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Review

Understanding GABAergic synapse diversity and its implications for GABAergic pharmacotherapy

Dilja Krueger-Burg [1,*]

Despite the substantial contribution of disruptions in GABAergic inhibitory neurotransmission to the etiology of psychiatric, neurodevelopmental, and neurodegenerative disorders, surprisingly few drugs targeting the GABAergic system are currently available, partly due to insufficient understanding of circuit-specific GABAergic synapse biology. In addition to GABA receptors, GABAergic synapses contain an elaborate organizational protein machinery that regulates the properties of synaptic transmission. Until recently, this machinery remained largely unexplored, but key methodological advances have now led to the identification of a wealth of new GABAergic organizer proteins. Notably, many of these proteins appear to function only at specific subsets of GABAergic synapses, creating a diversity of organizer complexes that may serve as circuit-specific targets for pharmacotherapies. The present review aims to summarize the methodological developments that underlie this newfound knowledge and provide a current overview of synapse-specific GABAergic organizer complexes, as well as outlining future avenues and challenges in translating this knowledge into clinical applications.

Highlights

GABAergic synapses contain an elaborate organizational protein machinery with a key role in regulating the properties of GABAergic synaptic transmission.

Recent methodological advances in imaging, electrophysiology, transcriptomics, and proteomics have enabled many new components of this organizational machinery to be identified.

Connection-specific analyses show that these GABAergic synapse organizer proteins are not distributed homogeneously, but that different GABAergic neuron subtypes utilize different molecular components at their synapses.

The resulting diversity of GABAergic organizer complexes offers a unique opportunity for the development of circuit-specific GABAergic therapeutics, although substantial further investigation will be necessary to translate these findings into clinical applications.

The GABAergic system in health and disease

Gamma-aminobutyric acid (GABA)ergic inhibitory synaptic transmission has a pivotal role in shaping the flow of information through neural circuits and, accordingly, disruptions in the GABAergic system contribute to the pathophysiology of a range of psychiatric, neurodevelopmental, and neurodegenerative disorders [1–4]. Despite this central importance, only a few pharmacological agents targeting the GABAergic system are in clinical use, many of which were developed more than 50 years ago [3]. While these therapeutics can be highly effective in treating acute attacks, their chronic use and wider application to other psychiatric disorders are precluded by their debilitating side effect profiles [5]. These side effects likely result from the fact that all known GABAergic therapeutics indiscriminately target GABAergic synaptic transmission throughout the central nervous system (CNS), resulting in pronounced actions in neuronal circuits unrelated to the psychiatric endophenotypes of interest. Therefore, novel therapeutic strategies that target the GABAergic system in a more circuit-specific manner are urgently needed [3].

A defining feature of the GABAergic system is its staggering complexity, which results from the enormous diversity of the cellular and molecular components that mediate GABAergic signaling (Box 1, Figure 1). This complexity has represented a major challenge in elucidating the organization of the GABAergic system, but it also offers an unparalleled opportunity for developing targeted strategies for specific interventions in behaviorally relevant circuits [3]. While the diversity

[1]Laboratory of Cell Biology and Neuroscience, Institute of Anatomy, University Medical Center of the Johannes Gutenberg University Mainz, Duesbergweg 6, 55128 Mainz, Germany

*Correspondence: dkruegerburg@uni-mainz.de (D. Krueger-Burg).

Box 1. Diversity in the GABAergic system

Diversity of GABAergic neuron subtypes

A plethora of GABAergic neuron subtypes exist that differ in their morphological, biochemical, and physiological properties (Figure 1A in the main text), resulting in fundamentally different influences on neuronal information flow [6–11,17]. For example, parvalbumin (PV)-positive basket cells (PV-BC) and chandelier cells (PV-CC) in the cortex specifically target the perisomatic region and axon initial segment of local pyramidal neurons (PNs), respectively, placing them in an ideal position to powerfully control output of the target neuron. Similarly, cholecystokinin (CCK)-positive interneurons target primarily the perisomatic region of pyramidal neurons. By contrast, somatostatin (SST)-positive interneurons primarily form axodendritic synapses that lie in close proximity to glutamatergic inputs, allowing them to fine-tune the integration of information from diverse inputs. Vasoactive intestinal peptide (VIP)-positive interneurons largely target other GABAergic neurons, resulting in disinhibitory circuits the wide-reaching behavioral consequences of which are only just beginning to be understood. Finally, long-range GABAergic projection neurons have a critical role in connecting regions throughout the brain. Disentangling the functions of these many categories of neurons, which are increasingly found to be differentially affected in psychiatric disorders, has been a major focus for understanding the role of the GABAergic system in health and disease [1,2,8,17].

Diversity of $GABA_A$ receptor subunits

$GABA_A$Rs, which mediate the effects of GABA at the postsynaptic membrane, are pentameric ionotropic chloride channels that result in hyperpolarization of the postsynaptic membrane in response to the binding of GABA to the extracellular domain (Figure 1B in the main text). Each $GABA_A$R comprises two α-subunits, two β-subunits and a γ/δ-subunit, with two GABA-binding sites at the interfaces between the α- and β-subunits [4,5,12,13]. A total of 19 isoforms of these subunits exist in the mammalian CNS, such that a large number of different subunit combinations can be formed, each with different anatomical, functional, and pharmacological properties. Therefore, elucidating the molecular logic by which these different $GABA_A$R subtypes are recruited to and utilized at different synapse subtypes is paramount to understanding synaptic inhibition.

Diversity of GABAergic synapse organizer proteins

A third aspect of GABAergic diversity arises from the structural and organizational components that anchor $GABA_A$Rs to the postsynaptic membrane opposing presynaptic GABAergic terminals (Figure 1C in the main text). Over the past decade, the advent of novel methodological approaches has led to the identification of a multitude of new components of the GABAergic synaptic machinery [14–17]. Determining how these various organizer proteins differentially shape physiological synaptic properties and information processing in a connection-specific manner is critical for understanding and targeting GABAergic synapse diversity in health and disease.

of GABAergic neuron subtypes [6–11] and of $GABA_A$ receptor subunits [4,5,12,13] have long been recognized and are well described (Box 1), a third factor, namely the diversity of GABAergic synapse organizer proteins [14–17], has begun to be appreciated only relatively recently.

The present review will focus on this latter aspect of GABAergic system diversity as a promising new avenue toward identifying circuit-specific targets for GABAergic pharmacotherapy. In the first part, an overview will be provided of the key methodological advances that have laid the groundwork for the recent surge in information on GABAergic synapse diversity, including imaging-based, electrophysiological, transcriptomic, and proteomic approaches. Subsequently, the current state of knowledge on the synapse-specific roles of individual GABAergic organizer proteins will be summarized. Finally, the implications of these findings for the development of more selective GABAergic drugs will be outlined, with the aim of highlighting future avenues toward improved therapeutics in psychiatry and neurology.

Methodological advances in the study of GABAergic synaptic complexes

The identification of proteins at GABAergic synaptic complexes has substantially lagged behind those at glutamatergic synapses for several reasons. First, there are fewer GABAergic than glutamatergic synapses in the brain, with estimates of 5–20% of total synapses depending on the brain region and species. Second, the postsynaptic density of GABAergic synapses is thinner and less elaborate than that of glutamatergic synapses (hence the classification as symmetric or Gray type II synapses) [18], making them more difficult to isolate by classical biochemical subcellular fractionation. Third, the enormous diversity of GABAergic neurons adds challenges of allocation not faced when studying glutamatergic synapses. In recent years, however, several key

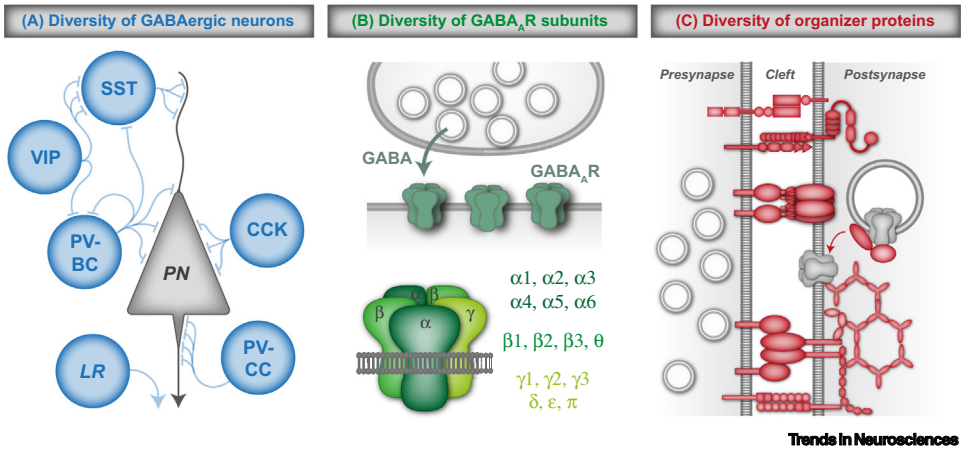

Figure 1. Diversity in the GABAergic system. (A) Diversity of GABAergic neuron subtypes (blue), simplified to represent only the major GABAergic neuron subtypes in cortex (see also [9]). (B) Diversity of $GABA_A$ receptor subunits. Upper panel: schematic of a GABAergic synapse, depicting the presynaptic terminal releasing GABA, as well as the postsynaptic membrane containing $GABA_A$Rs (green). Lower panel: $GABA_A$Rs are pentameric chloride channels comprising two α-subunits, two β-subunits, and one γ-subunit. Multiple different subforms of each subunit exist. (C) Diversity of GABAergic synapse organizer proteins. Schematic of the synaptic cleft, with the presynaptic terminal on the left and the postsynaptic membrane on the right. GABAergic organizer proteins (red) span the synaptic cleft and recruit $GABA_A$Rs to the postsynaptic membrane. For details of these proteins, see the main text and Figure 2 in the main text. Abbreviations: CCK, cholecystokinin-positive cell; LR, long-range GABAergic projection neuron; PN, pyramidal neuron; PV-BC, parvalbumin-positive basket cell; PV-CC, parvalbumin-positive chandelier cell; SST, somatostatin-positive cell; VIP, vasoactive intestinal polypeptide-positive cell.

methodological advances have opened highly promising new avenues toward finally resolving the composition of GABAergic synapses in a subtype-specific manner at a level of detail previously attainable only for glutamatergic synapses.

Imaging-based methods to assess the molecular diversity of GABAergic synapses

Analysis of the molecular diversity of GABAergic synapses using imaging-based methods faces two main challenges. First, it is necessary to selectively label the synapses of interest in a connection-specific manner (i.e., with a defined identity of both the pre- and the postsynaptic neuron). Second, at less than 1 µm in size, synapses fall below the resolution limit of conventional light microscopy [19], necessitating specialized imaging techniques to study the differential nanoarchitecture of these structures.

Connection-specific labeling of GABAergic synapses using classical antibody-based immunohistochemical approaches has traditionally presented substantial challenges. Many antibodies against GABAergic synaptic proteins do not work adequately in classical paraformaldehyde perfusion-fixed tissue due to epitope masking and steric hindrance, necessitating specialized fixation protocols that often limit the ability to co-stain with cell type-specific markers [20]. Moreover, there are few cell type-specific markers that localize directly at the pre- or postsynaptic compartment, and it is challenging to determine the identity of the postsynaptic neuron by antibody labeling alone. To overcome these issues, genetically encoded fluorescent tags have been developed that can be expressed conditionally in a cell type-specific manner using parvalbumin (PV)-, somatostatin (SST)-, vasoactive intestinal peptide (VIP)- or other GABAergic neuron-specific Cre driver mouse lines. In particular, cell type-specific presynaptic terminals can be selectively identified by overexpression of fluorescently labeled synaptophysin or synaptotagmin constructs [21,22]. Postsynaptically, gephyrin can be visualized in a cell type-specific manner using expression of a GFP-tagged fibronectin intrabody generated by mRNA display (FingR) [23] or CRISPR-

based fluorescent tags [24,25]. By combining these pre- and postsynaptic labels, it becomes possible to visualize GABAergic synapses in a connection-specific manner even in intact and complex brain tissues.

In addition to the development of such connection-specific labeling strategies, the past two decades have seen the advent of a range of technological developments that have allowed for the imaging of synapses at unprecedented resolution. These include not only super-resolution microscopy approaches, such as stimulated emission depletion (STED), stochastic optical reconstruction microscopy (STORM), and photoactivated localization microscopy (PALM), which allow for imaging at a resolution beyond the diffraction limit of light [19], but also array tomography, in which serial ultrathin sections are imaged at high resolution and subjected to volumetric reconstruction [26,27], correlative light and electron microscopy (CLEM), which combines the respective advantages of these two microscopy methods [28], expansion microscopy, in which biological samples are physically expanded to circumvent the resolution limit of the microscope [29], and advanced machine learning algorithms, which allow for brain-wide synaptome mapping [30,31]. These approaches have been used to image GABAergic synapses at high resolution, demonstrating, for example, that some, but not all, GABAergic postsynaptic sites are likely to be organized into nanoscale subsynaptic domains [32–36], and that spine and shaft synapses differ in their ultrastructure [37]. Whether differences in the organization and composition of nanoscale domains contribute to the diversity of GABAergic synapse subtypes, as proposed for glutamatergic synapses [38], remains an area of ongoing investigation.

Electrophysiological methods to assess connection-specific synaptic transmission properties

To determine the connection-specific functional consequences of GABAergic synapse organizer proteins, electrophysiological approaches are required that allow for stimulation of individual presynaptic neurons in a cell type-specific manner, while recording from a genetically, morphologically, or functionally defined postsynaptic neuron.

In recent years, significant advances have been made in the cell type-specific stimulation of presynaptic neurons. Classically, connections between specific GABAergic neurons and their postsynaptic targets have been investigated using paired recordings [10,39,40], in which two synaptically connected neurons are simultaneously assessed by whole-cell patch-clamp electrophysiology and stimulation of the GABAergic presynaptic neuron. However, due to technical challenges involved in performing multiple simultaneous patch-clamp recordings, optical approaches toward cell type-specific stimulation of the presynaptic neuron, such as neurotransmitter uncaging and optogenetic stimulation, are gaining in popularity [41,42]. In neurotransmitter uncaging, a caged and, hence, inactive glutamate compound is activated by flash photolysis specifically in the vicinity of a genetically defined presynaptic neuron, resulting in the generation of an evoked action potential [41]. Caged GABA compounds are also available that can be used to directly activate $GABA_A$Rs in a subcellular compartment-specific manner [43], although they do not provide information on the identity of the corresponding presynaptic neuron. The optogenetic approach is based on optical stimulation of the presynaptic neuron using a genetically encoded light-sensitive ion channel, such as Channelrhodopsin-2 [42]. This approach not only provides insights into the synapse subtype-specific properties of synaptic transmission, but also enables study of the molecular mechanisms underlying input-specific plasticity at inhibitory synapses [44–47].

While optical approaches have become standard in the cell type-specific stimulation of the presynaptic neuron, recordings from postsynaptic neurons are still commonly performed using patch-clamp electrophysiology due to the as yet unrivaled temporal precision of the recorded

responses. Nevertheless, definitive identification of the postsynaptic neuron using this approach is challenging and, hence, the use of genetically encoded optical tools to identify the recorded neurons is gaining traction in the precision mapping of neural circuits, particularly *in vivo*. These approaches include not only Ca^{2+} imaging and voltage imaging using genetically encoded activity sensors [48,49], but also the use of optogenetic tags for light-based identification of neurons for subsequent electrophysiological recordings [50]. Together, these approaches have vastly improved the ability to assess GABAergic synaptic transmission in a connection-specific manner.

Transcriptomic approaches to assess cell type-specific expression patterns of synaptic organizer proteins

Next to the aforementioned candidate-based strategies that specifically investigate the localization and function of known target molecules at GABAergic synapses, unbiased approaches have a key role in identifying new proteins that may contribute to GABAergic synapse diversity. Single cell transcriptomic analyses have offered unprecedented insights into the cell type-specific expression patterns of molecules that regulate the synaptic connectivity of GABAergic synapse subtypes [51–53]. Moreover, by combining single cell RNA sequencing with patch-clamp electrophysiology in the Patch-sequencing (Seq) approach, gene expression patterns can be correlated with large-scale functional characterization of neuronal subtypes [54–56]. Using these approaches, synapse subtype-specific pairings of cell adhesion proteins at GABAergic neuron subtypes have been identified [6,57], demonstrating the power of transcriptomics to define the molecular architecture of connectivity in the GABAergic system.

Proteomic approaches to assess synapse-specific GABAergic organizer complexes

One of the most promising methodological advances for assessing the biochemical composition of different GABAergic synapse subtypes in an unbiased manner are recently developed synapse-specific proteomic approaches [58]. Given that GABAergic synapses comprise only a small percentage of the total number of synapses in the brain, with an even tinier fraction belonging to each of the GABAergic synapse subtypes, the notion of specifically isolating these protein complexes for proteomic analysis appeared inconceivable until recently. Two key methodological developments have provided the means to address this challenge: proximity labeling-based proteomics [59] and fluorescence-activated synaptosome sorting (FASS) [60].

The proximity-labeling proteomic approach is based on the use of engineered enzymes, such as APEX, BioID, and TurboID, which convert an inert small-molecule substrate, such as biotin, into a reactive species that can covalently tag neighboring proteins [59]. Thus, these enzymes can be used to attach a biotin tag to all proteins within a short radius, generally 1–10 nm, around the enzyme. By fusing the engineered enzyme to an endogenous protein of interest (e.g., a core component of GABAergic synapses), it becomes possible to generate a precise spatial map of the surrounding protein complex, which can then be isolated using the biotin tag and subjected to mass spectrometry for protein identification [58,59]. Two seminal studies used this methodology to identify postsynaptic [61] and trans-synaptic [62] protein complexes at GABAergic synapses, and efforts are underway to expand this approach to synapse subtype-specific investigations.

The second methodology, FASS, involves labeling the axon terminal in a genetically defined population of presynaptic neurons with a fluorescent tag, followed by subcellular fractionation to obtain a synaptosome fraction (representing the synaptic compartment) and fluorescence-activated cell sorting (FACS) to specifically isolate the fluorescently tagged synaptosomes [58,60]. This approach was recently used in a key study to provide the first synapse subtype-specific proteome at GABAergic synapses [63], comparing PV-, SST-, and VIP-positive synapses in the cortex and paving the way for understanding the GABAergic proteome in unprecedented detail.

Using methodologies such as those outlined above, a steadily increasing number of components of GABAergic synaptic complexes are being identified, most notably synaptic adhesion proteins, but also scaffolding proteins, intracellular signaling proteins, and $GABA_AR$ auxiliary subunits (Figure 2). It should be noted that the aim of this review is not to discuss the detailed molecular function of each of these proteins, but to highlight recent studies defining their involvement in establishing GABAergic synaptic diversity. For a more comprehensive description of the individual proteins, the reader is referred to recent review articles [64–79].

Synapse-specific roles of GABAergic synaptic adhesion proteins

Neurexins (Nrxns)

The Neurexins (Nrxns) are a family of three presynaptic cell adhesion proteins, Nrxns1–3, which are characterized by the existence of a vast number of splice isoforms [64,65]. These splice isoforms bind differentially to dozens of different postsynaptic ligands, resulting in a highly complex combinatorial code that has been proposed to form a key basis for synapse-specific connectivity throughout the CNS. Due to this enormous heterogeneity as well as a lack of isoform-specific antibodies, it has been challenging to define the precise molecular logic by which Nrxns govern synaptic connectivity. Nevertheless, some general principles are emerging that illustrate the molecular mechanisms linking Nrxn splice isoform heterogeneity to GABAergic synapse diversity [80].

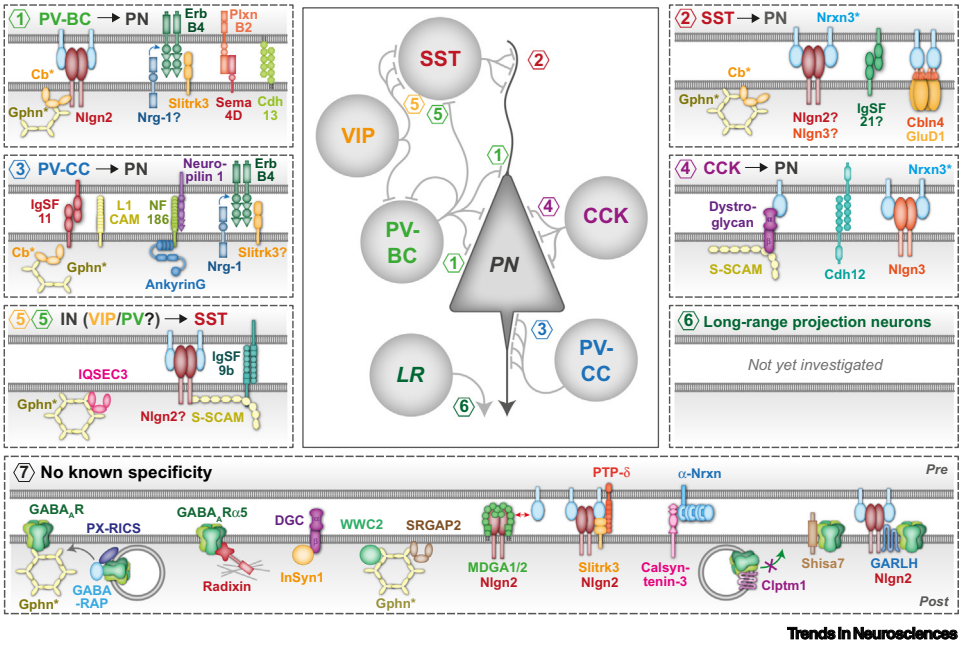

Figure 2. Proposed synapse subtype-specific function of GABAergic organizer proteins. Schematic of GABAergic neuron subtypes and their circuit-specific connections (central panel), as well as of GABAergic organizer proteins proposed to act at the different synapse subtypes (boxed insets 1–7). In each inset, the presynaptic side of the synapse is shown at the top and the postsynaptic side at the bottom. Information on synapse specificity originates primarily from studies in neocortex and hippocampus, and little is known about the organization and composition of GABAergic synapses in other brain regions. In addition, many of the proposed synapse allocations of the organizer proteins have yet to be verified and some of the organizer proteins are also expressed in cell types other than neurons. For protein names and abbreviations, please see the main text. Proteins marked with an asterisk (*) are likely to be regulated in a synapse-specific manner by alternative splicing and/or post-translational modifications. Abbreviations CCK, cholecystokinin-positive cell; IN, interneuron; LR, long-range GABAergic projection neuron; PN, pyramidal neuron; PV-BC, parvalbumin-positive basket cell; PV-CC, parvalbumin-positive chandelier cell; SST, somatostatin-positive cell; VIP, vasoactive intestinal polypeptide-positive cell.

Nrxns contain up to six alternatively spliced segments, AS1–6, which regulate their binding affinity to their numerous postsynaptic partners. Differential splicing of these sites, most notably of sites AS4 and AS5 in Nrxn3, has been reported in PV-, SST- and VIP-positive neurons in mice, defining the synaptic transmission properties of the corresponding synapse subtypes [80,81]. This differential splicing results from the cell type-specific expression of key splicing factors, including members of the STAR protein family, such as SAM68, SLM1, and SLM2 [82–84], placing these splicing factors in a central position in defining Nrxn-mediated GABAergic synapse diversity.

Neuroligin-2 (Nlgn2)

Neuroligin (Nlgn)-2 is a member of the Neuroligin family of postsynaptic adhesion molecules, which bind to presynaptic Nrxns to form trans-synaptic bridges that regulate synapse formation and function [66]. Nlgn2 was the first adhesion protein to be identified specifically at inhibitory synapses, and is a core component of almost all GABAergic synapses in the CNS, having a key role in recruiting $GABA_A$Rs to the synapse [67]. Despite this near-universal expression, deletion of Nlgn2 in mice was found to reduce GABAergic synapses specifically in the perisomatic region in hippocampus and amygdala [85–87], and paired recordings indicated that Nlgn2 deletion affected synaptic transmission specifically at PV-positive but not SST-positive synapses in somatosensory cortex [39]. Together, these observations indicated that synapses formed by PV-positive basket cells are selectively vulnerable to Nlgn2 loss-of-function, and that compensatory mechanisms may be involved at other synapse subtypes. However, some studies have also found consequences of Nlgn2 deletion on dendritic synapses formed by SST-positive neurons [88,89]. Conversely, deletion of Nlgn2 in mice had no effect on inhibitory synaptic transmission in GABAergic neurons in the centromedial amygdala and thalamic reticular nucleus [85,90,91], indicating that Nlgn2 may function differently at inhibitory synapses onto GABAergic neurons than onto glutamatergic neurons. Consistent with this notion, it was reported that deletion of Nlgn2 from glutamatergic and GABAergic neurons results in differential consequences for the excitatory/inhibitory balance [92].

Neuroligin-3 (Nlgn3)

Unlike Nlgns1 and 2, Nlgn3 is not restricted to either excitatory or inhibitory synapses, but can be found at either synapse type in a brain region-, phosphorylation-, and splice isoform-dependent manner [93–95]. In mouse hippocampal area CA1, deletion of Nlgn3 resulted in an endocannabinoid-dependent enhancement at cholecystokinin (CCK)-positive synapses, but not PV-positive synapses [40], while knock down of Nlgn3 in the same region selectively reduced inhibition by dendritic SST-positive synapses, but not perisomatic PV-positive synapses [88]. Alternative splicing has been proposed as one of the mechanisms differently regulating Nlgn3 function at different inhibitory synapse subtypes in CA1 [96].

Neuroligin-4 (Nlgn4)

Arguably the most enigmatic of the Nlgn family members, Nlgn4 has come under substantial scrutiny due to its link to autism spectrum disorders (ASD) [68]. In mice, Nlgn4 shows substantial functional similarity to Nlgn2, regulating the structure and function of inhibitory synapses, with a particular role at perisomatic synapses likely formed by PV-positive neurons [97]. However, *NLGN4* appears to have undergone unusually rapid evolution [98] and, in humans, Nlgn4X was subsequently found to contribute primarily to excitatory, but not inhibitory, synapse function [99,100]. Accordingly, the Nlgn4 pathway is unlikely to represent a relevant therapeutic target at GABAergic synapses.

Dystroglycan complex

Next to the Nlgns, another postsynaptic adhesion molecule at inhibitory synapses that was identified early on was dystroglycan [69]. Best known for its role in the dystrophin/dystroglycan

complex at the neuromuscular junction, dystroglycan was shown to additionally localize to GABAergic synapses in mice, where it interacts trans-synaptically with Nrxns [101] and contributes to the regulation of homeostatic plasticity [102]. Recent studies indicated that dystroglycan may have a particularly prominent role in regulating the formation and function of CCK-positive perisomatic synapses onto pyramidal neurons [103], and that alterations at this synapse subtype may contribute to the neurological dysfunction observed in dystroglycanopathies [104].

L1 immunoglobulin family proteins

The L1 family of cell adhesion molecules (CAMs) has four members, L1CAM, NrCAM, Neurofascin and CHL1, which share a common extracellular domain structure containing immunoglobulin and fibronectin domains that can mediate both homophilic and heterophilic interactions [70]. Interestingly, the members of the L1 family appear to be involved in the formation of axo-axonic synapses formed by PV-positive chandelier cells on the axon initial segment of pyramidal neurons through intracellular interactions with the axon initial segment scaffolding protein AnkyrinG [71,72]. Accordingly, deletion of Neurofascin [105,106] or L1CAM [107] resulted in a specific loss of axo-axonic synapses.

Immunoglobulin superfamily proteins

Several immunoglobulin superfamily (IgSF) proteins have been linked to the formation of specific subsets of GABAergic synapses. IgSF9b was initially proposed to interact with Nlgn2 to regulate formation of GABAergic synapses, likely those formed onto other GABAergic neuron (i.e., disinhibitory synapses) [73,108]. Subsequently, it was shown that, at least in the mouse amygdala, Nlgn2 and IgSF9b do not interact functionally, but act at separate subsets of GABAergic synapses, and that deletion of IgSF9b results in an increase in GABAergic synaptic transmission onto GABAergic neurons in the centromedial amygdala [90]. Another IgSF protein, IgSF11, was shown to be expressed preferentially by PV-positive chandelier cells compared with other interneuron subtypes, as well as by pyramidal neurons in the target layer [109]. In this manner, IgSF11 determines the layer-specific connectivity of axon initial segment-targeting chandelier cells in the cortex. Finally, IgSF21 was also reported to have a role at GABAergic synapses, where it may preferentially regulate the formation of dendritic synapses [110,111].

MDGA family proteins

The MDGA family comprises two proteins, MDGA1 and MDGA2, which are anchored in the synaptic cleft plasma membrane via a GPI anchor. These MDGA proteins have been proposed to act as suppressors of synapse formation and function by binding to Nlgns at their Nrxn-binding site and disrupting the Nlgn/Nrxn trans-synaptic complex [74,112]. However, neither the synapse specificity nor the precise mechanism of action of MDGAs are yet fully understood, with contradictory evidence alternatively pointing to functions at excitatory or inhibitory synapses for both MDGA1 and MDGA2 [62,113–115], and with some studies reporting that MDGAs can promote rather than suppress synapse formation [89,113] and can function through partners other than Nlgns [116]. Without additional evidence, the contribution of the MDGAs to GABAergic synapse subtype specificity is challenging to judge.

Cadherins (Cdhs)

The cadherins (Cdhs) constitute a large class of CAMs that form largely homophilic interactions and have an important role in defining synaptic connectivity [75]. While the network of Cdh interactions is highly complex, one example of a Cdh that has been found to show GABAergic synapse subtype specificity is CDH-13. CDH-13 expression and function in the hippocampus was found to be restricted to PV- and SST-positive GABAergic neurons in both mice and human tissue [117,118]. Consistent with this notion, CDH-13 and CDH-12 were recently

shown to selectively regulate PV- and CCK-positive synapses, respectively, onto two separate subpopulations of pyramidal neurons in somatosensory cortex of mice [57], defining connection specificity within this network.

Semaphorins/Plexins

Cell adhesion proteins of the Semaphorin family and their receptors, the Plexins, were originally identified as axon guidance molecules during development, but have since also been found to have a role at mature synapses [119]. In particular, Sema4D and its receptor Plexin-B were shown to be specifically involved in the formation and function of GABAergic synapses [120]. More recently, it was reported that Plexin-B2 (PlxnB2), but not Plexin-B1, is specifically expressed by PV-positive interneurons in mouse hippocampus, and that it binds to postsynaptic Sema4D specifically on pyramidal neurons, but not other PV-positive neurons [121].

ErbB4/Neuregulin (Nrg)

ErbB4 is a receptor tyrosine kinase that, in hippocampus and cortex, is expressed predominantly in PV-positive neurons (both basket and chandelier) [122], and contributes to the formation of synapses by PV-positive chandelier cells onto the axon initial segment through its interaction with its postsynaptic receptor, Neuregulin-1 [123]. More recently, ErbB4 has additionally been proposed to interact with Slitrk3, another postsynaptic protein at GABAergic synapses, at PV-positive perisomatic and axon initial segment synapses in mice, in a manner that is independent of its tyrosine kinase activity [124].

GluD1/Cerebellin-4 (Cbln4)

A surprising recent addition to the family of GABAergic synapse organizer proteins is the glutamate receptor delta-1 (GluD1) [125,126]. GluD1 is an ionotropic glutamate receptor that interacts trans-synaptically with presynaptic Nrxns through the secreted adaptor protein Cerebellin-4 (Cbln4). In mice, Cbln4 was shown to be secreted selectively by SST-positive interneurons in the cortex [6], and, hence, it has been proposed that the Nrxn/Cbln4/GluD1 complex specifies inhibitory cortical connectivity by mediating synaptogenesis between Cbln4-expressing SST-positive interneurons and pyramidal neurons [125].

Other synaptic adhesion proteins

In addition to the forementioned candidates, several other CAMs have been identified at GABAergic synapses for which little information is yet available on potential synapse subtype selectivity, including Slitrk3 [127,128] and Calsyntenin-2 and -3 [129–131]. It will be interesting to determine whether any of these proteins display synapse subtype specificity that may be of therapeutic relevance.

Synapse-specific roles of GABAergic postsynaptic scaffolding proteins

Gephyrin (Gphn)

A key component of most GABAergic synapses is the postsynaptic scaffolding protein gephyrin. This protein forms a lattice-like scaffolding structure below the GABAergic postsynaptic membrane, acting as a central hub that coordinates the binding and function of $GABA_A$Rs, synaptic adhesion proteins, and intracellular signaling pathways [76,77]. Despite this core function, or precisely because of it, gephyrin has been proposed to contribute to the diversity of GABAergic synapse subtypes by multiple mechanisms.

First, gephyrin is regulated by a range of post-translational modifications [16,44,76,132], and differential modification at different synapse subtypes may fundamentally alter the properties of these subtypes, although this has yet to be substantiated experimentally. Probes that detect

modification-specific states of gephyrin, such as antibodies or the newly developed anti-gephyrin DARPins [133], will be key to addressing this question. Second, a large number of splice variants of gephyrin exist, and early evidence indicates that these may contribute differentially to different GABAergic synapse subtypes [134–136]. Third, as described above, evidence from high-resolution imaging indicates that gephyrin is organized into nanoscale subsynaptic domains [32–36], which may be regulated by phase separation mechanisms [137], and that differences in this organization may contribute to synaptic diversity [38]. Finally, several studies have also found that gephyrin is not required for $GABA_AR$ clustering at all synapses, and that, for example, a subset of PV-positive synapses in mice may entirely lack a gephyrin scaffold [138]. A more detailed analysis of how these various mechanisms contribute to GABAergic synapse subtype-specific function will be essential with respect to future therapeutic approaches.

S-SCAM/MAGI-2

The only other scaffolding protein, to the author's knowledge, that has been reported to localize to GABAergic synapses to date is synaptic scaffolding molecule (S-SCAM), also known as MAGI-2 [108,139]. However, little is known about how S-SCAM may interact with the gephyrin scaffold, and its role at different GABAergic synapse subtypes remains largely unknown.

Synapse-specific roles of intracellular signaling proteins at GABAergic synapses

Collybistin/ARHGEF9

Collybistin (Cb), which is encoded by *ARHGEF9*, is a brain-specific GTP/GDP exchange factor (GEF) that interacts with gephyrin and Nlgn2 to recruit $GABA_ARs$ to the synapse. Accordingly, deletion of collybistin in mice results in a loss of GABAergic synapses, albeit in a brain-region specific manner, with profound effects in, for example, hippocampus, amygdala, and cerebellum, but not in cerebral cortex, striatum, or thalamus [140], possibly due to the existence of several splice isoforms of Cb with differential expression patterns [136,141]. Intriguingly, it has been proposed that, in the latter regions, the role of Cb may be taken over by the $GABA_AR$ subunit α3, which appears to be the only $GABA_AR$ subunit that can bind directly to gephyrin [142]. In terms of neuronal subtype specificity, deletion of Cb appears to affect PV- and SST-positive synapses in hippocampus equally [88,140]. Nevertheless, mutation of the Cb binding site on the $GABA_AR$ subunit α2 results in a specific loss of PV-positive synapses at the axon initial segment in cortex [143] as well as a loss of Cb specifically from CCK-positive synapses [144]. Together, these findings indicate that Cb shows a considerable diversity both in regional and in neuronal subtype specific function, the full extent of which remains to be characterized.

IQSEC3

Another GEF found to interact specifically with gephyrin and to regulate GABAergic synapse formation is the Arf-GEF IQSEC3 [145–147]. Interestingly, IQSEC3 appears to have a particularly prominent role in SST-positive interneurons in the mouse hippocampus, regulating levels of SST peptide expression [146] and showing NPAS4- and activity-dependent upregulation selectively in SST-positive neurons [148]. Accordingly, deletion of IQSEC3 selectively from SST-positive neurons resulted in impaired GABAergic synaptic transmission onto these neurons, as well as in anxiety-like behaviors [148]. Which roles IQSEC3 has at other GABAergic synapses is currently unknown.

Other signaling proteins

Additional signaling and trafficking proteins identified at GABAergic synapses include GABARAP [149,150], PX-RICS [151], radixin [152], InSyn1 [61,153], SRGAP2 [154], and WWC2 [155], although whether these proteins have any synapse subtype-specific functions remains to be investigated.

Synapse-specific roles of $GABA_AR$ transmembrane auxiliary subunits

GARLH

The most recent category of organizer proteins at GABAergic synapses to be identified are transmembrane auxiliary subunits of $GABA_ARs$ [79]. While glutamatergic ionotropic receptors have long been known to be regulated by auxiliary subunits, such as transmembrane AMPAR regulatory proteins (TARPs) and cornichons (CNIHs), the notion that $GABA_AR$ trafficking, kinetics, and pharmacological properties are also modulated by such auxiliary proteins arose relatively recently, with the characterization of Lipoma HMGIC fusion partner-like 4 (LHFPL4, also known as $GABA_AR$ regulatory Lhfpl4, or GARLH) [156–159]. GARLH/LHFPL4 was found to regulate $GABA_AR$ clustering in a subunit-dependent manner, without affecting cell surface trafficking or channel properties of $GABA_ARs$ [156,157,159]. Moreover, deletion of GARLH/LHFPL4 resulted in the loss of fast, but not slow, miniature inhibitory postsynaptic currents (mIPSCs) in dissociated mouse hippocampal pyramidal neurons, but not in interneurons, providing initial evidence for a synapse subtype-specific function in $GABA_AR$ clustering [157].

Other $GABA_AR$ transmembrane auxiliary subunits

Additional $GABA_AR$ transmembrane auxiliary subunits identified recently include Cleft lip and palate transmembrane protein 1 (Clptm1), which acts as a negative regulator of $GABA_AR$ cell surface trafficking [160], Shisa7/CKAMP59, which modulates channel kinetics and benzodiazepine sensitivity [161,162], and TMEM132B, which regulates cell surface expression and alcohol-dependent modulation of $GABA_ARs$ [163]. To the author's knowledge, however, no information is currently available on the putative synapse subtype specificity of these auxiliary proteins, necessitating further studies to fully identify their potential therapeutic properties.

Concluding remarks and future perspectives

Over the past decade, the development of sophisticated new methodologies for the study of synapse biology, as well as the resulting identification of a plethora of molecular components of GABAergic synaptic complexes, have opened new hopes in the search for novel strategies to therapeutically target the GABAergic system. However, despite this exciting progress, it is clear that substantial challenges still lie ahead before any attempts can be made at translating these targets into clinical applications.

In particular, next to the general translational considerations faced by all research findings originating from animal models (see also Outstanding questions), a specific challenge in this context lies in the fact that most of the GABAergic synapse organizer proteins discussed above are structural proteins without enzymatic activity or small-molecule ligand-binding sites and, as such, are not obvious 'druggable' targets. Accordingly, alternative strategies will need to be identified if these organizer proteins are to be targeted therapeutically. One promising approach involves targeting the protein–protein interactions that regulate the function of GABAergic synapses. In recent years, major efforts in the drug discovery field have been invested in developing modulators of protein–protein interactions for clinical use [164]. These efforts have so far focused largely on the field of cancer biology, but initial evidence indicates that similar approaches may also succeed in brain disorders [3,78]. For example, the antimalarial drug artemisinin was recently shown to disrupt binding between gephyrin and $GABA_ARs$, thereby modulating GABAergic synaptic transmission [165,166]. An analogous concept could be used to target synapse subtype-specific $GABA_AR$ interactions, particularly those with the recently identified $GABA_AR$ auxiliary subunits, thereby modulating the synaptic localization, channel kinetics, or drug sensitivity of $GABA_ARs$ in a potentially synapse-specific manner [3,79]. Similarly, it may be possible to modulate synaptic properties by targeting protein interactions between the pre- and postsynaptic terminals. An intriguing example of this strategy was recently reported at glutamatergic synapses,

Outstanding questions

How do the synapse-specific functions of the individual post- and trans-synaptic organizer proteins at GABAergic synapses translate into consequences for behavioral output? This knowledge will be essential in determining which proteins to target for which psychiatrically relevant endophenotypes, as well as in identifying potential adverse effects that could result from functions outside of the neural circuits of interest.

Can the function of GABAergic organizer proteins identified in animal models be validated in humans? GABAergic neurons in the human brain display some key differences compared with those in rodents, requiring identification of the most relevant protein complexes specifically in the human brain. Surgically resected human brain tissue and human-derived brain organoids, potentially paired with CRISPR-based targeting strategies, may offer avenues to address these questions.

How can the patients who will benefit from targeting specific GABAergic organizer proteins be identified? Given the etiological heterogeneity of psychiatric disorders, any successful therapeutic approach must take into account the GABAergic circuit profile of the individual patient.

How can GABAergic organizer proteins be targeted pharmacologically? Most are structural proteins without enzymatic activity and, as such, are not obvious 'druggable' targets. Potential alternative strategies include modulators of protein–protein interactions, regulators of cell type-specific splicing or post-translational modifications, and CRISPR/Cas-mediated gene therapy.

where an artificial trans-synaptic molecular bridge restored excitatory synaptic transmission and promoted functional recovery in mouse models of neurological disorders [167]. Alternatively, it may be possible to selectively target those components of the GABAergic synaptic machinery that do have enzymatic activity, such as collybistin or IQSEC3, or that undergo either cell type-specific splicing or post-translational modifications that can be regulated pharmacologically. Examples of the latter two strategies have again been reported for glutamatergic synapses, where targeting of Nrxn1 splicing [168] or of Nlgn3 downstream signaling pathways [169] normalized ASD-related behaviors in mouse models. Finally, with the advent of CRISPR/Cas-mediated gene therapy [170], it is conceivable that, in the long run, gene-editing approaches may be used to modify the function of specific GABAergic organizer proteins in circuit-specific manner. Further investigation of these different approaches will be critical to harnessing the power of circuit-specific GABAergic therapeutics in the development of next-generation treatments for psychiatric, neurodevelopmental, and neurodegenerative disorders.

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Declaration of interests

The author declares no competing interests.

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