



Published in final edited form as:

*Adv Pharmacol.* 2015 ; 72: 97–146. doi:10.1016/bs.apha.2014.11.008.

## Regulation of GABA<sub>A</sub>Rs by Phosphorylation

Yasuko Nakamura, Laura M. Darnieder, Tarek Z. Deeb, and Stephen J. Moss<sup>1</sup>

Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts, USA

### Abstract

$\gamma$ -Aminobutyric acid type A receptors (GABA<sub>A</sub>Rs) are the principal mediators of fast synaptic inhibition in the brain as well as the low persistent extrasynaptic inhibition, both of which are fundamental to proper brain function. Thus unsurprisingly, deficits in GABA<sub>A</sub>Rs are implicated in a number of neurological disorders and diseases. The complexity of GABA<sub>A</sub>R regulation is determined not only by the heterogeneity of these receptors but also by its posttranslational modifications, the foremost, and best characterized of which is phosphorylation. This review will explore the details of this dynamic process, our understanding of which has barely scratched the surface. GABA<sub>A</sub>Rs are regulated by a number of kinases and phosphatases, and its phosphorylation plays an important role in governing its trafficking, expression, and interaction partners. Here, we summarize the progress in understanding the role phosphorylation plays in the regulation of GABA<sub>A</sub>Rs. This includes how phosphorylation can affect the allosteric modulation of GABA<sub>A</sub>Rs, as well as signaling pathways that affect GABA<sub>A</sub>R phosphorylation. Finally, we discuss the dysregulation of GABA<sub>A</sub>R phosphorylation and its implication in disease processes.

### 1. INTRODUCTION

With kinases alone constituting about 2% of the human genome, it is perhaps unsurprising that phosphorylation plays a key role in all aspects of cellular activity and is one of the best characterized of posttranslational modifications (Manning, Whyte, Martinez, Hunter, & Sudarsanam, 2002; Ubersax & Ferrell, 2007). Phosphorylation is dynamically executed by the opposing functions of kinases and phosphatases, which lead to changes in protein conformation and function. Protein kinases contain a common catalytic domain that catalyses the transfer of  $\gamma$ -ATP to a serine, threonine, or tyrosine residue of the intended protein. In contrast, phosphatases remove phosphate groups from their substrates. Kinases are primarily divided into two main groups: (1) the *serine/threonine kinases* which include cyclic-AMP dependent protein kinase (PKA), phospholipid-dependent protein kinase C (PKC), and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CamKII) and (2) the *tyrosine kinases* which include Src family tyrosine kinases. The protein kinase family is considerably diverse, as exemplified by PKC, which consist of multiple isoforms with distinct responses to specific activation (Song & Messing, 2005; Tanaka & Nishizuka, 1994; Taylor, Buechler, & Yonemoto, 1990; Taylor, Knighton, Zheng, Ten Eyck, & Sowadski, 1992; Ubersax & Ferrell, 2007). PKC are composed of “classical” or “conventional” cPKC subgroup (isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$ ) which are activated by calcium, phosphatidylserine (PS), and

<sup>1</sup>Corresponding author: stephen.moss@tufts.edu.

diacylglycerol (DAG); the “novel” nPKC ( $\delta$ ,  $\epsilon$ ,  $\eta$ , and  $\theta$ ) which are activated by DAG and PS; and the “atypical” aPKC ( $\zeta$  and  $\lambda/\iota$ ), activated by other lipid messengers. Phorbol esters, often used to activate PKC, predominantly stimulate cPKC and nPKC (Song & Messing, 2005; Tanaka & Nishizuka, 1994; Taylor et al., 1990, 1992; Ubersax & Ferrell, 2007).

Different kinases recognize specific consensus sequences in the target polypeptide. However, as exemplified below with GABA<sub>A</sub>Rs, the presence of consensus sequences for a specific kinase does not ensure that the protein is a substrate for the kinase *in vivo*. Likewise, bona fide phosphorylation sites may not correspond to the consensus sequence (Ubersax & Ferrell, 2007). GABA<sub>A</sub>Rs in particular are accepted phosphoproteins and their phosphorylation governs numerous processes, including directly varying channel function, regulating receptor trafficking, affecting receptor-interacting proteins, and their sensitivity to pharmacological agents (Brandon, Jovanovic, & Moss, 2002; Houston, He, & Smart, 2009; Jacob, Moss, & Jurd, 2008; Luscher, Fuchs, & Kilpatrick, 2011). Thus, the interplay between kinases and phosphatases can dynamically regulate neuronal excitability and ultimately shape brain function.

## 2. THE $\gamma$ -AMINO BUTYRIC ACID TYPE A RECEPTORS

GABA<sub>A</sub>Rs are GABA-gated Cl<sup>-</sup>-channels responsible for the majority of inhibition in the mammalian brain and the major target for many clinically relevant drugs. Deficits in GABA<sub>A</sub>R function are increasingly implicated in numerous pathologies including anxiety (Lydiard, 2003; Rudolph & Mohler, 2004), cognitive deficits (D’Hulst & Kooy, 2007; DeLorey & Olsen, 1999; Thompson-Vest, Waldvogel, Rees, & Faull, 2003), depression (Luscher, Shen, & Sahir, 2011b), epilepsy (Benarroch, 2007; Fritschy, 2008), schizophrenia (Benes & Berretta, 2001; Charych, Liu, Moss, & Brandon, 2009), and substance abuse (Krystal et al., 2006). GABA<sub>A</sub>Rs are responsible for two forms of inhibition known as phasic (synaptic) and tonic (extrasynaptic) inhibition. Synaptic inhibition occurs via transient or “phasic” activation of GABA<sub>A</sub>Rs after the release of GABA from synaptic vesicles, whereas extrasynaptic inhibition necessitates low ambient levels of GABA for continual or “tonic” activation (Farrant & Nusser, 2005).

Structurally, GABA<sub>A</sub>Rs are heteropentameric channels (Fig. 1) composed from a pool of 19 possible subunits:  $\alpha$ 1–6,  $\beta$ 1–3,  $\gamma$ 1–3,  $\delta$ ,  $\epsilon$ ,  $\theta$ ,  $\pi$ , and  $\rho$ 1–3 (Olsen & Sieghart, 2008). However, general consensus holds that the vast majority of receptors are assembled from two  $\alpha$ , two  $\beta$  and one  $\gamma$  (or one  $\delta$ ) (Fig. 1). GABA<sub>A</sub>Rs composed of  $\alpha$ 1–3 and  $\gamma$ 2 are largely synaptically located, whereas  $\alpha$ 4–6 and  $\delta$  are predominantly expressed extrasynaptically (Farrant & Nusser, 2005; Luscher, Fuchs, & Kilpatrick, 2011; Nusser & Mody, 2002). In addition to the diversity of receptor subtypes, alternative splicing offers further heterogeneity. For instance, the  $\gamma$ 2 subunits occur as short ( $\gamma$ 2S) and long ( $\gamma$ 2L) splice variants due to an additional eight amino acids in  $\gamma$ 2L (Whiting, McKernan, & Iversen, 1990). Notably, differences in subunit compositions can have an impact on the physiological and pharmacological properties of these receptors (Jacob et al., 2008; Rudolph & Knoflach, 2011; Verdoorn, Draguhn, Ymer, Seeburg, & Sakmann, 1990). Moreover, specific subunits have distinct regional expression profiles which are developmentally

regulated (Hortnagl et al., 2013; Laurie, Wisden, & Seeburg, 1992). Indeed, not only is GABA<sub>A</sub>R subunit expression brain region-specific, but the expression of particular subunits is also found to be specific at a subcellular level. Finally, on top of this staggering level of complexity, there are also the added intricacies of subunit-specific phosphorylation and dephosphorylation by multiple kinases and phosphatases.

### 3. PHOSPHORYLATION SITES ON GABA<sub>A</sub>R

The major intracellular loop between TM3 and TM4 of GABA<sub>A</sub>R contains numerous consensus sites for phosphorylation by both serine/threonine and tyrosine protein kinases (Moss & Smart, 1996). Earlier work relied largely on glutathione *S*-transferase (GST) fusion proteins that encode for the large intracellular loop of specific GABA<sub>A</sub>R subunits. Several kinases were found to phosphorylate sites in this domain, the vast majority of which lie on  $\beta$  and  $\gamma$ 2 subunits (Brandon, Delmas, Hill, Smart, & Moss, 2001; McDonald & Moss, 1994; McDonald & Moss, 1997; Moss, Doherty, & Haganir, 1992; Moss, Gorrie, Amato, & Smart, 1995; Table 1b and c). For example, a conserved serine residue found in  $\beta$ 1–3 subunits can be phosphorylated by PKA, PKC, protein kinase G (PKG), and CamKII (McDonald & Moss, 1994, 1997; Moss, Doherty, et al., 1992). Both  $\gamma$ 2S and  $\gamma$ 2L can be phosphorylated on serine residue 327 (S327) by PKC, whereas  $\gamma$ 2L has an additional residue at S343 that can be phosphorylated by PKC and CamKII (McDonald & Moss, 1994, 1997; Moss, Doherty, et al., 1992).

#### 3.1. Phosphorylation in expression systems

The methods outlined above identified a number of sites that were later recapitulated in heterologous expression systems. Although studies of this manner may not account for the presence of proteins intimately associated with GABA<sub>A</sub>Rs, they allow for the direct examination of the effects of phosphorylation on specific residues with precise receptor combinations. These approaches have been carried out on the numerous phosphorylation sites first identified utilizing GST conjugated to the large intracellular loop of GABA<sub>A</sub>Rs (McDonald & Moss, 1994, 1997; Moss, Doherty, et al., 1992; Whiting et al., 1990). Human embryonic kidney (HEK293) cells lines have been extensively used as a vehicle for the expression of recombinant receptors for such studies, and generally these results have lent support to the *in vitro* studies, albeit with a few discrepancies (Table 1b and c).

**3.1.1 PKA**—As a specific example of such discrepancies, *in vitro* experiments have suggested that  $\beta$ 1–3 subunits are phosphorylated by PKA (McDonald & Moss, 1997; Moss, Doherty, et al., 1992). In contrast, studies in HEK293 cells showed that  $\beta$ 2-containing receptors were *not* phosphorylated and failed to be modulated by PKA activation (McDonald et al., 1998). However, PKA-induced phosphorylation did, in fact, differentially regulate  $\beta$ 1 and  $\beta$ 3 subunits expressed as  $\alpha$ 1 $\beta$ 1/3 $\gamma$ 2S in HEK293 cells. PKA-dependent phosphorylation of  $\beta$ 1-containing receptors at S409 led to an inhibition of GABA-activated responses (McDonald et al., 1998; Moss, Smart, et al., 1992). In contrast, similar PKA activation in  $\beta$ 3-containing receptors resulted in the potentiation of GABA-induced currents attributed to two adjacent serine residues at 408 and 409, both of which are phosphorylated by PKA. Moreover, mutation of S408 to an alanine (S408A) in the  $\beta$ 3 subunit transformed

the enhancement of activity to an inhibition analogous to  $\beta 1$  subunits that are exclusively phosphorylated on S409. The lack of  $\beta 2$  subunit regulation by PKA is most likely due to an inability of  $\beta 2$  to bind A-kinase anchoring proteins (AKAP) (see Section 4.3). These anchoring proteins are found endogenously in HEK293 cells (Gardner, Tavalin, Goehring, Scott, & Bahouth, 2006) and are crucial to subcellular targeting of PKA (Sanderson & Dell'Acqua, 2011).

Since PKA appears to exclusively phosphorylate  $\beta 1$  (S409) and  $\beta 3$  (S408/S409) subunits, it was generally expected that the regulation of GABA<sub>A</sub>R activity by PKA should depend on whether a population of receptors is largely  $\beta 1$ - or  $\beta 3$ -containing. However, contrary to experiments conducted with synaptically expressed  $\alpha 1$  receptors, extrasynaptically expressed  $\alpha 4\beta 3\gamma 2$  and  $\alpha 4\beta 3\delta$  receptors tell a different story. In HEK293 cells, PKA activation caused a larger increase of spontaneous activity in cells expressing  $\alpha 4\beta 3\delta$  receptors compared to  $\alpha 4\beta 3\gamma 2$  receptors; however in the same cells, PKA activation did not affect currents that were activated by 1  $\mu$ M GABA (Tang, Hernandez, & Macdonald, 2010). These results suggest that PKA could regulate GABA<sub>A</sub>R-mediated currents by an unknown process that depends on the GABA concentration.

**3.1.2 CamKII and Src**—Discrepancies also exist on residues that were thought to be phosphorylated by CamKII (McDonald & Moss, 1994, 1997). In these cases, it has been the choice of cell line that has appeared to underlie a particular kinase's ability to modulate receptor function (Houston et al., 2009; Houston & Smart, 2006). CamKII did not regulate HEK293 cells transfected with  $\alpha 1\beta 2$  and  $\alpha 1\beta 3$  receptors. Contrary to this, in the neuroblastoma-glioma hybrid (NG108-15) cell line, CamKII activity enhanced GABA-evoked current amplitudes of  $\alpha 1\beta 3$  and  $\alpha 1\beta 3\gamma 2$  receptor expressing cells, but not in cells expressing  $\alpha 1\beta 2$  and  $\alpha 1\beta 2\gamma 2$  receptors (Houston & Smart, 2006). Moreover, similar enhancements were observed upon CamKII activation in cerebellar granule neurons from both wild-type mice and those in which the  $\beta 2$  subunit was specifically knocked out. This result suggests that in cerebellar granule neurons, CamKII effects are mediated primarily by the  $\beta 3$  subunit.

CamKII-dependent enhancement of GABA-evoked currents in  $\alpha 1\beta 3$ -transfected NG108-15 cells (without  $\gamma 2$ ) was mediated solely by S383 on the  $\beta 3$  subunit (Houston, Lee, Hosie, Moss, & Smart, 2007). Other potential CamKII phosphorylation sites initially identified by GST pull-down experiments (McDonald & Moss, 1994, 1997) were not involved (Houston et al., 2007; Table 1b). Interestingly, this site not only deviates from the typical consensus site for CamKII, but, thus far, it is also a site exclusively phosphorylated by CamKII. Further, in  $\alpha 1\beta 3\gamma 2$  transfected NG108-15 cells, CamKII-mediated enhancement of GABA-evoked currents were not exclusively mediated by S383 on the  $\beta 3$  subunit, but were also mediated by tyrosine phosphorylation sites on residues Y365/Y367 of the  $\gamma 2$  subunit. This suggests that activation of CamKII not only leads to the direct phosphorylation of S383 on  $\beta 3$  but also activates endogenous tyrosine kinases to phosphorylate sites on  $\gamma 2$  (Houston et al., 2007). The tyrosine kinase Src can specifically interact with  $\beta$  and  $\gamma 2$  subunits (Brandon et al., 2001). Moreover, Y365/Y367 in the  $\gamma 2$  subunit can be phosphorylated by Src kinase in  $\alpha 1\beta 1\gamma 2L$ -expressing A293 cells, which in agreement, also resulted in an enhancement of GABA-activated currents (Moss et al., 1995). These observations were attributed to the

phosphorylation of  $\gamma 2$  residue at Y365/Y367 since mutations of these residues to phenylalanines (Y365/Y367F) ablated tyrosine phosphorylation and receptor modulation. Finally, while it is currently unknown why HEK293 cells expressing GABA<sub>A</sub>Rs lack modulation by CamKII, it has been postulated that the nonneuronal lineage of the cells might be the root. Alternatively, the lack of relevant anchoring proteins that would allow kinases to associate with GABA<sub>A</sub>Rs may also be a cause.

**3.1.3 PKC**—One of the first pieces of evidence that PKC modulates GABA<sub>A</sub>Rs came from experiments performed on *Xenopus* oocytes expressing chick brain mRNA. In this study, activation of PKC by phorbol esters decreased the amplitude of GABA<sub>A</sub>R-mediated currents (Sigel & Baur, 1988). The reduction in current amplitude was also observed in heterologous cells expressing  $\alpha 1\beta 1\gamma 2S/L$  and  $\alpha 1\beta 2\gamma 2S$  subunits. This effect was later discovered to be mediated by phosphorylation of S409/S410 in  $\beta 1/\beta 2$  subunits, S327 in  $\gamma 2S/\gamma 2L$  subunits, and S343 in  $\gamma 2L$  subunits (Kellenberger, Malherbe, & Sigel, 1992; Krishek et al., 1994). Moreover, the level of modulation by a particular phosphorylation site was site-specific. In particular, S343 in  $\gamma 2$  displayed the greatest effect on the GABA-induced response (Kellenberger et al., 1992; Krishek et al., 1994). In contrast, application of constitutively active PKC in L929 fibroblasts expressing  $\alpha 1\beta 1\gamma 2L$  enhanced GABA<sub>A</sub>Rs-mediated currents. These enhancements were prevented by mutations of either  $\beta 1$  S409 to alanine (S409A) or, similarly,  $\gamma 2L$  S327A and  $\gamma 2L$  S343A (Lin, Angelotti, Dudek, Browning, & Macdonald, 1996; Lin, Browning, Dudek, & Macdonald, 1994). Although the reason for the discrepancy is unclear, there may be numerous explanations: for example, the use of different experimental systems (Houston & Smart, 2006; Mercik, Pytel, & Mozrzymas, 2003), or differential activation of specific PKC isoforms due to the use of phorbol ester versus PKC isolated from bovine brain. Notably, the same phosphorylation sites abolished the observed decreases or increases in GABA<sub>A</sub>R currents, which perhaps indicates yet another PKC site may dictate the direction of the response.

Although the majority of studies generally concentrate on synaptic GABA<sub>A</sub>Rs, more recent evidence indicates that PKC activation may also affect extrasynaptic GABA<sub>A</sub>Rs. In COS7 cells, the activation of PKC by application of phorbol esters increases the phosphorylation of S443 on the  $\alpha 4$  subunit and enhances surface levels of  $\alpha 4\beta 3$  receptors (Abramian et al., 2010). Phosphomimetic mutations revealed that the rise in surface levels were due to increased stability, concurrent with enhanced levels of insertion at the cell surface. On the contrary, activation of PKC has also been found to result in decreases in surface levels of GABA<sub>A</sub>Rs in  $\alpha 4\beta 2\delta$ -expressing HEK293 cells. In parallel, a decrease in tonic GABA<sub>A</sub>R inhibition was observed which was dependent on the phosphorylation of S410 on the  $\beta 2$  subunit. Further, PKC activation did not differentially affect S443 mutants of the  $\alpha 4$  subunit when compared to wild type (Bright & Smart, 2013). The reasons for the apparent discrepancy are unclear, although they may be explained by the differential phosphorylation and/or recruitment of kinases and phosphatases to the  $\beta 2$  and  $\beta 3$  subunits as will be discussed below. This would suggest that PKC activity could bidirectionally regulate tonic inhibition depending on the  $\beta$  subunit subtype found in a given GABA<sub>A</sub>R.

**3.1.4 Lessons from expression systems**—The phosphorylation sites listed in Table 1a–c are by no means exhaustive; indeed, more recent work using mass spectrometric analysis has identified potential sites of phosphorylation on the  $\beta$  subunits of GABA<sub>A</sub>Rs (Kang, Heo, & Lubec, 2011). Moreover, the identification of the first phosphorylation site on an  $\alpha$  subunit was more recently found on residue S443 of the  $\alpha 4$  subunit (Abramian et al., 2010). Collectively, studies *in vitro* and in heterologous systems have highlighted important considerations when investigating phosphorylation. For example, the actions of kinases are not only receptor subunit specific, but also splice variants allow for additional sites of phosphorylation (McDonald & Moss, 1994; Moss, Doherty, et al., 1992; Whiting et al., 1990). Further, the determination of the existence of a phosphorylation site may depend on the experimental system utilized (Houston & Smart, 2006; Mercik et al., 2003) as well as the experimental conditions (Bright & Smart, 2013). Interestingly, there is the potential for multiple kinases to act on one site, or, alternatively, for a single kinase to be assigned to one particular site. Kinases can also phosphorylate neighboring sites to differentially regulate GABA<sub>A</sub>Rs (McDonald et al., 1998) and the phosphorylation on one site may affect phosphorylation of another site within the same subunit or on another subunit (Houston et al., 2007). Critically, these studies have made evident that subtype-specific phosphorylation of key residues can govern GABA<sub>A</sub>R activity and trafficking (Brandon, Jovanovic, et al., 2002; Kittler & Moss, 2003).

### 3.2. Divergent effects of kinases and phosphatases on neuronal GABA<sub>A</sub>Rs

Due to their diverse nature, the study of GABA<sub>A</sub>Rs in their neuronal environment has been, and still is, a challenge. Nevertheless, a large body of evidence suggests that phosphorylation can affect neuronal activity through the regulation of GABA<sub>A</sub>Rs. Similar to heterologous systems,  $\beta$  and  $\gamma 2$  subunits are the primary substrates for kinases in neurons. However, the effect of kinases on GABA<sub>A</sub>Rs in neuronal preparations is complex and often yields conflicting results. Disparate effects of phosphorylation on neurons should probably be expected due to the mixed populations of GABA<sub>A</sub>Rs composed of various subunits (Pirker, Schwarzer, Wieselthaler, Sieghart, & Sperk, 2000; Poisbeau, Cheney, Browning, & Mody, 1999). Indeed, and as exemplified with PKA, a single kinase can have divergent effects that are dependent on the  $\beta$ -subunit subtype. Moreover, kinases have a considerable number of effects on other substrates that may influence GABA<sub>A</sub>R activity, including the activation of signaling pathways and phosphorylation of associated proteins, which can make data interpretation challenging.

For example, PKA activation has been studied in a number of brain regions, and found to both increase and decrease GABA-evoked currents. Decreases in GABA<sub>A</sub>R-mediated currents were observed in cultured cerebellar granule cells, hippocampal pyramidal cells, neostriatal neurons, spinal cord neurons, and superior cervical ganglia (Flores-Hernandez et al., 2000; Moss, Smart, et al., 1992; Poisbeau et al., 1999; Porter, Twyman, Uhler, & Macdonald, 1990; Robello, Amico, & Cupello, 1993). However, increases in GABA<sub>A</sub>R activity were seen in cerebellar interneurons, hippocampal dentate granule cells and olfactory bulb granule cells (Kano & Konnerth, 1992; Nusser, Sieghart, & Mody, 1999; Poisbeau et al., 1999). Similar inconsistencies are apparent upon activation of PKC, with reports of decreased GABA<sub>A</sub>R activity in cortical neurons, hippocampal pyramidal neurons,

retinal rod bipolar cells, and thalamic neurons (Brandon et al., 2000; Bright & Smart, 2013; Chou et al., 2010; Gillette & Dacheux, 1996), increases in hippocampal dentate granule cells and hippocampal neurons (Abramian et al., 2010; Poisbeau et al., 1999), and no effect in hippocampal pyramidal cells (Poisbeau et al., 1999).

There are a plethora of reasons why such differences may be observed, from the known heterogeneity of GABA<sub>A</sub>Rs in both receptor subunit combinations and overall regional distribution to the presence of numerous isoforms of PKC that may mediate differential effects (which may or may not be directed at GABA<sub>A</sub>Rs). Indeed, studies have reported that PKA and PKC elicit differential effects even within *one* region—that of the hippocampus. In CA1 pyramidal cells, PKA activation enhanced miniature inhibitory postsynaptic current (mIPSC) amplitudes, whereas PKC activation showed no observable effects. On the other hand, in dentate gyrus granule cells it was PKA that was unresponsive, whereas PKC activation led to increases in peak mIPSC amplitudes (Poisbeau et al., 1999). Although it is tempting to speculate that these observed effects correspond to the prominence of specific GABA<sub>A</sub>R subunits, the situation is unclear (Pirker et al., 2000; Wisden, Laurie, Monyer, & Seeburg, 1992), and such conclusions are perhaps even premature considering novel phosphorylation sites are still being discovered (Abramian et al., 2010; Kang et al., 2011).

As well as the aforementioned experiments performed utilizing GST fusion proteins and heterologous expression systems, PKA and PKC have both been demonstrated to phosphorylate purified *neuronal* GABA<sub>A</sub>Rs (Brandon et al., 2000; Moss & Smart, 1996). In neurons,  $\beta 3$  and  $\gamma 2$  are basally phosphorylated at S408/S409 and Y365/Y367, respectively (Brandon et al., 2001, 2000; Jovanovic, Thomas, Kittler, Smart, & Moss, 2004). These results support earlier studies utilizing GST fusion proteins as well as those in HEK293 cells (McDonald et al., 1998; McDonald & Moss, 1997). At  $\beta 3$  subunits, activation of PKC leads to increased phosphorylation at S408/S409, while inhibition of PKC led to concomitant decreases in receptor phosphorylation. Of particular note is that the enhancement of  $\beta 3$  phosphorylation by PKA activation in cortical neurons was only observed when PKC activity was inhibited (Brandon et al., 2000).

GABA<sub>A</sub>Rs constitutively cycle between the neuronal surface and intracellular compartments. The endocytosis of receptors from the neuronal surface is a clathrin-dependent process (Kittler et al., 2000, 2001). There is a wealth of evidence in expression systems that PKC activation also leads to a parallel loss of  $\alpha 1\beta 2\gamma 2$  receptors from the surface (Chapell, Bueno, Alvarez-Hernandez, Robinson, & Leidenheimer, 1998; Connolly et al., 1999; Filippova, Sedelnikova, Zong, Fortinberry, & Weiss, 2000; Kittler et al., 2000). However, in heterologous cells, this is unlikely to be due to a direct phosphorylation of GABA<sub>A</sub>Rs since mutagenesis of all *known* PKC phosphorylation sites in recombinant receptors ( $\alpha 1$ ,  $\beta 2$  S410A,  $\gamma 2L$  S327A/S343A) did not inhibit PKC-mediated decreases in surface expression. Instead, PKC activation prevented receptors that are internalized constitutively from recycling back to the surface (Connolly et al., 1999; Filippova et al., 2000). Whether similar mechanisms are employed in PKC-mediated modifications of GABA<sub>A</sub>Rs in neurons is unclear. PKC activation resulting in decreases in GABA<sub>A</sub>R activity has reported to show variable effects in the level of surface receptors, with observed decreases in cerebellar granule cells (Balduzzi, Cupello, & Robello, 2002; Chou et al.,

2010), increases in cortical and hippocampal neurons (Jovanovic et al., 2004), or no change in cortical neurons (Brandon et al., 2000). Additionally, the constitutive cycling of neuronal receptors was observed to occur at a significantly reduced rate when compared to HEK293 cells, which suggests additional receptor regulation and anchoring at the synapse (Connolly et al., 1999). Thus, differential effects observed in neurons may be dependent on regional differences as well as the activation of specific isozymes, which then leads to the regulation of various receptor associated proteins. Indeed, PKC $\epsilon$  was observed to form a complex with *N*-ethylmaleimide sensitive factor (NSF) and  $\gamma$ 2-containing GABA $_A$ Rs. The activation of PKC $\epsilon$  was found to result in decreases in the surface levels of GABA $_A$ R, which was dependent upon PKC $\epsilon$  phosphorylation of NSF and their recruitment to inhibitory synapses (Chou et al., 2010). Notably, NSF has also been shown to bind directly to  $\beta$  subunits of GABA $_A$ Rs at residues 395–415, which contains the major phosphorylation site conserved within  $\beta$  subunits (Goto et al., 2005). Whether phosphorylation of this site affects GABA $_A$ R–NSF interactions remains to be seen. Although NSF stabilizes excitatory AMPA receptors at the surface by disrupting endocytosis (Hanley, Khatri, Hanson, & Ziff, 2002), similar characterization of NSF and GABA $_A$ Rs interactions have not been performed.

#### 4. GABA $_A$ R-INTERACTING PROTEINS AND PHOSPHORYLATION

GABA $_A$ R distribution and expression is under tight subtype-specific management, governed to some degree by its interacting partners. As with the phosphorylation of these receptors, GABA $_A$ R interactions primarily take place at the large intracellular loop, with the majority of interactions occurring through the  $\beta$  and  $\gamma$ 2 subunits. These interactions affect trafficking and surface stability as well as the phosphorylation state of specific subunits (Charych et al., 2009; Chen & Olsen, 2007; Jacob et al., 2008; Kneussel & Loebrich, 2007).

##### 4.1. Adaptor protein 2

The adaptor protein (AP2) is a heterotetrameric complex composed of  $\alpha$ ,  $\beta$ 2,  $\mu$ 2, and  $\sigma$ 2 subunits. AP2 binds membrane, cargo, and clathrin and is fundamental to clathrin-mediated endocytosis (McMahon & Boucrot, 2011). GABA $_A$ Rs  $\beta$ 1–3,  $\gamma$ 2, and  $\delta$  subunits are directly associated with the  $\mu$ 2 subunit of AP2. As will be discussed below, thus far three mechanisms for the binding of AP2 to GABA $_A$ Rs have been identified (Kittler et al., 2005, 2000; Vitlani & Moss, 2009).

Binding sites important for AP2, clathrin and dynamin-mediated internalization of GABA $_A$ Rs were first identified on the  $\beta$ 2 subunit. A dileucine motif on the  $\beta$ 2 was reported to be required for PKC-mediated endocytosis in heterologous cells (Herring, Huang, Singh, Dillon, & Leidenheimer, 2005; Herring et al., 2003).

A second atypical AP2 binding motif on GABA $_A$ Rs was later determined as a region of highly basic amino acids, which contains a major phosphorylation site for PKA and PKC in  $\beta$ 1 and  $\beta$ 3 subunits, and PKC in  $\beta$ 2 subunits (Brandon et al., 2003; Kittler et al., 2005). Phosphorylation of S408/S409 on the  $\beta$ 3 subunit resulted in decreased association with the AP2 complex. Thus, when this site is dephosphorylated, AP2 binds GABA $_A$ Rs, thereby prompting receptor endocytosis (Fig. 2). Consequently, introducing dephosphorylated  $\beta$ 3-derived peptides that compete for the AP2 interaction leads to an increase in mIPSC



amplitude and whole-cell current (Kittler et al., 2005). Additionally, mutations of S408/S409 that decrease AP2 binding to the  $\beta 3$  subunit were found to result in a concomitant decrease in endocytosis and an increase in surface-expressed receptors. Interestingly, these mutations resulted in an enhancement in size and number of inhibitory synapses was observed with parallel decreases in the number of excitatory synapses (Jacob et al., 2009).

Finally, the third AP2 interaction motif on GABA<sub>A</sub>Rs comprises dual sites in the major intracellular domain of the  $\gamma 2$  subunit. A tyrosine motif Y-x-x- $\Phi$  (where x is any amino acid and  $\Phi$  is hydrophobic) allows binding of AP2 to  $\gamma 2$ -containing receptors on Y<sup>365</sup>GY<sup>367</sup>ECL. Phosphorylation of Y365/Y367 inhibits binding to AP2, leading to an enhancement in levels of surface receptors (Fig. 2). These motifs can work separately or together to decrease receptor numbers (Kittler et al., 2008). Indeed, phosphorylation within this motif (Y365/Y367) is mediated by Src family members (Brandon et al., 2001; Moss et al., 1995) including Fyn. Moreover, the phosphorylation of Y367 within the  $\gamma 2$  subunit facilitates the direct binding of Fyn kinase (Jurd, Tretter, Walker, Brandon, & Moss, 2010). To this end, Fyn knockout (KO) mice exhibit decreased phosphorylation at this residue in addition to altered GABA<sub>A</sub>R function (Boehm, Peden, Harris, & Blednov, 2004; Jurd et al., 2010). Infusion of peptides that block AP2- $\gamma 2$  interaction in neurons resulted in an increase in mIPSC amplitude accompanied with an increase in receptors at the surface (Kittler et al., 2008). The importance of this site *in vivo* is highlighted by the embryonic lethality of knock-in mice where  $\gamma 2$  Y365/Y367 residues were mutated to phenylalanines (Y365F/Y367F). This observed phenotype is possibly due to increased levels of GABAergic excitation during early development (Ben-Ari, Khalilov, Kahle, & Cherubini, 2012). Experiments performed with viable heterozygous mice revealed increased surface levels of  $\gamma 2$ , enhanced levels of GABA<sub>A</sub>Rs at specific subdomains of the hippocampus as well as deficits in spatial object recognition (Tretter et al., 2009).

Notably,  $\delta$  subunits bind AP2 through sites containing both the classical Y-x-x- $\Phi$  motif as found in  $\gamma 2$  subunits and the atypical basic motif, which is found in  $\beta$  subunits. Since phosphorylation sites have not currently been identified within the  $\delta$  subunits, whether this association is also mediated by phosphorylation within these motifs remains to be seen (Gonzalez, Moss, & Olsen, 2012).

#### 4.2. Gephyrin

The important role of gephyrin as a postsynaptic scaffold protein underlying GABA<sub>A</sub>R synaptic clustering has been an area of intense study (Luscher, Fuchs, & Kilpatrick, 2011; Tretter et al., 2012; Tyagarajan & Fritschy, 2014). Gephyrin has been shown to bind GABA<sub>A</sub>R subunits  $\alpha 1$ - $\alpha 3$  (Mukherjee et al., 2011; Saiepour et al., 2010; Tretter et al., 2011) and more recently to  $\beta 2$  and  $\beta 3$  subunits (Kowalczyk et al., 2013). Importantly, this interaction may be regulated by the phosphorylation of specific subunits. For example, the mutation of a putative phosphorylation site, T375, in the  $\alpha 1$  subunit to a phosphomimetic, decreases its affinity to gephyrin. The decrease in gephyrin affinity subsequently leads to decreases in synaptic  $\alpha 1$ -containing clusters and reductions in the amplitude of mIPSCs (Mukherjee et al., 2011). In addition, phospho-deficient mutations of S410 in the  $\beta 2$  subunit lead to a decrease in affinity for gephyrin (Kowalczyk et al., 2013). Whether putative sites

such as T375 are altered by kinases and phosphatases are currently unknown, but it raises the intriguing possibility that phosphorylation may dynamically regulate the clustering of GABA<sub>A</sub>Rs at inhibitory synapses.

#### 4.3. A-kinase anchoring protein

The proper targeting of kinases and phosphatases such as PKA, PKC, and calcineurin (CaN, also known as PP2B) to their appropriate substrates at both excitatory and inhibitory synapses require a family of AKAP scaffolding proteins (Brandon et al., 2003; Klauck et al., 1996; Sanderson & Dell'Acqua, 2011).

The AKAP Yotiao has been shown to bind PKA and the type 1 protein phosphatases (PP1) (Westphal et al., 1999). Furthermore, application of dopamine D4 receptor agonists resulted in decreases in GABA<sub>A</sub>R-mediated currents which required an intact Yotiao–PKA–PP1 complex (Wang, Zhong, & Yan, 2002). However, whether these actions are mediated by the direct phosphorylation or dephosphorylation of GABA<sub>A</sub>Rs is unknown.

AKAP79/150 has been demonstrated to directly bind to GABA<sub>A</sub>R  $\beta$ 1 and  $\beta$ 3, but not  $\beta$ 2-subunits. PKA-mediated phosphorylation of the  $\beta$ 3 subunit was found to be AKAP dependent (Brandon et al., 2003). However, there has been disagreement as to whether this occurs at the Golgi apparatus instead of at inhibitory synapses in the hippocampus (Lilly, Alvarez, & Tietz, 2005). Nevertheless, more recently, it has been suggested that the PKA–AKAP–CaN signaling complex residing at GABAergic synapses is required for the induction of GABAergic long-term depression in dopamine (DA) neurons of the ventral tegmental area (VTA) (Dacher, Gouty, Dash, Cox, & Nugent, 2013). Unfortunately, specific phosphorylation sites on GABA<sub>A</sub>Rs have not been characterized in this study, but it is tempting to speculate that direct phosphorylation of these receptors may play a role in DA signaling required for reward-related learning.

#### 4.4. Phospholipase C-related inactive protein

The phospholipase C-related inactive protein (PRIP) family of proteins includes the ubiquitously expressed PRIP2, and PRIP1 that is principally expressed in the CNS. PRIPs were first discovered as proteins that bound to inositol 1,4,5-triphosphate (IP<sub>3</sub>) (Kanematsu, Mizokami, Watanabe, & Hirata, 2007; Kanematsu et al., 2000). PRIPs have multiple functions which include: (1) trafficking of  $\gamma$ 2-containing GABA<sub>A</sub>Rs to the cell surface through the ternary binding of PRIPs to GABA<sub>A</sub>R and the GABA<sub>A</sub>R-associated protein (GABARAP), (2) regulating constitutive AP2- and clathrin-mediated endocytosis of GABA<sub>A</sub>R, and (3) modulating GABA<sub>A</sub>R phosphorylation (Kanematsu, Fujii, et al., 2007; Kanematsu, Mizokami, et al., 2007).

The ability of PRIP to regulate dephosphorylation occurs through its binding to protein phosphatases PP1 $\alpha$ , PP2A, and the  $\beta$  subunits of GABA<sub>A</sub>Rs (Kanematsu, Mizokami, et al., 2007; Kanematsu et al., 2006; Terunuma et al., 2004; Yoshimura et al., 2001). The PRIP1–PP1 $\alpha$  association renders the phosphatase catalytically inactive. However, upon PKA activation, PRIP1 phosphorylation facilitates the dissociation of the PRIP1–PP1 $\alpha$  complex. The subsequent release of active PP1 $\alpha$  dephosphorylates the  $\beta$ 3 subunit specifically at the AP2 binding site, thereby resulting in GABA<sub>A</sub>R internalization (Kanematsu et al., 2006;

Terunuma et al., 2004; Fig. 2). Further, PRIP1 KO mice displayed increased PP1 $\alpha$  activity and functional deficits in PKA-mediated modulation of GABA<sub>A</sub>Rs (Terunuma et al., 2004). In contrast to PP1 $\alpha$ , PRIP1–PP2A association does not alter PP2A activity. Ultimately, the level of  $\beta$  subunit phosphorylation will depend on the balance between the rate of direct phosphorylation of  $\beta$  subunits and the rate of phosphorylation of PRIP1 by PKA.

#### 4.5. Receptor for activated C-kinase

The highly conserved, multifaceted adaptor protein receptor for activated C-kinase (RACK1) binds specifically to activated PKC and enables its trafficking to membrane locales, thereby allowing for PKC phosphorylation at precise receptor sites (Adams, Ron, & Kiely, 2011; Mochly-Rosen, Khaner, & Lopez, 1991; Ron et al., 1994). In addition to binding PKC, RACK1 also interacts with the major intracellular domain of GABA<sub>A</sub>R  $\beta$ 1 and  $\beta$ 3 subunits adjacent to a PKC binding site (Brandon, Jovanovic, Smart, et al., 2002; Brandon et al., 1999). The PKC isoform PKC $\beta$ II binds directly to  $\beta$ 1 and  $\beta$ 3 subunits and phosphorylates residues S410 and S408/S409, respectively. Binding assays with GST- $\beta$ 1 fusion proteins revealed that RACK1 bound to residues 395–404, immediately upstream of the PKC binding site (residues 405–415) (Brandon, Jovanovic, Smart, et al., 2002; Brandon et al., 1999). Although association of RACK1 is not essential for PKC binding to  $\beta$  subunits, it potentiates the phosphorylation of  $\beta$ 1 subunits at S409.

## 5. PHOSPHORYLATION AND ALLOSTERIC MODULATION

Pharmacological agents that target GABA<sub>A</sub>R for therapeutics are largely positive allosteric modulators used for their anesthetic, anticonvulsant, anxiolytic, or sedative-hypnotic actions. Positive allosteric modulators bind receptors at a site separate from the agonist binding site, enhancing the response of GABA<sub>A</sub>Rs to GABA. Importantly, allosteric modulation of GABA<sub>A</sub>Rs by barbiturates, benzodiazepines, and neurosteroids can be regulated by phosphorylation in a kinase- and subunit-specific manner. Further, allosteric modulators may also regulate the phosphorylation state of GABA<sub>A</sub>Rs.

### 5.1. Barbiturates and benzodiazepines

Benzodiazepines increase GABA<sub>A</sub>R current by increasing channel opening frequency, whereas barbiturates have dose-dependent differences in their effects. At low concentrations, barbiturates act to allosterically enhance the GABA response through increasing channel opening duration, while at higher concentrations they directly activate GABA<sub>A</sub>Rs (Korpi, Grunder, & Luddens, 2002; Macdonald & Olsen, 1994; MacDonald, Rogers, & Twyman, 1989).

Benzodiazepines bind the interface between  $\alpha$  (1, 2, 3, or 5) and  $\gamma$  subunits and regulate GABA<sub>A</sub>Rs by increasing channel opening frequency upon GABA binding (Goldschen-Ohm, Wagner, Petrou, & Jones, 2010; Jacob et al., 2008; Macdonald & Olsen, 1994). Evidence from synaptosomal preparations suggests CamKII activation results in an increase in benzodiazepine binding to the receptor (Churn et al., 2002). Furthermore, a number of studies have reported that the effects of these drugs can be regulated via PKC activation. Pretreatment of  $\alpha$ 1 $\beta$ 2 $\gamma$ 2L-expressing oocytes with PKC activators resulted in increases in

the ability of diazepam and pentobarbital to allosterically modulate GABA-induced currents (Leidenheimer, McQuilkin, Hahner, Whiting, & Harris, 1992). However, similar experiments performed on oocytes expressing  $\alpha 1\beta 2\gamma 2$  by another group could not reproduce the effect of diazepam or pentobarbital (Ghansah & Weiss, 2001). Nonetheless, further support of PKC regulation of GABA<sub>A</sub>R allosteric modulation, albeit in the other direction, stems from PKC $\epsilon$  KO mice. These studies showed that mice that lacked PKC $\epsilon$  were more sensitive to benzodiazepines and barbiturates (Harris et al., 1995; Hodge et al., 1999). PKC $\epsilon$  has been shown to phosphorylate the GABA<sub>A</sub>R  $\gamma 2$  subunit at S327. Indeed, in  $\alpha 1\beta 2\gamma 2$ -expressing HEK293 cells, phospho-null mutation of  $\gamma 2$  S327 enhances the actions of benzodiazepine (Qi et al., 2007). By comparison, PKC $\gamma$  KO mice did not have altered sensitivity to barbiturates or benzodiazepines (Harris et al., 1995; Hodge et al., 1999). These studies outlined above highlight an added layer of complexity and sophistication, whereby different kinase isozymes specifically and differentially mediate the allosteric modulation of GABA<sub>A</sub>Rs, presenting a fertile topic for future studies.

## 5.2. Neurosteroids

Neurosteroids are steroids synthesized *de novo* in the brain by glia and neurons (Compagnone & Mellon, 2000; Herd, Belelli, & Lambert, 2007). Neurosteroids are potent and selective allosteric modulators of GABA<sub>A</sub>Rs that increase the channel open duration and frequency at lower concentrations and directly activate GABA<sub>A</sub>Rs at higher concentrations (Lambert, Cooper, Simmons, Weir, & Belelli, 2009). Besides their allosteric modulation of GABA<sub>A</sub>Rs, neurosteroids have also been shown to exert their effects through the potentiation of PKC phosphorylation on S443 within  $\alpha 4$  subunits of GABA<sub>A</sub>Rs. Phosphorylation at this residue leads to a sustained up-regulation of  $\alpha 4$ -containing receptors through insertion of receptors into surface membranes, resulting in a selective enhancement of tonic current in hippocampal neurons (Abramian et al., 2014; Comenencia-Ortiz, Moss, & Davies, 2014). Although others have reported decreases in PKC-mediated tonic inhibition (Bright & Smart, 2013), this work nevertheless highlights a novel mechanism by which neurosteroids can alter neuronal inhibition.

The connection between PKC phosphorylation and neurosteroid modulation of GABA<sub>A</sub>Rs is complex. A number of studies have reported that neurosteroid modulation of GABA<sub>A</sub>Rs may be enhanced by PKC phosphorylation (Fáncsik, Linn, & Tasker, 2000; Harney, Frenguelli, & Lambert, 2003; Leidenheimer & Chapell, 1997; Vicini, Losi, & Homanics, 2002), while other studies report that the opposite is true (Hodge et al., 1999, 2002; Kia et al., 2011; Kokksma et al., 2003). For example, GABA<sub>A</sub>R  $\delta$ -subunit KO mice display reduced sensitivity to neurosteroids, which could be restored by activation of PKC with phorbol esters (Vicini et al., 2002). Further, inhibition of either PKA or PKC decreased the sensitivity of GABA<sub>A</sub>Rs to neurosteroids in hippocampal CA1 pyramidal neurons, whereas, in contrast, activation of PKC had no effect (Harney et al., 2003). Interestingly, and in the same preparation, PKC activation *did* enhance the sensitivity of GABA<sub>A</sub>Rs to neurosteroid in dentate gyrus granule cells. This suggests that even within a specific brain region, there are likely to be neuron-specific effects that depend upon both the circulating levels of neurosteroids as well as the phosphorylation status of GABA<sub>A</sub>Rs and/or their associated proteins. As with the enhanced response to benzodiazepine and barbiturate mentioned

above, PKC $\epsilon$ -deficient mice also exhibit increased sensitivity to neurosteroids (Hodge et al., 1999, 2002). Moreover, these mice were less anxious and had reduced response to stress hormones, consistent with the anxiolytic effects of neurosteroids (Hodge et al., 2002).

Further molecular mechanisms that impact GABA $_A$ R sensitivity to neurosteroids have emerged from studies of the hypothalamic oxytocin neurons of the supraoptic nuclei. These studies showed that substantial changes in activity around the time of parturition can be attributed to changes in circulating levels of progesterone and metabolites (Brussaard & Herbison, 2000; Concas et al., 1998). Recordings from these cells show that pregnant rats during late gestation are neurosteroid-sensitive, but that post-parturition, GABA $_A$ Rs become neurosteroid-insensitive (Brussaard, Wossink, Lodder, & Kits, 2000; Koksma et al., 2003). Moreover, these observations were ascribed to modifications in kinase and phosphatase activity. During late pregnancy, activation of PKC with phorbol esters or inhibition of phosphatases PP1 and PP2A reduced neurosteroid modulation of GABA $_A$ R. Conversely, after giving birth, when GABA $_A$ Rs are less sensitive to neurosteroid modulation, inhibitors of PP1 and PP2A or activation of PKC rescued neurosteroid sensitivity of GABA $_A$ R (Koksma et al., 2003).

Clearly, the impact of phosphorylation on allosteric modulation of GABA $_A$ Rs is complex, and information on the molecular mechanisms that regulate these processes is currently unavailable. Furthermore, it is still unclear from these studies whether kinases target receptors directly or one of its intimately associated proteins, and whether phosphorylation increases binding of allosteric modulators to receptors or whether it influences modulator-induced changes to channel gating. Nevertheless, it is evident that phosphorylation imparts further diversity to the interplay between GABA $_A$ Rs and allosteric modulators.

## 6. SIGNALING PATHWAYS THAT MODULATE GABA $_A$ R PHOSPHORYLATION

Excitation and inhibition in the brain is tightly and dynamically balanced and is crucial for proper brain function. Since GABA $_A$ Rs are the principal mediators of inhibition in the brain, it is unsurprising that multiple signaling pathways can regulate the phosphorylation status of these receptors.

### 6.1. Receptor tyrosine kinases

Receptor tyrosine kinases (RTKs) are an extensive class of cell-surface receptors that are essential to numerous cellular processes (Schlessinger, 2000). In particular, two ligand-receptor pairs of the RTK class include insulin and the insulin receptor, and brain-derived neurotrophic factor (BDNF) and its associated tyrosine kinase receptor (TrkB). Insulin and BDNF have both been implicated in the regulation of GABA $_A$ Rs, and will be discussed in more detail below.

**6.1.1 Brain-derived neurotrophic factor**—The neurotrophin tyrosine kinase receptor 2 (TrkB) exerts its effects through activation by BDNF (Klein et al., 1991). BDNF is abundant and ubiquitously expressed in the brain, playing key roles in neurogenesis, differentiation, survival, and synaptic plasticity (Boulle et al., 2012; Geral, Angelova, & Lesieur, 2013). More specifically, BDNF has been reported to be critical for the development of GABAergic

synapses and GABAergic inhibition (Seil, 2003; Vicario-Abejón, Collin, McKay, & Segal, 1998). Studies have implicated BDNF in the increase in cell-surface expression of  $\delta$  subunits of GABA<sub>A</sub>Rs. Although this effect was reported to be mediated by TrkB receptors, phospholipase C (PLC $\gamma$ ), and PKC, the substrate for the kinase was unidentified (Joshi & Kapur, 2009). Further, the actions of BDNF on GABA<sub>A</sub>Rs seem to be developmentally regulated and require the phosphorylation of residues on the  $\beta$ 3 and  $\gamma$ 2 subunits, as will be discussed below.

Exogenous application of BDNF led to an early, transient potentiation followed by a lasting decrease in GABA<sub>A</sub>R-mediated currents in cortical, hippocampal and superior colliculus neurons (Brünig, Penschuck, Berninger, Benson, & Fritschy, 2001; Henneberger, Jüttner, Rothe, & Grantyn, 2002; Jovanovic et al., 2004; Kanematsu et al., 2006). The biphasic nature of the response was attributed to the differential recruitment of PKC and RACK1 that was required for the initial rapid phosphorylation of GABA<sub>A</sub>R  $\beta$ 3 subunit at S408/S409 (Jovanovic et al., 2004), followed by PRIP1/2 and PP2A mediated dephosphorylation of this site (Kanematsu et al., 2006). Intriguingly, the phosphorylation of S408/S409 has been shown to reduce association of PKC, whereas it increased binding of PP2A, which may explain the transient nature of phosphorylation that has been observed at this site (Brandon, Jovanovic, Smart, et al., 2002; Jovanovic et al., 2004; Kanematsu et al., 2006). In addition, application of BDNF to neurons from PRIP1/2 double knockout (dKO) mice showed a slow consistent increase of  $\beta$ 3 phosphorylation complemented with an increase in GABA-induced current. This demonstrated that the initial PKC-dependent phosphorylation persisted, whereas the following PRIP-mediated PP2A dephosphorylation step was abrogated (Kanematsu et al., 2006). As mentioned previously, one would expect that the dephosphorylation of  $\beta$ 3 at Ser408/S409 by PP2A would result in AP2 binding and subsequent endocytosis of receptors (Kittler et al., 2005), which would explain the subsequent decrease in mIPSC amplitude. However, the effects of BDNF on surface levels of GABA<sub>A</sub>R are in conflict, where both increases (Jovanovic et al., 2004; Kuczewski et al., 2011) and decreases (Kanematsu et al., 2006) have been observed. The reason for this discrepancy is unknown but may be mediated by a number of factors that include methodology, neuronal cell-type, age of neurons and the species that the neurons originated from. Nevertheless, these studies emphasize the importance of BDNF-mediated phosphorylation of GABA<sub>A</sub>Rs and bring further support to the prominence of RACK1 and PRIPs as facilitators of the phosphorylation and dephosphorylation of GABA<sub>A</sub>Rs.

In contrast to the regulation of GABA<sub>A</sub>R by BDNF in relatively young animals/culture described above, application of BDNF to slices from the prefrontal cortex (PFC) has been shown to cause enhancements in GABA<sub>A</sub>R-dependent inhibition to older (2–4 months) animals. BDNF led to increased phosphorylation of Y365/Y367 of  $\gamma$ 2 subunits, a subsequent decrease in binding of AP2, and finally, an associated increase in levels of surface receptors. Further, BDNF was no longer able to modulate mIPSCs in mice where these residues had been mutated to Y365F/Y367F. Interestingly, these animals had increased antidepressant phenotype and increased neurogenesis. Although BDNF produced antidepressant-like actions and neurogenesis in wild-type animals, BDNF was unable to further modulate these effects in Y365F/Y367F mice (Vithlani et al., 2013).

Whether these mechanisms are truly different due to development, and not due to, for example, differences in experimental manipulations, deserves future attention. Indeed, BDNF application also increased surface levels of GABA<sub>A</sub>Rs (Porcher et al., 2011) in young developing cortical cultures at a stage when GABA<sub>A</sub>Rs are still excitatory (Ben-Ari et al., 2012; Owens, Boyce, Davis, & Kriegstein, 1996). Moreover, an additional complication that may be relevant to these studies is that BDNF also modulates K<sup>+</sup>-Cl<sup>-</sup> cotransporter activity, which could affect the efficacy of inhibitory transmission (Rivera et al., 2002; Shulga et al., 2008; Wardle & Poo, 2003).

**6.1.2 Insulin**—Although pancreatic insulin is well recognized for its regulation of blood glucose levels, insulin is also synthesized in the brain (Havrankova, Brownstein, & Roth, 1981) where it regulates synaptic plasticity, spine and dendritic morphogenesis, and neuronal survival (Bassil, Fernagut, Bezdard, & Meissner, 2014; Chiu & Cline, 2010). Insulin treatment results in a rapid increase in surface levels of GABA<sub>A</sub>Rs and an enhancement of mIPSC amplitude (Fujii et al., 2010; Vetiska et al., 2007; Wan et al., 1997; Wang, Liu, Pei et al., 2003). Work on the mechanisms regulating these effects has so far concentrated on the β2 subunit of GABA<sub>A</sub>Rs. More specifically, the presence of insulin results in the phosphorylation at residues Y372 and Y379 of the β2 subunit of GABA<sub>A</sub>Rs by an unidentified kinase and these residues were critical for the increases in surface levels of GABA<sub>A</sub>Rs upon stimulation with insulin. Furthermore, enhancement of GABA<sub>A</sub>R-mediated currents was found to be phosphoinositide 3-kinase (PI3K) dependent and correlated with an increase in binding between PI3K p85 subunit SH2 domain and GABA<sub>A</sub>Rs specifically at these phosphorylated residues on the β2 subunit (Vetiska et al., 2007).

Elsewhere, other groups have shown that the observed increases in surface receptors required the well-documented activation by insulin of the PI3K-Akt pathway (Hemmings & Restuccia, 2012; Wan et al., 1997; Wang, Liu, Pei, et al., 2003). Activated Akt solely phosphorylated the S410 residue on the β2 subunit in α1β2γ2-containing HEK293 cells. Insulin stimulation of Akt in α1β2γ2-expressing HEK293 cells led to an enhancement in surface receptors and an increase in the amplitude of GABA<sub>A</sub>R-mediated mIPSCs (Wan et al., 1997). Indeed, the phosphorylation of this residue by Akt was also crucial for the observed translocation of GABA<sub>A</sub>R in cultured neurons (Wang, Liu, Pei, et al., 2003). Notably, upon insulin application, activated Akt translocated specifically to more distal dendritic locales where it colocalized with GABA<sub>A</sub>Rs. In addition, PRIP1 was required to traffic active Akt to GABA<sub>A</sub>Rs upon stimulation by insulin, since disruption of this complex in PRIP dKO mice or PRIP interference peptide in wild-type mice resulted in a dearth in phosphorylated β subunit. This was also paralleled with a lack of insulin-mediated potentiation of GABA-induced currents (Fujii et al., 2010). The S410 phosphorylation site in the β2 subunit is conserved (in β1 S409 and β3 S408/S409), and again one would presume that its phosphorylation may result in less binding to AP2, thereby increasing surface levels of receptor. Instead, inhibition of ER to Golgi trafficking by brefeldin A resulted in a decrease in GABA-evoked currents beyond that of controls. This suggests that increases in surface GABA<sub>A</sub>R are mediated by insertion of newly synthesized receptors concurrent and to a lesser degree with a decrease in endocytosis (Fujii et al., 2010). Whether these mechanisms also apply to other β subunits has not been explored.

Thus, the insulin-mediated effects hinge on the phosphorylation of GABA<sub>A</sub>Rs by two seemingly different mechanisms. How these processes may converge will be a fascinating topic for future investigations. Moreover, these studies advocate phosphorylation of a specific site that has differential consequences and is contingent on the kinase concerned.

## 6.2. Glutamate receptors

The vast majority of synapses use glutamate as their excitatory neurotransmitter. Glutamate release from presynaptic terminals triggers rapid activation of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA<sub>R</sub>s) and *N*-methyl-D-aspartate receptors (NMDARs). This activation leads to AMPAR and NMDAR opening to allow an influx of cations, and stimulation of the postsynaptic cell. The strength of synaptic transmission is extremely plastic and can be increased (long-term potentiation, LTP) or decreased (long-term depression, LTD) (Malenka & Bear, 2004). The polarity of this plasticity is largely defined by the kinetics and amount of Ca<sup>2+</sup> influx through NMDARs. Strong, brief, high frequency stimulation (HFS) of the input neuron induces rapid and robust Ca<sup>2+</sup> entry into cells, resulting in LTP. Additionally, weak, prolonged, low frequency stimulation, allowing for lower concentrations of Ca<sup>2+</sup> entry, results in LTD (Bliss & Lømo, 1973; Dudek & Bear, 1992; Yang, Tang, & Zucker, 1999). The difference in the sensitivities of kinases and phosphatases to Ca<sup>2+</sup> provides one of the mechanisms that underlie this bidirectional expression of plasticity (Lee, 2006). Together, LTP and LTD are thought to represent the cellular correlate for learning and memory in the CNS (Bear & Abraham, 1996; Bliss & Collingridge, 1993).

Likewise, similar long-term changes in synaptic strength occur at GABAergic synapses (Gaiarsa, Caillard, & Ben-Ari, 2002). NMDAR-dependent HFS that induces LTP at excitatory synapses in the hippocampus also results in the concomitant LTD (LTD<sub>GABA</sub>) at inhibitory synapses (Lu, Mansuy, Kandel, & Roder, 2000). The decrease in efficacy of GABAergic transmission was accompanied with the dephosphorylation at S327 in the  $\gamma$ 2 subunit of GABA<sub>A</sub>Rs and was dependent upon the binding of the CaN catalytic domain to the intracellular loop of  $\gamma$ 2 subunits (Wang, Liu, Haditsch, et al., 2003). Moreover, increases in excitatory activity resulting in NMDA-dependent Ca<sup>2+</sup> influx lead to the fast and reversible diffusion of GABA<sub>A</sub>Rs from synaptic clusters and was found to be independent of receptor endocytosis (Bannai et al., 2009; Muir et al., 2010). The lateral diffusion of receptors was contingent upon CaN-dependent dephosphorylation of S327 on the  $\gamma$ 2 subunit (Fig. 3) (Muir et al., 2010).

Conversely, NMDAR-dependent chemically induced LTD (chemLTD) of excitatory transmission results in an increase of GABA<sub>A</sub>Rs at the surface and an enhancement of inhibitory transmission (LTP<sub>GABA</sub>). As has been found in excitatory synapses, the bidirectional regulation of GABA<sub>A</sub>Rs is dependent upon the strength of NMDAR activation and Ca<sup>2+</sup> influx. Strong NMDAR activation leads to CamKII $\alpha$  translocation to excitatory synapses, enhancing surface levels of AMPARs, whereas targeting of CamKII $\alpha$  is inhibited at inhibitory synapses by CaN. Weaker stimuli resulted in CamKII $\alpha$  localization at inhibitory synapses and an enhancement of surface levels of GABA<sub>A</sub>Rs (Marsden, Beattie, Friedenthal, & Carroll, 2007; Marsden, Shemesh, Bayer, & Carroll, 2010). More recent



evidence lends further support to the role of GABA<sub>A</sub>R phosphorylation in these mechanisms. Similar induction of chemLTD resulted in a CamKII-dependent LTP<sub>GABA</sub>, marked by increases in surface levels of GABA<sub>A</sub>Rs and the potentiation in the mIPSC and spontaneous inhibitory synaptic current amplitude. During LTP<sub>GABA</sub>, GABA<sub>A</sub>Rs were immobilized at the synapse and the scaffold protein gephyrin was recruited from nearby extrasynaptic sites. Moreover, the phosphorylation of  $\beta 3$  at S383 increased during LTP<sub>GABA</sub>. Phosphorylation of  $\beta 3$  S383 was required for GABA<sub>A</sub> Rimmobilization at synapses and the recruitment of gephyrin to synaptic sites. Thus, the phosphorylation of  $\beta 3$  S383 is considered critical for the expression of LTP<sub>GABA</sub>. Finally, similar accumulation of GABA<sub>A</sub>Rs and gephyrin is observed *in vivo* following an experience-dependent plasticity protocol (Petrini et al., 2014; Fig. 3).

### 6.3. Voltage-gated Ca<sup>2+</sup> channels

In cerebellar Purkinje neurons, activation of excitatory synapses induce long-lasting enhancement of GABA<sub>A</sub>R-mediated inhibitory current. This “rebound potentiation” (RP) occurs through activation of voltage-gated Ca<sup>2+</sup> channels (VGCCs) resulting in a transient Ca<sup>2+</sup> influx and is dependent upon CamKII (Kano, Kano, Fukunaga, & Konnerth, 1996) and  $\gamma 2$  subunit association with GABARAP (Kawaguchi & Hirano, 2007). Further, RP can be suppressed by GABA type B receptor (GABA<sub>B</sub>R) activation through decreasing levels of PKA. This reduction of PKA ultimately leads to an increase in PP1 activity through the PKA/DARPP-32/PP1 signaling pathway resulting in the inhibition of CamKII (Hirano & Kawaguchi, 2014; Kawaguchi & Hirano, 2002). Since PKA and CamKII are both kinases of the  $\beta 1$  and  $\beta 3$  subunit, and because the PKA/DARPP-32/PP1 signaling pathways have previously been reported to increase the phosphorylation of  $\beta 1$  and  $\beta 3$  subunits (Flores-Hernandez et al., 2000), it is possible that direct phosphorylation of these subunits may underlie RP in these neurons.

Activation of L-type VGCCs in hippocampal preparations results in CamKII-dependent increases in cell surface  $\alpha 5\beta 3$ -containing receptors and an enhancement of tonic current. Moreover, these observations were contingent on the phosphorylation of S383 on the  $\beta 3$  subunit, which subsequently led to the insertion of new receptors to the surface without affecting endocytosis. Increases or decreases in neuronal activity also resulted in respective increases and decreases in phosphorylated  $\beta 3$  and surface expression of GABA<sub>A</sub>Rs. Thus, neuronal activity can bidirectionally regulate surface-expressed GABA<sub>A</sub>Rs and tonic inhibition through the phosphorylation of a single residue on the  $\beta 3$  subunit (Saliba, Kretschmannova, & Moss, 2012).

### 6.4. Dopamine

Both fast synaptic glutamatergic (excitatory) and GABAergic (inhibitory) transmission can be modulated through G-protein-coupled mechanisms or by direct interactions with the comparatively slower neurotransmission of dopaminergic receptors. DA receptors are broadly classified into two distinct groups, the D1-like (D1 and D5) and D2-like (D2–4) classes of receptors. This distinction was based on the initial observations that DA could modulate adenylyl cyclase activity resulting in increased (D1-like) or decreased (D2-like) cyclic AMP (cAMP) production required for the subsequent activation of PKA (Beaulieu &

Gainetdinov, 2011; Greengard, 2001). Since dopamine receptors affect PKA activity, evidence for a role of DA-mediated phosphorylation of GABA<sub>A</sub>Rs is largely derived from studies on the  $\beta$  subunits (Chen, Kittler, Moss, & Yan, 2006; Flores-Hernandez et al., 2000; Goffin et al., 2010; Terunuma et al., 2004).

DA can modulate the excitability of striatal medium spiny neurons (MSNs) expressing a tonic GABA<sub>A</sub>R current via a PKA and  $\beta$ 3 subunit-dependent mechanism (Janssen, Ade, Fu, & Vicini, 2009; Janssen, Yasuda, & Vicini, 2011). In neostriatal neurons, DA activation of D1 and D5 receptors both decreased and enhanced GABA-evoked currents in MSNs (Flores-Hernandez et al., 2000) and cholinergic interneuron (Yan & Surmeier, 1997), respectively.

In particular, application of a D1 agonist in adult MSNs resulted in a PKA-dependent reduction in GABA-induced current. Membrane permeable analogues of cAMP mimicked the attenuation of the GABA-evoked response, which was further reduced by inhibition of PP1/PP2A. Additionally, DA was observed to increase the phosphorylation of  $\beta$ 1 and  $\beta$ 3 subunits (although  $\beta$ 1 subunits were more prevalent), which was dependent upon DA- and cAMP-regulated phosphoprotein, 32 kDa (DARPP-32). Thus, the observed decrease in GABA-induced current requires activation of the PKA/DARPP-32/PP1 signaling pathway, leading to the modulation of GABA<sub>A</sub>Rs through the phosphorylation of  $\beta$ 1 subunits (Flores-Hernandez et al., 2000).

In hippocampal cells, the activation of D1 receptors led to a PKA- and PRIP-dependent enhancement of GABA-evoked currents and a concomitant increase in phosphorylation of  $\beta$ 3 residues S408/S409 (Terunuma et al., 2004). Thus, these studies lend support to aforementioned work in heterologous systems whereby PKA was observed to reduce GABA-induced currents by phosphorylation of S409 in the  $\beta$ 1 subunit and increase currents by phosphorylation of two adjacent residues S408/S409 on the  $\beta$ 3 subunit (McDonald et al., 1998).

In addition to D1-like receptors, D3 receptor activation in the MSNs of the nucleus accumbens was shown to reduce GABA<sub>A</sub>R-mediated currents concurrent with increased internalization and a decrease in surface receptors. These effects were dependent on cAMP, PKA, the  $\beta$  subunit of GABA<sub>A</sub>R as well as clathrin-dependent endocytosis (Chen et al., 2006). In this case, the activation of D3 receptors leads to reduced PKA activity resulting in the dephosphorylation of  $\beta$  subunits and the subsequent endocytosis of these receptors, which reduces GABA<sub>A</sub>R function.

Activation of D2-like receptors in the VTA induces LTD of GABAergic synapses and was dependent on the clathrin-mediated endocytosis of GABA<sub>A</sub>Rs. Moreover, these observations required an IP<sub>3</sub>-dependent rise in Ca<sup>2+</sup>, activation of CaN, and parallel inhibition of PKA. Finally, disrupting the AKAP-PKA complex mimicked LTD<sub>GABA</sub> (Dacher et al., 2013).

## 6.5. Others

In addition to its ability to suppress RP, GABA<sub>B</sub>R activation increases  $\alpha$ 4 $\beta$  $\delta$ - and  $\alpha$ 6 $\beta$  $\delta$ -mediated tonic current in thalamocortical and dentate gyrus granule cells, respectively (Connelly, Errington, & Crunelli, 2013a). This effect was further dependent upon  $\delta$ -

containing receptors and PKA inhibitors mimicked GABA<sub>B</sub>R-induced enhancements (Connelly, Errington, Di Giovanni, & Crunelli, 2013; Naylor, Liu, Niquet, & Wasterlain, 2013).

Studies of serotonergic neurotransmission in the PFC show that activation of 5-HT<sub>2</sub> receptors decreases GABA-evoked currents, which is dependent upon PKC–RACK1 association and results in the PKC-dependent phosphorylation of GABA<sub>A</sub>R  $\gamma$ 2 subunit (Feng, Cai, Zhao, & Yan, 2001). In contrast, activation of 5-HT<sub>4</sub> receptors resulted in the reversible, bidirectional modulation of GABA-induced currents determined by basal PKA activity (Cai, Flores-Hernandez, Feng, & Yan, 2002).

Collectively, it is clear that multiple signaling pathways can, or have the potential to, orchestrate the phosphorylation state of specific residues on GABA<sub>A</sub>Rs. Further, these signaling molecules allows for specific modification determined by intricate cues and presents an additional layer of complexity in the regulation of GABA<sub>A</sub>R activity.

## 7. DYSREGULATION OF GABA<sub>A</sub>R PHOSPHORYLATION IN DISEASE

Although compromised trafficking of GABA<sub>A</sub>Rs are thought to play key roles in a number of pathological conditions (Benarroch, 2007; DeLorey & Olsen, 1999; Krystal et al., 2006; Rudolph & Mohler, 2004; Thompson-Vest et al., 2003), evidence for the importance of phosphorylation in these studies is relatively scarce.

### 7.1. Ischemia

Excessive release of glutamate in cerebral ischemia results in excitotoxicity and cell death (Lipton, 1999; Lo, Dalkara, & Moskowitz, 2003). Although the majority of research has focused on reducing the effects of the glutamatergic system, GABA<sub>A</sub>R trafficking is also considerably modified, which may exacerbate these effects (Schwartz-Bloom & Sah, 2001). Indeed, decreases in surface levels of GABA<sub>A</sub>Rs and an enhancement of ubiquitin-dependent lysosomal degradation have been reported in the *in vitro* oxygen-glucose deprivation (OGD) model of ischemia (Arancibia-Carcamo & Kittler, 2009; Liu et al., 2010; Mielke & Wang, 2005). Interestingly, the association of the  $\alpha$ 1 subunit to gephyrin decreased and could be rescued with a CaN inhibitor. Inhibitors of PP1 $\alpha$ /PP2A rescued the loss of  $\alpha$ 1 subunit by OGD. Further, ischemic insult resulted in decreased phosphorylation of S408/S409 of the  $\beta$ 3 subunit and phosphomimetic mutants of  $\beta$ 3 or mutants that blocked AP2 binding protected cells from neuronal death (Mele, Ribeiro, Inacio, Wieloch, & Duarte, 2014; Smith et al., 2012).

### 7.2. Epilepsy

Epilepsy is a common and often devastating neurological disorder based on a striking imbalance between excitatory and inhibitory activity. Prolonged, continuous seizures (status epilepticus, SE) in animal models and humans can induce the development of temporal lobe epilepsy (TLE). Modifications in GABA<sub>A</sub>R trafficking and expression have been reported in patients and animal models of SE and TLE (Brooks-Kayal, Shumate, Jin, Rikhter, & Coulter, 1998; Goodkin, Joshi, Mtchedlishvili, Brar, & Kapur, 2008; Loup, Wieser, Yonekawa, Aguzzi, & Fritschy, 2000; Naylor, Liu, & Wasterlain, 2005; Sperk, Drexel, &

Pirker, 2009; Terunuma et al., 2008). Increases and decreases in GABA<sub>A</sub>R expression have been observed, both of which are dependent upon specific subunits and the time period of epileptogenesis studied. Loss of GABA<sub>A</sub>Rs is thought to be one mechanism that underlies pharmacoresistance to benzodiazepines (Deeb, Maguire, & Moss, 2012). Furthermore, dephosphorylation of GABA<sub>A</sub>Rs has been implicated in the loss of these receptors during SE. In particular, induction of SE by pilocarpine resulted in the decrease of PKC-mediated phosphorylation of  $\beta$ 3 S408/S409 residues. This dephosphorylation enhanced binding to AP2, resulting in receptor endocytosis during SE (Terunuma et al., 2008). Moreover, other reports have suggested kainate and pilocarpine-induced SE results in an NMDAR-dependent increase in CaN activity and expression resulting in the dephosphorylation of  $\beta$ 2/3 subunits of GABA<sub>A</sub>Rs. Whether this phosphorylation status also resulted in changes to surface expression was not explored (Kurz et al., 2001; Wang, Chi, Wang, Wang, & Sun, 2009).

### 7.3. Drug abuse

Although ligands such as benzodiazepines may have clinically important uses, there is substantial evidence for a role of GABA<sub>A</sub>Rs in the regulation of the addictive properties of drugs of abuse (Kalivas, 2007; Tan, Rudolph, & Lüscher, 2011; Trudell, Messing, Mayfield, & Harris, 2014).

Increased BDNF levels after cocaine withdrawal results in decreased GABAergic inhibition and reduced GABA<sub>A</sub>Rs surface expression, concurrent with the facilitation of activity-induced LTP. These effects were mediated by the BDNF–TrkB–PP2A signaling pathway, which also resulted in decreases in phosphorylated  $\beta$ 3 subunits. These effects have been suggested to underlie behavioral modifications after cocaine withdrawal (Lu, Cheng, Lim, Khoshnevisrad, & Poo, 2010). Application of BDNF has reported to lead to lasting decreases in GABA<sub>A</sub>R-mediated currents through the PRIP1/2 and PP2A mediated dephosphorylation of  $\beta$ 3 S408/S409 (Kanematsu et al., 2006), but whether similar mechanisms apply here remains to be seen.

GABA<sub>A</sub>R subunits composition could be altered reversibly within minutes after a single intoxicating dose of alcohol, resulting in decreased surface levels of extrasynaptic  $\alpha$ 4 and  $\delta$ -containing GABA<sub>A</sub>Rs (Gonzalez et al., 2012; Liang et al., 2007; Shen et al., 2011). This may contribute to the rapid tolerance observed after acute ethanol exposure (LeBlanc, Kalant, & Gibbins, 1975; Liang et al., 2007; Ludvig, George, Tang, Gonzales, & Bungay, 2001; Wallace et al., 2007). Further changes in subunit expression were observed at later time points with increases in  $\alpha$ 4 and  $\gamma$ 2 subunits and decreases in  $\alpha$ 1 and  $\delta$  subunits (Liang et al., 2007). Moreover, these changes persisted after chronic intermittent administration of alcohol followed by withdrawal (Liang et al., 2007, 2006). The ethanol-induced decreases in  $\delta$ -containing receptors were attributed to increased AP2 binding and subsequent endocytosis (Gonzalez et al., 2012). Chronic ethanol exposure was also found to lead to changes in PKC $\gamma$  association with GABA<sub>A</sub>R subunits, with decreases in  $\alpha$ 1 subunit association and enhancements in  $\alpha$ 4 subunit association. The reduction in PKC $\gamma$ - $\alpha$ 1 binding correlated with increased  $\alpha$ 1 subunit in clathrin-coated vesicles (Kumar, Kralic, O'Buckley, Grobin, & Morrow, 2003; Kumar, Sieghart, & Morrow, 2002). Further, inhibition of PKC $\gamma$  (but not PKC $\beta$ ) prevented ethanol-mediated modulations in  $\alpha$ 1 and  $\alpha$ 4 subunit expression (Kumar et

al., 2010; Werner et al., 2011). Intriguingly, PKC phosphorylation of  $\alpha 4$  at S443 has been shown to increase receptor numbers at the surface (Abramian et al., 2010). Whether this site can also be modulated through ethanol administration remains to be seen.

Phosphorylation of GABA<sub>A</sub>R may well underlie some of these changes, but evidence showing direct modifications of GABA<sub>A</sub>R phosphorylation states by ethanol comes from transgenic mice lacking specific isoforms of PKC. As well as altering the previously discussed positive allosteric effects of benzodiazepines, PKC $\epsilon$  also modifies the allosteric effects of alcohol. As with benzodiazepines, PKC $\epsilon$  KO mice display increased sensitivity to the effects of ethanol as well as a reduction in phosphorylated  $\gamma 2$  S327. In  $\alpha 1\beta 2\gamma 2$  expressing HEK293 cells, phospho-null mutation of this site enhanced the effect of ethanol (Hodge et al., 1999; Qi et al., 2007). It is interesting to note that PKC $\epsilon$  KO animals self-administered considerable less ethanol compared to their wild-type counterparts (Hodge et al., 1999; Olive, Mehmert, Messing, & Hodge, 2000). Strikingly, the modulation of ethanol sensitivity and self-administration in PKC $\epsilon$  KO mice could be rescued to wild-type levels by suppression of the transgene (Choi, Wang, Dadgar, Chang, & Messing, 2002).

PKC $\delta$  KO mice are more resilient to the intoxicating effects of ethanol, an effect that is believed to be mediated by extrasynaptic,  $\delta$  subunit-containing GABA<sub>A</sub>Rs. PKC $\delta$  KO mice showed no response to doses of ethanol which were shown to increase GABA-mediated tonic currents in thalamic and hippocampal neurons of wild-type animals (Choi et al., 2008). Although direct phosphorylation of  $\delta$  subunits of GABA<sub>A</sub>Rs by PKC $\delta$  has been suggested, as yet, there is no direct evidence of  $\delta$  subunit phosphorylation.

## 8. CONCLUSION

There has been a great deal of progress in understanding the molecular mechanisms that regulate GABA<sub>A</sub>Rs. The significance of the dynamic regulation of GABA<sub>A</sub>Rs by phosphorylation is unquestionable. Notwithstanding, there are still considerable gaps and inconsistencies in our knowledge as exemplified in the large number of conflicting results that have arisen from activation of kinases in neuronal preparations.

GABA<sub>A</sub>R may be phosphorylated on multiple sites and on multiple subunits, but our knowledge of how the phosphorylation of a specific site may impact on other sites within the same subunit, or on different subunits, is tenuous. Additionally, it is not certain how phosphorylation on *multiple* sites impacts the fate of receptors. Ultimately, addressing such gaps in our knowledge will provide much-needed clarity to the many differences that are observed in the roles of phosphorylation.

GABA<sub>A</sub>R-interacting proteins allow a further refinement of its regulation. Given the plethora of different GABA<sub>A</sub>R subunits, it is perhaps surprising that there is such a paucity of known interacting proteins. Thus, possible interactions that may be regulated by phosphorylation remain virtually unknown. In particular,  $\alpha$  subunits are the most diverse of the GABA<sub>A</sub>Rs subtypes, and differentiating the particular interaction partners of specific  $\alpha$  subunits may tell us, for example, how different subunits are targeted to particular subcellular regions. Of the  $\alpha$  subtypes, only  $\alpha 4$  has been recently shown to be

phosphorylated at a particular residue. However, the established phosphorylation sites are unlikely to be comprehensive, and with this, it is likely that more phospho-dependent interactions will emerge. Indeed, more recently discovered sites (Abramian et al., 2010; Kang et al., 2011) suggest that seemingly disparate results in the activation of kinases could simply arise from gaps in our current knowledge. Therefore, further examination into this area is necessary in order to enhance our current knowledge on this topic.

Evidence suggests that phosphorylation can increase insertion of receptors to the membrane (Abramian et al., 2014, 2010) and although the mechanisms of GABA<sub>A</sub>Rs endocytosis are becoming more apparent, our understanding of receptor recycling and insertion is still in its infancy. It will be important to determine the consequence of these internalized receptors, and the phosphorylation of GABA<sub>A</sub>Rs seems ideally poised to mediate these trafficking decisions.

Studies on the impact of ethanol clearly demonstrate the need to selectively examine specific PKC isoforms to understand their role in the phosphorylation of GABA<sub>A</sub>Rs (Song & Messing, 2005). Moreover, the different effects of kinases and phosphatases could also be due to differences in development and/or regional expression of specific GABA<sub>A</sub>R subunits, kinases, phosphatases and/or associated proteins. Ultimately, entire pathways will have to be collectively examined to refine the full picture. Further challenges arise from the multiple signaling pathways that converge upon GABA<sub>A</sub>Rs, yielding another level of complexity. Indeed, the effects of BDNF on GABA<sub>A</sub>Rs are far from clear, probably due to developmental and region-specific expression of both proteins (Ip, Cheung, & Ip, 2001; Mizoguchi, Ishibashi, & Nabekura, 2003; Sathanoori et al., 2004; Webster, Herman, Kleinman, & Shannon, 2006).

The overwhelming complexity of GABA<sub>A</sub>Rs is multifaceted, owing to their diversity in subunit composition, differential localization and expression as well as posttranslational modifications. However, it is exactly this mechanistic complexity that makes GABA<sub>A</sub>Rs such opportune drug targets, since it favors the intricate tuning by pharmacological interventions. Accordingly, further research into this diversity through mechanisms of GABA<sub>A</sub>R phosphorylation will surely clarify matters of contention and provide new and exciting insights to understanding neuronal function in health and disease.

## References

- Abramian AM, Comenencia-Ortiz E, Modgil A, Vien TN, Nakamura Y, Moore YE, et al. Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic GABA<sub>A</sub> receptors. *Proceedings of the National Academy of Sciences*. 2014; 111:7132–7137.
- Abramian AM, Comenencia-Ortiz E, Vithlani M, Tretter EV, Sieghart W, Davies PA, et al. Protein kinase C phosphorylation regulates membrane insertion of GABA<sub>A</sub> receptor subtypes that mediate tonic inhibition. *Journal of Biological Chemistry*. 2010; 285:41795–41805. [PubMed: 20940303]
- Adams DR, Ron D, Kiely PA. RACK1, A multifaceted scaffolding protein: Structure and function. *Cell Communication and Signaling: CCS*. 2011; 9:22. [PubMed: 21978545]
- Arancibia-Carcamo IL, Kittler JT. Regulation of GABA<sub>A</sub> receptor membrane trafficking and synaptic localization. *Pharmacology & Therapeutics*. 2009; 123:17–31. [PubMed: 19374920]

- Balduzzi R, Cupello A, Robello M. Modulation of the expression of GABA<sub>A</sub> receptors in rat cerebellar granule cells by protein tyrosine kinases and protein kinase C. *Biochimica et Biophysica Acta (BBA): Biomembranes*. 2002; 1564:263–270. [PubMed: 12101021]
- Bannai H, Lévi S, Schweizer C, Inoue T, Launey T, Racine V, et al. Activity-dependent tuning of inhibitory neurotransmission based on GABA<sub>A</sub>R diffusion dynamics. *Neuron*. 2009; 62:670–682. [PubMed: 19524526]
- Bassil F, Fernagut PO, Bezard E, Meissner WG. Insulin, IGF-1 and GLP-1 signaling in neurodegenerative disorders: Targets for disease modification? *Progress in Neurobiology*. 2014; 118C:1–18.
- Bear MF, Abraham WC. Long-term depression in hippocampus. *Annual Review of Neuroscience*. 1996; 19:437–462.
- Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews*. 2011; 63:182–217. [PubMed: 21303898]
- Ben-Ari Y, Khalilov I, Kahle KT, Cherubini E. The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. *The Neuroscientist*. 2012; 18:467–486. [PubMed: 22547529]
- Benarroch EE. GABA<sub>A</sub> receptor heterogeneity, function, and implications for epilepsy. *Neurology*. 2007; 68:612–614. [PubMed: 17310035]
- Benes FM, Berretta S. GABAergic interneurons: Implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology*. 2001; 25:1–27. [PubMed: 11377916]
- Bliss TV, Collingridge GL. A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature*. 1993; 361:31–39. [PubMed: 8421494]
- Bliss TVP, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology*. 1973; 232:331–356. [PubMed: 4727084]
- Boehm SL, Peden L, Harris RA, Blednov YA. Deletion of the fyn-kinase gene alters sensitivity to GABAergic drugs: Dependence on  $\beta 2/\beta 3$  GABA<sub>A</sub> receptor subunits. *Journal of Pharmacology and Experimental Therapeutics*. 2004; 309:1154–1159. [PubMed: 14764659]
- Bouille F, Kenis G, Cazorla M, Hamon M, Steinbusch HWM, Lanfumey L, et al. TrkB inhibition as a therapeutic target for CNS-related disorders. *Progress in Neurobiology*. 2012; 98:197–206. [PubMed: 22705453]
- Brandon NJ, Delmas P, Hill J, Smart TG, Moss SJ. Constitutive tyrosine phosphorylation of the GABA<sub>A</sub> receptor  $\gamma 2$  subunit in rat brain. *Neuropharmacology*. 2001; 41:745–752. [PubMed: 11640929]
- Brandon NJ, Delmas P, Kittler JT, McDonald BJ, Sieghart W, Brown DA, et al. GABA<sub>A</sub> receptor phosphorylation and functional modulation in cortical neurons by a protein kinase C-dependent pathway. *Journal of Biological Chemistry*. 2000; 275:38856–38862. [PubMed: 10978327]
- Brandon NJ, Jovanovic JN, Colledge M, Kittler JT, Brandon JM, Scott JD, et al. A-kinase anchoring protein 79/150 facilitates the phosphorylation of GABA<sub>A</sub> receptors by cAMP-dependent protein kinase via selective interaction with receptor  $\beta$  subunits. *Molecular and Cellular Neuroscience*. 2003; 22:87–97. [PubMed: 12595241]
- Brandon NJ, Jovanovic JN, Moss SJ. Multiple roles of protein kinases in the modulation of  $\gamma$ -aminobutyric acid A receptor function and cell surface expression. *Pharmacology & Therapeutics*. 2002; 94:113–122. [PubMed: 12191597]
- Brandon NJ, Jovanovic JN, Smart TG, Moss SJ. Receptor for activated C kinase-1 facilitates protein kinase C-dependent phosphorylation and functional modulation of GABA<sub>A</sub> receptors with the activation of G-protein-coupled receptors. *Journal of Neuroscience*. 2002; 22:6353–6361. [PubMed: 12151513]
- Brandon NJ, Uren JM, Kittler JT, Wang H, Olsen R, Parker PJ, et al. Subunit-specific association of protein kinase C and the receptor for activated C kinase with GABA type A receptors. *The Journal of Neuroscience*. 1999; 19:9228–9234. [PubMed: 10531426]
- Bright DP, Smart TG. Protein kinase C regulates tonic GABA<sub>A</sub> receptor-mediated inhibition in the hippocampus and thalamus. *European Journal of Neuroscience*. 2013; 38:3408–3423. [PubMed: 24102973]

- Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA<sub>A</sub> receptor subunit expression and function in temporal lobe epilepsy. *Nature Medicine*. 1998; 4:1166–1172.
- Brüning I, Penschuck S, Berninger B, Benson J, Fritschy JM. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA<sub>A</sub> receptor surface expression. *European Journal of Neuroscience*. 2001; 13:1320–1328. [PubMed: 11298792]
- Brussaard AB, Herbison AE. Long-term plasticity of postsynaptic GABA<sub>A</sub>-receptor function in the adult brain: Insights from the oxytocin neurone. *Trends in Neurosciences*. 2000; 23:190–195. [PubMed: 10782120]
- Brussaard AB, Wossink J, Lodder JC, Kits KS. Progesterone-metabolite prevents protein kinase C-dependent modulation of  $\gamma$ -aminobutyric acid type A receptors in oxytocin neurons. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97:3625–3630. [PubMed: 10716707]
- Cai X, Flores-Hernandez J, Feng J, Yan Z. Activity-dependent bidirectional regulation of GABA<sub>A</sub> receptor channels by the 5-HT<sub>4</sub> receptor-mediated signalling in rat prefrontal cortical pyramidal neurons. *The Journal of Physiology*. 2002; 540:743–759. [PubMed: 11986365]
- Chapell R, Bueno OF, Alvarez-Hernandez X, Robinson LC, Leidenheimer NJ. Activation of protein kinase C induces  $\gamma$ -aminobutyric acid type A receptor internalization in *Xenopus* oocytes. *Journal of Biological Chemistry*. 1998; 273:32595–32601. [PubMed: 9829997]
- Charych EI, Liu F, Moss SJ, Brandon NJ. GABA<sub>A</sub> receptors and their associated proteins: Implications in the etiology and treatment of schizophrenia and related disorders. *Neuropharmacology*. 2009; 57:481–495. [PubMed: 19631671]
- Chen GJ, Kittler JT, Moss SJ, Yan Z. Dopamine D-3 receptors regulate GABA<sub>A</sub> receptor function through a phospho-dependent endocytosis mechanism in nucleus accumbens. *Journal of Neuroscience*. 2006; 26:2513–2521. [PubMed: 16510729]
- Chen ZW, Olsen RW. GABA<sub>A</sub> receptor associated proteins: A key factor regulating GABA<sub>A</sub> receptor function. *Journal of Neurochemistry*. 2007; 100:279–294. [PubMed: 17083446]
- Chiu SL, Cline HT. Insulin receptor signaling in the development of neuronal structure and function. *Neural Development*. 2010; 5:7. [PubMed: 20230616]
- Choi DS, Wang D, Dadgar J, Chang WS, Messing RO. Conditional rescue of protein kinase C epsilon regulates ethanol preference and hypnotic sensitivity in adult mice. *Journal of Neuroscience*. 2002; 22:9905–9911. [PubMed: 12427847]
- Choi DS, Wei W, Deitchman JK, Kharazia VN, Lesscher HMB, McMahon T, et al. Protein kinase C delta regulates ethanol intoxication and enhancement of GABA-stimulated tonic current. *Journal of Neuroscience*. 2008; 28:11890–11899. [PubMed: 19005054]
- Chou WH, Wang D, McMahon T, Qi ZH, Song M, Zhang C, et al. GABA<sub>A</sub> receptor trafficking is regulated by PKC $\epsilon$  and the N-ethylmaleimide-sensitive factor. *The Journal of Neuroscience*. 2010; 30:13955–13965. [PubMed: 20962217]
- Churn SB, Rana A, Lee K, Parsons JT, De Blas A, Delorenzo RJ. Calcium/calmodulin-dependent kinase II phosphorylation of the GABA<sub>A</sub> receptor  $\alpha$ 1 subunit modulates benzodiazepine binding. *Journal of Neurochemistry*. 2002; 82:1065–1076. [PubMed: 12358754]
- Comenencia-Ortiz E, Moss SJ, Davies PA. Phosphorylation of GABA<sub>A</sub> receptors influences receptor trafficking and neurosteroid actions. *Psychopharmacology*. 2014; 231:3453–3465. [PubMed: 24847959]
- Compagnone NA, Mellon SH. Neurosteroids: Biosynthesis and function of these novel neuromodulators. *Frontiers in Neuroendocrinology*. 2000; 21:1–56. [PubMed: 10662535]
- Concas A, Mostallino MC, Porcu P, Follesa P, Barbaccia ML, Trabucchi M, et al. Role of brain allopregnanolone in the plasticity of  $\gamma$ -aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proceedings of the National Academy of Sciences*. 1998; 95:13284–13289.
- Connelly WM, Errington AC, Crunelli V. gamma-Hydroxybutyric acid (GHB) is not an agonist of extrasynaptic GABA<sub>A</sub> receptors. *PLoS One*. 2013; 8:e79062. [PubMed: 24244421]
- Connelly WM, Errington AC, Di Giovanni G, Crunelli V. Metabotropic regulation of extrasynaptic GABA<sub>A</sub> receptors. *Frontiers in Neural Circuits*. 2013; 7



- Connolly CN, Kittler JT, Thomas P, Uren JM, Brandon NJ, Smart TG, et al. Cell surface stability of  $\gamma$ -aminobutyric acid type A receptors. *Journal of Biological Chemistry*. 1999; 274:36565–36572. [PubMed: 10593956]
- D'Hulst C, Kooy RF. The GABA<sub>A</sub> receptor: A novel target for treatment of fragile X? *Trends in Neurosciences*. 2007; 30:425–431. [PubMed: 17590448]
- Dacher M, Gouty S, Dash S, Cox BM, Nugent FS. A-kinase anchoring protein–calcineurin signaling in long-term depression of GABAergic synapses. *The Journal of Neuroscience*. 2013; 33:2650–2660. [PubMed: 23392692]
- Deeb TZ, Maguire J, Moss SJ. Possible alterations in GABA<sub>A</sub> receptor signaling that underlie benzodiazepine-resistant seizures. *Epilepsia*. 2012; 53:79–88.
- DeLorey TM, Olsen RW. GABA and epileptogenesis: Comparing gabrb3 gene-deficient mice with Angelman syndrome in man. *Epilepsy Research*. 1999; 36:123–132. [PubMed: 10515160]
- Dudek SM, Bear MF. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; 89:4363–4367. [PubMed: 1350090]
- Fáncsik A, Linn DM, Tasker JG. Neurosteroid modulation of GABA IPSCs is phosphorylation dependent. *The Journal of Neuroscience*. 2000; 20:3067–3075. [PubMed: 10777770]
- Farrant M, Nusser Z. Variations on an inhibitory theme: Phasic and tonic activation of GABA<sub>A</sub> receptors. *Nature Reviews Neuroscience*. 2005; 6:215–229. [PubMed: 15738957]
- Feng J, Cai X, Zhao J, Yan Z. Serotonin receptors modulate GABA<sub>A</sub> receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2001; 21:6502–6511. [PubMed: 11517239]
- Filippova N, Sedelnikova A, Zong Y, Fortinberry H, Weiss DS. Regulation of recombinant  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> and GABA<sub>C</sub> receptors by protein kinase C. *Molecular Pharmacology*. 2000; 57:847–856. [PubMed: 10779366]
- Flores-Hernandez J, Hernandez S, Snyder GL, Yan Z, Fienberg AA, Moss SJ, et al. D1 dopamine receptor activation reduces GABA<sub>A</sub> receptor currents in neostriatal neurons through a PKA/DARPP-32/PP1 signaling cascade. *Journal of Neurophysiology*. 2000; 83:2996–3004. [PubMed: 10805695]
- Fritschy JM. Epilepsy, E/I balance and GABA<sub>A</sub> receptor plasticity. *Frontiers in Molecular Neuroscience*. 2008; 1:5. [PubMed: 18946538]
- Fujii M, Kanematsu T, Ishibashi H, Fukami K, Takenawa T, Nakayama KI, et al. Phospholipase C-related but catalytically inactive protein is required for insulin-induced cell surface expression of gamma-aminobutyric acid type A receptors. *Journal of Biological Chemistry*. 2010; 285:4837–4846. [PubMed: 19996098]
- Gaiarsa JL, Caillard O, Ben-Ari Y. Long-term plasticity at GABAergic and glycinergic synapses: Mechanisms and functional significance. *Trends in Neurosciences*. 2002; 25:564–570. [PubMed: 12392931]
- Gardner LA, Tavalin SJ, Goehring AS, Scott JD, Bahouth SW. AKAP79-mediated targeting of the Cyclic AMP-dependent protein kinase to the  $\beta$ 1-adrenergic receptor promotes recycling and functional resensitization of the receptor. *Journal of Biological Chemistry*. 2006; 281:33537–33553. [PubMed: 16940053]
- Geral C, Angelova A, Lesieur S. From molecular to nanotechnology strategies for delivery of neurotrophins: Emphasis on brain-derived neurotrophic factor (BDNF). *Pharmaceutics*. 2013; 5:127–167. [PubMed: 24300402]
- Ghansah E, Weiss DS. Modulation of GABA<sub>A</sub> receptors by benzodiazepines and barbiturates is autonomous of PKC activation. *Neuropharmacology*. 2001; 40:327–333. [PubMed: 11166325]
- Gillette MA, Dacheux RF. Protein kinase modulation of GABA<sub>A</sub> currents in rabbit retinal rod bipolar cells. *Journal of Neurophysiology*. 1996; 76:3070–3086. [PubMed: 8930256]
- Goffin D, Ali AB, Rampersaud N, Harkavyi A, Fuchs C, Whitton PS, et al. Dopamine-dependent Tuning of striatal inhibitory synaptogenesis. *The Journal of Neuroscience*. 2010; 30:2935–2950. [PubMed: 20181591]

- Goldschen-Ohm MP, Wagner DA, Petrou S, Jones MV. An epilepsy-related region in the GABA<sub>A</sub> receptor mediates long-distance effects on GABA and benzodiazepine binding sites. *Molecular pharmacology*. 2010; 77:35–45. [PubMed: 19846749]
- Gonzalez C, Moss SJ, Olsen RW. Ethanol promotes clathrin adaptor-Mediated endocytosis via the intracellular domain of delta-containing GABA<sub>A</sub> receptors. *Journal of Neuroscience*. 2012; 32:17874–17881. [PubMed: 23223306]
- Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of GABA<sub>A</sub> receptors during status epilepticus. *The Journal of Neuroscience*. 2008; 28:2527–2538. [PubMed: 18322097]
- Goto H, Terunuma M, Kanematsu T, Misumi Y, Moss SJ, Hirata M. Direct interaction of N-ethylmaleimide-sensitive factor with GABA<sub>A</sub> receptor  $\beta$  subunits. *Molecular and Cellular Neuroscience*. 2005; 30:197–206. [PubMed: 16095914]
- Greengard P. The neurobiology of slow synaptic transmission. *Science*. 2001; 294:1024–1030. [PubMed: 11691979]
- Hanley JG, Khatri L, Hanson PI, Ziff EB. NSF ATPase and  $\alpha$ -/ $\beta$ -SNAPs disassemble the AMPA receptor-PICK1 complex. *Neuron*. 2002; 34:53–67. [PubMed: 11931741]
- Harney SC, Frenguelli BG, Lambert JJ. Phosphorylation influences neurosteroid modulation of synaptic GABA<sub>A</sub> receptors in rat CA1 and dentate gyrus neurones. *Neuropharmacology*. 2003; 45:873–883. [PubMed: 14529725]
- Harris RA, McQuilkin SJ, Paylor R, Abeliovich A, Tonegawa S, Wehner JM. Mutant mice lacking the gamma isoform of protein kinase C show decreased behavioral actions of ethanol and altered function of gamma-aminobutyrate type A receptors. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92:3658–3662. [PubMed: 7731960]
- Havrankova J, Brownstein M, Roth J. Insulin and insulin receptors in rodent brain. *Diabetologia*. 1981; 20(Suppl):268–273.
- Hemmings BA, Restuccia DF. PI3K-PKB/Akt pathway. *Cold Spring Harbor Perspectives in Biology*. 2012; 4:a011189. [PubMed: 22952397]
- Henneberger C, Jüttner R, Rothe T, Grantyn R. Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus. *Journal of Neurophysiology*. 2002; 88:595–603. [PubMed: 12163512]
- Herd MB, Belelli D, Lambert JJ. Neurosteroid modulation of synaptic and extrasynaptic GABA<sub>A</sub> receptors. *Pharmacology & Therapeutics*. 2007; 116:20–34. [PubMed: 17531325]
- Herring D, Huang RQ, Singh M, Dillon GH, Leidenheimer NJ. PKC modulation of GABA<sub>A</sub> receptor endocytosis and function is inhibited by mutation of a dileucine motif within the receptor beta 2 subunit. *Neuropharmacology*. 2005; 48:181–194. [PubMed: 15695157]
- Herring D, Huang R, Singh M, Robinson LC, Dillon GH, Leidenheimer NJ. Constitutive GABA<sub>A</sub> receptor endocytosis is dynamin-mediated and dependent on a dileucine AP2 adaptin-binding motif within the  $\beta$ 2 subunit of the receptor. *Journal of Biological Chemistry*. 2003; 278:24046–24052. [PubMed: 12707262]
- Hirano T, Kawaguchi SY. Regulation and functional roles of rebound potentiation at cerebellar stellate cell-Purkinje cell synapses. *Frontiers in Cellular Neuroscience*. 2014; 8:42. [PubMed: 24600347]
- Hodge CW, Mehmert KK, Kelley SP, McMahon T, Haywood A, Olive MF, et al. Supersensitivity to allosteric GABA<sub>A</sub> receptor modulators and alcohol in mice lacking PKC $\epsilon$ . *Nature Neuroscience*. 1999; 2:997–1002. [PubMed: 10526339]
- Hodge CW, Raber J, McMahon T, Walter H, Sanchez-Perez AM, Olive MF, et al. Decreased anxiety-like behavior, reduced stress hormones, and neurosteroid supersensitivity in mice lacking protein kinase C epsilon. *Journal of Clinical Investigation*. 2002; 110:1003–1010. [PubMed: 12370278]
- Hortnagl H, Tasan RO, Wieselthaler A, Kirchmair E, Sieghart W, Sperk G. Patterns of mRNA and protein expression for 12 GABA<sub>A</sub> receptor subunits in the mouse brain. *Neuroscience*. 2013; 236:345–372. [PubMed: 23337532]
- Houston CM, He Q, Smart TG. CaMKII phosphorylation of the GABA<sub>A</sub> receptor: Receptor subtype- and synapse-specific modulation. *The Journal of Physiology*. 2009; 587:2115–2125. [PubMed: 19332484]

- Houston CM, Lee HHC, Hosie AM, Moss SJ, Smart TG. Identification of the sites for CaMK-II-dependent phosphorylation of GABA<sub>A</sub> receptors. *Journal of Biological Chemistry*. 2007; 282:17855–17865. [PubMed: 17442679]
- Houston CM, Smart TG. CaMK-II modulation of GABA<sub>A</sub> receptors expressed in HEK293, NG108-15 and rat cerebellar granule neurons. *European Journal of Neuroscience*. 2006; 24:2504–2514. [PubMed: 17100839]
- Ip FC, Cheung J, Ip NY. The expression profiles of neurotrophins and their receptors in rat and chicken tissues during development. *Neuroscience Letters*. 2001; 301:107–110. [PubMed: 11248434]
- Jacob TC, Moss SJ, Jurd R. GABA<sub>A</sub> receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nature reviews. Neuroscience*. 2008; 9:331–343. [PubMed: 18382465]
- Jacob TC, Wan Q, Vithlani M, Saliba RS, Succol F, Pangalos MN, et al. GABA<sub>A</sub> receptor membrane trafficking regulates spine maturity. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:12500–12505. [PubMed: 19617557]
- Janssen MJ, Ade KK, Fu Z, Vicini S. Dopamine modulation of GABA tonic conductance in striatal output neurons. *Journal of Neuroscience*. 2009; 29:5116–5126. [PubMed: 19386907]
- Janssen M, Yasuda RP, Vicini S. GABA<sub>A</sub> receptor  $\beta$ 3 subunit expression regulates tonic current in developing striatopallidal medium spiny neurons. *Frontiers in Cellular Neuroscience*. 2011; 5:15. [PubMed: 21847370]
- Joshi S, Kapur J. Slow intracellular accumulation of GABA<sub>A</sub> receptor  $\delta$  subunit is modulated by BDNF. *Neuroscience*. 2009; 164:507–519. [PubMed: 19665523]
- Jovanovic JN, Thomas P, Kittler JT, Smart TG, Moss SJ. Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA<sub>A</sub> receptor phosphorylation, activity, and cell-surface stability. *Journal of Neuroscience*. 2004; 24:522–530. [PubMed: 14724252]
- Jurd R, Tretter V, Walker J, Brandon NJ, Moss SJ. Fyn kinase contributes to tyrosine phosphorylation of the GABA<sub>A</sub> receptor  $\gamma$ 2 subunit. *Molecular and cellular neurosciences*. 2010; 44:129–134. [PubMed: 20233604]
- Kalivas PW. Neurobiology of cocaine addiction: Implications for new pharmacotherapy. *American Journal on Addictions*. 2007; 16:71–78. [PubMed: 17453607]
- Kanematsu T, Fujii M, Mizokami A, Kittler JT, Nabekura J, Moss SJ, et al. Phospholipase C-related inactive protein is implicated in the constitutive internalization of GABA<sub>A</sub> receptors mediated by clathrin and AP2 adaptor complex. *Journal of Neurochemistry*. 2007; 101:898–905. [PubMed: 17254016]
- Kanematsu T, Mizokami A, Watanabe K, Hirata M. Regulation of GABA<sub>A</sub>-receptor surface expression with special reference to the involvement of GABARAP (GABA<sub>A</sub> receptor-associated protein) and PRIP (phospholipase C-related, but catalytically inactive protein). *Journal of Pharmacological Sciences*. 2007; 104:285–292. [PubMed: 17690529]
- Kanematsu T, Yasunaga A, Mizoguchi Y, Kuratani A, Kittler JT, Jovanovic JN, et al. Modulation of GABA<sub>A</sub> receptor phosphorylation and membrane trafficking by phospholipase C-related inactive protein/protein phosphatase 1 and 2A signaling complex underlying brain-derived neurotrophic factor-dependent regulation of GABAergic inhibition. *Journal of Biological Chemistry*. 2006; 281:22180–22189. [PubMed: 16754670]
- Kanematsu T, Yoshimura K, Hidaka K, Takeuchi H, Katan M, Hirata M. Domain organization of p130, PLC-related catalytically inactive protein, and structural basis for the lack of enzyme activity. *European Journal of Biochemistry*. 2000; 267:2731–2737. [PubMed: 10785396]
- Kang SU, Heo S, Lubec G. Mass spectrometric analysis of GABA<sub>A</sub> receptor subtypes and phosphorylations from mouse hippocampus. *Proteomics*. 2011; 11:2171–2181. [PubMed: 21538884]
- Kano M, Kano M, Fukunaga K, Konnerth A. Ca<sup>2+</sup>-induced rebound potentiation of  $\gamma$ -aminobutyric acid-mediated currents requires activation of Ca<sup>2+</sup>/calmodulin-dependent kinase II. *Proceedings of the National Academy of Sciences*. 1996; 93:13351–13356.
- Kano M, Konnerth A. Potentiation of GABA-mediated currents by cAMP-dependent protein kinase. *Neuroreport*. 1992; 3:563–566. [PubMed: 1421107]

- Kawaguchi S, Hirano T. Signaling cascade regulating long-term potentiation of GABA<sub>A</sub> receptor responsiveness in cerebellar Purkinje neurons. *Journal of Neuroscience*. 2002; 22:3969–3976. [PubMed: 12019316]
- Kawaguchi, S-y, Hirano, T. Sustained structural change of GABA<sub>A</sub> receptor-associated protein underlies long-term potentiation at inhibitory synapses on a cerebellar purkinje neuron. *The Journal of Neuroscience*. 2007; 27:6788–6799. [PubMed: 17581966]
- Kellenberger S, Malherbe P, Sigel E. Function of the alpha 1 beta 2 gamma 2S gamma-aminobutyric acid type A receptor is modulated by protein kinase C via multiple phosphorylation sites. *Journal of Biological Chemistry*. 1992; 267:25660–25663. [PubMed: 1334482]
- Kia A, Ribeiro F, Nelson R, Gavrilovici C, Ferguson SSG, Poulter MO. Kindling alters neurosteroid-induced modulation of phasic and tonic GABA<sub>A</sub> receptor-mediated currents: Role of phosphorylation. *Journal of Neurochemistry*. 2011; 116:1043–1056. [PubMed: 21175618]
- Kittler JT, Chen G, Honing S, Bogdanov Y, McAinsh K, Arancibia-Carcamo IL, et al. Phospho-dependent binding of the clathrin AP2 adaptor complex to GABA<sub>A</sub> receptors regulates the efficacy of inhibitory synaptic transmission. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:14871–14876. [PubMed: 16192353]
- Kittler JT, Chen G, Kukhtina V, Vahedi-Faridi A, Gu Z, Tretter V, et al. Regulation of synaptic inhibition by phospho-dependent binding of the AP2 complex to a YECL motif in the GABA<sub>A</sub> receptor gamma 2 subunit. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:3616–3621. [PubMed: 18305175]
- Kittler JT, Delmas P, Jovanovic JN, Brown DA, Smart TG, Moss SJ. Constitutive endocytosis of GABA<sub>A</sub> receptors by an association with the Adaptin AP2 complex modulates inhibitory synaptic currents in hippocampal neurons. *Journal of Neuroscience*. 2000; 20:7972–7977. [PubMed: 11050117]
- Kittler JT, Moss SJ. Modulation of GABA<sub>A</sub> receptor activity by phosphorylation and receptor trafficking: Implications for the efficacy of synaptic inhibition. *Current Opinion in Neurobiology*. 2003; 13:341–347. [PubMed: 12850219]
- Kittler JT, Rostaing P, Schiavo G, Fritschy JM, Olsen R, Triller A, et al. The subcellular distribution of GABARAP and its ability to interact with NSF suggest a role for this protein in the intracellular transport of GABA<sub>A</sub> receptors. *Molecular and Cellular Neuroscience*. 2001; 18:13–25. [PubMed: 11461150]
- Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, Scott JD. Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. *Science (New York, NY)*. 1996; 271:1589–1592.
- Klein R, Nanduri V, Jing S, Lamballe F, Tapley P, Bryant S, et al. The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell*. 1991; 66:395–403. [PubMed: 1649702]
- Kneussel M, Loeblich S. Trafficking and synaptic anchoring of ionotropic inhibitory neurotransmitter receptors. *Biology of the Cell/Under the Auspices of the European Cell Biology Organization*. 2007; 99:297–309.
- Koksma JJ, van Kesteren RE, Rosahl TW, Zwart R, Smit AB, Luddens H, et al. Oxytocin regulates neurosteroid modulation of GABA<sub>A</sub> receptors in supraoptic nucleus around parturition. *Journal of Neuroscience*. 2003; 23:788–797. [PubMed: 12574407]
- Korpi ER, Grunder G, Luddens H. Drug interactions at GABA<sub>A</sub> receptors. *Progress in Neurobiology*. 2002; 67:113–159. [PubMed: 12126658]
- Kowalczyk S, Winkelmann A, Smolinsky B, Foerster B, Neundorff I, Schwarz G, et al. Direct binding of GABA<sub>A</sub> receptor beta 2 and beta 3 subunits to gephyrin. *European Journal of Neuroscience*. 2013; 37:544–554. [PubMed: 23205938]
- Krishek BJ, Xie X, Blackstone C, Haganir RL, Moss SJ, Smart TG. Regulation of GABA<sub>A</sub> receptor function by protein kinase C phosphorylation. *Neuron*. 1994; 12:1081–1095. [PubMed: 8185945]
- Krystal JH, Staley J, Mason G, Petrakis IL, Kaufman J, Harris RA, et al. gamma-Aminobutyric acid type A receptors and alcoholism - Intoxication, dependence, vulnerability, and treatment. *Archives of General Psychiatry*. 2006; 63:957–968. [PubMed: 16952998]

- Kuczewski N, Fuchs C, Ferrand N, Jovanovic JN, Gaiarsa JL, Porcher C. Mechanism of GABAB receptor-induced BDNF secretion and promotion of GABA<sub>A</sub> receptor membrane expression. *Journal of Neurochemistry*. 2011; 118:533–545. [PubMed: 21255015]
- Kumar S, Kralic JE, O'Buckley TK, Grobin AC, Morrow AL. Chronic ethanol consumption enhances internalization of alpha 1 subunit-containing GABA<sub>A</sub> receptors in cerebral cortex. *Journal of Neurochemistry*. 2003; 86:700–708. [PubMed: 12859683]
- Kumar S, Sieghart W, Morrow AL. Association of protein kinase C with GABA<sub>A</sub> receptors containing alpha 1 and alpha 4 subunits in the cerebral cortex: Selective effects of chronic ethanol consumption. *Journal of Neurochemistry*. 2002; 82:110–117. [PubMed: 12091471]
- Kumar S, Suryanarayanan A, Boyd KN, Comerford CE, Lai MA, Ren Q, et al. Ethanol reduces GABA<sub>A</sub> alpha 1 subunit receptor surface expression by a protein kinase C gamma-dependent mechanism in cultured cerebral cortical neurons. *Molecular Pharmacology*. 2010; 77:793–803. [PubMed: 20159950]
- Kurz JE, Sheets D, Parsons JT, Rana A, Delorenzo RJ, Churn SB. A significant increase in both basal and maximal calcineurin activity in the rat pilocarpine model of status epilepticus. *Journal of Neurochemistry*. 2001; 78:304–315. [PubMed: 11461966]
- Lambert JJ, Cooper MA, Simmons RDJ, Weir CJ, Belelli D. Neurosteroids: Endogenous allosteric modulators of GABA<sub>A</sub> receptors. *Psychoneuroendocrinology*. 2009; 34(1 Supplement):S48–S58. [PubMed: 19758761]
- Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen GABA<sub>A</sub> receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *Journal of Neuroscience*. 1992; 12:4151–4172. [PubMed: 1331359]
- LeBlanc AE, Kalant H, Gibbins RJ. Acute tolerance to ethanol in the rat. *Psychopharmacologia*. 1975; 41:43–46. [PubMed: 1124268]
- Lee HK. Synaptic plasticity and phosphorylation. *Pharmacology & Therapeutics*. 2006; 112:810–832. [PubMed: 16904750]
- Leidenheimer NJ, Chapell R. Effects of PKC activation and receptor desensitization on neurosteroid modulation of GABA<sub>A</sub> receptors. *Molecular Brain Research*. 1997; 52:173–181. [PubMed: 9495538]
- Leidenheimer NJ, McQuilkin SJ, Hahner LD, Whiting P, Harris RA. Activation of protein kinase C selectively inhibits the gamma-aminobutyric acidA receptor: Role of desensitization. *Molecular Pharmacology*. 1992; 41:1116–1123. [PubMed: 1319547]
- Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I. Mechanisms of reversible GABA<sub>A</sub> receptor plasticity after ethanol intoxication. *The Journal of Neuroscience*. 2007; 27:12367–12377. [PubMed: 17989301]
- Liang J, Zhang N, Cagetti E, Houser CR, Olsen RW, Spigelman I. Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal GABA<sub>A</sub> receptors. *The Journal of Neuroscience*. 2006; 26:1749–1758. [PubMed: 16467523]
- Lilly SM, Alvarez FJ, Tietz EI. Synaptic and subcellular localization of A-kinase anchoring protein 150 in rat hippocampal CA1 pyramidal cells: Co-localization with excitatory synaptic markers. *Neuroscience*. 2005; 134:155–163. [PubMed: 15951119]
- Lin YF, Angelotti TP, Dudek EM, Browning MD, Macdonald RL. Enhancement of recombinant alpha 1 beta 1 gamma 2 L gamma-aminobutyric acidA receptor whole-cell currents by protein kinase C is mediated through phosphorylation of both beta 1 and gamma 2 L subunits. *Molecular Pharmacology*. 1996; 50:185–195. [PubMed: 8700112]
- Lin YF, Browning MD, Dudek EM, Macdonald RL. Protein kinase C enhances recombinant bovine  $\alpha 1\beta 1\gamma 2L$  GABA<sub>A</sub> receptor whole-cell currents expressed in L929 fibroblasts. *Neuron*. 1994; 13:1421–1431. [PubMed: 7993633]
- Lipton P. Ischemic cell death in brain neurons. *Physiological Reviews*. 1999; 79:1431–1568. [PubMed: 10508238]
- Liu B, Li L, Zhang Q, Chang N, Wang D, Shan Y, et al. Preservation of GABA<sub>A</sub> receptor function by PTEN inhibition protects against neuronal death in ischemic. *Stroke*. 2010; 41:1018–1026. [PubMed: 20360540]

- Lo EH, Dalkara T, Moskowitz MA. Mechanisms, challenges and opportunities in stroke. *Nature Reviews Neuroscience*. 2003; 4:399–414. [PubMed: 12728267]
- Loup F, Wieser HG, Yonekawa Y, Aguzzi A, Fritschy JM. Selective alterations in GABA<sub>A</sub> receptor subtypes in human temporal lobe epilepsy. *The Journal of Neuroscience*. 2000; 20:5401–5419. [PubMed: 10884325]
- Lu H, Cheng P-l, Lim BK, Khoshnevisrad N, Poo M-m. Elevated BDNF after cocaine withdrawal facilitates LTP in medial prefrontal cortex by suppressing GABA inhibition. *Neuron*. 2010; 67:821–833. [PubMed: 20826313]
- Lu YM, Mansuy IM, Kandel ER, Roder J. Calcineurin-mediated LTD of GABAergic inhibition underlies the increased excitability of CA1 neurons associated with LTP. *Neuron*. 2000; 26:197–205. [PubMed: 10798404]
- Ludvig N, George MA, Tang HM, Gonzales RA, Bungay PM. Evidence for the ability of hippocampal neurons to develop acute tolerance to ethanol in behaving rats. *Brain Research*. 2001; 900:252–260. [PubMed: 11334805]
- Luscher B, Fuchs T, Kilpatrick CL. GABA<sub>A</sub>R trafficking-mediated plasticity of inhibitory synapses. *Neuron*. 2011; 70:385–409. [PubMed: 21555068]
- Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. *Molecular Psychiatry*. 2011; 16:383–406. [PubMed: 21079608]
- Lydiard RB. The role of GABA in anxiety disorders. *The Journal of Clinical Psychiatry*. 2003; 64(Suppl 3):21–27. [PubMed: 12662130]
- Macdonald RL, Olsen RW. Gaba<sub>A</sub> receptor channels. *Annual Review of Neuroscience*. 1994; 17:569–602.
- MacDonald RL, Rogers CJ, Twyman RE. Barbiturate regulation of kinetic properties of the GABA<sub>A</sub> receptor channel of mouse spinal neurones in culture. *Journal of Physiology*. 1989; 417:483–500. [PubMed: 2482885]
- Malenka RC, Bear MF. LTP and LTD: An embarrassment of riches. *Neuron*. 2004; 44:5–21. [PubMed: 15450156]
- Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science*. 2002; 298:1912–1934. [PubMed: 12471243]
- Marsden KC, Beattie JB, Friedenthal J, Carroll RC. NMDA receptor activation potentiates inhibitory transmission through GABA receptor-associated protein-dependent exocytosis of GABA<sub>A</sub> receptors. *Journal of Neuroscience*. 2007; 27:14326–14337. [PubMed: 18160640]
- Marsden KC, Shemesh A, Bayer KU, Carroll RC. Selective translocation of Ca<sup>2+</sup>/calmodulin protein kinase II alpha (CaMKII alpha) to inhibitory synapses. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:20559–20564. [PubMed: 21059908]
- McDonald BJ, Amato A, Connolly CN, Benke D, Moss SJ, Smart TG. Adjacent phosphorylation sites on GABA<sub>A</sub> receptor  $\beta$  subunits determine regulation by cAMP-dependent protein kinase. *Nature Neuroscience*. 1998; 1:23–28. [PubMed: 10195104]
- McDonald BJ, Moss SJ. Differential phosphorylation of intracellular domains of gamma-aminobutyric acid type A receptor subunits by calcium/calmodulin type 2-dependent protein kinase and cGMP-dependent protein kinase. *Journal of Biological Chemistry*. 1994; 269:18111–18117. [PubMed: 8027073]
- McDonald BJ, Moss SJ. Conserved phosphorylation of the intracellular domains of GABA<sub>A</sub> receptor  $\beta$ 2 and  $\beta$ 3 subunits by cAMP-dependent protein kinase, cGMP-dependent protein kinase, protein kinase C and Ca<sup>2+</sup>/calmodulin type II-dependent protein kinase. *Neuropharmacology*. 1997; 36:1377–1385. [PubMed: 9423925]
- McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nature Reviews Molecular Cell Biology*. 2011; 12:517–533. [PubMed: 21779028]
- Mele M, Ribeiro L, Inacio AR, Wieloch T, Duarte CB. GABA<sub>A</sub> receptor dephosphorylation followed by internalization is coupled to neuronal death in in vitro ischemia. *Neurobiology of Disease*. 2014; 65:220–232. [PubMed: 24513087]
- Mercik K, Pytel M, Mozrzymas JW. Recombinant  $\alpha$ 1 $\beta$ 2 $\gamma$ 2 GABA<sub>A</sub> receptors expressed in HEK293 and in QT6 cells show different kinetics. *Neuroscience Letters*. 2003; 352:195–198. [PubMed: 14625018]

- Mielke JG, Wang YT. Insulin exerts neuroprotection by counteracting the decrease in cell-surface GABA<sub>A</sub> receptors following oxygen-glucose deprivation in cultured cortical neurons. *Journal of Neurochemistry*. 2005; 92:103–113. [PubMed: 15606900]
- Mizoguchi Y, Ishibashi H, Nabekura J. The action of BDNF on GABA<sub>A</sub> currents changes from potentiating to suppressing during maturation of rat hippocampal CA1 pyramidal neurons. *Journal of Physiology-London*. 2003; 548:703–709.
- Mochly-Rosen D, Khaner H, Lopez J. Identification of intracellular receptor proteins for activated protein kinase C. *Proceedings of the National Academy of Sciences of the United States of America*. 1991; 88:3997–4000. [PubMed: 1850844]
- Moss SJ, Doherty CA, Haganir RL. Identification of the cAMP-dependent protein kinase and protein kinase C phosphorylation sites within the major intracellular domains of the beta 1, gamma 2S, and gamma 2 L subunits of the gamma-aminobutyric acid type A receptor. *Journal of Biological Chemistry*. 1992; 267:14470–14476. [PubMed: 1321150]
- Moss SJ, Gorrie GH, Amato A, Smart TG. Modulation of GABA<sub>A</sub> receptors by tyrosine phosphorylation. *Nature*. 1995; 377:344–348. [PubMed: 7566089]
- Moss SJ, Smart TG. Modulation of amino acid-gated ion channels by protein phosphorylation. *International Review of Neurobiology*. 1996; 39:1–52. [PubMed: 8894843]
- Moss SJ, Smart TG, Blackstone CD, Haganir RL. Functional modulation of GABA<sub>A</sub> receptors by cAMP-dependent protein phosphorylation. *Science (New York, NY)*. 1992; 257:661–665.
- Muir J, Arancibia-Carcamo IL, MacAskill AF, Smith KR, Griffin LD, Kittler JT. NMDA receptors regulate GABA<sub>A</sub> receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the gamma 2 subunit. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:16679–16684. [PubMed: 20823221]
- Mukherjee J, Kretschmannova K, Gouzer G, Maric HM, Ramsden S, Tretter V, et al. The residence time of GABA<sub>A</sub>Rs at inhibitory synapses is determined by direct binding of the receptor alpha 1 subunit to gephyrin. *Journal of Neuroscience*. 2011; 31:14677–14687. [PubMed: 21994384]
- Naylor DE, Liu H, Niquet J, Wasterlain CG. Rapid surface accumulation of NMDA receptors increases glutamatergic excitation during status epilepticus. *Neurobiology of Disease*. 2013; 54:225–238. [PubMed: 23313318]
- Naylor DE, Liu HT, Wasterlain CG. Trafficking of GABA<sub>A</sub> receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *Journal of Neuroscience*. 2005; 25:7724–7733. [PubMed: 16120773]
- Nusser Z, Mody I. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *Journal of Neurophysiology*. 2002; 87:2624–2628. [PubMed: 11976398]
- Nusser Z, Sieghart W, Mody I. Differential regulation of synaptic GABA<sub>A</sub> receptors by cAMP-dependent protein kinase in mouse cerebellar and olfactory bulb neurones. *The Journal of Physiology*. 1999; 521:421–435. [PubMed: 10581313]
- Olive MF, Mehmert KK, Messing RO, Hodge CW. Reduced operant ethanol self-administration and in vivo mesolimbic dopamine responses to ethanol in PKCepsilon-deficient mice. *The European journal of neuroscience*. 2000; 12:4131–4140. [PubMed: 11069609]
- Olsen RW, Sieghart W. International union of pharmacology. LXX. Subtypes of gamma-aminobutyric acid A receptors: Classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacological Reviews*. 2008; 60:243–260. [PubMed: 18790874]
- Owens DF, Boyce LH, Davis MBE, Kriegstein AR. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. *The Journal of Neuroscience*. 1996; 16:6414–6423. [PubMed: 8815920]
- Petrini EM, Ravasenga T, Hausrat TJ, Iurilli G, Olcese U, Racine V, et al. Synaptic recruitment of gephyrin regulates surface GABA<sub>A</sub> receptor dynamics for the expression of inhibitory LTP. *Nature Communications*. 2014; 5
- Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. GABA<sub>A</sub> receptors: Immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience*. 2000; 101:815–850. [PubMed: 11113332]

- Poisbeau P, Cheney MC, Browning MD, Mody I. Modulation of synaptic GABA<sub>A</sub> receptor function by PKA and PKC in adult hippocampal neurons. *The Journal of Neuroscience*. 1999; 19:674–683. [PubMed: 9880588]
- Porcher C, Hatchett C, Longbottom RE, McAinch K, Sihra TS, Moss SJ, et al. Positive feedback regulation between  $\gamma$ -aminobutyric acid type A (GABA<sub>A</sub>) receptor signaling and brain-derived neurotrophic factor (BDNF) release in developing neurons. *Journal of Biological Chemistry*. 2011; 286:21667–21677. [PubMed: 21474450]
- Porter NM, Twyman RE, Uhler MD, Macdonald RL. Cyclic AMP-dependent protein kinase decreases GABA<sub>A</sub> receptor current in mouse spinal neurons. *Neuron*. 1990; 5:789–796. [PubMed: 2176510]
- Qi ZH, Song M, Wallace MJ, Wang D, Newton PM, McMahon T, et al. Protein kinase C epsilon regulates gamma-aminobutyrate type A receptor sensitivity to ethanol and benzodiazepines through phosphorylation of gamma 2 subunits. *Journal of Biological Chemistry*. 2007; 282:33052–33063. [PubMed: 17875639]
- Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, et al. BDNF-induced TrkB activation down-regulates the K<sup>+</sup>-Cl<sup>-</sup> cotransporter KCC2 and impairs neuronal Cl<sup>-</sup> extrusion. *The Journal of Cell Biology*. 2002; 159:747–752. [PubMed: 12473684]
- Robello M, Amico C, Cupello A. Regulation of GABA<sub>A</sub> receptor in cerebellar granule cells in culture: Differential involvement of kinase activities. *Neuroscience*. 1993; 53:131–138. [PubMed: 7682295]
- Ron D, Chen CH, Caldwell J, Jamieson L, Orr E, Mochly-Rosen D. Cloning of an intracellular receptor for protein kinase C: A homolog of the beta subunit of G proteins. *Proceedings of the National Academy of Sciences*. 1994; 91:839–843.
- Rudolph U, Knoflach F. Beyond classical benzodiazepines: Novel therapeutic potential of GABA<sub>A</sub> receptor subtypes. *Nature Reviews Drug Discovery*. 2011; 10:685–697. [PubMed: 21799515]
- Rudolph U, Mohler H. Analysis of GABA<sub>A</sub> receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annual Review of Pharmacology and Toxicology*. 2004; 44:475–498.
- Saiepour L, Fuchs C, Patrizi A, Sassoè-Pognetto M, Harvey RJ, Harvey K. Complex role of collybistin and gephyrin in GABA<sub>A</sub> receptor clustering. *Journal of Biological Chemistry*. 2010; 285:29623–29631. [PubMed: 20622020]
- Saliba RS, Kretschmannova K, Moss SJ. Activity-dependent phosphorylation of GABA<sub>A</sub> receptors regulates receptor insertion and tonic current. *Embo Journal*. 2012; 31:2937–2951. [PubMed: 22531784]
- Sanderson JL, Dell'Acqua ML. AKAP signaling complexes in regulation of excitatory synaptic plasticity. *The Neuroscientist*. 2011; 17:321–336. [PubMed: 21498812]
- Sathanoori M, Dias BG, Nair AR, Banerjee SB, Tole S, Vaidya VA. Differential regulation of multiple brain-derived neurotrophic factor transcripts in the postnatal and adult rat hippocampus during development, and in response to kainate administration. *Molecular Brain Research*. 2004; 130:170–177. [PubMed: 15519687]
- Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2000; 103:211–225. [PubMed: 11057895]
- Schwartz-Bloom RD, Sah R.  $\gamma$ -Aminobutyric acidA neurotransmission and cerebral ischemia. *Journal of Neurochemistry*. 2001; 77:353–371. [PubMed: 11299298]
- Seil FJ. TrkB receptor signaling and activity-dependent inhibitory synaptogenesis. *Histology and Histopathology*. 2003; 18:635–646. [PubMed: 12647813]
- Shen Y, Lindemeyer AK, Spigelman I, Sieghart W, Olsen RW, Liang J. Plasticity of GABA<sub>A</sub> receptors after ethanol pre-exposure in cultured hippocampal neurons. *Molecular Pharmacology*. 2011; 79:432–442. [PubMed: 21163967]
- Shulga A, Thomas-Crusells J, Sigl T, Blaesse A, Mestres P, Meyer M, et al. Posttraumatic GABA<sub>A</sub>-mediated [Ca<sup>2+</sup>]<sub>i</sub> increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2008; 28:6996–7005. [PubMed: 18596173]



- Sigel E, Baur R. Activation of protein kinase C differentially modulates neuronal Na<sup>+</sup>, Ca<sup>2+</sup>, and gamma-aminobutyrate type A channels. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:6192–6196. [PubMed: 2457909]
- Smith KR, Muir J, Rao Y, Browarski M, Gruenig MC, Sheehan DF, et al. Stabilization of GABA<sub>A</sub> receptors at endocytic zones is mediated by an AP2 binding motif within the GABA<sub>A</sub> receptor β3 subunit. *The Journal of Neuroscience*. 2012; 32:2485–2498. [PubMed: 22396422]
- Song M, Messing RO. Protein kinase C regulation of GABA<sub>A</sub> receptors. *Cellular and Molecular Life Sciences: CMLS*. 2005; 62:119–127. [PubMed: 15666084]
- Sperk G, Drexel M, Pirker S. Neuronal plasticity in animal models and the epileptic human hippocampus. *Epilepsia*. 2009; 50:29–31.
- Tan KR, Rudolph U, Lüschner C. Hooked on benzodiazepines: GABA<sub>A</sub> receptor subtypes and addiction. *Trends in Neurosciences*. 2011; 34:188–197. [PubMed: 21353710]
- Tanaka C, Nishizuka Y. The protein kinase C family for neuronal signaling. *Annual Review of Neuroscience*. 1994; 17:551–567.
- Tang X, Hernandez CC, Macdonald RL. Modulation of spontaneous and GABA-evoked tonic α4β3δ and α4β3γ2L GABA<sub>A</sub> receptor currents by protein kinase A. *Journal of Neurophysiology*. 2010; 103:1007–1019. [PubMed: 19939957]
- Taylor SS, Buechler JA, Yonemoto W. Camp-dependent protein kinase: Framework for a diverse family of regulatory enzymes. *Annual Review of Biochemistry*. 1990; 59:971–1005.
- Taylor SS, Knighton DR, Zheng J, Ten Eyck LF, Sowadski JM. Structural framework for the protein kinase family. *Annual Review of Cell Biology*. 1992; 8:429–462.
- Terunuma M, Jang IS, Ha SH, Kittler JT, Kanematsu T, Jovanovic JN, et al. GABA<sub>A</sub> receptor phospho-dependent modulation is regulated by phospholipase C-related inactive protein type 1, a novel protein phosphatase 1 anchoring protein. *Journal of Neuroscience*. 2004; 24:7074–7084. [PubMed: 15306641]
- Terunuma M, Xu J, Vitlhani M, Sieghart W, Kittler J, Pangalos M, et al. Deficits in phosphorylation of GABA<sub>A</sub> receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. *Journal of Neuroscience*. 2008; 28:376–384. [PubMed: 18184780]
- Thompson-Vest NM, Waldvogel HJ, Rees MI, Faull RLM. GABA<sub>A</sub> receptor subunit and gephyrin protein changes differ in the globus pallidus in Huntington's diseased brain. *Brain Research*. 2003; 994:265–270. [PubMed: 14642653]
- Tretter V, Kerschner B, Milenkovic I, Ramsden SL, Ramerstorfer J, Saiepour L, et al. Molecular basis of the γ-aminobutyric acid a receptor α3 subunit interaction with the clustering protein gephyrin. *Journal of Biological Chemistry*. 2011; 286:37702–37711. [PubMed: 21880742]
- Tretter VE, Mukherjee J, Maric HM, Schindelin H, Sieghart W, Moss SJ. Gephyrin, the enigmatic organizer at GABAergic synapses. *Frontiers in Cellular Neuroscience*. 2012; 6:23. [PubMed: 22615685]
- Tretter V, Revilla-Sanchez R, Houston C, Terunuma M, Havekes R, Florian C, et al. Deficits in spatial memory correlate with modified gamma-aminobutyric acid type A receptor tyrosine phosphorylation in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:20039–20044. [PubMed: 19903874]
- Trudell JR, Messing RO, Mayfield J, Harris RA. Alcohol dependence: Molecular and behavioral evidence. *Trends in Pharmacological Sciences*. 2014; 35:317–323. [PubMed: 24865944]
- Tyagarajan SK, Fritschy JM. Gephyrin: A master regulator of neuronal function? *Nature Reviews Neuroscience*. 2014; 15:141–156. [PubMed: 24552784]
- Ubersax JA, Ferrell JE Jr. Mechanisms of specificity in protein phosphorylation. *Nature Reviews Molecular Cell Biology*. 2007; 8:530–541. [PubMed: 17585314]
- Verdoorn TA, Draguhn A, Ymer S, Seeburg PH, Sakmann B. Functional properties of recombinant rat GABA<sub>A</sub> receptors depend upon subunit composition. *Neuron*. 1990; 4:919–928. [PubMed: 1694446]
- Vetiska SM, Ahmadian G, Ju W, Liu L, Wymann MP, Wang YT. GABA<sub>A</sub> receptor-associated phosphoinositide 3-kinase is required for insulin-induced recruitment of postsynaptic GABA<sub>A</sub> receptors. *Neuropharmacology*. 2007; 52:146–155. [PubMed: 16890252]

- Vicario-Abejón C, Collin C, McKay RDG, Segal M. Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *The Journal of Neuroscience*. 1998; 18:7256–7271. [PubMed: 9736647]
- Vicini S, Losi G, Homanics GE. GABA<sub>A</sub> receptor delta subunit deletion prevents neurosteroid modulation of inhibitory synaptic currents in cerebellar neurons. *Neuropharmacology*. 2002; 43:646–650. [PubMed: 12367609]
- Vithlani M, Hines RM, Zhong P, Terunuma M, Hines DJ, Revilla-Sanchez R, et al. The ability of BDNF to modify neurogenesis and depressive-like BEHAVIORS Is dependent UPON phosphorylation of tyrosine residues 365/367 in the GABA<sub>A</sub>-receptor gamma 2 subunit. *Journal of Neuroscience*. 2013; 33:15567–15577. [PubMed: 24068823]
- Vithlani M, Moss Stephen J. The role of GABA<sub>A</sub>R phosphorylation in the construction of inhibitory synapses and the efficacy of neuronal inhibition. *Biochemical Society Transactions*. 2009; 37:1355. [PubMed: 19909275]
- Wallace MJ, Newton PM, Oyasu M, McMahon T, Chou WH, Connolly J, et al. Acute functional tolerance to ethanol mediated by protein kinase C epsilon. *Neuropsychopharmacology*. 2007; 32:127–136. [PubMed: 16541084]
- Wan Q, Xiong ZG, Man HY, Ackerley CA, Braunton J, Lu WY, et al. Recruitment of functional GABA<sub>A</sub> receptors to postsynaptic domains by insulin. *Nature*. 1997; 388:686–690. [PubMed: 9262404]
- Wang A, Chi Z, Wang S, Wang S, Sun Q. Calcineurin-mediated GABA<sub>A</sub> receptor dephosphorylation in rats after kainic acid-induced status epilepticus. *Seizure*. 2009; 18:519–523. [PubMed: 19497770]
- Wang J, Liu S, Haditsch U, Tu W, Cochrane K, Ahmadian G, et al. Interaction of calcineurin and type-A GABA receptor  $\gamma$ 2 subunits produces long-term depression at CA1 inhibitory synapses. *The Journal of Neuroscience*. 2003; 23:826–836. [PubMed: 12574411]
- Wang Q, Liu L, Pei L, Ju W, Ahmadian G, Lu J, et al. Control of synaptic strength, a novel function of Akt. *Neuron*. 2003; 38:915–928. [PubMed: 12818177]
- Wang X, Zhong P, Yan Z. Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. *The Journal of Neuroscience*. 2002; 22:9185–9193. [PubMed: 12417643]
- Wardle RA, Poo MM. Brain-derived neurotrophic factor modulation of GABAergic synapses by postsynaptic regulation of chloride transport. *The Journal of Neuroscience*. 2003; 23:8722–8732. [PubMed: 14507972]
- Webster MJ, Herman MM, Kleinman JE, Shannon Weickert C. BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. *Gene Expression Patterns*. 2006; 6:941–951. [PubMed: 16713371]
- Werner DF, Kumar S, Criswell HE, Suryanarayanan A, Fetzer JA, Comerford CE, et al. PKC gamma is required for ethanol-induced increases in GABA<sub>A</sub> receptor alpha 4 subunit expression in cultured cerebral cortical neurons. *Journal of Neurochemistry*. 2011; 116:554–563. [PubMed: 21155805]
- Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser IDC, Langeberg LK, et al. Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science*. 1999; 285:93–96. [PubMed: 10390370]
- Whiting P, McKernan RM, Iversen LL. Another mechanism for creating diversity in gamma-aminobutyrate type A receptors: RNA splicing directs expression of two forms of gamma 2 phosphorylation site. *Proceedings of the National Academy of Sciences*. 1990; 87:9966–9970.
- Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 GABA<sub>A</sub> receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *The Journal of Neuroscience*. 1992; 12:1040–1062. [PubMed: 1312131]
- Yan Z, Surmeier DJ. D5 dopamine receptors enhance Zn<sup>2+</sup>-sensitive GABA<sub>A</sub> currents in striatal cholinergic interneurons through a PKA/PP1 cascade. *Neuron*. 1997; 19:1115–1126. [PubMed: 9390524]
- Yang SN, Tang YG, Zucker RS. Selective induction of LTP and LTD by postsynaptic [Ca<sup>2+</sup>]<sub>i</sub> elevation. *Journal of Neurophysiology*. 1999; 81:781–787. [PubMed: 10036277]

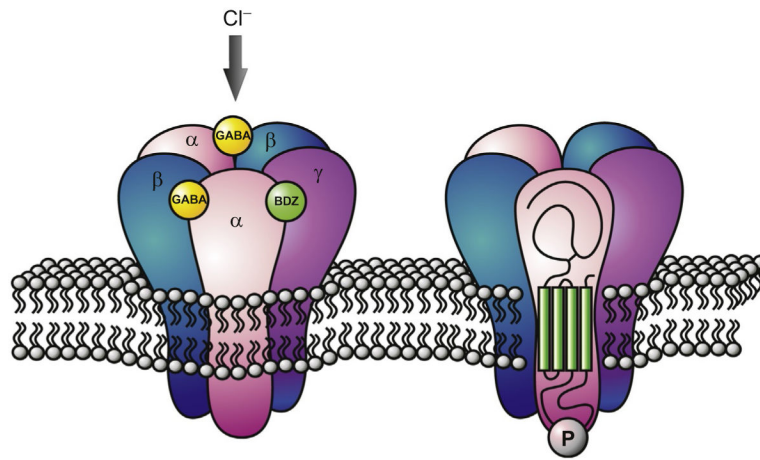
Yoshimura K, Takeuchi H, Sato O, Hidaka K, Doira N, Terunuma M, et al. Interaction of p130 with, and consequent inhibition of, the catalytic subunit of protein phosphatase 1 $\alpha$ . *Journal of Biological Chemistry*. 2001; 276:17908–17913. [PubMed: 11278544]

Author Manuscript

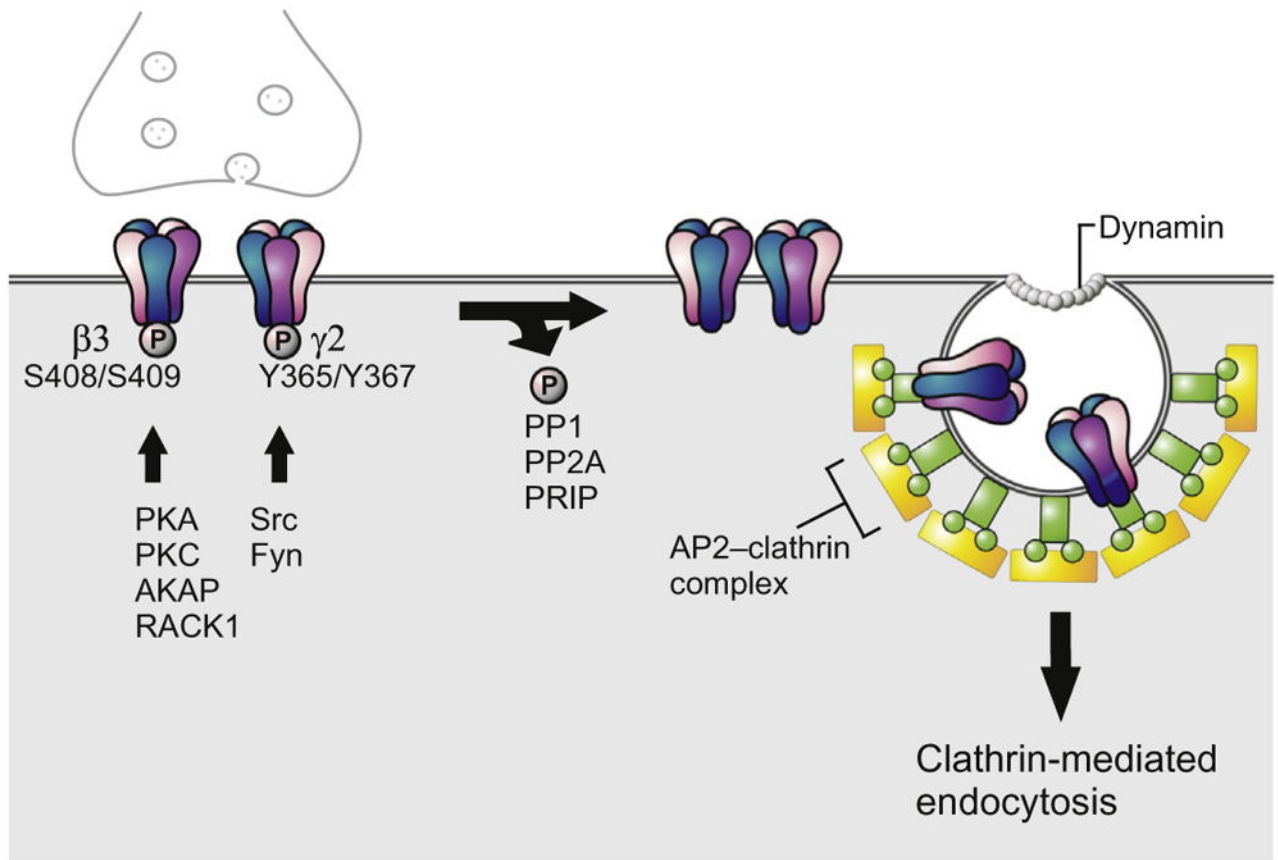
Author Manuscript

Author Manuscript

Author Manuscript

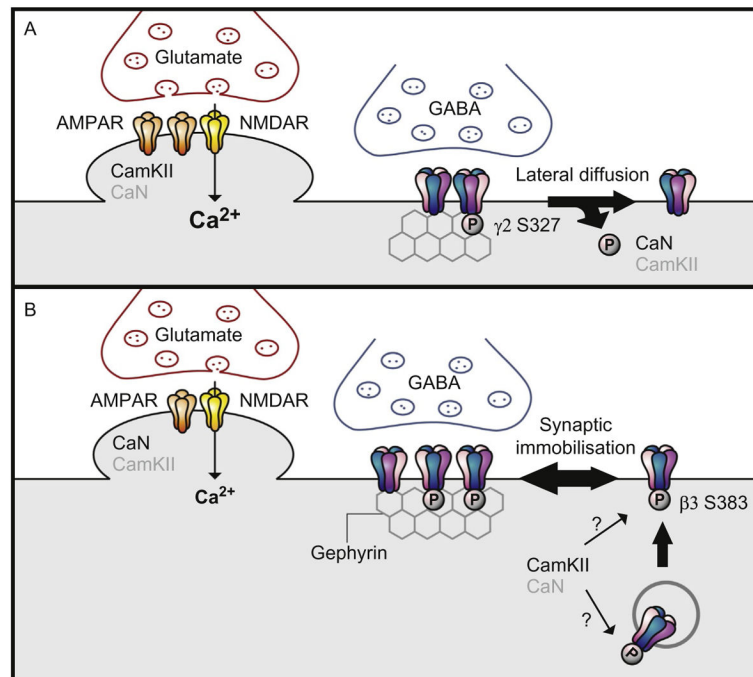


**Figure 1.** Schematic representation of the pentameric GABA<sub>A</sub>R. GABA<sub>A</sub>Rs are composed of a large extracellular N-terminal domain, four-transmembrane domains (TM1–4), and a major intracellular loop between TM3 and TM4 where phosphorylation (Ⓟ) primarily occurs. Binding of regions for benzodiazepines (BDZ) and GABA are depicted.



**Figure 2.**

Phosphoregulation of GABA<sub>A</sub>R endocytosis. The endocytosis of GABA<sub>A</sub>Rs is regulated by the interaction of the AP2 complex with β and γ2 subunits. Phosphorylation of β3 (S408/S409) and γ2 (Y365/Y367) by PKA/PKC and Src/Fyn, respectively, prevents binding to AP2 and thus stabilizes these receptors at the cell surface. Phosphorylation of β1 and β3 subunits is facilitated by AKAP and RACK1. Dephosphorylation of β3 (S408/409) by PP1 and PP2A, and γ2 by an unknown phosphatase enables binding to AP2, triggering dynamin-dependent clathrin-mediated endocytosis. PRIP regulates dephosphorylation through binding PP1/PP2A and β subunits. The phosphorylation state of PRIP1 determines the release of active PP1α.



**Figure 3.**

Phosphoregulation of GABA<sub>A</sub>R trafficking. GABA<sub>A</sub>R are postsynaptically localized through its binding to gephyrin. (A) Strong NMDAR activation results in the influx of Ca<sup>2+</sup>. CamKII activation at excitatory synapses results in an increase of AMPARs at the cell surface. Calcineurin dependent dephosphorylation of GABA<sub>A</sub>R subunit γ2 (S327) enables diffusion of GABA<sub>A</sub>Rs away from the synapse. (B) Weaker stimuli induced by an NMDAR-dependent chemLTD protocol results in a moderate influx of Ca<sup>2+</sup> and CamKII is targeted to inhibitory synapses. CamKII phosphorylation of β3 (S383) is critical for recruitment of gephyrin to synaptic sites, immobilization of GABA<sub>A</sub>Rs at synapses and an enhancement of cell surface levels of GABA<sub>A</sub>Rs.

Table 1

Known phosphorylation sites of GABA<sub>A</sub>Rs subunits

(a)  $\alpha$  and  $\delta$  subunits

Subunit Site	Kinase or phosphatase		Neuron	Effect of phosphorylation, phosphomimetic	Selected publications
	GST	Cell line			
$\alpha$ 1	<input checked="" type="checkbox"/> Akt <input checked="" type="checkbox"/> PKA <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> Src	HEK: $\alpha$ 1 $\beta$ 1 $\gamma$ 2 <input checked="" type="checkbox"/> PKA	FB synaptosome: <input checked="" type="checkbox"/> CamKII	<input checked="" type="checkbox"/> -	HEK (Moss, Smart, et al., 1992) FB (Churn et al., 2002) GST (Moss, Doherty, et al., 1992; Vetiska et al., 2007; Wang, Liu, Pei, et al., 2003)  (Mukherjee et al., 2011)
Putative T375	-	-	-	Hip: Phosphomimetic $\downarrow$ gephyrin binding, $\downarrow$ synaptic clustering, and $\downarrow$ mIPSC amplitude.	
$\alpha$ 4	-	COS7: $\alpha$ 4 $\beta$ 3 <input checked="" type="checkbox"/> PKC	Hip: <input checked="" type="checkbox"/> PKC	COS7 ( $\alpha$ 4 $\beta$ 3): $\uparrow$ surface, $\uparrow$ insertion, $\uparrow$ surface stability, block I <sub>GABA</sub> rundown. Hip: $\uparrow$ surface, $\uparrow$ insertion, $\uparrow$ tonic current.	(Abramian et al., 2014, 2010)
$\delta$	-	COS7: <input checked="" type="checkbox"/> PKC	-	-	(Abramian et al., 2010)

(b)  $\beta$  subunits

Subunit Site	Kinase or phosphatase		Neuron	Effect of phosphorylation	Selected publications
	GST	Cell line			
$\beta$ 1	T227, Y230	-	Hip	-	Mass spectrometry (Kang et al., 2011)
S384	<input checked="" type="checkbox"/> CamKII	-	-	-	GST (McDonald & Moss, 1994)
S409	<input checked="" type="checkbox"/> CamKII <input checked="" type="checkbox"/> PKA <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PKG	HEK: $\alpha$ 1 $\beta$ 1 $\gamma$ 2 <input checked="" type="checkbox"/> PKA; $\alpha$ 1 $\beta$ 1 $\gamma$ 2S/L <input checked="" type="checkbox"/> PKC	SCG	HEK: <input checked="" type="checkbox"/> PKA $\downarrow$ I <sub>GABA</sub> amplitude. HEK: <input checked="" type="checkbox"/> PKC $\downarrow$ I <sub>GABA</sub> amplitude.	GST (McDonald & Moss, 1994; Moss, Doherty, et al., 1992) HEK (Krishhek et al., 1994; Moss, Smart, et al., 1992) SCG (Brandon, Jovanovic, Smart, & Moss, 2002)
$\beta$ 2	-	-	Hip, Ctx: <input checked="" type="checkbox"/> PKC (via BDNF)	Hip, Ctx (via BDNF): <input checked="" type="checkbox"/> PKC no $\oplus$ stability, block I <sub>GABA</sub> rundown. PFC (via 5HT <sub>2</sub> ): <input checked="" type="checkbox"/> PKC no change in $\oplus$	BDNF (Jovanovic et al., 2004) PFC (Feng et al., 2001)
Y215, T439	-	-	Hip	-	Mass spectrometry (Kang et al., 2011)
Y372/Y379	<input checked="" type="checkbox"/> Src	HEK: $\alpha$ 1 $\beta$ 1 $\gamma$ 2	-	HEK (via insulin): $\uparrow$ surface.	(Vetiska et al., 2007)
S410	<input checked="" type="checkbox"/> Akt <input checked="" type="checkbox"/> CamKII <input checked="" type="checkbox"/> PKA <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PKG	HEK: $\alpha$ 1 $\beta$ 2 $\gamma$ 2 <input checked="" type="checkbox"/> Akt, <input checked="" type="checkbox"/> PKA Oocyte: $\alpha$ 1 $\beta$ 2 $\gamma$ 2S <input checked="" type="checkbox"/> PKC HEK: $\alpha$ 4 $\beta$ 26 <input checked="" type="checkbox"/> PKC	Hip: <input checked="" type="checkbox"/> Akt CGC: $\beta$ 2 KO <input checked="" type="checkbox"/> CamKII	GST: Phosphomimetic binds gephyrin. HEK, Hip: <input checked="" type="checkbox"/> Akt $\uparrow$ surface, $\uparrow$ mIPSC amplitude & frequency. Oocyte: <input checked="" type="checkbox"/> PKC $\downarrow$ I <sub>GABA</sub> amplitude (with $\gamma$ 2 S327 for full affect). CGC $\beta$ 2 KO: No affect of CamKII activation compared to WT. HEK $\alpha$ 4 $\beta$ 26: <input checked="" type="checkbox"/> PKC $\downarrow$ tonic inhibition, $\downarrow$ surface	GST (Kowalczyk et al., 2013; McDonald & Moss, 1997) Akt (Wang, Liu, Pei, et al., 2003) Not PKA (McDonald et al., 1998) Oocyte (Kellenberger et al., 1992) $\beta$ 2 KO (Houston & Smart, 2006) Tonic (Bright & Smart, 2013)

(b) $\beta$ subunits		Kinase or phosphatase			Neuron		Effect of phosphorylation		Selected publications	
Subunit	Site	GST	Cell line	Neuron	Neuron	Effect of phosphorylation	Effect of phosphorylation	Effect of phosphorylation	Selected publications	Selected publications
$\beta 3$	T282, S406	-	-	Hip	Hip	-	-	-	Mass spectrometry (Kang et al., 2011)	
	S383	<input checked="" type="checkbox"/> CamKII	HEK: $\alpha 1\beta 3$ <input checked="" type="checkbox"/> CamKII NG108-15: $\alpha 1\beta 3(\gamma 2)$ <input checked="" type="checkbox"/> CamKII	Hip: <input checked="" type="checkbox"/> CamKII	Hip: <input checked="" type="checkbox"/> CamKII	NG108-15: <input checked="" type="checkbox"/> CamKII $\uparrow$ $I_{GABA}$ amplitude. Hip (via VGCCs): <input checked="" type="checkbox"/> CamKII $\uparrow$ surface, $\uparrow$ insertion, $\uparrow$ tonic current. Hip: <input checked="" type="checkbox"/> CamKII LTP <sub>GABA</sub> $\uparrow$ synaptic clusters of gephyrin and GABA <sub>A</sub> R.			GST (McDonald & Moss, 1997) NG108-15 (Houston et al., 2007) VGCC (Saliba et al., 2012) LTP <sub>GABA</sub> (Petrini et al., 2014)	
S408	<input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> CamKII	(see S408/S409)	(see S408/S409)	(see S408/S409)	(see S408/S409)	GST: <input checked="" type="checkbox"/> S409 $\downarrow$ binding to PKC.			GST (McDonald & Moss, 1997)	
S409	<input checked="" type="checkbox"/> CamKII <input checked="" type="checkbox"/> PKA <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PKG	(see S408/S409)	(see S408/S409)	(see S408/S409)	(see S408/S409)	GST: <input checked="" type="checkbox"/> S409 $\downarrow$ binding to PKC.			GST (Brandon, Jovanovic, Smart, et al., 2002; McDonald & Moss, 1997)	
S408/S409	(As above)	HEK: $\alpha 1\beta 3\gamma 2$ <input checked="" type="checkbox"/> PKA NG108-15: $\alpha 1\beta 3$ <input checked="" type="checkbox"/> CamKII	Ctx, Hip: RACK1- <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PP2A Ctx, Hip: PRIP- <input checked="" type="checkbox"/> PP2A Hip: <input checked="" type="checkbox"/> PKA PRIP1- <input checked="" type="checkbox"/> PP1 $\alpha$	Ctx, Hip: RACK1- <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PP2A Ctx, Hip: PRIP- <input checked="" type="checkbox"/> PP2A Hip: <input checked="" type="checkbox"/> PKA PRIP1- <input checked="" type="checkbox"/> PP1 $\alpha$	Ctx, Hip: RACK1- <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PP2A Ctx, Hip: PRIP- <input checked="" type="checkbox"/> PP2A Hip: <input checked="" type="checkbox"/> PKA PRIP1- <input checked="" type="checkbox"/> PP1 $\alpha$	HEK: <input checked="" type="checkbox"/> PKA $\uparrow$ $I_{GABA}$ . CamKII NG108-15: Mutant does not change $I_{GABA}$ . <input checked="" type="checkbox"/> inhibits binding to AP2. Ctx, Hip (via BDNF): <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PP2A, transient $\uparrow$ then $\downarrow$ mIPSC amplitude and <input checked="" type="checkbox"/> $\uparrow$ surface but also reported to $\uparrow$ then $\downarrow$ surface in WT and $\uparrow$ surface in PRIP dKO. Hip: <input checked="" type="checkbox"/> PKA $\uparrow$ $I_{GABA}$ in WT but not in PRIP1 KO. PKA activity dissociates PRIP1-PP1 $\alpha$ which de <input checked="" type="checkbox"/> S408/S409.		HEK (McDonald et al., 1998) NG108-15 (Houston et al., 2007) AP2 (Kittler et al., 2005a) BDNF (Jovanovic et al., 2004; Kanematsu et al., 2006) PP1 $\alpha$ (Terunuma et al., 2004)		
(c) $\gamma$ subunits		Kinase or phosphatase			Neuron		Effect of phosphorylation		Selected publications	
Subunit	Site	GST	Cell line	Neuron	Neuron	Effect of phosphorylation	Effect of phosphorylation	Effect of phosphorylation	Selected publications	Selected publications
$\gamma 2S/L$	-	<input checked="" type="checkbox"/> Akt <input checked="" type="checkbox"/> PKG <input checked="" type="checkbox"/> PKA <input checked="" type="checkbox"/> PKC $\gamma 2L$ <input checked="" type="checkbox"/> PKC $\gamma 2S$	HEK: $\alpha 1\beta 1\gamma 2$ <input checked="" type="checkbox"/> PKA	Hip, Ctx: <input checked="" type="checkbox"/> PKC (via BDNF) PFC: RACK1- <input checked="" type="checkbox"/> PKC Hip Fyn: <input checked="" type="checkbox"/> Fyn (KO $\downarrow$ phospho)	Hip, Ctx: <input checked="" type="checkbox"/> PKC (via BDNF) PFC: RACK1- <input checked="" type="checkbox"/> PKC Hip Fyn: <input checked="" type="checkbox"/> Fyn (KO $\downarrow$ phospho)	Hip, Ctx (via BDNF): <input checked="" type="checkbox"/> PKC no <input checked="" type="checkbox"/> PFC (via 5HT2): <input checked="" type="checkbox"/> PKC $\downarrow$ $I_{GABA}$ , $\gamma$ $\uparrow$ 2 <input checked="" type="checkbox"/>			GST (McDonald & Moss, 1994; Moss, Doherty, et al., 1992; Wang, Liu, Pei, et al., 2003; Whiting et al., 1990) HEK (Moss, Smart, et al., 1992), BDNF (Jovanovic et al., 2004). PFC (Feng et al., 2001), and Fyn (Jurd et al., 2010)	
	S327	<input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PKCe <input checked="" type="checkbox"/> CaN	HEK: $\alpha 1\beta 1\gamma 2S/L$ <input checked="" type="checkbox"/> PKC <input checked="" type="checkbox"/> PKCe Oocyte: $\alpha 1\beta 2\gamma 2S$ <input checked="" type="checkbox"/> PKC	Hip: <input checked="" type="checkbox"/> CaN Hip, Ctx: <input checked="" type="checkbox"/> PKCe (PKCe KO $\downarrow$ phospho)	Hip: <input checked="" type="checkbox"/> CaN Hip, Ctx: <input checked="" type="checkbox"/> PKCe (PKCe KO $\downarrow$ phospho)	HEK: <input checked="" type="checkbox"/> PKC $\downarrow$ $I_{GABA}$ amplitude. HEK: <input checked="" type="checkbox"/> PKCe modulates actions of benzodiazepine and ethanol Oocyte: <input checked="" type="checkbox"/> PKC $\downarrow$ $I_{GABA}$ amplitude (with $\beta 2$ S410 for full affect). Hip (via LTD <sub>GABA</sub> ): <input checked="" type="checkbox"/> CaN $\downarrow$ S327 <input checked="" type="checkbox"/> required for LTD <sub>GABA</sub> , $\uparrow$ lateral diffusion			GST (Moss, Doherty, et al., 1992) HEK (Krishek et al., 1994), PKCe (Qi et al., 2007), oocyte (Kellenberger et al., 1992), and LTD <sub>GABA</sub> (Muir et al., 2010; Wang, Liu, Haditsch, et al., 2003)	
S348, T350	<input checked="" type="checkbox"/> CamKII	NG108-15: $\alpha 1\beta 3$ <input checked="" type="checkbox"/> CamKII	NG108-15: $\alpha 1\beta 3$ <input checked="" type="checkbox"/> CamKII	NG108-15: $\alpha 1\beta 3$ <input checked="" type="checkbox"/> CamKII	NG108-15: $\alpha 1\beta 3$ <input checked="" type="checkbox"/> CamKII	NG108-15: <input checked="" type="checkbox"/> CamKII <input checked="" type="checkbox"/> mutant does not change $I_{GABA}$ .			GST (McDonald & Moss, 1994) NG108-15 (Houston et al., 2007)	



(c)  $\gamma$  subunits

Subunit	Site	Kinase or phosphatase		Neuron	Effect of phosphorylation	Selected publications
		GST	Cell line			
$\gamma 2L$	S343	<input checked="" type="checkbox"/> Fyn	HEK; $\alpha 1\beta 1\gamma 2L$	Ctx, WB: basally	<b>HEK:</b> <input checked="" type="checkbox"/> Src $\uparrow$ I <sub>GABA</sub> amplitude. <input checked="" type="checkbox"/> Y365/Y367 binds Src. <b>Hip:</b> <input checked="" type="checkbox"/> Y367 binds Fyn. <input checked="" type="checkbox"/> Y365/Y367 $\downarrow$ AP2 binding. <b>Y365/7F<sup>+/+</sup></b> : embryonic lethality. <b>Y365/7F<sup>-/-</sup></b> : $\uparrow$ surface, $\uparrow$ size inhibitory synapse, $\uparrow$ mIPSC amplitude and frequency, $\downarrow$ AP2 binding, $\downarrow$ spatial object recognition	HEK (Moss et al., 1995) GST, Ctx, and WB (Brandon et al., 2001) Fyn (Jurd et al., 2010) Y365/7F mouse (Tretter et al., 2009) AP2 (Kittler et al., 2008)
		<input checked="" type="checkbox"/> CamKII	HEK; $\alpha 1\beta 1\gamma 2S/L$	<input checked="" type="checkbox"/> PKC		

Whole-cell GABA-activated currents (IGABA); miniature inhibitory post synaptic current (mIPSC); Cerebellar granule cells (CGC); Cortex (Ctx); Hippocampus (Hip); Prefrontal cortex (PFC); Superior cervical ganglion (SCG); Whole brain (WB);  $\uparrow$  increase;  $\downarrow$  decrease;  phosphorylation; (de) dephosphorylation;  phosphorylated/dephosphorylated by specific kinase/phosphatase;  not phosphorylated/not dephosphorylated by specific kinase/phosphatase.