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Molecular and functional heterogeneity of GABAergic synapses

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Abstract

Knowledge of the functional organization of the GABAergic system, the main inhibitory neurotransmitter system, in the CNS has increased remarkably in recent years. In particular, substantial progress has been made in elucidating the molecular mechanisms underlying formation and plasticity of GABAergic synapses. Evidence available ascribes a key role to the cytoplasmic protein gephyrin to form a postsynaptic scaffold anchoring GABA_A receptors along with other transmembrane proteins and signaling molecules in the postsynaptic density. However, the mechanisms of gephyrin scaffolding remain elusive, notably because gephyrin can auto-aggregate spontaneously and lacks PDZ protein interaction domains found in a majority of scaffolding proteins. In addition, the structural diversity of GABA_A receptors, which are pentameric channels encoded by a large family of subunits, has been largely overlooked in these studies. In this review, we discuss recent results derived mainly from the analysis of mutant mice lacking a specific GABA_A receptor subtype or a core protein of the GABAergic postsynaptic density (neuroligin-2, collybistin), which highlight the molecular diversity of GABAergic synapses and its relevance for brain plasticity and function. In addition, we discuss the contribution of the dystrophin-glycoprotein complex to the molecular and functional heterogeneity of GABAergic synapses.

Keywords: GABA_A receptor; gephyrin; neuroligin, collybistin, dystrophin-glycoprotein complex; synaptic plasticity

Introduction

Early electron microscopy studies uncovered a fundamental dichotomy in the ultrastructure of synapses in vertebrate CNS, classified as type I (asymmetric) and type II (symmetric) synapses, based on the width of their electron-dense postsynaptic density (PSD) [1]. Subsequently, identification of glutamate (and aspartate) and GABA (and glycine) as neurotransmitters in the CNS, combined with the advent of immunohistochemistry, showed that this dichotomy corresponds to excitatory and inhibitory synapses, respectively. This structural and neurochemical distinction reflects major differences in biochemical composition of the PSD in both types of synapses. The PSD contains distinct sets of scaffolding proteins associated with neurotransmitter receptors and signaling molecules. Biochemical fractionation revealed hundreds of proteins in the PSD of glutamatergic synapses. These include numerous PDZ domain-containing scaffolding proteins, notably PSD-95 and other members of the family of membrane-associated guanylate kinase proteins (MAGUKs) [2]. The PDZ interaction motifs are a characteristic feature of many scaffolding proteins, allowing to build modular protein complexes [3], thereby ensuring rapid, efficient, and site-specific propagation of incoming synaptic inputs [4]. The phototransduction complex assembled by INAD in *Drosophila* photoreceptors is a prototypical example of such signaling scaffold organized by PDZ motifs [5].

In glutamatergic synapses, MAGUKs play a key role in assembly and regulation of the PSD [6,7]. MAGUKs are membrane-anchored by specific lipid modifications (e.g., palmitoylation) and/or interact with components of the cytoskeleton to ensure long-term structural stability of the PSD [8,9]. In addition, they contribute to plasticity of synaptic transmission, mainly by binding to specific effector proteins, which induce posttranslational modifications regulating the trafficking and function of neurotransmitter receptors and other signaling molecules. Given the central role of PSD scaffolding molecules, even minor alterations in their expression, trafficking, regulation, or function have been associated with brain disease [7,10-12]. Therefore, identifying and correcting these dysfunctions might provide promising new avenues for drug therapy.

In striking contrast with type I synapses, the PSD of GABAergic and glycinergic synapses contains only few proteins with a PDZ domain. Rather, they are organized around a core scaffolding protein, gephyrin, which forms multimeric complexes by auto-aggregation and by interacting with other postsynaptic proteins, including GABA_A receptors (GABA_AR), glycine receptors (GlyR), neuroligins (NL), and collybistin (CB) [13]. Models to explain the formation

and maintenance of either glycinergic or GABAergic synapses are still fragmentary [14,15], notably because the biochemical composition of their PSD is poorly characterized and because gephyrin structure is only partially resolved. The paucity of PDZ domains in proteins forming GABAergic and glycinergic PSDs implies, however, that protein-protein interactions are based largely on other motifs. While the binding site of the GlyR β subunit intracellular loop to gephyrin has been well characterized, much less is known about GABA_AR subunits, although information available suggests direct interactions with members of the α subunit family [16-18]. A major challenge for studying GABA_AR-gephyrin interactions is the presence of multiple receptor subtypes differing in subunit composition, each of them likely interacting differently with gephyrin and its partners.

PSDs typically are associated with specific subcellular compartments; glutamatergic synapses being formed on dendritic spines whereas GABAergic synapses occurring mainly on neuronal somata, dendritic shafts, and axon initial segments. Therefore, association with specific cytoskeletal elements and/or transmembrane proteins linked to extracellular matrix proteins likely is of primary relevance for the formation and maintenance of PSDs. Although gephyrin was characterized initially as a tubulin-binding protein, the role of the cytoskeleton for anchoring GABAergic and glycinergic PSDs remains largely obscure. Furthermore, PSDs interact closely with presynaptic terminals by means of trans-synaptic ligand/receptor protein complexes, such as neuroligins/NLs, integrins, ephrins, cadherins, etc., raising the possibility that the subcellular localization of synapses is determined by specific molecular components of the PSD.

The purpose of this review is to highlight current knowledge of proteins interacting with gephyrin at GABAergic synapses. We will discuss how molecular and functional heterogeneity of GABAergic synapses arises from these differential interactions. We will also present evidence to reconsider the role of gephyrin as anchoring molecule in favor of gephyrin being the hub of a signaling scaffold organized in concert with specific GABA_AR subtypes to regulate GABAergic synapse function.

Overview of major PSD proteins at inhibitory synapses

Gephyrin

At the PSD of inhibitory synapses, anchoring GABA_AR and GlyR is ensured, at least in part, by a scaffold assembled upon self-oligomerization of gephyrin. These interactions will be discussed in

more detail in the following sections. Additional well-characterized proteins associated with gephyrin include the transmembrane protein NL2 and CB, a member of the Dbl family of guanine nucleotide exchange factors (GEF), discovered upon its ability to translocate gephyrin to the cell surface in non-neuronal cells (reviewed in [13]). A number of additional gephyrin-interacting proteins have been reported, which are involved mainly in regulation of the cytoskeleton and gephyrin trafficking. So far, however, proteins directly regulating gephyrin auto-aggregation have not been described, despite the fact this aggregation exclusively occurs at postsynaptic sites.

Gephyrin is a highly conserved molecule, whose primary role in the living kingdom is to catalyze the synthesis of Molybdenum cofactor (Moco) [19]. In vertebrates, gephyrin acts in addition as a postsynaptic scaffolding molecule, and current evidence from rodents indicates that Moco synthesis in brain is restricted to astrocytes [20]. Gephyrin has two major functional domains, E and G, linked by an unstructured C-domain, which contains most gephyrin regulatory sites and binding sites for interacting proteins. While the crystal structure of the G and E domains has been partially determined, the instability of full-length recombinant gephyrin in solution and the lack of information about the structure of the C-domain are major obstacles for elucidating gephyrin function and the mechanism of gephyrin auto-aggregation [13]. Current models posit either formation of hexameric gephyrin scaffolds, with 6 gephyrin molecules binding to 3 GlyR [21], or aggregation of gephyrin trimers, each of which is bound to one GlyR [13].

These models of gephyrin aggregation are based on partial structural information, localization of the GlyR binding site in the E-domain, and identification of surface-exposed loops regulating gephyrin postsynaptic aggregation [13,22,21]. However, available experimental evidence does not allow distinguishing between them. Data from GlyR single-particle tracking experiments and gephyrin fluorescence recovery after photobleaching revealed that gephyrin modulates synaptic retention time and lateral mobility of these receptors, and that GlyR diffusion properties modulate gephyrin clustering [23,24]. Therefore, a dynamic exchange of gephyrin and receptors occurs in apparently stable postsynaptic structures. Furthermore, time-lapse recordings from neurons expressing fluorescent tagged gephyrin revealed that gephyrin clusters are constantly remodeled and can move in dendrites over distances of several μm in concert with apposed presynaptic terminals [25]. Collectively, these observations are consistent with both views that gephyrin units

(e.g., trimer) can be added and removed from the scaffold and that the gephyrin scaffold acts as a stable but mobile entity in dendrites.

There are multiple splice-variants of gephyrin generated by alternative splicing of cassettes localized mainly in the G- and C-domain (see [13] for nomenclature). The splice variants capable of Moco synthesis have been identified, but they do not necessarily differ from those forming postsynaptic clusters in neurons; further, insertion of the G2 splice cassette, a gephyrin isoform expressed in both neuronal and non-neuronal tissues, prevents gephyrin aggregation and Moco synthesis, and has been proposed to regulate gephyrin function, synaptic localization, and clustering [20,23,26]. Further characterization of gephyrin splice variants, notably in the C-domain, will be necessary to better understand the regulation of gephyrin enzymatic activity and clustering in different types of synapses.

Recent evidence points to gephyrin posttranslational modification by phosphorylation as a mechanism regulating its postsynaptic function. Although gephyrin is a known phospho-protein [27], it is only in 2007 that a first report suggested that phosphorylation-dependent recruitment of the peptidyl-prolyl isomerase Pin1 to a site located in the C-domain regulates GlyR binding [28]. We have identified a novel phosphorylation site on gephyrin, Ser270, and shown that it selectively regulates formation of postsynaptic gephyrin clusters in vitro and in vivo under the control of GSK3 β [29]. These findings provide a novel mechanism to regulate GABAergic synapse formation and function by a phosphorylation cascade activated by a plethora of extra- and intracellular signals and controlling basic cellular functions.

Neuroigin2

Neurexins and NLs gained major interest upon discovery that they induce formation of synapses in recombinant non-neuronal cell systems. Reports of preferential distribution of NL1 in glutamatergic synapses and NL2 in GABAergic synapses [30-32] fuelled considerable efforts to elucidate their role in synapse formation and specification in the CNS. Importantly, in the context of the present review, NL2 has been shown to interact directly with gephyrin and this interaction is necessary for postsynaptic localization of gephyrin clusters at GABAergic sites [33]. NL3 and NL4 also have been reported to be present in a subset of GABAergic and glycinergic synapses, respectively [34,35]. The significance of a segregated distribution of NLs to different types of synapses is not fully understood [36], because this apparent selectivity is lost upon

overexpression and because NL3 has been reported to be present in both glutamatergic and GABAergic synapses *in vivo*. It is also likely that NLs are functionally interchangeable to some degree, as seen in targeted gene deletion experiments, in which the loss of a particular NL isoform appears to be compensated in part by another isoform [37]. Nevertheless, the significance of the NL2-gephyrin interaction was highlighted by a study showing that NL2 also interacts with CB, in a manner that favors formation and regulation of gephyrin clusters at postsynaptic sites (see below).

Collybistin

CB is a neuron-specific Rho-GEF encoded by a single gene (*ARHGEF9*) subject to alternative splicing. In rat, there are three main CB variants, CB1-3, differing in the C-terminal region. They all possess three major functional domains: an N-terminal SH3 domain, encoded by a spliced exon (SH3⁺ or SH3⁻), a DH (or GEF) domain selectively activating the small GTPase Cdc42, and a PH domain (Fig. 1). Available literature is somewhat confusing about the existence and structure of CB variants in other species. In human, CB is also known as hPEM2 and only one sequence, with a C-terminus identical to rat CB3 has been described [38]. In mouse, four transcripts have been identified, which are largely conserved compared to human and rat CB3, but differ in their N-terminus (Fig. 1). The relative abundance of CB splice variants in the CNS and their expression pattern across different brain regions or developmental stages have not been investigated systematically. A recent report using antibodies recognizing the majority of CB isoforms (notably CB3) confirmed the localization of CB at GABAergic PSDs in most brain regions [39].

The significance of CB for glycinergic and GABAergic synaptic function is underscored by the discovery of several loss of function mutations in *ARHGEF9* underlying severe forms of X-linked mental retardation and hyperekplexia [40,41]. In recombinant expression systems, CB is essential for cell-surface translocation of gephyrin [42]. However, this function is only assumed by CB variants lacking the SH3 domain, mainly CB2_{SH3-}, suggesting that the SH3 domain somehow controls CB activity. A plausible model posits that, in analogy to other GEFs, the SH3 domain exerts an auto-inhibitory effect on the DH domain (and hence on activation of Cdc-42) [33]. CB binds gephyrin in the C-domain, whereas the binding site of gephyrin on CB partially overlaps with the binding of Cdc42, which led to speculation that they might be mutually

exclusive [43](but see [44]). A recent study suggested, however, that CB-mediated gephyrin postsynaptic clustering is not dependent on a functional DH domain, but requires the PH domain [45]. Because gephyrin postsynaptic clusters were observed in the brain of conditional Cdc42-deficient mice, this study even concluded that Cdc42 is dispensable for gephyrin aggregation.

In addition to gephyrin, CB interacts directly with NL2 via its SH3 domain. Based on data from recombinant systems, this interaction was suggested to cause a conformational change relieving the auto-inhibition of the DH domain, thereby facilitating cell-surface translocation of gephyrin and GABA_AR [33]. In neurons, the activation of CB_{SH3+} by NL2 was postulated to facilitate gephyrin clustering at postsynaptic sites upon binding to phosphoinositol-3-phosphate in the cell membrane via its PH domain. This model received further support in a report showing that CB binds to GABA_AR α 2 (and α 3) subunit and that this interaction leads to CB activation and translocation of trimeric CB-gephyrin- α 2 subunit complexes to the cell membrane [46]. However, the role of NL2 was not examined in this study. Furthermore, in view of the multiplicity of CB isoforms and of the heterogeneity of GABA_ARs, which form multiple subtypes differing in subunit composition and cellular/subcellular localization [47], such models are necessarily fragmentary and do not take into account the diversity and exquisite functional specialization of GABAergic synapses across the CNS.

Insight into the roles of CB isoforms for gephyrin clustering was derived from over-expression studies in transfected neurons, which aimed to distinguish between CB2_{SH3+} and CB2_{SH3-} [48,44], as well as to elucidate the contribution of Cdc42, the only known substrate of CB. In both studies, CB over-expression contributed to stabilize gephyrin, as evidenced by a marked increase in size and density of postsynaptic gephyrin clusters. Interestingly, CB2_{SH3-} facilitated the formation of supernumerary postsynaptic clusters, whereas CB2_{SH3+} favored formation of intracellular gephyrin aggregates, as illustrated in Figure 2. Further, deletion of the PH domain of CB2 led to gephyrin retention along the dendritic cytoskeleton, whereas deletion of the DH domain strongly interfered with gephyrin postsynaptic clustering. Finally, over-expression of Cdc42, using dominant negative and constitutively active mutants for comparison, revealed that Cdc42 cooperates with CB2_{SH3-} for postsynaptic targeting of gephyrin and that it regulates gephyrin cluster size at postsynaptic sites [44]. These data add to the model proposed by [33], by unraveling distinct functions of CB2 isoforms for gephyrin transport, postsynaptic targeting, and regulation of postsynaptic clustering in concert with Cdc42. However, they suggest that the

postulated conformation-dependent activation of CB_{SH3+} by NL2 might not be crucial for proper CB function at GABAergic PSDs.

Differences between GABAergic and glycinergic synapses

Because gephyrin is found in both glycinergic and GABAergic synapses, it is generally considered that the assembly of signaling scaffolds by gephyrin is similar in both types of synapses. This concept is reinforced by the homology between GABA_AR and GlyR, which are members of the family of Cys-loop ligand-gated ion channels. However, despite being closely related, GABA_ARs and GlyRs differ fundamentally in their affinity for gephyrin. Recent reports identified the binding site of the GABA_AR α 1- α 3 subunits on the gephyrin E-domain and showed substantial overlap with the GlyR β subunit, albeit with a >30-fold lower affinity [16,49]. Nevertheless, expression of a mutant GABA_AR lacking the interaction site in the M3-M4 loop of the α 3 subunit disrupted its postsynaptic clustering in cultured hippocampal neurons. These findings confirm and extend a previous report, which identified a gephyrin-interaction motif in the M3-M4 intracellular loop of the α 2 subunit, sufficient to direct chimeric GABA_AR to gephyrin-rich postsynaptic sites [17].

As discussed in the next section, postsynaptic clustering of some GABA_AR subtypes is possible in the absence of gephyrin. In contrast, GlyR postsynaptic clustering appears to be dependent on the presence of gephyrin clusters [50], mirroring the high affinity of their interaction and confirming observations that GlyR trafficking in neurons is facilitated by association with gephyrin [51].

A second major difference between GABAergic and glycinergic synapses is the dependence of gephyrin on CB for postsynaptic aggregation. Unexpectedly, CB-knockout (KO) mice exhibit no apparent morphological and functional alteration of glycinergic synapses in the brainstem and spinal cord [52]. In particular, they do not show any signs of spasticity typical of hypoglycinergic function at birth, which are prominent in mice lacking the type 1 glycine transporter (GlyT1) [53]. These results indicated that CB either is dispensable for gephyrin/GlyR clustering at glycinergic PSDs or is functionally replaced by a homologous protein. In contrast, as detailed in the next section, CB deficiency produces marked alterations of gephyrin clustering at GABAergic postsynaptic sites.

A third difference is the differential targeting of NLs to GABAergic and glycinergic synapses. While NL2 is apparently localized in the former synapses (and lacking in the latter), NL4 was reported to be selectively localized to glycinergic synapses, at least in the retina [35].

A fourth difference is the existence of multiple, presumably dozens of GABA_AR subtypes, differing in subunit composition in adult brain, compared to a few GlyR subtypes, assembled mainly from α 1 or α 3 and β subunit variants. The multiplicity of GABA_AR underlies molecular and functional heterogeneity of GABAergic synapses, as well as differential targeting to extrasynaptic versus postsynaptic sites. There is evidence that receptors differing in α subunit variant are functionally not interchangeable, and that only GABA_AR targeted postsynaptically contribute to formation of gephyrin clusters [54,55]. Therefore, interactions of GABA_AR subtypes with gephyrin and other GABAergic PSD proteins are likely more heterogeneous and synapse-specific than GlyR-gephyrin interactions. These limitations are well evident in single-particle tracking studies of GABA_AR cell surface mobility, which cannot be performed on a uniquely identified receptor subtype. Nevertheless, evidence available indicates that diffusion properties and synaptic confinement of GABA_ARs containing the γ 2 subunit are modulated by enhanced excitatory synaptic activity, in a Ca⁺⁺-dependent manner [56].

Several reports suggested that, despite the tight biochemical association between GlyRs and gephyrin, there is no direct correlation between gephyrin availability at postsynaptic sites and synaptic retention time or cell-surface diffusion parameters of GlyRs in neurons. Therefore, the high affinity of gephyrin for the GlyR β loop trafficking does not imply a one-to-one relationship. It is conceivable that gephyrin scaffolds contain a limited number of binding sites for GlyR [57,23,58]. Further evidence for a dissociation between GlyR and gephyrin clustering dynamics was provided by analyzing the interaction between gephyrin and heat-shock cognate protein 70 (Hsc70), a chaperone modulating ubiquitination of its substrates [59]. These authors showed that Hsc70 selectively regulates gephyrin clustering in cultured spinal cord neurons, without affecting GlyR clustering and cell-surface diffusion kinetics.

Heterogeneity of GABAergic synapses revealed in mice with targeted gene deletions

Initial observations of a functional interaction between gephyrin and GABA_AR were made in γ 2-KO mice, in which postsynaptic clustering of GABA_AR and gephyrin was strongly impaired [60-62]. Conversely, GABA_AR clustering was widely disrupted in neurons from gephyrin-KO mice [63]. This interdependence was later shown not to be absolute, as GABA_AR containing the α 1 subunit, for instance, can form postsynaptic clusters mediating synaptic transmission in the absence of gephyrin [64,65]. However, ablation of postsynaptic GABA_AR by targeted deletion of an α subunit variant often results in the disruption of gephyrin clusters and formation of large intra-cellular gephyrin aggregates [54,66-70], confirming the dependence of gephyrin on GABA_ARs for postsynaptic localization. Importantly, in thalamic neurons expressing both α 1-GABA_AR postsynaptically and α 4-GABA_AR extrasynaptically, gephyrin clustering was not rescued following α 1 subunit deletion, indicating that α 4-GABA_AR cannot interact with gephyrin [69,54]. Beyond GABA_ARs, evidence for multiple mechanisms of gephyrin postsynaptic clustering at GABAergic synapses was provided by the analysis of CB- and NL2-KO mice. In particular, in GABAergic synapses of the forebrain and cerebellum, multiple phenotypes were observed in CB-KO mice, ranging from disruption of both gephyrin and GABA_AR clusters (e.g., CA1 pyramidal cells) to complete preservation of these clusters (e.g., PV⁺ interneurons in CA1), passing by partial alteration (loss of gephyrin, but not GABA_AR, clusters in Purkinje cells of the cerebellum) [52,71]. Although preservation of gephyrin clusters might point to a functional homologue of CB, the phenotype of Purkinje cells rather indicates that clustering of gephyrin and of GABA_AR depends on different mechanisms, with only the former requiring the presence of CB. In NL2-KO mice, differential loss of postsynaptic clusters was observed in different subcellular compartments of CA1 pyramidal cells and dentate gyrus granule cells, with the main disruption of gephyrin and GABA_AR clusters occurring on the soma, at synapses formed by basket cells [72,73,33]. Thus, these reports pointed to distinct clustering mechanisms for postsynaptic proteins of perisomatic versus dendritic GABAergic synapses.

Further insight into GABAergic synapse heterogeneity was provided by the analysis of postsynaptic proteins in the hippocampal formation of α 2-KO mice [68]. Focusing on CA1 pyramidal cells, where α 2-GABA_AR predominate in the perisomatic area and the axon-initial segment [74,75], a pronounced reduction of gephyrin clusters was observed, notably on cell somata, on the axon-initial segment, as well as in stratum radiatum and stratum oriens (Fig. 3A). While this loss of gephyrin clusters was suggestive of a disruption of the PSD, only moderate

changes in GABAergic miniature inhibitory postsynaptic currents (mIPSC) were observed in mice lacking $\alpha 2$ -GABA_AR, with unchanged amplitude and 40% reduction in frequency. Therefore, these results indicated preservation of GABAergic function despite strongly altered gephyrin clustering at postsynaptic sites. Analysis of $\alpha 1$ -GABA_AR and NL2 revealed that their presence at perisomatic postsynaptic sites was preserved in CA1 pyramidal cells of $\alpha 2$ -KO mice (Fig. 3B), whereas they were absent from the axon-initial segment. This striking subcellular difference was correlated with the presence of the dystrophin-glycoprotein complex (DGC) in perisomatic synapses (Fig. 3C), suggesting that this protein complex represents an alternate mechanism contributing to formation and maintenance of PSD in a subset of GABAergic synapses [68], and confirming that perisomatic and dendritic GABAergic PSDs are molecularly distinct.

The dystrophin-glycoprotein complex

The DGC is a transmembrane signaling complex present in striate and cardiac muscle cells, kidney tubular epithelial cells, neurons, and astrocytes, linking the extracellular matrix to the actin cytoskeleton (reviewed in [76-78]). The DGC is essential for normal brain development and synaptic function and for maintaining the structural integrity of the sarcolemma. Mutations affecting the formation, post-translational modification, or function of the DGC cause multiple forms of hereditary muscle dystrophies. While the contribution of the DGC to muscle disease has been thoroughly investigated, patients with Duchenne and Becker muscular dystrophies also show signs of cognitive impairments and learning disabilities that have been related to its functions in the CNS [79,77]. In neurons, dystrophin was shown initially to be present in PSD fraction of purified brain membranes [80]. Subsequent studies demonstrated its association with a subset of GABAergic synapses [81,82], where it regulates GABA_AR clustering and GABAergic synaptic function, as well as glutamatergic synaptic plasticity (reviewed in [77,76]). In mdx mice, direct involvement of dystrophin in synaptic alterations has been demonstrated by dystrophin rescue experiments, which normalize mIPSC amplitude and frequency, as well as LTP in CA1 pyramidal cells [83,84].

The molecular composition of the DGC differs between muscle sarcolemma, neuromuscular junction, brain and retina, and astrocytes [85,86], where, for instance, the DGC is involved in

proper membrane targeting of aquaporin 4 at end-feet in contact with brain blood vessels [87,88]. Common components of the DGC include α - and β -dystroglycan, full-length dystrophin (Dp427), sarcoglycans, a dystrobrevin isoform, and syntrophins. The predominant dystrophin isoform found in astrocytes is the short N-terminal variant Dp71.

Localization of the DGC by immunofluorescence has been documented in neurons, using antibodies to full-length dystrophin, Dp71, utrophin, α -/ β -dystroglycan, dystrobrevin, and syntrophins [76,89]. These studies reveal a systematic colocalization with proteins of the GABAergic PSD in a subset of neurons of cortical areas, including the entire cerebral cortex, hippocampal formation (where Dp71 is selectively present in dentate gyrus granule cells), tectum, and cerebellum. In the hippocampus, the DGC appears to be restricted to principal cells, and is present selectively on the soma and proximal dendrites, suggesting an association with perisomatic synapses (formed by basket cells) [90,81].

Association of the DGC with cytoplasmic signaling molecules is ensured by direct protein-protein interactions with β -dystroglycan or dystrophin or by means of PDZ domains present in syntrophin isoforms. Identified partners of syntrophins include nNOS, aquaporin 4, voltage-gated Na^+ and K^+ channels [77], and NLs [91]. Interactions with extracellular signaling molecules critically depends on α -dystroglycan posttranslational modification by glycosylation. Consequently, mutations in glycosylating enzymes contribute to the pathogenesis of a subset of congenital muscle dystrophies associated with lissencephaly and mental retardation [78,92]. α -dystroglycan function on glial end-feet appears to be especially crucial for normal brain development, whereas neuronal α -dystroglycan glycosylation is necessary for proper synaptic plasticity [93].

In the context of the present review, interaction of DGC members with GABAergic PSD proteins deserves particular attention. Interaction between α -dystroglycan and α -/ β -neurexins by means of laminin-neurexin-sex hormone-binding globulin (LNS)/laminin G domains has been reported several years ago [94], and was proposed to compete with α -latrotoxin binding on neurexins. In addition, binding of synaptic cell adhesion molecule (SCAM) to β -dystroglycan and NL2 has been characterized in studies showing the selective presence of SCAM at inhibitory synaptic sites [95,96]. SCAM has been identified for its synaptogenic role and interactions with NMDA-receptor subunits and NLs. Its binding to β -dystroglycan involves one of two WW domains, whereas binding to NL2 occurs through the second of its three PDZ domains. Recently SCAM

was shown to bind also SynArfGEF, which selectively activates Arf6, a GTP-binding protein member of the ADP ribosylation factor family. SCAM is able to interact also with several MAGUKs (including PSD-95, SAP97 and Homer) [96]. Further, these authors showed that SynArfGEF also interacts with dystrophin and utrophin via its PDZ domain and that it co-localizes with dystrophin and gephyrin at GABAergic synapses. Besides CB, SynArfGEF is only the second GEF identified so far in GABAergic synapses. Owing to the multiple effects of Arf6, the role of SynArfGEF is not yet determined. However, it is clear that it cannot substitute CB for gephyrin or GABA_AR clustering at synapses containing the DGC, as shown in CB-KO mice.

While interactions with SCAM appear important for the association of the DGC with NL2 at GABAergic postsynaptic sites, they also provide for the possibility of cross-talk with glutamatergic synapses, in particular via activation of Arf6. Further, the selective association of SCAM with NL2 might be of relevance to explain the selective loss of inhibitory PSD proteins in perisomatic synapses of NL2-KO mice (see next section).

Model of PSD protein clustering at GABAergic synapses

Differences in PSD protein clustering properties in GABAergic synapses in relation to the presence or absence of the DGC indicate that models of GABAergic synapse formation and maintenance need to incorporate the role DGC proteins to account for published results. However, since the DGC is present in a subset of GABAergic synapses only, and is dispensable for GABAergic synapse formation in cultured hippocampal neurons [97], models of clustering that do not involve the DGC are also required.

The models proposed here (Fig. 4) take into account the following considerations:

- 1) Gephyrin clustering, but not NL2 clustering, requires the presence of postsynaptic GABA_AR [98], in particular those containing the $\alpha 2$ subunit [68].
- 2) Disruption of gephyrin clusters in perisomatic synapses of hippocampal neurons of NL2-KO mice [73,33] suggests a mandatory association between NL2 and SCAM/ β -dystroglycan [95] and indicates that postsynaptic clustering of GABA_AR requires the presence of a NL isoform. As a corollary, gephyrin cluster preservation in dendritic synapses in these mutant mice suggests compensation by another NL isoform.

- 3) Gephyrin-independent $\alpha 1$ -GABA_AR clustering has been observed in CB-KO mice [52], indicating that these GABA_AR do not require CB for their proper localization.
- 4) Distinct CB isoforms, SH3⁺ and SH3⁻, contribute to gephyrin stabilization, transport, and postsynaptic clustering in concert with Cdc42 activity [44,48].
- 5) The DGC, as visualized with antibodies to dystrophin and dystroglycans, is present exclusively at GABAergic synapses [99,81,97,100].
- 6) The DGC is not altered in neurons lacking functional GABAergic transmission or gephyrin clusters [98,68], indicating that its formation and postsynaptic localization is not dependent on GABA_AR or gephyrin.

Accordingly, the models are elaborated on the basis of the phenotype of mutant mice lacking critical components of GABAergic PSDs:

- Starting with $\alpha 1$ -KO mice, in which two populations of neurons lacking functional synaptic GABAergic transmission were identified (relay cells in the ventrobasal complex of thalamus and Purkinje cells in cerebellum), we propose that GABA_AR are required for clustering of gephyrin (Fig. 4A). In Purkinje cells, the presence of the DGC is independent of GABA_AR and is sufficient for NL2 clustering (Fig. 4A'), presumably by means of SCAM-mediated interaction. The presence/absence of CB has not been determined.
- In $\alpha 2$ -KO mice (Fig. 4B), the presence of the DGC in perisomatic synapses was sufficient to rescue clustering of NL2, as well as $\alpha 1$ -GABA_AR. These observations suggest a possible direct interaction between $\alpha 1$ -GABA_AR and NL2 to explain preservation of $\alpha 1$ -GABA_AR clusters. The loss of gephyrin clusters reveals their dependence on $\alpha 2$ -GABA_AR, which interact directly with both gephyrin and CB2_{SH3+} [46].
- In NL2-KO mice (not shown), loss of perisomatic GABAergic PSDs suggests that NL2 is the only isoform capable of interacting with SCAM and the DGC in these cells. However, this observation cannot be generalized throughout the brain, as, for example, there is no loss of $\alpha 1$ -GABA_AR and gephyrin clusters in Purkinje cells of NL2-KO mice (personal observation); the nature of the mechanisms compensating for the absence of NL2 in these cells is not known.

- In *mdx* mice (Fig. 4C), lacking the DGC in GABAergic synapses of CA1 pyramidal cells, clustering of GABA_AR is partially affected, whereas gephyrin is preserved. These observations underscore on one hand the significance of the association between NL2 and the DGC for stabilization of GABA_AR clusters, and on the other hand, the relevance of the NL2/CB2_{SH3+}/α2-GABA_AR interaction for formation of gephyrin clusters, independently of the DGC. Therefore, it is conceivable that the function of NL2 associated with the DGC is distinct from that of NL2/3 associated with CB and gephyrin.
- Finally, in WT mice, the postulated obligatory association between NL2 and β-dystroglycan is underscored in perisomatic synapses (Fig. 4D), whereas in synapses devoid of DGC (Fig. 4E), it is conceivable that NL2 is replaced (or associated with) NL3, explaining their preservation in NL2-KO mice.
- In CA1, perisomatic synapses contain both α1- and α2-subunits [68,75]; however, since GABA_A R pentamers contain two α subunits, it is not known whether α1 and α2 correspond to different receptor subtypes, or whether they are intermingled within single receptors [68].

The model also incorporates the postulated functions of CB2 isoforms and Cdc42 [44]: 1) the ability of CB2_{SH3-} to form trimeric complexes with gephyrin and Cdc42 likely contributes to gephyrin postsynaptic targeting; 2) the role of membrane anchored CB2_{SH3+}, interacting with NL2, gephyrin, and α2-GABA_AR for PSD formation and gephyrin clustering; 3) the stabilization of gephyrin clusters by membrane-anchored CB2_{SH3-}; 4) the regulation of gephyrin postsynaptic cluster size and shape by activated (GTP-bound) Cdc-42.

To explain the phenotype of CB-KO mice, in which a differential loss of gephyrin and GABA_AR occurs in various cell types, the presence or absence of the DGC is not sufficient. For instance, α1-GABA_AR are retained in Purkinje cells of CB-KO mice in the absence of gephyrin [52], suggesting their synaptic anchoring via the DGC and NL2. In contrast, in CA1 pyramidal cells, gephyrin, α1- and α2-GABA_AR disappear in CB-KO mice, whereas in PV⁺ interneurons (and in thalamic VB neurons), gephyrin and α1-GABA_AR are retained [71]. However, since the α2-subunit interacts directly with CB2 [46], one might hypothesize that GABAergic synapses containing α2-GABA_AR are selectively affected in CB-KO mice. Consequently, in CA1

pyramidal cells, perisomatic synapses might be made of heteromeric $\alpha 1/\alpha 2/\beta/\gamma 2$ receptors, which would fail to cluster in the absence of CB.

Conclusions

Taken together, the results discussed in this review indicate that GABAergic synapses are molecularly heterogeneous, and that this heterogeneity arises from the multiple proteins regulating gephyrin clustering and its function as signaling scaffold at the PSD. Moreover, the view that gephyrin forms an inert structure anchoring GABA_AR needs to be replaced by the concept that these receptors are required at postsynaptic sites to enable gephyrin clustering and to establish a signaling hub in the GABAergic PSD. Therefore, the diversity of GABA_AR subunits likely matches a diversity of signaling pathways operating at GABAergic synapses. While the precise mechanisms underlying GABA_AR postsynaptic clustering remain to be elucidated, the model of PSD formation proposed here imparts a major role to the neurexin/NL2 complex for specifying the location of GABAergic PSDs in relation to presynaptic terminals. The model is compatible with the proposed seeding role of CB/NL2 interaction for initiating GABA_AR and gephyrin clustering. However, the differential clustering of $\alpha 1$ - and $\alpha 2$ -GABA_AR observed in mutant mice raises the possibility that these two receptor subtypes belong to functionally distinct signaling scaffolds in GABAergic synapses. Finally, extending previous concepts, our model introduces the DGC and its interaction with NL2 via SCAM as key elements enabling gephyrin-independent $\alpha 1$ -GABA_AR clustering. Although the DGC is present at a subset of GABAergic synapses only, it might fulfill at least three major functions: stabilization and maintenance of GABAergic synapses, anchoring signaling molecules, notably those containing a PDZ domain, such as SynArfGEF or nNOS, in close vicinity to GABAergic synapses, transducing extracellular signals acting on dystroglycan to regulate the function and plasticity of GABAergic transmission.

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Figure legends

Figure 1: Comparison of CB isoforms in rat, human, and mouse. The three main functional domains are indicated; different colors denote distinct sequences in the N- or C-termini. The number of residues present in each domain or in each isoform is given on top of each variant. CB isoforms are best characterized in rat, where the existence of three splice variants (CB1-CB3) differing in the C-terminal domain is well established. In addition, each of CB1-CB3 variants is believed to be N-terminally spliced to include or exclude the SH3 domain. The C-terminal region of CB3 is identical to hPEM2, the only CB isoform described in human. The N-terminus of CB3 differs slightly from that of CB1/2. In mice, the CB1-CB3 nomenclature does not apply because the sequence closest to rat CB3 is encoded in three variants with different N-termini, whereas the sequence closest to either CB1 or CB2 has a unique C-terminus.

Figure 2: Differential effects of CB2_{SH3+} and CB2_{SH3-} on the postsynaptic clustering of gephyrin; adapted from [44]. Primary cultures from hippocampal embryonic neurons were transfected with tagged cDNA constructs after 11 days-in-vitro (DIV) and processed for immunofluorescence staining with the markers indicated 4-7 days later. A) Control cell showing extensive co-localization of gephyrin and $\alpha 2$ subunit immunofluorescence at postsynaptic sites. B) Upon transfection with mycCB2_{SH3-}, there is a marked increase in the density and size of gephyrin/ $\alpha 2$ clusters in the soma and on dendrites, suggesting that CB2_{SH3-} stabilizes gephyrin and favors its postsynaptic clustering. C-D) Similar effects were observed in neurons co-transfected with eGFP-gephyrin and myc-CB2_{SH3+} (C) or myc-CB2_{SH3-} (D); however, CB2_{SH3+} favored the formation of non-synaptic aggregates (arrows), whereas CB2_{SH3-} led to increased density of postsynaptic aggregates, recognized by apposition to the presynaptic marker synapsin 1 (arrowhead). The formation of non-synaptic aggregates suggests saturation of molecules, such as NL2, which normally ensure postsynaptic localization of CB2_{SH3+}. Scale bar, 20 μm .

Figure 3: Loss of gephyrin clusters, but preservation of $\alpha 1$ -subunit, NL2, and dystrophin clusters in perisomatic synapses of $\alpha 2$ -KO mice; adapted from [68]. A-A') Distribution of gephyrin clusters in the CA1 region of the hippocampus, illustrating the strong reduction in $\alpha 2$ -KO mice and the appearance of large intracellular gephyrin aggregates. B-B') Despite this effect, clustering of $\alpha 1$ subunit and NL2 was not affected around the pyramidal cell body in mutant mice, as seen

by triple immunofluorescence staining. The framed area is shown below in color-separated images. C-C') Similarly, the postsynaptic clustering of dystrophin and its colocalization with the $\alpha 1$ subunit and NL2 is not affected in $\alpha 2$ -KO mice. Scale bars: A, 50 μm ; B-C, 10 μm .

Figure 4: Models of GABAergic PSD scaffold structure in distinct cell types of $\alpha 1$ -KO, $\alpha 2$ -KO, *mdx* (lacking full-length dystrophin), and WT mice. A-A') Absence of the $\alpha 1$ subunit causes disruption of the gephyrin cluster in neurons expressing $\alpha 1$ -GABA_AR, but preservation of NL2 clustering in the presence of the DGC, presumably via binding to S-SCAM. Question marks point to proteins whose presence has not been investigated so far. B) In $\alpha 2$ -KO mice, gephyrin clustering is disrupted in synapses containing $\alpha 2$ -GABA_AR. In perisomatic synapses of CA1 pyramidal cells, the DGC anchors NL2 via S-SCAM, thereby preserving NL2 and $\alpha 1$ subunit clustering. The latter posits a possible interaction between NL2 and $\alpha 1$ -GABA_AR, for example via extracellular domains. C) Conversely, in *mdx* mice, the partial reduction of GABA_AR at perisomatic synapses might be due to absence of NL2 linked to the DGC. Preservation of gephyrin clustering is best explained by the interaction between CB2/NL2/ $\alpha 2$ subunit. Maintenance of the gephyrin cluster also implies proper function of CB2 variants: CB2_{SH3-} contributes to targeting gephyrin to postsynaptic sites (along with cdc-42) and maintaining gephyrin clusters; CB2_{SH3+} regulates gephyrin attachment to the cluster, along with NL2 and $\alpha 2$. In addition to acting with CB2, cdc-42 also regulates gephyrin cluster shape at postsynaptic sites (see [44] for details). D-D') Distinct situations are present in different subcellular sites in CA1 pyramidal cells of WT mice, depending on the presence or absence of the DGC. In these cells, it is not clear, however, whether $\alpha 1$ and $\alpha 2$ subunit correspond to different receptors, or to receptors containing both subunits. Our model postulates a tight interaction between NL2 (black) and the DGC, whereas NL isoforms interacting with CB2_{SH3+} might be either NL2 or NL3. A possible role for phosphorylation sites on gephyrin and on GABA_AR is indicated. Finally, the presence of SynArfGEF linked to dystrophin and S-SCAM is also included, although its function at GABAergic synapses has not been determined.

References

1. Gray EG (1969) Electron microscopy of excitatory and inhibitory synapses: a brief review. *Prog Brain Res* 31:141-155
2. Collins MO, Husi H YL, Brandon JM, Anderson CN, Blackstock WP, Choudhary JS, Grant SG (2006) Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *J Neurochem* 97 Suppl 1:16-23
3. Sheng M, Sala C (2001) PDZ domains and the organization of supramolecular complexes. *Annu Rev Neurosci* 24:1-29
4. Kennedy MB (1998) Signal transduction molecules at the glutamatergic postsynaptic membrane. *Brain Res Rev* 26 (2-3):243-257
5. Tsunoda S, Zuker CS (1999) The organization of INAD-signaling complexes by a multivalent PDZ domain protein in *Drosophila* photoreceptor cells ensures sensitivity and speed of signaling. *Cell Calcium* 26:165-171
6. Oliva C, Escobedo P, Astorga C, Molina C, Sierralta J (2011) Role of the MAGUK protein family in synapse formation and function. *Dev Neurobiol* Jul 7. doi: 10.1002/dneu.20949.
7. Gardoni F, Marcello E, Di Luca M (2009) Postsynaptic density-membrane associated guanylate kinase proteins (PSD-MAGUKs) and their role in CNS disorders. *Neuroscience* 158:324-333
8. Ho GP, Selvakumar B, Mukai J, Hester LD, Wang Y, Gogos JA, Snyder SH (2011) S-nitrosylation and S-palmitoylation reciprocally regulate synaptic targeting of PSD-95. *Neuron* 71:131-141
9. Sturgill JF, Steiner P, Czervionke BL, Sabatini BL (2009) Distinct domains within PSD-95 mediate synaptic incorporation, stabilization, and activity-dependent trafficking. *J Neurosci* 29:12845-12854
10. Südhof TC (2008) Neuroligins and neurexins link synaptic function to cognitive disease. *Nature* 455:903-911
11. Siddiqui TJ, Craig AM (2011) Synaptic organizing complexes. *Curr Opin Neurobiol* 21:132-143

12. Lin YC, Koleske AJ (2010) Mechanisms of synapse and dendrite maintenance and their disruption in psychiatric and neurodegenerative disorders. *Annu Rev Neurosci* 33:349-378
13. Fritschy JM, Harvey RJ, Schwarz G (2008) Gephyrin, where do we stand, where do we go? *Trends Neurosci* 31:257-264
14. Papadopoulos T, Soykan T (2011) The role of collybistin in gephyrin clustering at inhibitory synapses: facts and open questions. *Front Cell Neurosci* 5:11
15. Sassoè-Pognetto M, Frola E, Pregno G, Briatore F, Patrizi A (2011) Understanding the molecular diversity of GABAergic synapses. *Front Cell Neurosci* 5:4
16. Tretter V, Kerschner B, Milenkovic I, Ramsden SL, Ramerstorfer J, Saiepour L, Maric HM, Moss SJ, Schindelin H, Harvey RJ, Sieghart W, Harvey K (2011) Molecular basis of the GABA_A receptor α 3 subunit interaction with gephyrin. *J Biol Chem* Sep 1. [Epub ahead of print]
17. Tretter V, Jacob TC, Mukherjee J, Fritschy JM, Pangalos MN, Moss SJ (2008) The clustering of GABA_A receptor subtypes at inhibitory synapses is facilitated via the direct binding of receptor α 2 subunits to gephyrin. *J Neurosci* 28:1356-1365
18. Mukherjee J, Kretschmannova K, Gouzer G, Maric HM, Ramsden SL, Tretter V, Harvey K, Davies PA, Triller A, Schindelin H, Moss SJ (2011) The residence time of GABA_ARs at inhibitory synapses is determined by direct binding of the receptor α 1 subunit to gephyrin. *J Neurosci* 31:14677-14687
19. Schwarz G, Mendel RR, Ribbe MW (2009) Molybdenum cofactors, enzymes and pathways. *Nature* 460:839-847
20. Smolinsky B, Eichler SA, Buchmeier S, Meier JC, Schwarz G (2008) Splice-specific functions of gephyrin in molybdenum cofactor biosynthesis. *J Biol Chem* 283:17370-17379
21. Sola M, Bavro VN, Timmins J, Franz T, Ricard-Blum S, Schoehn G, Ruigrok RW, Paarmann I, Saiyed T, O'Sullivan GA, Schmitt B, Betz H, Weissenhorn W (2004) Structural basis of dynamic glycine receptor clustering by gephyrin. *EMBO J* 23:2510-2519
22. Saiyed T, Paarmann I, Schmitt B, Haeger S, Sola M, Schmalzing G, Weissenhorn W, Betz H (2007) Molecular basis of gephyrin clustering at inhibitory synapses: role of G- and E-domain interactions. *J Biol Chem* 282:5625-5632

23. Calamai M, Specht CG, Heller J, Alcor D, Machado P, Vannier C, Triller A (2009) Gephyrin oligomerization controls GlyR mobility and synaptic clustering. *J Neurosci* 29:7639-7648
24. Bedet C, Bruusgaard JC, Vergo S, Groth-Pedersen L, Eimer S, Triller A, Vannier C (2006) Regulation of gephyrin assembly and glycine receptor synaptic stability. *J Biol Chem* 281:30046-30056
25. Dobie FA, Craig AM (2011) Inhibitory synapse dynamics: coordinated presynaptic and postsynaptic mobility and the major contribution of recycled vesicles to new synapse formation. *J Neurosci* 31:10481-10493
26. Meier J, Grantyn R (2004) A gephyrin-related mechanism restraining glycine receptor anchoring at GABAergic synapses. *J Neurosci* 24 (6):1398-1405
27. Langosch D, Hoch W, Betz H (1992) The 93 kDa protein gephyrin and tubulin associated with the inhibitory glycine receptor are phosphorylated by an endogenous protein kinase. *FEBS Lett* 298:113-117
28. Zita MM, Marchionni I, Bottos E, Righi M, Del Sal G, Cherubini E, Zacchi P (2007) Post-phosphorylation prolyl isomerisation of gephyrin represents a mechanism to modulate glycine receptors function. *EMBO J* 26:1761-1771
29. Tyagarajan SK, Ghosh H, Yevenes GE, Nikonenko I, Ebeling C, Schwerdel C, Sidler C, Zeilhofer HU, Gerrits B, Muller D, Fritschy JM (2011) Regulation of GABAergic synapse formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. *Proc Natl Acad Sci USA* 108:379-384
30. Varoqueaux F, Jamain S, Brose N (2004) Neuroligin 2 is exclusively localized to inhibitory synapses. *Eur J Cell Biol* 83:449-456
31. Song JY, Ichtchenko K, Sudhof TC, Brose N (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc Natl Acad Sci (USA)* 96 (3):1100-1105
32. Hoon M, Bauer G, Fritschy JM, Moser T, Falkenburger BH, Varoqueaux F (2009) Neuroligin 2 controls the maturation of GABAergic synapses and information processing in the retina. *J Neurosci* 29:8039-8050

33. Pouloupoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, Jedlicka P, Schwarzacher SW, Betz H, Harvey RJ, Brose N, Zhang W, Varoqueaux F (2009) Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. *Neuron* 63:628-642
34. Budreck EC, Scheiffele P (2007) Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses. *Eur J Neurosci*:1738-1748
35. Hoon M, Soykan T, Falkenburger BH, Hammer M, Patrizi A, Schmidt KF, Sassoè-Pognetto M, Löwel S, Moser T, Taschenberger H, Brose N, Varoqueaux F (2011) Neuroligin-4 is localized to glycinergic postsynapses and regulates inhibition in the retina. *Proc Natl Acad Sci USA* 108:3053-3058
36. Lise MF, El-Husseini A (2006) The neuroligin and neurexin families: from structure to function at the synapse. *Cell Mol Life Sci* 63:1833-1849
37. Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Südhof TC, Brose N (2006) Neuroligins determine synapse maturation and function. *Neuron* 51:741-754
38. Harvey K, Duguid IC, Alldred MJ, Beatty SE, Ward H, Keep NH, Lingenfelter SE, Pearce BR, Lundgren J, Owen MJ, Smart TG, Luscher B, Rees MI, Harvey RJ (2004) The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. *J Neurosci* 24:5816-5826
39. Patrizi A, Viltono L, Frola E, Harvey K, Harvey RJ, Sassoè-Pognetto M (2011) Selective localization of collybistin at a subset of inhibitory synapses in brain circuits. *J Comp Neurol* 520:130-141
40. Kalscheuer VM, Musante L, Fang C, Hoffmann K, Fuchs C, Carta E, Deas E, Venkateswarlu K, Menzel C, Ullmann R, Tommerup N, Dalpra L, Tzschach A, Selicorni A, Luscher B, Ropers HH, Harvey K, Harvey RJ (2009) A balanced chromosomal translocation disrupting ARHGAP9 is associated with epilepsy, anxiety, aggression, and mental retardation. *Hum Mutat* 30:61-68
41. Shimojima K, Sugawara M, Shichiji M, Mukaida S, Takayama R, Imai K, Yamamoto T (2011) Loss-of-function mutation of collybistin is responsible for X-linked mental retardation associated with epilepsy. *J Hum Genet* 56:561-565

42. Kins S, Betz H, Kirsch J (2000) Collybistin, a newly identified brain-specific GEF, induces submembrane clustering of gephyrin. *Nature Neurosci* 3:22-29
43. Xiang S, Young Kim E, Connelly JJ, Nassar N, Kirsch J, Winking J, Schwarz G, Schindelin H (2006) The crystal structure of Cdc42 in complex with collybistin II, a gephyrin-interacting guanine nucleotide exchange factor. *J Mol Biol* 359:35-46
44. Tyagarajan SK, Ghosh H, Harvey K, Fritschy JM (2011) Collybistin splice variants differentially interact with gephyrin and Cdc42 to regulate gephyrin clustering at GABAergic synapses. *J Cell Sci* 124:2786-2796
45. Reddy-Alla S, B. S, Birkenfeld J, Eulenburg V, Dutertre S, Böhringer C, Götz M, Betz H, Papadopoulos T (2010) PH-Domain-driven targeting of collybistin but not Cdc42 activation is required for synaptic gephyrin clustering. *Eur J Neurosci* 31:1173-1184
46. Saiepour L, Fuchs C, Patrizi A, Sassoè-Pognetto M, Harvey RJ, Harvey K (2010) Complex role of collybistin and gephyrin in GABA_A receptor clustering. *J Biol Chem* 285:29623-29631
47. Fritschy JM, Brünig I (2003) Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol Ther* 98:299-323
48. Chiou TT, Bonhomme B, Jin H, Miralles CP, Xiao H, Fu Z, Harvey RJ, Harvey K, Vicini S, De Blas AL (2011) Differential regulation of the postsynaptic clustering of γ -aminobutyric acid type A (GABA_A) receptors by collybistin isoforms. *J Biol Chem* 286:22456-22468
49. Maric HM, Mukherjee J, Tretter V, Moss SJ, Schindelin H (2011) Gephyrin-mediated GABA_A and glycine receptor clustering relies on a common binding site. *The Journal of Biological Chemistry* Oct 17. [Epub ahead of print]
50. Kirsch J, Betz H (1998) Glycine-receptor activation is required for receptor clustering in spinal neurons. *Nature* 392:717-720
51. Hanus C, Vannier C, Triller A (2004) Intracellular association of glycine receptor with gephyrin increases its plasma membrane accumulation rate. *J Neurosci* 24 (5):1119-1128
52. Papadopoulos T, Korte M, Eulenburg V, Kubota H, Retiounskaia M, Harvey RJ, Harvey K, O'sullivan GA, Laube B, Hulsmann S, Geiger JR, Betz H (2007) Impaired GABAergic

transmission and altered hippocampal synaptic plasticity in collybistin-deficient mice. *EMBO J* 26 ():3888-3899

53. Gomeza J, Hülsmann S, Ohno K, Eulenburg V, Szöke K, Richter DW, Betz H (2003) Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. *Neuron* 40:785-796
54. Kralic JE, Sidler C, Parpan F, Homanics G, Morrow AL, Fritschy JM (2006) Compensatory alteration of inhibitory synaptic circuits in thalamus and cerebellum of GABA_A receptor α 1 subunit knockout mice. *J Comp Neurol* 495:408-421
55. Schneider Gasser EM, Duveau V, Prenosil GA, Fritschy JM (2007) Reorganization of GABAergic circuits maintains GABA_A receptor-mediated transmission onto CA1 interneurons in α 1-subunit-null mice. *Eur J Neurosci* 25:3287-3304
56. Bannai H, Lévi S, Schweizer C, Inoue T, Launey T, Racine V, Sibarita JB, Mikoshiba K, Triller A (2009) Activity-dependent tuning of inhibitory neurotransmission based on GABA_AR diffusion dynamics. *Neuron* 62:670-682
57. Specht CG, Grünewald N, Pascual O, Rostgaard N, Schwarz G, Triller A (2011) Regulation of glycine receptor diffusion properties and gephyrin interactions by protein kinase C. *EMBO J* Aug 9. [Epub ahead of print]
58. Lévi S, Schweizer C, Bannai H, Pascual O, Charrier C, Triller A (2008) Homeostatic regulation of synaptic GlyR numbers driven by lateral diffusion. *Neuron* 59:261-273
59. Machado P, Rostaing P, Guigonis JM, Renner M, Dumoulin A, Samson M, Vannier C, Triller A (2011) Heat shock cognate protein 70 regulates gephyrin clustering. *J Neurosci* 31:3-14
60. Gunther U, Benson J, Benke D, Fritschy JM, Reyes GH, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y, Bluethmann H, Mohler H, Luscher B (1995) Benzodiazepine-insensitive mice generated by targeted disruption of the γ 2-subunit gene of γ -aminobutyric acid type A receptors. *Proc Natl Acad Sci USA* 92:7749-7753
61. Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B (1998) Postsynaptic clustering of major GABA_A receptor subtypes requires the γ 2 subunit and gephyrin. *Nature Neurosci* 1:563-571

62. Schweizer C, Balsiger S, Bluethmann H, Mansuy IM, Fritschy JM, Mohler H, Luscher B (2003) The $\gamma 2$ subunit of GABA_A receptors is required for maintenance of receptors at mature synapses. *Mol Cell Neurosci* 24:442-450
63. Kneussel M, Brandstatter JH, Laube B, Stahl S, Muller U, Betz H (1999) Loss of postsynaptic GABA_A receptor clustering in gephyrin-deficient mice. *J Neurosci* 19:9289-9297
64. Levi S, Logan SM, Tovar KR, Craig AM (2004) Gephyrin is critical for glycine receptor clustering but not for the formation of functional GABAergic synapses in hippocampal neurons. *J Neurosci* 24 (1):207-217
65. Kneussel M, Brandstätter JH, Gasnier B, Feng G, Sanes JR, Betz H (2001) Gephyrin-independent clustering of postsynaptic GABA_A receptor subtypes. *Mol Cell Neurosci* 17:973-982
66. Studer R, von Boehmer L, Haenggi T, Schweizer C, Benke D, Rudolph U, Fritschy JM (2006) Alteration of GABAergic synapses and gephyrin clusters in the thalamic reticular nucleus of GABA_A receptor $\alpha 3$ subunit-null mice. *Eur J Neurosci* 24:1307-1315
67. Fritschy JM, Panzanelli P (2006) Molecular and synaptic organization of GABA_A receptors in the cerebellum: Effects of targeted subunit gene deletions. *Cerebellum* 5:275-285
68. Panzanelli P, Gunn BG, Schlatter MC, Benke D, Tyagarajan SK, Scheiffele P, Belelli D, Lambert JJ, Rudolph U, Fritschy JM (2011) Distinct mechanisms regulate GABA_A receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. *J Physiol* 589:4959-4980
69. Peden DR, Petitjean CM, Herd MB, Durakoglugil M, Rosahl TW, Wafford K, Homanics GE, Belelli D, Fritschy JM, Lambert JJ (2008) Developmental maturation of synaptic and extrasynaptic GABA_A receptors in mouse thalamic ventrobasal neurones. *J Physiol* 586:965-987
70. Fritschy JM, Panzanelli P, Kralic JE, Vogt KE, Sassoè-Pognetto M (2006) Differential dependence of axo-dendritic and axo-somatic GABAergic synapses on GABA_A receptors containing the $\alpha 1$ subunit in Purkinje cells. *J Neurosci* 26:3245-3255
71. Papadopoulos T, Eulenburg V, Reddy-Alla S, Mansuy IM, Li Y, Betz H (2008) Collybistin is required for both the formation and maintenance of GABAergic postsynapses in the hippocampus. *Mol Cell Neurosci* 39:161-169

72. Gibson JR, Huber KM, Südhof TC (2009) Neuroligin-2 deletion selectively decreases inhibitory synaptic transmission originating from fast-spiking but not from somatostatin-positive interneurons. *J Neurosci* 29:13883-13897
73. Jedlicka P, Hoon M, Papadopoulos T, Vlachos A, Winkels R, Pouloupoulos A, Betz H, Deller T, Brose N, Varoqueaux F, Schwarzacher SW (2011) Increased dentate gyrus excitability in neuroligin-2-deficient mice in vivo. *Cereb Cortex* 21:357-367
74. Prenosil GA, Schneider Gasser EM, Rudolph U, Keist R, Fritschy JM, Vogt KE (2006) Specific subtypes of GABA_A receptors mediate phasic and tonic forms of inhibition in hippocampal pyramidal neurons. *J Neurophysiol* 96:846-857
75. Kasugai Y, Swinny JD, Roberts JD, Dalezios Y, Fukazawa Y, Sieghart W, Shigemoto R, Somogyi P (2010) Quantitative localisation of synaptic and extrasynaptic GABA_A receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. *Eur J Neurosci* 32 (11):1868-1888
76. Haenggi T, Fritschy JM (2006) Role of dystrophin and utrophin for assembly and function of the dystrophin glycoprotein complex in non-muscle tissue. *Cell Mol Life Sci* 63:1614-1631
77. Perronnet C, Vaillend C (2010) Dystrophins, utrophins, and associated scaffolding complexes: role in mammalian brain and implications for therapeutic strategies. *J Biomed Biotechnol* ID 849426
78. Barresi R, Campbell KP (2006) Dystroglycan: from biosynthesis to pathogenesis of human disease. *J Cell Sci* 119:199-207
79. Waite A, Tinsley CL, Locke M, Blake DJ (2009) The neurobiology of the dystrophin-associated glycoprotein complex. *Ann Med* 41:344-359
80. Lidov HGW (1996) Dystrophin in the nervous system. *Brain Pathol* 6:63-77
81. Knuesel I, Mastrocola M, Zuellig RA, Bornhauser B, Schaub MC, Fritschy JM (1999) Altered synaptic clustering of GABA_A-receptors in mice lacking dystrophin (*mdx* mice). *Eur J Neurosci* 11:4457-4462

82. Knuesel I, Zuellig RA, Schaub MC, Fritschy JM (2001) Alterations in dystrophin and utrophin expression parallel the reorganization of GABAergic synapses in a mouse model of temporal lobe epilepsy. *Eur J Neurosci* 13:1113-1124
83. Dallérac G, Perronnet C, Chagneau C, Leblanc-Veyrac P, Samson-Desvignes N, Peltekian E, Danos O, Garcia L, Laroche S, Billard J, Vaillend C (2011) Rescue of a dystrophin-like protein by exon skipping normalizes synaptic plasticity in the hippocampus of the mdx mouse. *Neurobiol Dis* 43:635-641
84. Vaillend C, Perronnet C, Ros C, Gruszczynski C, Goyenvalle A, Laroche S, Danos O, Garcia L, Peltekian E (2010) Rescue of a dystrophin-like protein by exon skipping in vivo restores GABA_A-receptor clustering in the hippocampus of the mdx mouse. *Mol Ther* 18:1683-1688
85. Pilgram GS, Potikanond S, Baines RA, Fradkin LG, Noordermeer JN (2010) The roles of the dystrophin-associated glycoprotein complex at the synapse. *Mol Neurobiol* 41:1-21
86. Bragg AD, Das SS, Froehner SC (2010) Dystrophin-associated protein scaffolding in brain requires α -dystrobrevin. *Neuroreport* 21:695-699
87. Adams ME, Mueller HA, Froehner SC (2001) In vivo requirement of the α -syntrophin PDZ domain for the sarcolemmal localization of nNOS and aquaporin-4. *J Cell Biol* 155 (1):113-122
88. Haenggi T, Soontornmalai A, Schaub MC, Fritschy JM (2004) The role of utrophin and Dp71 for assembly of different dystrophin-associated protein complexes (DPCs) in the choroid plexus and microvasculature of the brain. *Neuroscience* 129:403-413
89. Grady RM, Wozniak DF, Ohlemiller KK, Sanes JR (2006) Cerebellar synaptic defects and abnormal motor behavior in mice lacking α - and β -dystrobrevin. *J Neurosci* 26:2841-2851
90. Knuesel I, Züllig RA, Bornhauser B, Schaub MC, Fritschy JM (2000) Differential expression of utrophin and dystrophin in CNS neurons: an in situ hybridization and immunohistochemical study. *J Comp Neurol* 422:594-611
91. Yamakawa H, Oyama S, Mitsuhashi H, Sasagawa N, Uchino S, Kohsaka S, Ishiura S (2007) Neuroigins 3 and 4X interact with syntrophin-gamma2, and the interactions are affected by autism-related mutations. *Biochem Biophys Res Com* 355:41-46

92. Michele DE, Barresi V, Kanagawa M, Saito F, Cohn RD, Satz JS, Dollar J, Nishino I, Kelley RI, Somer H, Straub V, Mathews KD, Moore SA, Campbell KP (2002) Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* 418:417-422
93. Satz JS, Ostendorf AP, Hou S, Turner A, Kusano H, Lee JC, Turk R, Nguyen H, Ross-Barta SE, Westra S, Hoshi T, Moore SA, Campbell KP (2010) Distinct functions of glial and neuronal dystroglycan in the developing and adult mouse brain. *J Neurosci* 30:14560-14572
94. Sugita S, Saito F, Tang J, Satz JS, Campbell KP, Südhof TC (2001) A stoichiometric complex of neurexins and dystroglycan in brain. *J Cell Biol* 154:435-445
95. Sumita K, Sato Y, Iida J, Kawata A, Hamano M, Hirabayashi S, Ohno K, Peles E, Hata Y (2007) Synaptic scaffolding molecule (S-SCAM) membrane-associated guanylate kinase with inverted organization (MAGI)-2 is associated with cell adhesion molecules at inhibitory synapses in rat hippocampal neurons. *J Neurochem* 100:154-166
96. Fukaya M, Kamata A, Hara Y, Tamaki H, Katsumata O, Ito N, Takeda S, Hata Y, Suzuki T, Watanabe M, Harvey RJ, Sakagami H (2011) SynArfGEF is a guanine nucleotide exchange factor for Arf6 and localizes preferentially at post-synaptic specializations of inhibitory synapses. *J Neurochem* 116:1122-1137
97. Levi S, Grady M, Henry M, Campbell K, Sanes J, Craig A (2002) Dystroglycan is selectively associated with inhibitory GABAergic synapses but is dispensable for their differentiation. *J Neurosci* 22 (11):4274-4285
98. Patrizi A, Scelfo B, Viltono L, Briatore F, Fukaya M, Watanabe M, Strata P, Varoqueaux F, Brose N, Fritschy JM, Sassoè-Pognetto M (2008) Synapse formation and clustering of neuroligin-2 in the absence of GABA_A receptors. *Proc Natl Acad Sci USA* 105:13151-13156
99. Briatore F, Patrizi A, Viltono L, Sassoè-Pognetto M, Wulff P (2010) Quantitative organization of GABAergic synapses in the molecular layer of the mouse cerebellar cortex. *PLoS ONE* 5 (8):e12119
100. Brünig I, Suter A, Knuesel I, Luscher B, Fritschy JM (2002) GABAergic presynaptic terminals are required for postsynaptic clustering of dystrophin, but not of GABA_A receptors and gephyrin. *J Neurosci* 22:4805-4813

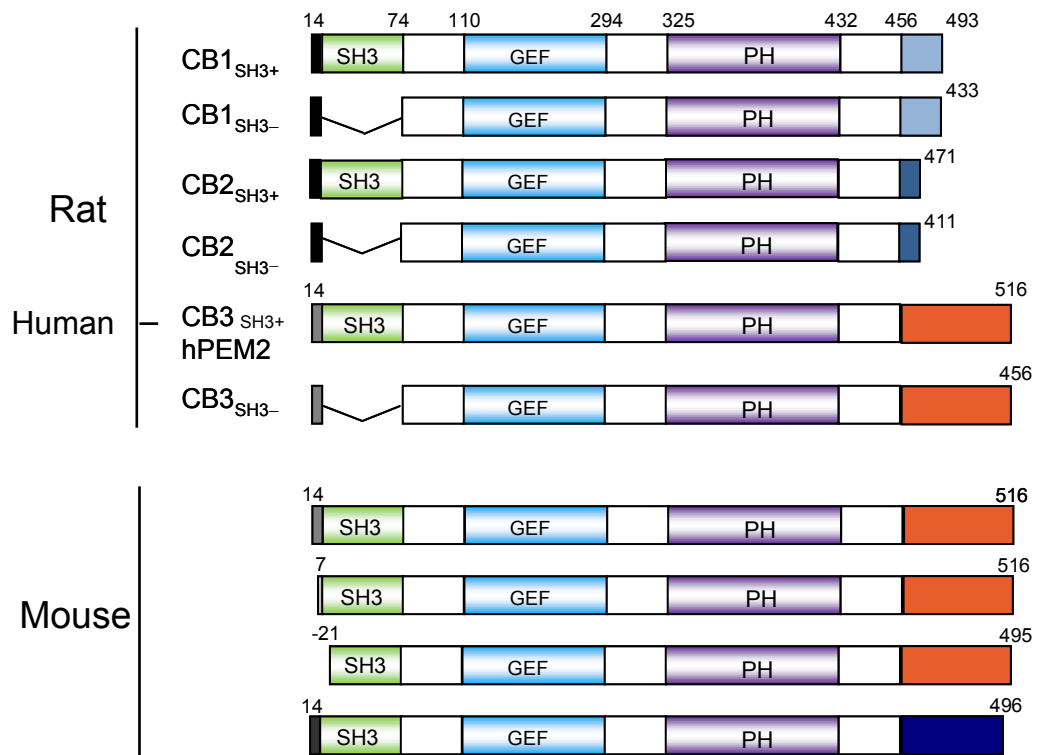


Figure 1

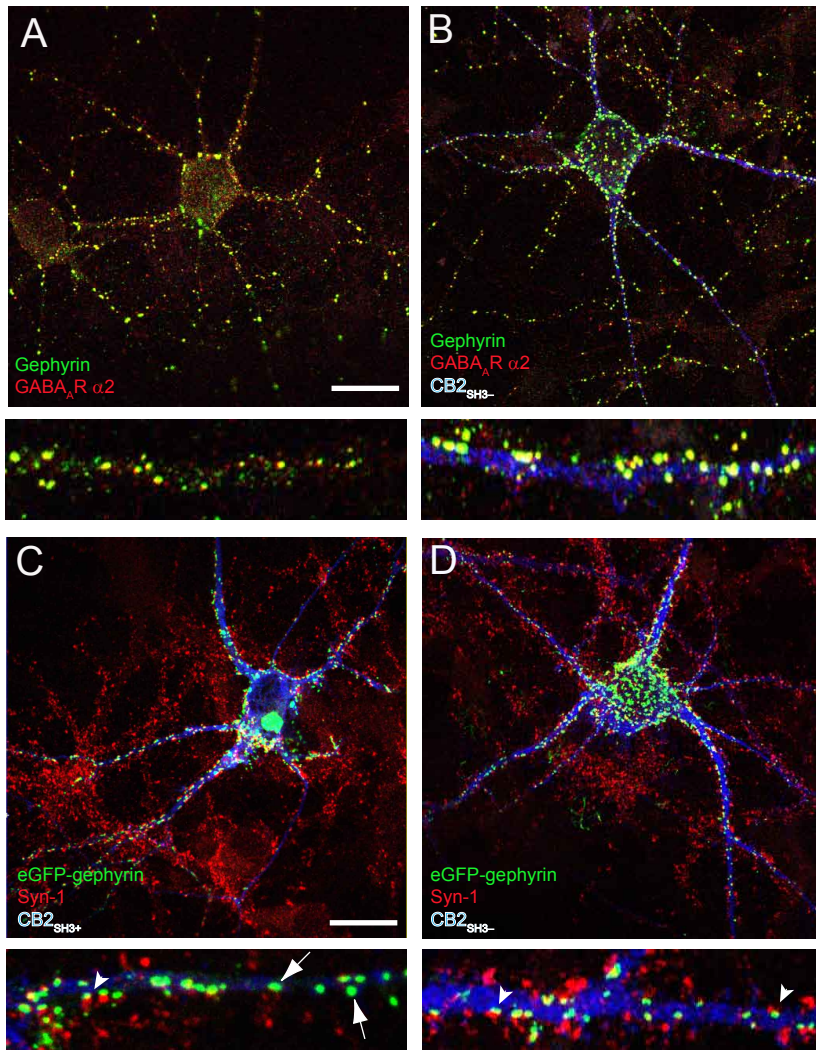


Figure 2

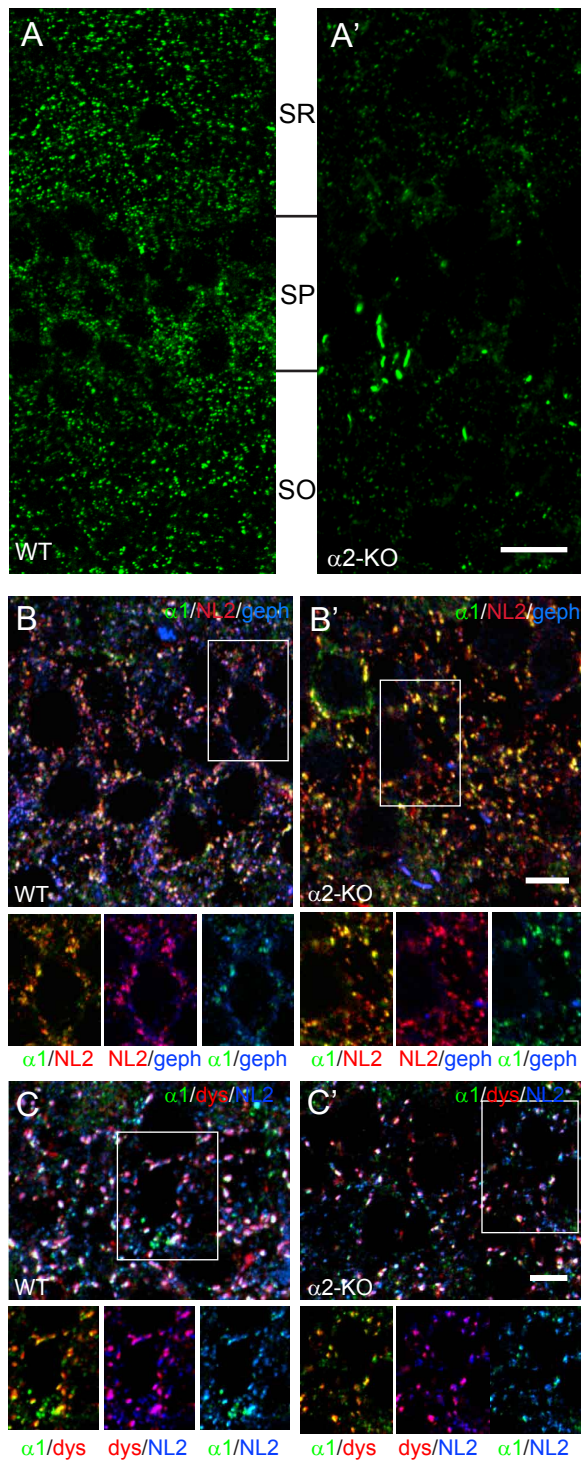
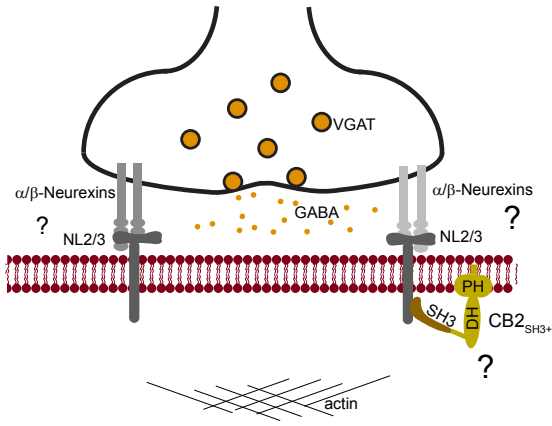
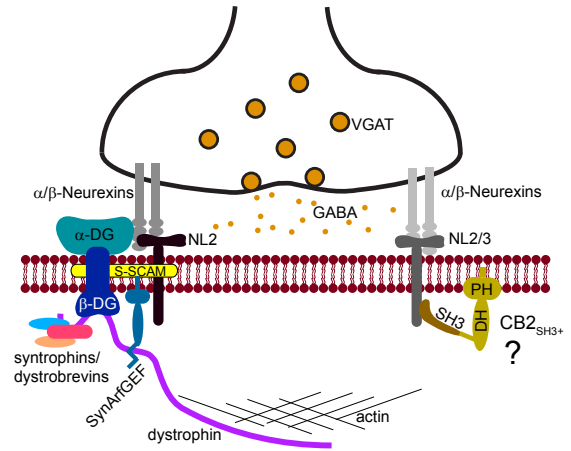


Figure 3

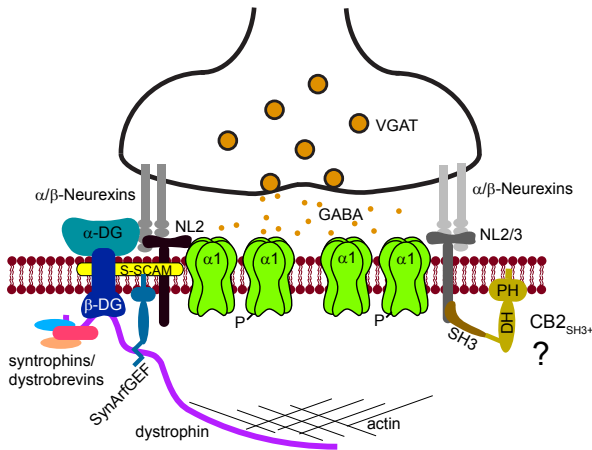
A $\alpha 1$ -KO, thalamic relay cell



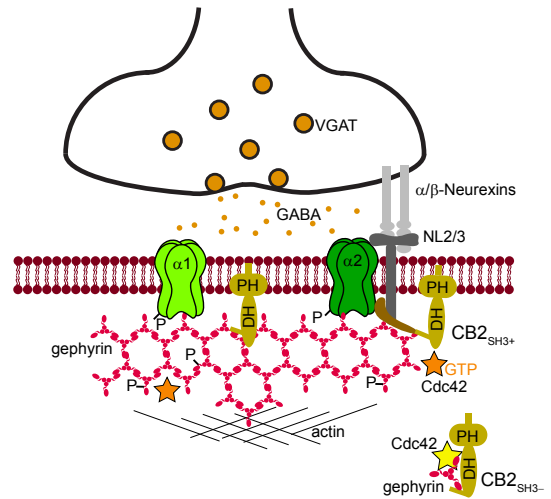
A' $\alpha 1$ -KO, Purkinje cell



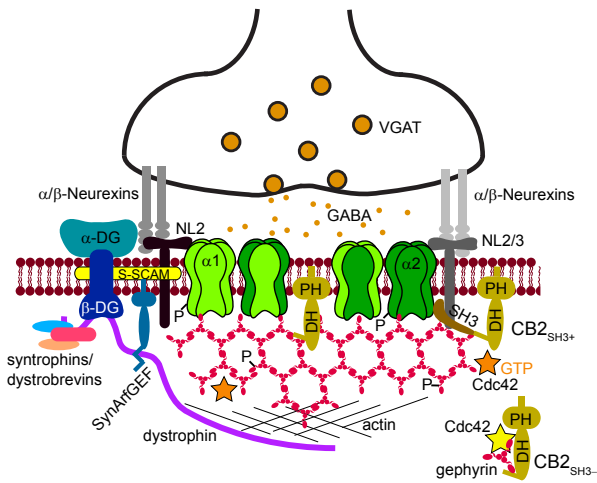
B $\alpha 2$ -KO, CA1 perisomatic synapse



C *mdx*, CA1 perisomatic synapse



D WT, CA1 perisomatic synapse



D' WT, CA1 dendritic synapse

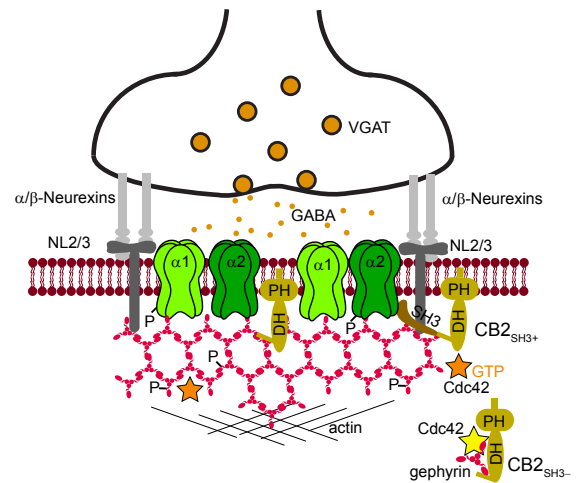


Figure 4