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Review

$GABA_A$-ρ Receptors in the CNS: Their Functional, Pharmacological, and Structural Properties in Neurons and Astroglia

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Abstract: Gamma-aminobutyric acid (GABA) is known as the main inhibitory transmitter in the central nervous system (CNS), where it hyperpolarizes mature neurons through activation of $GABA_A$ receptors, pentameric complexes assembled by combination of subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3). $GABA_A$-ρ subunits were originally described in the retina where they generate non-desensitizing Cl- currents that are insensitive to bicuculline and baclofen. However, now is known that they are widely expressed throughout the brain including glial cells. For example, whole-cell patch-clamp recordings demonstrated the functional expression of $GABA_A$-ρ receptors in primary cultures of cerebellar astrocytes, as well as in cerebellar ependymal cells and striatal astrocytes. In these cells GABA-currents were partially blocked by TPMPA and insensitive to barbiturates. These receptors are proposed to be involved in extrasynaptic communication and dysfunction of the signaling is accompanied by reduced expression of $GABA_A$-ρ receptors in Huntington's disease and autism spectrum disorders (ASD). Thus, the aim of this review is to present an overview about $GABA_A$-ρ receptors including their structure and function, as well as their importance in the excitatory/inhibitory (E/I) balance in neurodevelopment and in disease.

Keywords: astroglia; gamma-aminobutyric acid; $GABA_{A\rho}$ receptors; central nervous system

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1. Introduction

Gamma-aminobutyric acid (GABA) is a non-protein amino acid present in invertebrates and vertebrates, where is considered the main neural transmitter exerting inhibitory activity on neurons and their intricate networks [1,2]. However, this neurotransmitter depolarizes astrocytes and neural precursors through the activation of ionotropic $GABA_A$ receptors, pentameric proteins that are modulated by barbiturates and benzodiazepines [3,4]. Interneuron–astrocyte communication regulates excitatory/inhibitory (E/I) balance and gliotransmission is accepted to play an important role. Astroglia setting includes the expression of $GABA_A$ receptors to detect interneuron signaling and calcium transients derived from this communication promote the release of gliotransmitters like GABA, known as an important extrasynaptic source for tonic inhibition. In this scenario, the expression of $GABA_A$-ρ subunits becomes relevant under physiological and pathological conditions. For example, during earlier postnatal neurodevelopment [5–10] and as a potential therapeutic target for post-stroke motor recovery [11].

2. Structural Properties, Characterization, and Functions of $GABA_A$-ρ Receptors

The $GABA_A$ receptor is an ion channel member of the ligand-gated ion channel (pLGIC) superfamily that opens transiently, inducing the inhibition of action potentials

through an inward membrane chloride conductance [4,12]. It was identified through bicuculline sensitivity in autonomic brain terminals and in mammalian brain slices [13]. This receptor is widely distributed in the central nervous system (CNS) and combinations of nineteen subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3) occur to coordinate differential neural inhibitions, such as phasic or tonic receptors, depending on the stoichiometry of each channel [8–10]. In the adult brain, the most common arrangement is: α1 (2), β2/3 (1), and γ2 (2) [14–16]. Topologically, each subunit contains an amino-terminal domain, where the neurotransmitter recognition site is located, a short extracellular carboxy-terminal domain, two cytoplasmic loops, and four transmembrane regions (Figure 1).

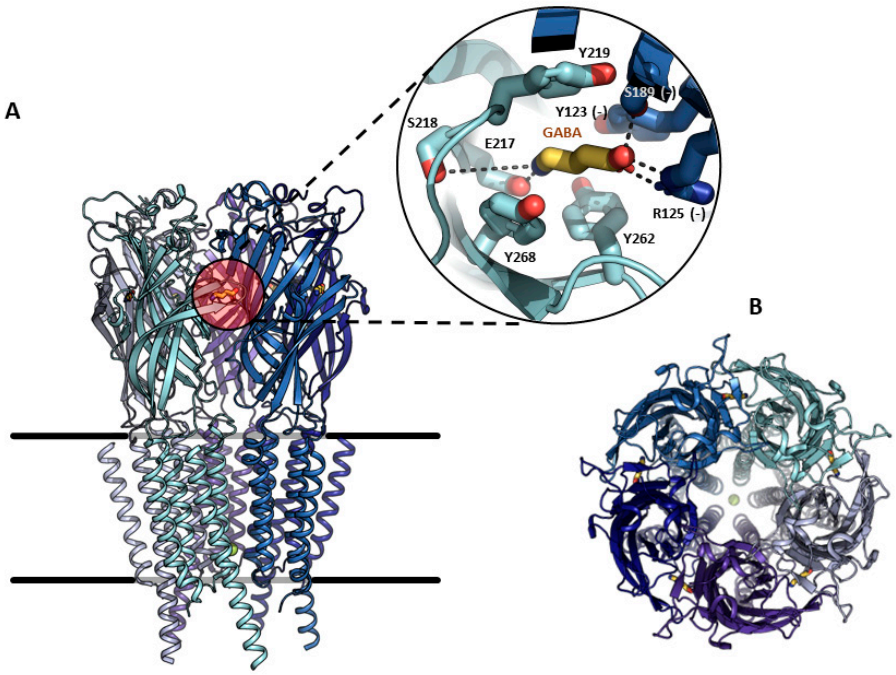

Figure 1. Molecular structure and binding-site of the human $GABA_A$-ρ1 receptor. (**A**). View of the pentameric receptor embedded in the plasma membrane, expanding in detail the orthosteric GABA binding site formed by the interface of two subunits in the amino-terminal extracellular domain. Residues interacting with GABA (yellow) from the main subunit (cyan), or complementary (–) subunit (deep blue), are indicated. (**B**). View of the pentamer from the outside of the membrane, showing the central ion-conducting pore. Structure representation was prepared using PyMOL v 2.5.2 (PDB file: 8OP9, from [17]).

Each subunit is predicted to have a molecular weight between 48 and 64 kDa, based on the amino acid sequence, and form a central pore or ion channel. Pentameric complexes weigh approximately 275 kDa [4]. Classical $GABA_A$ receptors are known to desensitize upon continuous exposure to the ligand; however, $GABA_A$-ρ subunits form functional homomeric receptors that do not desensitize [18]. $GABA_A$-mediated signaling is a key element for the E/I balance of the brain and its dysfunction is linked to motor, cognitive, and psychiatric disorders [16]. $GABA_A$-ρ was originally described as a retinal component (Table 1). There are three genes of $GABA_A$-ρ1-3 subunits; the first two were originally cloned from human retinal cDNA libraries [18,19], and the third from a rat retinal cDNA library [20]. The human $GABA_A$-ρ1 subunit shows alternative spliced variants and two deletions of 51 and 450 nucleotides, respectively ($GABA_A$-ρ1-51 and $GABA_A$-ρ1-450), which brought speculations as to the regulation of the expression of full-length $GABA_A$-ρ1 subunits, possibly co-assembling with some of the alternative spliced subunits and leading to variants with tissue-specific properties [21]. The first electrophysiological characterization was performed with the archetypical heterologous expression system for the study of ion channels, developed by Ricardo Miledi and collaborators, by injecting exogenous mRNA in

Xenopus oocytes and using a two-electrode voltage clamp (TEVC) [22]. This pioneer group injected mRNA preparations from either the brain or retina in *Xenopus* oocytes and found different GABA responses, which were kinetically and pharmacologically different [4]. For example, a slight decay and the magnitude of the current remained almost at maximum as long as the agonist was present in the perfusion bath. On the other hand, GABA responses decayed quickly near the basal level [4]. The sensitivity to the agonist was also very different, as the retinal $GABA_A$ receptor presented an EC_{50} of 1.48 μM, in contrast to the brain cortex, (EC_{50} = 84.6 μM). The cloned cDNA coding for the $GABA_A$-ρ1 subunit expressed in *Xenopus* oocytes displayed a GABA EC_{50} of 2 μM and was insensitive to bicuculline [4,18]; these results were like those obtained from oocytes injected with retinal mRNA. Several reports have shown heteromerization in different $GABA_A$-ρ subunits [23,24], as well as in other $GABA_A$ subunits [8,25–27], or even in subunits belonging to different members of the pLGIC superfamily [28]. The homomeric $GABA_A$-ρ1 subunit is insensitive to bicuculline and baclofen, commonly used to discriminate between heteromeric $GABA_A$ channels and metabotropic $GABA_B$ receptors, respectively [29–31]. The pharmacological distinctiveness that allowed the discriminating of $GABA_A$-ρ subunits from different subunits of heteromeric $GABA_A$ receptors was the synthesis of conformationally restricted GABA analogues [32]. The *cis*-isomer, *cis*-4-amino-crotonic acid (CACA) and *trans*-4-amino-crotonic acid (TACA) are $GABA_A$-ρ1 partial and potent agonists, respectively. Nonetheless, TACA is not selective and is also able to activate typical heteromeric $GABA_A$ receptors; this gives an advantage to using CACA to clearly identify $GABA_A$-ρ subunits [33,34]. Homomeric $GABA_A$-ρ1 receptors are insensitive to many of the heteromeric $GABA_A$ allosteric modulators, such as benzodiazepines and barbiturates. However, some drugs have powerful allosteric properties, like the anticonvulsant loreclezole [35] or drugs designed for specific purposes, such as (1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid (TPMPA), -cis-/trans-3-aminocyclopentane) butylphosphinic acid ([±]-*cis* and [±]-*trans*-3-ACPBuPA, Piperidin-4-yl) methylphosphinic acid (P4MPA), Piperidin-4-yl seleninic acid (SEPI), and 3-(guanido)-1-oxo-1-hydroxy-phospholane (3-GOHP) [36]; all of them have been used as a source to investigate the involvement of $GABA_A$-ρ receptors in sleep–waking behavior and antinociception in the peripheral nervous system or in learning, and also to investigate memory enhancers and other cognitive activities [36,37]. The importance of the $GABA_A$-ρ receptor becomes increasingly clear through its wide expression in the CNS (Table 1). Additionally, it is highly related to ammonia-induced apoptosis, retinitis pigmentosa, myopia, and may be important for some specific in vivo effects of ethanol and Huntington's disease (HD) [16,38]. At the last stage of the preparation of this manuscript the cryogenic electron microscopy structures of $GABA_A$-ρ1 subunits was reported, in apo state as well as in the presence of TPMPA, picrotoxin (PTX), and GABA, generating different conformations [17]. This milestone is an exciting point in the state-of-the-art of the current research that will help us to understand the intricated mechanisms that pharmacologically and functionally define this family of proteins, as a natural step following the pioneering experimental achievements described here.

Table 1. Distribution of $GABA_A$-ρ receptors in the CNS.

CNS Region or Cell Type	$GABA_A$-ρ1	$GABA_A$-ρ2	$GABA_A$-ρ3	References
Retina				[20,39–45]
Outer nuclear layer	X	X	X	
Retinal rod bipolar cells				
Outer plexiform layer	X	X		
Dendritic tree of bipolar cells				
Inner nuclear layer	X	X		
Bodies of horizontal cells	X	X		
Bodies of bipolar cells				
Inner plexiform layer	X	X		
Axons of bipolar cells				

Table 1. *Cont.*

CNS Region or Cell Type	$GABA_A$-ρ1	$GABA_A$-ρ2	$GABA_A$-ρ3	References
Amygdala	X	X		[46–51]
Basolateral	x	x	x	
Central	X	X		
Cortical basomedial	x	x	x	
Intercalated paracapsular islands	X	X		
Lateral	X	X		
Postmortem nuclei				
Brainstem	X			[7,26,52–56]
Hypoglosal motoneurons	X			
Dorsal vagal nucleus		X		
Lateral parabrachial nucleus	X	X	X	
Medial nucleus of trapezoid body	X			
Motoneurons				
Rachidian bulb	X	X		
Olivar	X	X		
Cuneiform	X	X		
Cuneiform accessory	X	X		
Reticular nuclei	X			
Solitary tract nucleus	X	X		
Varolio's pons				[6,53,57–60]
Cerebellum		X		
Astrocytes	X	X		
Basket cells	X		X	
Ependymal cells			X	
(paraventricular zone)	X	X		
Purkinje cell layer			X	
Cerebral cortex		X		[49–51,61–64]
Entorhinal		X		
Frontal			X	
Layer II (pyramidal-parvalbumin neurons				
Lateral		X		
Medial		X	X	
Mediolateral	X	X	X	
Layer VI (pyramidal-parvalbumin neurons				
Mediolateral		X		
Parietal	X	X	X	
Somatosensory		X		
Barrel (principal and adjacent whisker)				
Temporal				
Visual	x	x		
Cornea		X	x	
Corpus callosum	X	X		
Hippocampus	X	X	X	[49]
CA1	X	X	X	[65]
CA2				[52]
CA3	X	X		[50–52,66–70]
Dendate gyrus	X	X	X	
Granular	X	X	X	
Polymorph			X	
Molecular	X	X		
Stratum oriens	X	X		
Subiculum	X	X		
Stratum radiatum	X	X	X	
Isthmo-optic	X	X		

Table 1. *Cont.*

CNS Region or Cell Type	$GABA_A$-ρ1	$GABA_A$-ρ2	$GABA_A$-ρ3	References
Neostriatum	X	X		
Polygonal and fusiform cells	X	X		
Calbindin interneurons				[56]
Calretinin interneurons	X	X		[5,9,51,52,54,71]
D2 projection neurons	X	X		
Astrocytes	X	X		
Olfactory bulb		X		
Mitral cells	X	X		
Optic tectum			X	
Optic nerve and tract	X			[72]
Pituitary gland	X	X		
Retinal pigment epithelium	X	X		[73]
Spinal cord	X	X	X	[52]
Dorsal root ganglion	X	X		[52]
Laminae I			X	[74]
Laminae II	X			[51–53,67,75,76]
Motoneurons	X		X	
Interneurons	X			
Ventral horn	X	X		
Superior colliculus	X	X		[51,53,57,77–79]
Dorsal lateral geniculate nucleus	X			
Superficial gray layer				
Calbindin neurons	X	X		
Thalamus	X	X	X	
Dorsal			X	
	X			
x *Only determined by functionality*				[51,56]

3. Trafficking and Scaffold Proteins of $GABA_A$-ρ Receptors

It has been hypothesized that the assembly of a complete pentameric $GABA_A$-ρ receptor occurs in the rough endoplasmic reticulum and is followed by trafficking via the Golgi network to the cell surface. Accordingly, $GABA_A$-ρ2 tagged with green fluorescent protein was observed in clusters distributed at the soma and along the axons of retinal bipolar neurons, while in cerebellar astrocytes it was restricted only to the soma [60].

The N-terminus of the $GABA_A$-ρ contains signals that are critical for the correct assembly of subunits into functional pentameric $GABA_A$ receptors, as revealed via heterologous expression studies in *Xenopus leavis* [30]. A key element supporting this assumption are the first 46 residues of the N-terminal domain, prior to the extracellular α-helix, known as the N-terminal extension. In the $GABA_A$-ρ1 receptor the N-terminal extension is much larger than other members of the pLGIC superfamily, and its function has been tested and confirmed through the functional impact of consecutive serial deletions [80]. Nevertheless, our understanding of the whole process of trafficking remains rudimentary, but it is becoming increasingly clear that trafficking is facilitated by the proteins described below.

The cytoskeletal protein microtubule-associated protein 1B (MAP1B) is a critical factor for the development and function of the nervous system and is viewed as the most significant $GABA_A$-ρ receptor subunit anchoring protein. A 12 amino acid sequence (RI(D/N)THAIDKYSR) at the C-terminal end of the second intracellular domain of $GABA_A$-ρ1 interacts physically with MAP1B [81,82]. Clustering of $GABA_A$-ρ subunits occurs at synapses on bipolar axon terminals of the retina [39,40]. $GABA_A$-ρ subunits can also bind to a glycine transporter splice variant, GLYT-1E/F, in a distinct region of the MAP1B binding site. This transporter protein is also involved in the anchoring and signaling of excitatory and metabotropic receptors that interact with different proteins, thus playing pivotal roles in linking membrane proteins to the actin cytoskeleton [83].

The $GABA_A$ receptor-associated protein (GABARAP) interacts with the intracellular domain of $GABA_A$ receptors [84]. It binds to microtubules and to the N-ethylmaleimide-sensitive factor (NSF), a protein that is linked to events of intracellular vesicular fusion [85]. GABARAP is abundant in the Golgi apparatus and in intracellular vesicles, but it is not present at GABAergic synapses, suggesting its participation in the intracellular transport of both homomeric and heteromeric $GABA_A$ receptors [86].

Some evidence suggests that the crosstalk between $GABA_A$-ρ1 and $GABA_A$-ρ2 receptors and purinergic P_2X_2 receptors co-localize in the same clusters in the plasma membrane [87]. On the other hand, $GABA_A$-ρ1 directly interacts with protein kinase C-ζ (PKCζ) via adapter proteins of the ZIP protein family, as well as with protein phosphatase 1 (PP1). This interaction is important in promoting (or not) the cellular internalization of $GABA_A$-ρ subunits [88]. ZIP3 is a C-terminal splice variant of the ZIP protein family, and it is ubiquitously expressed in non-neuronal and neuronal tissues, including the retina. The $GABA_A$-ρ intracellular binding regions of ZIP3 contain ZZ-zinc finger domains that interact with 10 amino acids conserved in $GABA_A$-ρ1–3 subunits but not in heteromeric $GABA_A$ receptors. Consistently, only $GABA_A$-ρ1–3 subunits can bind to ZIP3, which dimerizes with another ZIP subunit (ZIP1–3), interacting with PKC-ζ. This complex is considered determinant for the formation of the scaffold of $GABA_A$-ρ subunits in the cytoskeleton [89].

4. $GABA_A$-ρ Receptors in Astroglia

Neurons are considered the basic unit for information processing associated with perception and cognition. However, this neurocentric definition has been challenged because they only represent a fraction of the brain cells. Glial cells, on the other hand, are the dominant population and respond to neuronal activity through neurotransmitter receptor activation [90]. Although the precise decoding of neuron–glia communication in brain function is unknown, glial cells contribute to synapse formation, cell development, myelination, brain microcirculation, neuroprotection, and the modulation of neural activity [91,92]. In this context, the $GABA_A$ receptors mediating synaptic (phasic) or extrasynaptic (tonic) transmission are molecularly and functionally distinct for both neurons and glial cells [93]. Astroglial cells include astrocytes, ependymal cells, Bergmann and Múller glia; all of them are identified by the expression of the cytoskeletal glial fibrillary acidic protein (GFAP). Astroglia express functional $GABA_A$ receptors, but their role in the CNS physiology is intriguing, since glial cells do not produce action potentials. Nevertheless, GABA depolarizes astrocytes through $GABA_A$ receptors but has unitary conductances and gating properties similar to those recorded in neurons [94]. Furthermore, specific differences in $GABA_A$ subunit expression were observed between primary cultures of rat cerebellar astrocytes and granular neurons [95]. Initial studies with RT-PCR reported that the total amount of $GABA_A$ receptor subunit mRNA in astrocytes was two orders of magnitude lower than in neuronal cells. Moreover, of all the $GABA_A$ subunits expressed by granular neurons, only α6 and γ2 subunits were not expressed by astrocytes. Another difference was that $GABA_A$-α1, α3, and α5 subunits were abundantly expressed in granular neurons, while $GABA_A$-α1 and α2 were predominant in cerebellar astrocytes. Likewise, $GABA_A$-β1 and β3 were abundantly expressed in both cell types. Finally, $GABA_A$-γ2 and $GABA_A$-γ1 were prevalent in cerebellar granular neurons and astrocytes, respectively [95]. Studies in astrocytes isolated from the thalamus showed that all of them express functional $GABA_A$ receptors that responded to the selective agonist muscimol [96]. The same study showed a $GABA_A$ subunit expression profile through single cell RT-PCR studies. The prevalent subunits were $GABA_A$-α2, $GABA_A$-α5, $GABA_A$-β1, and $GABA_A$-γ1 [96]. Overall, these studies reported the heterogeneous expression of $GABA_A$ subunits associated with a brain region. Accordingly, astroglia showed differences in their responses to pharmacological modulators of $GABA_A$ receptors such as benzodiazepines and barbiturates [9,93,97]. For example, DMCM, an inverse benzodiazepine agonist that enhanced $GABA_A$-mediated currents in astrocytes and inhibited them in neurons, suggests a different subunit composition [93]. Electrophysiological recordings on cerebellar astrocytes in situ showed a

heterogeneous array of $GABA_A$ subunits because modulation by benzodiazepines was absent in Bergmann glia [97]. Moreover, modulation by barbiturates, such as pentobarbital, was present in Bergmann glia but absent in ependymal glial cells of the cerebellum [6,97]. These differences are related to a specific $GABA_A$ subunit array that includes $GABA_A$-Δ or $GABA_A$-ρ subunits in Bergmann glia and ependymal cells, respectively [6,97]. Additionally, the functional expression of $GABA_A$ receptors was reported in situ via in striatal astrocytes; pentobarbital modulation was absent and $GABA_A$-ρ subunits were found in more than half of them [9] (Figure 2).

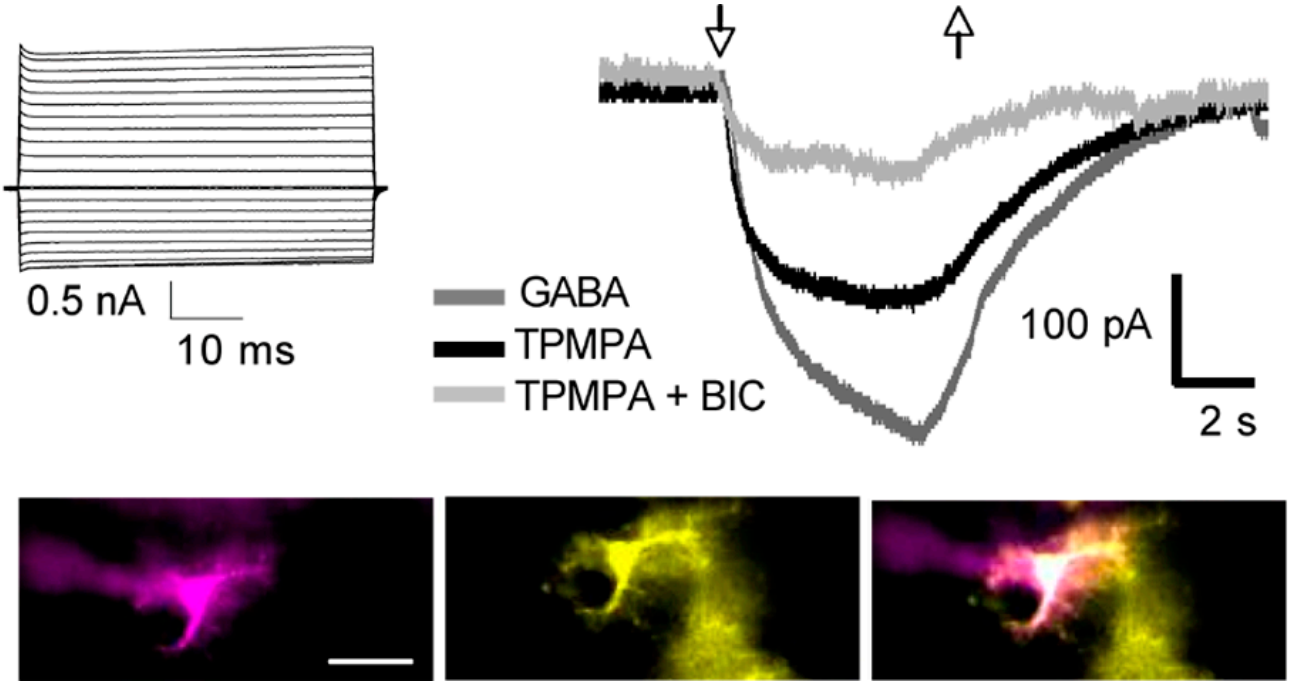

Figure 2. Functional expression of the $GABA_A$-ρ subunit in astrocytes. Whole-cell patch-clamp recording of an astrocyte in a coronal slice of the dorsal striatum of a GFAP-EGFP mouse. A classic passive current profile obtained from a sulforhodamine B (magenta)-injected cell that was also GFAP-EGFP+ (yellow). The overlay of both signals confirms cell identity. Scale bar represents 20 μm. Membrane currents were evoked in 50 ms voltage steps ranging from −160 to +40 mV, from a holding potential of −70 mV. Astrocytes responded to GABA (10 μM) and the current was partially reduced by the selective $GABA_A$-ρ antagonist TPMPA (100 μM); a further inhibition was observed when the selective GABA-A antagonist bicuculline (BIC, 100 μM) was added. The arrows indicate the start (up) and end (down) of the agonist/antagonist application.

Although a role for the $GABA_A$-ρ subunit expressed in glial cells is not yet evident, the three genes have been localized by means of immunogold electron microscopy in astrocytes from the neostriatum and cerebellum [5,9] (Figure 3).

The role of the $GABA_A$-ρ subunit in cerebellar or neostriatal astroglia during early postnatal development or in the control of precise movements in adults remains unexplored.

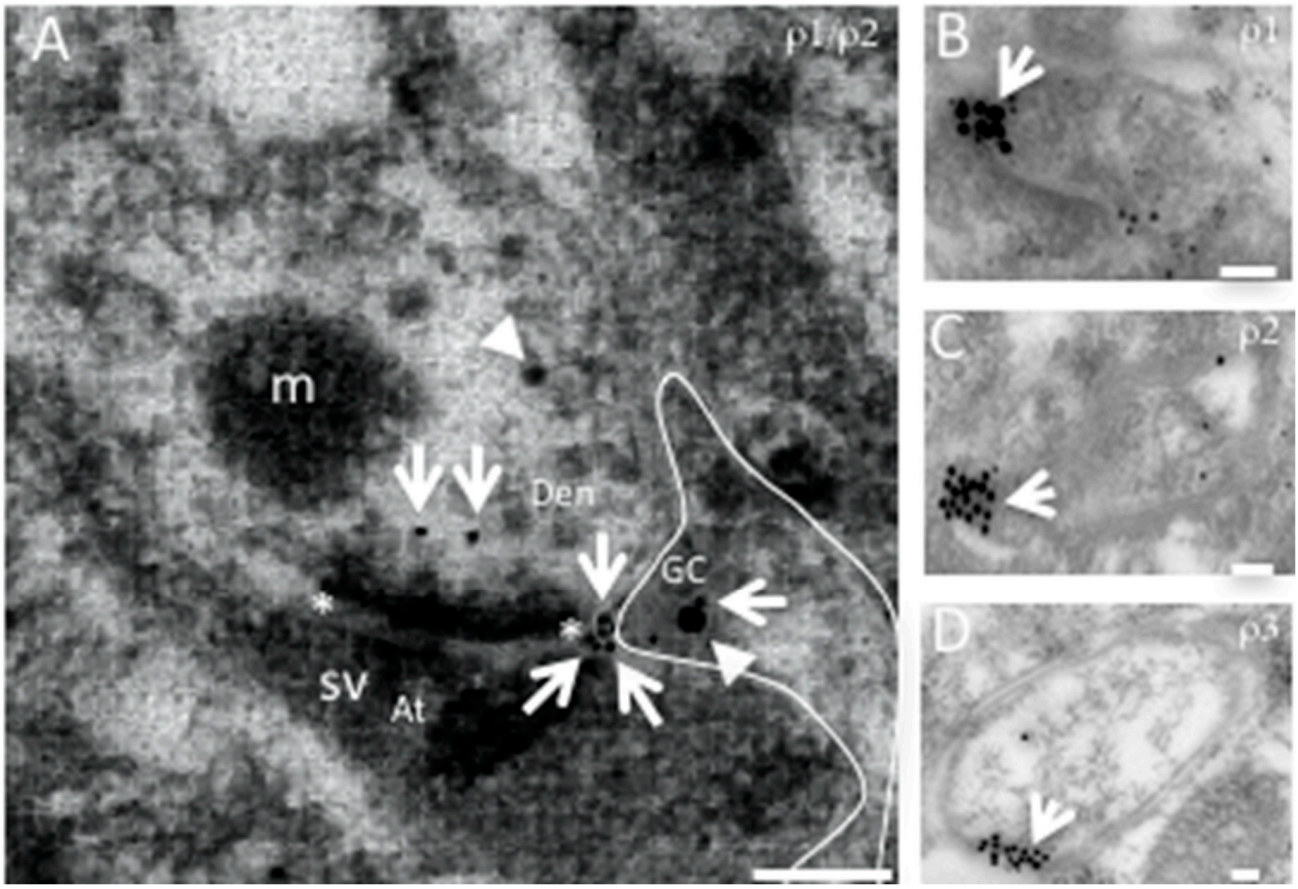

Figure 3. Representative electron micrographs of $GABA_A$-ρ1-3 immunogold localization in mouse neostriatum. (**A**). Axon–dendrite synaptic connection co-expressing $GABA_A$-ρ1 (arrow) and -ρ2, (arrowhead) and a positive glial cell process (GC) for both subunits (outlined). $GABA_A$-ρ1 is located at peri- and extrasynaptic spaces. The synaptic active zone is marked with asterisks; presynaptic region is marked with (At: axon terminal), and postsynaptic with (Den: dendrite). GCs with perisynaptic processes showing $GABA_A$-ρ1 (**B**), $GABA_A$-ρ2 (**C**) and $GABA_A$-ρ3 (**D**) immunogold localization. Scale bar 100 nm. (**B**–**D**). Expression of $GABA_A$-ρ subunits in glial processes. Abbreviations: m: mitochondria, sv: synaptic vesicles. Scale bar 200 nm.

5. $GABA_A$ Receptors and Extrasynaptic Communication in Brain Diseases

Huntington disease (HD). Patients with HD show motor, cognitive, and mental illnesses. The synthesis and release of GABA is compromised by the loss of striatal neurons, resulting in an increase in thalamo-cortical activity that produces "dance-like" movements. The expression of $GABA_A$ receptors displays a variability expression in HD [98] and the use of agonists is a common therapeutic strategy. Moreover, experimental evidence in HD transgenic R6/2 mouse brain showed that $GABA_A$-ρ3 immunoreactivity decreases significantly in the striatum, hippocampus, and cortex [50]. Accordingly, RT-PCR studies reported that the mRNA expression of $GABA_A$-ρ3 subunits occurs through different stages of the mouse postnatal development and double immunofluorescence studies showed its expression in calretinin+ interneurons and astrocytes, indicating a relevant role of this subunit in the physiology and pathology of the striatum [9].

Parkinson's disease (PD). The main symptoms of PD are tremor and motor problems, a hallmark of the disease is the increased expression of $GABA_A$ receptors in the susbstantia nigra and caudate nucleus in postmortem brains of diagnosed patients. GABAergic transmission through ionotropic receptors has two modalities known as phasic and tonic. The first is involved in the regulation of neuronal activity while the second is mediated by extrasynaptic receptors that are activated mainly by astrocytes and influence the gain

in neuronal excitability. Thus, astrocytes exhibit the augmented synthesis and release of GABA in PD, resulting in an increased tonic inhibition because of the augmented expression of GABA transporters (GATs) and high-affinity $GABA_A$ receptors [99].

Alzheimer's disease (AD). This mental illness is associated with elderly people and memory loss is evident because of the accumulation of β-amyloid plaques in the brain. The number of GABAergic terminals contacting cortical neurons is reduced, while $GABA_A$ receptors are increased in AD. Furthermore, GABA synthesis (GAD67 or MAOB) by astrocytes is increased and released through GAT3 or bestrophin 1 channels, causing an increased tonic inhibition in the dentate gyrus of a mouse model of AD. Thus, astrocytic GABA maybe relevant in the pathology of AD [99].

Autism spectrum disorder (ASD). This developmental disorder is characterized by stereotyped and repetitive behaviors, social interaction deficits, and communication impairments. Dysfunction of the GABAergic signaling is associated with ASD, particularly, the $GABA_A$ receptors are involved in neuronal proliferation and differentiation [10]. The depolarization induced by GABA during early neurodevelopment leads to a decrease in DNA synthesis and the proliferation of GFAP+ progenitor cells in the subventricular zone (SVZ). This mechanism was also observed in the adult hippocampus where progenitor cells showed tonic currents induced by $GABA_A$ receptors containing the α4 subunit. The signaling requires an autocrine/paracrine release of GABA. Lastly, the agonist muscimol induces the proliferation of granule cells in the developing cerebellum of the rat. Moreover, postmortem studies in patients diagnosed with ASD reported an increased expression of GAD67 in cerebellar interneurons, together with Purkinje cell loss and diminished expression of the $GABA_A$ subunits α1 and β3, resulting in a significant E/I imbalance ([100]). The preclinical model of autism induced by prenatal exposure to valproic acid (VPA) reproduced Purkinje cell loss and the increased expression of the astrocytic marker GFAP [10]. The expression of $GABA_A$-ρ3 increases linearly throughout neurodevelopment of the cerebellum and homomeric receptors show high affinity for the neurotransmitter (EC50 ∼ 3 μM). Immunofluorescence studies reported that, besides Purkinje cells, $GABA_A$-ρ3 is expressed in ependymal cells and whole-cell patch-clamp recordings confirmed the functional expression of $GABA_A$-ρ receptors, because GABA-evoked currents were partially blocked with TPMPA and completely abolished when bicuculline was added, pentobarbital potentiation was absent. Interestingly, GABAρ3 expression is disrupted in the VPA model, suggesting that this subunit is relevant in ASD [6,10].

6. Discussion

Interneuron–astrocyte communication through GABAergic signaling induces calcium transients that can actively regulate E/I balance through gliotransmission [101]. Indeed, extracellular chloride concentration is important for inhibitory transmission and is partly modulated by astrocytic depolarization, resulting from chloride efflux after $GABA_A$ receptor activation. The Cl-mediated depolarization favors the influx of Ca^{2+} from the extracellular space through voltage-gated calcium channels, promoting additional calcium release from intracellular stores and consequently, the release of different gliotransmitters [102] (Figure 4).

The astrocytic release of GABA was identified as one of the main extrasynaptic sources for tonic inhibition [103] and it controls the migration of neuronal precursors through $GABA_A$ receptor activation at the subventricular zone, while a similar mechanism operates for cortical development through $GABA_A$-ρ receptors [61,104]. Moreover, interneurons and astrocytes express $GABA_A$-ρ subunits during early postnatal development of the striatum [5,9], while GABAρ3 is expressed before the onset of hearing and later replaced by $GABA_A$-ρ1 and $GABA_A$-ρ2 subunits in the medial nucleus of the trapezoid body [6]. The $GABA_A$-ρ3 subunit in cerebellar ependymal cells is reduced in a preclinical model of autism induced by prenatal exposure to valproic acid, suggesting a key role of $GABA_A$-ρ receptors in neurodevelopment disorders [10]. On the other hand, therapeutic strategies targeting astroglial $GABA_A$-ρ receptors were recently explored in post-stroke motor recovery [11].

Thus, this review highlights $GABA_A$-ρ expressing astroglia as another population that can potentially modulate synaptic function. Accordingly, astrocytes with a specific molecular signature were recently shown to contribute to LTP strengthening and hippocampal memories, opposing hyperexcitation during seizures and possible sub-thalamic nucleus overactivation in Parkinson's disease after astrocyte-targeted genetic VGLUT deletion [105]. Future studies should explore the precise inhibitory synapse–astrocyte organization at the molecular (scRNAseq) and functional level (3D Ca2+ imaging).

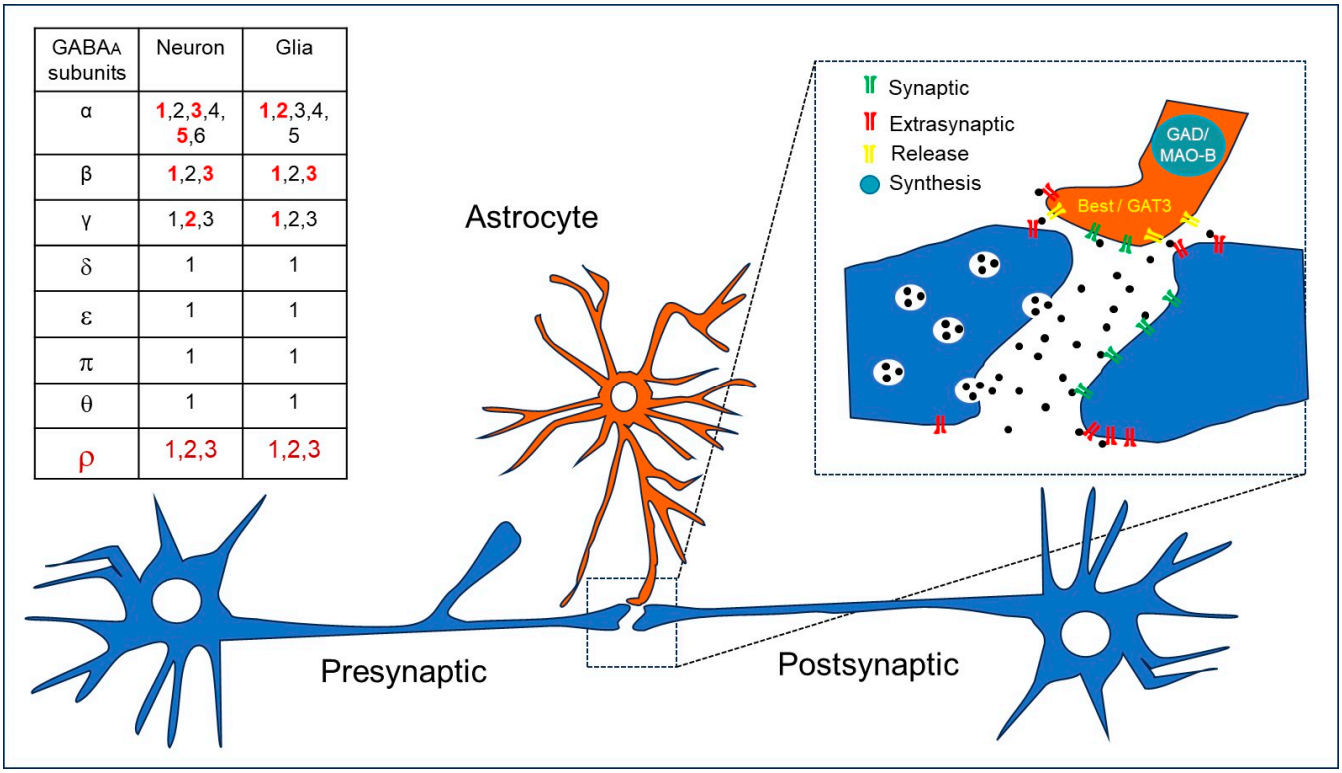

Figure 4. GABAergic Astrocytes modulate synaptic communication. The table summarizes $GABA_A$ subunits expressed by neurons and astrocytes; notice the common expression of GABAρ subunits. Thus, astrocytes express functional $GABA_A$ receptors, although with different subunit arrangements, as suggested by pharmacological evidence. Synaptic release of GABA by interneurons activates postsynaptic and extrasynaptic receptors. Activation of astrocytic $GABA_A$ receptors results in chloride efflux and depolarization. This opens voltage-gated calcium channels (VGCC) leading to an influx of extracellular Ca^{2+}. Activation of $GABA_B$ receptors induces release of Ca^{2+} from the intracellular IP_3-sensitive stores, resulting in calcium transients that promote gliotransmission. Astrocytes fine-tune synaptic communication through the release of gliotransmitters. Among them, GABA synthesis is mediated by glutamate decarboxylase (GAD) or monoamino oxidase B (MAO-B) and the release of this gliotransmitter is through bestrophin channels (Best) or the GABA transporter 3 (GAT-3). The release of astrocytic GABA can activate the presynaptic receptors of excitatory neurons and/or tonic currents through extrasynaptic receptors, regulating the excitability of interneurons.

7. Conclusions

$GABA_A$-ρ receptors are functionally expressed by neurons and astroglia. They are involved in extrasynaptic-mediated tonic currents and are relevant in earlier postnatal neurodevelopment. However, further studies are required to understand their role in the physiology and pathology of the central nervous system.

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