GABAA receptors, gephyrin and homeostatic synaptic plasticity

Tyagarajan, S K; Fritschy, J M

GABA$_A$ receptors, gephyrin & homeostatic synaptic plasticity

Shiva K. Tyagarajan and Jean-Marc Fritschy*

Institute of Pharmacology and Toxicology, University of Zurich, CH – 8057 Zurich, Switzerland

*Corresponding author (e-mail: Fritschy@pharma.uzh.ch; Tel. +41 44 635 5926; Fax. +41 44 635 6874)

Abstract

Homeostatic synaptic plasticity describes the changes in synapse gain and function that occur in response to global changes in neuronal activity to maintain the stability of neuronal networks. In this review, we argue that a coordinated regulation of excitatory and inhibitory synaptic transmission is essential for maintaining CNS function while allowing both global and local changes in synaptic strength and connectivity. Therefore, we postulate that homeostatic synaptic plasticity depends on signaling cascades regulating in parallel the efficacy of glutamatergic and GABAergic transmission. Since neurotransmitter receptors interact closely with scaffolding proteins in the postsynaptic density, this coordinated regulation of excitatory and inhibitory synaptic transmission likely involves posttranslational modifications of scaffolding proteins, which in turn modulate local synaptic function. Here we review the current state of knowledge on the regulation of $GABA_A$ receptors and their main scaffolding protein gephyrin by posttranslational modifications; we outline future lines of research that might contribute to further our understanding of the molecular mechanisms regulating GABAergic synapse function and homeostatic plasticity.

Abbreviations

CaMKII, Ca^{++}/calmodulin-dependent protein kinase II; $GABA_A R$, $GABA_A$ receptor; GlyR, glycine receptor; PSD, postsynaptic density

Introduction

Maintaining a balance between excitatory and inhibitory synaptic transmission is essential for long-term stability and function of neuronal networks. Global changes in network activity are counteracted by homeostatic compensation changing synaptic gain and adapting intrinsic neuronal properties. Likewise, local changes in the strength or number of excitatory synapses induce adaptations to inhibitory synapses with the aim of maintaining a level of network activity within preset limits (Turrigiano, 2007). This homeostatic plasticity is essential to ensure overall stability of neuronal networks while allowing local changes to synaptic strength and connections. Thus, even under pathological conditions, such as epileptic seizures, the strength of inhibitory transmission is enhanced in an effort to confine the overly-increased excitatory drive (Nusser *et al.*, 1998). Perturbations of synaptic homeostasis will cause profound alterations of brain function, and unsurprisingly, this has been linked with several pathological conditions, including epilepsy, anxiety, and developmental neuropsychiatric disorders (Dani *et al.*, 2005; Fritschy, 2008; Südhof, 2008). Of equal significance, the delayed therapeutic onset of psychoactive drugs, typically observed in patients treated for mood disorders, anxiety, or schizophrenia, might well reflect homeostatic adaptations of neuronal circuits to compensate for the chronic effects that these drugs have on synaptic transmission.

Homeostatic synaptic plasticity likely involves iterative synapse formation and removal, as well as dynamic regulation of synaptic strength, either presynaptically (e.g., regulation of transmitter release) and/or postsynaptically (e.g., posttranslational regulation of receptor function and trafficking; adaptation of downstream signal transduction; changes in synaptic structure) to counteract any global change in network activity (Kilman *et al.*, 2002; Nelson & Turrigiano, 2008; Bannai *et al.*, 2009). These changes are relayed by specific signaling cascades onto postsynaptic protein scaffolds - which serve as a crucial hub for holding functionally related proteins in close proximity – to produce a coordinated adaptation of synaptic strength and function.

So far, considerable knowledge has been gained about the regulation of glutamatergic synapses, whose postsynaptic density (PSD) can be isolated biochemically and visualized morphologically on dendritic spines (Boeckers, 2006). These synapses are characterized by a core scaffolding protein, PSD-95, which forms a complex assembly of proteins interacting via specific PDZ domains (Kim & Sheng, 2004). Studies of bidirectional synaptic plasticity (long-term potentiation and long-term depression), a hallmark of learning and memory, have uncovered rapid but long-lasting changes in glutamatergic synaptic function triggered by activity-dependent processes. These changes involve the activation of protein phosphorylation networks that regulate PSD-

95 and signaling complexes to achieve the required adaptations of the glutamatergic synapses. For example, a recent study has revealed that PSD-95 phosphorylation by Ca^{++}/calmodulin-dependent protein kinase 2 (CaMKII) regulates its stability and trafficking and underlies changes in spine morphology that accompany LTP (Steiner *et al.*, 2008).

By comparison, little is known about molecular mechanisms underlying compensatory adaptations at GABAergic synapses. $GABA_A$ receptors ($GABA_A R$), which mediate the bulk of fast synaptic inhibition in the CNS, are regulated by phosphorylation mechanisms, affecting both their functional properties and their cell surface mobility and trafficking (Jacob *et al.*, 2008). Their synaptic distribution is intimately linked with that of gephyrin, the main scaffolding protein of inhibitory postsynaptic sites (Fritschy *et al.*, 2008). However, the regulation of the GABAergic PSD has received little attention so far, notably because gephyrin has long been considered as a mere cytoskeletal anchoring protein, despite being a phospho-protein (Langosch *et al.*, 1992) and a substrate for calpain (Kawasaki *et al.*, 1997). Therefore, in analogy to PSD-95, studying the effects of gephyrin phosphorylation might reveal key aspects of GABAergic synapse regulation in homeostatic plasticity.

Mechanistically, changes in excitability involving glutamatergic synapses have to be paralleled by corresponding changes at GABAergic synapses to avoid hyperexcitation (or silencing) of the network. Here, we hypothesize that synaptic homeostasis depends on signaling cascades regulating in parallel the efficacy of glutamatergic and GABAergic transmission. According to this view, these signals might converge onto postsynaptic protein scaffolds and regulate synaptic function by means of posttranslational modifications of specific target proteins. Such convergence is apparent in the concerted changes in synaptic function produced by specific protein kinases and phosphatases. An alternative (or complementary view) is that synaptic homeostasis might depend also on multifunctional proteins that regulate intrinsic neuronal properties along with excitatory and inhibitory neurotransmission. In particular, given the importance of Cl^- gradients in determining $GABA_A R$ function (Blaesse *et al.*, 2009), a potential role of chloride transporters in synaptic homeostasis appears as an attractive possibility.

The aim of this review is to discuss evidence in support of this hypothesis of a concerted regulation of glutamatergic and GABAergic synapse strength in homeostatic synaptic plasticity.

$GABA_A$ receptor structure and regulation

$GABA_A R$ belong to the family of ligand-gated ion channels and form multimeric GABA-gated channels selectively permeable to Cl^- and HCO_3^- ions. They are encoded by a large family of subunit genes (α1-6, β1-3, γ1-3, δ, ε, π, θ, ρ1-3), whose differential assembly into receptor complexes gives rise to multiple $GABA_A$ receptor subtypes

4

differing in functional and pharmacological properties (Sieghart & Ernst, 2005). This heterogeneity is a major facet of their regulation, since distinct GABA$_A$ receptor subtypes are localized at different subcellular sites (e.g., synaptic/extrasynaptic) and are integrated in specific neuronal circuits subserving distinct brain functions and pharmacological roles (Fritschy & Brünig, 2003). While the γ2 subunit is well known for its role in postsynaptic clustering of GABA$_A$R (Essrich et al., 1998; Li et al., 2005), the mechanisms that specify extrasynaptic GABA$_A$ receptor localization, notably of α4- and a subpopulation of α5- and α6-GABA$_A$R are not known. However, analysis of α1-subunit null mice suggested that the two pools of receptors are not interchangeable, as extrasynaptic α4-GABA$_A$R did not substitute for missing synaptic α1-GABA$_A$R, as shown in thalamic neurons (Peden et al., 2007). Therefore, it is unlikely that homeostatic compensations of synaptic function involve major redistribution of synaptic and extrasynaptic GABA$_A$R subtypes.

The subunit heterogeneity of GABA$_A$R also provides considerable room for subtype-specific regulation of receptor function and trafficking by posttranslational modification (Jacob et al., 2008; Arancibia-Cárcamo & Kittler, 2009). Thus, subunit-specific phosphorylation of GABA$_A$R by CaMKII, notably on the β2 or β3 subunit, produces major effects on their functional properties in a synapse-dependent manner (Houston et al., 2008). In turn, phosphorylation of the γ2 subunit regulates GABA$_A$R cell surface expression by modulating binding to AP2 and clathrin-dependent internalization (Kittler et al., 2008). The significance of the γ2 subunit for intracellular and membrane trafficking on GABA$_A$R is also underscored by its palmitoylation (Keller et al., 2004; Rathenberg et al., 2004) and its interaction with calcium-modulating cyclophilin ligand, which is determinant for the recycling of endocytosed GABA$_A$R (Yuan et al., 2008). In the context of homeostatic synaptic plasticity, the multiple mechanisms involved in the regulation of GABA$_A$ receptor expression and postsynaptic clustering provide a framework for differential activation by specific signaling pathways.

Gephyrin structure and regulation

Gephyrin, a cytoplasmic protein initially isolated as a constituent of purified glycine receptor (GlyR) preparations, is now considered as the "master" scaffolding molecule of inhibitory synapses. In neurons, gephyrin is selectively clustered to the PSD of glycinergic and GABAergic synapses, contributing to GlyR and GABA$_A$R clustering (Fritschy et al., 2008). The selective localization of gephyrin and its capacity to auto-oligomerize and form postsynaptic clusters underscores its role as a scaffolding molecule. This idea was strengthened further once the crystal structure of the N-terminal G-domain and the C-terminal E-domain of gephyrin showed that they form trimers and dimers in solution, respectively. The G- and E-domain of gephyrin are interconnected by a linker region, the C-domain, which is sensitive to proteolytic

5

degradation and whose structure remains elusive. A leading model based on these partial crystallography results suggested that gephyrin forms a hexagonal lattice by G-domain trimerization and E-domain dimerization linking GlyR (or GABA$_A$R) in the membrane with the cytoskeleton (Sola *et al.*, 2004). This model is attractive because it explains how a protein scaffold can emerge in the absence of protein-protein interaction domains equivalent to the PDZ-domains of PSD-95. However, auto-aggregation of gephyrin in a hexagonal lattice does not provide much room for dynamic regulation of the postsynaptic scaffold, a prerequisite of homeostatic plasticity. An alternative model, based on observations that native gephyrin forms trimers in solution, suggests that the gephyrin scaffold is formed by regulated aggregation of trimeric units (Fritschy *et al.*, 2008). Accordingly, in glycinergic synapses, a gephyrin trimer would be linked to a single GlyR, with each E-domain binding to the intracellular loop of a β-subunit. In this model, a key role for the C-domain, which contains most of the interacting sites with other proteins, is proposed for the regulation of gephyrin aggregation. However, while several functionally diverse proteins have been identified as gephyrin-binding partners (Fritschy *et al.*, 2008), it is unclear how they regulate gephyrin aggregation, and by extension, contribute to the plasticity of inhibitory postsynaptic sites. Therefore, it will be essential to identify and characterize the protein-protein interaction sites on the C-domain to understand how trimeric units assemble in a regular scaffold.

The model of trimeric gephyrin units as basic building blocks of gephyrin scaffolds is well supported by evidence from GlyR tracking studies that a large fraction of extrasynaptic GlyR is bound to gephyrin (Ehrensperger *et al.*, 2007) and that their cell surface dynamics is regulated by gephyrin clustering properties (Calamai *et al.*, 2009). Further evidence for this model stems from mutagenesis studies, in which a surface-exposed loop, named L2, containing residues differing between vertebrate and plant or bacterial gephyrin homologues was found to be essential for regulating gephyrin clustering (Lardi-Studler *et al.*, 2007). Mutations in this loop result either in impaired gephyrin aggregation (L2B) or formation of supernumerary postsynaptic gephyrin clusters (L2C) in cultured neurons. The latter observation is particularly intriguing, because it implies that regulation of gephyrin clustering is directly related to synapse formation. Furthermore, it identifies specific residues that play a key role in gephyrin aggregation at postsynaptic sites.

GABA$_A$ receptor – gephyrin interactions

In striking contrast to AMPA and NMDA receptors, whose interaction with PSD-95 has been extensively analyzed in the context of synaptic plasticity and homeostasis, the interaction between GABA$_A$R and gephyrin remains largely elusive. Direct binding has long been considered unlikely, in the absence of conclusive biochemical evidence. However, analysis the cell surface dynamics of GABA$_A$R revealed that gephyrin

6

negatively regulates their diffusion coefficient (Jacob *et al.*, 2005), allowing the formation of postsynaptic GABA$_A$R clusters, implicating at least an indirect interaction. Recently, a possible direct interaction between gephyrin and α2-GABA$_A$R has been reported and postulated to play a key role for targeting of these receptors to the axon-initial segment of pyramidal cells (Tretter *et al.*, 2008). Numerous studies have attempted to clarify the significance of gephyrin-GABA$_A$ receptor interaction by up or down-regulation experiments. Thus, overexpression of gephyrin has little or no effect on the size and number of gephyrin clusters and GABAergic synapses, but causes a significant reduction in glutamatergic PSD size and NMDA receptor density, suggestive of heterotypic effects on excitatory synapses (Yu & De Blas, 2008). Conversely, down-regulation of gephyrin expression by gene targeting or gene silencing leads to rapid disappearance of postsynaptic GABA$_A$ receptor clustering and loss of IPSCs (Essrich *et al.*, 1998; Yu *et al.*, 2007), without affecting the number of GABA$_A$R at the cell surface (Jacob *et al.*, 2005), suggesting lateral diffusion to extrasynaptic sites. Despite these effects, there is near consensus that the formation of postsynaptic GABA$_A$ receptor clusters is gephyrin-independent in developing neurons (Danglot *et al.*, 2003).

Counter-intuitively for a scaffolding protein, postsynaptic clustering of gephyrin is disrupted in the absence of GABA$_A$R, as shown in vitro and in vivo (Essrich *et al.*, 1998; Schweizer *et al.*, 2003; Li *et al.*, 2005; Kralic *et al.*, 2006; Studer *et al.*, 2006). This observation provides compelling arguments in favor of the view that gephyrin clustering is a regulated process, likely dependent on local synaptic activity, and might be a crucial mechanism to adjust the strength and gain of inhibitory transmission.

An avenue that has so far remained unexplored is the possibility that gephyrin phosphorylation might regulate GABA$_A$ receptor binding and their postsynaptic localization or trafficking. Preliminary evidence is only available for GlyR, notably the demonstration that proline-directed phosphorylation of gephyrin can induce a conformational change favoring GlyR binding (Zita *et al.*, 2007). Furthermore, since NMDA receptor activation reduces the surface mobility of GlyR in a Ca^{2+}-dependent fashion, the authors of this study postulated that gephyrin or GlyR phosphorylation, possibly by PKC, might contribute to this effect (Lévi *et al.*, 2008).

Regulation of postsynaptic scaffolds by protein phosphorylation

Protein kinases and phosphatases form intricate networks integrating multiple signals to regulate excitatory and inhibitory synapses. So far, most attention has been given to the respective neurotransmitter receptors (AMPA/NMDA and GABA$_A$R), and to a lesser extent to the regulation of excitatory PSD proteins (Kim & Sheng, 2004). Importantly, protein phosphorylation modulates not only protein-protein interactions within the PSD, but also the stability of target proteins through the activation of specific proteases, such

7

as calpain-1. Therefore, bidirectional effects (increase or decrease of synapse strength) can be expected in dependence of local and global neuronal activity.

Here, we postulate that intracellular signaling cascades act in concert on the postsynaptic protein complexes of glutamatergic and GABAergic synapses to maintain the balance of excitation and inhibition. Such coordinated regulation would imply extensive convergence of signal transduction cascades acting on PSD-95 and gephyrin scaffolds. Therefore, it will be of primary interest to identify and characterize putative phosphorylation sites on gephyrin to determine their significance for the regulation of postsynaptic clustering, interaction with GABA$_A$R (and GlyR), and other proteins of the GABAergic PSD. Next, the protein kinases and phosphatases involved will have to be identified. Given the relevance of Ca^{++}-dependent mechanisms for intracellular signaling, a major open question to resolve in GABAergic synapses will be the identification of the source of calcium, and its relationship to GABA$_A$ receptor function. However, evidence for cross-talk between metabotropic receptors and GABA$_A$R, involving Ca^{++}-dependent phosphorylation mechanisms is accumulating rapidly (e.g., (Chen *et al.*, 2006; Janssen *et al.*, 2009)), indicating that multiple pathways likely contribute to the regulation of GABAergic transmission in different populations of neurons. Ultimately, it will be necessary to distinguish the respective roles of GABA$_A$R versus gephyrin phosphorylation, and notably the possibility that the gephyrin scaffold serves to anchor functionally inter-related signaling proteins that mediate homeostatic synaptic plasticity.

Role of cation-chloride cotransporters

As a complementary view to the main hypothesis discussed in the previous sections, synaptic homeostasis might also depend to a large degree on the action of multifunctional proteins, such as KCC2, which exert multiple, independent effects on intrinsic neuronal properties, synapse formation and synaptic plasticity (Blaesse *et al.*, 2009). Thus, KCC2 expression levels in developing neurons regulate the formation of GABAergic and glutamatergic synapses (Chudotvorova *et al.*, 2005), the latter being independent of its cotransporter function but achieved by interactions with specific cytoskeletal proteins (Li *et al.*, 2007). Deficiency in KCC2 affects dendritic spine morphology, in line with the selectively high concentration of KCC2 proteins in the vicinity of spines in mature neurons (Gulyas *et al.*, 2001). Finally, be regulating intracellular Cl$^-$ gradients, KCC2 has an immediate impact on the strength of GABA function, which might be set dynamically by phosphorylation-dependent cell surface trafficking (Lee *et al.*, 2007). Therefore, rather than requiring parallel or concerted action of signaling cascades onto distinct postsynaptic complexes, the multifunctionality of KCC2 offers another powerful and versatile mechanism of homeostatic synaptic plasticity that might complement the regulation of scaffolding proteins in GABAergic and glutamatergic PSDs. It will be of interest to determine whether these two principle

8

pathways are interconnected, for example via an interaction between KCC2 and gephyrin (and/or PSD-95).

References

Arancibia-Cárcamo IL & Kittler JT. (2009). Regulation of GABA$_A$ receptor membrane trafficking and synaptic localization. *Pharmacol Ther* **123**, 17-31.

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$_A$R diffusion dynamics. *Neuron* **62**, 670-682.

Blaesse P, Airaksinen MS, Rivera C & Kaila K. (2009). Cation-chloride cotransporters and neuronal function. *Neuron* **61,** 820-838.

Boeckers TM. (2006). The postsynaptic density. *Cell Tissue Res* **326**, 409-422.

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.

Chen G, Kittler JT, Moss SJ & Yan Z. (2006). Dopamine D3 receptors regulate GABA$_A$ receptor function through a phospho-dependent endocytosis mechanism in nucleus accumbens. *J Neurosci* **26**, 2513-2521.

Chudotvorova I, Ivanov A, Rama S, Hübner CA, Pellegrino C, Ben Ari Y & Medina I. (2005). Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. *J Physiol* **566,** 671-679.

Danglot L, Triller A & Bessis A. (2003). Association of gephyrin with synaptic and extrasynaptic GABA$_A$ receptors varies during development in cultured hippocampal neurons. *Mol Cell Neurosci* **23**, 264-278.

Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R & Nelson SB. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc Natl Acad Sci USA* **102**, 12560-12565.

Ehrensperger MV, Hanus C, Vannier C, Triller A & Dahan M. (2007). Multiple association states between glycine receptors and gephyrin identified by SPT analysis. *Biophys J* **92**, 3706-3718.

Essrich C, Lorez M, Benson JA, Fritschy JM & Luscher B. (1998). Postsynaptic clustering of major GABA$_A$ receptor subtypes requires the γ2 subunit and gephyrin. *Nature Neurosci* **1**, 563-571.

Fritschy JM. (2008). Epilepsy, E/I balance and GABA$_A$ receptor plasticity. *Front Mol Neurosci* **1**.

Fritschy JM & Brünig I. (2003). Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol Ther* 98, 299-323.

Fritschy JM, Harvey RJ & Schwarz G. (2008). Gephyrin, where do we stand, where do we go? *Trends Neurosci* **31**, 257-264.

Gulyás AI, Sík A, Payne JA, Kaila K & Freund TF. (2001). The KCl cotransporter, KCC2, is highly expressed in the vicinity of excitatory synapses in the rat hippocampus. *Eur J Neurosci* **13,** 2205-2217.

Houston CM, Hosie AM & Smart TG. (2008). Distinct regulation of $\beta 2$ and $\beta 3$ subunit-containing cerebellar synaptic GABA$_A$ receptors by calcium/calmodulin-dependent protein kinase II. *J Neurosci* **28**, 7574-7584.

Jacob TC, Bogdanov YD, Magnus C, Saliba RS, Kittler JT, Haydon PG & Moss SJ. (2005). Gephyrin regulates the cell surface dynamics of synaptic GABA$_A$ receptor. *J Neurosci* **25**, 10469-10478.

Jacob TC, Moss SJ & Jurd R. (2008). GABA$_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nature Rev Neurosci* **9**, 331-343.

Janssen MJ, Ade KK, Fu Z & Vicini S. (2009). Dopamine modulation of GABA tonic conductance in striatal output neurons. *J Neurosci* **29**, 5116-5126.

Kawasaki BT, Hoffman KB, Yamamoto RS & Bahr BA. (1997). Variants of the receptor/channel clustering molecule gephyrin in brain: distinct distribution patterns, developmental profiles, and proteolytic cleavage by calpain. *J Neurosci Res* **49**, 381-388.

Keller CA, Yuan X, Panzanelli P, Martin ML, Alldred M, Sassoe-Pognetto M & Luscher B. (2004). The $\gamma 2$ subunit of GABA$_A$ receptors is a substrate for palmitoylation by GODZ. *J Neurosci* **24**, 5881-5891.

Kilman V, van Rossum MC & Turrigiano GG. (2002). Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA$_A$ receptors clustered at neocortical synapses. *J Neurosci* **22**, 1328-1337.

Kim E & Sheng M. (2004). PDZ domain proteins of synapses. *Nature Rev Neurosci* **5**, 771-781.

Kittler JT, Chen G, Kukhtina V, Vahedi-Faridi A, Gu Z, Tretter V, Smith KR, McAinsh K, Arancibia-Carcamo IL, Saenger W, Haucke V, Yan Z & Moss SJ. (2008). Regulation of synaptic inhibition by phospho-dependent binding of the AP2 complex to a YECL motif in the GABA$_A$ receptor $\gamma 2$ subunit. *Proc Natl Acad Sci USA* **105**, 3616-3621.

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 $\alpha 1$ subunit knockout mice. *J Comp Neurol* **495**, 408-421.

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.

Lardi-Studler B, Smolinsky B, Petitjean CM, Koenig F, Sidler C, Meier JC, Fritschy JM & Schwarz G. (2007). Vertebrate-specific sequences in the gephyrin E-domain regulate cytosolic aggregation and postsynaptic clustering. *J Cell Biol* **120**, 1371-1382.

Lee HH, Walker JA, Williams JR, Goodier RR, Payne JA & Moss SJ. (2007). Direct PKC-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter, KCC2. *J Biol Chem* **282,** 29777-29784.

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.

Li H, Khirug S, Cai C, Ludwig A, Blaesse P, Kolikova J, Afzalov R, Coleman SK, Lauri S, Airaksinen MS, Keinänen K, Khiroug L, Saarma M, Kaila K & Rivera C. (2007). KCC2 interacts with the dendritic cytoskeleton to promote spine development. *Neuron* **56,** 1019-1033.

Li RW, Yu W, Christie SB, Miralles CP, Bai J, Loturco JJ, De Blas AL (2005). Disruption of postsynaptic GABA receptor clusters leads to decreased GABAergic innervation of pyramidal neurons. *J Neurochem* **95,**756-770.

Nelson SB & Turrigiano GG. (2008). Strength through diversity. *Neuron* **60**, 477-482.

Nusser Z, Hajos N, Somogyi P & Mody I. (1998). Increased number of synaptic $GABA_A$ receptors underlies potentiation at hippocampal synapses. *Nature* **395**, 172-177.

Peden DR, Petitjean CM, Herd MB, Durakoglugil M, Rosahl TW, Wafford K, Homanics GE, Belelli D, Fritschy JM & Lambert JJ. (2007). Developmental maturation of synaptic and extrasynaptic $GABA_A$ receptors in mouse thalamic ventrobasal neurones. *J Physiol* **586**, 965-987.

Rathenberg J, Kittler JT & Moss SJ. (2004). Palmitoylation regulates the clustering and cell surface stability of $GABA_A$ receptors. *Mol Cell Neurosci* **26**, 251-257.

Schweizer C, Balsiger S, Bluethmann H, Mansuy IM, Fritschy JM, Mohler H & Luscher B. (2003). The γ2 subunit of $GABA_A$ receptors is required for maintenance of receptors at mature synapses. *Mol Cell Neurosci* **24**, 442-450.

Sieghart W & Ernst M. (2005). Heterogeneity of $GABA_A$ receptors: revived interest in the development of subtype-selective drugs. *Curr Med Chem* **5**, 217-242.

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.

Steiner P, Higley MJ, Xu W, Czervionke BL, Malenka RC & Sabatini BL. (2008). Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. *Neuron* **60**, 788-802.

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 α3 subunit-null mice. *Eur J Neurosci* **24**, 1307-1315.

Südhof TC. (2008). Neuroligins and neurexins link synaptic function to cognitive disease. *Nature* **455**, 903-911.

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 α2 subunits to gephyrin. *J Neurosci* **28**, 1356-1365.

Turrigiano G. (2007). Homeostatic signaling: the positive side of negative feedback. *Cur Opin Neurobiol* **17**, 318-324.

Yu W & De Blas AL. (2008). Gephyrin expression and clustering affects the size of glutamatergic synaptic contacts. *J Neurochem* **104**, 830-845.

Yu W, Jiang M, Miralles CP, Li R, Chen G & de Blas A. (2007). Gephyrin clustering is required for the stability of GABAergic synapses. *Mol Cell Neurosci* **36**, 484-500.

Yuan X, Yao J, Norris D, Tran DD, Bram RJ, Chen G & Luscher B. (2008). Calcium-modulating cyclophilin ligand regulates membrane trafficking of postsynaptic GABA$_A$ receptors. *Mol Cell Neurosci* **38**, 277-289.

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.

Author contributions
S.K.T. and J.M.F. have jointly written this review and have approved the final version.