Published in final edited form as:
Sci Signal. ; 1(41): ra9. doi:10.1126/scisignal.1162396.

BDNF Selectively Regulates $GABA_A$ Receptor Transcription by Activation of the JAK/STAT Pathway

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Abstract

The γ-aminobutyric acid (GABA) type A receptor ($GABA_AR$) is the major inhibitory neurotransmitter receptor in the brain. Its multiple subunits show regional, developmental, and disease-related plasticity of expression; however, the regulatory networks controlling $GABA_AR$ subunit expression remain poorly understood. We report that the seizure-induced decrease in $GABA_AR$ α1 subunit expression associated with epilepsy is mediated by the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway regulated by brain-derived neurotrophic factor (BDNF). BDNF- and seizure-dependent phosphorylation of STAT3 cause the adenosine 3′,5′-monophosphate (cAMP) response element–binding protein (CREB) family member ICER (inducible cAMP early repressor) to bind with phosphorylated CREB at the *Gabra1*:CRE site. JAK/STAT pathway inhibition prevents the seizure-induced decrease in $GABA_AR$ α1 abundance in vivo and, given that BDNF is known to increase the abundance of $GABA_AR$ α4 in a JAK/STAT-independent manner, indicates that BDNF acts through at least two distinct pathways to influence $GABA_AR$-dependent synaptic inhibition.

INTRODUCTION

The subunit composition of $GABA_ARs$, which mediate most fast synaptic inhibition in the mature brain, determines their function and their response to drugs and other modulators (1, 2). Most $GABA_ARs$ are heteropentamers composed of isoforms of α, β,and γ subunits, with the most common subtype being α1β2γ2. Alterations in $GABA_AR$ subunit expression occur

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Citation: I. V. Lund, Y. Hu, Y. H. Raol, R. S. Benham, R. Faris, S. J. Russek, A. R. Brooks-Kayal, BDNF selectively regulates $GABA_A$ receptor transcription by activation of the JAK/STAT pathway. *Sci. Signal*. **1**, ra9 (2008).

during development (1-5) and in animal models of epilepsy (6-9), alcohol dependence (10), anxiety (8,11), and stress (12-14), but the molecular pathways that regulate these changes are largely unknown. Prolonged seizures [status epilepticus (SE)] result in alterations in the expression and membrane localization of several $GABA_AR$ subunits (α1, α4, γ2, δ) in hippocampal dentate granule neurons (9,15-17). These alterations, which are associated with changes in phasic and tonic $GABA_AR$-mediated inhibition and in $GABA_AR$ modulation by benzodiazepines, neurosteroids, and zinc, begin soon after SE and continue as animals become epileptic (9,15,17,18). After SE in adult rodents, $GABA_AR$ α1 subunit messenger RNA (mRNA) expression decreases, α4 subunit mRNA expression increases, and animals develop the recurrent spontaneous seizures that define epilepsy (9,15). In contrast, neonatal SE (postnatal day 10) results in increased $GABA_AR$ α1 subunit expression and does not lead to the subsequent development of epilepsy (19). Increasing $GABA_AR$ α1 expression in adults by viral-mediated gene transfer reduces the incidence of spontaneous seizures during the first 4 weeks after SE by 60% (20). Together, these data support a role for $GABA_AR$ α subunit changes in the process of epilepsy development (epileptogenesis).

Although the mechanisms mediating regulation of the α4 subunit after SE have been characterized (21,22), the molecular signals that regulate seizure-induced changes in expression of the gene encoding the $GABA_AR$ α1 subunit (abbreviated *GABRA1* for the human gene and *Gabra1* for the rat gene) have not been identified. The human *GABRA1* promoter (*GABRA1-p*) contains a functional adenosine 3′,5′-monophosphate (cAMP) response element (CRE) (23). Cyclic AMP response element–binding protein (CREB) is a transcription factor that is activated by phosphorylation at Ser^{133} (24,25) in response to various stimuli (26-29). Phosphorylation of CREB at Ser^{133} is often associated with an increase in transcriptional activation of target genes (24,30,31); among these are various CREB family members, including inducible cAMP early repressor (ICER), a group of four proteins transcribed from an internal promoter of the gene encoding CRE modulator (CREM) (32,33). ICER binds CREs to repress target gene expression (34,35), and transcription from *GABRA1-p* is bi-directionally regulated by phosphorylated CREB (pCREB) and ICER (23). In transfected cultured cortical neurons, pCREB enhances the activity of *GABRA1-p*–containing reporter constructs, whereas ICER—or co-binding of ICER and pCREB—represses their activity. Increased phosphorylation of CREB and transcription of ICER may occur after seizures (36-40), but it is not known whether pCREB and ICER mediate a seizure-induced decrease in the activity of the rat $GABA_AR$ α1 subunit gene promoter (*Gabra1-p*). Moreover, the upstream pathways mediating increases in the transcription of ICER in response to seizures have not been identified.

We report that CREB and ICER regulate *Gabra1-p* activity in vivo, showing an increase in CREB phosphorylation and ICER expression in the hippocampal dentate gyrus (DG) after SE. This leads to enhanced binding of pCREB and ICER to the *Gabra1-p* CRE, resulting in transcriptional repression and a subsequent reduction in α1 mRNA expression and the number of α1γ2-containing $GABA_ARs$. Furthermore, using primary cultured hippocampal neurons, we show that pCREB is required for the ICER-mediated decrease in *GABRA1-p* activity and that brain-derived neurotrophic factor (BDNF) activation of the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway is the endogenous signal that drives the process. In particular, blockade of the JAK/STAT pathway inhibits SE-induced ICER up-regulation and the subsequent decrease in abundance of $GABA_AR$ α1 subunit mRNA in vivo, and small interfering RNA (siRNA) knockdown of STAT3 in primary hippocampal neurons blocks BDNF-induced activation of ICER transcription. Taken together, these data show that BDNF and the JAK/STAT pathway control $GABA_AR$ α1 subunit expression in the brain. Identification of signaling pathways regulating the most abundant $GABA_AR$ α subunit in the central nervous system (CNS) may facilitate the development of novel molecular therapies for

disorders such as epilepsy, alcoholism, anxiety, and stress that are associated with reduced $GABA_AR$ α1 expression.

RESULTS

SE increases CREB phosphorylation and ICER expression in DG

To determine if CREB family transcription factors are involved in regulating *Gabra1* expression in response to SE, we looked for evidence of seizure-induced activation of the CREB pathway with the use of an antibody directed against Ser^{133} pCREB as an indicator. The abundance of pCREB normalized to β-actin in DG increased to 270% of controls [$n = 4$ controls (CTRL), $n = 5$ SE, $P < 0.05$] at 1 hour and 310% of controls ($n = 4$ CTRL, $n = 5$ SE, $P < 0.05$) at 24 hours after SE (Fig. 1, A and B). Western blot analysis indicated no changes in total CREB protein at 1 hour ($n = 3$ CTRL, $n = 5$ SE) or 24 hours ($n = 4$ CTRL, $n = 5$ SE) after SE (Fig. 1, C and D). We also measured CREB mRNA expression with real-time reverse transcription polymerase chain reaction (RT-PCR). CREB mRNA was not increased at 1 hour ($n = 3$ CTRL, $n = 4$ SE), 6 hours ($n = 4$ CTRL, $n = 6$ SE), 48 hours ($n = 4$ CTRL, $n = 6$ SE), or 1 week after SE ($n = 4$ CTRL, $n = 5$ SE) (Fig. 1E). There was an increase in CREB mRNA 24 hours after SE to 220% of controls ($n = 4$ CTRL, $n = 6$ SE, $P < 0.05$), but, as noted above, this was not associated with a significant change in the abundance of CREB protein.

Because SE in adult rats results in dentate granule cell (DGC) neuro-genesis and CREB phosphorylation has been shown in newly born DGCs during development (41), we used immunostaining to see whether pCREB activation occurred predominantly in regions of newborn DGCs. Immunostaining of hippocampal slices with antibody directed against pCREB at 1 and 24 hours after SE showed increased pCREB staining in DGCs throughout the DGC layer (Fig. 1F). pCREB staining extended beyond the inner third of the DGC layer where newborn DGCs are predominantly found, suggesting that the increase in pCREB was not limited to newborn DGCs.

Phosphorylation of most CREB isoforms in the brain is associated with increased transcriptional activation of target genes (25,29) and is thus unlikely to be solely responsible for decreased transcription of the $GABA_AR$ α1 gene (*Gabral*) after SE. We therefore examined whether increased phosphorylation of CREB is accompanied by increased expression of the CREB-family repressors CREM and ICER. Using RT-PCR, we found robust increases in ICER mRNA abundance in DG after SE (Fig. 2A) starting at 1 hour (1360% of controls; $n = 4$ CTRL, $n = 6$ SE, $P < 0.05$), peaking at 6 hours (3270% of controls; $n = 4$ CTRL, $n = 6$ SE, $P < 0.01$), and continuing at 24 hours (1050% of controls; $n = 4$ CTRL, $n = 6$ SE, $P < 0.01$) and 48 hours (250% of controls; $n = 5$ CTRL, $n = 6$ SE, $P < 0.01$). By 1 week after SE, ICER mRNA expression was similar to that in control animals ($n = 4$ CTRL, $n = 6$ SE). ICER protein abundance was also increased 6 and 24 hours after SE as measured by Western blot (at 6 hours: 1900% of controls, $n = 5$ SE, $n = 4$ CTRL, $P < 0.05$; at 24 hours: 3330% of controls, $n = 4$ SE, $n = 4$ CTRL, $P < 0.05$; Fig. 2, B and C) and by immunostaining in the DGC layer (Fig. 2D). As with pCREB immunostaining, enhanced staining for ICER after SE was present diffusely throughout the DGC layer, suggesting that it was not limited exclusively to newborn DGCs. As would be expected if ICER is regulating *Gabra1* transcription, the increase in ICER abundance precedes the increase in $GABA_AR$ α_1 subunit mRNA expression (fig. S1). CREM mRNA abundance was found by RT-PCR to slightly increase 24 hours after SE (Fig. 2E; 137% of controls; $n = 4$ CTRL, $n = 5$ SE, $P < 0.05$), but not at 1, 6, or 48 hours or 1 week. No changes were observed in CREM protein abundance at 6 hours ($n = 4$ CTRL, $n = 5$ SE) or 24 hours ($n = 4$ CTRL, $n = 4$ SE) after SE (Fig. 2, F and G).

Increased binding of CREB and ICER to the *Gabra1*:CRE site is associated with reduced abundance of α1γ2-containing $GABA_A$Rs

Changes in the expression of the $GABA_A$R α_1 and α_4 subunits occur in a temporally coincident fashion after SE and have been hypothesized to result in changes in $GABA_A$R composition that underlie deficits in receptor function found in animal models of temporal lobe epilepsy (TLE) (9,21,22,42-44). To confirm whether SE-induced changes in α1 and α4 subunit abundance are associated with a change in the composition of $GABA_A$Rs, we immunoprecipitated receptors from DG with antibodies directed against γ2 [which confers synaptic localization by virtue of its binding to synaptic scaffolding proteins such as gephyrin (45,46)] and then determined the abundance of α1γ2- and α4γ2-containing $GABA_A$Rs by Western blot analysis. Twenty-four hours after SE, the abundance of α1γ2-containing $GABA_A$Rs decreased by 33%, whereas that of α4γ2-containing $GABA_A$Rs increased by 41% (Fig. 3, A and B). Taken together with earlier study results that SE produces altered physiological and pharmacological properties and changes in subcellular localization of $GABA_A$Rs in DGCs (9,15-18) as well as the finding that α4γ2-containing receptors have kinetics that make them less effective than α1γ2-containing receptors at inhibiting neurons during repetitive stimulation (42), our finding of seizure-induced changes in α subunit composition of γ2-containing $GABA_A$Rs provides evidence that transcriptional regulation of α subunit genes after SE results in functional changes in $GABA_A$R populations.

A consensus CRE site (*GABRA1*:CRE) is present in the human and rat α1 promoters (*GABRA1-p* and *Gabra1-p*, respectively; Fig. 3C). To determine if SE-induced increases in pCREB and ICER mediate decreased *Gabra1* transcription, we investigated whether SE produces a change in the binding of these factors to the rat *Gabra1-p*:CRE site. We immunoprecipitated the endogenous rat *Gabra1*–protein complex with antibodies directed against pCREB (Ser^{133}) and detected precipitated *Gabra1* genomic fragments by PCR. We used two sets of primers to detect precipitated *Gabra1*: one pair that flanks the CRE site and one that flanks a potential CCAAT–enhancer binding protein (C/EBP) site that is 545 base pairs (bp) upstream of the CRE. Although both primer pairs detected *Gabra1* sequence in DNA subjected to sonication but not immunoprecipitated (called "input" DNA), only the primer pair amplifying the region of *Gabra1* that contained a CRE site amplified DNA after precipitation with anti-pCREB antibody (Fig. 3, D and E). The C/EBP primer pair did not amplify any DNA after precipitation with anti-pCREB antibody (Fig. 3F), confirming the specificity of pCREB binding to the region of *Gabra1* containing the CRE. Results of these chromatin immunoprecipitation (ChIP) assays show that there is a greater than 200% increase in the amount of pCREB at the *Gabra1*:CRE site 24 hours after SE (Fig. 3E).

All available antibodies to ICER also recognize CREM variants, making it impossible to specifically identify ICER binding to *Gabra1* by standard ChIP. We therefore developed a DNA pull-down assay to purify nuclear proteins in DG tissue that specifically recognize the *Gabra1*:CRE so that these proteins could be identified by gel electrophoresis. Nuclear binding proteins prepared from the DG of animals 24 hours after SE and controls were incubated with streptavidin beads containing oligonucleotide duplexes corresponding to the *Gabra1*:CRE. ICER was identified from the bound proteins by size on an SDS–polyacrylamide gel electrophoresis (SDS-PAGE) Western blot. As shown in Fig. 3G (left panel), CREB bound to the *Gabra1*:CRE site in both the control and SE-exposed animals 24 hours after SE. ICER, however, was barely detectable in control animals but was abundant after SE. Additional CREM variants were not detected at the *Gabra1*:CRE site (Fig. 3G, right panel). Mutant A1 CRE containing oligonucleotide duplexes do not purify CREB or ICER proteins from nuclear extracts of cultured neurons (fig. S2).

Gabra1 transcriptional repression relies on pCREB, ICER, and BDNF

GABRA1-p is differentially regulated by the binding of CREB and ICER to the CRE site, and overexpression of recombinant ICER in neurons decreases the presence of α1 subunits at the cell surface (23). Given that SE causes an increase in CREB phosphorylation and transcriptional induction of ICER and that ICER can bind as a heterodimer or as a homo-dimer, we determined whether pCREB is required for the ICER-mediated decrease in *GABRA1* transcription. Overexpression of ICER in the presence and in the absence of a dominant-negative form of CREB (MCREB), which contains a mutation in the Ser^{133} phosphorylation site of pCREB, suggests that ICER-induced endogenous repression of *Gabra1* promoter activity in embryonic hippocampal neurons (Fig. 4A) depends on pCREB. Taken together with the presence of pCREB at *Gabra1-p* after SE, these results suggest that CREB–ICER heterodimers repress *Gabra1* transcription in vivo.

Seizures increase BDNF expression (22,47-50) and limiting activation of the TrkB receptor inhibits development of kindling, suggesting that BDNF may play a role in epileptogenesis (51). Twenty-four hours of BDNF exposure increases the abundance of $GABA_AR$ α_4 subunits and decreases the abundance of α_1 subunits in primary hippocampal neurons in a manner similar to that seen in animal models of TLE (22). Here, we found that BDNF treatment of hippocampal neurons for 6 hours decreased α1 mRNA expression by 33% (Fig. 4B). We further found that this decrease was associated with an increase in ICER mRNA expression and protein abundance (Fig. 4, C and D), suggesting that BDNF-induced decreases in transcription of α1 may be mediated by an increase in ICER. The protein kinase A (PKA) inhibitor H-89 failed to block induction of ICER, as did inhibitors of calmodulin and phosphoinositide 3 (PI 3)-kinases previously shown to play a role in CREB activation (fig. S3), indicating that BDNF-induced ICER in primary hippocampal neurons is not PKA dependent.

JAK/STAT inhibition restores α1 subunit mRNA abundance in culture and in vivo

Because the JAK/STAT pathway can be activated by cytokines and has been implicated in multiple diseases associated with the increased release of cytokines (52,53), we examined whether this signaling pathway controls synthesis of ICER in response to BDNF and SE. BDNF treatment induced the activation of STAT3 (Fig. 4), and pretreatment with the pan JAK inhibitor pyridone 6 (P6, 500 nM) (54) prevented BDNF-induced ICER synthesis (Fig. 5, A, B, and C) and the decrease in α1 subunit mRNA expression (Fig. 5D) in primary hippocampal neurons. P6 failed to inhibit forskolin-induced increases in ICER expression (fig. S5), suggesting that JAK/STAT activation is essential for increases in ICER expression mediated by BDNF but not those mediated by other signaling pathways. P6 alone had no effect on ICER expression (Fig. 5, A, B, and C) or on α1 expression (Fig. 5D), nor did it have any effect on the amount of pCREB with or without BDNF stimulation (Fig. 5, E and F). To directly examine the contribution of the JAK/STAT pathway to the altered gene expression controlled by BDNF, hippocampal neurons were transfected with siRNAs that specifically target STAT3. As shown in Fig. 5G, when compared to scrambled siRNA controls, STAT3 siRNAs specifically attenuated the amount of BDNF-induced ICER mRNAs (43.1 ± 3.6%; $n = 6$, CTRL, $n = 5$, STAT3, $P < 0.05$) in a manner consistent with a 41.3 ± 0.5% transfectional efficiency of siRNA transfection in primary hippocampal neurons (see Materials and Methods).

We next determined whether activation of the JAK/STAT pathway elicits the decrease in transcription of *Gabra1* that occurs in vivo in the pilocarpine model of epilepsy. We examined the activation of the JAK/STAT pathway at several time points after SE by measuring phosphorylated STAT3 (pSTAT3) and phosphorylated STAT1 (pSTAT1) abundance (Fig. 6, A to D). pSTAT3 abundance in DG increased to 380% of controls at 1 hour ($n = 4$ CTRL, $n = 5$ SE, $P < 0.05$) to 2190% of controls at 6 hours ($n = 4$ CTRL, $n = 5$ SE, $P < 0.05$), to 890% of controls at 24 hours ($n = 4$ CTRL, $n = 6$ SE, not significant), and to 860% of controls at 48

hours (n = 4 CTRL, n = 5 SE, $P < 0.05$) after SE (Fig. 6, A and B). pSTAT1 abundance in DG did not increase at 1 hour (n = 4 CTRL, n = 5 SE), but increased to 520% of controls at 6 hours (n = 4 CTRL, n = 5 SE, $P < 0.05$), to 1004% of controls at 24 hours (n = 4 CTRL, n = 6 SE, $P > 0.05$), and was not significantly different from controls by 48 hours (n = 4 CTRL, n = 5 SE) after SE (Fig. 6, C and D). The time points for these increases in STAT phosphorylation correlate temporally with the observed increases in ICER mRNA and protein abundance and precede α1 subunit changes (Fig. 2 and fig. S1), thereby supporting the hypothesis that the JAK/STAT pathway may be an upstream regulator of ICER-dependent decreases in *Gabra1* transcription after SE.

To directly determine if the JAK/STAT pathway mediates ICER synthesis and α1 mRNA expression after SE, we used Alzet (Cupertino, CA) mini osmotic pumps to slowly infuse 1 mM of the JAK/STAT pathway inhibitor P6 directly into the DG beginning 48 hours before SE induction. Given that P6 was administered before SE was induced, we first determined whether P6 affects the acquisition of SE itself and found that pump implantation and slow infusion of P6 did not affect SE (fig. S4). The total dose of pilocarpine required to induce SE (fig. S4A), the latency to develop stage 5 seizures (fig. S4B), and the total dose of diazepam (milligrams per kilogram) required to stop SE (fig. S4C) were not different between the SE only group (SE), the group that was implanted with dimethyl sulfoxide (DMSO, vehicle)–filled pumps before SE (veh-SE), or the group that was implanted with P6-filled pumps before SE (P6-SE) (for all: n = 6 SE, n = 8 veh-SE, n = 8 P6-SE; $P > 0.05$).

P6 infusion blocked JAK/STAT pathway activation after SE as shown by the absence of an increase in P6-SE animals 48 hours after SE (P6-SE; Fig. 6, E and F). pSTAT3 abundance was 980% higher in the combined group that contained rats that underwent SE only and rats that underwent SE after implantation with DMSO vehicle pumps (SE + veh-SE) than in controls (n = 6 rats per group, $^*P < 0.05$) but there was no significant difference in pSTAT3 abundance between P6-SE (n = 4) and controls ($P > 0.05$). Slow infusions of P6 also blocked increases in ICER mRNA expression and protein abundance that occur at 48 hours after SE (Fig. 6, E, G, and H). ICER protein was 510% higher in the SE + veh-SE group than in controls (n = 6 CTRL, n = 6 SE + veh-SE, $^*P < 0.05$; Fig. 6, E and G). ICER protein abundance in the P6-SE group was not significantly different from that in controls (n = 6 CTRL, n = 8 P6-SE, $P > 0.05$; Fig. 6, E and G). ICER mRNA expression in DG increased to 990% of control values in the SE + veh-SE group (n = 6, $^*P < 0.05$) but did not change in P6 pump–implanted rats (P6-SE, n = 4, $P > 0.05$) compared to controls (Fig. 6H).

Activated STATs bind to STAT consensus sequences in the promoters of target genes to increase their transcription, so we determined whether pSTAT3 might exert its effects on ICER expression by binding to a consensus site on the ICER promoter (*ICER-p*). We identified one STAT site in the proximal region of the proposed rat and human *ICER-p* (Fig. 6I) and used ChIP to determine whether there is an increase in associated pSTAT3 after SE (Fig. 6J). pSTAT3 binding to *ICER-p* increased nearly 1000% after SE (control, 0.016 ± 0.015 relative units; SE, 0.168 ± 0.01 relative units; Fig. 6, J and K), providing further evidence that the JAK/STAT pathway regulates ICER in vivo and identifying pSTAT3 as a mediator of ICER transcription in the brain.

Finally, we asked whether the decrease in *Gabra1* transcription after SE was reversed on inhibition of JAK/STAT signaling in vivo. Animals subjected to SE and killed 9 days after implantation of DMSO vehicle or P6-containing Alzet pumps were used to determine whether JAK/STAT inhibition prevented decreases in *Gabra1* transcription that persist in animals that become chronically epileptic after SE (Fig. 6L). Animals in the SE + veh-SE group showed a 69% reduction in α1 mRNA expression (n = 6 CTRL, n = 8 SE + veh-SE, $P < 0.05$), but there

was no significant decrease in the P6-SE group ($n = 4$, $P > 0.05$; Fig. 6L), suggesting that JAK/STAT pathway activation is necessary for seizure-induced decreases in *Gabra1* transcription.

DISCUSSION

Changes in $GABA_AR$ α subunit composition may be important in the molecular pathogenesis of several common CNS conditions including epilepsy (6-9), alcoholism (10,55), anxiety (8, 11), and stress (12-14). Decreases in $GABA_AR$ α1 subunit expression and increases in α4 subunit expression in hippocampal DG after prolonged seizures result in altered $GABA_AR$ localization and function, which may facilitate the development of spontaneous seizures (20). BDNF mediates increases in $GABA_AR$ α4 transcription by stimulating the protein kinase C–mitogen-activated protein kinase (MAPK) pathway to activate early growth factor 3 (Egr3) binding to the early growth factor response element site of *Gabra4* (21,22); however, the mechanisms underlying the coordinate decreases in transcription of *Gabra1* have been unclear. We now elucidate the signaling pathways that regulate *Gabra1*, showing that increases in pCREB and ICER occurring after SE mediate the repression of *Gabra1* transcription through CRE site binding, resulting in reduced expression of α1 mRNAs and a loss of α1γ2-containing $GABA_ARs$. We further show that the JAK/STAT pathway drives enhanced ICER transcription after SE and that activation of this pathway is stimulated by BDNF. These findings suggest that the interplay of the CREB, JAK/STAT, and BDNF signaling pathways are critical for $GABA_AR$ α1 gene regulation and that these pathways may provide novel therapeutic targets for the treatment of epilepsy and possibly for other CNS disorders in which $GABA_AR$ α1 subunit decreases are a pathogenic mechanism.

Modulation of neuronal activity, particularly through the neuro-transmitter receptors for $GABA_AR$, glutamate, and serotonin, can influence α1 subunit abundance (56-59), but the regulatory pathways mediating these effects are not known. A study using microarrays to screen for changes in multiple mRNAs in response to overexpressed dominant-negative CREB (MCREB) in vivo suggested that *GABRA1* might be regulated directly or indirectly by CREB (60), but whether this mechanism was important for *GABRA1-p* regulation after SE was not known.

Either homodimerization of ICER or heterodimerization of CREB with ICER could repress promoter activity by blocking CREB homodimerization that would result in enhanced transcription (34,35). In the current study, we find that in vivo in DG, SE stimulates increased binding of pCREB and ICER to *Gabra1-p*, suggesting that CREB and ICER bind as heterodimers at the *Gabra1*:CRE site to repress transcription. Using transfected *GABRA1* promoter–reporter constructs with expression vectors for CREB, MCREB, and ICER, we provide further evidence that the repressive effect of ICER on *GABRA1* promoter activity in transfected hippocampal neurons depends on pCREB (Fig. 4A).

No evidence was found that other repressors of the CREB family are involved in the transcriptional regulation of α1 subunit expression. The CREM gene encodes multiple truncated proteins that can bind CRE sites but lack the activation domain necessary to support transcription. The ICER antibody used in our studies detects other CREM isoforms, but no increases in these isoforms occurred after SE. Other CREB family repressors, such as activating transcription factor-4 (ATF-4), were not specifically examined, so their effects on *Gabra1* regulation after SE remain unknown.

The excessive neuronal activity associated with SE stimulates many different signaling pathways that could result in enhanced phosphorylation of CREB and expression of ICER (51). Determining which of these pathways mediates the increase in ICER expression critical for decreased transcription of the $GABA_AR$ α1 subunit is crucial to understanding how cross

talk between different signaling pathways can lead to specific changes in inhibitory neurotransmission. We focused on BDNF as a potential regulator of ICER because BDNF expression increases markedly after SE and because BDNF differentially regulates the abundance of α1 and α4 subunits in cultured neurons (22). We here confirm that BDNF induces ICER expression in primary hippocampal cultures, but that this induction does not depend on PKA activation (fig. S1), as expected based on our previous finding that PKA activation by forskolin increases transcription of ICER and decreases α1 mRNA abundance in cortical cultures (23). BDNF activates several cAMP–PKA–independent signaling pathways that could regulate ICER synthesis, including the Ras-MAPK, calmodulin (CaM)-dependent protein kinase IV, PI 3-kinase–protein kinase C, and phospholipase C-γ pathways (61-64). However, BDNF-induced ICER expression is not blocked by either the PI 3-kinase inhibitor LY294002 or the CaM kinase inhibitor KN93 (fig. S1), suggesting that another pathway must underlie BDNF-mediated ICER induction in primary cultured hippocampal neurons.

Because the JAK/STAT pathway has been implicated in stress-related cellular changes in function and has been shown to be activated in CNS disorders, including ischemia and one form of seizure (65-67), we tested whether BDNF might activate this pathway to induce ICER and decrease $GABA_AR$ α1 subunit expression in primary cultures. The JAK/STAT signaling pathway is activated by cytokines binding to their specific receptors, resulting in transphosphorylation of JAK kinases that then lead to phosphorylation of STAT proteins (68-72). Phosphorylation of STATs on tyrosine residues leads to STAT homo- or heterodimerization, trans-location from the cytoplasm to the nucleus, and binding to specific DNA elements (STAT-recognition sites) to regulate target gene expression (69,70,73). Such an element is found in the ICER promoter (Fig. 6I) and here we show that pSTAT3 association with this site is enhanced after SE in DG (Fig. 6, J and K). We further show that siRNA knockdown of STAT3 inhibits BDNF-induced ICER expression, as does blockade of JAK/STAT signaling with P6 in primary hippocampal cultures (Fig. 5G). Using P6 as a tool in vivo to explore the relationship of the JAK/STAT pathway to seizure-induced α1 subunit changes, we show that ICER induction and decreased transcription of α1 mRNA are pathway dependent. Our findings are consistent with recent studies showing that BDNF induces phosphorylation of JAK2, STAT1, and STAT3 to promote neurite growth in the major pelvic ganglion of the rat (74).

Numerous changes in $GABA_AR$ trafficking, localization, and subunit expression have been reported in TLE models (8,9,15,17,21,75). In hippocampal DGCs, decreases in α1 subunit transcription and increases in α4 subunit transcription may play a critical role in epileptogenesis. Increases in α4 subunit expression in DGCs have been reported in different epilepsy models (3,9,75-77), and two recent studies have found increased colocalization of α4 and γ2 subunits in synaptic and perisynaptic locations on DGCs in epileptic animals (16, 17). Our current receptor pull-down studies show that α1 is replaced by the α4 subunitin γ2-containing receptors after SE. This provides further support for the concept that during epileptogenesis, altered expression of α subunits leads to changes in synaptic $GABA_AR$ composition and localization that may underlie impairments in synaptic inhibition in DGCs, including diminished benzodiazepine sensitivity, enhanced zinc sensitivity, reduced neurosteroid modulation, and diminished phasic inhibition in dendrites (9,16-18).

The JAK/STAT and CREB/ICER pathways regulate a myriad of genes with diverse functions and modulation of either of these pathways may thus have a multitude of downstream effects, many of which may involve cell- and region-specific responses. Therefore, the final impact of global blockade of these pathways on epileptogenesis may be difficult to predict. Although the enhanced $GABA_AR$ α1 subunit expression in DG 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 (78) and develop more severe epilepsy

after pilocarpine-induced SE (40). Consistent with this finding, ICER-overexpressing mice show retardation of kindling development (78). Whether the effects of short-term and transient blockade of ICER increases at the time of SE specifically in the hippocampal formation, such as done in the current study, will have a similar effect on epileptogenesis as constitutive under- or overexpression of ICER in the brain examined previously remains to be determined.

In summary, we show that seizure-induced decreases in $GABA_AR$ α1 subunit expression are mediated by JAK/STAT pathway stimulation of ICER expression, resulting in binding of CREB and ICER to the *Gabra1* promoter. Our findings that BDNF treatment of hippocampal neurons stimulates phosphorylation of STAT3 and induces increases in ICER expression, and decreased transcription of *Gabra1* similar to those seen after SE, suggest that seizure-associated increases in BDNF are likely part of the upstream signals stimulating the JAK/STAT pathway. These results are particularly interesting in light of the demonstration that BDNF up-regulates $GABA_AR$ α4 expression by stimulating expression of Egr3 via protein kinase C–MAPK pathway activation (21,22). Taken together, these findings identify BDNF as a "master" transcriptional regulator that works through at least two distinct signaling pathways to coordinately influence $GABA_AR$ subunit expression.

MATERIALS AND METHODS

Induction of SE

Adult rats underwent pilocarpine-induced SE according to standard protocols (9). All rats treated with full-dose pilocarpine were confirmed to have prolonged stage 5 behavioral seizures (79). Control rats were given subconvulsive doses of pilocarpine. Rats were killed at 1, 6, 24, or 48 hours or 1 week after onset of SE and the DG was rapidly dissected and frozen for protein and RNA isolation for Western blots and real-time RT-PCR, respectively, or animals were rapidly perfused with 4% paraformaldehyde for immunocytochemistry and ChIP. All animal procedures were performed in accordance with Institutional Animal Care and Use Committee regulations and approved protocols.

RT-PCR

RNA was extracted from microdissected DG with the use of the Trizol reagent protocol (Invitrogen, Carlsbad, CA). To synthesize complementary DNA (cDNA), 1 μg of RNA was separated and processed with the SuperScript II reverse transcription kit (Invitrogen) according to the manufacturer's instructions and then diluted 1:4 for storage and subsequent RT-PCR. For RT-PCR reactions, each sample was run in triplicate and each 25-μl reaction contained 1.25 μl ICER, CREB, CREM, or $GABA_AR$ α1 Taqman primer and probe sets from Applied Biosystems (Foster City, CA) or 1.25 μl Taqman cyclophilin probe (Applied Biosystems) with 1.25 μl of each cyclophilin primer (cycloREV, 5′-CCC AAG GGC TCG CCA-3′; cycloFWD, 5′-TGC AGA CAT GGT CAA CCC C-3′; IDT Technologies, Coralville, IA), and 12.5 μl Taqman Master mix, and 10 μl of sample cDNA. RT-PCR was performed on the SDS-7500 PCR machine (Applied Biosystems). The RT-PCR runs consisted of first 1 cycle of 50°C for 2 min, then 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. All values were normalized to cyclophilin expression in the same samples to control for loading variability, then expressed as percent change with respect to mean control values in the same run (defined as 1). Statistical significance was defined as a *P* value of less than 0.05 and was calculated with InStat software with the Mann–Whitney or Kruskal–Wallis tests as indicated.

Receptor immunoprecipitation

Coimmunoprecipitation experiments were carried out with the use of the Pro-found mammalian coimmunoprecipitation kit (Pierce, Rockford, IL). Affinity-purified rabbit polyclonal antibodies (50 μg) raised against the γ2 subunit (Alpha Diagnostics, San Antonio,

TX), were coupled to AminoLink Plus gels as specified by the manufacturer. Hippocampi from each rat were homogenized in 1 ml of M-PER buffer (mammalian protein extraction reagent) plus protease inhibitor cocktail (Roche, Indianapolis, IN) 1× and 1 mM phenylmethylsulfonyl fluoride (PMSF). Homogenized lysates were passed five times through a 21-gauge needle fitted to a syringe. Samples were centrifuged at 12,000*g* for 30 min at 4°C. The supernatant was recovered and protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA). Supernatants were incubated with the antibody-coupled gel overnight at 4°C with gentle rocking. Gels were washed five times with buffer and complexes were recovered with 100 μl of the elution buffer provided with the kit. Controls were performed by quenching the same gel before coupling antibodies to test for proteins that may bind nonspecifically to the gel. Approximately 35 μl of the eluate was analyzed by Western blot with anti-α1 or anti-α4 (both 1:1000 dilution, Novus Biologicals, Littleton, CO) and γ2 (1:1,000 dilution, Alpha Diagnostic) polyclonal antibodies.

Immunocytochemistry

Immunocytochemistry was performed with standard methods (80). Cryostat sections (10 μM) were blocked with horse serum, incubated with hydrogen peroxide, and then treated with rabbit polyclonal antibody raised against CREB phosphorylated at Ser^{133} (anti-pCREB) (Upstate, Lake Placid, NY; 1:500) or rabbit polyclonal antibody raised against CREM-1 (anti-CREM/ICER) (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1000), which recognizes multiple CREM isoforms including ICER. After overnight incubation, the slices were treated with polyclonal anti-rabbit immunoglobulin G (IgG) antibody labeled with rhodamine (1:1000) or avidin conjugated and reacted with diaminobenzene (Sigma, St. Louis, MO; 1:1000).

Western blot

Western blot was performed with modifications of published protocols (80). Protein (25 μg) extracted from DG was loaded into 10% (for pCREB and pSTATs) or 12% (for CREM/ICER and pSTATs) SDS–polyacrylamide gels and run for 1.5 hours at 110 V. Blots were then transferred to nitrocellulose membranes and blocked in 2% milk/tris-buffered saline with Tween-20 (TBS-T) (for pCREB) or 5% milk/phosphate-buffered saline (PBS) (for CREM/ICER). Membranes were incubated with rabbit polyclonal antibodies raised against CREB phosphorylated at Ser^{133} (anti-pCREB) (Upstate, 1:400), total CREB (anti-CREB) (Upstate, 1:500), CREM-1 (anti-CREM/ICER) (Santa Cruz Biotechnology; 1:1000), STAT3 phosphorylated at Tyr^{705} (anti-pSTAT3) (Cell Signaling Technologies; 1:1000), total STAT3 (anti-STAT3) (Cell Signaling Technologies; 1:1000), STAT1 phosphorylated at Tyr^{701} (anti-pSTAT1) (Cell Signaling Technologies; 1:1000) or total STAT1 (anti-STAT1) (Cell Signaling Technologies; 1:1000) overnight at 4°C in 1.5% milk/TBS-T (for pCREB or CREB) or 5% milk/PBS (for CREM/ICER), or 5% bovine serum albumin/TBS-T (for pSTATs), then washed and incubated with anti-rabbit antibody (1:5000) conjugated to horseradish peroxidase (HRP) for 2 hours. CREM and ICER bands were differentiated based on size (CREM, 31 kDa; ICER/ICERγ, 19/17 kDa). Protein bands were detected with the use of chemiluminescent solution (Pierce), then membranes were stripped and reprobed with rabbit polyclonal antibody raised against β-actin (1:5000, Sigma) in 5% milk/TBS-T. All values were normalized to β-actin expression in the same samples to control for loading amount variability and then expressed as percent change with respect to mean control values in the same run (defined as 1). (Exception: Fig. 4, E and F data from pSTAT3 Western blot were normalized to total STAT3 expression that does not change with BDNF treatment.) Densitometry was performed with NIH Image version 1.63. Statistical significance was defined as a *P* value of less than 0.05 and was calculated with InStat software with the Mann–Whitney or Kruskal–Wallis tests as indicated.

Chromatin immunoprecipitation

After the animal was perfused with 4% formaldehyde, DG tissue was dissected, homogenized, and kept immersed in formaldehyde to cross-link protein–DNA interactions. DNA was sheared, producing about 300-bp fragments. Size was confirmed by gel electrophoresis. DNA was immunoprecipitated with CREB and pCREB antibodies (both 1:150; Cell Signaling), then isolated and dissolved in 100 μl TE or for pSTAT3 antibody (Santa Cruz Biotechnology) in 25 μl nuclease-free water. Real-time PCR was performed to determine amplification in the linear range with the use of primers that flank the CRE site and an internal sequence-specific Taqman probe: forward, 5′TGG TAC CAC CTT CCT TTC TAA AAT AAA3′; reverse, 5′ ATA CGT CCC AGC GCA AAC C3′; Taqman probe, 5′TCT CTC TGG CAT GAA GTC ACC GCC T3′. Data were normalized as percentage of antibody/input signal and expressed as percent change with respect to control (defined as 100%). Input is the signal from the DNA preparation before precipitation. Standard PCR for detection of precipitated genomic DNA was performed with the following primer pairs: for CREB binding to *Gabra1-p*, forward 5′-AGA GGA ATG CTT GCA GCA G-3′ and reverse 5′-GCT TCC CAA TAT CCA ATC TG-3′; for C/EBP binding to *Gabra1-p* (as a control for DNA fragmentation and site specificity of CREB binding), forward 5′-TGT CTG GCT GTC CTC TAT GC-3′ and reverse 5′AAT GAA CGC CAA GCT ACG AA-GC-3′; for STAT3 binding to *ICER-p*, forward 5′-GTT CAG TCC CTG AAA TGT GG-3′ and reverse 5′-GCC GTA CCA CAG TCC AAA AT-3′. Nonradioactive PCR products were visualized with standard agarose gel electrophoresis (*Gabra1-p*) and p^{33}-labeled radioactive PCR products were visualized with 5% polyacrylamide gel electrophoresis followed by exposure to film (*ICER-p*). Negative controls for nonspecific DNA precipitation were either IgG, standard in the field, or an isotype antibody such as chicken polyhistidine tag (fig. S6). We see no difference between the negative controls for their ability to detect nonspecific binding.

Nuclear extract preparation

Hippocampi from three control and three SE-exposed rats were individually homogenized in 1 ml of 1× PBS. The cell pellet was gently resus-pended in 500 μl buffer A [10 mM tris–Cl, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), 1 mM PMSF, 1× Protease inhibitor cocktail] and incubated for 15 min, after which 25 μl of a 10% NP-40 solution (American Bioanalytical, Natick, MA) was added. The cell suspension was vortexed for 5 s and centrifuged at 12,000*g* for 30 s to pellet nuclei. The supernatant was removed and the nuclear pellet was resuspended in 400 μl of buffer C (20 mM tris–Cl, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1× Protease inhibitor cocktail) and incubated for 1 hour at 4°C. The nuclear debris was then removed by centrifugation at 12,000*g* for 5 min at 4°C and the supernatant was transferred to a fresh tube. Glycerol was added to a final concentration of 20%. Before the nuclear extracts were used for DNA pull-down assay, buffer exchange was performed with the use of Zeba Desalt Spin Columns (Pierce). The nuclear extract buffer was changed to DNA pull-down buffer C (20 mM tris, pH 8.0, 1 mM EDTA, 10% glycerol, 1 mM DTT, 50 mM NaCl) and then protein concentration was determined by the Bradford assay (Bio-Rad Laboratories).

DNA pull-down assay

The DNA pull-down assay was performed as previously described (81). Oligonucleotide duplexes corresponding to the sequence of the CRE site in the α1 promoter (as well as the mutant form containing a 2-bp mutation; see fig. S2) and its flanking region were covalently linked to a biotin moiety at their 5′ ends. Sequence of sense strand: wild type-CTC TGG CAT GAA GTC ACC GCC TAT; mutant-CTC TGG CAT GAA CAC ACC GCC TAT. Streptavidin Dynabeads (DynaL A.S.A.) were washed three times in buffer A (5 mM tris, pH 8.0, 0.5 mM EDTA, 1 M NaCl). Annealed oligonucleotides were incubated with beads (200 μmol/mg of

beads) for 15 min at room temperature in buffer A. Beads were then washed twice with 500 μl buffer A and three times with 500 μl buffer C (20 mM tris, pH 8.0, 1 mM EDTA, 10% glycerol, 1 mM DTT, 50 mM NaCl). Nuclear extract (600 μg) from hippocampal tissues in buffer C was incubated with 1 mg of streptavidin Dynabeads bound to the appropriate oligonucleotides. After 15 min incubation at room temperature, beads were washed three times with buffer C and eluted in 1 M NaCl. Eluted proteins were then analyzed by SDS-PAGE followed by Western blot with anti-CREB (Cell Signaling) and anti-CREM1 antibody (which detects multiple CREM isoforms including ICER) (Santa Cruz Biotechnology).

Cell culture and transfections

Primary rat hippocampal cultures were prepared from embryonic day 18 embryos (Charles River Laboratories) and transfected 6 to 8 days after dissociation with a modified calcium phosphate precipitation method (23).

siRNA transfection

siRNAs corresponding to STAT3 were chemically synthesized by Ambion as were negative control scrambled siRNAs. Transfections were carried out with RNAiFect transfection reagent (Qiagen). Primary cultured hippo-campal neurons were plated in six-well plates as described (21-23). Seven days after plating, siRNAs (1 μg) were diluted 1:100 with buffer EC-R to a total volume of 100 μl. Complex formation was formulated with 12 μlof RNAiFect transfection reagent. After 15 min of incubation, complexes were directly added onto the cells and incubated for 24 hours under normal growth conditions. The culture medium was replaced with Neuro-basal medium. The transfection was repeated for another 24 hours. After the second transfection, cells were treated with BDNF for 4 hours to stimulate ICER induction, and further analysis was performed. Transfectional efficiency of around 40% was measured by quantitation of fluorescent siRNA controls through microscopy.

Reporter assays

The human GABRA1 promoter fragment (894/+70) in the pGL2 luciferase reporter vector (Promega, Madison, WI) was a generous gift of the D. H. Farb laboratory. The promoter fragment confers full promoter activity on primary neocortical neurons. The dominant-negative MCREB expression vector was a generous gift of the M. E. Greenberg laboratory. Wild-type CREB was generated with the use of MCREB as a template for site-directed mutagenesis reestablishing the Ser^{133} phosphorylation site. The ICER expression vector was generated from the CREM cDNA. ICER IIγ cDNA was amplified from an IMAGE human cDNA clone (accession no. BC090051, Open Biosystems, Huntsville, AL) by PCR. The sequences of the primers were as follows: ICER IIγ,forward 5′-CGG GAT CCA TGG CTG TAA CTG GAG ATG ACA CAG CTG CCA CTG GTG ACA TGC CAA C-3′; ICER IIγ, reverse 5′-GCT CTA GAC TAG TAA TCT GTT TTG GGA GAA CAA ATG-3′. The amplified fragments were cloned into the *BamH I* (5′ end) and *Xba I* (3′ end) sites of the expression vector pcDNA3 (Invitrogen). The construct was sequenced to confirm identity. A Western blot analysis was performed after overexpression of the ICER IIγ cDNA construct in human embryonic kidney–293 cells to confirm expression and identity. Eight micrograms of total DNA was transfected into each well of a six-well dish and luciferase activity was assayed 12 to 16 hours after transfection with the Victor 1420 detection system (E. G. Wallace). Luciferase counts were normalized to protein in each dish (counts per minute per microgram).

Osmotic pump implantation and P6 infusions

Sprague–Dawley male rats were obtained from Zivic Miller (New Castle, PA), preimplanted with mini osmotic pumps (model 1002; Alzet) that were prefilled to deliver 1 mM P6 or DMSO vehicle at a rate of 0.5 μl/hour. Pumps were attached to unilateral cannulae to deliver P6 or

vehicle into the right DG at the following bregma coordinates: anterior/posterior, −4.5 mm; medial/lateral, 2.0 mm; dorsal/ventral, 3.8 mm. SE was induced with pilocarpine as described above 48 hours after pump implantation and the start of P6 or vehicle infusion. Rats were killed at 48 hours or 9 days after SE along with subconvulsive pilocarpine control rats that did not undergo surgery (CTRL group) and rats subjected to pilocarpine-induced SE that did not undergo pump implantation surgery (SE group). After decapitation, brains were removed, then hippocampi were rapidly dissected and fast-frozen as described above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES AND NOTES

1. Rudolph U, Mohler H. GABA-based therapeutic approaches: GABAA receptor sub-type functions. Curr. Opin. Pharmacol 2006;6:18–23. [PubMed: 16376150]
2. Sieghart W. Structure, pharmacology, and function of GABAA receptor subtypes. Adv. Pharmacol 2006;54:231–263. [PubMed: 17175817]
3. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. gamma-Aminobutyric acid(A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. J. Neurochem 2001;77:1266–1278. [PubMed: 11389177]
4. Brooks-Kayal AR, Jin H, Price M, Dichter MA. Developmental expression of GABA(A) receptor subunit mRNAs in individual hippocampal neurons in vitro and in vivo. J. Neurochem 1998;70:1017–1028. [PubMed: 9489721]
5. Mohler H. Molecular recognition of cognitive functions and developmental plasticity: Impact of GABA (A) receptors. J. Neurochem 2007;102:1–12. [PubMed: 17394533]
6. Lauren HB, Pitkanen A, Nissinen J, Soini SL, Korpi ER, Holopainen IE. Selective changes in gamma-aminobutyric acid type A receptor subunits in the hippocampus in spontaneously seizing rats with chronic temporal lobe epilepsy. Neurosci. Lett 2003;349:58–62. [PubMed: 12946586]
7. Sperk G, Furtinger S, Schwarzer C, Pirker S. GABA and its receptors in epilepsy. Adv. Exp. Med. Biol 2004;548:92–103. [PubMed: 15250588]
8. Mohler H. GABAA receptors in central nervous system disease: Anxiety, epilepsy, and insomnia. J. Recept. Signal Transduct. Res 2006;26:731–740. [PubMed: 17118808]
9. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat. Med 1998;4:1166–1172. [PubMed: 9771750]
10. Grobin AC, Fritschy JM, Morrow AL. Chronic ethanol administration alters immunoreactivity for GABA(A) receptor subunits in rat cortex in a region-specific manner. Alcohol. Clin. Exp. Res 2000;24:1137–1144. [PubMed: 10968650]
11. Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS. Reversal of neurosteroid effects at α4β2δ $GABA_A$ receptors triggers anxiety at puberty. Nat. Neurosci 2007;10:469–477. [PubMed: 17351635]
12. Montpied P, Weizman A, Weizman R, Kook KA, Morrow AL, Paul SM. Repeated swim-stress reduces $GABA_A$ receptor alpha subunit mRNAs in the mouse hippocampus. Brain. Res. Mol. Brain. Res 1993;18:267–272. [PubMed: 7684486]
13. Hsu FC, Zhang G, Raol YS, Valentino RJ, Coulter DA, Brooks-Kayal AR. Repeated neonatal handling with maternal separation permanently alters hippocampal $GABA_A$ receptors and behavioral stress responses. Proc. Natl. Acad. Sci. U.S.A 2003;100:12213–12218. [PubMed: 14530409]
14. Orchinik M, Weiland NG, McEwen BS. Chronic exposure to stress levels of corticosterone alters $GABA_A$ receptor subunit mRNA levels in rat hippocampus. Brain. Res. Mol. Brain. Res 1995;34:29–37. [PubMed: 8750858]
15. Peng Z, Huang CS, Stell BM, Mody I, Houser CR. Altered expression of the delta subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. J. Neurosci 2004;24:8629–8639. [PubMed: 15456836]

16. Sun C, Mtchedlishvili Z, Erisir A, Kapur J. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the a4 subunit of GABA(A) receptors in an animal model of epilepsy. J. Neurosci 2007;27:12641–12650. [PubMed: 18003843]

17. Zhang N, Wei W, Mody I, Houser CR. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. J. Neurosci 2007;27:7520–7531. [PubMed: 17626213]

18. Cohen AS, Lin DD, Quirk GL, Coulter DA. Dentate granule cell GABA(A) receptors in epileptic hippocampus: Enhanced synaptic efficacy and altered pharmacology. Eur. J. Neurosci 2003;17:1607–1616. [PubMed: 12752378]

19. Zhang G, Raol YH, Hsu FC, Coulter DA, Brooks-Kayal AR. Effects of status epilepticus on hippocampal $GABA_A$ receptors are age-dependent. Neuroscience 2004;125:299–303. [PubMed: 15062973]

20. Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. Enhancing GABA(A) receptor α1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. J. Neurosci 2006;26:11342–11346. [PubMed: 17079662]

21. Roberts DS, Raol YH, Bandyopadhyay S, Lund IV, Budreck EC, Passini MA, Wolfe JH, Brooks-Kayal AR, Russek SJ. Egr3 stimulation of GABRA4 promoter activity as a mechanism for seizure-induced up-regulation of GABA(A) receptor α4 subunit expression. Proc. Natl. Acad. Sci. U.S.A 2005;102:11894–11899. [PubMed: 16091474]

22. Roberts DS, Hu Y, Lund IV, Brooks-Kayal AR, Russek SJ. Brain-derived neurotrophic factor (BDNF)–induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor α4 subunits in hippocampal neurons. J. Biol. Chem 2006;281:29431–29435. [PubMed: 16901909]

23. Hu Y, Lund IV, Gravielle MC, Farb DH, Brooks-Kayal AR, Russek SJ. Surface expression of GABA (A) receptors is transcriptionally controlled by the interplay of CREB and its binding partner ICER. J. Biol. Chem 2008;283:9328–9340. [PubMed: 18180303]

24. Montminy MR, Sevarino KA, Wagner JA, Mandel G, Goodman RH. Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc. Natl. Acad. Sci. U.S.A 1986;83:6682–6686. [PubMed: 2875459]

25. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. Neuron 2002;35:605–623. [PubMed: 12194863]

26. Shaywitz AJ, Greenberg ME. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu. Rev. Biochem 1999;68:821–861. [PubMed: 10872467]

27. Sheng M, Greenberg ME. The regulation and function of c-*fos* and other immediate early genes in the nervous system. Neuron 1990;4:477–485. [PubMed: 1969743]

28. Frank DA, Greenberg ME. CREB: A mediator of long-term memory from mollusks to mammals. Cell 1994;79:5–8. [PubMed: 7923377]

29. Mayr B, Montminy M. Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat. Rev. Mol. Cell Biol 2001;2:599–609. [PubMed: 11483993]

30. Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH. Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 1993;365:855–859. [PubMed: 8413673]

31. Gonzalez GA, Montminy MR. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 1989;59:675–680. [PubMed: 2573431]

32. Molina CA, Foulkes NS, Lalli E, Sassone-Corsi P. Inducibility and negative autoregulation of CREM: An alternative promoter directs the expression of ICER, an early response repressor. Cell 1993;75:875–886. [PubMed: 8252624]

33. Foulkes NS, Borrelli E, Sassone-Corsi P. CREM gene: Use of alternative DNA-binding domains generates multiple antagonists of camp-induced transcription. Cell 1991;64:739–749. [PubMed: 1847666]

34. Jaworski J, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, Gozdz A, Proszynski T, Hetman M, Mallet J, Kaczmarek L. Inducible camp early repressor, an endogenous antagonist of camp responsive element–binding protein, evokes neuronal apoptosis in vitro. J. Neurosci 2003;23:4519–4526. [PubMed: 12805292]

35. Mioduszewska B, Jaworski J, Kaczmarek L. Inducible camp early repressor (ICER) in the nervous system—A transcriptional regulator of neuronal plasticity and programmed cell death. J. Neurochem 2003;87:1313–1320. [PubMed: 14713288]
36. Fitzgerald LR, Vaidya VA, Terwilliger RZ, Duman RS. Electroconvulsive seizure increases the expression of CREM (cyclic AMP response element modulator) and ICER (inducible cyclic AMP early repressor) in rat brain. J. Neurochem 1996;66:429–432. [PubMed: 8522985]
37. Moore AN, Waxham MN, Dash PK. Neuronal activity increases the phosphorylation of the transcription factor camp response element–binding protein (CREB) in rat hippocampus and cortex. J. Biol. Chem 1996;271:14214–14220. [PubMed: 8662977]
38. Lee B, Dziema H, Lee KH, Choi YS, Obrietan K. CRE-mediated transcription and COX-2 expression in the pilocarpine model of status epilepticus. Neurobiol. Dis 2007;25:80–91. [PubMed: 17029965]
39. Tanis KQ, Duman RS, Newton SS. CREB binding and activity in brain: Regional specificity and induction by electroconvulsive seizure. Biol. Psychiatry 2008;63:710–720. [PubMed: 17936724]
40. Porter BE, Lund IV, Varodayan FP, Wallace RW, Blendy JA. The role of transcription factors cyclic-AMP responsive element modulator (CREM) and inducible cyclic-AMP early repressor (ICER) in epileptogenesis. Neuroscience 2008;152:829–836. [PubMed: 18295410]
41. Bender RA, Lauterborn JC, Gall CM, Cariaga W, Baram TZ. Enhanced CREB phosphorylation in immature dentate gyrus granule cells precedes neurotrophin expression and indicates a specific role of CREB in granule cell differentiation. Eur. J. Neurosci 2001;13:679–686. [PubMed: 11207803]
42. Lagrange AH, Botzolakis EJ, Macdonald RL. Enhanced macroscopic desensitization shapes the response of α4 subtype–containing $GABA_A$ receptors to synaptic and extrasynaptic GABA. J. Physiol 2007;578:655–676. [PubMed: 17124266]
43. Schwarzer C, Tsunashima K, Wanzenbock C, Fuchs K, Sieghart W, Sperk G. GABA(A) receptor subunits in the rat hippocampus II: Altered distribution in kainic acid–induced temporal lobe epilepsy. Neuroscience 1997;80:1001–1017. [PubMed: 9284056]
44. Sperk G, Schwarzer C, Tsunashima K, Kandlhofer S. Expression of GABA(A) receptor subunits in the hippocampus of the rat after kainic acid–induced seizures. Epilepsy Res 1998;32:129–139. [PubMed: 9761315]
45. Moss SJ, Smart TG. Constructing inhibitory synapses. Nat. Rev. Neurosci 2001;2:240–250. [PubMed: 11283747]
46. Essrich C, Lorez M, Benson JA, Fritschy JM, Luscher B. Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the g2 subunit and gephyrin. Nat. Neurosci 1998;1:563–571. [PubMed: 10196563]
47. Binder DK, Routbort MJ, McNamara JO. Immunohistochemical evidence of seizure-induced activation of trk receptors in the mossy fiber pathway of adult rat hippocampus. J. Neurosci 1999;19:4616–4626. [PubMed: 10341259]
48. Rudge JS, Mather PE, Pasnikowski EM, Cai N, Corcoran T, Acheson A, Anderson K, Lindsay RM, Wiegand SJ. Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. Exp. Neurol 1998;149:398–410. [PubMed: 9500963]
49. Mudo G, Jiang XH, Timmusk T, Bindoni M, Belluardo N. Change in neurotrophins and their receptor mRNAs in the rat forebrain after status epilepticus induced by pilocarpine. Epilepsia 1996;37:198–207. [PubMed: 8635431]
50. Altar CA, Laeng P, Jurata LW, Brockman JA, Lemire A, Bullard J, Bukhman YV, Young TA, Charles V, Palfreyman MG. Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. J. Neurosci 2004;24:2667–2677. [PubMed: 15028759]
51. McNamara JO, Huang YZ, Leonard AS. Molecular signaling mechanisms underlying epileptogenesis. Sci. STKE 2006;2006:re12. [PubMed: 17033045]
52. O'Neill LA. Targeting signal transduction as a strategy to treat inflammatory diseases. Nat. Rev. Drug Discov 2006;5:549–563. [PubMed: 16773072]
53. Campbell IL. Cytokine-mediated inflammation, tumorigenesis, and disease-associated JAK/STAT/SOCS signaling circuits in the CNS. Brain Res. Brain Res. Rev 2005;48:166–177. [PubMed: 15850655]

54. Thompson JE, Cubbon RM, Cummings RT, Wicker LS, Frankshun R, Cunningham BR, Cameron PM, Meinke PT, Liverton N, et al. Photochemical preparation of a pyridone containing tetracycle: A Jak protein kinase inhibitor. Bioorg. Med. Chem. Lett 2002;12:1219–1223. [PubMed: 11934592]
55. Devaud LL, Fritschy JM, Sieghart W, Morrow AL. Bidirectional alterations of GABA(A) receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. J. Neurochem 1997;69:126–130. [PubMed: 9202302]
56. Montpied P, Ginns EI, Martin BM, Roca D, Farb DH, Paul SM. gamma-Aminobutyric acid (GABA) induces a receptor-mediated reduction in $GABA_A$ receptor alpha subunit messenger RNAs in embryonic chick neurons in culture. J. Biol. Chem 1991;266:6011–6014. [PubMed: 1848843]
57. Henneberger C, Juttner R, Schmidt SA, Walter J, Meier JC, Rothe T, Grantyn R. GluR- and TrkB-mediated maturation of GABA receptor function during the period of eye opening. Eur. J. Neurosci 2005;21:431–440. [PubMed: 15673442]
58. Sibille E, Pavlides C, Benke D, Toth M. Genetic inactivation of the serotonin(1A) receptor in mice results in downregulation of major GABA(A) receptor alpha subunits, reduction of GABA(A) receptor binding, and benzodiazepine-resistant anxiety. J. Neurosci 2000;20:2758–2765. [PubMed: 10751426]
59. Brown MJ, Bristow DR. Molecular mechanisms of benzodiazepine-induced down-regulation of $GABA_A$ receptor α1 subunit protein in rat cerebellar granule cells. Br. J. Pharmacol 1996;118:1103–1110. [PubMed: 8818332]
60. McClung CA, Nestler EJ. Regulation of gene expression and cocaine reward by CREB and DeltaFosB. Nat. Neurosci 2003;6:1208–1215. [PubMed: 14566342]
61. Kaplan DR, Miller FD. Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol 2000;10:381–391. [PubMed: 10851172]
62. Segal RA, Greenberg ME. Intracellular signaling pathways activated by neurotrophic factors. Annu. Rev. Neurosci 1996;19:463–489. [PubMed: 8833451]
63. Huang EJ, Reichardt LF. Trk receptors: Roles in neuronal signal transduction. Annu. Rev. Biochem 2003;72:609–642. [PubMed: 12676795]
64. Segal RA. Selectivity in neurotrophin signaling: Theme and variations. Annu. Rev. Neurosci 2003;26:299–330. [PubMed: 12598680]
65. Choi JS, Kim SY, Park HJ, Cha JH, Choi YS, Kang JE, Chung JW, Chun MH, Lee MY. Upregulation of gp130 and differential activation of STAT and p42/44 MAPK in the rat hippocampus following kainic acid–induced seizures. Brain Res. Mol. Brain Res 2003;119:10–18. [PubMed: 14597225]
66. Suzuki S, Tanaka K, Nogawa S, Dembo T, Kosakai A, Fukuuchi Y. Phosphorylation of signal transducer and activator of transcription-3 (Stat3) after focal cerebral ischemia in rats. Exp. Neurol 2001;170:63–71. [PubMed: 11421584]
67. Planas AM, Soriano MA, Berruezo M, Justicia C, Estrada A, Pitarch S, Ferrer I. Induction of Stat3, a signal transducer and transcription factor, in reactive microglia following transient focal cerebral ischaemia. Eur. J. Neurosci 1996;8:2612–2618. [PubMed: 8996811]
68. Darnell JE Jr. Kerr IM, Stark GR. Jak–STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994;264:1415–1421. [PubMed: 8197455]
69. Zhong Z, Wen Z, Darnell J. STAT3: A STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994;264:95–98. [PubMed: 8140422]
70. Zhong Z, Wen Z, Darnell J. STAT3 and STAT4: Members of the family of signal transducers and activators of transcription. Proc. Natl. Acad. Sci. U.S.A 1994;91:4806–4810. [PubMed: 7545930]
71. Schindler C, Darnell JE Jr. Transcriptional responses to polypeptide ligands: The JAK–STAT pathway. Annu. Rev. Biochem 1995;64:621–651. [PubMed: 7574495]
72. Ihle JN. STATs: Signal transducers and activators of transcription. Cell 1996;84:331–334. [PubMed: 8608586]
73. Aaronson DS, Horvath CM. A road map for those who don't know JAK–STAT. Science 2002;296:1653–1655. [PubMed: 12040185]
74. Bella AJ, Lin G, Tantiwongse K, Garcia M, Lin CS, Brant W, Lue TF. Brain-derived neurotrophic factor (BDNF) acts primarily via the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: Part I. J. Sex. Med 2006;3:815–820. [PubMed: 16942526]

75. Kapur J. Hippocampal neurons express $GABA_A$ receptor insensitive to diazepam in hyperexcitable conditions. Epilepsia 2000;41(Suppl 6):S86–S89. [PubMed: 10999526]

76. Hsu FC, Waldeck R, Faber DS, Smith SS. Neurosteroid effects on GABAergic synaptic plasticity in hippocampus. J. Neurophysiol 2003;89:1929–1940. [PubMed: 12612040]

77. Shieh PB, Ghosh A. Molecular mechanisms underlying activity-dependent regulation of BDNF expression. J. Neurobiol 1999;41:127–134. [PubMed: 10504200]

78. Kojima N, Borlikova G, Sakamoto T, Yamada K, Ikeda T, Itohara S, Niki H, Endo S. Inducible camp early repressor acts as a negative regulator for kindling epileptogenesis and long-term fear memory. J. Neurosci 2008;28:6459–6472. [PubMed: 18562617]

79. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. Clin. Neurophysiol 1972;32:281–294. [PubMed: 4110397]

80. Raol YH, Zhang G, Lund IV, Porter BE, Maronski MA, Brooks-Kayal AR. Increased GABA(A)-receptor alpha1-subunit expression in hippocampal dentate gyrus after early-life status epilepticus. Epilepsia 2006;47:1665–1673. [PubMed: 17054689]

81. Drewett V, Molina H, Millar A, Muller S, von Hesler F, Shaw PE. DNA-bound transcription factor complexes analysed by mass-spectrometry: Binding of novel proteins to the human c-*fos* SRE and related sequences. Nucleic Acids. Res 2001;29:479–487. [PubMed: 11139618]

82. This work was supported by NIH grants R01 NS051710, R01 NS38595, and R01 NS050393 and an American Epilepsy Society Research Initiative grant to A.B.K. and S.J.R. I.V.L. was supported by NIH F31 NS51943. Y.H. was supported by a stipend from the Boston University School of Medicine (BUSM) Program in Biomedical Neurosciences. We are grateful to D. H. Farb for his gift of the human GABRA1 promoter region and for the collegial environment of his BUSM research laboratory. We thank M. E. Greenberg for his gift of the dominant negative MCREB. We also thank R. Wallace and M. Maronski for technical assistance on immunocytochemistry work. I.V.L., Y.H., Y.H.R., R. S. B., S.J.R., and A.R.B.-K. contributed ideas and planning of experiments; I.V.L., Y.H., and R.S.B. carried out experiments; Y.H.R. shared his expertise as a consultant and assisted with the development and implementation of the animal model; R. F. provided all primary brain cultures and assisted with these studies; and I.V.L., Y.H., R.S.B, S.J.R., and A.R.B.-K. wrote the manuscript.

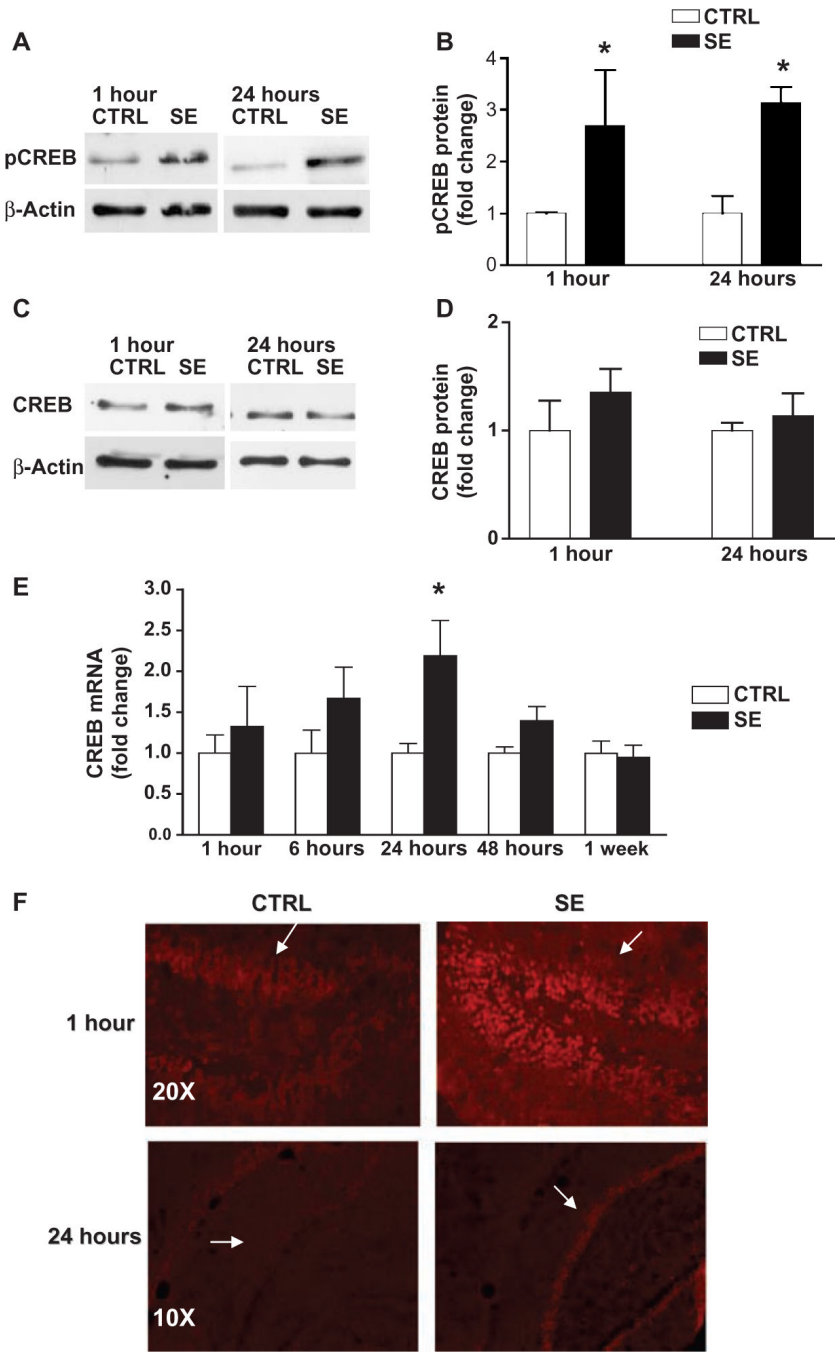

Fig. 1.

pCREB increases in DG after pilocarpine-induced SE. (**A**) Representative Western blot of protein homogenates from DG tissue from SE and control rats 1 hour (left) and 24 hours (right) after onset of SE probed with anti-pCREB and anti–β-actin antibodies. (**B**) Bar graph of densitometry analysis of pCREB protein abundance. pCREB abundance was normalized to β-actin expression to control for loading variability and expressed as fold change relative to mean control values (defined as 1). pCREB protein abundance in DG increased 2.7-fold at 1 hour ($n = 4$ CTRL, $n = 5$ SE, $^*P < 0.05$) and 3.1-fold at 24 hours ($n = 4$ CTRL, $n = 6$ SE, $^*P < 0.05$) after SE. (**C**) Representative Western blot of protein homogenates from DG tissue from SE and control rats 1 hour (left) and 24 hours (right) after onset of SE probed with anti-CREB

and anti–β-actin antibodies. (**D**) Bar graph of densitometry analysis of total CREB protein abundance in DG. CREB abundance was normalized to β-actin and evaluated as described for (A). CREB abundance was unchanged at 1 hour (n = 3 CTRL, n = 5 SE) or 24 hours (n = 4 CTRL, n = 5 SE) after SE. (**E**) Quantification of RT-PCR analysis of CREB mRNA expression in DG from SE and Control rats. CREB expression was normalized to cyclophilin expression in the same samples and expressed as fold change compared to controls (defined as 1). CREB mRNA expression did not change at 1 hour (n = 3 CTRL, n = 4 SE), 6 hours (n = 4 CTRL, n = 6 SE), 48 hours (n = 4 CTRL, n = 6 SE), or 1 week after SE (n = 4 CTRL, n = 5 SE). SE did result in an increase in CREB mRNA expression at 24 hours after SE (2.2-fold, n = 4 CTRL, n = 6 SE, $^*P < 0.05$). (**F**) Immunocytochemical staining of the DG performed with anti-pCREB primary antibody and rhodamine-conjugated secondary antibody at 1 hour (top, 20× magnification) and 24 hours (bottom, 10× magnification) after SE shows an increase in pCREB expression throughout the DGC layer (right) compared to control (left). All data are presented as mean ± SEM and all statistics were performed with the Mann–Whitney test.

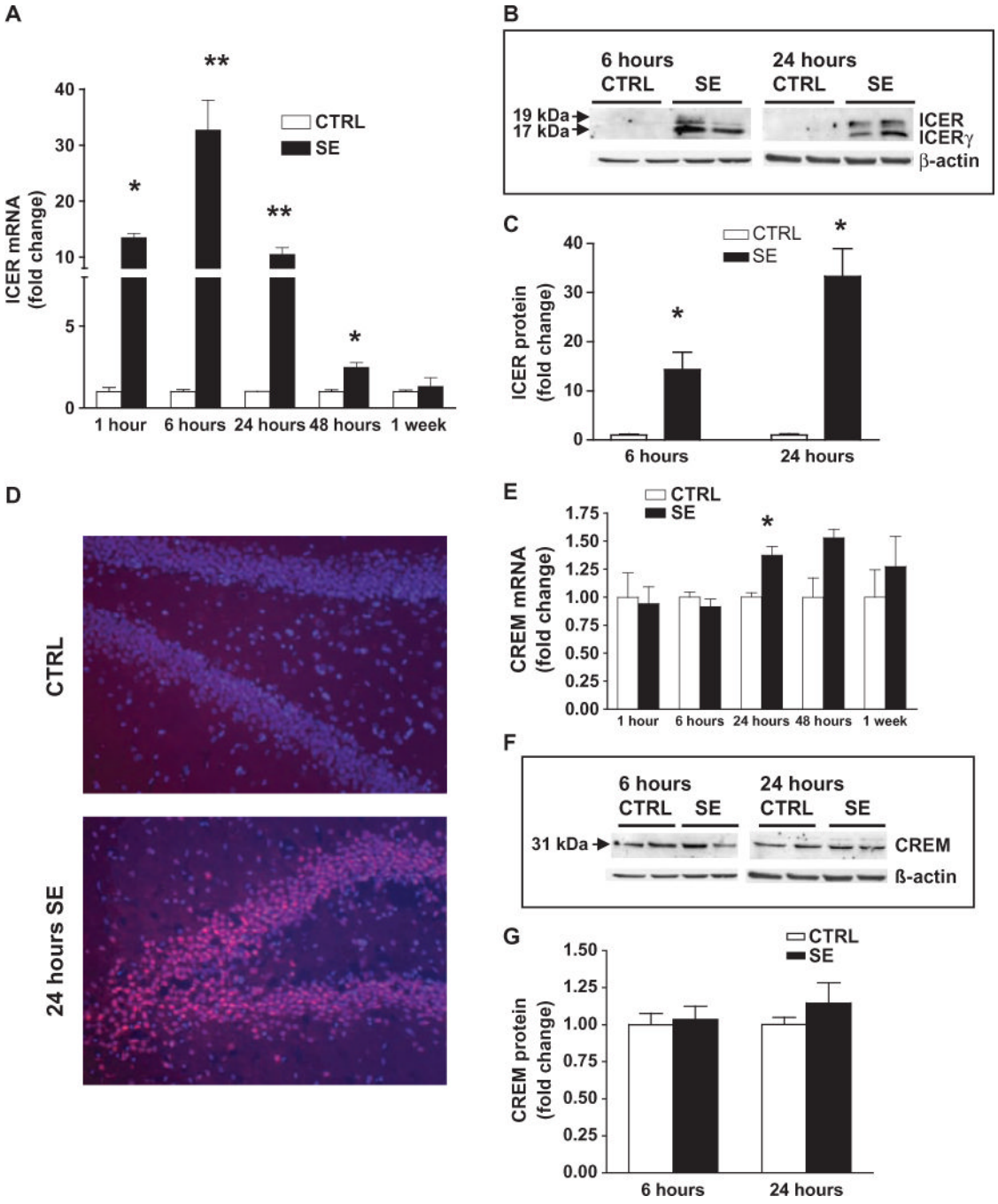

Fig. 2.

ICER increases in DG after SE. (**A**) Quantification of RT-PCR analysis of ICER mRNA expression in DG at 1, 6, 24, and 48 hours and 1 week after SE. ICER expression was normalized to cyclophilin expression and expressed as fold change compared to controls (defined as 1). ICER showed robust increases in mRNA expression in DG from SE compared to control rats starting at 1 hour after SE and continuing until 48 hours after SE (1 hour: 13.6-fold, $n = 4$ CTRL, $n = 4$ SE, $^{*}P < 0.05$; 6 hours: 32.8-fold, $n = 4$ CTRL, $n = 6$ SE, $^{**}P < 0.01$; 24 hours: 10.5-fold, $n = 4$ CTRL, $n = 6$ SE, $^{**}P < 0.01$; 48 hours: 2.5-fold, $n = 5$ CTRL, $n = 6$ SE, $^{*}P < 0.05$). (**B**) Representative Western blot of protein homogenates from DG of control and SE adult rats at 6 hours (left) and 24 hours (right) after SE reacted with anti-CREM/ICER

antibody (top gel) or anti–β-actin antibody (bottom gel). Note the bands at 19 and 17 kDa corresponding to the size of ICER and ICERγ present in the SE rats that are absent in controls. (**C**) Bar graph of densitometry analysis of ICER protein. Expression of both bands corresponding to ICER and ICERγ were added and normalized to β-actin expression in the same samples and expressed as fold change relative to controls (defined as 1). ICER protein increased at 6 hours (17.3-fold, $n = 4$ CTRL, $n = 5$ SE, $^*P < 0.05$) and at 24 hours (33.3-fold, $n = 4$ SE, $n = 4$ CTRL, $^*P < 0.05$). (**D**) Immunocytochemical staining of the DG performed with rhodamine-tagged anti-CREM/ICER antibody (red) and co-stained with 4′,6′-diamidino-2-phenylindole (blue; 10× magnification). The DGC layer shows widespread ICER/CREM immunostaining at 24 hours after SE (bottom) compared to the nearly absent ICER/CREM staining in controls (top). (**E**) Quantification of RT-PCR analysis of CREM mRNA expression in DG at 1, 6, 24 and 48 hours and 1 week after SE. CREM expression was normalized to cyclophilin expression and evaluated as described for (A). A modest increase in CREM mRNA was detected in DG only at 24 hours after SE (1.37-fold, $n = 4$ CTRL, $n = 6$ SE, $^*P < 0.05$). (**F**) Representative Western blot of protein homogenates from DG of control and SE adult rats at 6 hours (left) and 24 hours (right) after SE reacted with anti-CREM/ICER antibody (top gel), or anti−β-actin antibody (bottom gel). Note the band at 31 kDa corresponding to CREM-1. G) Bar graph of densitometry analysis of CREM protein. CREM abundance was normalized to β-actin as described for Panel B. CREM protein did not change at either 6 hours ($n = 6$ CTRL, $n = 6$ SE) or 24 hours ($n = 6$ CTRL, $n = 6$ SE) after SE. All data are presented as mean ± SEM and all statistics were performed with the Mann–Whitney test.

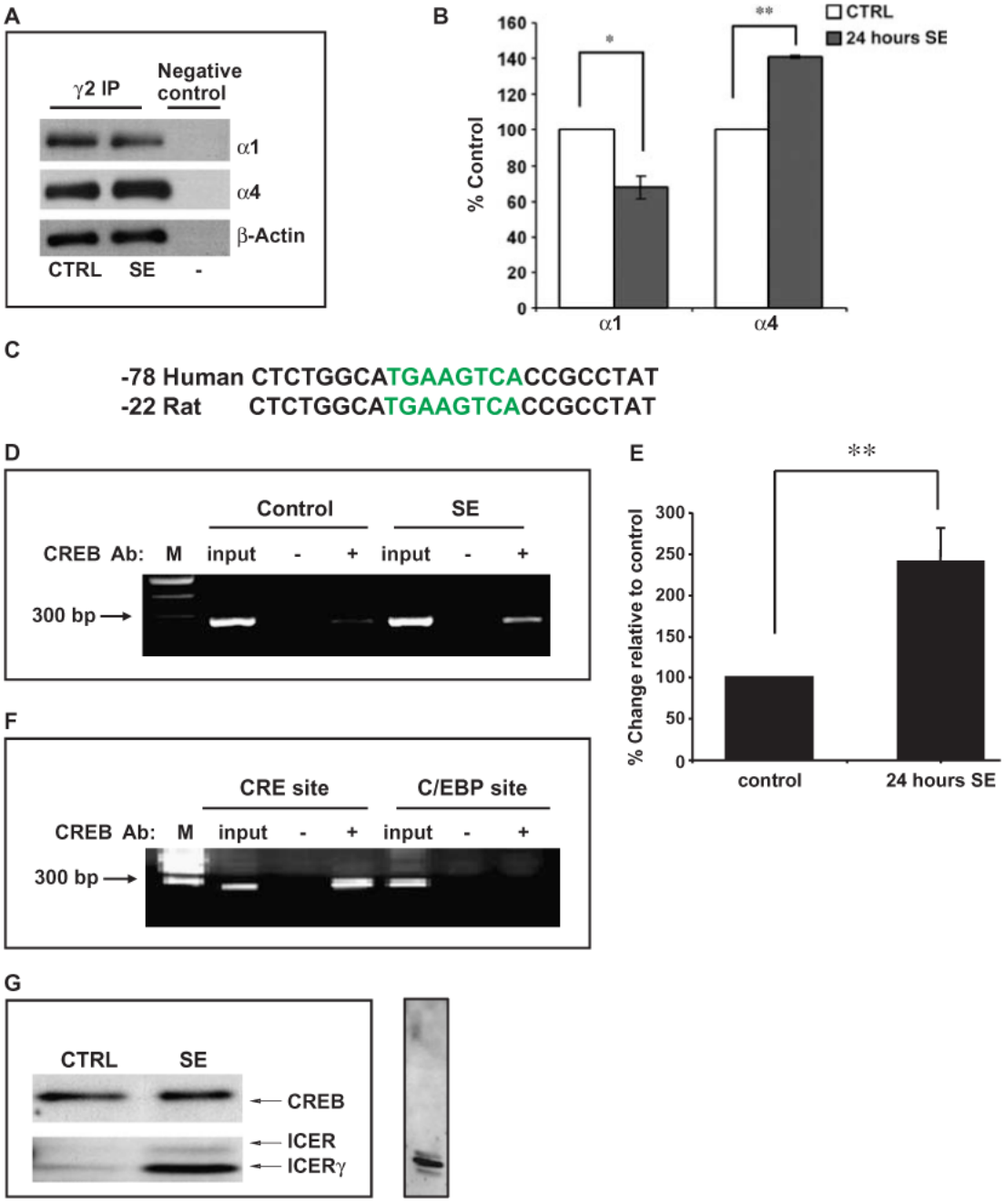

Fig. 3.

Increased pCREB and ICER binding to *Gabra1-p* after SE is associated with a reduction in the abundance of α1γ2-containing $GABA_A$Rs in DG. (**A**) SE alters the abundance of α1 and α4 subunits in γ2-containing $GABA_A$Rs of the DG. Representative Western blot shows a decrease in α1 and increase in α4 subunit abundance of γ2-containing $GABA_A$Rs after pilocarpine-induced SE. Coimmunoprecipitation (IP) was performed on DG from animals 24 hours after SE and from controls. Whole-cell protein extracts from DG were applied to the γ2 subunit antibody–coupled AminoLink Plus gel. After overnight incubation, the γ2 subunit antibody–coupled protein complexes were eluted and separated by SDS-PAGE and then followed by Western blot with anti-α1, anti-α4, and anti-γ2 subunit antibodies. Negative controls were performed by coupling normal rabbit IgG to AminoLink Plus gel to test for

proteins that may bind nonspecifically. (**B**) Abundance of α1, α4, and γ2 subunits was quantified by densitometry (*$P < 0.05$, **$P < 0.01$; $n = 3$ rats per group). Normalized data (α1 subunit/γ2 subunit, α4 subunit/γ2 subunit) are presented as mean ± SEM and expressed as percent change with respect to control animals (defined as 100%). (**C**) Sequence conservation of the CRE site (in gray) in the human and rat α1 promoters. (**D** and **E**) Association of Ser^{133} pCREB with endogenous *Gabra1* increases after SE as assayed by ChIP. Genomic DNA and associated protein complexes were collected from the DG of rats 24 hours after SE or controls and immunoprecipitated with an anti-pCREB antibody (Ab). Immunoprecipitated *Gabra1* genomic DNA fragments were detected by PCR (D) or real-time PCR (E) with the use of specific primers that flank the CRE site in the rat Gabra1 gene ($n = 4$ rats per group, **$P < 0.01$). "Input" lane shows PCR band from genomic DNA before immunoprecipitation (positive control); "−" lane is PCR band from genomic DNA immunoprecipitated with IgG only (negative control); and "+" lane is PCR band from genomic DNA immunoprecipitated with anti-pCREB antibody. (**F**) As a control for specific antibody precipitation and for the specificity of binding of pCREB to the CRE site in *Gabra1*, PCR was performed with specific primers that flank a potential C/EBP site that is 545 bp upstream of the *Gabra1*:CRE. Template for amplification came from genomic DNA fragments immunoprecipitated with anti-pCREB antibody from pooled control and SE DG samples. Note the absence of any detectable binding of pCREB to this region upstream of *Gabra1*:CRE site (representative result from $n = 3$). (**G**) CREB and ICER both bind directly to the CRE site in the *Gabra1-p* as detected by DNA pull-down assay followed by Western blot. Nuclear extract was obtained from hippocampal tissue 24 hours after SE. Nuclear extract (600 μg) was incubated with 1 mg of streptavidin Dynabeads bound to *Gabra1*:CRE oligonucleotides (see Materials and Methods). Western blot analysis was performed with anti-CREB antibody and anti–CREM-1 antibody (that recognizes multiple CREM isoforms including ICER). Note detection of ICER and ICERγ after SE (left panel). No other CREM isoforms were detected (right panel). Each binding assay (per condition, control and SE) was performed on 600 μg of nuclear extracts pooled from three control or three SE animals. Data are representative of two independent experiments (three animals pooled per condition per experiment) that both showed a marked increase in the binding of ICER to the *GABRA1*:CRE 24 hours after SE.

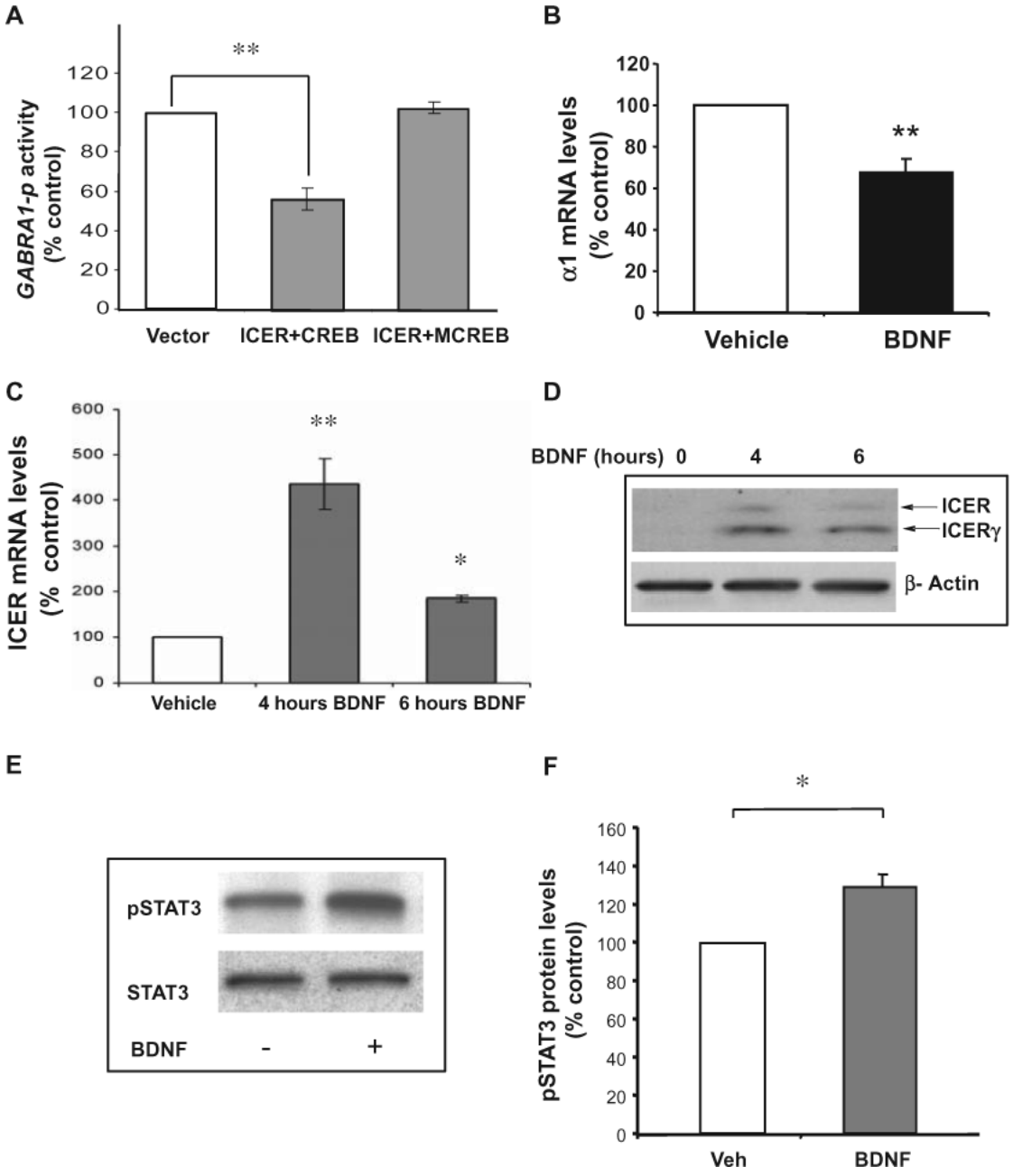

Fig. 4.

GABRA1-p activity depends on pCREB, ICER, and BDNF. (**A**) Bar graph displaying *GABRA1* promoter activity from *GABRA1* promoter/luciferase construct transfected into primary cultured hippocampal neurons in comparison with promoterless basic vector. Luciferase activity is shown as counts per minute per microgram protein. (**$P < 0.01$, mean ± SE; $n = 4$ per group). Overexpression of ICER or CREB and ICER with the *GABRA1* promoter/luciferase reporter construct decreases promoter activity in primary cultured hippocampal neurons compared to control. Luciferase activity (counts per minute) was normalized to the amount of protein (micrograms) in each sample. Results are expressed as percent activity measured from control [*GABRA1* promoter/luciferase reporter construct cotransfected with expression vector backbone alone (Vector)], defined as 100% (**$P < 0.01$;

mean ± SE; $n = 4$ per group). Ser^{133} phosphorylation of CREB is required for the repression of *GABRA1* promoter activity in neurons cotransfected with ICER expression constructs. Hippocampal neurons were cotransfected with ICER and/or the dominant-negative MCREB construct. 24 hours after transfection, cultures were harvested and luciferase assays were performed. Luciferase activity (counts per minute) was normalized to the amount of protein (micrograms) in each sample. Results are expressed as percent activity measured from control [*GABRA1* promoter/reporter constructs cotransfected with expression vector backbone alone (Vector)], defined as 100%. (**$P < 0.01$; mean ± SE; $n = 4$ per condition). (**B**) Cultures were treated with either vehicle (H_2O) or BDNF (50 ng/ml) for 6 hours. Total RNA was extracted and real-time PCR was performed with PCR primers and probes specific for $GABA_AR$ α1 subunit and cyclophilin mRNAs. Experimental data were normalized to cyclophilin. mRNA abundance in each treatment group is expressed relative to the vehicle (H_2O) (**$P < 0.01$; mean ± SE; $n = 5$). (**C** and **D**) Primary cultured hippocampal neurons were treated with either vehicle (H_2O) or BDNF (50 ng/ml) for the indicated time. Total RNA or protein was extracted and ICER mRNA or protein abundance was measured by real-time RT-PCR (C) or Western blot (D). Data were normalized to cyclophilin for mRNA measurement (*$P < 0.05$, **$P < 0.01$; mean ± SE; $n = 4$). Proteins were visualized by enhanced chemiluminescence (ECL). β-Actin protein abundance did not vary with BDNF treatment and was used as an internal control. (**E** and **F**) BDNF stimulation of primary cultured hippocampal neurons increases pSTAT3 abundance. Hippocampal cultures were treated with vehicle (H_2O) or BDNF (50 ng/ml) for 30 min. Cellular protein was extracted and Western blot (E) was performed to determine pSTAT3 abundance. Total STAT3 did not vary with BDNF treatment and was used as a loading control. Normalized data (pSTAT3/STAT3) are presented as a histogram (F) and expressed as percent change with respect to vehicle (Veh)-treated samples (vehicle control is set at 100%; *$P < 0.05$, $n = 4$).

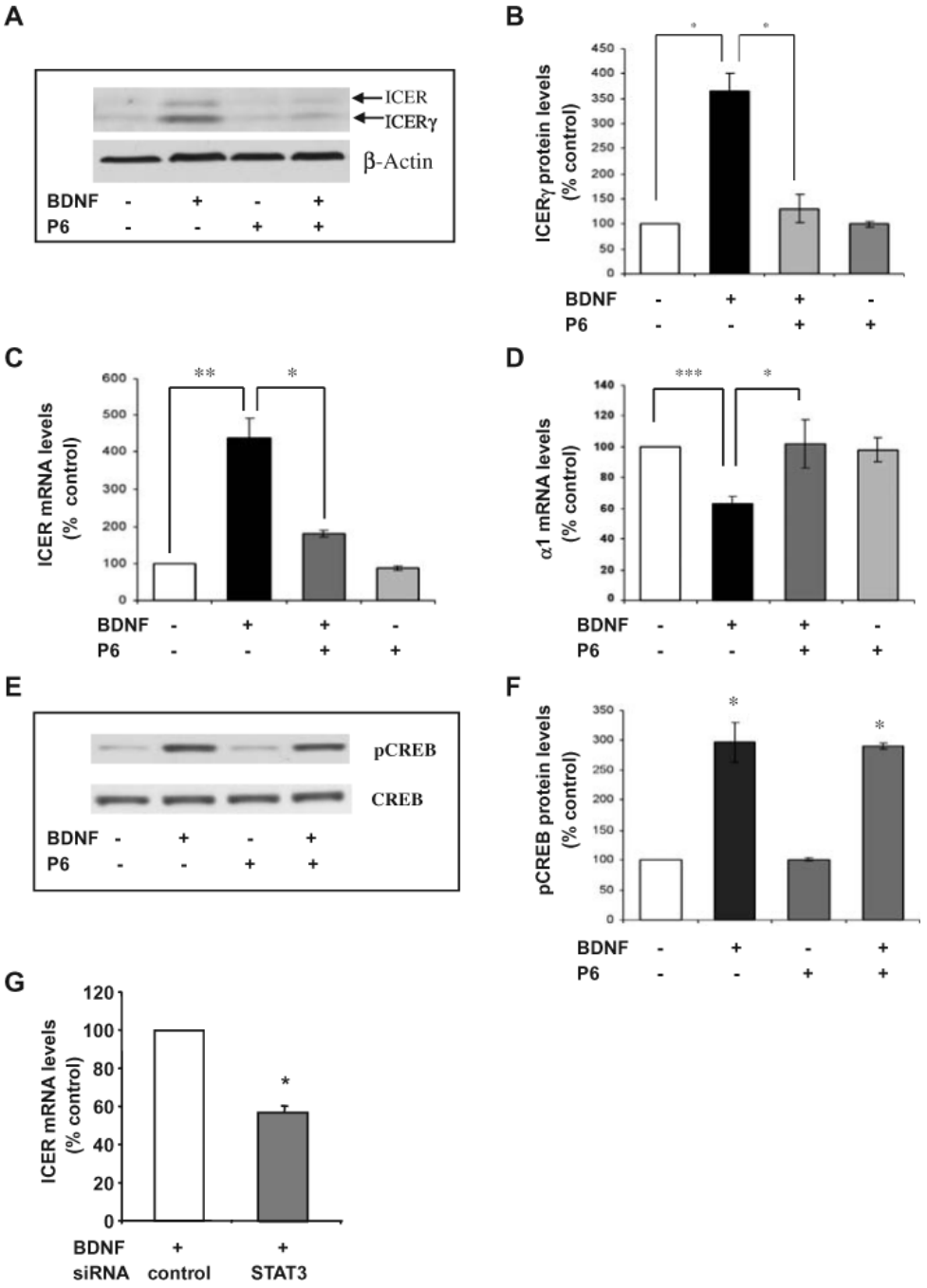

Fig. 5.

JAK/STAT inhibition restores α1 subunit expression in cultured hippocampal neurons. (**A** and **B**) ICER protein/hippocampal cultures were pretreated with a pan JAK inhibitor P6 (500 nM) or vehicle (DMSO) for 1 hour. Cultures were then treated with either vehicle (H_2O) or BDNF (50 ng/ml) for an additional 4 hours. Total cellular proteins were extracted. Western blot was performed to determine the abundance of ICER protein. β-Actin abundance did not vary with BDNF treatment and was used as an internal control. A representative Western blot is shown (A). Normalized data (ICER/β-actin) are presented as a bar graph (B) and expressed as percent change with respect to vehicle-treated cultures (defined as 100%). Significant changes are as indicated (*$P < 0.05$; mean ± SEM; $n = 3$). Proteins were visualized by ECL. (**C**) ICER mRNAs:

hippocampal cultures were treated as described above. Cultures were harvested and total RNA was extracted. ICER mRNA abundance was measured by real-time RT-PCR and data were normalized to cyclophilin expression (*$P < 0.05$, **$P < 0.01$; mean ±SE; $n = 4$). (**D**) *GABRA1* mRNAs: cultures were treated as described above with the exception that the exposure to BDNF was for 6 hours. α1 mRNA abundance was measured by RT-PCR (*$P < 0.05$, **$P < 0.01$; mean ±SE; $n = 5$). (**E** and **F**) pCREB protein: cultures were treated as described above with the exception that exposure to BDNF was for 30 min. Total cellular proteins were analyzed by Western blot with polyclonal CREB and pCREB antibodies. Proteins were visualized by ECL after incubation with an anti-rabbit HRP-conjugated antibody (E). Normalized data (pCREB/CREB) are presented as mean ± SEM and expressed as percent change with respect to vehicle-treated cultures (defined as 100%) (F). Total CREB abundance did not vary with BDNF treatment and was used as an internal control. Significant changes are as indicated (*$P < 0.05$; mean ± SEM; $n = 3$). (**G**) STAT3 siRNA knockdown: BDNF-induced ICER mRNA synthesis is reversed after treatment with STAT3-specific siRNAs (*$P < 0.05$; $n = 5$). STAT3 and scramble (control) siRNAs were transfected into primary hippocampal neurons before BDNF treatment (50 ng/ml). After 4 hours of BDNF treatment, RNA was isolated and mRNA expression of ICER was determined by RT-PCR. ICER mRNA expression was normalized to cyclophilin expression to control for loading variability and expressed as percentage change from scramble control (control set at 100%).

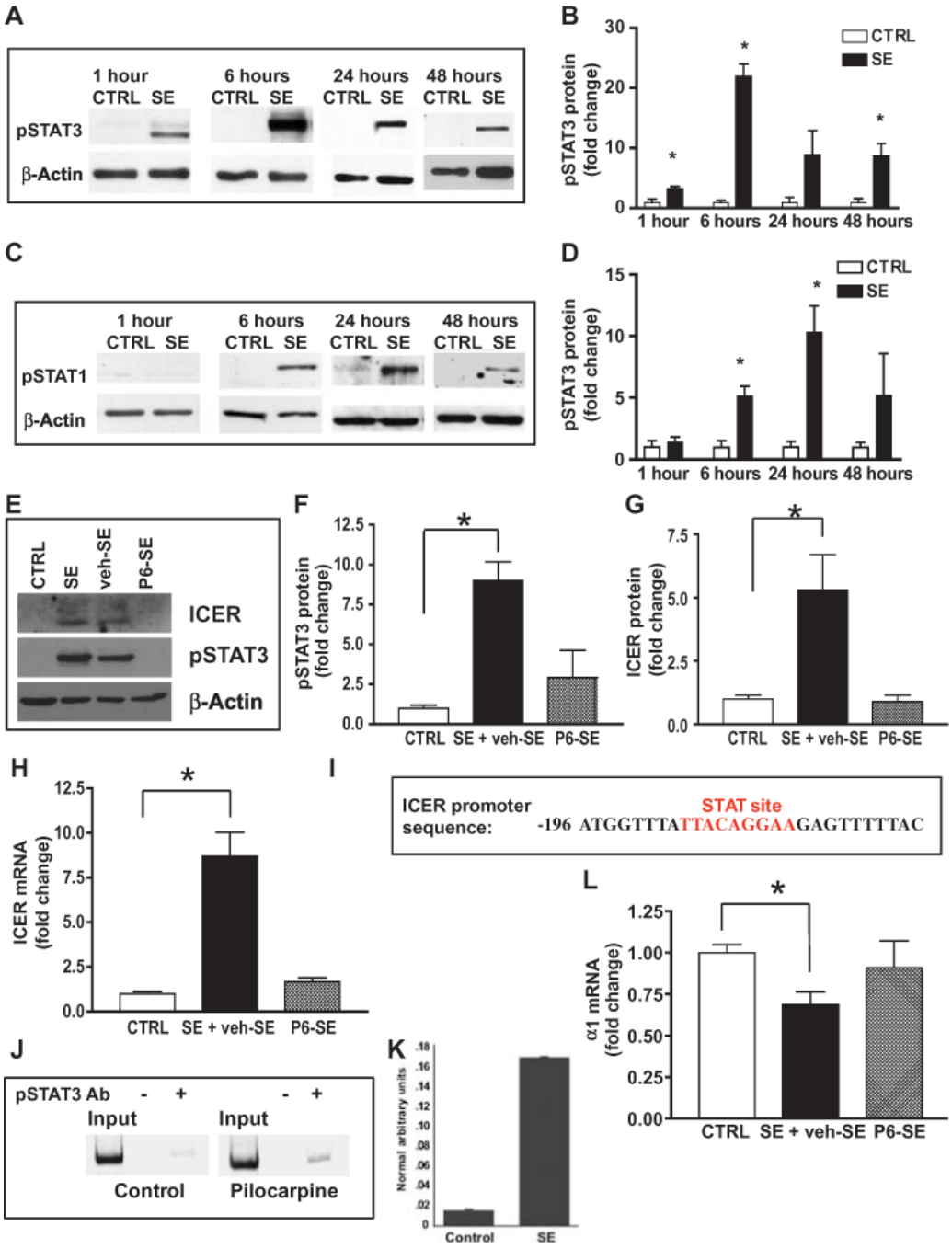

Fig. 6.

JAK/STAT inhibition blocks ICER abundance and restores α1 subunit expression in DG after SE. pSTAT3 and pSTAT1 increase after SE. Representative Western blot of protein homogenates of DG tissue from SE and control rats 1, 6, 24, and 48 hours after onset of SE reacted with (**A**) anti-pSTAT3, (**C**) anti-pSTAT1, and anti–β-actin antibodies. Bar graphs of densitometry analysis of pSTAT3 (**B**) and pSTAT1 (**D**) protein abundance. pSTAT3 and pSTAT1 abundance was normalized to β-actin to control for loading variability and expressed as fold change relative to mean control values (defined as 1). pSTAT3 protein abundance in DG increased 3.8-fold at 1 hour ($n = 4$ CTRL, $n = 5$ SE, $^*P < 0.05$), 21.9-fold at 6 hours ($n = 4$ CTRL, $n = 5$ SE, $^*P < 0.05$), 8.9-fold at 24 hours ($n = 4$ CTRL, $n = 6$ SE, not significant),

and 8.6-fold at 48 hours ($n = 4$ CTRL, $n = 5$ SE, $^*P < 0.05$) after SE. pSTAT1 protein abundance in DG did not increase at 1 hour ($n = 4$ CTRL, $n = 5$ SE) but increased 5.2-fold at 6 hours ($n = 4$ CTRL, $n = 5$ SE, $^*P < 0.05$), 10.4-fold at 24 hours ($n = 4$ CTRL, $n = 6$ SE, $^*P < 0.05$), and were not significantly different at 48 hours ($n = 4$ CTRL, $n = 5$ SE) after SE. All data for (B) and (D) are represented as mean ± SE and all statistics were measured by Mann–Whitney test. (**E**) Representative Western blot showing amounts of pSTAT3 and ICER in DG tissue 48 hours after administration of a subconvulsive dose of pilocar-pine (CTRL), pilocarpine-induced SE alone (SE), SE after 48 hours of Alzet pump continuous slow infusion (0.5 μl / hour) of DMSO vehicle (veh-SE) or 1 mM P6 infusion into the DG (P6-SE). Animals that were injected with vehicle before undergoing SE (veh-SE) showed amounts of pSTAT3 and ICER comparable to noninjected SE (SE) animals and significantly increased versus control, so SE and veh-SE groups were pooled. (**F**) Densitometry analysis of pSTAT3 protein. pSTAT3 was normalized to β-actin expression to control for loading amount variability and expressed as fold change relative to mean control values (defined as 1). pSTAT3 abundance was 9.0-fold higher in the SE + veh-SE group versus control ($n = 4$ CTRL, $n = 5$ SE + veh-SE, $^*P < 0.05$) but was not significantly higher in P6-SE ($n = 4$) versus CTRL ($P > 0.05$). (**G**) Bar graph of densitometry analysis of ICER protein. ICER was normalized to β-actin expression to control for loading amount variability and expressed as fold change relative to mean control values (defined as 1). ICER abundance was 5.7-fold higher in the SE + veh-SE group versus control ($n = 4$ CTRL, $n = 5$ SE + veh-SE, $^*P < 0.05$). Abundance in the P6-SE group was not significantly different from that of control ($n = 4$ P6-SE, $P > 0.05$). (**H**) β-Actin of RT-PCR analysis of ICER mRNA 48 hours after SE. ICER mRNA was normalized to cyclophilin expression to control for loading amount variability and expressed as fold change relative to mean control ($n = 4$) values (defined as 1). ICER mRNA in DG increased 8.7-fold in rats in the SE + veh-SE group ($n = 4$ CTRL, $n = 6$ SE + veh-SE, $^{**}P < 0.01$) but did not change in the P6-SE group ($n = 4$ P6-SE, $P > 0.05$) compared to control. (**I**) A STAT site in *ICER-p*. (**J**) SE increases pSTAT3 binding to endogenous *ICER-p* in DG of adult rats. ChIP was performed on slices of DG 24 hours after pilocarpine-induced SE or controls. In vivo cross-linked fragments of chromatin-associated DNA were precipitated with antibodies to pSTAT3. Fragmented DNA was amplified with specific primers flanking the STAT sites on the promoter. A representative sample is shown for each treatment group (J). Input is genomic DNA before precipitation with antibody. Presence or absence of antibody is represented as "+" or "−", respectively. Bar graph showing quantification of pSTAT3 precipitation (**K**). $^*P = 0.001$ (Student's *t* test, $n = 3$ animals per condition. (**L**) α1 mRNA expression in DG decreased 69% in rats in the SE + veh-SE group ($n = 4$ CTRL, $n = 6$ SE + veh-SE, $^*P < 0.05$) but did not change in the P6-SE group ($n = 4$ P6-SE, $P > 0.05$) compared to control. Data are represented as mean ± SE and were analyzed statistically with Kruskal–Wallis nonparametric analysis of variance with Dunn posttest to correct for multiple comparisons.