Extrasynaptic δ-subunit containing GABA$_A$ receptors

Ayla Arslan[1],*

[1] *School of Advanced Studies, University of Tyumen, 9 Marta Street 2k1, 625000 Tyumen, Russia*

*Correspondence: a.arslan@utmn.ru (Ayla Arslan)

γ-Aminobutyric acid type A receptors (GABA$_A$Rs) are GABA gated heteropentameric chloride channels responsible for the adult brain's primary inhibition. In specific brain cells, such as in the hippocampus, one of the subtypes of GABA$_A$Rs, the δ subunit containing GABA$_A$Rs (δ-GABA$_A$Rs), is predominantly expressed and located in extrasynaptic or perisynaptic positions. δ-GABA$_A$Rs mediate a slow constant inhibitory current called tonic inhibition. While δ-GABA$_A$Rs and tonic inhibition is critical for the excitability of single neurons, accumulating data suggest that the function of δ-GABA$_A$Rs are broader and includes an integrative role in the network oscillations. While these open new horizons on the neurobiology of δ-GABA$_A$Rs, the complexity continues to challenge the analysis of GABA$_A$Rs and their subtypes. This review will summarize the current knowledge of molecular, cellular and physiological characteristics of δ-GABA$_A$Rs during health and disease.

Keywords

GABA; Inhibition; Tonic; Phasic; Synaptic; Extrasynaptic; Cys-loop receptors; δ-subunit; GABA(A) receptor; GABA receptor; Ion channel; GABRD; Hippocampus; Dentate gyrus; GABAergic interneurons; Granule cells; Epilepsy; Anxiety; Post-partum; Depression

1. Introduction

Hippocampus is a unique structure. One aspect of this uniqueness is its special anatomy. Concealed between the mesencephalon and the medial temporal lobe, this deep cortical structure extends through the lateral ventricle's inferior horn, where it lies at the posterior border of the amygdala [1]. Critical for learning and memory, the hippocampus is segmented into several regions [2], including the hippocampus proper, CA3, CA2, CA1 regions and the dentate gyrus (DG), a key region in hippocampal memory formation (reviewed in [3]).

A principal cell type in the DG is the dentate gyrus granule cells (DGGCs), characterized by unique anatomical features (reviewed in [4]). The cone-shaped spiny dendritic arbors of the DGGCs are innervated by different neuronal ensembles such as the input from the entorhinal cortex via the perforant path and contralateral hippocampus via the commissural path [5–7]. Diverse GABAergic interneurons synapse on the soma, axon initial segment, proximal and distal dendrites of DGGCs. For example, parvalbumin-positive interneurons (PPI) synapse on the axon- initial segment and

the perisomatic domain [8]. These GABAergic interneuron inputs to the DGGCs are involved in the synchronization of the network activities during theta- frequency (4-10 Hz) and gamma-frequency (30-150 Hz) oscillations [9], sharp waves-ripples (SWRs) [10], and dentate spikes [11].

The critical network operations for neuronal synchronization require the presynaptic terminals of the GABAergic interneurons (such as PPIs) to precisely match their molecular counterparts at the postsynaptic sites of the DGGCs. Here, γ-Aminobutyric acid type A receptors (GABA$_A$Rs), the GABA gated heteropentameric chloride channels, are massively clustered in the postsynaptic sites of the symmetric inhibitory synapses. GABA$_A$Rs belong to the superfamily of ligand-gated ion channels (Cys-loop receptors) [12], which also includes the nicotinic acetylcholine receptors (nAChRs), the 5-hydroxytryptamine type 3 (5-HT3) receptors, the zinc-activated ion channel (ZAC) and the glycine receptors in vertebrates [13]. Upon GABA release, the postsynaptic GABA$_A$Rs in the mature granule cells become active and elicit hyperpolarizing inhibitory postsynaptic currents (IPSCs), during which chloride and bicarbonate ions will travel through the receptor channel depending on their electrochemical gradient. Known to be benzodiazepine (BZ) sensitive [14, 15], these IPSCs are called phasic inhibition. The phasic signals are typically generated rapidly (often with sub-millisecond rise times), with the stimulus-evoked synaptic currents being in the range of less than 10 to 200 pA at a holding potential of -50 mV, which is known to vary in a typical quantal fashion [16].

In addition to the phasic synaptic inhibition, the DGGCs and PPIs have some other spots where a subset of high-affinity extrasynaptic GABA$_A$Rs is strategically located in the hippocampus mediate a different tone of GABAergic inhibition than phasic inhibition. This type of GABAergic signal is called tonic inhibition. Tonic inhibition is characterized by constant, slow currents, high GABA affinity, slow desensitization and BZ insensitivity [17]. The tonic current is about four times larger than the total phasic current in the DGGCs [18]. Like cerebellar granule cells [19], where the tonic inhibition was first described, distinct subtypes of extrasynaptic GABA$_A$Rs appear to mediate these relatively constant and slow inhibitory currents [18], which have also been shown in
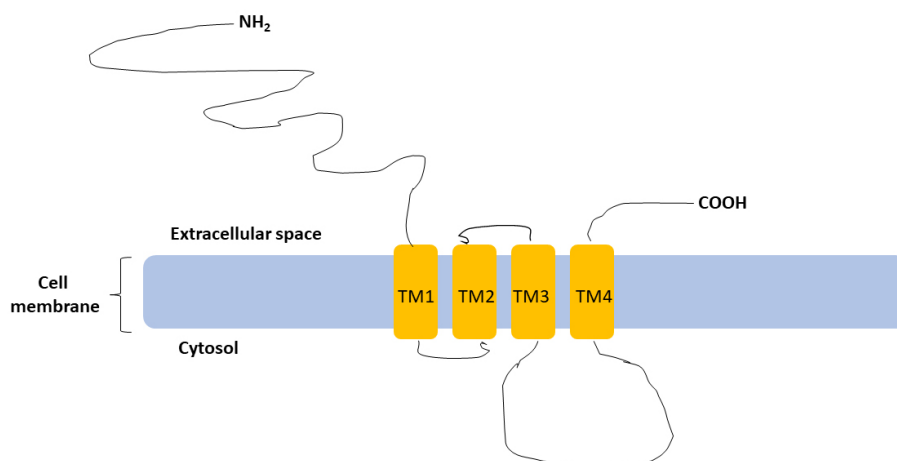
Fig. 1. Basic structure of δ subunit. δ subunit shares a standard topological structure with other subunits of GABA$_A$Rs: a long extracellular N-terminal, a short extracellular C-terminal, four transmembrane domains (TM1, TM2, TM3, TM4), cytoplasmic domain located between the third (TM3) and the fourth (TM4) transmembrane domains (Figure not to scale).

the neurons found almost all other major brain areas: neocortex, thalamus, hypothalamus and brain stem [20]. This distributed fashion of tonic inhibition, mediated by GABA$_A$Rs, represents distinct subunit composition, which involves either $\alpha5$ or δ subunits [21]. This review will focus on the δ subunit containing GABA$_A$Rs (δ-GABA$_A$Rs), which mediate a significant fraction of tonic current.

2. Molecular and cellular properties of δ-GABA$_A$Rs

For GABA$_A$R research, the late 80s and 90s were exciting years. Almost the entire GABA$_A$R subunit family was cloned by Seeburg and his colleagues [22–24]. The cloning strategy was based on the classical approach: Screening the brain cDNA libraries by synthetic DNA probes derived from purified receptors' peptides. Thus, eventually, it became clear that GABA$_A$Rs were assembled from 19 subunit isoforms (α(1-6), β(1-3), γ(1-3), δ, ϵ, θ, π and ρ(1-3)) which correspond to 11 structurally and functionally distinct receptor subtypes [22–24]. In general, all these subunits share a common topological structure: a peptide sequence which is about 450 amino acids long, made up of a long extracellular N-terminal, a short C-terminal, four transmembrane domains, intracellular or cytoplasmic domain located between the third and the fourth transmembrane domains (Fig. 1). This organization was originally based on the structural studies of acetylcholine-binding protein and nAChRs [25]. In particular, the subunits of acetylcholine receptors and the human GABA$_A$R $\beta3$ homopentamer's crystal structure at 3Å resolution confirmed this prediction [25, 26]. In contrast to the subunits' above-described properties, the hetero-pentameric receptor structure was not fully known until recently. In recent years, oligomerized heteropentameric receptor structure has also been resolved in detail [27–30].

It is well known that the GABA$_A$R subunit composition

determines their differential distribution and functionality [31–38]. Among the possible subunit combinations, typically, there is a combination of 2α and 2β subunits and a single $\gamma2$ or δ subunit (Fig. 2), the 2α, 2β and $\gamma2$ combinations being the most abundant. Indeed, about 90% of all GABA$_A$Rs are made up of $\gamma2$-GABA$_A$Rs [33]. Thus, the most GABA$_A$R research is directed to α, β and γ subunits, which are found both in the postsynaptic and extrasynaptic locations [39]. Among the α subunits, the BZ insensitive $\alpha4$ and $\alpha6$ subunits form a unique partnership with the δ subunit (together with β subunit isoforms) in the forebrain and cerebellum, respectively [36]. Thus, in the arrangement of δ-GABA$_A$Rs, δ subunit has been hypothesized as a replacement of the $\gamma2$ subunit in the receptor heteropentamer recruited exclusively to extrasynaptic or perisynaptic locations [40]. In the DGGCs, $\alpha4\beta\delta$ receptors, the most common isoform of δ-GABA$_A$Rs, are expressed. Also, $\alpha4\beta\delta$ receptors have been identified in several other neuronal cell types (see also Table 1) [41, 42], and like other δ-GABA$_A$R isoforms, localized in the extrasynaptic and perisynaptic positions but never in the postsynaptic sites [43, 44].

By *in situ* hybridization analysis, the regions of the adult rat brain in which δ subunits are expressed have been studied in detail [45]: The δ-subunit is expressed weakly or moderately in the regions of the olfactory bulb (granule cells and periglomerular), neocortex (layer II/III, layer IV, layer V/VI and pyriform cortex), hippocampus (DGGCs, stratum pyramidalis CA1 and stratum pyramidalis CA3), basal ganglia (caudate, putamen, nucleus accumbens, claustrum), thalamus (mediodorsal, ventral posterior nucleus, medial-, dorso- and ventrolateral geniculate nucleus). In Table 1, a summary of δ-GABA$_A$R isoforms (e.g., $\alpha4\beta\delta$, $\alpha6\beta\delta$, or $\alpha1\beta\delta$) and their cell type specific distribution are shown. This specific distribution is well reflected with the tonic inhibition. For example, $\alpha4\beta\delta$ receptors mediate the larger fraction ($> 70\%$) of the tonic inhibition in the DGGCs [21].

Table 1. Summary of δ-GABA$_A$R isoforms found in specific cells in the forebrain and cerebellum.

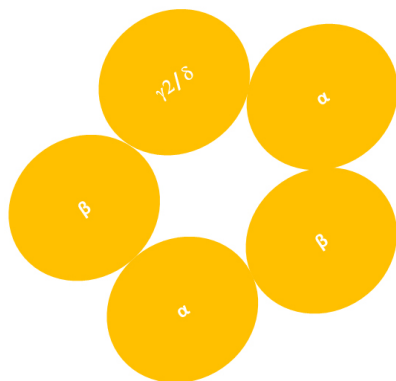| Subunit composition	Cell type	Reference
$\alpha 6b\delta$	Cerebellar granule cells	[46]
$\alpha 1b\delta$	Hippocampal interneurons, Neocortical interneurons	[47, 48]
$\alpha 4\beta 2\delta$	Thalamic relay neurons, Striatal spiny neurons, Hippocampal dentate granule cells, Neocortical pyramidal cells	[41, 42, 49–53]

Fig. 2. Heteropentameric structure of δ-GABA$_A$Rs. The diagram showing the heteropentameric structure of GABA$_A$Rs, which are typically composed of two α, two β and one γ or δ subunit. Experimental studies suggest that the subunit arrangement of $\gamma 2$-subunit containing GABA$_A$Rs ($\gamma 2$-GABA$_A$Rs) is counterclockwise when viewed from the extracellular space. It is not known if this arrangement also applies to δ-subunit containing GABA$_A$Rs (δ-GABA$_A$Rs) (Figure not to scale).

3. Variety of δ-GABA$_A$R mediated inhibition

It is known that GABA mediates multiple forms of postsynaptic inhibitory signals, such as fast and slow inhibitory postsynaptic currents [54, 55]. Additionally, δ-GABA$_A$Rs, mediating tonic inhibition and characterized by relatively constant, slow IPSC, has been known for the last few decades. However, tonic inhibition with these characteristics has been considered uniform and the only inhibition associated with the δ-GABA$_A$Rs. For example, the $\alpha 4\beta\delta$ receptors in DG-GCs and thalamic relay neurons mediate such tonic currents [41, 42]. These BZ insensitive GABA$_A$Rs have a high affinity for the GABA diffused from the synaptic cleft [56], besides the GABA released from GABA transporters [57, 58]. Interestingly, the literature has started to dissect the tonic inhibition: Depending on the subunit co-assembly, δ-GAB$_A$Rs have different GABA sensitivity, desensitization, and kinetics [59]. For example, it was shown that the $\alpha 4\beta\delta$ GABA$_A$Rs are the most sensitive to GABA levels ranging from $\sim$ 100 nM to 800 nM. Whereas $\alpha 1\beta 2\delta$ and $\alpha 5\beta 3\gamma 2$ (in addition to $\alpha 1\beta 2\gamma 2$) receptors detect GABA levels 1-10 μM range [59].

Accumulating data suggest that extrasynaptic GABA$_A$Rs might mediate a significant part of tonic inhibition, independent of gating by GABA; thus, spontaneous activity could occur [60, 61]. Such spontaneous activity also applies to δ-GABA$_A$Rs mediated tonic inhibition [62]. This phenomenon's functional significance is not understood, and it is probably dependent on the specific cell types and isoforms

of δ-GABA$_A$Rs, such as $\alpha 1\beta\delta$ and $\alpha 4\beta\delta$ expressed in these cells.

In addition to studies focusing on δ-GABA$_A$Rs, some studies dissect the physiological roles of GABAergic inhibition without explicitly indicating the associated subunit. So far, a few types of GABA$_A$Rs such as the ones containing either the δ, $\alpha 5$ subunits or receptors containing only $\alpha\beta$ subunits have been shown to mediate the tonic inhibition [63]. Thus, it is hard to predict the role of δ-GABA$_A$Rs in these studies. For example, in mice, in the reticular thalamic neurons, a phasic inhibition with slowed-down kinetics is mediated by GABA$_A$Rs [64]. This association is linked to $\alpha 4$ containing GABA$_A$Rs, but the exact receptor co-assembly is not clear. Possibly δ-GABA$_A$Rs might mediate this activity because, in the thalamus, most of the $\alpha 4$ containing receptors involve δ-subunit [41]. This is supported by some other findings, too. For example, the δ subunit is expressed explicitly in the thalamus [45], including the reticular thalamic nucleus [38]. However, this latter study represents the monkey brain, reflecting some differences compared to the rodent brain. It turns out that, in rat and mouse brains, δ-subunit is not expressed in the reticular thalamic nucleus [38, 65], whereas in the monkey, it is [38]. Thus, it is not clear if the $\alpha 4$ subunit linked phasic inhibition with slowed-down kinetics [64] is mediated by δ-GABA$_A$Rs even though the specific partnership of δ subunit with $\alpha 4$ subunit in the forebrain, including the thalamus, is well known [31, 41, 42, 66, 67].

Nevertheless, there is a collection of data supporting an additional GABAergic inhibition representing an intermediate form between the classical phasic (GABA, fast) and tonic inhibition, which is called GABA$_A$, slow [54, 55] some of which may be mediated by δ-GABA$_A$Rs as experimental evidence supports that δ-GABA$_A$Rs contribute to postsynaptic inhibition. Postsynaptic inhibition contributed by δ-GABA$_A$Rs was observed in the cerebellum, thalamus and neocortex [68]; in DGGCs of the mouse hippocampus [69, 70]. Thus, Fig. 3 shows different types of inhibition mediated by δ-GABA$_A$Rs as a proposition. For reference, postsynaptic $\gamma 2$-GABA$_A$Rs, which mediate phasic inhibition, are also shown (Fig. 3).

4. Variety of functions

As mentioned above, at the neuronal level, δ-GABA$_A$Rs mediated tonic inhibition, which is important for the threshold of action potential generation [36, 71–73]. It is generally hypothesized that tonic inhibition decreases neuronal excitability. Recent evidence-based computer models revealed that tonic inhibition might also increase excitability [74].
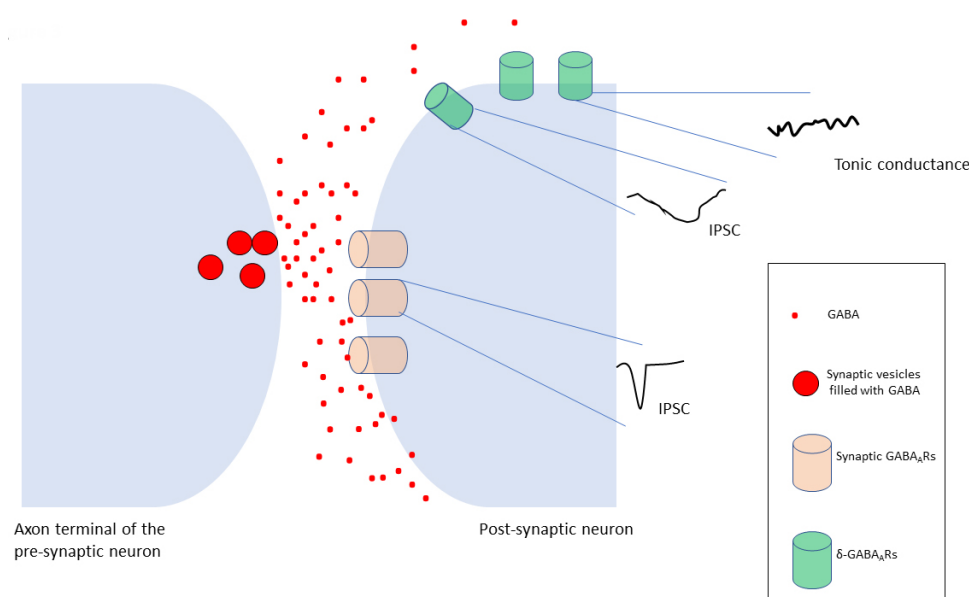
Fig. 3. Variations of GABAergic inhibition. Fast, point to point, phasic inhibition is typically mediated by synaptic GABA$_A$Rs, clustered in the postsynaptic membrane of the inhibitory synapses. These receptors evoke inhibitory postsynaptic current (IPSC) (phasic inhibition) in a millisecond range upon GABA binding. The GABA spillover from the synaptic region (black arrows) results in extrasynaptic receptors, which mediate a slow inhibitory conductance, the tonic inhibition. Phasic and tonic inhibition of synaptic and extrasynaptic GABA$_A$Rs has led to a functional distinction of these receptor subtypes. On the other hand, subsets of GABA$_A$Rs, including δ-GABA$_A$Rs may have intermediate activation, desensitization, and deactivation rates determined by the receptor subunit isoforms between these two states. This leads to the idea that δ-GABA$_A$Rs may contribute to postsynaptic inhibitory currents (IPSCs) (Figure not to scale).

Increasing literature shows the critical role of the nonsynaptic GABA$_A$R and/or tonic inhibition in various functions, including network oscillations [64, 75, 76], synaptic plasticity [77], synaptic pruning during adolescence [78], neurogenesis [79, 80], neuronal development [81], information processing, and cognition [81]. For example, in the dentate gyrus, δ-subunit is linked to enhanced memory and neurogenesis [82].

δ-GABA$_A$Rs mediated tonic inhibition is indicated for modulation of γ oscillations in the mouse hippocampal CA3 interneurons [75]. Also, coupling presynaptic activity to postsynaptic Inhibition in the somatosensory thalamus involved a process that influenced the δ-selective allosteric modulator, DS2 [76]. These take δ-GABA$_A$Rs from being the mediators of "shunting" inhibition involved in controlling neuronal excitability to additional roles in the network level activities, including but not limited to the thalamocortical system and neurogenesis in the hippocampus.

5. δ-GABA$_A$Rs and associated pathophysiology

The δ subunit modulators such as sedative and hypnotic agents [83], anxiolytic and anticonvulsive agents [84, 85] suggest that δ subunit may play a role in the etiology of the relevant disorders. Alterations of δ subunit or their modulation as therapeutic targets have been linked to sex specific behavioral disruption [86], Alzheimer's disease [87], stress induced deficiency in learning and memory [88], fragile X syndrome [89] schizophrenia [90], epilepsy [91], mood disorders [92–94], childhood mood disorders [95], anxiety in methamphetamine dependence [96], major depression [97]; post-partum depression, and post-partum psychosis [94, 98], consumption of opioids [99], menstrual cycle related problems [100, 101], stroke [102], Fragile X Syndrome [89, 103], traumatic brain injury [104, 105], Huntington's disease [106], pain [107], insomnia [83, 108–110], alcohol use disorders [111].

In animal studies, alcohol use disorders or associated behavioral alterations have been linked to δ-GABA$_A$Rs [112, 113] and sex-dependent [114] as well as developmental [115] factors seem to play a role in the underlying mechanisms. At the molecular level, ethanol impacts the modulation of the clathrin adaptor-mediated endocytosis of δ-GABA$_A$Rs [116], and its withdrawal influences δ-GABA$_A$Rs via PKCδ Activation [117]. Due to the estrous cycle-dependent plasticity of δ-GABA$_A$Rs, which was previously shown as associated with seizure susceptibility and anxiety [100], one study, using the model of "Drinking-in-the-Dark binge-drinking", showed that δ-GABA$_A$Rs are a critical target for binge drinking in females, a phenomenon observed at higher rates among women and girls [118]. The methylation pattern of δ subunit was also suggested as a diagnostic biomarker for alcohol use disorders [111].

It is important to talk about the special link between the δ-subunit and epilepsy. Various mutations (missense, nonsense, and frameshift mutations in coding DNA sequences besides mutations in the intronic, 3' downstream, or 5' upstream mutations) in GABA receptor subunit encoding genes have been linked to consequences such as the distortion of protein structure, conformation, abundance, or localization. Some of these mutations, which are detected in $\alpha 1$, $\beta 3$, $\gamma 2$, and δ subunits, have been associated with idiopathic generalized epilepsies (IGEs). For example, mutations in the $\gamma 2$ subunit are characterized by change of a single amino acid ($\gamma 2$(Q351X [119], $\gamma 2$(R43Q) [120], and premature translation-termination codon (PTC)-generating mutations $\gamma 2$(Q351X) [121]) are associated with different IGEs. Two δ subunit missense mutations, namely δ(E177A) and δ(R220H), were reported [122, 123]. Due to the distortion in the coding sequence, missense mutations lead to an altered amino acid sequence in the signal peptide regions of mature peptide regions. Dibbens *et al.* [123] reported mutations in the genomic region (1p36.3) of the δ subunit, representing susceptibility locus for generalized epilepsies. The δ subunit missense mutations, located in the subunit's extracellular N-terminus, are associated with generalized epilepsy with febrile seizures plus (GEFS+), a type of IGEs. These mutations alter the channel conductance [123], gating and surface expression of δ-GABA$_A$Rs [122]. Thus, δ-GABA$_A$Rs are considered as targets in the treatment of epilepsy.

Neurosteroids are endogenous substances synthesized from cholesterol into pregnenolone, which is then converted to compounds such as allopregnanolone and allotetrahydrodeoxycorticosterone [124]. It is suggested that fluctuations in neurosteroid interactions, such as those seen during stress or the ovarian cycle, determine the seizure threshold, a phenomenon that is partially mediated by δ-GABA$_A$Rs [100]. This and other evidence [125, 126] suggest that neurosteroids are novel drug candidates for epileptic disorders [125, 128]. Consequently, due to their potent actions on δ-GABA$_A$Rs [128, 129], δ-GABA$_A$Rs are novel therapeutic targets for the treatment of epileptic disorders and maybe a future perspective to control epileptogenesis [91, 130]. Ganaxolone, the synthetic analog of endogenous neurosteroid, is used as an antiepileptic agent (catamenial epilepsy), although it is the modulator of all GABA$_A$Rs, it shows a higher effect on δ-GABA$_A$Rs [131–134].

Interestingly, the modulation and pharmacology of δ-GABA$_A$Rs have become more critical recently. In addition to their modulation by insulin [135] and oxytocin [136], recently in 2019, the allopregnanolone brexanolone (Zulresso^{TM}, the brand of Sage Therapeutics, Inc.), one of the neurosteroids known as a potent modulator of δ-GABA$_A$Rs has been approved by the Food and Drug Admini-

stration (FDA) for postpartum depression[1] as a result of successful clinical trials [137–139]. Brexanolone seems to be effective on other mood disorders, such as major unipolar depression and post-traumatic stress disorder [140]. A synthetic GABA$_A$R modulator that shares a similar molecular pharmacological profile as brexanolone, the zuranolone (SGE-217), resulted in a reduction in depressive symptoms according to a recent phase 2 clinical trial [141].

Despite the progress, the field is dominated by many unknowns, which is a significant bottleneck. For example, the above-mentioned preferential modulation of δ-GABA$_A$Rs by neurosteroids is controversial and requires further validation. Regarding this, some studies suggested that the neurosteroid sensitivity of $\alpha 4/\delta$-containing extrasynaptic receptors may not be different than that of $\alpha/\beta/\gamma 2$-containing receptors [142, 143]. Along with the other inconsistencies, which will be summarized in the section *"7. The basics of unknowns"*, more research is needed for δ-GABA$_A$Rs.

6. A circuit pharmacology for δ-GABA$_A$Rs

The variations and specificities of δ-GABA$_A$Rs in terms of their isoforms, inhibitory action, distribution, sensitivity, modulation and spontaneous activity, which have been described so far, lead to the question to ask whether these properties can be utilized for circuit pharmacology. The idea of GABA$_A$R circuit pharmacology has probably gained momentum when the diversity of subunits and their specific pharmacology in the subunit assembly have started to be shown [144, 145]. However, the focus was mainly on the modulators of α subunit isoforms [23, 144–146] such as $\alpha 5$ inverse agonists RO4938581 [147]; S44819 [148], L-655,708 [149], Alpha5IA [150]. RO4938581 is under preclinical investigation for its potential to cure cognitive deficits in people with Down syndrome [151], for example.

Since the subunit-specific function and specific modulation are key to the strategy of circuit pharmacology, δ-GABA$_A$R seem to fit into this strategy. Among the isoforms of δ-GABA$_A$Rs, two population receptors are expressed in the hippocampus. $\alpha 1\beta\delta$ receptors are expressed predominantly in hippocampal interneurons, whereas $\alpha 4\beta\delta$ receptors are expressed predominantly in granule cells of the dentate gyrus (DGGCs) (Table 1). One study selectively silenced one population of these isoforms: $\alpha 1\beta\delta$ expressed in the Parvalbumin positive interneurons [152]. Thus, using the "PV/Cre-Gabrd/floxed system", it was reported that *in vitro* γ oscillations in the CA3 region were altered in both PV-Gabrd(+/-) and PV-Gabrd(-/-) mice in these interneurons. Interestingly, the increased γ oscillations were lowered to control PV-Gabrd(+/-) levels when 100 nM allopregnanolone ($3\alpha,5\alpha$-tetrahydroprogesterone) was used. But when 10 μM synthetic δ-GABA$_A$R positive allosteric modu-

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[1] Drug Approval Package, FDA (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/211371Orig1s000TOC.cfm), accessed in 28.01.2021.

lator 4-Chloro-N-[2-(2-thienyl)imidazo[1,2-a]pyridin-3-yl] benzamide (DS-2) was used, this was not observed. DS-2 selectively targets $\alpha4\beta\delta$ receptors but not the $\alpha1\beta\delta$ receptors, which are expressed in the interneurons. These suggest the specific role of $\alpha1\beta\delta$ isoform in the hippocampus's integrative network operations, in a way that can be modulated by selective agents. In line with this, another study examined the paired whole-cell recordings from synaptically coupled reticular thalamus and thalamocortical neurons of the ventrobasal complex in brain slices of $\alpha4$ knock-out ($\alpha4(0/0)$) mice. Results suggest a dynamic and activity-dependent engagement of δ-GABA$_A$Rs receptors for the coupling of presynaptic activity to postsynaptic excitability, a process sensitive to DS2, the specific modulator $\alpha4/\beta/\delta$ receptors [76]. The resolution of the three-dimensional structure of GABA$_A$R subtypes in recent years will trigger the design of novel drugs targeting specific δ-GABA$_A$R isoforms, which will likely aid the treatment of network disorders by circuit pharmacology approach.

7. The remaining unknowns

GABA$_A$R research has been challenged by the receptors' unusual molecular and cellular diversity and thus a huge effort is required to fully understand the properties of GABA$_A$Rs . Here, we will briefly mention the unknowns related to molecular and modulatory features of δ-GABA$_A$Rs, only. The nonsynaptic localization δ-GABA$_A$Rs is well established by electron microscopy studies [43, 44]. However, it is unknown if a passive or an active mechanism mediates this specific nonsynaptic localization pattern. Previously, it was suggested that the subunit's intracellular domain might play a role in this process [153]. The intracellular domain, which is found in between the third and the fourth transmembrane domains, is a large cytoplasmic domain, highly conserved across the whole span of vertebrate evolution [153].

Despite new studies [154–156], the current knowledge about the assembly and stoichiometry δ- GABA$_A$Rs is limited. Several studies have shown the stoichiometry of δ-GABA$_A$Rs as 2α, 2β and δ [157, 158]. For example, one recent study suggested that recombinant $\alpha1\beta3\delta$ receptors have the same stoichiometry and subunit arrangement with $\alpha1\beta3\gamma2$ receptors. However, these results are not entirely conclusive [155]. Thus, the basics such as assembly rules, stoichiometry, and arrangement of δ-GABA$_A$Rs, and their membrane trafficking, maintenance and modulation are not precisely known. For instance, in the *in vitro* live neuroblastoma cells, our group reported that recombinant δ subunits require both α and β subunits for membrane targeting [159], confirming the previously hypothesized analogy ($\gamma2$ subunit is replaced by δ in the δ-GABA$_A$R arrangement) between δ subunit and $\gamma2$ subunit: it is known that $\gamma2$ cannot assemble into receptors inserted in the cell membrane without α and/or β subunits [160, 161]. In contrast to our findings [159], some other previous studies suggest that $\beta\gamma$ and $\beta\delta$ containing receptors exist and show functionality in

Xenopus oocytes [162, 163]. So, there is no consensus. This may arise from the methodological variations used during *in vitro* studies: use of different vectors, cell types, or subunit isoforms, experimental strategy (such as fluorescent protein tagging location) may impact on these results. For example, in HEK-293T cells, quantification of fluorescent alpha-bungarotoxin bound subunits on Western blots of surface immunopurified tagged GABA$_A$Rs led to the conclusion that the cell surface expression of $\alpha_42\beta_2\delta$- GABA$_A$Rs was regulated by the ratio of subunit cDNAs transfected [164].

The distribution of δ subunit has been shown in different species, which shows species-specific variations. For instance, in the reticular thalamus, caudate, putamen and globus pallidus, there is an expression of δ subunit in the monkey, while this expression is absent in the rat [38]. The human brain distribution of δ subunit is not known fully. At the same time, some studies reported the distribution of $\alpha1$-$\alpha3$, $\beta2/\beta3$, and $\gamma2$ subunits in the human striatum [165] and thalamus [37].

Sensitivity to neuroactive steroids has also been questioned. Neuroactive steroids such as allopregnanolone ($3\alpha5\alpha$P) and allotetrahydrodeoxycorticosterone (THDOC) are considered to selectively affect δ-GABA$_A$Rs over $\gamma2$-GABA$_A$Rs. δ-GABA$_A$Rs sensitivity to neurosteroids in specific brain regions [166] is hypothesized to be very specific such that the endogenous neurosteroid THDOC at physiologically relevant concentrations (10-100 nM) selectively increases the tonic current, with almost no effect on the phasic current in mouse dentate gyrus granule cells and cortical granule cells [128, 167]. Thus, selective interaction of δ-GABA$_A$Rs with neurosteroids has been hypothesized to have clinical significance due to tonic inhibition's modulation, impacting excitability, seizure susceptibility, and behavior [100]. On the other hand, the neurosteroid binding site has been identified in the transmembrane domain of the α-subunit [168]. Moreover, a recent study suggests that neurosteroids act through both δ-containing and non-δ-containing receptors [143]. Thus, the degree of neurosteroid selectivity of δ-GABA$_A$Rs is questionable [142, 143].

Similarly, the mechanism by which ethanol potentiates GABA$_A$Rs is still not fully understood, and several publications have reported contradicting results. In general, $\gamma2$-GABA$_A$R subtypes are sensitive to ethanol at amounts required for high intoxication, whereas the extra-synaptic δ-GABA$_A$Rs are hypothesized to be most sensitive to ethanol at levels of social drinking, that is less than 30 mM [47, 70, 113, 169, 170]. However, this has been challanged by some publications [171, 172].

8. Conclusions

Increasing studies open new horizons on the δ-GABA$_A$R's neurobiology; however, the complexity continues to be a challenge. On the one hand, it could turn out that δ-GABA$_A$Rs function may be broader than previously hypothesized. This is well reflected with studies showing the pos-

sible contribution of δ-GABA$_A$R mediated inhibition to the control of major thalamocortical oscillations. Also, the possibility of some other forms of phasic inhibition, with roles in the integrative function and network oscillations, may underlie an even broader spectrum of physiological functions of δ-GABA$_A$R during health and disease.

On the other hand, knowledge is deficient in the level of "basics". There is uncertainty regarding the knowledge about the assembly [155], membrane targeting [159], clustering [153] and modulation of δ-GABA$_A$Rs [142], for example. Without elucidation of the mechanisms involved in these basic receptor mechanisms, precisely, it will be challenging to unravel the δ-GABA$_A$ receptor physiological significance and plasticity during health and disease. Thus, there is a need for a focused establishment of these "basics" in a subtype-specific fashion. Such an effort requires novel methodologies and careful consideration of experimental subject design. Experimental parameters appear to have a critical impact on the GABA$_A$R research illustrated by the lack of convergent findings obtained by the experimentation on the same subject by different methods.

Abbreviations

BZs, Benzodiazepines; CNS, Central nervous system; DGGC, Dentate gyrus granule cell; DS-2, 4-Chloro-N-[2-(2-thienyl)imidazo[1,2-a]pyridin-3-yl] benzamide; FDA, Food and Drug Administration; GABA, γ-Aminobutyric acid; GABA$_A$Rs, γ-Aminobutyric acid type A receptors; GABA(A) receptor, γ-Aminobutyric acid type A receptor; GABA-d, GABA receptor δ subunit; δ-GABA$_A$Rs, δ subunit containing GABA$_A$Rs; γ2-GABA$_A$Rs, γ2 subunit containing GABA$_A$Rs; HEK293T cells, Human embryonic kidney 293T cells; IPSC, Inhibitory postsynaptic currents; μM, Micro molar; N, Asparagine; nM, Nano molar; nAChRs, Nicotinic acetylcholine receptors; NMDA, N-methyl-D-aspartate receptor; pA, pico Amper; PPI, Parvalbumin positive interneurons; Q, Glutamine; THDOC, Allotetrahydrodeoxycorticosterone; W, Tryptophan.

Author contributions

AA conceptualized the study, identified the purpose and the scope, analyzed the literature, synthesized the knowledge and wrote the paper.

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Conflict of interest

The authors declare no conflict of interest. Given her role as the Editorial Board Member of JIN, Prof. Ayla Arslan had no involvement in the peer-review of this article and has no access to information regarding its peer-review.

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