends on Ca^{2+} but does not depend on action potentials [30] (Figure 2a).

Short-term changes in GABA uptake can also occur if cells are depolarized and electrochemical gradients for transportable substances (e.g. GABA and inorganic ions) are perturbed [35]. Glial cell coverage (and therefore neurotransmitter uptake) has also been shown to change with hormonal status in the rat supraoptic nucleus [36]. The full impact of these forms of modulation remains to be established.

The observation that δ-subunit-containing $GABA_A$ receptors are highly sensitive to endogenous neurosteroids has considerable implications for modulation of tonic currents mediated by these receptors [37]. This was highlighted by a recent study in which the tonic conductance in dentate gyrus and cerebellar granule cells was approximately doubled by the neurosteroid allotetrahydrodeoxycorticosterone (THDOC) at physiological concentrations (10 nM) [16] (Figure 2b,c). Because progesterone metabolites act as neurosteroids, the finding that this class of molecule modulates tonic signalling provides a potential explanation for fluctuations in the incidence of seizures in patients with catamenial epilepsy, and even possibly for affective disorders due to a fall in

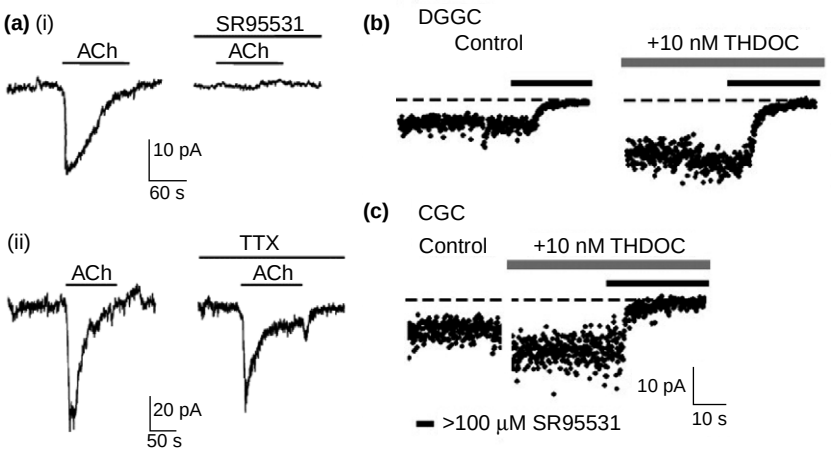

Figure 2. Modulation of $GABA_A$-receptor-mediated tonic current by ACh and allotetrahydrodeoxycorticosterone (THDOC). **(a)** Application of ACh (100 μM) resulted in an inward current mediated by $GABA_A$ receptor activation (the current was abolished by the $GABA_A$ receptor antagonist SR95531) (i). Sequential responses to ACh decreased only slightly with repeated applications, and blocking action potential generation with tetrodotoxin (TTX, 1 μM) had no effect, consistent with action-potential-independent release of GABA by ACh (ii). Reproduced, with permission, from Ref. [30]. **(b,c)** The tonic $GABA_A$-receptor-mediated current was enhanced by the neurosteroid THDOC (10 nM, grey bar) both in dentate gyrus granule cells (DGGC; b) and in cerebellar granule cells (CGC; c). Black horizontal bars indicate the time when SR95531 was directly injected into the recording chamber in excess of 100 μM. The dashed line is the mean current after complete block of $GABA_A$ receptors. Reproduced, with permission, from Ref. [16].

progesterone levels that can accompany the menstrual cycle. However, whether this results in a decrease in tonic inhibition in dentate granule cells is unclear, because an increase in expression of α_4 and δ subunits has also been reported in an experimental model of progesterone withdrawal [38].

The modulation of tonic $GABA_A$-receptor-mediated currents by neurosteroids is influenced by local neurosteroid metabolism, inhibition of which can greatly enhance the response of the tonic current in dentate granule cells to endogenous neurosteroids, while having no effect on the response to the synthetic metabolically stable neurosteroid ganaxalone [39]. Such neurosteroid metabolism demonstrates regional specificity, which could further contribute to the regional specificity of the tonic $GABA_A$-receptor-mediated currents, and their modulation. This raises the intriguing possibility of selectively regulating network excitability with inhibitors of neurosteroid metabolism; these could thus have an antiepileptic action. Another potentially important modulator of high affinity $GABA_A$ receptors is ethanol, which has been reported to potentiate currents mediated by $\alpha_4\delta$-containing receptors at socially relevant concentrations [38].

Tonic signalling can also be modulated by mechanisms that determine the relative expression of high- and low-affinity $GABA_A$ receptors: genetic overexpression of α_6 subunits in hippocampal pyramidal cells increases the tonic current [14]. Although this example reflects experimental perturbation of receptors, there is abundant evidence that $GABA_A$ receptor subunits can be modulated by seizures. Indeed, experimental epileptogenesis and human epilepsy are accompanied by changes in subunits that may either contribute to, or compensate for, the hyperexcitable state [40].

Effect of tonic inhibition on computations within individual neurons

The membrane conductance plays an important role in neuronal processing because it determines both the voltage response to a current and the membrane time constant. The changes in the membrane time constant and input resistance that occur during changes in tonic inhibition therefore alter the time window over which synaptic integration occurs [11,41]. These alterations in membrane properties can modulate both the conductance threshold (and current threshold) for firing and the firing pattern. Two recent studies that have investigated the effect of tonic inhibition in cerebellar granule cells suggest that it could play an important role in processing rate-coded sensory information as it flows through the cerebellar cortex [30,41].

Processing of rate-coded information can be defined in terms of the relationship between the mean excitation (i.e. current, conductance or input firing rate) and mean output firing rate (Figure 3). A change in slope of this input–output relationship corresponds to a multiplicative operation (or gain change), whereas a shift along the *x*-axis corresponds to an additive operation [42,43]. Mitchell and Silver [41] studied the effects of tonic inhibition on synaptic integration and the input–output relationship using dynamic clamp [44] to inject excitatory and inhibitory conductances into granule cells. The granule cell is particularly suited to this approach because the soma and dendrites form a single electrical compartment [45]. Conductances injected through a patch pipette at the soma are therefore integrated in a similar manner to synaptic conductances. The mathematical operations performed on the granule cell input–output relationship by modulating the level of tonic inhibition were found to depend on the properties of the excitatory input. When excitation was mediated by a constant conductance, increasing tonic inhibition performed an almost perfect subtraction on the input–output relationship [41] (Figure 3a,b), as predicted from theory [46] and observed previously for step current injections [5]. However, when excitation was mediated by random trains of synaptic conductance waveforms, which mimic physiological

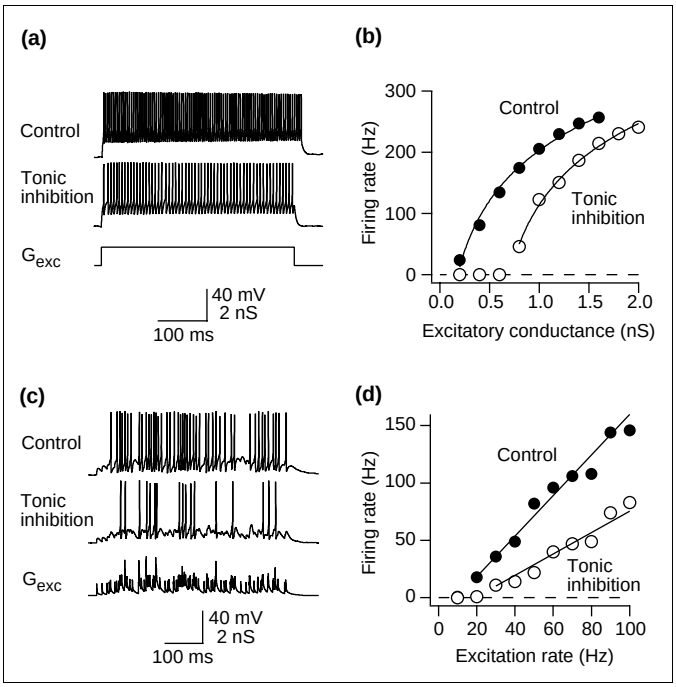

Figure 3. Mathematical operations performed by tonic inhibition depend on the properties of excitation. **(a)** Recording from a cerebellar granule cell in slice at 37 °C, firing in response to a 1 nS tonic excitatory conductance (G_{exc}) in the absence (control) and presence of a 1 nS tonic inhibitory conductance with a reversal potential of −75 mV. **(b)** Relationship between output firing frequency and tonic excitatory conductance amplitude under control conditions and in the presence of 1 nS tonic inhibition. The slope, or gain, of the relationship at each output frequency was similar for control and inhibition. Tonic inhibition therefore performed a subtractive operation on the input–output relationship under these conditions. **(c)** Granule cell firing in response to four independent 50 Hz Poisson trains of excitatory synaptic conductance waveforms (G_{exc}) for control conditions and in the presence of 1 nS tonic inhibition. **(d)** Relationship between output frequency and excitation frequency under control conditions and in the presence of 1 nS tonic inhibition. In contrast to (b), the gain of the relationship at each output frequency was reduced by tonic inhibition. Tonic inhibition therefore performed a multiplicative scaling operation on the input–output relationship under conditions that mimic rate-coded inputs *in vivo*. Reproduced, with permission, from Ref. [41].

excitation *in vivo* [47], tonic inhibition changed the slope of the input–output relationship at each output frequency (Figure 3c,d) – a multiplicative (divisive) gain change [41]. This change in gain alters the sensitivity of a neuron to changes in the input frequency, while the accompanying shift along the *x*-axis allows subtraction of baseline levels of excitation. Gain increases were also observed when tonic inhibition was blocked with furosemide and granule cells were excited with synchronized mossy fibre stimulation [21]. These multiplicative scaling operations are analogous to changing the volume on a hi-fi and are an important feature of almost any system that can perform useful computations, whether it is implemented in the brain or in an electronic circuit [48].

Modelling predicts that the mathematical operations performed by tonic inhibition depend on the variability of the excitatory rate-coded input [41]. Changes in the level of tonic inhibition in neurons with large fast excitatory inputs, which generate noisy excitation, are likely to produce robust changes in gain. This occurs because the gain of the input–output relationship depends on the variability of the input conductance [49,50]. Increasing the level of tonic inhibition shifts, to a higher level, the excitatory input frequency required to attain a given output firing rate. This increases the variance of the input conductance at each output firing frequency, thus reducing the gain of the input–output relationship [41]. It will be interesting to investigate whether tonic inhibition-mediated gain modulation is present in the other neuronal types because this would establish a general role for this form of inhibition.

Effects on network excitability

Modulation of neuronal gain and firing threshold are important for maintaining the firing rate within the operational range over a wide range of excitatory drive. This allows cells that have a limited dynamic range to operate over a wide range of network conditions without saturating. For example, if tonic inhibition in the cerebellar granule cell layer changes from one level to another, either through a change in Golgi cell firing or through an action-potential-independent release mechanism, it will scale granule cell responses to incoming excitatory mossy fibre activity. Furthermore, if the level of inhibition increases as a function of the excitatory mossy fibre drive (via mossy fibre synapses onto Golgi cells), tonic inhibition could ensure that only a small fraction of granule cells are active, thus maintaining sparse coding onto Purkinje cells even during high levels network excitation, as proposed in Marr's classic theory of cerebellar function [51,52]. Because sparse coding is thought to be important in pattern separation [53,54], tonic inhibition-mediated reductions in granule cell gain are likely to enhance the number of motor control programs that could be stored in the cerebellar cortex.

Subtler insight into the significance of tonic $GABA_A$-receptor-mediated conductances comes from examining their cell-type-dependent expression in the context of network behaviour. Modelling of the cerebellar granule cell layer suggests that tonic inhibition in granule cells desynchronizes the oscillatory behaviour generated by the Golgi cell–granule cell feedback during random mossy fibre input [55]. Because hippocampal interneurons play a crucial role in oscillatory network behaviour [56], their tonic inhibition might also modulate rhythm generation. By simply adjusting the excitability of the network, it is possible that tonic inhibition could control the extent of oscillations, which are thought to be important for distributed signal processing [57].

Under baseline conditions, the $GABA_A$-receptor-mediated tonic current is considerably larger than the current mediated by spontaneous IPSCs, and an important determinant of interneuronal excitability and firing. In keeping with this, a low concentration of picrotoxin (1 μM) that relatively selectively inhibits tonic current significantly increased the frequency of spontaneous IPSCs in hippocampal pyramidal cells [11] (Figure 4). Thus picrotoxin, conventionally considered to be a powerful pro-convulsant, at low concentrations paradoxically enhances the inhibitory drive to principal neurons. These findings suggest that a possible role of tonic conductance in interneurons is to act as a homeostatic regulator of synaptic inhibition of principal cells: if the ambient GABA concentration decreases, this renders interneurons more excitable, resulting in a compensatory increase in the

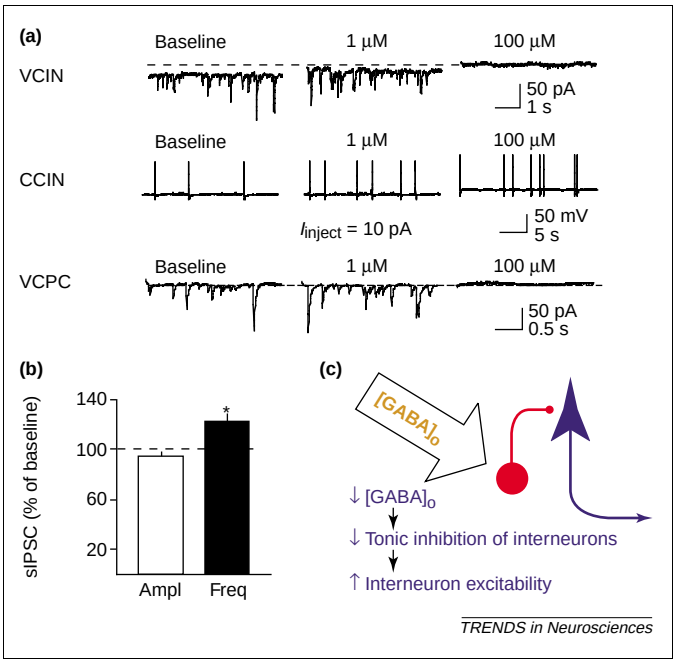

Figure 4. Inhibition of the interneuron-specific tonic current increases the excitability of interneurons and the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in pyramidal cells. **(a)** Recordings from CA1 stratum radiatum interneurons in voltage clamp (VCIN) and current clamp (CCIN), and from CA1 pyramidal cells in voltage clamp (VCPC), under baseline condition and in 1 μM and 100 μM picrotoxin (PTX; a $GABA_A$ receptor antagonist). $GABA_B$ receptors and ionotropic glutamate receptors were blocked in all experiments. PTX at high concentrations (100 μM) inhibited both the tonic current and sIPSCs. PTX at a low concentration (1 μM), however, relatively selectively reduced the tonic current (VCIN) and so increased the excitability of interneurons (increased interneuronal firing – CCIN) with no significant effect on sIPSC amplitude. Because interneurons start to fire more often, the selective inhibition of the interneuronal tonic current increased the frequency of sIPSCs in pyramidal cells (VCPC). **(b)** Mean sIPSC amplitude (ampl) and frequency (freq), recorded in pyramidal neurons in the presence of 1 μM PTX. **(c)** Interneuron (red) excitability varies inversely with the extracellular concentration of GABA ($[GABA]_o$) because of tonic inhibition, thereby providing a homeostatic control of the inhibitory tone in the circuit (pyramidal cell in blue). Reproduced, with permission, from Ref. [11] © Nature Publishing Group (http://www.nature.com/).

frequency of GABA-receptor-mediated IPSCs in pyramidal cells (Figure 4). Conversely (although this remains to be tested), an increase in ambient GABA concentration would be expected to render interneurons relatively unexcitable, leading to a decrease in inhibition of pyramidal neurons.

The presence of a tonic $GABA_A$-receptor-mediated current could also have implications for communication among different cell types in the brain, because glia can under certain conditions release GABA [58] and also express $GABA_A$ receptors [59]. Glial networks are capable of maintaining Ca^{2+} oscillations and are organized as an extended network via gap-junctions. Although its role is still largely speculative, it seems that tonic GABA-receptor-mediated signalling is well placed for integrating the neuronal and glial networks.

Concluding remarks

Tonic $GABA_A$-receptor-mediated conductances are large in cerebellar and dentate granule cells and in hippocampal interneurons, but are much smaller or undetectable in pyramidal cells. This cell-type specificity probably reflects differences in the distribution of high-affinity $GABA_A$ receptors and the level of receptor expression, and local differences in the extracellular GABA concentration. Tonic inhibition is developmentally regulated and can increase or decrease with age depending on the cell type. Modulation of tonic inhibition on short time scales by neuronal activity and by endogenous neuromodulators changes the properties of synaptic integration by changing the membrane input conductance and time constant. In cells with highly variable excitatory inputs, tonic inhibition can modulate neuronal gain, allowing rate-coded signals to be multiplicatively scaled. Moreover, its robust expression in hippocampal interneurons potentially reflects a role in homeostatic regulation of inhibitory tone. Because of the major impact of tonic inhibition on neuronal excitability, and its pharmacological profile, this form of inhibition could be an important novel pharmacological target for a wide range of disorders, including epilepsy.

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