Implications for Anesthesia and Beyond

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Abstract
Gamma-aminobutyric acid (GABA), a nonpeptide amino acid transmitter, is a major component of modern neuropharmacology and one of the most crucial target sites for general anesthetics and therapeutic drugs. GABA type A receptors (GABAARs) are the most abundant inhibitory neurotransmitter receptors in the central nervous system. They are part of the rapid-acting, ligand-gated ion channel (LGIC) receptor category, a pentameric Cys-loop superfamily member that mediates inhibitory neurotransmission in the mature brain. GABAARs mainly consist of two α subunits, two β subunits, and one additional subunit from either γ or δ arranged around a central chloride (Cl-) selective channel. Multiple GABAAR subunit subtypes and splice variants have been identified. Each variant of GABAAR exhibits distinct biophysical and pharmacologic properties. Several compounds allosterically modulate the GABAAR positively or negatively. The widely used positive GABAAR modulators include benzodiazepines (anxiolytic and anticonvulsant), general anesthetics (volatile agents like isoflurane, and intravenous agents like barbiturates, etomidate, and propofol), long-chain alcohols, some anti-convulsants, and neuroactive steroids. The binding sites for each drug are distinctly different. The anesthetic drugs enhance receptor-mediated synaptic transmission and thus interrupt the thalamocortical transmission, which controls the sleep–wake patterns. Abnormality in the GABAAR function has been implicated in several neurological conditions, such as sleep disorders, seizures, depression, cognitive function, neurological recovery after injury, and neuroplasticity. Understanding the GABAAR lays the foundation for the development of highly specific drugs in the treatment of neurological disorders and general anesthesia.

Keywords
- GABA
- GABAAR receptor
- isoforms
- general anesthesia
- modulation
- neurological disorders

Introduction
Gamma-aminobutyric acid (GABA), a nonpeptide amino acid, is the primary inhibitory neurotransmitter in the brain and a major inhibitory transmitter in the spinal cord, acting through the GABA receptor. The various levels of amnesia and loss of consciousness produced by many current general anesthetics such as benzodiazepines, barbiturates, propofol, etomidate, and volatile anesthetics are also mediated via their effects at the GABA receptor, notably the type A GABA receptor (GABAAR). The advances in modern molecular pharmacology and neuroscience have enabled investigators to understand the role of GABAAR in physiological and pathological conditions. Modulation of GABAAR is also one of the major components of modern neuropharmacology for several disorders. Understanding GABAAR has received a great deal of attention in the search for highly specific drug targets in the central nervous system (CNS). This narrative review gives a brief overview of the biochemistry
of GABA<sub>R</sub>, including structure, function, and modulation by drugs and disease.

**The GABAergic System**

The GABAergic system of the brain consists of GABA-releasing cells and receptors that bind GABA. The GABA-releasing cells are incredibly diverse. They control the activity of local networks (interneurons) and form the output of some areas of the brain and nuclei (e.g., striatal medium spiny neurons and cerebellar Purkinje cells). GABA neurons are involved in the transmission of afferent pain signals and descending pain-modulating pathways. The GABA receptors are virtually located on every neuron in the brain and represent a diverse array of receptor types. GABA signaling also plays a vital role in controlling neuronal differentiation during development.

In the spinal cord, GABA neurons have ubiquitous distribution with maximal concentration in the dorsal gray matter, followed by the ventral gray and white matter.

**GABA Receptors**

Three types of GABA receptors are described: type A (GABA<sub>A</sub>R), type B (GABA<sub>B</sub>R), and type C (GABA<sub>C</sub>R). GABA<sub>A</sub>Rs are fast-acting, ligand-gated, chloride ion channel (LGIC) receptors that mediate inhibition in the brain. GABA<sub>B</sub>Rs are relatively slow, class C of G-protein-coupled receptors. GABA<sub>C</sub> R, also named GABA-A-rho, is now classified as a subtype of GABA<sub>A</sub>R. GABA<sub>C</sub> is more selective and nearly 10 times more potent at GABA<sub>C</sub> than GABA<sub>A</sub> receptors due to the higher number of agonist-binding sites in the GABA<sub>C</sub> complex. The structural and pharmacological action of these three receptors is illustrated in - Table 1.

GABA<sub>A</sub>R is the most abundant fast inhibitory neurotransmitter receptor in the CNS. It is a member of the pentameric Cys-loop superfamily. The other receptors of this family are the nicotinic acetylcholine, glycine, 5-HT<sub>3</sub>, and zinc-activated receptors. The intercellular communication mediated by GABA receptor activation differs from the “point-to-point” communication that underlies the synaptic transmission or the gap junction-mediated electrical coupling. It is more akin to the paracrine transmission associated with the actions of neuromodulators such as serotonin, histamine, dopamine, acetylcholine, and peptides in the brain.

**Structure and Distribution of GABA<sub>A</sub>R Receptor**

GABA<sub>A</sub>R mainly consists of two α subunits, two β subunits, and one additional subunit from either a γ or δ, arranged as a pentameric ring around a central chloride selective channel (- Fig. 1A). When the receptor is activated, this ring serves as a channel through which chloride ions pass (- Fig. 1B). The receptor has extracellular, transmembrane, and cytosolic domains. Each subunit comprises of a long N-terminal extracellular hydrophilic domain, four transmembrane-α-helices (TM1–TM4), three inter-helix loops, and a short C-terminal extracellular domain (- Fig. 1C).

The GABA<sub>A</sub> pentamer receptor includes various isoforms, and the possible arrangement of these isoforms is illustrated in - Fig. 2A. The common GABA<sub>A</sub>R isoforms in the brain are αβγ and αβδ receptors. About 19 GABA<sub>A</sub>R subunit subtypes and splice variants have been identified: α (1–6), β (1 to 3), γ (1 to 3), δ, ε, π, θ and ρ (1–3) ( - Fig. 2B). Each of the receptor subtypes exhibits distinct pharmacological and electrophysiological properties. These physiological and pharmacological properties of a receptor are determined by subunit composition, their arrangement, and developmental expression pattern.

The properties of the subunits of α are mentioned in - Table 2. Recently, Laverty et al developed a high-resolution cryo-electron microscopy structure of the full-length human α1β3γ2L isoform of the synaptic GABA<sub>A</sub>R. The cryo-EM structure demonstrates the organization of heterooligomeric GABA<sub>A</sub>R receptors and provides a reference framework for the future of molecular principles of GABAergic signaling and pharmacology. The stoichiometry and subunit arrangement of αβγ receptors are well established, but the αβδ receptors need further research.

The distribution and function of the receptor subtypes are varied. The α1β2γ2 GABA<sub>A</sub>R subtype is distributed in the thalamus. The α5β2 GABA<sub>A</sub>R subunits are distributed in the hippocampus and neocortical pyramidal cells. The δ subunits coassemble with α6 subunits in the cerebellum and with α4 subunits in the hippocampus, striatum, thalamus, and cortex. The vital role of maintaining an inhibitory tone is contributed by the β3 subunit. Both GABA<sub>A</sub>R GABA<sub>B</sub>R have been located in the spinal cord. GABA<sub>B</sub>Rs are uniformly distributed in the gray matter (on dorsal and ventral interneurons), while GABA<sub>B</sub>Rs are spread in the dorsal horn (lamine I-III), both having a presynaptic location on primary afferent fibers and mediate synaptic inhibition. These GABA neurons enable excitatory proprioceptive signal integration, which permits the spinal cord to amalgamate sensory information and create smooth movements. Direct GABA<sub>A</sub>R or GABA<sub>B</sub>R-mediated inhibition of opioid-containing neurons facilitates pain transmission by reducing the release of these endogenous analgesics. GABAergic neurons located in the gray matter, anterior horn, and the substantia gelatinosa of Rolando explain the muscle relaxant effect of benzodiazepines.

**GABAergic Inhibition**

The GABA<sub>A</sub>Rs are most prevalent, localized mainly in the synapses. However, GABA<sub>B</sub>Rs do not exclusively locate to synapses. A small portion of the receptor subtypes, like α5β4 GABA<sub>A</sub>R and others containing the δ subunit like the αβδ receptors, has been found in the extrasynaptic regions (- Fig. 3).

Three kinetically distinct forms of GABA<sub>A</sub>R-mediated inhibition are exhibited:

(i) Rapid phasic inhibition at synaptic GABA<sub>A</sub>Rs—The α1β2γ2 GABA<sub>A</sub>R mediates phasic inhibition in response to transient high concentrations of synaptic GABA release (- Fig. 4A).

(ii) Persistent tonic inhibition at extrasynaptic receptors—Mediated by α4β2δ GABA<sub>A</sub>Rs. When activated by low-concentration extrasynaptic GABA, they produce tonic inhibitory currents (- Fig. 4B).

(iii) A prolonged albeit phasic “spillover” inhibitory postsynaptic current. GABA spilling from the synaptic cleft can activate presynaptic terminals.
receptors or neighboring synapses on the same or adjacent neurons to produce inhibitory postsynaptic currents (IPSCs) (► Fig. 4C).

Any disturbance in the phasic or tonic inhibition is associated with many neurological and psychiatric diseases. Thus, modulating these signals has led to the basis of drug therapy as well as anesthesia.

Role of Extrasynaptic GABA<sub>A</sub> Receptors
Tonic inhibition produced by extrasynaptic inhibition is vital in regulating states of consciousness. The extrasynaptic GABA<sub>A</sub>Rs are essential targets for anesthetics, sleep-promoting drugs, neurosteroids, and alcohol. Disorders such as schizophrenia, epilepsy, and Parkinson’s disease are found to involve disruptions in network dynamics associated with alterations in the tonic GABA<sub>A</sub>R-mediated conductance. The extrasynaptic GABA<sub>A</sub>Rs are potential therapeutic targets for the treatment of these diseases to enhance cognition and aid post-stroke functional recovery.

Desensitization of GABA<sub>A</sub> Receptor
A variety of kinases and phosphatases are involved in the regulation of GABA<sub>A</sub>R. Phosphorylation plays a crucial role in the allosteric modulation of GABA<sub>A</sub>Rs and governs its trafficking, expression, and interaction partner. The initial binding

### Table 1 Types of GABA receptors

<table>
<thead>
<tr>
<th>Pharmacology</th>
<th>GABA&lt;sub&gt;A&lt;/sub&gt; receptor</th>
<th>GABA&lt;sub&gt;B&lt;/sub&gt; receptor</th>
<th>GABA&lt;sub&gt;C&lt;/sub&gt;/rho receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Fast, short-acting ionotropic (cys-loop ligand-gated chloride ion channel) transmembrane receptor</td>
<td>Slow, metabotropic (G protein-coupled), seven transmembrane receptor</td>
<td>Slow, sustained ionotropic (ligand-gated chloride ion channel) transmembrane receptor</td>
</tr>
<tr>
<td>Structure</td>
<td>Heteropentamer (2α, 2β and 1 γ/δ subunits) with Cl&lt;sup&gt;-&lt;/sup&gt; in the center.</td>
<td>Heterodimer (R1, R2)</td>
<td>Homo/heteropentamer (3 p subunits: p1, p2, p3) with Cl&lt;sup&gt;-&lt;/sup&gt; in the center.</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Postsynaptic inhibition by (±) of Cl&lt;sup&gt;-&lt;/sup&gt; influx</td>
<td>Inhibits adenyl cyclase, (- cAMP), Presynaptic inhibition by (−) of voltage gated Ca&lt;sup&gt;2+&lt;/sup&gt; channels and postsynaptic inhibition by (±) of K&lt;sup&gt;+&lt;/sup&gt; channels</td>
<td>Postsynaptic inhibition by (±) of Cl influx</td>
</tr>
<tr>
<td>Distribution</td>
<td>CNS: Widespread The postsynaptic membrane of CNS High concentration in the limbic system and the retina Others: liver, endocrine pancreas, placenta</td>
<td>CNS: Widespread The presynaptic and postsynaptic membrane of CNS. High concentration in thalamic pathways and cerebral cortex Others: PNS</td>
<td>Brain: Widespread postsynaptic Spinal cord, retina, superior colliculus, and pituitary gland Others: PNS, GIT, sperm cells</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>300 kDa</td>
<td>80 kDa</td>
<td>Similar to GABA&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td>Site of action:</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; site: brain: IPSP</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; site: spin cord: (slow IPSP polysynaptic and monosynaptic reflex)</td>
<td>Similar to GABA&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td>Endogenous agonist</td>
<td>GABA</td>
<td>GABA</td>
<td>GABA</td>
</tr>
<tr>
<td>Agonists</td>
<td>Muscimol</td>
<td>Baclofen</td>
<td>Muscimol, CACA, CAMP</td>
</tr>
<tr>
<td>Modulators</td>
<td>Neuroactive steroids, barbiturates, benzodiazepine (anxiolytic, anticonvulsant) Long chain alcohol Muscle relaxants (thiocarbamide) Propofol, isofluran Eutidate</td>
<td>–</td>
<td>Neuroactive steroids, Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antagonists</td>
<td>Flumazenil, bicuculline, picrotoxinin (Cl&lt;sup&gt;-&lt;/sup&gt;channel blocker)</td>
<td>THIP</td>
<td>TPMPA, picrotoxinin</td>
</tr>
<tr>
<td>Insensitive to</td>
<td>Baclofen</td>
<td>Bicuculline</td>
<td>GABA&lt;sub&gt;A&lt;/sub&gt; or GABA&lt;sub&gt;B&lt;/sub&gt; agonist, saclofen or bicuculline</td>
</tr>
<tr>
<td>Pharmacological effects</td>
<td>Sedation, amnesia, hypnosis, anticonvulsant, muscle relaxation</td>
<td>Epileptogenesis, central muscle, relaxation</td>
<td>Analgesia, visual image processing</td>
</tr>
</tbody>
</table>

Abbreviations: CACA, cis-4-aminoacetoctic acid; CAMP, cis-2-amino-methylcyclopropane-carboxylic acid; Cl, chloride ion; CNS, central nervous system; GABA<sub>A</sub>R, gamma-aminobutyric acid type A receptor; K<sup>+</sup>, potassium ion; GIT, gastrointestinal system; IPSP, inhibitory postsynaptic potential current; PNS, peripheral nervous system; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridine-3-ol; TPMPA, 1,2,5,6-tetrahydropyridine-4-yl methyl-phosphonic acid; Zn<sup>2+</sup>, zinc.
of an agonist to GABA<sub>A</sub>R causes activation of the LGIC (which facilitates the selective flow of permeant ions across the plasma membrane) and affects cell excitability. But sustained binding of the agonist renders LGICs to enter a shut state, which is refractory to activation, called the desensitized state.<sup>19</sup> The exact roles of desensitization in vivo are still controversial. Still, they may include the prolongation of synaptic currents, decrement of responses during high-frequency neurotransmitter release, and modulation of extrasynaptic receptors subjected to tonic activation by low ambient concentrations of neurotransmitters.<sup>20–23</sup>
Pharmacological Modulation of GABA Receptors

Several compounds allosterically modulate the GABA\textsubscript{A}R positively or negatively in the presence of GABA. The widely used positive GABA\textsubscript{A}R modulators include benzodiazepines (anxiolytic and anticonvulsant), general anesthetics (volatile agents like isoflurane, and intravenous agents like barbiturates, etomidate, and propofol), long-chain alcohols, some anticonvulsants, and some neuroactive steroids.\textsuperscript{7} The notable negative GABA\textsubscript{A}R modulator includes proconvulsant flumazenil.

Mechanism of Modulation
GABA binding to the GABA\textsubscript{A}R increases the opening of the chloride ion channel. Many potent general anesthetics allosterically enhance the activation of GABA\textsubscript{A}R\textsubscript{s} by decreasing the kind of the receptor.\textsuperscript{24,25} This enhanced GABA\textsubscript{A}R function comes about by at least four actions: enhanced affinity at the GABA binding site, enhanced channel opening, conductance, and modulation. At high concentrations, they cause direct activation of GABA\textsubscript{A}R. General anesthetics also inhibit GABA uptake into neurons and glia, thus increasing GABA concentrations at postsynaptic GABA\textsubscript{A}R\textsubscript{s}.\textsuperscript{26} (\textsuperscript{►}Fig. 5)

Differences in the GABA\textsubscript{A}R Enhancement by General Anesthetics
Although all anesthetics have principal effects on the GABA\textsubscript{A}R, the binding sites are distinctly different. The details of the binding sites of various drugs on the subunits

### Table 2 Pharmacological actions of isoforms of \(\alpha\) subunits of GABA\textsubscript{A}R

<table>
<thead>
<tr>
<th>(\alpha) subunit</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha 1)</td>
<td>Amnesia/sedative + muscle relaxant effects</td>
</tr>
<tr>
<td>(\alpha 2)</td>
<td>Anxiolytic + anticonvulsant effects</td>
</tr>
<tr>
<td>(\alpha 3)</td>
<td>Anxiolytic + anticonvulsant effects</td>
</tr>
<tr>
<td>(\alpha 4, \alpha 6)</td>
<td>Augment benzodiazepine action</td>
</tr>
<tr>
<td>(\alpha 5)</td>
<td>Augment cognitive effects</td>
</tr>
</tbody>
</table>

Abbreviation: GABA\textsubscript{A}R, gamma-aminobutyric acid type A receptor.
of GABA<sub>4</sub>R are mentioned in Table 3 and Fig. 6A. There is good evidence that the intravenous anesthetics act near the extracellular end of the membrane-spanning domain (M) of various subunits. Amino acid residues located in the nonchannel lining face of the M1, M3, and M4 α-helices have been proposed as the binding sites for a range of compounds, including neurosteroids and general anesthetics. The differences in their effects are explained by the differences in affinity for the high agonist efficacy of GABA αβγ receptors and intermediate action at the αβδ receptors.

The general anesthetics act by selectively binding to the transmembrane intersubunit pockets of αβγ receptors. The α1β2γ2 GABA<sub>4</sub>R has five subunit interfaces that harbor sites for drug binding and functional modulation of GABA<sub>4</sub>R, and each compound uses a different set of subunit interfaces: (i)
### Table 3 Pharmacological effects of anesthetic agents mediated by GABA_{A}R subtypes

<table>
<thead>
<tr>
<th>Anesthetic agent</th>
<th>GABA_{A}R subtype</th>
<th>Pharmacological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etomidate</td>
<td>β2</td>
<td>Hypnosis, sedation</td>
</tr>
<tr>
<td></td>
<td>β3</td>
<td>Hypnosis, anesthesia, immobility</td>
</tr>
<tr>
<td>Propofol</td>
<td>α1β3γ2</td>
<td>Sedation</td>
</tr>
<tr>
<td></td>
<td>α6β3γ2</td>
<td>Sedation</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>α1βγ2</td>
<td>Antiepileptic effects, sedation, anterograde amnesia</td>
</tr>
<tr>
<td></td>
<td>α2βγ2</td>
<td>Anxiolysis, myorelaxation, analgesia</td>
</tr>
<tr>
<td></td>
<td>α3βγ2</td>
<td>Myorelaxation, analgesia</td>
</tr>
<tr>
<td></td>
<td>α5βγ2</td>
<td>Impaired cognition, myorelaxation</td>
</tr>
</tbody>
</table>

Abbreviation: GABA_{A}R, gamma-aminobutyric acid type A receptor.

### Table 4 Influence of isoforms of α subunits on anesthetic effects of drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Isoform</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzodiazepines</td>
<td>Combination of γ and β subunits with α1/2 isoforms</td>
<td>Sensitivity Insensitivity Sedation Anxiolysis at the limbic system</td>
</tr>
<tr>
<td></td>
<td>Combination of γ and β subunits with α 4/6 isoforms</td>
<td>α1 isoform α2 isoform</td>
</tr>
<tr>
<td>Propofol</td>
<td>α6 isoform</td>
<td>α1 isoform Higher gating Lower gating</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>α6 isoform</td>
<td>α1-5 isoforms Highest agonist efficacy Lower agonist efficacy</td>
</tr>
</tbody>
</table>

γβ+/α-β (interface 1), (ii) αβ+/α-γ (interface 2), (iii) α +/β-, (iv) α +/γ, and (v) γ +/β. Etomidate acts predominantly at interface 1 and γ +/β subunit interfaces, propofol acts predominantly at interface 1, interface 2, γ +/β, and pentobarbital acts predominantly at interface 2 and additionally at α +/β-, and/or α +/γ subunit interfaces. The asymmetry in anesthetic potentiation of the α 1β2γ2 GABA_{A}R contributes to the differences in their effects.²⁹ (Fig. 6B).

### Influence of Isoform on Anesthetic Drug Effects

The subunit composition of the GABA_{A}R plays a key role in determining the sensitivity to agonists, antagonists and modulators. The effect of the drug varies with the isoforms.³⁰–³⁵ The influence of the isoforms on anesthetic drug effects is shown in Table 4.

### Functional Effects of Anesthetics on GABA_{A}R in the Thalamocortical Pathway

The thalamus has a pivotal role in controlling conscious state transitions and has been recognized as an essential locus for anesthetic-induced sedation and hypnosis. There is impairment of thalamocortical (GABAergic neurons projecting from the thalamic reticular nucleus (TRN) toward the ventral basalis [VB1] and corticocortical projections during the general-anesthetic-induced unconscious state. Glutamatergic cells from the ventro-postero-medial nucleus (VPM) and cortex loop with the GABAergic TRN neurons. The excitatory glutamatergic pathway offers a tonic depolarization of the VPM neurons in the wakeful, activated state. It prevents them from entering synchronized, oscillatory states, which close the “gate” of the information processing.³⁶ The anesthetic drugs enhance the GABA_{A}R-mediated synaptic transmission and inhibit these glutamatergic pathways, thus interrupting the thalamocortical transmission. The extent of this inhibition determines the level of consciousness. The neural reactivity of the primary sensory cortices to external stimuli is preserved at the sedation level of anesthesia.
Functional Effects of Anesthetics on Extrasynaptic $\alpha\beta\delta$ GABA$_A$Rs

The possibility of extrasynaptic GABA$_A$R site of action of general anesthetics is suggested by enhanced tonic inhibition at hippocampal neurons, thalamic relay neurons, and neocortical neurons by anesthetics like propofol and isoflurane. Thus, both $\alpha\beta\delta$ and $\alpha\beta\gamma$ receptors are targets for several general anesthetics. General anesthetics such as barbiturates, benzodiazepines, propofol, and etomidate have been shown to induce changes in GABA-dependent receptor activation mediated by $\alpha\beta\gamma$ and $\alpha\beta\delta$ receptors. The application of general anesthetics increases the mean channel open time by inducing long-lived open states in the GABA$_A$Rs. General anesthetics at high concentrations also directly activate $\alpha\beta\delta$ receptors.

The allosteric modulation of GABA$_A$Rs by the general anesthetics disrupts the normal physiologic circuits, which require precise timing of GABA-ergic input. The GABA$_A$Rs are involved in mediating some of the standard components of general anesthesia: hypnosis, depression of spinal reflexes, and amnesia. However, the contribution of GABA$_A$Rs in mediating immobility and analgesia is less clear. Different classes of anesthetics can have differing effects on these pathways.

Functional Effect on Synaptic Phasic Inhibition

**Spill Over Inhibition**

Etomidate, propofol, the barbiturate, pentobarbital, and the neurosteroids tetrahydro deoxycorticosterone (THDOC) and alfalfxalone, all prolong neuronal IPSCs decay by phasic inhibition associated with $\alpha\beta\gamma$ receptors. The drug effects are studied for desensitization (current reduction during agonist application) and deactivation (current return to baseline after terminating agonist application). Propofol decreases the extent of desensitization of $\alpha_3\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ receptors, while THDOC and etomidate do not alter desensitization of $\alpha_1\beta_2/3\gamma_2$ receptors. General anesthetics prolong the deactivation of $\alpha_1\beta_2/3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ receptors. Hence, desensitization may contribute to the differences in the apparent maximal intrinsic efficacy at $\alpha\beta\gamma$ receptors.

**Functional Effect on Spillover Inhibition**

The GABA spills over from the synapse to activate extrasynaptic or perisynaptic GABA$_A$Rs at relatively high frequencies of presynaptic stimulation, producing IPSCs. Pentobarbital, propofol, the steroidal anesthetic alfalfxalone, and etomidate are known to act as positive allosteric modulators of both synaptic GABA$_A$Rs and extrasynaptic $\delta$-GABA$_A$Rs, and they are predicted to enhance “spillover” inhibition. In contrast, benzodiazepines, such as diazepam or midazolam, do not affect $\delta$-GABA$_A$Rs and, therefore, are predicted to have only a modest influence on “spillover” inhibition. (*Table 5*)

**Effects of General Anesthetics on GABA$_A$ Receptor**

**Barbiturates:** The direct effect of pentobarbital on the $\alpha_3\beta_2\gamma_2$ GABA$_A$Rs appears biphasic, with maximal currents due to direct agonism and inhibition at higher concentrations via a distinct inhibitory site. The anticonvulsant effect is mediated at the $\gamma_+/β$–$\gamma$-interfaces on $\alpha_3\beta_2\gamma_2$ GABA$_A$Rs. The $\gamma_+/β$–$\gamma$-interface can mediate allosteric channel gating shifts in opposing directions, perhaps depending on the specific orientation of hypnotic and convulsant barbiturates within the site (– Fig. 6B).

**Etomidate:** Etomidate is a potent stereoselective imidazole ester anesthetic. The GABA$_A$R site of effect for etomidate is suggested by enhanced tonic inhibition at GABA-induced chloride currents. The former site lies within the outer third of the transmembrane domain of the GABA$_A$R and is located between subunits. The latter exists within the transmembrane helical bundle of the subunit. Evidence suggests that etomidate binds selectively in the two $\beta_+$/α–$\gamma$-interfaces of $\alpha_3\beta_2/3\gamma$ GABA$_A$Rs in the transmembrane domain (– Fig. 6B). R(+)–etomidate positively modulates and directly activates $\alpha_3\beta_2\gamma_2$ receptors about 20-fold more potently than S(−) etomidate.

Etomidate has a more substantial effect on the β3 subunit at GABA$_A$ slow synapses than on GABA$_A$ fast receptors. Thus, etomidate effects lower-frequency electroencephalogram rhythms (i.e., $\delta$ and $\theta$ oscillations) more than higher-frequency activity (i.e., $\gamma$ oscillations). The amnesic effects of etomidate are mediated through $\alpha_5$–containing receptors forming “tonic” GABA$_A$Rs, but this does not produce the sedative or immobilizing effects. The loss of recall, sedation, loss of consciousness, and surgical immobility effects of etomidate may be mediated by actions on other ion channels and signaling pathways. Etomidate analogs have been developed with selective GABA$_A$R effect and avoidance of prolonged adrenocortical suppression.

**Propofol:** The alkylphenol propofol (2,6-di-isopropyl phenol) has both GABA-potentiating effects and direct effects on GABA$_A$Rs. The property of direct activation of the GABA receptor by propofol depends on the β subunit, while

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**Table 5** The effects of anesthetic drugs on phasic, tonic, and spillover inhibition

<table>
<thead>
<tr>
<th>Type of inhibition</th>
<th>Effect</th>
<th>By agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phasic inhibition</td>
<td>Augmented</td>
<td>Benzodiazepine, neuroactive steroid, etomidate, propofol, pentobarbital</td>
</tr>
<tr>
<td>Tonic inhibition</td>
<td>Augmented</td>
<td>Neuroactive steroid, etomidate, propofol, pentobarbital</td>
</tr>
<tr>
<td></td>
<td>No effect</td>
<td>Benzodiazepine</td>
</tr>
<tr>
<td>Spillover inhibition</td>
<td>Modest augmented</td>
<td>Benzodiazepine</td>
</tr>
<tr>
<td></td>
<td>Augmented</td>
<td>Neuroactive steroid, etomidate, propofol, pentobarbital</td>
</tr>
</tbody>
</table>
the modulatory effects were considered to involve α and β subunits. The α, β, and γ subunits contribute to the sensitivity of GABAAR to propofol. Propofol was shown to be less efficacious at β1-containing receptors than at those containing β2 or β3 subunits.45

**Benzodiazepines:** The drugs of the benzodiazepine family, including the newer drugs like remimazolam, bind to the interface between α and γ subunits, while barbiturates bind to the β and γ interface subunit of GABAAR (Fig. 6A). So, there is the additive effect between benzodiazepine and barbiturate and no competitive effect. Benzodiazepines are GABA facilita-
tory and increase the frequency of chloride ion channel opening, while barbiturates are GABA mimetic and increase the duration of chloride ion opening.

**Volatile Anesthetics:** Isoflurane, desflurane, and sevo-
flurane enhance the amplitude and prolong the duration of GABA-mediated synaptic inhibition at low concentrations. At supraclinical concentrations, they can cause “direct activa-
tion” by opening the receptor’s anion channel even in the absence of GABA.

**Ketamine:** The primary target site for ketamine is the N-
methyl-D-aspartate (NMDA) receptor, but it also inhibits GABAergic-enhanced conductance arising from α6-containing GABAARs. Ketamine has a high affinity for NMDA receptors on the inhibitory GABAergic interneurons. Thus, ketamine may also share the exact hypnotic mechanism as that of the GABAergic anesthetics.

**Modulation of GABAAR by Nonanesthetic Drugs**
Several nonanesthetic drugs that modulate GABAAR positively and negatively are used in the treatment of neurological conditions such as seizures, pain, cognitive dysfunction, and sleep disorders. The drug gabapentin is used to treat partial-onset seizures, sleep disorders, and alcohol withdrawal. Its mechanism of action is still unclear; it possibly acts by enhancement of GABA synthesis. Vigabatrin increases the ambient GABA levels by an irreversible block of GABA transaminase and is used to manage refractory complex partial seizures and infantile spasms but has the drawback of visual field loss. Pregabalin enhances the activity of glutamic acid decarboxylase, leading to increased GABA synthesis and higher ambient GABA levels. Pregabalin is used in the management of partial seizures (with or without secondary generalization), neuropathic pain (diabetes, postherpetic neuralgia), and anxiety disorder. Ganaxolone and alphaxalone are the positive allosteric modulators of most GABAARs with greater potency at δ-GABAARs, leading to selective enhancement of the tonic conductance. Ganaxolone is used for catamenial epilepsy management, while alphaxalone is used for anesthetic and long-term sedation in the intensive care unit.

**GABA Antagonists**
These drugs bind to GABA and inhibit its action, exhibiting convulsant and stimulant effects. They are used to treat the overdose of sedative drugs. They act at the GABA receptor site and are classified as competitive, noncompetitive antagonists and negative allosteric modulators. The details of these drugs are summarized in Table 6.

### Role of GABAAR in Neurological Conditions
Abnormality in the GABAAR function has been implicated in several neurological conditions.

#### Sleep Disorders
GABAARs play a pivotal role in the control of sleep rhythms. The alterations in the dynamics of the thalamo-striatal-cortical network and the alterations in extrasynaptic GABAAR function play a vital role in sleep. The alterations in ambient GABA levels may contribute to the sleep disturbances commonly associated with several neurological disorders, including depression.

Sleep abnormalities are the frequent nonmotor and early symptoms of Parkinson’s disease.47 The caudate-putamen of the striatum that is linked to Parkinson’s disease also expresses high levels of extrasynaptic αβδ subunit-containing GABAARs. In Parkinson’s disease, the loss of dopaminergic drive enhances the GABA concentrations in the striatum, and this change may underlie the sleep disruptions associated with Parkinson’s disease.48

<table>
<thead>
<tr>
<th>Type of GABA antagonist</th>
<th>Mechanism</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competitive/orthosteric antagonists</td>
<td>Bind to the active/orthosteric receptor site of the GABAR complex (but do not activate it) Compete with GABA and block its binding to GABAR</td>
<td>GABA, antagonists: bicuculline, gabazine, suramin</td>
</tr>
<tr>
<td>Negative allosteric modulators</td>
<td>Bind to an allosteric site on the GABAR complex in a negative manner. Reduce the efficiency of the leading active site by reducing Cl- conductance</td>
<td>GABA, antagonists: flumazenil, samazenil, zinc</td>
</tr>
<tr>
<td>Noncompetitive channel Blockers</td>
<td>Bind to the central pore of the GABA receptor and inhibit Cl- ion conductance</td>
<td>GABA, antagonists: picrotoxinin, fipronil</td>
</tr>
<tr>
<td>Inverse benzodiazepine agonists</td>
<td>Inhibit GABA binding. Can induce seizures</td>
<td>β-carbolines</td>
</tr>
</tbody>
</table>

Abbreviations: Cl-: chloride ion; GABAAR, gamma-aminobutyric acid type A receptor; THIP, tetrahydroisoxazolidopyridinol; TPMPA, 1,2,5,6-Tetrahydrodipyridin-4-yl methylphosphonic acid.
Drugs that potentiate GABAA-R currents, such as benzodiazepines and zolpidem, are the mainstay in the treatment of insomnia.

The problems of producing tolerance, addiction, and withdrawal prompt the search for more refined drug interventions; δ-selective GABAA-Rs such as gaboxadol have failed phase III clinical trials as an alternative to benzodiazepines for sleep promotion due to side effects such as hallucinations and disorientation. More potent δ-GABAA-R selective agonists are under development.

**Epilepsy**
Disturbances in synaptic and extrasynaptic GABAA-R function have been implicated in many forms of epilepsy. Maintaining appropriate levels of tonic inhibition is vital for controlling neuronal network behavior. δ-GABAA-Rs are often targeted in the treatment of specific forms of epilepsy, and drugs altering ambient GABA levels in the brain are used as antiepileptics. The mechanism of modulation of GABA by the antiepileptics is tabulated (Table 7).

All epilepsies do not respond to enhancing tonic inhibition. The defining feature of absence seizures is slow-wave discharges within the thalamocortical network, and this correlates with increased levels of tonic inhibition due to dysfunction of the GABA transporter (GAT-1) and the resulting elevated ambient GABA levels within the thalamus. This type of seizure is triggered by enhanced δ-GABAA-R with drugs like tiagabine and vigabatrin.

**Memory and Cognition**
Neuronal plasticity is regarded as the mechanism underlying learning and memory. Long-term potentiation at glutamatergic synapses plays a role in neuronal plasticity, and GABAergic inhibition obstructs this plasticity. Drugs that modulate tonic inhibition mediated by δ-GABAA-Rs have potential as novel treatments for Alzheimer’s disease or other neurological and psychiatric disorders characterized by deficits in learning, memory, or cognition.

**Anesthetic-Induced Neurocognitive Changes**
Anesthetics are known to produce a prolonged effect on cognition, which is maintained long after the agent is eliminated. Animal studies revealed a persistent increase of the CA1 neuron tonic current mediated by α5-GABAA-Rs and an associated decrease in the magnitude of long-term potentiation. The inflammation triggered by surgical trauma, by the anesthetic per se, or both may increase circulating concentrations of IL-1b, which has been shown to increase cell surface expression of α5-GABAA-Rs and, consequently, to increase the CA1 tonic current. The anesthetics may act synergistically with IL-1b to enhance the CA1 tonic current mediated by GABAA-Rs.

**Neuroprotection and Recovery of Function after Brain Injury**
The adult brain comprises a remarkable structural and functional plasticity, but some barriers may impede its plasticity once a developmental window is closed. Enhanced tonic inhibition has a role in acute neuroprotective quality. The mechanisms involving an enhanced tonic inhibition of GABAA-Rs may impede functional plasticity during recovery from cerebral insult. Recovery of function following acute cerebral injury may be controlled by the availability of GABA.

The pathogenesis of several chronic neurological and psychiatric disorders involving neuroplasticity is also attributed to the defects of GABAergic neurotransmission. The mechanism of anesthetic-induced plasticity is not yet entirely known.

**Neurosteroids**
Neurosteroids are brain-synthesized metabolites of ovarian and adrenal cortical steroid hormones. The glial cells synthesize endogenous neurosteroids like THDOC. δ-GABAA-Rs are a preferred site of action for neurosteroids and at physiological concentrations, they selectively enhance tonic currents mediated by αδ receptors. The neurosteroid sensitivity of the extrasynaptic GABAA-Rs may explain their

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**Table 7** GABA receptor modulation by antiepileptics

<table>
<thead>
<tr>
<th>Drug</th>
<th>GABA receptor modulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzodiazepines: clobazam, clonazepam</td>
<td>Enhance the frequency of chloride channel opening and increase the binding of GABA to the GABA receptor</td>
</tr>
<tr>
<td>Benzodiazepines: topiramate and felbamate</td>
<td>Cause GABA modulation</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>Prolong the open time of the chloride channel burst opening. Effective in GTCS</td>
</tr>
<tr>
<td>Valproate</td>
<td>Enhances sodium channel inactivation and reduction in both T-type Ca2⁺ channel currents and release of gamma-hydroxybutyric acid. Effective in partial onset and absence seizures</td>
</tr>
<tr>
<td>Tiagabine</td>
<td>Inhibit GABA reuptake into neurons and glia through presynaptic membrane. Enhance GABA catabolism resulting in higher synaptic GABA concentration. Effective in complex-partial seizures</td>
</tr>
<tr>
<td>Vigabatrin</td>
<td>Unique permanent suicide inhibitor of GABA transaminase enzyme required for GABA catalysis. Enhance GABA catabolism, resulting in higher synaptic GABA concentration.</td>
</tr>
</tbody>
</table>

Abbreviations: GABA, gamma-aminobutyric acid type A; GTCS, generalized tonic–clonic seizures.
importance in stress-, ovarian cycle, and pregnancy-related mood disorders. Stress hormones heavily regulate GABA<sub>A</sub>Rs, and changes in extrasynaptic GABA<sub>A</sub>R expression are often associated with stress-related disorders.

**Future Research Areas**

The cryoelectron microscopy of the receptor structure offers critical, novel insights into structure-based drug design that may facilitate the development of better molecules to treat neurological diseases and safer general anesthetics. Neurosteroids binding sites are distinct from etomidate, propofol, and barbiturates binding sites. Neurosteroids enhance GABA<sub>A</sub>R activation by etomidate and barbiturate and synergize with etomidate in anesthetizing animals. The syner-gism of neurosteroids and anesthetics has the potential for clinical research. Understanding the actions of the anesthetic drugs on the receptors may enable the development of selective anesthetic drugs devoid of adverse effects, such as etomidate, with no adrenocortical effects.

**Conclusion**

GABA<sub>A</sub>, the most prominent fast inhibitory neurotransmitter in the CNS, has various subunits and split variants exhibiting different structures and pharmacology. Various drugs, though bind to the same GABA receptor, produce different effects due to the structural heterogeneity of the receptors, the presence of multiple allosteric binding sites, and a broad range of ligands that can bind to them. Abnormalities of GABA<sub>A</sub>R have been associated with neurological disorders like epilepsy, neurocognitive disorders, and insomnia. Significant progress in understanding the mechanisms of general anesthetic action at the molecular, cellular, and neural systems levels is essential.

**Conflicts of Interest**
None declared.

**References**
Lam DW, Reynolds JN. Modulatory and direct effects of propofol.

Steinbach JH, Akk G. Modulation of GABA(A) receptor channel.


Garcia PS, Kolesky SE, Jenkins A. General anesthetic actions on GABA(A) receptors. Curr Neuropharmacol 2010;8(01):2–9


Drafts BC, Fisher JL. Identification of structures within GABA(A) receptor alpha subunits that regulate the agonist action of pentobarbital. J Pharmacol Exp Ther 2006;318(03):1094–1101


Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL. Isoflurane is a potent modulator of extrasynaptic GABA(A) receptors in the thalamus. J Pharmacol Exp Ther 2008;324(03):1127–1135

Garcia PS, Kolesky SE, Jenkins A. General anesthetic actions on GABA(A) receptors. Curr Neuropharmacol 2010;8(01):2–9


Koyanagi Y, Oi Y, Yamamoto K, Kobayashi M. Fast-spiking cell to pyramidal cell connections are the most sensitive to propofol-induced facilitation of GABAergic currents in rat insular cortex. Anesthesiology 2014;121(01):68–78


