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GABA and neuroligin signaling: linking synaptic activity and adhesion in inhibitory synapse development

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Abstract

GABA-mediated synaptic inhibition is crucial in neural circuit operations. In mammalian brains, the development of inhibitory synapses and innervation patterns is often a prolonged postnatal process, regulated by neural activity. Emerging evidence indicates that GABA acts beyond inhibitory transmission and regulates inhibitory synapse development. Indeed, $GABA_A$ receptors not only function as chloride channels that regulate membrane voltage and conductance but also play structural roles in synapse maturation and stabilization. The link from $GABA_A$ receptors to post- and pre- synaptic adhesion is likely mediated, in part, by neuroligin-reurexin interactions, which are potent in promoting GABAergic synapse formation. Therefore, similar to glutamate signaling at excitatory synapse, GABA signaling may coordinate maturation of pre- and post-synaptic sites at inhibitory synapses. Defining the many steps from GABA signaling to receptor trafficking/stability and neuroligin function will provide further mechanistic insights into activity-dependent development and possibly plasticity of inhibitory synapses.

Introduction

In many areas of the vertebrate brain, neural circuits rely on inhibition mediated by γ-aminobutyric acid (GABA) from diverse cell types to control the spatiotemporal patterns of electrical signaling [1]. The inhibitory output is distributed in the network through GABAergic axons and synapses, which constitute elaborate and cell type-specific inhibitory innervation patterns [2]. GABAergic neurons are generated during mid-embryonic stages and influence many aspects of early neural development. The subsequent maturation of GABAergic synapses and innervation patterns into a potent inhibitory network is often a protracted process, extending well into postnatal life, and is regulated by neural activity and experience [3–6]. Such activity-dependent development of inhibitory synapses and innervation patterns is a major component of neural circuit assembly, yet the underlying cellular and molecular mechanisms are poorly understood.

As key mediators of neural activity, neurotransmitters are particularly well suited to couple synaptic transmission with synaptic growth and refinement [7,8]. Although the initial steps of nascent synapse formation are likely independent of neurotransmitter release [9,10], there is substantial evidence that neurotransmitters and synaptic activity regulate nearly all aspects of synapse formation [11–13], plasticity [14–17], and the development of axonal and dendritic arbors [7,18,19]. Such synaptotrophic functions have been most extensively studied for glutamate, the main excitatory neurotransmitter in the brain, and for acetylcholine at the neuromuscular junction [20,21]. Therefore, in addition to synaptic transmission, excitatory transmitters also function as trophic signals for regulating synapse formation, maturation, and plasticity.

Initially discovered as an inhibitory transmitter, GABA has since been implicated in multiple processes of neural development, from cell proliferation to circuit formation [22]. The trophic effects of GABA on neuronal migration and neurite growth during the embryonic and perinatal period are largely explained by its depolarizing action in immature neurons, which triggers calcium influx and signaling [23,24,25]. In fact, GABA signaling in the neonatal brain represents an early form of neural communication, which precedes and promotes the formation of glutamatergic synapses [26–28]. GABA signals are transduced through two general classes of receptors, the metabotropic $GABA_B$ receptors and ionotropic $GABA_A$Rs which are permeable to chloride ions. The early, depolarizing function of GABA results from chloride ion efflux through the $GABA_A$ receptor. During the postnatal period, the up-regulation of the chloride transporter KCC2 in neurons results in increased extrusion of intracellular chloride [29], and GABA assumes its classic role as an inhibitory transmitter [30]. Recently, several studies suggest that, in addition to mediating synaptic inhibition in the more mature circuits, GABA signaling may promote and coordinate pre- and post-synaptic maturation during activity-dependent development of inhibitory synapses. In this review, we will first discuss recent genetic studies that revealed critical roles for both GABA and $GABA_A$ receptor complexes in inhibitory synapse development. We will then explore the possibility that the neuroligin-neurexin trans-synaptic adhesion system participates in this activity-dependent inhibitory synapse formation and maturation processes.

GABA signaling: linking inhibitory transmission to synaptic wiring

The rodent visual and somatosensory cortices are excellent model systems for studying activity-dependent development of GABAergic connectivity. For example, the maturation of inhibitory innervation [3,4] and transmission [4,6] proceeds well into postnatal weeks and is regulated by sensory experience [3,6,31]. Interestingly, the maturation of many features of interneuron axonal arbors and inhibitory synapses can be recapitulated in cortical organotypic cultures [32] and is strongly regulated by neuronal activity [3,33]. By genetic knockdown of GABA synthesis in cortical organotypic cultures and in vivo, a recent study implicates GABA signaling itself in the development of inhibitory synapses [34]. Unlike glutamate which is both the precursor and product of many essential metabolic and signaling processes in the cell, GABA can only be synthesized by two glutamate decarboxylases (GAD67 and GAD65), and the main function of GABA is intercellular signaling [35]. GAD67 is the rate-limiting enzyme and influences cellular GABA contents in a dosage dependent manner [36,37]. Knockdown of GAD67 in single GABAergic interneurons,

which should have minimum impact on circuit activity levels, results in profound cell autonomous deficits in synapse formation, axon branching, and innervation field; such deficits were partially rescued by blocking GABA re-uptake or enhancing $GABA_A$ or $GABA_B$ receptor function [34]. These results demonstrate that GABA acts beyond its classic role in inhibitory transmission in the *adolescent brain* and regulates the maturation of inhibitory synapses and innervation patterns (Figure 1), thus revealing a new facet of GABA function distinct from its early tropic action in neonatal brain.

Structural role of $GABA_A$ receptors: coupling synaptic transmission to synapse maturation and stability

A second set of findings that highlights a role for GABA signaling in the development of inhibitory synapses come from the analysis of knock-out mice lacking individual subunits of the $GABA_A$ receptor ($GABA_AR$). $GABA_ARs$ are heteropentameric chloride channels composed of several classes of subunits [38]. Although over 19 subunits have been identified, giving rise to a large number of possible subunit combinations, the vast majority of $GABA_ARs$ consist of α, β, and γ2 subunits in a 2:2:1 stoichiometry. In the mature brain, $GABA_ARs$ are primarily localized at postsynaptic and extrasynaptic membranes where they mediate phasic and tonic inhibition, respectively.

Purkinje cells in the mouse cerebellum provide a unique opportunity to test the role of $GABA_AR$ in synapse formation as deletion of the α1 subunit gene results in a complete loss of functional $GABA_ARs$ by postnatal day 18 [39]. Purkinje cells are themselves GABAergic neurons but also receive two types of GABAergic inputs: the axo-somatic synapses from basket interneurons and the axo-dendritic synapses from stellate interneurons, both with $GABA_ARs$ containing the α1 subunit. In α1−/− mice, GABAergic terminals from stellate axons are initially formed normally onto the Purkinje dendritic shaft. However, starting from postnatal day 7, synapse formation and stabilization on the dendritc shaft is severely perturbed [40]. Instead, the stellate cell terminals form aberrant and mismatched contacts with postsynaptic specialization on the spines of Purkinje dendrites. These results suggest that initial steps of GABAergic synapse formation can proceed in the absence of α1, but synapse maturation and maintenance require postsynaptic $GABA_ARs$. Notably, basket cell synapses on the Purkinje cell soma are not altered in α1−/− mice. Therefore, the two presynaptic inputs differ in their molecular requirements for synapse formation and stabilization onto Purkinje cells, even with respect to the postsynaptic $GABA_AR$ that is common to both types of synapses.

Similar conclusions regarding the role of $GABA_ARs$ in synapse formation were derived from studies examining the γ2 subunit. The γ2 subunit is essential for accumulation of cell surface $GABA_ARs$ at postsynaptic sites [41,42]. Interestingly, acute suppression of γ2 expression in cultured hippocampal neurons not only disrupts $GABA_AR$ clustering but also results in a profound reduction of GABAergic innervation of γ2 deficient neurons [43,44]. Moreover, when palmitoylation of the γ2 subunit was suppressed by knockdown of the DHHC-family palmitoyltransferase GODZ, trafficking of $GABA_ARs$ to postsynaptic sites was perturbed and GABAergic innervation was reduced [43]. Interestingly, no comparable presynaptic defect is evident in neuron cultures and brain sections from germ-line γ2−/−

mice, which exhibit a uniform deficit in postsynaptic $GABA_A$Rs in all neurons. These data suggest the presence of a retrograde signal that allows GABAergic axons to preferentially innervate target neurons expressing higher levels of $GABA_A$R at the cell surface. Because both presynaptic GABA and postsynaptic $GABA_A$ receptors influence GABAergic synapse development, a simple hypothesis is that activity-dependent GABA signaling promotes the differentiation of pre- and post- synaptic sites, and coordinates the maturation and stabilization of inhibitory synapses. The failure to form and stabilize presynaptic terminals after postsynaptic loss of $GABA_A$Rs strongly suggests the presence of a retrograde signal that is regulated by synaptic activity or by association with postsynaptic $GABA_A$Rs. Amongst the molecular mechanisms that may contribute to such an activity-regulated trans-synaptic signal, the neuroligin and neurexin complex represents one of the plausible candidates.

Trans-synaptic signaling by the neuroligin-neurexin adhesion system

The neuroligin-neurexin complex is a heterophilic adhesion system broadly expressed in the central nervous system [45]. Both neuroligins and neurexins are encoded by multiple genes and alternative splicing generates further isoform diversity from each gene. Cell biological studies have revealed potent “synaptogenic” or synapse-organizing activities for these proteins (recently reviewed in [46,47]. Postsynaptic neuroligins promote assembly of functional presynaptic specializations in axons. Conversely, presynaptic neurexins - through interaction with neuroligins - recruit postsynaptic scaffolding proteins and neurotransmitter receptors in dendrites.

While neuroligin-neurexin complexes are common building blocks of glutamatergic and GABAergic synapses and are required for normal glutamatergic and GABAergic transmission, the morphological loss of function data available so far most strongly support their critical roles in the organization of GABAergic synapses. Triple knockout mice lacking the three alpha-neurexin transcripts show a 50% reduction in the density of GABAergic synapses in the brainstem [48]. While triple knock-out animals die at birth, an analysis of more mature neuronal circuits could be performed in double knockout mice, some of which reach adulthood. In these mice, GABAergic synapse density is reduced by 30% whereas glutamatergic synapse density is apparently unchanged, although principal neurons display shortened dendritic branches and lower spine density compared to wild-type mice [49]. As for the neuroligins, mice lacking the three major isoforms (NL1,2, and 3) show perinatal lethality. Although there is only a relatively small (15–20%) reduction in the number synapses in the brain stem of these mutant mice, they show a severe loss of $GABA_A$Rs and the scaffolding protein gephyrin from postsynaptic sites [50]. These studies uncovered a critical function of neuroligin proteins in postsynaptic differentiation in vivo, especially at GABAergic synapses.

More recent studies began to dissect potential synapse-specific functions of individual neuroligin and neurexin isoforms. In cell culture assays, alpha- and beta-neurexin splice variants differ significantly in their biochemical interactions with neuroligins as well as in their ability to organize glutamatergic versus GABAergic postsynaptic structures [51–54]. This selectivity might reflect a trans-synaptic adhesive code regulated by gene and splice

variant choice of the pre- and postsynaptic partners. Further analysis in the neuroligin single knock-out mice strongly support an important function of the NL2 isoform at GABAergic synapses. Layer 2/3 neurons in acute cortical slices from NL2 −/− mice show a selective impairment of GABAergic transmission whereas glutamatergic transmission is normal. In addition, NL2−/− mice display a selective decrease in the number of inhibitory synapses in the postnatal neocortex [55]. Overexpression of NL2 in cultured neurons increases the density of glutamatergic and GABAergic terminals but has a higher activity towards GABAergic axons [56]. At a functional level, NL2 overexpression selectively increases the amplitude of inhibitory postsynaptic currents suggesting that the postsynaptic coupling of this neuroligin isoform is specific for inhibitory synapses [55]. Notably, this overexpression-induced increase in GABAergic transmission is blocked by pharmacological inhibition of network activity in the culture. Therefore, neuronal and synaptic activity might either regulate the presynaptic response to NL2 or postsynaptic stabilization induced by NL2.

Taken all together, there is substantial evidence to indicate that both neuroligins and $GABA_A$ receptors play critical roles in the maturation of postsynaptic specializations and the differentiation and stabilization of presynaptic terminals at inhibitory synapses. These observations raise an obvious question: how do GABA/$GABA_A$R-mediated synaptic signaling and neuroligin/neurexin-mediated synaptic adhesion interact and cooperate to regulate activity-dependent development of inhibitory synapse?

From $GABA_A$ receptors to synaptic adhesion and activity-dependent retrograde signaling

It is currently unknown at what stage of the biosynthetic pathway $GABA_A$Rs first interact with NLs, and how such interactions might be regulated. In the plasma membrane, $GABA_A$Rs exist as dispersed populations and as synaptic or extrasynaptic clusters, depending on their subunit compositions; lateral diffusion in the plasma membrane allows continual exchange among these groups [57]. Pentameric $GABA_A$Rs are assembled in the endoplasmic reticulum (ER) from appropriate subunits, and are delivered into the plasma membrane through a highly regulated trafficking process. Most $GABA_A$Rs are first delivered to extrasynaptic locations, and their subsequent diffusion and trapping increase their accumulation at postsynaptic sites. The scaffolding protein gephyrin promotes the synaptic targeting of $GABA_A$Rs in part by direct binding to the α2 subunit [58], and the synaptic localization and function of gephyrin can be regulated by neural activity [59].

In one model (Figure 2), NL2 and synaptic $GABA_A$Rs would stabilize each other, either through intracellular reciprocal interactions aided by scaffolding proteins such as gephyrin or through extracellular *cis* interactions. In addition, GABA activation of $GABA_A$Rs might further stabilize $GABA_A$Rs at synapses through as yet unknown structural or signaling mechanisms. Such activity- and GABA-mediated stabilization of $GABA_A$Rs might further increase the levels of NL2 at postsynaptic sites; this, in turn, would stabilize the presynaptic terminals through trans-synaptic interactions with neurexins. Evidence consistent with this model include: 1) in vitro studies demonstrated a co-aggregation of NL2 and the $GABA_A$R γ2 subunit in heterologous cells [60]; 2) the residence time of $GABA_A$Rs on the plasma membrane and their targeting to synapses is regulated by synaptic activity [61]; 3)

pharmacological blockade of neuronal activity in cultured neurons diminish the synaptogenic activity of NL2 [55]; 4) reduced GABA synthesis and release result in a reduction of inhibitory synapses [34]. Moreover, there is precedent for such mechanisms in activity-dependent recruitment of transmitter receptor and trans-synaptic signaling at glutamatergic synapses. Local spontaneous activity and glutamate release reduce diffusion exchange of GluR1 between synaptic and extrasynaptic domains, resulting in postsynaptic accumulation of GluR1 [62]. In addition, PSD-95 and NL1 retrogradely modulate presynaptic release probability and may coordinate post- and pre- synaptic morphological changes [63,64]. It remains to be seen whether analogous mechanisms for GABA and NL2 signaling exist at inhibitory synapses.

In an alternative model, it is not the $GABA_AR$ levels but rather the expression and/or localization of NL2 itself that might be regulated by GABA signaling, either through regulating NL2 protein levels or NL2-interacting proteins involved in its synaptic localization. It is also possible that GABA binding to $GABA_ARs$ might modulate their coupling to NL2, thereby increasing the potency and affinity of NL2 towards neurexin in the presynaptic terminals.

Conclusion

Evidence from several lines of experiments are converging to suggest that synaptic signaling by GABA-$GABA_A$ receptors may engage the neuroligin-neurexin cell adhesion system to regulate activity-dependent development of inhibitory synapses and innervation patterns. Clearly, the models described above are highly speculative at this time but they provide exciting opportunities to test the coupling of $GABA_ARs$ to synaptic adhesion complexes. A key question in understanding this molecular coupling will be to determine how $GABA_ARs$ interact with NLs. A conventional model would include the interaction of NL2 with GABAergic scaffolding molecules which in turn couple to the receptors. However, other models such as direct interactions between NL2 and $GABA_AR$ subunits can not be ruled out. Another major question is whether and how GABA binding to $GABA_ARs$ regulates their conformation and/or biochemical interactions with other synaptic proteins, thereby influencing receptor stability, trafficking, and turnover. The significant advances made during the past years have implicated the neuroligin-neurexin system as well as $GABA_ARs$ as key players in inhibitory synapse development. Exploring whether these two systems organize GABAergic synapses through direct interactions or parallel pathways will be a challenge for the future.

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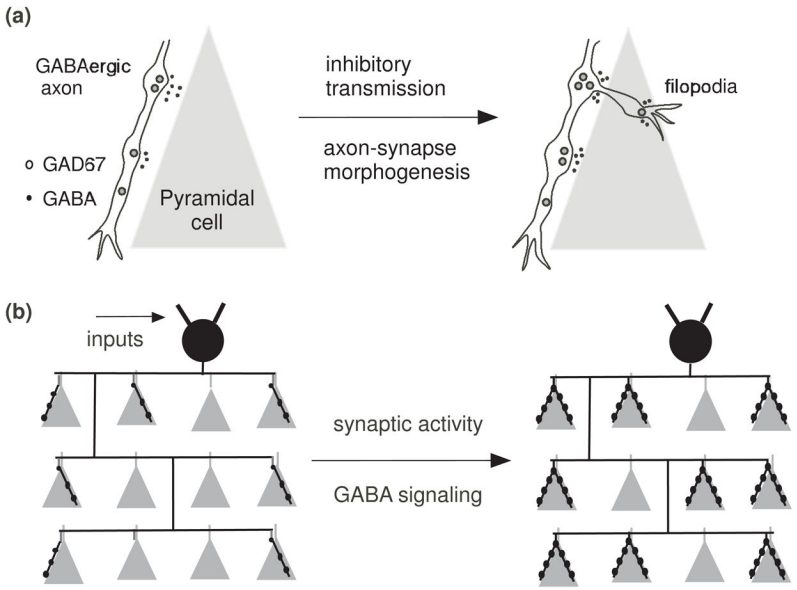

Figure 1.

GAD67 and GABA act beyond inhibitory transmission and regulate inhibitory synapse development. (a) GABA signaling may regulate the morphogenesis (e.g. growth and stability) of inhibitory synapses. (b) Since synapse formation is an integral part of axon growth and branching, activity-dependent GABA signaling may further influence the development of GABAergic axon arbor and innervation pattern.

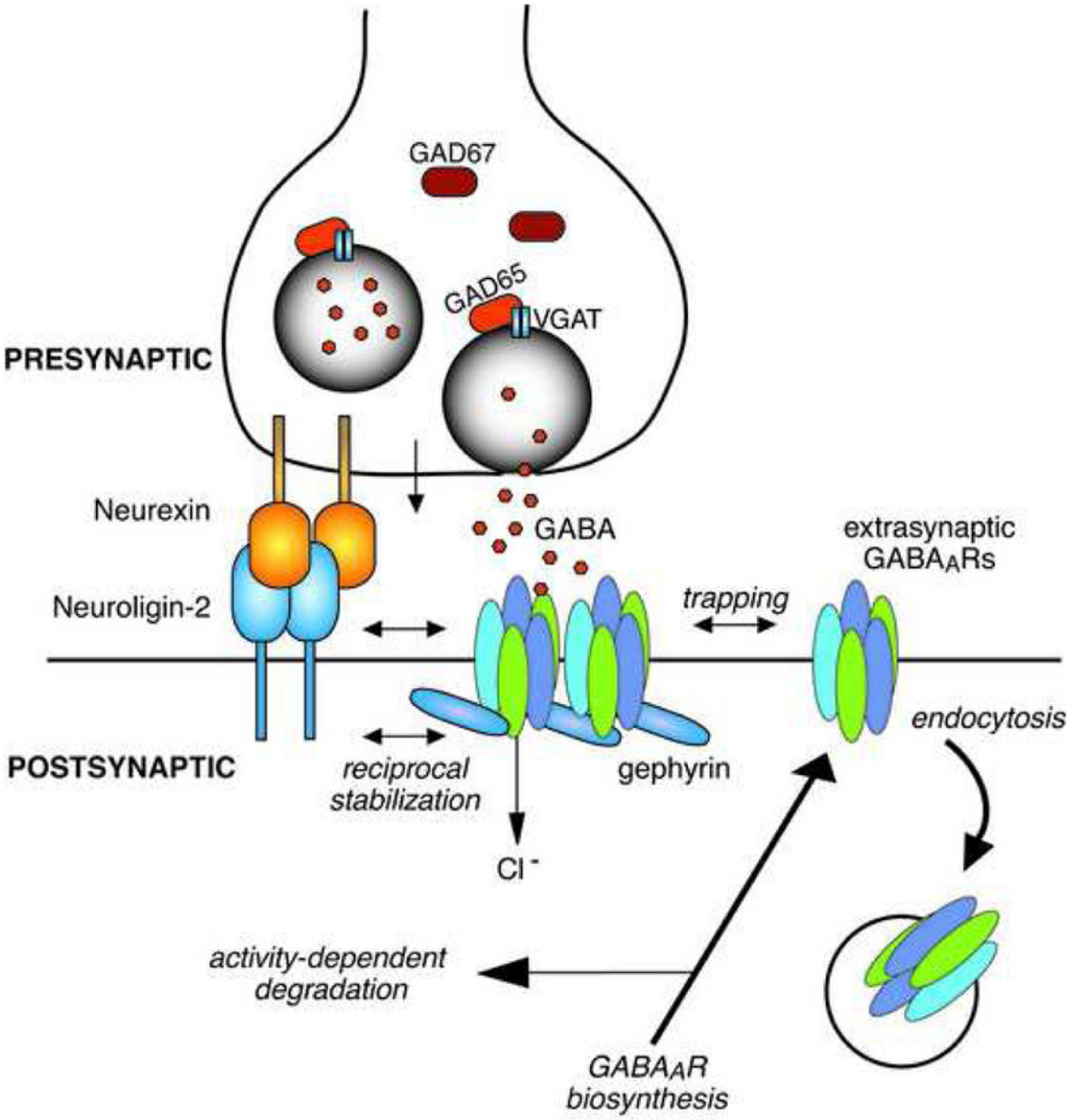

Figure 2.

A hypothetical model depicting how GABA-GABA$_A$R signaling and neuroligin-neurexin adhesion may interact and cooperate to regulate the development of inhibitory synapses. Pentameric GABA$_A$Rs are assembled in the endoplasmic reticulum and are subject to activity-dependent proteasomal degradation. Most GABA$_A$Rs are first delivered to extrasynaptic locations, they then either diffuse to and become trapped at postsynaptic sites or undergo endocytosis. NL2 and synaptic GABA$_A$R stabilize each other, either through intracellular reciprocal interactions aided by scaffolding proteins such as gephyrin or through extracellular cis interaction. In addition, GABA activation of GABAARs might further stabilize synaptic GABA$_A$Rs through structural changes or signaling mechanisms. Such activity- and GABA-mediated stabilization of GABA$_A$R might further increase the

levels of NL2 at cell-cell contacts and, in turn, stabilize presynaptic terminals through trans-synaptic interactions with neurexins.