

Mechanisms and functions of GABA co-release

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Abstract | The ‘one neuron, one neurotransmitter’ doctrine states that synaptic communication between two neurons occurs through the release of a single chemical transmitter. However, recent findings suggest that neurons that communicate using more than one classical neurotransmitter are prevalent throughout the adult mammalian CNS. In particular, several populations of neurons previously thought to release only glutamate, acetylcholine, dopamine or histamine also release the major inhibitory neurotransmitter GABA. Here, we review these findings and discuss the implications of GABA co-release for synaptic transmission and plasticity.

Neurons in the vertebrate and invertebrate nervous systems contain and release several small-molecule and peptide transmitters, each capable of signalling through a variety of receptors. A distinction is often made between classical small-molecule neurotransmitters, including glutamate, GABA, glycine, acetylcholine (ACh), purines and monoamines, which are the primary means of transferring electrical signals between synaptic partners, and neuropeptides such as somatostatin, neuropeptide Y, substance P and enkephalin (among many others), which slowly alter the properties of target neurons by activating G protein-coupled receptors (GPCRs). It has been established for several decades that exocytosis of one or more neuropeptides accompanies the release of classical small-molecule neurotransmitters in most neurons^{1,2}.

Nevertheless, our understanding of neural function and rapid synaptic communication has been dominated by the idea that a neuron releases a single classical neurotransmitter at all of its synapses — a notion commonly known as Dale’s principle (BOX 1). This principle has helped reduce the complexity of the nervous system by assigning each neuron to one of three functional categories: excitatory, inhibitory, or modulatory. However, several important facts complicate the simple characterization of neurons in this way. First, excitatory and inhibitory neurons are also

capable of modulatory functions, because glutamate, GABA, ACh and purines can activate GPCRs in addition to ligand-gated ion channels. Second, the synaptic actions of individual neurons vary with developmental age, membrane potential, cellular identity and the biochemical state of the postsynaptic cell. Third, in the nervous system of invertebrates and in the peripheral nervous system and developing CNS of vertebrates, many neuronal populations have been shown to release more than one classical neurotransmitter^{1–4}. However, the prevalence and physiological role of such co-release in the adult mammalian CNS are less well established. In this Progress article, we discuss recent reports of neuronal populations in the rodent adult CNS that co-release GABA, examine the molecular mechanisms involved and consider the functional significance of GABAergic co-transmission for neuronal communication and circuit computations.

Defining co-release

The term neurotransmitter co-release describes the process by which two (or more) neurotransmitters are released by a single neuron in response to an action potential. This description emphasizes the mechanism involved and is restricted to the release of neurotransmitters by vesicular exocytosis⁴. Co-release results from packaging multiple transmitters

into individual synaptic vesicles or from the coincident membrane fusion of different vesicles filled with distinct neurotransmitters (FIG. 1a–d). Because small molecule transmitters and neuropeptides are often distributed to separate vesicular compartments (clear synaptic vesicles and large dense core vesicles) and differentially mobilized by intracellular Ca^{2+} (see REF. 2), the term co-release typically applies to exocytosis of two classical neurotransmitters.

The term co-transmission also refers to synaptic release of multiple substances from a single cell, but it does not specify mechanism and, therefore, also extends to gaseous transmitters. It does, however, emphasize function, as it implies that both transmitters contribute to synaptic transmission, either by evoking postsynaptic electrical responses or by modulating the excitability of pre- and postsynaptic membranes¹. Hence, co-release might not result in co-transmission if the target cell does not express receptors for both transmitters (FIG. 1e). We note that the relative ease with which fast synaptic currents can be detected experimentally compared with the detection of slow modulatory influences has biased the study of co-transmission to classical neurotransmitters acting on ionotropic receptors.

Examples of GABA co-release

At a minimum, the co-release of two neurotransmitters requires that presynaptic terminals express the molecular machinery necessary to acquire both transmitters and to package them into synaptic vesicles. GABA is typically synthesized from glutamate by glutamate decarboxylase 65 kDa isoform (GAD65; encoded by *Gad2*) and/or glutamate decarboxylase 67 kDa isoform (GAD67; encoded by *Gad1*), and it is transported into synaptic vesicles using the vesicular GABA transporter (VGAT; also known as VIAAT, which is encoded by *Slc32a1*)⁵. The degree of plasma membrane uptake of GABA and glutamate has also been shown to influence the amount of GABA that is exocytosed, particularly during sustained synaptic stimulation^{6–8}. GABA signals through Cl^- -permeable type A GABA (GABA_A) and GABA_C receptors, as well as $\text{G}\alpha_{i/o}$ -coupled GABA_B receptors. In this section, we summarize the classical

Box 1 | In defence of Sir Henry Dale

In the early 1900s, Otto Loewi (1873–1961) observed that stimulation of frog parasympathetic and sympathetic nerves evoked the release of substances that inhibited or accelerated heart rate, respectively. Henry Dale (1875–1968) subsequently helped identify the released transmitters — acetylcholine and noradrenaline — and coined the terms ‘cholinergic’ and ‘adrenergic’ to describe nervous systems that liberated these molecules. These findings, which earned Loewi and Dale the 1936 Nobel Prize in Physiology or Medicine, established the chemical basis of neurotransmission and fostered the concept that neurons have distinct chemical identities that specify their function. Dale is often cited for proposing that each neuron releases a single classical transmitter — a rule that is increasingly proving not to hold. However, in his defence, Dale recognized the possibility that nerves release more than one molecule and never explicitly formulated or embraced the principle that has become synonymous with his name²⁰. In fact, the phrase ‘Dale’s principle’ was introduced by John Eccles (1903–1997) in 1954 in reference to Dale’s hypothesis that neurons release the same transmitter (or set of transmitters) across all of their synapses. Interestingly, recent work^{10,38,71} suggests that this may not be universally true.

neurotransmitter systems in which the co-release of GABA has been observed in the adult mammalian CNS and discuss the molecular and cellular mechanisms involved in each example.

Glycine–GABA. Functional co-release of GABA and glycine in the adult CNS was first reported in the late 1990s and has since been extensively documented in several neuronal populations in the spinal cord, brainstem and cerebellum (reviewed in REF. 4). Glycine and GABA are released from the same synaptic vesicles, as both neurotransmitters utilize VGAT for packaging into synaptic vesicles⁵ (FIG. 1a). The relative contribution of each of these transmitters to neurotransmission at individual synapses varies with the local presynaptic concentration of GABA and glycine and with the density and composition of postsynaptic receptors^{8–10}.

Glutamate–GABA. Classically, it is thought that glutamate and GABA are released from distinct sets of excitatory and inhibitory neurons in the adult CNS. However, despite the opposing functions of these neurotransmitters, two recent reports describe the co-release of glutamate and GABA from individual CNS axons. Neurons in the ventral tegmental area (VTA) and entopeduncular nucleus (EP) were recently shown to release both glutamate and GABA onto neurons in the lateral habenula (LHb)^{11,12}. Activation of fibres from VTA most frequently resulted in short latency suppression of firing in LHb neurons. By contrast, stimulation of EP inputs reliably evoked spiking in LHb neurons, reflecting a predominantly excitatory mode of action. Interestingly, the ratio of GABAergic to glutamatergic signalling from EP is decreased in animal models of depression and is increased in response to treatment

with the antidepressant citalopram¹¹. These findings add to a growing literature implicating LHb dysfunction in major depressive disorders¹³ and suggest that changes in glutamate–GABA co-release within the LHb might be a therapeutic target for these illnesses.

Most axons from EP and VTA that terminate in LHb co-express vesicular transporters for GABA and glutamate^{11,12}, indicating that individual synapses are capable of packaging and releasing both transmitters. Quantal synaptic events with biphasic waveforms have occasionally been observed¹¹, providing compelling functional evidence that GABA and glutamate are co-packaged at some release sites in LHb (FIG. 1b). However, it is difficult to experimentally exclude the possibility that there are also intermingled synaptic vesicles preferentially enriched with one or the other transmitter (FIG. 1c). Interestingly, individual mesohabenular boutons establish symmetric and asymmetric postsynaptic contacts enriched in GABA_A and glutamate receptors, respectively, which is suggestive of a postsynaptic division of labour¹². The finding that multiple synaptic inputs into this small brain area share the peculiar property of releasing both GABA and glutamate suggests that co-release may confer a specific function on this region.

ACh–GABA. Cholinergic neurons in the CNS have long been suspected of co-releasing GABA, on the basis of extensive co-expression of GABAergic and cholinergic markers in many vertebrate species¹⁴. Unambiguous functional evidence of co-release was found in an elegant study of the mouse retina that demonstrated, using paired whole-cell recordings, that monosynaptic GABAergic and cholinergic transmission occurs

from starburst amacrine cells (SACs) onto direction-sensitive retinal ganglion cells (DSGCs)¹⁵. Interestingly, this study revealed that vesicular release of GABA and ACh is differentially regulated by intracellular Ca²⁺ and is spatially uncoupled; ACh transmission is reliably evoked in all SAC–DSGC pairs whereas GABA release occurs preferentially in response to visual stimuli moving in one particular direction and is significantly biased to one half of the radial dendritic tree of a DSGC¹⁵. The spatial bias emerges from the asymmetric development of SAC GABAergic synapses onto DSGCs^{16,17}. These data strongly suggest that SACs package GABA and ACh into distinct populations of synaptic vesicles that distribute to non-overlapping or partially-overlapping presynaptic terminals (FIG. 1d).

More recently, functional evidence of co-release of GABA and ACh has been found in the cortex of mice, which receives most of its cholinergic input from basal forebrain projection neurons. Optogenetic activation of cholinergic fibres in general¹⁸, or of cholinergic fibres originating in globus pallidus specifically¹⁹, evoked postsynaptic responses in inhibitory cortical interneurons mediated by both GABA_A and nicotinic ACh receptors. The short onset latency and pharmacological properties of GABAergic currents suggest that cholinergic fibres directly release GABA. As in the retina, cortical ACh–GABA co-release seems to occur through separate populations of synaptic vesicles because individual cortical interneurons often exhibited responses to only one transmitter, and labelling of cholinergic presynaptic terminals from the globus pallidus showed spatial segregation of vesicular transporters for GABA and ACh^{18,19}. However, further research is required to determine the precise presynaptic mechanism and postsynaptic targets of ACh–GABA co-release in cortex.

Dopamine–GABA. Dopamine is synthesized from tyrosine by the enzymes tyrosine hydroxylase (TH) and aromatic-L-amino-acid decarboxylase (AADC) and is loaded into synaptic vesicles via the vesicular monoamine transporter 2 (VMAT2; encoded by *Slc18a2*). The identification of neurons capable of releasing dopamine has historically been carried out by labelling TH in neurons and, more recently, by using mice expressing transgenes under the control of the TH promoter. These approaches revealed that a surprisingly high number of TH-expressing cells also express GADs

and might therefore co-release GABA²⁰. However, careful analyses revealed that low levels of TH mRNA are transcribed in many neurons during development and in adulthood without generating detectable amounts of TH protein or dopamine, and that several populations of TH-expressing neurons in the cortex, striatum, hypothalamus and midbrain lack expression of AADC and VMAT2 and the ability to release dopamine^{20–23}. Therefore, care should be taken when determining the neurochemical identity of a cell and identifying neuronal candidates for co-release in the adult brain. This is important in all optogenetic studies, as nonspecific opsin expression in even a small number of off-target cells could yield results that may easily be misconstrued as reflecting co-release from one population of neurons²⁴.

Nevertheless, functional evidence of co-release of dopamine and GABA has been found in several brain areas. The olfactory bulb and retina contain populations of cells that express GADs, TH, VGAT and VMAT2. Depolarization of these neurons results in the release of both transmitters from small clear vesicles in a Ca²⁺-dependent fashion^{25–28}. Interestingly, quantal release of dopamine and GABA is mostly asynchronous — the release of dopamine outlasts GABA exocytosis by several seconds^{26,27} — suggesting that packaging into largely non-overlapping synaptic vesicles occurs (FIG. 1c).

We recently reported that dopamine-containing neurons in the VTA and substantia nigra pars compacta (SNc) monosynaptically inhibit spiny projection neurons (SPNs) in the striatum through Ca²⁺-dependent release of a GABA_A receptor agonist^{29,30}. Dopamine does not gate Cl⁻ conductances in striatal neurons^{29,31}, and several lines of evidence instead suggest that GABA is the released transmitter³⁰. However, whereas GABA synthetic enzymes have been observed in a small fraction of SNc and VTA neurons in the rat³², striatum-projecting dopaminergic neurons in mice do not contain detectable levels of GAD65, GAD67 or VGAT mRNA or protein^{23,29,30,33}, which raises the question of how SNc and VTA neurons acquire and package GABA for exocytosis. Our studies revealed that VMAT2 is necessary for GABAergic transmission from nigrostriatal afferents and restores vesicular release of GABA when re-expressed in GABAergic neurons from which VGAT had been knocked out²⁹. VMAT2 shows homology to a large family of transporters that expel

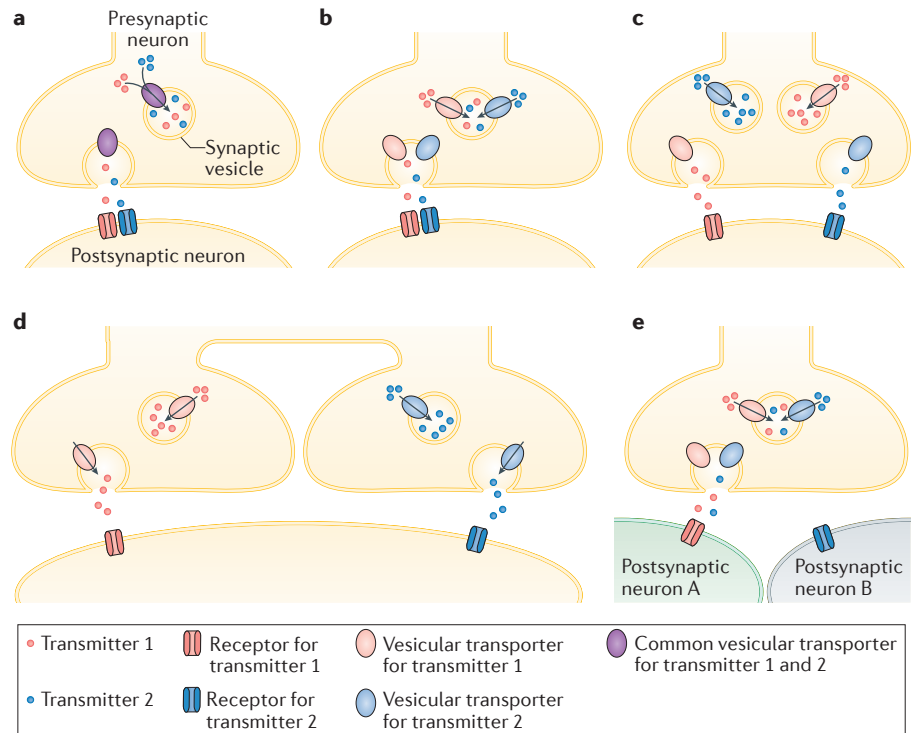


Figure 1 | Distinct cellular and molecular mechanisms of co-transmission. There are several possible mechanisms by which co-transmission of two neurotransmitters might occur, each offering distinct functionality and plasticity. Unfortunately, these mechanisms can be difficult to distinguish experimentally using electrophysiological and anatomical approaches. **a** | The transmitters may be loaded into individual synaptic vesicles using a common vesicular transporter. The abundance of the transmitters in the cytosol and the affinity of the transporter for those transmitters will determine the relative content of synaptic vesicles. Such co-packaging ensures that both transmitters are released at the same time and location, and that they are subject to similar presynaptic short-term plasticity. **b** | The transmitters may be packaged into the same vesicles using distinct vesicular transporters. The relative abundance of each co-transmitter in vesicles varies with cytosolic transmitter availability and with the expression levels of the vesicular transporters. Unless all vesicles express both transporters, this type of co-packaging probably occurs together with the co-packaging described in part **c**. **c** | The transmitters may be packaged into separate vesicles that are found within individual presynaptic boutons. Although both transmitters would be exocytosed from the same presynaptic bouton, their release could be modulated independently over short and long timescales, depending on transmitter abundances, synaptic vesicle release probability and the rate of synaptic vesicle recycling and loading for each transmitter. **d** | The transmitters may be packaged into separate vesicles that distribute to distinct presynaptic release sites. Physical separation would allow the presynaptic release of both transmitters to be modulated separately and the targeting of each transmitter to different postsynaptic compartments. For example, glutamate release may target dendritic spines while GABA is exocytosed onto dendritic shafts. **e** | An example of transmitter co-release that does not result in synaptic co-transmission is depicted, in which postsynaptic membranes (neuron A and neuron B) express receptors for only one of the two transmitters. This mechanism enables presynaptic neurons to broadcast a signal that is differentially interpreted by distinct neuronal elements.

chemicals from cells, and it is the most promiscuous of the vesicular neurotransmitter transporters, with over a dozen reported substrates, including methylenedioxymethamphetamine (MDMA), ethidium and rhodamine³⁴. VMAT2 — or a molecular complex incorporating VMAT2 — may therefore transport GABA into synaptic vesicles, raising the possibility that GABA and dopamine are co-packaged in SNc and VTA axons (FIG. 1a). In agreement with this possibility, ultrastructural

studies detected GABA associated with VMAT2-containing synaptic vesicles within dopaminergic terminals³⁵. Additional biochemical studies are needed to determine the relative affinity of GABA for VMAT2 and whether other proteins also enable VMAT2-dependent transport of GABA. The absence of such a cofactor in neurons containing dopamine and GABA in the retina and olfactory bulb could explain why co-packaging is not observed more frequently in these cells^{26,27}.

Midbrain dopaminergic neurons express several genes typically found in neurons that release GABA³⁶, including plasma membrane GABA transporters, which are involved in terminating the synaptic actions of GABA³⁰. In mice, these transporters are also required to sustain GABAergic transmission from SNc neurons³⁰, suggesting that reuptake is an important means of supplying GABA for exocytosis. This mechanism may allow the degree of GABAergic transmission to vary along dopaminergic axons, depending on extracellular GABA levels or the membrane distribution of the GABA transporter³⁷. The latter may explain why striatal cholinergic neurons do not receive GABAergic co-transmission from SNc axons³⁸, despite expressing GABA_A receptors and receiving dopaminergic innervation³⁹.

A recent study by Kim and colleagues⁴⁰ supports the finding that mouse midbrain dopaminergic neurons do not express GADs^{23,29,30,33}, and it reveals a non-canonical pathway for the synthesis of GABA by aldehyde dehydrogenase 1A1 (ALDH1A1). Pharmacological inhibition, global genetic deletion, and midbrain knockdown of ALDH1A1 in mice all reduced GABA release from SNc axons by half, and the administration of GABA reuptake blockers abolished the remaining GABA release, indicating that ALDH1A1 and membrane GABA transporters both contribute to the cytosolic accumulation of GABA for exocytosis⁴⁰. Interestingly, dopamine release is also dependent on both *de novo* synthesis and membrane reuptake⁴¹. In addition, the study by Kim *et al.*⁴⁰ reported that prolonged incubation of acute brain slices in ethanol depresses GABA co-release from SNc axons, and that this effect requires ALDH1a1. Furthermore, during a continuous two-bottle-choice test in their home cage, mice lacking ALDH1A1 consumed more alcohol than wild-type controls, suggesting a functional role for GABA co-release in regulating behavioural responses to alcohol.

Co-release of GABA may also occur in other populations of dopaminergic neurons in the mammalian CNS, as dopaminergic nuclei A11–A14 all express GADs²⁰. Although the presence of VMAT2 alone should render these cells capable of releasing both transmitters, they may also express VGAT. Co-expression of both vesicular transporters might allow for the presence of separate pools of vesicles enriched with each neurotransmitter. These observations support a large body of work

in several species indicating that there is substantial overlap in the expression of dopaminergic and GABAergic markers in the developing and mature nervous systems, under both physiological and pathological conditions^{42–45}, which collectively suggest that GABA is a ubiquitous co-transmitter in dopaminergic neurons.

Histamine–GABA. Neurons of the tuberomammillary nucleus (TMN), which are the only source of histamine in the vertebrate CNS, exhibit a GABAergic phenotype characterized by expression of GABA, GAD67 and VGAT^{46,47}. However, functional evidence of GABA release from these cells has remained elusive. Electrical stimulation of TMN neurons elicits inhibitory postsynaptic potentials in supraoptic nucleus neurons that are insensitive to the GABA_A receptor antagonist bicuculline⁴⁸, and optogenetic activation of TMN neurons fails to evoke phasic GABAergic responses locally within TMN, in preoptic nucleus target neurons or in cortical pyramidal neurons, despite evidence of histamine release in those areas^{47,49}. This may reflect the fact that TMN axonal varicosities are not typically found closely apposed to postsynaptic structures⁴⁶, preventing the efficient detection of co-released GABA. Alternatively, GABA and histamine may be packaged into distinct vesicles in TMN neurons⁵⁰ and released onto different targets that remain to be identified.

However, it was recently shown that GABA co-release might participate in the control of arousal and wakefulness by neurons of the TMN by ablating VGAT from these cells in mice⁴⁷. Recordings from cortical and striatal neurons in slices from wild-type mice revealed a slow and sustained increase in holding current on prolonged optical stimulation of TMN axons (900 light pulses over 3 min) that was mediated by GABA_A receptors. This current was dependent on transmitter release from TMN axons, was partially blocked by the inclusion of H1 and H2 histamine receptor antagonists and was not observed in slices from mice with genetic deletion of VGAT in the TMN, providing the first functional evidence that GABA may be co-released by TMN neurons. However, two caveats should be noted that prevent more definitive conclusions being drawn from this study. First, the authors did not exclude a contribution of H3 histamine receptors to the postsynaptic response to TMN stimulation; H3 histamine receptors

are widely expressed, and might contribute to the accumulation of extracellular GABA by stimulating the release of GABA from cortical interneurons or by preventing its reuptake. Second, the experiments that established the dependence of TMN stimulation-induced cortical and striatal responses on the expression of VGAT involved comparisons between two separate strains of mice, viral vectors and specificities of opsin expression. It is therefore difficult to assess whether TMN neurons were recruited to a similar extent under both conditions. In addition, the small magnitude of the induced GABAergic currents complicates the assessment of the contribution by VMAT2 to these currents as, presumably, VMAT2 does not transport GABA as efficiently as VGAT.

Functions of GABA co-release

GABA is a remarkably versatile neurotransmitter: it can exert excitatory, inhibitory or trophic influences, it may act either tonically or phasically, and can bind to either ionotropic or metabotropic receptors that may be localized pre- and postsynaptically. This flexibility, combined with the diversity of GABA co-transmitters, neural circuit architectures and co-release mechanisms, suggest it is unlikely that GABA co-transmission serves a single, universal function. Here, we propose some interesting possible functions of GABA co-release in synaptic transmission that we hope will stimulate further investigation. We note that in addition to the possible roles discussed below, GABA co-release might have a role in regulating the neurotransmitter cycle in the developing and diseased CNS⁵, the specification of neurotransmitters^{44,45}, and the functional establishment and reorganization of synapses (see REFS 4,51–53).

Inhibition. A major function of GABAergic signalling is the inhibition of activity in target neurons by decreasing exocytosis, hyperpolarizing membranes and shunting depolarization^{54–56} (FIG. 2a,b). When paired with excitatory or modulatory co-transmitters, GABA co-release may provide a faster and more targeted form of inhibition than that resulting from typical disynaptic mechanisms. For instance, in the LHB, a region lacking local interneurons, GABA co-release may balance glutamatergic excitation from EP¹¹ and VTA¹², and possibly provide a form of gain control to the LHB that in other brain areas arises from feedforward recruitment of fast-spiking interneurons.

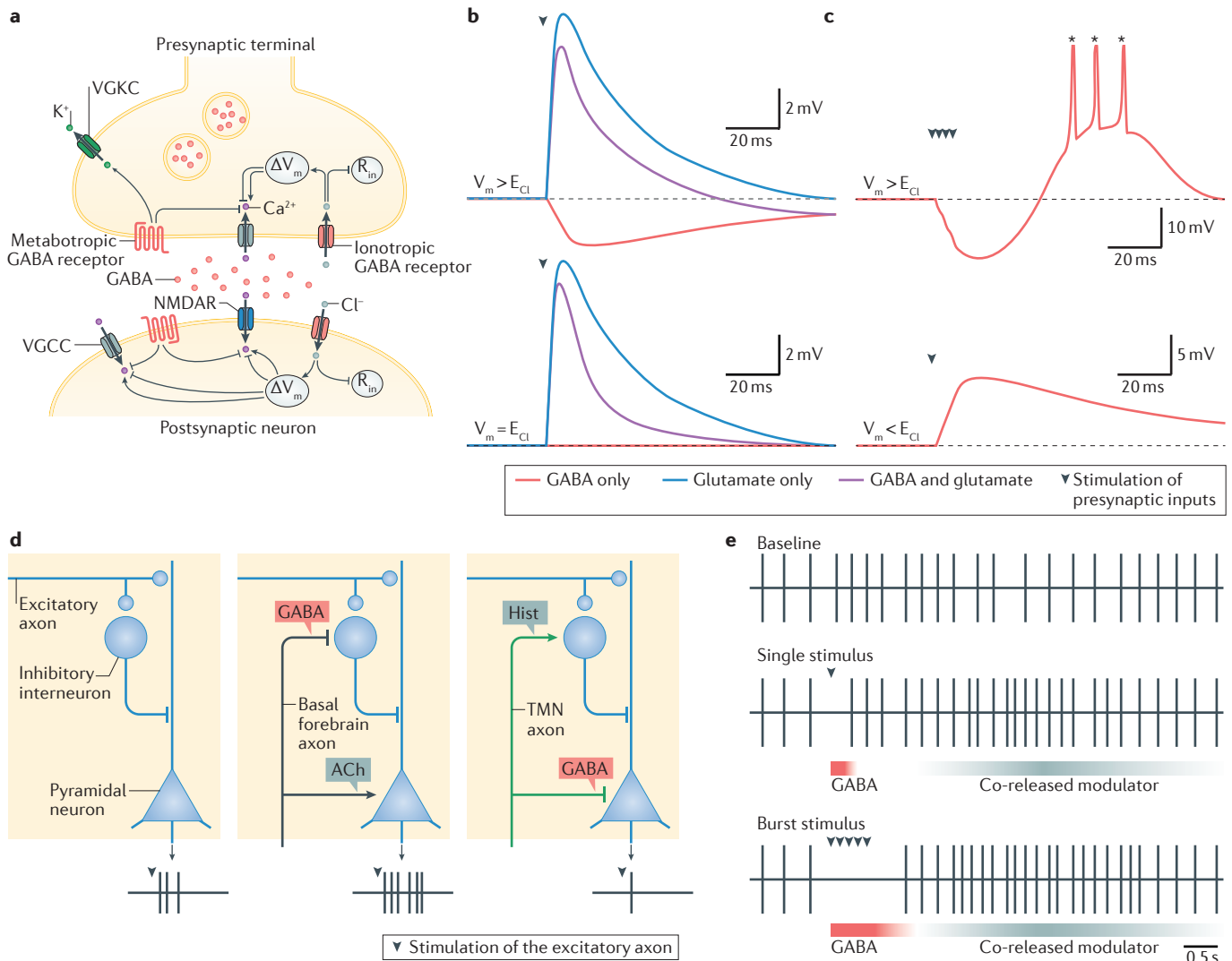


Figure 2 | Functions of GABA co-release. **a** | Once released in the synaptic cleft, GABA can signal pre- and postsynaptically, homo- and heterosynaptically and via ionotropic type A GABA (GABA_A) receptors and GABA_C receptors and metabotropic GABA_B receptors. Gating of GABA_A and GABA_C receptors shunts synaptic conductances by decreasing input resistance (R_{in}). Net flow of Cl⁻ through these receptors alters the membrane potential (V_m), which affects Ca²⁺ influx through voltage-gated Ca²⁺ channels (VGCCs) and NMDA receptors (NMDARs). GABA_B receptors act to inhibit presynaptic exocytosis and postsynaptic depolarization through modulation of voltage gated potassium channels (VGKCs), VGCCs and the presynaptic release machinery. **b** | Depending on the Cl⁻ reversal potential (E_{Cl}) relative to the resting V_m of a neuron, GABA co-release may dampen cellular excitability by physically hyperpolarizing postsynaptic membranes (top) or by shunting synaptic depolarizations (bottom). To illustrate this, the postsynaptic potentials evoked by presynaptic release of glutamate only, GABA only and co-release of glutamate and GABA are depicted. **c** | GABA release can exert a depolarizing influence if the postsynaptic cell expresses hyperpolarization-activated channels that depolarize membranes (top) or if intracellular Cl⁻ is elevated, such that E_{Cl} lies above the resting V_m (bottom). Each example

depicts fluctuations in membrane potential upon stimulation of a presynaptic GABAergic synapse (asterisk denotes truncated action potential). **d** | Co-transmission can be directed in space to achieve synergistic effects in complex neuronal circuits. Three cortical microcircuits are shown under different conditions, each composed of a pyramidal neuron, a local inhibitory interneuron and an excitatory afferent. Under baseline conditions (left), excitatory afferent stimulation evokes three spikes in the pyramidal cell. Target-specific co-release of GABA at some synapses, but not others, enables basal forebrain neurons to enhance (middle) and enables tuberomammillary nucleus (TMN) neurons to dampen (right) the output of pyramidal cells. **e** | GABA co-release provides a temporally precise signal. Schematic representation of the spontaneous action potential discharge of a target cell under baseline conditions (top) or upon stimulation of afferents that co-release GABA along with a neuromodulator that increases membrane excitability by activating G protein-coupled receptors (middle and bottom). Phasic inhibition of action potential firing by GABA co-release (the phasic inhibitory influence is shown in red) allows the postsynaptic cell to readily distinguish between single and burst stimulation of presynaptic afferents. ACh, acetylcholine; Hist, histamine.

What function might the co-release of two inhibitory transmitters, such as GABA and glycine (which both gate Cl⁻ conductances) serve? Glycinergic currents are considerably shorter than GABAergic currents and are

accelerated by GABA⁵⁷. Co-transmission of two inhibitory neurotransmitters may therefore allow fine-tuning of the magnitude and duration of synaptic inhibition. Because metabotropic receptors for glycine have not

been identified in mammals, co-release of GABA also confers on glycinergic synapses the ability to modulate transmission in a homosynaptic and heterosynaptic manner, via metabotropic GABA_B receptors⁵⁸.

Providing a depolarizing influence. GABA can evoke action potentials in target neurons if intracellular Cl^- is elevated or if the target neurons express ion channels that promote rebound excitation (FIG. 2c). In mature neurons, the Cl^- equilibrium potential (E_{Cl}) is typically below the spike threshold, precluding GABA from directly evoking firing. However, it is not uncommon for E_{Cl} to lie above the resting membrane potential of a neuron. In such cases, activation of GABA_A receptors would cause a subthreshold membrane depolarization, with important consequences for somatic excitability and the induction of synaptic plasticity⁵³. Co-release of GABA along with excitatory or modulatory transmitters may therefore confer flexibility on synaptic partners to fine-tune the membrane potential of target cells so as to optimize the ionotropic or metabotropic actions of co-transmitters.

Providing a spatially targeted signal. GABA co-transmission, where it does occur, is not necessarily uniform across all synapses, either because presynaptic neurons restrict GABA release to particular sites, or because some postsynaptic targets do not express GABA receptors. Thus, rather than broadcasting the same information to all of the postsynaptic targets of a presynaptic neuron, GABA co-transmission may serve several synergistic functions in complex circuits (FIG. 2d). For example, the fact that some terminals of individual SACs predominantly release ACh, whereas others within the same cell release both ACh and GABA, enables downstream DSGCs to encode both motion sensitivity and direction selectivity¹⁵. As another example, in the cortex, coincident ACh-mediated excitation of layer I disinhibitory interneurons and GABA-mediated inhibition of deeper layer interneurons may account for the role of basal forebrain neurons in arousal⁵⁹. Similarly, TMN neurons may increase cortical inhibitory synaptic transmission via the release of histamine, while simultaneously providing slow and sustained GABAergic inhibition to pyramidal neurons⁴⁷.

GABAergic co-transmission might be further restricted to distinct subcellular regions of a target neuron, enabling fine spatial control of presynaptic release and postsynaptic activity. This may be particularly important for co-transmitter systems that signal by volume transmission. Midbrain dopaminergic neurons, for example, form synapses on dendritic

spines that receive glutamatergic inputs⁶⁰; because dopamine signals in a diffuse manner, GABA co-release may specifically shunt excitatory potentials within individual spines.

Providing a temporally precise signal. Monoamines signal almost exclusively through GPCRs. Their synaptic effects are therefore achieved over hundreds of milliseconds to minutes, notably slower than those of transmitters acting on ionotropic receptors. Therefore in monoaminergic neurons, GABA co-release may provide the means to rapidly convey signals time-locked to sensory or motor events (FIG. 2e). SNc and VTA neurons fire tonically and modulate their discharge in response to salient stimuli by adding or omitting a few spikes. GABA co-release might have an important role in communicating these phasic changes in firing to striatal neurons. Interestingly, worms and insects have evolved Cl^- channels gated by biogenic amines in addition to metabotropic receptors^{61–63}, suggesting an important role for coincident fast inhibitory and slow modulatory signalling by aminergic cells.

Regulation of synaptic plasticity.

By directly and indirectly influencing axonal and dendritic Ca^{2+} influx (FIG. 2a), GABAergic signalling plays an important role in the induction of short- and long-term synaptic plasticity at GABAergic and non-GABAergic synapses^{54,55}. Like any mild depolarization, subthreshold GABA_A-mediated depolarization might favour Ca^{2+} influx and synaptic plasticity by reducing the efficacy of NMDA-type glutamate receptor Mg^{2+} blockade and increasing the opening probability of voltage-gated Ca^{2+} channels (VGCCs)^{64–66}, while simultaneously dampening excitatory potentials via shunting inhibition. These mechanisms would be predicted to facilitate the induction of long-term potentiation, although such an effect not yet been demonstrated for GABA. Conversely, activation of GABA_B receptors, often localized at excitatory postsynaptic terminals, can reduce Ca^{2+} influx through NMDA receptors and VGCCs by a variety of mechanisms^{54,67–69}, which would limit the induction of synaptic plasticity. Thus, GABA co-release could be a complementary signal to the metabotropic actions of transmitters such as ACh, dopamine and histamine in modulating the plasticity of synapses and neuronal circuits.

Conclusions

It has long been appreciated that individual neurons have the capacity to express and release multiple small-molecule, peptide and gaseous transmitters^{1,2}. However, the notion that the primary synaptic actions of many neurons in the adult CNS are not limited to the release of a single classical transmitter has only recently been widely recognized^{3,4}. Rather than representing an evolutionary oversight, it is likely that co-release of multiple classical transmitters has emerged because of its ability to provide additional means of modulating and fine-tuning synaptic transmission between individual synaptic partners over many different timescales. As discussed in this article, there is now compelling functional evidence that GABAergic transmission can occur from neurons that also release glycine, glutamate, ACh and monoamines in the adult CNS. Many of the functions of GABA co-release remain to be determined, but the diversity and flexibility of GABAergic signalling are likely to factor greatly in the prevalence of GABA as a co-transmitter.

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