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Molecular pathways controlling inhibitory receptor expression

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SUMMARY

Epilepsy is a disease of complex etiology and multiple molecular mechanisms contribute to its development. Temporal lobe epilepsy (TLE) may result from an initial precipitating event such as hypoxia, head injury, or prolonged seizure (i.e., status epilepticus (SE)), that is followed by a latent period of months to years before spontaneous seizures occur. GABA_A receptor (GABA_AR) subunits changes occur during this latent period and may persist following the onset of spontaneous seizures. Research into the molecular mechanisms regulating these changes and potential targets for intervention to reverse GABA_AR subunit alterations have uncovered seizure-induced pathways that contribute to epileptogenesis. Several growth or transcription factors are known to be activated by SE, including (but not limited to): Brain Derived Neurotrophic Factor (BDNF), cAMP response element binding protein (CREB), Inducible cAMP Early Repressor (ICER), and Early Growth Response factors (Egrs). Results of multiple studies suggest that these factors transcriptionally regulate GABA_AR subunit gene expression in a way that is pertinent to the development of epilepsy. This article will focus on these signaling elements and describe their possible roles in gene regulatory pathways that may be critical in the development of chronic epilepsy.

Keywords

Brain Derived Neurotrophic Factor (BDNF); cAMP response element binding protein (CREB); dentate gyrus (DG); Early Growth Response factors (EGRs); GABA_{A γ} receptor 1 subunit; GABA_A receptor 4 subunit; hippocampus; Inducible cAMP Early Repressor (ICER); Status Epilepticus (SE)

GABA_A RECEPTORS AND EPILEPSY

Post-synaptic GABA_ARs mediate most fast synaptic inhibition in the forebrain. GABA_ARs are heteromeric protein complexes composed of multiple subunits that form ligand-gated, anion-selective channels whose properties are modulated by barbiturates, benzodiazepines, zinc, ethanol, anesthetics and neurosteroids. There are several different GABA_AR subunit families and multiple subtypes exist within each classification (α 1– α 6, β 1– β 4, γ 1– γ 3, δ , ϵ , ϕ).

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The most common GABA_AR is the $\alpha 1 \beta 2 \gamma 2$ subtype, but subtype combinations vary in different brain regions and cell types, and during different times in development (Laurie et al., 1992; Sieghart et al., 2006; Rudolph et al., 2006). Subunit composition of GABA_ARs determines the intrinsic properties of each channel, including GABA affinity, kinetics, conductance, allosteric modulation, probability of channel opening, interaction with modulatory proteins, and subcellular distribution (Sieghart et al., 2006; Pritchett et al., 1989; Mohler et al., 2006; Mehta et al., 1999; Macdonald et al., 1994). For example, alterations in the $\gamma 2$ -subtype results in differences in GABA_AR modulation by benzodiazepines, neurosteroids, and zinc (Vicini et al., 1991; Smith et al., 2007; Pritchett et al., 1989; Nusser et al., 2002). Alterations in GABA_AR subunit composition occur during epileptogenesis in animal models of epilepsy (Brooks-Kayal et al., 1998; Feng et al., 2008; Lauren et al., 2003; Lund et al., 2008; Peng 2004; Sperk et al., 2004; Zhang et al., 2007). Status epilepticus results in changes in the expression and membrane localization (i.e., extrasynaptic vs. synaptic) of several GABA_AR subunits (e.g., $\alpha 1$, $\alpha 4$, $\beta 2$, and $\gamma 2$) in hippocampal dentate granule cell neurons (DGN). Beginning soon after the prolonged seizures of SE and continuing at least until the animals become epileptic, these alterations are associated with changes in phasic and tonic GABA_AR-mediated inhibition, and in GABA_AR pharmacology (Gibbs et al., 1997; Cohen et al., 2003; Buhl et al., 1996). After pilocarpine-induced SE, GABA_AR $\alpha 1$ subunit mRNA expression decreases, GABA_AR $\alpha 4$ subunit mRNA expression increases and animals develop recurrent spontaneous seizures (Brooks-Kayal et al., 2001; Brooks-Kayal et al., 1998). Changes in GABA_AR function and subunit expression have also been observed in neurons from surgically resected hippocampus of patients with intractable temporal lobe epilepsy (TLE) (Brooks-Kayal et al., 1999; Sperk et al., 2009). These alterations are associated with an increased abundance of $\alpha 4 \beta 2$ containing receptors, a reduction in $\alpha 1 \beta 2$ containing receptors in dentate gyrus (Lund, et al., 2008) and shift of $\beta 2$ -containing receptors from synaptic to perisynaptic locations, likely as part of $\alpha 4 \times \beta 2$ receptors (Zhang et al., 2007). This provides additional support for the concept that during epileptogenesis altered expression and localization of γ -subunits lead to changes in synaptic GABA_AR composition that may underlie previously reported impairments in synaptic inhibition in dentate granule cell neurons, including diminished benzodiazepine sensitivity, enhanced zinc sensitivity, reduced neurosteroid modulation, and diminished phasic inhibition in dendrites (Gibbs et al., 1997; Cohen et al., 2003; Buhl et al., 1996; Sun et al., 2007). To directly address the relevance GABA_AR subunit alterations to epileptogenesis, viral-mediated gene transfer in adult rodents was used to mitigate changes in GABA_AR subunit expression and the effect on epilepsy development was examined. A bicistronic RNA containing the coding information for the $\alpha 1$ subunit and the yellow enhanced fluorescent protein was delivered under control of the $\alpha 4$ subunit promoter, a promoter that is markedly activated in the dentate gyrus after SE. Delivery of this transgene using an adeno-associated viral vector (AAV) prior to SE resulted in a robust increase in expression of GABA_AR $\alpha 1$ after SE, a three-fold increase in the mean time to the first spontaneous seizure, and only 39% of AAV- $\alpha 1$ -injected rats were observed to develop spontaneous seizures in the first 4 weeks after SE compared to 100% of rats receiving sham injections (Raol et al., 2006). Together, these data support a role for GABA_AR γ -subunit changes in the process of epileptogenesis. This article will elucidate our current understanding of the molecular mechanisms that regulate aberrant GABA_AR receptor subunit expression in dentate gyrus associated with epilepsy and will discuss potential therapeutic targets this research has identified.

MECHANISMS REGULATING GABA_A RECEPTOR SUBUNIT EXPRESSION

Signaling Proteins involved in GABA_A receptor subunit regulation

BDNF—BDNF, a member of the mammalian neurotrophins (NTs), although classically recognized for its effects on the growth and survival of neurons during development through the activation of Trk receptors, is also known for its striking influence on neuronal excitability. Expression levels of BDNF and TrkB increase in kindling, kainic acid (KA) and pilocarpine models of TLE. BDNF mRNA and protein levels are elevated in temporal lobe tissue from human epileptic brains. (Murray et al., 2000; Mathern et al., 1997).

Evidence that BDNF up-regulation is associated with seizure activity suggests that BDNF may play a role in epileptogenesis. BDNF^{+/-} mice show a marked reduction in the rate of kindling acquisition and transgenic mice with increased BDNF signaling have more severe seizures in response to KA (Kokaia et al., 1995; Lahtinen et al., 2003).

Intracerebroventricular administration of TrkB-Fc receptor bodies, to sequester endogenous BDNF, inhibits kindling acquisition, and kindling development is also impaired in conditional TrkB null mice (He et al., 2004; Binder et al., 1999). Additional mechanisms by which BDNF may contribute to epileptogenesis include BDNF-induced modulation of inhibitory transmission, such as phosphorylation of the GABA_ARs, alteration of GABA_AR trafficking, and regulation of GABA_AR subunit gene expression (Lund et al., 2008; Brunig et al., 2001; Jovanovic et al., 2004). Specifically, it has been shown that 24 hours of BDNF exposure directly regulates the levels of α -subunits in hippocampal neurons in culture in a manner similar to that seen in TLE models, causing a decrease in GABA_AR α 1 subunit expression and an increase in GABA_AR α 4 expression (Lund et al., 2008). BDNF has also been shown to activate the JAK/STAT (Janus kinase /signal transducer and activators of transcription) pathway in primary hippocampal neurons in culture, and this pathway has been shown to be a critical mediator of BDNF's effects on GABA_AR subunit expression as detailed below.

Janus kinase (JAK)/signal transducer and activators of transcription (STAT) proteins

—The JAK/STAT pathway is composed of 4 JAK proteins (JAK1,2,3, and TYK) and 7 STAT proteins (STAT 1, 2, 3, 4, 5A, 5B, and 6) in mammals (Aaronson et al., 2002). These proteins are inactive in the cytoplasm until membrane-bound receptors are bound by a variety of extracellular signaling proteins (e.g., IL-6, gp-130, PDGF, FGF, BDNF) to initiate the signal transduction cascade. JAKs are consequently recruited to the membrane where they are phosphorylated by the integral receptor kinases, and are activated to recruit and phosphorylate target STAT proteins. Phosphorylated STAT proteins (pSTATs) dimerize and translocate to the nucleus where they bind specific STAT DNA regulatory elements in the promoters of target genes to alter their transcription (Darnell et al., 1994; Levy et al., 2002; Ihle et al., 1996; Schindler et al., 1995). The JAK/STAT pathway is dynamically controlled at many levels by phosphorylation, nuclear trafficking, and dimerization. Proteins called suppressors of cytokine signaling (SOCS) also serve as negative feedback regulators of the pathway and are turned on in response to excessive STAT activation (Krebs et al., 2001).

JAK/STAT proteins may have important roles in regulating cell survival in both neuronal development and brain disorders. JAK and STAT proteins are differentially expressed in the brain throughout development. STAT5 and STAT6 are abundant in embryonic stages in rats, but decrease in adulthood, whereas STAT1 expression is highest in adulthood with STAT3 highly expressed at all ages (De-Fraja et al., 1998; Cattaneo et al., 1999). Likewise, JAK 1 and JAK2 are abundant in the brain, but JAK3 and TYK are barely detectable (De-Fraja et al., 1998). Several laboratories have demonstrated that the JAK/STAT pathway is activated in the hippocampus in response to both pilocarpine and kainate induced SE (Xu et al., 2011; Lund et al., 2008; Choi et al., 2003) and we have recently demonstrated that this activation

plays a critical role in the presence of the inducible cAMP early repressor (ICER) that controls GABA_AR $\alpha 1$ subunit downregulation, as detailed below.

ICER and CREB—The cAMP response element binding protein (CREB) is another key transcription factor that may mediate the observed gene expression changes in epilepsy. CREB is a stimulus-induced bZIP transcription factor that is activated by phosphorylation at its Ser 133 site (Gonzalez et al., 1989). Phosphorylated CREB (pCREB) dimerizes and binds to cAMP response element (CRE) motifs on promoters that contain the consensus sequence TGACGTCA. Together with its anchoring binding partner, CREB binding protein (CBP), pCREB upregulates transcription of target genes. Transcriptional regulation through CREB has been implicated in mechanisms of cell survival, plasticity, and learning and memory paradigms (Frank et al., 1994). Target genes of pCREB include CREB family members, which consist of cAMP response element modulator (CREM), inducible cAMP early repressor (ICER) and activating transcription factor (ATF). These para- and homologs of CREB bind CRE elements to modulate the transcription of particular genes. The CREM gene produces many spliced isoforms that can act as transcriptional repressors or activators. One of these repressor forms is ICER, a group of 4 proteins made from an internal promoter of the CREM gene. ICER can heterodimerize with CREB and directly block CREB-induced transcription (Foulkes et al., 1991; Shaywitz et al., 1999; Molina et al., 1993; Mioduszevska et al., 2003; Jaworski et al., 2003).

Several studies using adult animal models of epilepsy suggest that seizures upregulate CREB or CREM/ICER activity for varying durations. For example, PTZ induced seizures in adults increase pCREB levels transiently, and electroconvulsive seizures have been found to cause rapid increases of CREM and ICER mRNA expression (Kojima et al., 2008; Tanis et al., 2008; Fitzgerald et al., 1996). Following pilocarpine-induced SE, pCREB and ICER increases in the DG of the hippocampus persist for 48 hours (Lund et al., 2008). Increases in pCREB and ICER subsequently mediate the GABA_AR $\alpha 1$ subunit decreases that occur after SE in the DG, as detailed below. Seizures may influence CREB phosphorylation and also the association of CREB with CRE sequences on promoters of target genes, a necessary component of CREB transcriptional control (Chrivia et al., 1993; Tanis et al., 2008). Taken together, these results suggest that increases in the levels or activation state of CREB and its family members, seen after multiple types of seizure induction, may be important mechanisms responsible for the changes in gene expression that occur during epileptogenesis.

Early growth response factors (Egrs)—Egrs, members of the immediate early genes, are a family of four proteins (Egr1, 2, 3 and 4) that share nearly identical zinc finger DNA binding domains and bind to a common Egr response element consensus sequence (EREs), GCG T/GGG GCG. Studies in Egr1–4-deficient animals illustrate quite divergent physiological roles for each protein. There is sequential induction of Egr1 and Egr3 transcription factors after electroconvulsive shock (ECS) and gel shift assays demonstrate that Egr-1 and Egr-3 DNA binding activities follow the same pattern (O'Donovan et al., 1998). These findings indicate that Egr-1 and Egr-3 act in concert to mediate early and late phases, respectively, of the transcriptional response regulated by their cognate response element and may play different regulatory roles. Presence of Egr consensus sites in the Egr3 gene suggest that Egr1 may regulate the transcription of its family member and/or that Egr3 gene expression is homologously regulated (Kim et al., 2012).

Induction of Egr family transcription factors occurs after SE, with protein levels of Egr3 increasing in the dentate gyrus (DG) of the hippocampus 24 hours after pilocarpine induced SE (Roberts et al., 2005). Egr proteins may have multiple roles in the process of epileptogenesis, although this remains to be demonstrated. Egr1 has been shown to increase

promoter activity and expression levels for several N-methyl-D-aspartate receptor (NMDAR) subunits (Bai and Kusiak, 1997). Egr3 is a specific regulator of GABA_AR 4 subunits, as discussed below, as well as the levels of NMDAR1 in primary cortical neurons (Kim et al., 2012).

Regulation of GABA_AR α 1

The human 1 promoter (*GABRA1-p*) contains a functional CRE site (Hu et al., 2008), and several studies using adult animal models of epilepsy suggest that seizures increase levels of the activated form of CREB (pCREB) and CREM/inducible cAMP early repressor (ICER) activity (Tanis et al., 2008; Moore et al., 1996; Fitzgerald et al., 1996; Porter et al., 2008). Using chromatin immunoprecipitation (ChIP) and DNA pulldown studies, it was determined that there was increased binding of pCREB and ICER to the endogenous *GABRA1-p* in dentate gyrus after SE (Lund et al., 2008). Additionally, results of *GABRA1-p*/luciferase reporter assays in transfected primary hippocampal neurons show that overexpression of CREB and ICER produces robust decreases in *GABRA1-p* activity, and overexpression of ICER alone produces a marked decrease in the levels of endogenous 1 subunits at the cell surface (Lund et al., 2008). These findings suggest that CREB and ICER are important regulators of seizure-induced changes in 1 subunit expression.

The excessive neuronal activity associated with SE stimulates many different signaling pathways that could lead to enhanced phosphorylation of CREB and expression of ICER (Chrivia et al., 1993; Tanis et al., 2008). As previously discussed, Brain-derived neurotrophic factor (BDNF) levels increases markedly after SE and BDNF differentially regulates the abundance of both 1 and 4 subunits in cultured primary hippocampal neurons similar to the changes observed after SE (Lund et al., 2008). BDNF is known to enhance CREB phosphorylation through binding to TrkB receptors and stimulation of the MEK-ERK pathway (Ying et al., 2002) as well as through a nitric oxide-dependent pathway (Riccio et al., 2006), among others. Further, BDNF has been found to be a potent regulator of ICER synthesis in hippocampal neurons, increasing ICER expression similar to what is seen *in vivo* after SE (Hu et al., 2008). BDNF regulation of ICER expression is mediated by JAK/STAT pathway activation (Lund et al., 2008).

The combination of in vitro and in vivo evidence suggests that activation of the JAK/STAT pathway by the increased release of BDNF that occurs following seizures results in transphosphorylation of JAKs, phosphorylation of STAT proteins, STAT homo- or heterodimerization, translocation from the cytoplasm to the nucleus, and binding to specific DNA elements (STAT-recognition sites) to regulate target gene expression (Zhong et al., 1994). The receptor that mediates BDNF induced activation of the JAK/STAT pathway has not been established. Using chromatin immunoprecipitation, it has been shown that pSTAT3 association with the STAT-recognition site on the ICER promoter is enhanced after SE in the dentate gyrus (Lund et al., 2008). Furthermore, siRNA knockdown of STAT3 inhibits BDNF-induced ICER increases, as does blockade of the JAK/STAT signaling pathway with pyridone 6 (P6) in primary hippocampal cultures (Lund et al., 2008). Most importantly, P6 administration *in vivo* into rat dentate gyrus prior to SE blocks both ICER induction and decreased transcription of *GABRA1* (Lund et al., 2008). These findings suggest a specific signaling cascade involving BDNF, JAK/STAT, and CREB that is critical to the reported decreases in 1 subunit levels following SE that may contribute to epileptogenesis. These pathways may provide novel therapeutic targets for the future prevention or treatment of epilepsy.

Regulation of GABA_AR α 4

Increases in GABA_A α 4 subunit are transcriptionally regulated by BDNF activation of the TrkB receptor, leading to upregulation of the early growth response factor (Egr3) pathway. BDNF activation of the TrkB receptor leads to induction of Egr3 synthesis via a PKC/MAPK-dependent pathway. Induction of Egr family transcription factors occurs 24 h after SE in the dentate gyrus of the hippocampus, where increases in mRNA and protein levels of Egr3 are associated with enhanced binding of Egr3 to the Egr response element (ERE) consensus sequence in the core promoter region of the GABA_A α 4 receptor subunit gene (*GABRA4*) (Roberts et al., 2006). Most importantly, BDNF-treated neuronal cultures and dentate gyrus tissue harvested shortly after SE demonstrate an increase in α 4 and a decrease in α 1- α 2-containing receptors (Lund et al., 2008). These results are supported by studies demonstrating that mice devoid of Egr3 have significantly lower levels of α 4 mRNA, strongly suggesting that Egr3 may be a critical regulator of endogenous GABA_A receptors that contain α 4 subunits (Roberts et al., 2005). As pharmacological inhibition of Egr3 control over GABA_A α 4 expression is difficult because there are no available Egr3 inhibitors or modulators, viral-mediated delivery of silencing molecules directed against Egr3 transcripts may provide a novel strategy to determine whether this particular transcription factor is critical for epileptogenesis. These studies are currently underway in our laboratories.

Other mechanisms of GABA_AR subunit regulation

Neurosteroids—Upregulation of α 4 subunits has been demonstrated following the withdrawal of progesterone-derived neurosteroids, such as allopregnanolone and pregnanolone (Smith et al., 2007; Smith et al., 1998; Kokate et al., 1996), resulting in enhanced neuronal excitability, seizure susceptibility, and benzodiazepine resistance. This increase in α 4 subunit expression is thought to be a potential molecular basis of catamenial epilepsy, a neuroendocrine condition that occurs at around the perimenstrual period and is characterized by neurosteroid withdrawal-linked seizure exacerbations in women with epilepsy. Recently, neurosteroid withdrawal-induced α 4-subunit upregulation was found to be mediated by Egr3 inhibition, similar to the role played by this transcription factor in upregulating α 4 after SE (Gangisetty et al., 2010).

In addition to changes in α 1 and α 4 expression, an increase in α 2-subunit and a decrease in α 3-subunit surface expression in dentate granule cells, with associated diminished neurosteroid sensitivity of tonic currents, has also been demonstrated in rodent models of TLE (Zhang et al., 2007; Peng et al., 2002; Peng et al., 2004; Sun et al., 2007; Rajasekaran et al., 2010). As for the α 4 subunit, studies support an important role for the neurosteroids in the regulation of α 1 and α 4 subunit expression during the ovarian cycle and in pregnancy (Maguire et al., 2005; Maguire et al., 2009a; Maguire et al., 2009b; Smith et al., 2007) although the specific molecular mechanisms regulating changes in expression of these subunits during epileptogenesis remain to be determined. Neurosteroid regulation of GABA_AR subunits will be covered more thoroughly in other articles within this supplement.

BDNF and cell surface expression of GABA_ARs—The critical role of BDNF in regulating the decreased expression of α 1-containing GABA_ARs via JAK/STAT signaling (Lund et al., 2008), and in the induction of Egr3 synthesis leading to overexpression of α 4 subunits via protein kinase C (PKC) and mitogen activated protein kinase (MAPK) has already been discussed (Roberts et al., 2005; Roberts, 2006). In addition to its role in the regulation of α 1 and α 4 subunit gene transcription after SE (for a summary, see Fig. 1), BDNF, acting via activation of TrkB receptors, has been shown to play an important role in determining the surface expression of the α 2, α 2/3, α 2, and α 3 subunits (Jovanovic et al.,

2004; Brunig et al., 2001). In combination, these findings establish BDNF's role as a multifunctional regulator of altered inhibition during epileptogenesis.

CONCLUSION

GABA_ARs that contain $\alpha 1$ subunits are sensitive to benzodiazepines and generally located at the synapse, contributing to phasic inhibition. GABA_ARs that contain $\alpha 4$ subunits have unique pharmacological properties, such as insensitivity to benzodiazepines and increased sensitivity to zinc blockade. Receptors containing $\alpha 4$ subunits are most often found with the $\beta 2$ rather than the $\beta 3$ subunit in combination with $\gamma 2$. These $\alpha 4\beta 2\gamma 2$ GABA_ARs are localized to extrasynaptic sites and contribute to tonic inhibition. Under physiological conditions, only a minor population of $\alpha 4\beta 2\gamma 2$ GABA_ARs are found within dentate gyrus synapses, where they are proposed to affect both the rise time and decay of synaptic currents (Lagrange et al., 2007). There is a significant decrease in $\alpha 1$ subunit expression and a marked increase in $\alpha 4$ subunit expression in dentate granule cells during epileptogenesis in TLE models (Brooks-Kayal et al., 2001; Brooks-Kayal et al., 1998; Lund et al., 2008) that results in an increase in the abundance of $\alpha 4\beta 2$ -containing receptors (Sun et al., 2007; Zhang et al., 2007) and a reduction in $\alpha 1\beta 2$ -containing receptors (Lund et al., 2008). The change in receptor subtype from $\alpha 1\beta 2$ to $\alpha 4\beta 2$ may contribute to epileptogenesis, as $\alpha 4$ -containing GABA_ARs have been shown to desensitize rapidly, especially when assembled with $\gamma 3$ subunits (Lagrange et al., 2007). In addition, GABA_ARs containing the $\alpha 4$ subunit are very sensitive to zinc blockade, as are GABA_ARs on dentate granule cells in the epileptic brain (Buhl et al., 1996; Cohen et al., 2003). Zinc containing mossy fiber terminals sprout from the granule cell layer of the hippocampus onto other granule cells and into CA3, likely depositing zinc onto the newly formed $\alpha 4\beta 2$ receptors causing a decreased response to GABA. Collectively these alterations may contribute to epilepsy development, pharmacoresistance and further epilepsy progression.

The full range of gene expression changes that are involved in epileptogenesis and the molecular mechanisms that underlie them are just beginning to be characterized. Recent work characterizing the functions of the BDNF, JAK/STAT, CREB/ICER, and Egr3 signaling pathways in GABA_A $\alpha 1$ and $\alpha 4$ subunit changes in the dentate gyrus after SE provides an important lead for the future development of molecular therapies aimed at restoring the balance of excitation and inhibition in the nervous system. However, the upstream components of these pathways, including the receptor that mediates BDNF induced changes in GABA_A $\alpha 1$ expression, and the exact means through which they confer vulnerability to epilepsy, must be further elucidated. Further, although the signaling pathways that mediate regulation of some GABA_A subunits immediately after SE have been established, it remains unknown if these same mechanisms regulate the long term differences in GABA_A expression that has been shown to persist in chronically epileptic animals. Finally, as many signaling pathways are activated simultaneously by SE and other brain insults that lead to epilepsy and as each of these signaling pathways regulate myriad genes with diverse functions, modulation of any of these pathways may have a multitude of downstream effects, many of which may involve cell- and region-specific responses throughout the brain. Therefore, the final impact of pathway blockade on epileptogenesis may be difficult to predict. For example, although the enhanced GABA_A $\alpha 1$ -subunit expression in dentate gyrus that results from JAK/STAT pathway blockade and subsequent ICER inhibition would be expected to have an antiepileptic effect, mutant mice lacking ICER have accelerated kindling (Kojima, 2008) and develop more severe epilepsy following pilocarpine-induced SE (Porter et al., 2008). Consistent with this finding, ICER-overexpressing mice show delayed kindling acquisition (Kojima et al., 2008). The effects on epileptogenesis of blocking ICER upregulation after SE via inhibition of STAT3 phosphorylation is currently under investigation. As several of these signaling pathways

have been implicated in the regulation of learning, memory, and cell survival, the effects of modulation of these pathways on these critical parameters will need to be closely monitored. It is likely that activation of immediate early genes and JAK/STAT proteins have multiple roles in epileptogenesis in addition to GABA_A receptor regulation, including mechanisms related to aberrant cell proliferation and cell death. Thus, the identification of the contribution of JAK/STAT and Egr3 signaling to the regulation of brain inhibition has opened new areas for investigation that may lead to multiple opportunities for therapeutic intervention in the future.

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Figure 1.

GABA_A receptor subunit regulation after status epilepticus (SE). Both *in vivo* and *in vitro* evidence suggest that increased levels of BDNF release following SE results in activation of at least two different signaling pathways, JAK/STAT and PKC/MAPK, that differentially regulate expression of GABA_AR subunits. Specifically, JAK/STAT pathway activation leads to increases in ICER and enhanced binding of ICER and phosphorylated CREB to the $\alpha 1$ promoter region, down-regulating $\alpha 1$ subunit expression (Left). PKC/MAPK pathway activation results in increased binding of Egr3 to the promoter region of the $\alpha 4$ subunit and subsequent increases in expression of this subunit (Right). Resulting changes in receptor

subunit composition within dentate granule neurons from predominantly $\alpha 1 \alpha 2$ to $\alpha 4 \alpha 2$ may alter inhibitory function and contribute to epileptogenesis.