

# Extrasynaptic and postsynaptic receptors in glycinergic and GABAergic neurotransmission: a division of labor?

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Glycine and GABA mediate inhibitory neurotransmission in the spinal cord and central nervous system. The general concept of neurotransmission is now challenged by the contribution of both phasic activation of postsynaptic glycine and GABA<sub>A</sub> receptors (GlyRs and GABA<sub>A</sub>Rs, respectively) and tonic activity of these receptors located at extrasynaptic sites. GlyR and GABA<sub>A</sub>R kinetics depend on several parameters, including subunit composition, subsynaptic localization and activation mode. Postsynaptic and extrasynaptic receptors display different subunit compositions and are activated by fast presynaptic and slow paracrine release of neurotransmitters, respectively. GlyR and GABA<sub>A</sub>R functional properties also rely on their aggregation level, which is higher at postsynaptic densities than at extrasynaptic loci. Finally, these receptors can co-aggregate at mixed inhibitory postsynaptic densities where they cross-modulate their activity, providing another parameter of functional complexity. GlyR and GABA<sub>A</sub>R density at postsynaptic sites results from the balance between their internalization and insertion in the plasma membrane, but also on their lateral diffusion from and to the postsynaptic loci. The dynamic exchange of receptors between synaptic and extrasynaptic sites and their functional adaptation in terms of kinetics point out a new adaptive process of inhibitory neurotransmission.

**Keywords:** glycine receptors, GABA<sub>A</sub> receptors, postsynaptic, extrasynaptic, lateral diffusion, aggregation, cross-talk, presynaptic

## INTRODUCTION

The idea that, in the adult, non-synaptic release of neurotransmitters occurs in addition to classical release from presynaptic terminals, gains more significance in recent years. Accordingly, the contribution of both synaptic and extrasynaptic receptors to neuronal activity extends the general concept of neurotransmission. Recent findings demonstrated that the density of receptors at postsynaptic sites does not solely depend on the balance between their internalization and insertion in the plasma membrane, but also on their lateral diffusion from and to the postsynaptic loci (Bruneau and Akaaboune, 2006; Kneussel and Loeblich, 2007; Triller and Choquet, 2005). These observations introduced the new notion that receptor accumulation at postsynaptic loci is a dynamic process, resulting from receptor oscillation between a diffuse state at extrasynaptic sites and a more confined state within the postsynaptic density. While this has been first demonstrated for N-Methyl-D-Aspartate receptors (NMDARs) at glutamatergic excitatory synapses, it was also recently shown to occur at inhibitory synapses.

Glycine and GABA are the main inhibitory neurotransmitters in the central nervous system (CNS) of vertebrates. They activate two distinct

families of ionotropic ligand-gated chloride channels, glycine receptors (GlyRs) (Legendre, 2001) and GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) (Macdonald and Olsen, 1994), which accumulate at postsynaptic sites to allow effective fast inhibitory neurotransmission. The postsynaptic aggregation and stabilization of GlyRs and GABA<sub>A</sub>Rs require interactions with intracellular anchoring proteins, which are part of a subsynaptic protein scaffold linked to the cytoskeleton (Legendre, 2001). In the recent years, however, it has become apparent that inhibitory receptors are not necessarily facing presynaptic terminals. During synaptogenesis and even in the adult, GlyRs and GABA<sub>A</sub>Rs are localized both at extrasynaptic and postsynaptic sites. These receptors are likely to display different functional properties, according to their subcellular distribution. Indeed, it is now well established that postsynaptic receptors are mostly responsible for fast and phasic neurotransmission while activation of extrasynaptic receptors would rather be involved in slow and tonic modulation of neuronal activity. Moreover, several evidences indicate that even inhibitory receptors trapped at postsynaptic loci can have different functional properties, depending on their aggregation level. Thus, the functional properties of inhibitory GlyRs and GABA<sub>A</sub>Rs will vary as a function of their subcellular.

Despite these functional differences, glycinergic and GABAergic synapses display morphological similarities. Glycine and GABA notably share the same presynaptic vesicular transporter (the vesicular inhibitory amino acid transporter VIAAT) (Dumoulin et al., 1999; Sagné et al., 1997) while the anchoring protein gephyrin is involved in the aggregation and postsynaptic stabilization of both GlyRs and GABA<sub>A</sub>Rs (Legendre, 2001). Accordingly, it is now well established that glycinergic and GABAergic neurotransmission can be intermingled to provide inhibition of neuronal activity. Indeed, in the spinal cord and several distinct brainstem areas, glycine and GABA can be co-released from the same presynaptic terminal to activate postsynaptic GlyRs and GABA<sub>A</sub>Rs, as demonstrated by recording mixed miniature inhibitory postsynaptic currents. Interestingly,

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recent findings indicate that the co-activation of GlyRs and GABA<sub>A</sub>Rs at mixed inhibitory synapses does not simply display a summation of currents mediated by both receptor subtypes, resulting from functional cross-talk between these co-aggregated postsynaptic receptors.

The purpose of this review is to present a coherent synthesis of recent findings focusing on the variability of GlyR and GABA<sub>A</sub>R functional properties, depending on their postsynaptic/extrasynaptic localization, their aggregation level and/or their level of interaction. In particular, the possible functional significance of this variability will be discussed.

## MOLECULAR HETEROGENEITY OF GlyRs AND GABA<sub>A</sub>Rs

As nicotinic acetylcholine and 5-hydroxytryptamine type 3 receptors, GlyRs and GABA<sub>A</sub>Rs belong to the cysteine-loop receptor family (Lynch, 2004). GlyRs and GABA<sub>A</sub>Rs are pentameric assemblies of subunits, whereby each subunit contains four transmembrane domains (TM1–TM4). The transmembrane domain TM2 forms the channel pore permeable to chloride ions. Subunit expression is developmentally regulated and varies depending on the brain region studied (Lynch, 2004; Mohler et al., 1995; Paysan and Fritschy, 1998).

### GlyR composition

GlyRs are composed of  $\alpha$  (1–4) and  $\beta$  subunits which assemble to form  $\alpha(2)\beta(3)$  heteromeric or  $\alpha(5)$  homomeric receptors. The agonist binding site is mainly carried by the  $\alpha$  subunit (Lynch, 2004) while the  $\beta$  subunit binds the anchoring protein gephyrin with high affinity (Kirsch and Betz, 1993; Meyer et al., 1995). GlyR subunits are differentially expressed depending on the brain area studied. In the adult, heteromeric  $\alpha1\beta$  GlyRs are predominant and uniformly expressed in the CNS, while the expression of the  $\alpha2$  subunit has been mainly detected in the cortex, the olfactory bulb, the thalamus, the central gray matter, the retina and the hippocampus (Malosio et al., 1991; Sato et al., 1992). The expression of the  $\alpha3$  subunit is restricted to the spinal cord ventral horn, the cerebellum, the olfactory bulb and the retina (Malosio et al., 1991). Finally, the  $\alpha4$  subunit is predominantly expressed in the inner plexiform layer of the retina (Heinze et al., 2007).

In addition to their regional distribution, GlyR subunit expression has been shown to be developmentally regulated (Lynch, 2004). The  $\alpha2$  subunit is already detected at the onset of synaptogenesis in the developing CNS, followed by the expression of  $\beta$ ,  $\alpha1$  and  $\alpha3$  subunits around birth. The expression of  $\alpha1$  and  $\alpha3$  subunits progressively increases during the first postnatal weeks while the expression of the  $\alpha2$  subunit decreases to reach a basal level during the same period. Thus, it is assumed that immature GlyRs would be homomeric  $\alpha2$  receptors, progressively replaced by  $\alpha2\beta$  heteromeric GlyRs and then by  $\alpha1\beta$  and  $\alpha3\beta$  heteromeric GlyRs. This developmental pattern has been determined in the spinal cord and several brainstem nuclei of rodents (Malosio et al., 1991; Piechotta et al., 2001; Singer et al., 1998).

The diversity of GlyR subunits is increased by the alternative splicing of  $\alpha1$ ,  $\alpha2$  and  $\alpha3$  subunits (Kuhse et al., 1991; Malosio et al., 1991; Nikolic et al., 1998). Interestingly,  $\alpha2$ - or  $\alpha3$ -containing GlyRs display strong differences in their functional properties depending on splice variants. In case of the  $\alpha2$  subunit, two alternative splice variants were detected, namely the  $\alpha2A$  and the  $\alpha2B$  subunits (Kuhse et al., 1991). Compared to the  $\alpha2A$  subunit,  $\alpha2B$  contains an isoleucine (instead of valine) at position 58 and an alanine (instead of threonine) at position 59. From a functional point of view,  $\alpha2A$ -containing GlyRs display a lower potency for glycine (higher EC<sub>50</sub> value) than those containing the  $\alpha2B$  subunit. In case of the  $\alpha3$  subunit, two alternative splice variants, namely  $\alpha3K$  and  $\alpha3L$ , were isolated from human fetal brain (Nikolic et al., 1998). Compared to the  $\alpha3K$  variant,  $\alpha3L$  contains an additional sequence of 15 amino acids, inserted within the cytoplasmic loop that connects transmembrane domains TM3 and TM4 (Nikolic et al., 1998). Interestingly, this alternative insert contains a consensus amino acid sequence for casein kinase-II

dependent phosphorylation (serine at position 370) (Nikolic et al., 1998). Functionally,  $\alpha3L$ -containing GlyRs desensitize more slowly than those containing the  $\alpha3K$  subunit. These results clearly show that alternative splicing alters the functional properties of GlyRs.

### Large diversity of GABA<sub>A</sub>Rs

In contrast to GlyRs, GABA<sub>A</sub>Rs are composed of a large variety of different subunits. Indeed, nineteen GABA<sub>A</sub>R subunits have been cloned so far ( $\alpha1$ –6,  $\beta1$ –3,  $\gamma1$ –3,  $\delta$ ,  $\epsilon$ ,  $\theta$ ,  $\pi$  and  $\rho1$ –3; the  $\rho1$ –3 subunits can also be part of GABA<sub>C</sub> receptors) (Macdonald and Olsen, 1994). While theoretically this subunit diversity allows for many different subunit combinations, only a dozen were detected so far (Farrant and Nusser, 2005). The most abundantly expressed GABA<sub>A</sub>R in the CNS is composed of two  $\alpha1$ , two  $\beta$  and one  $\gamma2$  subunit. The agonist binding site is carried mainly by the  $\alpha$  subunits, while the  $\gamma$  subunits are responsible for linking GABA<sub>A</sub>Rs to the postsynaptic cytoskeleton. Similarly to GlyRs, GABA<sub>A</sub>R subunit expression is developmentally regulated (Paysan and Fritschy, 1998) and depends on the brain area studied (Wisden et al., 1992). Finally, as for GlyRs, the diversity of GABA<sub>A</sub>R subunits is increased by alternative splicing (Farrant and Nusser, 2005).

## CORRESPONDENCE BETWEEN GlyR AND GABA<sub>A</sub>R SUBCELLULAR DISTRIBUTIONS AND SUBUNIT COMPOSITIONS

### Homomeric and heteromeric GlyRs

The GlyR  $\beta$  subunit directly binds gephyrin, which immobilizes postsynaptic GlyRs through gephyrin-mediated anchoring at the subsynaptic cytoskeleton. Thus, heteromeric  $\alpha\beta$  GlyRs are expected to be mainly postsynaptically located, although it has been suggested that GlyRs and gephyrin could bind before GlyR insertion into postsynaptic sites (Hanus et al., 2004). To the contrary, homomeric  $\alpha$  GlyRs, which do not contain the  $\beta$  subunit, are expected to freely diffuse in the plasma membrane rather than to accumulate at postsynaptic densities, thus representing extrasynaptic GlyRs. However, low-affinity interactions between the  $\alpha2$  subunit and gephyrin suggest that homomeric  $\alpha2$  GlyRs could also aggregate at postsynaptic loci (Takagi et al., 1992). Indeed, synaptic currents displaying functional characteristics of homomeric GlyRs have been recorded from the hindbrain of zebrafish larvae (Legendre, 1997), suggesting that these receptor subtypes could also be synaptically activated.

### Extrasynaptic localization of GABA<sub>A</sub>Rs: $\alpha5$ and $\delta$ subunits

GABA<sub>A</sub>Rs composed of  $\alpha1$ –4,  $\alpha6$ ,  $\beta2/3$  or  $\gamma2$  subunits can be found both at postsynaptic loci and at extrasynaptic sites, albeit at lower density (Fritschy et al., 1992; Nusser et al., 1995). The  $\gamma2$  subunit appears to be essential for postsynaptic accumulation of GABA<sub>A</sub>Rs (Essrich et al., 1998; Sassoè-Pognetto et al., 1995; Sur et al., 1995). Therefore, replacement of the  $\gamma2$  subunit by the  $\delta$  subunit may have a profound impact on the subcellular localization of GABA<sub>A</sub>Rs. Indeed,  $\alpha4\beta\delta$  and  $\alpha6\beta\delta$  GABA<sub>A</sub>Rs were found exclusively at extrasynaptic sites (Nusser et al., 1998). Moreover, the  $\alpha5$  subunit also seems to contribute to the pool of extrasynaptic receptors because in hippocampal pyramidal cells these GABA<sub>A</sub>Rs are found diffusely distributed and do not co-localize with postsynaptic gephyrin (Brünig et al., 2002). Accordingly, it was postulated that the  $\alpha5$  subunit prevents GABA<sub>A</sub>Rs from postsynaptic accumulation by impairment of the  $\gamma2$ -gephyrin interaction interface (Crestani et al., 2002).

## SYNAPTIC AND NON-SYNAPTIC RELEASE OF GLYCINE AND GABA

GlyRs and GABA<sub>A</sub>Rs display different functional properties according to whether they are accumulated at postsynaptic densities or diffuse at extrasynaptic sites. To some extent, these differences result from different receptor activation modes, according to fast presynaptic or slow paracrine neurotransmitter release.



### Presynaptic release and fast inhibitory transmission

Fast synaptic transmission is mediated by the transient activation of postsynaptic receptors, which allows the conversion of presynaptic activity into postsynaptic signals that shape the activity of the postsynaptic cell. When an action potential reaches the presynaptic terminal, a local calcium influx rapidly increases the release probability of presynaptic vesicles. In case of inhibitory synapses, each vesicle can release a large number (several thousands) of GABA and/or glycine molecules into the synaptic cleft in a very short time interval. Consequently, the neurotransmitter concentration in the vicinity of postsynaptic receptors increases quickly and allows their synchronous activation or opening. Neurotransmitter clearance either by diffusion out of the synaptic cleft or by re-uptake through specific transporter proteins then stops receptor activation.

Thus, particular features of phasic synaptic transmission are the short duration and the transient high neurotransmitter concentrations within the synaptic cleft. In fact, using low-affinity competitive GABA<sub>A</sub>R antagonists, the decay time constant of GABA clearance from the synaptic cleft was estimated to be ~100 microseconds (Barberis et al., 2004; Mozrzymas et al., 1999). This parameter has not been determined for glycinergic phasic synaptic transmission. However, according to the simple geometry of glycinergic synaptic clefts (Vannier and Triller, 1997), to the diffusion coefficient of glycine and to the lack of effect of glycine re-uptake inhibitors on the time course of glycinergic synaptic events (Titmus et al., 1996), synaptic glycine clearance is likely to be as fast as synaptic GABA clearance.

Another important parameter modulating postsynaptic receptor activation is their occupancy level by neurotransmitters released from presynaptic terminals. The occupancy level at individual postsynaptic site was investigated at glycinergic synapses in the hindbrain of zebrafish (Rigo et al., 2003) as well as at GABAergic synapses formed between basket and stellate interneurons in the cerebellum (Auger and Marty, 1997). In both studies, application of  $\alpha$ -Latrotoxin (a neurotoxin purified from black widow spider venom) triggered massive vesicular neurotransmitter release at active zones and induced bursts of miniature synaptic events that apparently originated at single release sites. The postsynaptic receptor occupancy level was then extracted from miniature postsynaptic current analysis. Although in some cases, postsynaptic receptors were saturated by the release of a single vesicle, the occupancy level was shown to strongly vary between individual synaptic sites and even at the same synapse, which is due to variations in the number of neurotransmitter molecules released per vesicle.

### Paracrine release and tonic inhibitory transmission

While phasic activation of postsynaptic GlyRs and GABA<sub>A</sub>Rs has been considered for decades to represent the main activation mode of inhibitory receptors, recent reports provide strong evidence for a slower receptor activation when located at extrasynaptic sites. These observations point to a tonic activation of extrasynaptic receptors, which is very likely to also contribute to inhibitory neurotransmission. The slow time course of extrasynaptic receptor activation can be explained by spillover of neurotransmitters from the synaptic cleft and by paracrine release of neurotransmitters from adjacent non-neuronal cells. Indeed, neurotransmitters released from astrocytes through vesicular dependent pathways were shown to modulate synaptic activity (Evanko et al., 2004). In fact, glycine uptake into synaptosomes through glycine transporters was shown to mediate carrier-dependent release of GABA (Raiteri et al., 2001), and since these transporters are expressed by astrocytes, a reverse activity of specific re-uptake transporters could provide a mechanism for non-synaptic release of neurotransmitters and modulation of inhibitory transmission through tonic receptor activation.

Slow activation of GABA<sub>A</sub>Rs has been extensively studied and recently reviewed by Farrant and Nusser (Farrant and Nusser, 2005). Continuous or tonic activation of GABA<sub>A</sub>Rs occurs before and during synaptogenesis at embryonic stages (LoTurco et al., 1995). However, this tonic activation has also been demonstrated at more mature stages in thalamocortical

neurons (Cope et al., 2005), in the hippocampal dentate gyrus, cerebellar granule cells (Brickley et al., 1996), in pyramidal neurons from the fifth layer of the somatosensory cortex (Salin and Prince, 1996), as well as in pyramidal cells and interneurons in the CA1 region of the hippocampus (Bieda and MacIver, 2004; Marchionni et al., 2007). Spillover of GABA in response to high presynaptic activity can slowly activate peri-synaptic GABA<sub>A</sub>Rs, reinforcing inhibitory GABAergic transmission. In the dentate gyrus of the hippocampus, however, vesicular release of GABA does not play a role in tonic GABA<sub>A</sub>R activation (Carta et al., 2004), suggesting a paracrine release of unknown origin and mechanism. Indeed, it was proposed that GABA re-uptake transporters, which usually remove GABA from the extracellular space, could function in a reverse manner depending on the sodium gradient in their vicinity, thereby providing ambient GABA responsible for continuous GABA<sub>A</sub>R activation (Attwell et al., 1993). However, this hypothesis has been challenged by studies describing an increase in the amplitude of GABA-mediated tonic currents in the presence of GABA re-uptake inhibitors (Jensen et al., 2003) as well as in mice deficient for these transporters (Wall and Usowicz, 1997).

Tonic activation of GlyRs is less documented than that of GABA<sub>A</sub>Rs. Rather than glycine, the endogenous GlyR agonist taurine appears to be involved in tonic activation of extrasynaptic GlyRs both at embryonic and adult stages. High taurine levels are detected in non-neuronal cells, such as astrocytes (Dutton et al., 1991), in immature neurons (Flint et al., 1998), as well as in specific neuronal subtypes like Purkinje cells in the cerebellum (Madsen et al., 1985). Initially, a non-synaptic release of taurine, sensitive to hypo-osmotic shock, was described in the immature cortex of mice. Although the release mechanism was not investigated in this study, the main source of taurine was discussed to be immature cortical neurons (Flint et al., 1998). While the concentration of taurine present in the immature cortex is not known, it was estimated to be ~0.3 mM, which roughly corresponds to the EC<sub>50</sub> (1.1 mM) of GlyRs expressed by these neurons (Flint et al., 1998). Another example of non-synaptic hypo-osmotic- or high-potassium-sensitive taurine release was described in the neurohypophysis. Furthermore, taurine release from pituicytes was shown to activate GlyRs expressed by supraoptic neurons, inhibiting vasopressin neuronal release (Hussy et al., 2001). Finally, while a few glycine-immunostained fibers were detected in the substantia nigra pars compacta (Rampon et al., 1996), taurine is assumed to be the major endogenous GlyR agonist in this brain area (Doretto et al., 1994; Häusser et al., 1992; Rampon et al., 1996). Besides these reports, a recent microdialysis study revealed an increase in the extracellular glycine concentration following calcium-dependent high potassium-mediated depolarization in the substantia nigra pars compacta of the rat (Doretto et al., 1994). Blocking either the tricarboxylic cycle with fluorocitrate or the glutamine synthetase activity markedly increased the extracellular level of glycine, suggesting that glycine could be released from astrocytes under specific conditions (Dopico et al., 2006). However, as the experimental conditions (application of hypo-osmotic or high extracellular potassium solutions) used to evoke this kind of neurotransmitter release failed to activate GlyRs expressed by dopaminergic neurons, the functional significance of this putative non-synaptic glycine release remains to be elucidated in the substantia nigra pars compacta (Mangin et al., 2002).

## SYNAPTIC NEUROTRANSMITTER RELEASE AND INTRINSIC FUNCTIONAL PROPERTIES OF POSTSYNAPTIC GlyRs AND GABA<sub>A</sub>Rs

Fast release of neurotransmitters within the synaptic cleft induces the synchronous activation of postsynaptic receptors underlying the fast onset of synaptic events. At single release site, the amplitude of synaptic currents will therefore depend on several pre- and postsynaptic factors, such as the number of neurotransmitter molecules released per vesicle (Kruk et al., 1997), the number (Kruk et al., 1997) and the single conductance levels (Robinson and Kawai, 1993) of postsynaptic receptors and the intrinsic receptor activation properties.

## Deactivation

A common property of postsynaptic GlyRs and GABA<sub>A</sub>Rs is their fast intrinsic kinetics, allowing them to respond to transient neurotransmitter release. The time course of the deactivation phase of glycinergic and GABAergic postsynaptic currents varies from 5 to 50 ms. These values also fit with the deactivation phase of miniature postsynaptic currents, which represent the activation of few postsynaptic receptors in response to the release of a single vesicle. Since glycine and GABA clearance from the synaptic cleft is considerably faster than the deactivation time course of miniature postsynaptic events, the deactivation time course is expected to be mainly governed by intrinsic functional properties of receptor channels. These intrinsic kinetic properties of postsynaptic receptors largely depend on their subunit composition. For example, postsynaptic currents resulting from the activation of  $\alpha 1\beta$  heteromeric GlyRs display a faster decay time constant than those mediated by  $\alpha 2\beta$  heteromeric GlyRs (Takahashi et al., 1992). In case of postsynaptic GABA<sub>A</sub>R, the length of the burst of channel openings varies according to the subtype of GABA<sub>A</sub>R  $\alpha$  subunits present within the channel (Gingrich et al., 1995). However, at inhibitory synapses the deactivation time course of postsynaptic currents can be lengthened through activation of peri-synaptic receptors by spillover of glycine (Ahmadi et al., 2003) or GABA (Rossi and Hamann, 1998) from the synaptic cleft. This prolonged deactivation phase then reflects the integrative image of bursts of channel re-openings, which occur before the agonist dissociates from its binding site (Betz, 1990; Unwin, 1993).

## Desensitization

Prolonged application of agonist induces receptor channel desensitization, a state in which the channels remain closed in the continued presence of the agonist. The rates of onset and recovery from desensitization are crucial in the control of size, decay time and frequency of postsynaptic currents (Jones and Westbrook, 1996).

In case of postsynaptic GlyRs,  $\alpha\beta$  heteromeric receptors display slow desensitization kinetics (Legendre, 2001). It is therefore unlikely that receptor desensitization kinetics influence the deactivation time course of glycinergic postsynaptic currents (Legendre, 1998, 2001). However, a recent study has shown that GlyR desensitization can influence the amplitude of glycinergic synaptic currents at stimulation rates  $>1$  Hz (Rigo and Legendre, 2006). This finding highlights the first evidence that GlyR desensitization might influence glycinergic synaptic function. Two recent modeling studies that incorporated desensitized states into GlyR kinetic schemes concluded that desensitized states can be reached from both single- and double-bound ligand open states (Gentet and Clements, 2002; Rigo and Legendre, 2006). Interestingly, the phosphorylation of  $\alpha 1$ -containing GlyRs controls its desensitization level (Gentet and Clements, 2002; Webb and Lynch, 2007), suggesting that synaptic strength can be modified by the activation of metabotropic receptors (Legendre, 2001).

Fast desensitization kinetics of specific postsynaptic GABA<sub>A</sub>R subtypes can shape the deactivation time course of postsynaptic currents. When the rates of onset and recovery from desensitization are fast enough, the receptors enter in a desensitized state during its transitory activation evoked by synaptic vesicular release (Scanziani, 2000). Considering that GABA<sub>A</sub>Rs with pronounced desensitization kinetics have functional significance only when these receptors are located postsynaptically, their desensitization properties could promote negative cross-talk during GABA spillover, between GABAergic synapses and/or between synaptic and non-synaptic GABA release. Although GABA<sub>A</sub>R accumulation in fast desensitized states reduces the amplitude of subsequent inhibitory synaptic currents, slow desensitization is likely to confer a high-pass character in the spatial domain since GABA can diffuse between inhibitory synapses to activate postsynaptic (Ahmadi et al., 2003) or extrasynaptic GABA<sub>A</sub>Rs (Scanziani, 2000). Indeed, GABA reaching distant sites during its spillover, in addition to its small and slowly-rising non-synaptic release (Barbour and Häusser, 1997; Bergles et al., 1999; Rusakov and

Kullmann, 1998), will result predominantly in slow desensitization, rather than activation, of GABA<sub>A</sub>Rs at neighboring synapses (Overstreet et al., 2000). This will therefore reduce the efficiency of neighboring GABAergic synapses during sustained presynaptic activity.

## CROSS-TALK BETWEEN GlyRs AND GABA<sub>A</sub>Rs

Several recent reports indicate that simultaneous activation of distinct co-aggregated receptors can induce cross-modulation of their respective activation properties. “Cross-talk” mediated by direct or indirect structural interactions between receptors might represent a fast adaptive process involved in the control of synaptic transmission efficacy. A direct “cross-talk” involving protein-protein interactions was demonstrated for several couples of metabotropic and ionotropic receptors, such as D1-dopamine and A1-adenosine receptors (Ginés et al., 2000), D1-dopamine and NMDARs (Lee et al., 2002), D2-dopamine and AMPA receptors (Zou et al., 2005) and D5-dopamine and GABA<sub>A</sub>Rs (Liu et al., 2000). “Cross-talk” also exists between ionotropic receptors, as demonstrated for ATP-P2X2 and  $\alpha 3/\beta 4$  nicotinic receptors (Khakh et al., 2000), ATP-P2X2 and GABA<sub>A</sub>Rs (Sokolova et al., 2001) and ATP-P2X3 and GABA<sub>A</sub>Rs (Toulmé et al., 2007). The cross-modulation of functional receptor properties can be directly mediated through a molecular interaction between receptors. ATP-P2X2/GABA<sub>A</sub>Rs and ATP-P2X3/GABA<sub>A</sub>Rs “cross-talk” involves a direct interaction between the C-terminal domain of the P2X2 subunit and the intracellular loop of the GABA<sub>A</sub>R  $\beta 3$  subunit (Boué-Grabot et al., 2004), or an intracellular QST (386–388) motif in the P2X3 subunit (Toulmé et al., 2007), respectively. Cross-modulation can also be indirect, as described for ATP-P2X2 and nicotinic receptors which require CAMKII activation (Díaz-Hernández et al., 2006).

## Functional interaction between GlyRs and GABA<sub>A</sub>Rs

The idea of a functional interaction between GlyRs and GABA<sub>A</sub>Rs has been proposed more than 20 years ago (Barker and McBurney, 1979). In spinal cord cell culture, sequential application of glycine and GABA induces a decrease in the amplitude of glycinergic or GABAergic-evoked responses, suggesting that glycine and GABA could share the same conductance channel (Barker and McBurney, 1979). However, this hypothesis has been challenged by the complete cloning of both GlyRs and GABA<sub>A</sub>Rs, which shows that these receptors are separate entities. In 1992, Grassi and colleagues demonstrated that cross-inhibitory effects observed in cultured rat hippocampal neurons were due to alterations in the chloride gradient and therefore in the driving force of chloride ions (Grassi, 1992). Since these neurons displayed no evidence for GlyR-GABA<sub>A</sub>R co-aggregation, a direct functional cross-interaction between these receptors was concluded to be unlikely.

The demonstration that cross-inhibition between glycinergic- and GABAergic-evoked responses does not simply result from a shift in the equilibrium potential of chloride ions was provided by Trombley and co-workers (1999). The authors clearly showed that inhibition of the GABAergic response in rat olfactory bulb neurons by pre-application of glycine was voltage-independent and can not be related to a change in the reversal potential of the evoked current. Interestingly, they also observed that this cross-inhibition was asymmetrical, with the glycinergic-evoked response being more efficient to inhibit the GABAergic current than vice-versa. Finally, it has been suggested that this “cross-talk” could be cell type-specific since it was not observed in all cells analyzed (Trombley et al., 1999). As relatively high concentrations of glycine and GABA (~1 mM) were used in this study, it was proposed that cross-inhibition mostly reflects the activation of specific inhibitory amino acid receptors that could equally bind glycine and GABA. A similar conclusion was obtained by Baev and co-workers (1992) from isolated lamprey spinal cord neurons. However, the existence of such a receptor remains to be determined. Moreover, GlyRs and GABA<sub>A</sub>Rs respectively displayed high and specific affinity for their respective endogenous agonist, glycine



and GABA. Although homomeric  $\alpha 1$  and  $\alpha 2$  GlyRs could be activated by GABA, this activation required high neurotransmitter concentrations, strongly above the physiological range (De Saint Jan et al., 2001), and there was no evidence for GABA<sub>A</sub>R activation by glycine.

An asymmetric cross-talk between glycinergic and GABAergic responses mediated by distinct GlyRs and GABA<sub>A</sub>Rs has been finally demonstrated on freshly dissociated dorsal horn neurons of juvenile rat spinal cords using the whole-cell patch-clamp recording technique (Li et al., 2003). First, the amplitude of inhibitory currents induced by simultaneous application of glycine and GABA was smaller than the sum of those mediated by separate applications of both neurotransmitters, clearly indicating a cross-modulation of glycine- and GABA-evoked responses. Then, sequential application of glycine and GABA revealed that the GABA-evoked current was more affected by glycine pre-application than was the glycinergic one by pre-application of GABA. GlyR activation notably decreases the amplitude and accelerates the rate of desensitization of GABA-evoked currents. This cross-inhibition does not result from a shift in the chloride equilibrium potential and is specifically mediated by GlyRs. Indeed, it was still observed at low glycine concentration (30  $\mu$ M) and could be prevented by the application of the GlyR antagonist strychnine, without affecting GABA-evoked response. These data rule out the existence of a single inhibitory amino acid receptor sensitive to both glycine and GABA, at least in dorsal horn neurons (Li et al., 2003). In these neurons, recovery from cross-inhibition is slow and displays a half-life ranging from 30 to 40 seconds for GlyR-dependent inhibition of GABA-evoked response, and a two-time shorter half-life for GABA<sub>A</sub>R-dependent inhibition of glycine-evoked response.

Several mechanisms are thought to be involved in this asymmetrical inhibitory cross-modulation, including different intracellular signaling pathways and probably direct protein-protein interactions between GlyRs and GABA<sub>A</sub>Rs. GlyR-dependent inhibition of GABA-evoked responses is mediated by dephosphorylation of GABA<sub>A</sub>Rs by the phosphatase 2B, also known as calcineurin. Indeed, this cross-modulation was prevented by CSPN, a specific calcineurin inhibitor. As a control, application of okadaic acid, which specifically blocks phosphatases 1 and 2A, had no effect (Li et al., 2003). Accordingly and since it was irreversible in presence of the kinase inhibitor staurosporine, recovery from cross-modulation is likely to involve GABA<sub>A</sub>R re-phosphorylation. Because a direct activation of phosphatase 2B through GlyRs awaits experimental confirmation, it is still unclear whether GlyR activation results in direct dephosphorylation of GABA<sub>A</sub>Rs or whether this is a consequence of dephosphorylation of GABA<sub>A</sub>R-associated subsynaptic proteins. Accordingly, Li and co-workers (2003) showed that the increase in intracellular calcium concentration induced by co-application of glycine and GABA was unlikely to activate this phosphatase. Instead, it was proposed that GlyR activation induces a change in the GABA<sub>A</sub>R conformation through receptor-receptor interaction, resulting in the exposure of phosphorylated GABA<sub>A</sub>R sites to phosphatase 2B. Accordingly, phosphatase 2B was shown to be mostly associated with the plasma membrane (Yakel, 1997), suggesting its close association with GABA<sub>A</sub>Rs (Jones and Westbrook, 1997). Moreover, it was found to modulate GABA<sub>A</sub>R desensitization (Li et al., 2003; Martina et al., 1996).

In contrast to GlyR-dependent inhibition of GABA-evoked responses, GABA<sub>A</sub>R-dependent inhibition of glycine-evoked currents does not involve phosphorylation mechanisms. Instead, this cross-modulation could be mediated by direct coupling between GlyRs and GABA<sub>A</sub>Rs, although the contribution of an unknown signaling protein mediating this interaction could not be excluded.

#### Cross-talk at mixed inhibitory glycinergic/GABAergic synapses

During synaptic vesicular release, postsynaptic GlyRs and GABA<sub>A</sub>Rs are specifically activated by glycine and GABA, respectively. Although glycinergic and GABAergic neurotransmissions could both contribute to inhibition in several CNS areas, it was assumed for decades that inhibitory synapses were purely glycinergic or purely GABAergic and functioned

through distinct pathways. In 1998, the pioneer work of Jonas and co-workers (1998) elegantly demonstrated that, in rat ventral spinal cord, GABA and glycine can be co-released from the same presynaptic terminal to co-activate postsynaptic GlyRs and GABA<sub>A</sub>Rs. Functional mixed inhibitory synapses were further identified from interneurons of distinct spinal cord layers (Geiman et al., 2002; González-Forero and Alvarez, 2005; Keller et al., 2001), from neurons of the trapezoid body (Awatramani et al., 2005; Smith et al., 2000), the abducens nucleus (Russier et al., 2002) or the lateral superior olive (Nabekura et al., 2004), as well as from motoneurons of the hypoglossal nucleus (Muller et al., 2006; O'Brien and Berger, 1999, 2001).

The possibility that mixed glycinergic/GABAergic synapses could represent an immature form of inhibitory synapses remains controversial since their detection at different developmental stages largely depends on the brain area studied. While mixed inhibitory events are recorded at an intermediate stage of synaptic maturation in the trapezoid body (Awatramani et al., 2005), the lateral superior olive (Nabekura et al., 2004), the lamina 1 and lamina 2 of the dorsal spinal cord (Keller et al., 2001) and motoneurons of the hypoglossal nucleus (Muller et al., 2006), they are still detected in Renshaw cells and several distinct interneurons in the spinal cord of mature animals (Geiman et al., 2002; González-Forero and Alvarez, 2005). Functional mixed inhibitory synapses were also detected during the whole postnatal development of rat abducens motoneurons (Russier et al., 2002). While mixed inhibitory events can still be detected at mature stages, a developmental decrease in their contribution to whole inhibition is frequently observed. Interestingly in the hypoglossal nucleus, this developmental decrease has been shown to result from the lost ability of presynaptic terminals to release both neurotransmitters, while GlyRs and GABA<sub>A</sub>Rs remain co-aggregated at postsynaptic loci (Muller et al., 2006).

Co-aggregation of GlyRs and GABA<sub>A</sub>Rs at postsynaptic densities of mixed inhibitory synapses suggests that "cross-talk" between GlyRs and GABA<sub>A</sub>Rs could play a crucial role at these synapses. The occurrence of a possible asymmetrical inhibitory cross-talk at mixed synapses has recently been described in neurons of lamina II of rat spinal cord slices (Mitchell et al., 2007). In these cells, the mean peak amplitude of mixed miniature inhibitory postsynaptic currents was lower than the sum of individual GABAergic and glycinergic synaptic components, and only slightly higher than the averaged amplitude of pure glycinergic synaptic events (Mitchell et al., 2007). The cross-inhibition of glycinergic and GABAergic inhibitory transmissions could also have profound functional implications during neuronal development. In immature animals, glycinergic and GABAergic synaptic activities are depolarizing. Since mixed inhibitory synapses highly contribute to inhibition at these developmental stages, it has been proposed that co-release of glycine and GABA results in a more efficient depolarization and a higher calcium influx in postsynaptic cells (Russier et al., 2002). Indeed, fast glycinergic currents tend to charge the cell capacitance and in turn enhance the strength of the slow GABA component (Russier et al., 2002). In this case, a cross-inhibition will represent an alternative way of limiting excessive depolarization at mixed synapses. In addition to its effect on the amplitude of synaptic events, GlyR activation would decrease the time course of GABAergic postsynaptic currents by speeding up GABA<sub>A</sub>R desensitization kinetics.

## EXTRASYNAPTIC GlyRs AND GABA<sub>A</sub>Rs: A FUNCTIONAL ADAPTATION

GlyRs and GABA<sub>A</sub>Rs that cannot accumulate at postsynaptic sites display different intrinsic functional properties than postsynaptic ones. This is mainly due to the difference in their activation mode. Indeed, extrasynaptic receptors involved in tonic neurotransmission are slowly activated by spillover and/or non-synaptic release of neurotransmitters and therefore necessitate ongoing activation for a long time.

GlyRs are stabilized at postsynaptic densities through binding of their  $\beta$  subunit to the anchoring protein gephyrin. Thus, homomeric  $\alpha$  GlyRs

are expected to be mainly extrasynaptically located.  $\alpha 2$  homomeric receptors represent the immature form of GlyRs expressed at embryonic stages, before the onset of  $\beta$  subunit expression and the subsequent formation of heteromeric  $\alpha\beta$  receptors. These receptors are believed to be involved in cell-to-cell interaction, neuronal differentiation and synaptogenesis (Nguyen et al., 2001). Interestingly, homomeric  $\alpha$  GlyRs composed of the  $\alpha 2A$  subunit display biophysical properties which strongly differ from those of GlyRs composed of  $\alpha 1$ ,  $\alpha 2B$  or  $\alpha 3$  subunits with or without the  $\beta$  subunit (Miller et al., 2004).  $\alpha 2A$  homomeric GlyRs display very slow kinetics which are not compatible with fast synaptic activation by transitory vesicular release of glycine (Mangin et al., 2003). Indeed,  $\alpha 2A$  homomeric GlyRs open only when their glycine binding site is fully occupied and their slow activation rate results from desensitization states linked to bound close states (Mangin et al., 2003). Moreover, their slow closed rate constant, fast opening rate constant and slow deactivation rate strongly suggest that these receptors are well adapted to non-synaptic release of glycine (Mangin et al., 2003). Finally,  $\alpha 2A$  homomeric GlyRs display slower and smaller desensitization kinetics when compared to postsynaptic GlyRs, allowing them to remain functional in the continuous presence of glycine.

Another major functional specificity of  $\alpha 2A$  homomeric GlyRs is its ten times lower apparent affinity for glycine than other GlyR subtypes. Consequently, these receptors necessitate a glycine concentration  $\geq 10 \mu\text{M}$  to be activated (Mangin et al., 2003). This concentration limit might act as a high pass filter to prevent unspecific GlyR activation by a high level of glycine in the extracellular space. This mechanism might serve notably in the developing CNS in which glycine re-uptake transporters are not fully active (Wall and Usowicz, 1997).

Homomeric  $\alpha 2A$  GlyR is probably not the only GlyR subtype displaying functional properties adapted to non-synaptic release of glycine. Recently, a new isoform of the  $\alpha 3$  GlyR subunit resulting from cystidine 554 deamination (RNA editing) has been identified (Meier et al., 2005). The subsequent substitution of a proline by a leucine at position 185 confers high glycine sensitivity to this receptor (submicromolar concentration), thereby promoting the generation of sustained chloride conductances associated with tonic inhibition. Interestingly, the level of GlyR  $\alpha 3$  subunit C554U RNA editing has been shown to be enhanced in response to brain lesion, indicating that the conditional regulation of GlyR  $\alpha 3$  subunit P185L is likely to be part of a post-transcriptional adaptive mechanism in neurons.

Also in case of GABA<sub>A</sub>Rs, extrasynaptic  $\delta$ -containing receptors ( $\alpha\beta\delta$ ) show a higher efficiency (lower  $EC_{50}$ ) and desensitize more slowly and less extensively than postsynaptic  $\alpha\beta\gamma$  GABA<sub>A</sub>Rs (Stórustovu and Ebert, 2006). Another example is given by  $\alpha 5$ -containing extrasynaptic GABA<sub>A</sub>Rs that display a reduction in their desensitization kinetics when compared with GABA<sub>A</sub>Rs containing other  $\alpha$  subunits (Gingrich et al., 1995). Since extrasynaptic GABA<sub>A</sub>Rs desensitize slowly and incompletely, they remain functional when exposed to a continuous presence of their agonist within the extracellular space, as well as during spillover or non-synaptic release of GABA.

## IS THE LATERAL DIFFUSION OF GlyRs AND GABA<sub>A</sub>RS FROM AND TO THE SYNAPTIC CLEFT ASSOCIATED WITH CHANGES IN THEIR FUNCTIONAL PROPERTIES?

Recent reports clearly establish the dynamic feature of receptor accumulation at postsynaptic sites and, especially, their lateral diffusion from and to the postsynaptic loci. The fact that receptor trapping by the anchoring protein is fast and reversible implies that the density of receptors within a synaptic cluster depends on the dynamic equilibrium between the pools of receptors trapped at postsynaptic loci and those diffusing out of the synapse. The lateral diffusion of receptors in the plasma membrane has been well established in case of highly mobile acetylcholine receptors in

developing neuromuscular synapses (Burden, 1998). This mechanism has been recently described for AMPARs and NMDARs at excitatory synapses (Borgdorff and Choquet, 2002; Groc et al., 2004), as well as for GlyRs and GABA<sub>A</sub>Rs at inhibitory synapses (Dahan et al., 2003). Lateral diffusion of receptors implies that they can oscillate between postsynaptic and extrasynaptic sites. Accordingly, they will be differentially activated depending on their subcellular distribution. Indeed, they will be phasically activated at postsynaptic sites and tonically activated at extrasynaptic sites.

### GlyR aggregation and functional properties

Postsynaptic and extrasynaptic receptors have different intrinsic functional properties (see above). Since postsynaptic receptors display fast desensitization properties, which are not compatible with tonic activation, the functional significance of their lateral diffusion to extrasynaptic sites is questionable unless one considers that these properties could change with the subcellular receptor localization. Interestingly, the functional receptor properties were shown to change according to their aggregation level, which tends to be higher at postsynaptic densities than at extrasynaptic sites.

Using heterologous expression of the human GlyR  $\alpha 1$  subunit, Taleb and Betz demonstrated that functional properties of  $\alpha 1$ -containing GlyRs depend on their density at the cellular membrane (Taleb and Betz, 1994). The injection of the GlyR  $\alpha 1$  subunit cDNA into *Xenopus* oocyte induced the expression of homomeric  $\alpha 1$  GlyRs over a wide membranous density range. The GlyR density was estimated by measuring the maximum amplitude of glycinergic currents evoked by the application of a saturating glycine concentration. A significant change in GlyR pharmacology could be correlated with different GlyR density levels. When highly concentrated at the oocyte membrane, GlyRs displayed a  $\approx 5$ -fold lower  $EC_{50}$  value for glycine,  $\beta$ -alanine and taurine than poorly aggregated receptors (Taleb and Betz, 1994). Highly concentrated receptors were also more resistant to strychnine antagonism. The authors proposed that increasing the density of GlyRs might increase the receptor affinity for its agonists. However, a change in  $EC_{50}$  value does not necessarily imply a change in receptor affinity.  $EC_{50}$ , or apparent affinity, reflects both ligand binding and conformational changes leading to channel opening. This parameter depends on the agonist microscopic dissociation constant  $K_A$  ( $K_A = k_{off}/k_{on}$ , where  $k_{off}$  is the dissociation rate constant in  $\text{second}^{-1}$  and  $k_{on}$  is the association rate constant in  $\text{M}^{-1}\text{second}^{-1}$ ) and the open/close isomerization equilibrium constant or efficacy  $E$  ( $E = \beta/\alpha$  where  $\beta$  is the opening rate constant and  $\alpha$  the closing rate constant).  $EC_{50}$  can be estimated when  $= K_A/(1 + E)$ . Interestingly, the value of the  $EC_{50}$  can change independently of  $E$  or  $K_A$  (Colquhoun, 1998; Legendre, 2001). Other parameters, such as desensitization kinetics, might also influence  $EC_{50}$ .

A functional significance of this phenomenon has been recently proposed in a study combining fast flow application techniques and outside-out patch clamp single channel recordings from cultured transfected human embryonic kidney (HEK) 293 cells expressing a chimer form of the GlyR  $\alpha 1$  subunit (Legendre et al., 2002). Fast flow application technique allows solution exchange in the vicinity of an outside-out patch in less than 0.1 ms and provides a direct estimation of receptor conformational changes over time. Legendre and co-workers (2002) compared the impact of GlyR density on their kinetics when GlyR expression was globally increased in HEK cell membranes or when local GlyR aggregation was induced by gephyrin co-transfection. Gephyrin interacts with the  $\beta$  GlyR subunit, implying the study of aggregated heteromeric  $\alpha\beta$  GlyRs. However, it is not possible to obtain a pure population of  $\alpha\beta$  GlyRs in HEK cells co-transfected with  $\alpha$  and  $\beta$  subunits. To overcome this problem, the authors used a chimeric GlyR  $\alpha 1$  subunit ( $\alpha 1\beta_{ge}$ ) bearing the gephyrin-binding site in its TM3–TM4 intracellular loop. As previously observed (Taleb and Betz, 1994), Legendre and co-workers described changes in GlyR functional properties depending on their aggregation level. However, in contrast to what was previously reported, they showed that increasing an GlyR aggregation level involved a parallel increase in GlyR desensitization without affecting the apparent affinity. Since fast



flow techniques allow the study of fast changes in receptor kinetics, they further showed that the changes in  $EC_{50}$  previously observed were directly related to changes in receptors desensitization kinetics. Finally, since the large TM3–TM4 intracellular domain of the GlyR  $\alpha 1$  subunit can interact with its TM1–TM2 intracellular loop (Nikolic et al., 1998), it has been hypothesized that changes in desensitization properties with higher receptor density could arise from a higher level of intracellular interactions. Thus, gephyrin binding to  $\alpha 1\beta_{gb}$  subunit could change the conformation of the GlyR TM3–TM4 intracellular loop and modify its interaction with the TM1–TM2 intracellular domain, mimicking a higher GlyR density. It is noteworthy that  $\alpha 1\beta_{gb}$ -containing GlyRs expressed at high level in HEK cells display desensitization kinetics which are considerably faster than those described for  $\alpha 1\beta$  GlyRs expressed in neurons. However, insertion of the  $\beta_{gb}$  sequence into the TM3–TM4 intracellular loop of the GlyR  $\alpha 1$  subunit is unlikely to account for these difference since desensitization kinetics of  $\alpha 1$ - and  $\alpha 1\beta_{gb}$ -containing GlyRs were similar (Legendre et al., 2002).

As an adaptative mechanism of neurotransmission, the lateral diffusion of GlyRs might therefore have important functional consequences. For example, in case of sustained presynaptic activity, extrasynaptic slightly desensitizing GlyRs will be continuously activated by glycine spillover and in turn enhance inhibitory synaptic strength.

### GlyR aggregation and gephyrin

GlyRs form clusters when binding to gephyrin or become diffuse when they dissociate from it (Meier and Grantyn, 2004; Meier et al., 2000). A similar mechanism could occur in neurons under physiological conditions. However, since synaptic gephyrin clusters also display a fast dynamic behavior (Hanus et al., 2006), GlyRs diffusing out of the synaptic cleft would not necessarily dissociate from gephyrin. If so, extrasynaptic GlyRs would remain aggregated and conserve similar desensitization kinetics than postsynaptic receptors. Accordingly, such extrasynaptic receptors would be poorly efficient in response to a slow release of glycine. But, GlyR diffusion and lateral motion of gephyrin clusters are two distinct dynamic processes occurring within the postsynaptic density, with gephyrin clusters moving on longer time scales (Hanus et al., 2006). The decreased diffusion of GlyRs at postsynaptic loci reflects their “confinement” by postsynaptic scaffold proteins (Choquet and Triller, 2003), arguing in favor of a binding–unbinding postsynaptic mechanism between GlyRs and gephyrin. This hypothesis is supported by the remaining presence of GlyRs in membranes of neurons in which gephyrin expression is impaired (Kirsch et al., 1993; Kneussel et al., 1999). Accordingly, Charrier and co-workers (2006) recently demonstrated that synaptic expression and maintenance of GlyRs and gephyrin are controlled by distinct mechanisms involving cytoskeleton dynamics. GlyR and gephyrin were tagged with different fluorophores and co-transfected in cultured spinal cord neurons. During cytoskeleton depolymerization, GlyR-associated fluorescence was shown to decrease before the gephyrin-associated one, therefore suggesting that receptor exchanges between synaptic and extrasynaptic sites do not reflect an increased mobility of the GlyR–gephyrin complex only (Charrier et al., 2006).

### GABA<sub>A</sub>R aggregation and functional properties

Similarly to GlyRs, GABA<sub>A</sub>Rs display lateral mobility and exchange between postsynaptic and extrasynaptic sites (Jacob et al., 2005). Therefore, it is not surprising that the GABA<sub>A</sub>R functional properties also depend on their aggregation level. The functional consequence of this dependency has been analyzed in cultured hippocampal neurons using fast flow application techniques and patch clamp outside-out single channel recordings (Petrini et al., 2003). GABA<sub>A</sub>R aggregation is dramatically reduced under microtubule disruption (Kneussel and Betz, 2000; Petrini et al., 2003). Contrary to what was observed for GlyRs, a decrease in GABA<sub>A</sub>R aggregation in response to microtubule depolymerization by nocodazole resulted in increased GABA<sub>A</sub>R desensitization kinetics. Indeed, the rise time of GABA-evoked currents and the onset of desensitization were faster

(Petrini et al., 2003). These observations were coherent with a previous report showing that GABARAP-induced aggregation of GABA<sub>A</sub>Rs induces faster deactivation and slower desensitization when compared to diffuse receptors (Chen et al., 2000). GABA<sub>A</sub>Rs displaying fast desensitization kinetics are expected to be poorly efficient in response to a continuous presence of GABA (Jones and Westbrook, 1995; Scanziani, 2000). This suggests that, in contrast with GlyRs, GABA<sub>A</sub>Rs moving to extrasynaptic sites through lateral diffusion would not be functional anymore.

## PERSPECTIVES

Inhibitory neurotransmission is subject to continuous and complex readjustments. Recently acquired knowledge and ongoing investigations are uncovering mechanisms that specify the role and the contribution of extrasynaptic and postsynaptic GlyRs and GABA<sub>A</sub>Rs to inhibition, as well as their functional adaptation during lateral mobility. Although the functional properties of extrasynaptic GlyRs and GABA<sub>A</sub>Rs have been extensively studied, the mechanisms underlying non-synaptic release of glycine and GABA are still poorly understood. So far, only non-synaptic release of taurine has been described to activate GlyRs in the developing cortex (Flint et al., 1998) and in the neurohypophysis of the adult rat (Hussy et al., 2001). GlyRs and GABA<sub>A</sub>Rs are widely expressed in the developing CNS, at both postsynaptic and extrasynaptic loci. Impairment in their expression strongly alters neuronal developmental processes. Thus, it is of crucial importance to determine the origin and release mechanism of neurotransmitters, which activate these receptors during synaptogenesis.

Another challenge is to investigate the molecular mechanisms underlying changes in the functional properties of GlyRs and GABA<sub>A</sub>Rs, depending on their aggregation level. This would be particularly relevant in case of mixed inhibitory synapses, where GlyRs and GABA<sub>A</sub>Rs co-aggregate at postsynaptic densities and cross-modulate their activity. Moreover, although a direct crosstalk between GlyRs and GABA<sub>A</sub>Rs has now been demonstrated, the molecular pathway and the physiological role of this mechanism remain to be clearly determined.

## CONFLICT OF INTEREST STATEMENT

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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